Advanced Nutrition and Human Metabolism

SEVENTH EDITION

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Dietary Reference Intakes (DRI)

The Dietary Reference Intakes (DRI) include two sets of values that serve as goals for nutrient intake—Recommended Dietary Allowances (RDA) and Adequate Intakes (AI). The RDA reflect the average daily amount of a nutrient considered adequate to meet the needs of most healthy people. If there is insufficient evidence to determine an RDA, an AI is set. AI are more tentative than RDA, but both may be used as goals for nutrient intakes. (Chapter 9 provides more details.)

In addition to the values that serve as goals for nutrient intakes (presented in the tables on these two pages), the DRI include a set of values called Tolerable Upper Intake Levels (UL). The UL represent the maximum amount of a nutrient that appears safe for most healthy people to consume on a regular basis. Turn the page for a listing of the UL for selected vitamins and minerals.

Estimated Energy Requirements (EER), Recommended Dietary Allowances (RDA), and Adequate Intakes (AI) for Water, Energy, and the Energy Nutrients

Age (yr)	Reference BMI (kg/m²)	Reference Height cm (in)	Reference Weight kg (lb)	Water[a] AI (L/day)	Energy EER[b] (kcal/day)	Carbohydrate RDA (g/day)	Total Fiber AI (g/day)	Total Fat AI (g/day)	Linoleic Acid AI (g/day)	Linolenic Acid[c] AI (g/day)	Protein RDA (g/day)[d]	Protein RDA (g/kg/day)
Males												
0–0.5	—	62 (24)	6 (13)	0.7[e]	570	60	—	31	4.4	0.5	9.1	1.52
0.5–1	—	71 (28)	9 (20)	0.8[f]	743	95	—	30	4.6	0.5	11	1.20
1–3[g]	—	86 (34)	12 (27)	1.3	1046	130	19	—	7	0.7	13	1.05
4–8[g]	15.3	115 (45)	20 (44)	1.7	1742	130	25	—	10	0.9	19	0.95
9–13	17.2	144 (57)	36 (79)	2.4	2279	130	31	—	12	1.2	34	0.95
14–18	20.5	174 (68)	61 (134)	3.3	3152	130	38	—	16	1.6	52	0.85
19–30	22.5	177 (70)	70 (154)	3.7	3067[h]	130	38	—	17	1.6	56	0.80
31–50	22.5[i]	177 (70)[i]	70 (154)[i]	3.7	3067[h]	130	38	—	17	1.6	56	0.80
>50	22.5[i]	177 (70)[i]	70 (154)[i]	3.7	3067[h]	130	30	—	14	1.6	56	0.80
Females												
0–0.5	—	62 (24)	6 (13)	0.7[e]	520	60	—	31	4.4	0.5	9.1	1.52
0.5–1	—	71 (28)	9 (20)	0.8[f]	676	95	—	30	4.6	0.5	11	1.20
1–3[g]	—	86 (34)	12 (27)	1.3	992	130	19	—	7	0.7	13	1.05
4–8[g]	15.3	115 (45)	20 (44)	1.7	1642	130	25	—	10	0.9	19	0.95
9–13	17.4	144 (57)	37 (81)	2.1	2071	130	26	—	10	1.0	34	0.95
14–18	20.4	163 (64)	54 (119)	2.3	2368	130	26	—	11	1.1	46	0.85
19–30	21.5	163 (64)	57 (126)	2.7	2403[j]	130	25	—	12	1.1	46	0.80
31–50	21.5[i]	163 (64)[i]	57 (126)[i]	2.7	2403[j]	130	25	—	12	1.1	46	0.80
>50	21.5[i]	163 (64)[i]	57 (126)[i]	2.7	2403[j]	130	21	—	11	1.1	46	0.80
Pregnancy												
1st trimester				3.0	+0	175	28	—	13	1.4	46	0.80
2nd trimester				3.0	+340	175	28	—	13	1.4	71	1.10
3rd trimester				3.0	+452	175	28	—	13	1.4	71	1.10
Lactation												
1st 6 months				3.8	+330	210	29	—	13	1.3	71	1.30
2nd 6 months				3.8	+400	210	29	—	13	1.3	71	1.30

NOTE: For all nutrients, values for infants are AI. Dashes indicate that values have not been determined.

[a]The water AI includes drinking water, water in beverages, and water in foods; in general, drinking water and other beverages contribute about 70 to 80 percent, and foods, the remainder. Conversion factors: 1 L = 33.8 fluid oz; 1 L = 1.06 qt; 1 cup = 8 fluid oz.

[b]The Estimated Energy Requirement (EER) represents the average dietary energy intake that will maintain energy balance in a healthy person of a given gender, age, weight, height, and physical activity level. The values listed are based on an "active" person at the reference height and weight and at the midpoint ages for each group until age 19. Chapter 8 provides equations and tables to determine estimated energy requirements.

[c]The linolenic acid referred to in this table and text is the omega-3 fatty acid known as alpha-linolenic acid.

[d]The values listed are based on reference body weights.

[e]Assumed to be from human milk.

[f]Assumed to be from human milk and complementary foods and beverages. This includes approximately 0.6 L (~2½ cups) as total fluid including formula, juices, and drinking water.

[g]For energy, the age groups for young children are 1–2 years and 3–8 years.

[h]For males, subtract 10 kcalories per day for each year of age above 19.

[i]Because weight need not change as adults age if activity is maintained, reference weights for adults 19 through 30 years are applied to all adult age groups.

[j]For females, subtract 7 kcalories per day for each year of age above 19.

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Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) for Vitamins

Age (yr)	Thiamin RDA (mg/day)	Riboflavin RDA (mg/day)	Niacin RDA (mg/day)[a]	Biotin AI (µg/day)	Pantothenic acid AI (mg/day)	Vitamin B_6 RDA (mg/day)	Folate RDA (µg/day)[b]	Vitamin B_{12} RDA (µg/day)	Choline AI (mg/day)	Vitamin C RDA (mg/day)	Vitamin A RDA (µg/day)[c]	Vitamin D RDA (IU/day)[d]	Vitamin E RDA (mg/day)[e]	Vitamin K AI (µg/day)
Infants														
0–0.5	0.2	0.3	2	5	1.7	0.1	65	0.4	125	40	400	400 (10 µg)	4	2.0
0.5–1	0.3	0.4	4	6	1.8	0.3	80	0.5	150	50	500	400 (10 µg)	5	2.5
Children														
1–3	0.5	0.5	6	8	2	0.5	150	0.9	200	15	300	600 (15 µg)	6	30
4–8	0.6	0.6	8	12	3	0.6	200	1.2	250	25	400	600 (15 µg)	7	55
Males														
9–13	0.9	0.9	12	20	4	1.0	300	1.8	375	45	600	600 (15 µg)	11	60
14–18	1.2	1.3	16	25	5	1.3	400	2.4	550	75	900	600 (15 µg)	15	75
19–30	1.2	1.3	16	30	5	1.3	400	2.4	550	90	900	600 (15 µg)	15	120
31–50	1.2	1.3	16	30	5	1.3	400	2.4	550	90	900	600 (15 µg)	15	120
51–70	1.2	1.3	16	30	5	1.7	400	2.4	550	90	900	600 (15 µg)	15	120
>70	1.2	1.3	16	30	5	1.7	400	2.4	550	90	900	800 (20 µg)	15	120
Females														
9–13	0.9	0.9	12	20	4	1.0	300	1.8	375	45	600	600 (15 µg)	11	60
14–18	1.0	1.0	14	25	5	1.2	400	2.4	400	65	700	600 (15 µg)	15	75
19–30	1.1	1.1	14	30	5	1.3	400	2.4	425	75	700	600 (15 µg)	15	90
31–50	1.1	1.1	14	30	5	1.3	400	2.4	425	75	700	600 (15 µg)	15	90
51–70	1.1	1.1	14	30	5	1.5	400	2.4	425	75	700	600 (15 µg)	15	90
>70	1.1	1.1	14	30	5	1.5	400	2.4	425	75	700	800 (20 µg)	15	90
Pregnancy														
≤18	1.4	1.4	18	30	6	1.9	600	2.6	450	80	750	600 (15 µg)	15	75
19–30	1.4	1.4	18	30	6	1.9	600	2.6	450	85	770	600 (15 µg)	15	90
31–50	1.4	1.4	18	30	6	1.9	600	2.6	450	85	770	600 (15 µg)	15	90
Lactation														
≤18	1.4	1.6	17	35	7	2.0	500	2.8	550	115	1200	600 (15 µg)	19	75
19–30	1.4	1.6	17	35	7	2.0	500	2.8	550	120	1300	600 (15 µg)	19	90
31–50	1.4	1.6	17	35	7	2.0	500	2.8	550	120	1300	600 (15 µg)	19	90

NOTE: For all nutrients, values for infants are AI.

[a]Niacin recommendations are expressed as niacin equivalents (NE), except for recommendations for infants younger than 6 months, which are expressed as preformed niacin.

[b]Folate recommendations are expressed as dietary folate equivalents (DFE).

[c]Vitamin A recommendations are expressed as retinol activity equivalents (RAE).

[d]Vitamin D recommendations are expressed as cholecalciferol and assume an absence of adequate exposure to sunlight.

[e]Vitamin E recommendations are expressed as α-tocopherol.

Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) for Minerals

Age (yr)	Sodium AI (mg/day)	Chloride AI (mg/day)	Potassium AI (mg/day)	Calcium RDA (mg/day)	Phosphorus RDA (mg/day)	Magnesium RDA (mg/day)	Iron RDA (mg/day)	Zinc RDA (mg/day)	Iodine RDA (µg/day)	Selenium RDA (µg/day)	Copper RDA (µg/day)	Manganese AI (mg/day)	Fluoride AI (mg/day)	Chromium AI (µg/day)	Molybdenum RDA (µg/day)
Infants															
0–0.5	120	180	400	200	100	30	0.27	2	110	15	200	0.003	0.01	0.2	2
0.5–1	370	570	700	260	275	75	11	3	130	20	220	0.6	0.5	5.5	3
Children															
1–3	1000	1500	3000	700	460	80	7	3	90	20	340	1.2	0.7	11	17
4–8	1200	1900	3800	1000	500	130	10	5	90	30	440	1.5	1.0	15	22
Males															
9–13	1500	2300	4500	1300	1250	240	8	8	120	40	700	1.9	2	25	34
14–18	1500	2300	4700	1300	1250	410	11	11	150	55	890	2.2	3	35	43
19–30	1500	2300	4700	1000	700	400	8	11	150	55	900	2.3	4	35	45
31–50	1500	2300	4700	1000	700	420	8	11	150	55	900	2.3	4	35	45
51–70	1300	2000	4700	1000	700	420	8	11	150	55	900	2.3	4	30	45
>70	1200	1800	4700	1200	700	420	8	11	150	55	900	2.3	4	30	45
Females															
9–13	1500	2300	4500	1300	1250	240	8	8	120	40	700	1.6	2	21	34
14–18	1500	2300	4700	1300	1250	360	15	9	150	55	890	1.6	3	24	43
19–30	1500	2300	4700	1000	700	310	18	8	150	55	900	1.8	3	25	45
31–50	1500	2300	4700	1000	700	320	18	8	150	55	900	1.8	3	25	45
51–70	1300	2000	4700	1200	700	320	8	8	150	55	900	1.8	3	20	45
>70	1200	1800	4700	1200	700	320	8	8	150	55	900	1.8	3	20	45
Pregnancy															
≤18	1500	2300	4700	1300	1250	400	27	12	220	60	1000	2.0	3	29	50
19–30	1500	2300	4700	1000	700	350	27	11	220	60	1000	2.0	3	30	50
31–50	1500	2300	4700	1000	700	360	27	11	220	60	1000	2.0	3	30	50
Lactation															
≤18	1500	2300	5100	1300	1250	360	10	13	290	70	1300	2.6	3	44	50
19–30	1500	2300	5100	1000	700	310	9	12	290	70	1300	2.6	3	45	50
31–50	1500	2300	5100	1000	700	320	9	12	290	70	1300	2.6	3	45	50

NOTE: For all nutrients, values for infants are AI.

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Tolerable Upper Intake Levels (UL) for Vitamins

Age (yr)	Niacin (mg/day)[a]	Vitamin B_6 (mg/day)	Folate (µg/day)[a]	Choline (mg/day)	Vitamin C (mg/day)	Vitamin A (µg/day)[b]	Vitamin D (IU/day)	Vitamin E (mg/day)[c]
Infants								
0–0.5	—	—	—	—	—	600	1000 (25 µg)	—
0.5–1	—	—	—	—	—	600	1500 (38 µg)	—
Children								
1–3	10	30	300	1000	400	600	2500 (63 µg)	200
4–8	15	40	400	1000	650	900	3000 (75 µg)	300
9–13	20	60	600	2000	1200	1700	4000 (100 µg)	600
Adolescents								
14–18	30	80	800	3000	1800	2800	4000 (100 µg)	800
Adults								
19–70	35	100	1000	3500	2000	3000	4000 (100 µg)	1000
>70	35	100	1000	3500	2000	3000	4000 (100 µg)	1000
Pregnancy								
≤18	30	80	800	3000	1800	2800	4000 (100 µg)	800
19–50	35	100	1000	3500	2000	3000	4000 (100 µg)	1000
Lactation								
≤18	30	80	800	3000	1800	2800	4000 (100 µg)	800
19–50	35	100	1000	3500	2000	3000	4000 (100 µg)	1000

[a]The UL for niacin and folate apply to synthetic forms obtained from supplements, fortified foods, or a combination of the two.
[b]The UL for vitamin A applies to the preformed vitamin only.
[c]The UL for vitamin E applies to any form of supplemental α-tocopherol, fortified foods, or a combination of the two.

Tolerable Upper Intake Levels (UL) for Minerals

Age (yr)	Sodium (mg/day)	Chloride (mg/day)	Calcium (mg/day)	Phosphorus (mg/day)	Magnesium (mg/day)[d]	Iron (mg/day)	Zinc (mg/day)	Iodine (µg/day)	Selenium (µg/day)	Copper (µg/day)	Manganese (mg/day)	Fluoride (mg/day)	Molybdenum (µg/day)	Boron (mg/day)	Nickel (mg/day)	Vanadium (mg/day)
Infants																
0–0.5	—	—	1000	—	—	40	4	—	45	—	—	0.7	—	—	—	—
0.5–1	—	—	1500	—	—	40	5	—	60	—	—	0.9	—	—	—	—
Children																
1–3	1500	2300	2500	3000	65	40	7	200	90	1000	2	1.3	300	3	0.2	—
4–8	1900	2900	2500	3000	110	40	12	300	150	3000	3	2.2	600	6	0.3	—
9–13	2200	3400	3000	4000	350	40	23	600	280	5000	6	10	1100	11	0.6	—
Adolescents																
14–18	2300	3600	3000	4000	350	45	34	900	400	8000	9	10	1700	17	1.0	—
Adults																
19–50	2300	3600	2500	4000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	1.8
51–70	2300	3600	2000	4000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	1.8
>70	2300	3600	2000	3000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	1.8
Pregnancy																
≤18	2300	3600	3000	3500	350	45	34	900	400	8000	9	10	1700	17	1.0	—
19–50	2300	3600	2500	3500	350	45	40	1100	400	10,000	11	10	2000	20	1.0	—
Lactation																
≤18	2300	3600	3000	4000	350	45	34	900	400	8000	9	10	1700	17	1.0	—
19–50	2300	3600	2500	4000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	—

[d]The UL for magnesium applies to synthetic forms obtained from supplements or drugs only.
NOTE: An Upper Limit was not established for vitamins and minerals not listed and for those age groups listed with a dash (—) because of a lack of data, not because these nutrients are safe to consume at any level of intake. All nutrients can have adverse effects when intakes are excessive.

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To my children Michelle and Michael, and to my husband, Daniel, for their ongoing encouragement, support, faith, and love and to the students who continue to impress and inspire me.

Sareen Gropper

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Tim Carr

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PREFACE

Since the first edition was published in 1990, much has changed in the science of nutrition. But the purpose of the text—to provide thorough coverage of normal metabolism for upper-division nutrition students—remains the same. We continue to strive for a level of detail and scope of material that satisfy the needs of both instructors and students. With each succeeding edition, we have responded to suggestions from instructors, content reviewers, and students that have improved the text by enhancing the clarity of the material and by ensuring accuracy. In addition, we have included the latest and most pertinent nutrition science available to provide future nutrition professionals with the fundamental information vital to their careers and to provide the basis for assimilating new scientific discoveries.

Just as the body of information on nutrition science has increased, so has the team of authors working on this text. Dr. James Groff and Dr. Sara Hunt coauthored the first edition. In subsequent editions, Dr. Sareen Gropper became a coauthor as Dr. Hunt entered retirement. In the fourth edition, Dr. Jack L. Smith joined the author team now led by Dr. Gropper. In this seventh edition, Dr. Tim Carr has provided additional expertise and co-authorship on several chapters.

NEW TO THIS EDITION

All chapters of the seventh edition have been updated, and many feature new or enhanced tables and illustrations. The organization of the content among the chapters has remained similar to the sixth edition.

Chapter 1 The Cell: A Microcosm of Life

- expanded the discussion of the components of the cytoskeleton
- elaborated on the mechanisms of apoptosis
- condensed and focused chapter content

Chapter 2 The Digestive System: Mechanism for Nourishing the Body

- expanded coverage of saliva, the regulation of gastric secretions and motility, and the roles of colonic microflora
- added information on tight junctions
- incorporated discussion of disorders causing malfunction of the gastrointestinal tract from the Perspective into the Chapter
- included new figures to enhance presentation of hydrochloric acid secretion and hepatic physiology
- added a new Perspective addressing the nutritional impact of gastric bypass surgery

Chapter 3 Carbohydrates

- added a new Perspective on the trends in carbohydrate intake in the United States over the past several decades
- expanded coverage on dextrins in the food supply and their digestion and metabolism
- added information on glycolysis and updated the relevant figures
- expanded coverage on the role of insulin and added a new figure on insulin signaling

Chapter 4 Fiber

- added new fiber definitions
- refocused and condensed the discussion of the properties of fiber to reflect current trends
- added 2015 Dietary Guidelines recommendations related to dietary patterns

Chapter 5 Lipids

- provided more thorough coverage on lipid digestion and absorption
- added a new figure depicting the major fat sources in the American diet
- expanded coverage on cholesterol, phytosterols, phospholipids, and sphingolipids
- added a new section on dietary sources of lipids and recommended intake
- reorganized and expanded the section on lipid transport and metabolism
- added a new figure depicting ethanol oxidation in the liver

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Chapter 6 Protein

- expanded the section on protein synthesis to include amino acid signaling, mTOR, and distribution of protein intake
- added information on new methodology used to evaluate protein quality
- provided a more detailed discussion of protein malnutrition and its diagnosis
- redirected the Perspective to address the impact of stress and inflammation on protein

Chapter 7 Integration and Regulation of Metabolism and the Impact of Exercise

- added a new Perspective on sports nutrition and supplementation
- expanded coverage on the distribution of fuel molecules and tissue-specific energy utilization
- added new section on muscle function and energy requirements during exercise, including new and updated figures
- expanded coverage on regulatory hormones
- expanded coverage on the fed-fast cycle, including new and updated figures

Chapter 8 Energy Expenditure, Body Composition, and Healthy Weight

- reorganized the chapter sections to improve flow and readability
- added a new figure depicting obesity prevalence in the United States
- added a new table describing the Institute of Medicine's Physical Activity Level (PAL) categories
- updated photographs illustrating methods of assessing body composition
- added new tables summarizing ideal body weight formulas and body mass index categories
- reorganized and updated the discussion on field and laboratory methods used to measure body composition
- provided more thorough coverage of factors regulating energy balance and body weight
- added a new section on the health implications of altered body weight

Chapter 9 Water-Soluble Vitamins

- provided new tables addressing the water-soluble vitamin contents of foods
- added information on the amounts and forms of the vitamins used in supplements
- included new tables addressing common manifestations of water-soluble vitamin deficiencies and an overview of water-soluble vitamin absorption and storage
- expanded coverage of the metabolic roles of thiamin, niacin, and pantothenic acid
- added sections on selected pharmacological uses of the vitamins
- updated and expanded coverage of water soluble vitamin deficiencies including those at risk for deficiency and the treatment of deficiencies

Chapter 10 Fat-Soluble Vitamins

- added tables to more thoroughly cover the fat-soluble vitamin content of foods
- included a new table addressing common manifestations of fat-soluble vitamin deficiencies
- expanded the coverage of fat-soluble vitamin deficiencies including those at risk of deficiencies and the treatment of deficiencies

Chapter 11 Major Minerals

- provided more thorough coverage of the major mineral contents of foods
- added information on the amounts and forms of the major minerals used in supplements
- expanded information on the manifestations associated with deficiencies of the major minerals and treatment of deficiencies
- added to the Perspective information on new tools used in assessing risk of osteoporosis

Chapter 12 Water and Electrolytes

- added new sections addressing water sources, absorption, and recommendations
- reorganized and expanded the discussion of water and sodium balance as well as acid base balance
- elaborated on the roles of the kidneys in maintaining fluid and sodium balance as well as acid base balance
- included several new figures depicting the role of the kidneys and hormones in maintaining fluid and sodium balance

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Chapter 13 Essential Trace and Ultratrace Minerals

- provided more thorough coverage of the trace and ultratrace mineral contents of foods including the addition of new tables
- added information on the forms and amounts of trace minerals found in supplements
- added new sections on the pharmacological uses of minerals as appropriate
- expanded the discussion of the regulation of body and cellular iron along with a new figure showing controls on hepcidin
- included more information on manifestations of trace mineral deficiencies and their treatment

Chapter 14 Nonessential Trace and Ultratrace Minerals

- expanded the discussion of the roles of fluoride
- added information about the arsenic content of foods and arsenic toxicity

PRESENTATION

The presentation of the text is designed to make the book easy for the reader to use. The second color draws attention to important elements in the text, tables, and figures and helps generate reader interest. The Perspectives provide applications of the information in the chapter text.

Because this book focuses on normal human nutrition and physiological function, it is an effective resource for students majoring in either nutrition sciences or dietetics. Intended for a course in advanced nutrition, the text presumes a sound background in the biological sciences. At the same time, however, it provides a review of the basic sciences—particularly biochemistry and physiology, which are important to understanding the material. This text applies biochemistry to nutrient use from consumption through digestion, absorption, distribution, and cellular metabolism, making it a valuable reference for health care providers. Health practitioners may use it as a resource to refresh their memories with regard to metabolic and physiological interrelationships and to obtain a concise update on current concepts related to human nutrition.

We continue to present nutrition as the science that integrates life processes from the molecular to the cellular level and on through the multisystem operation of the whole organism. Our primary goal is to give a comprehensive picture of cell reactions at the tissue, organ, and system levels. Subject matter has been selected for its relevance to meeting this goal.

ORGANIZATION

Each of the 14 chapters begins with a topic outline, followed by a brief introduction to the chapter's subject matter. These features are followed in order by the chapter text, a brief summary that ties together the ideas presented in the chapter (in Chapters 1–8 and 12), a reference list, and a Perspective with its own reference list.

The text is divided into three sections. Section I (Chapters 1 and 2) focuses on cell structure, gastrointestinal tract anatomy, and function with respect to digestion and absorption.

Section II (Chapters 3–8) discusses metabolism of the macronutrients. This section reviews primary metabolic pathways for carbohydrates, lipids, and proteins, emphasizing those reactions particularly relevant to issues of health. Since most of the body's energy production is associated with glycolysis or the tricarboxylic acid cycle by the way of the electron transport chain and oxidative phosphorylation, the carbohydrates chapter (Chapter 3) covers these aspects of energy transformation. We include a separate chapter (Chapter 4) on fiber. The metabolism of alcohol, which contributes to the caloric intake of many people, is discussed within the lipids chapter (Chapter 5). Alcohol's chemical structure more closely resembles that of carbohydrates, but its metabolism is more similar to that of lipids. Chapter 7 discusses the interrelationships among the metabolic pathways that are common to the macronutrients. This chapter also includes a discussion of the regulation of the metabolic pathways and a description of the metabolic dynamics of the fed-fast cycle, along with a presentation of the effects of physical exertion on the body's metabolic pathways. Chapter 8 focuses on energy expenditure, energy balance, and healthy weight and also includes a brief discussion of measuring body composition and the health implications of altered body weight.

Section III (Chapters 9–14) concerns those nutrients considered regulatory in nature: the water- and fat-soluble vitamins and the minerals, including the major minerals, trace minerals, and ultratrace minerals. These chapters cover nutrient features such as digestion, absorption, transport, function, metabolism, excretion, deficiency, toxicity, and assessment of nutriture, as well as the latest Recommended Dietary Allowances or Adequate Intakes for each nutrient. Information about the major minerals has been split into two chapters: Chapter 11 addresses calcium, phosphorus, and magnesium, and Chapter 12 discusses sodium, potassium, and chloride. Chapter 12 integrates coverage of the maintenance of the body's homeostatic environment—including discussions of body fluids, electrolyte balance, and pH maintenance—with the presentation of the electrolytes.

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SUPPLEMENTARY MATERIAL

New to this edition, MindTap is a digital learning platform that works alongside your campus LMS to deliver course curriculum across the range of electronic devices in your life. MindTap is built on an "app" model, allowing enhanced digital collaboration and delivery of engaging content across a spectrum of Cengage and non-Cengage resources. Additionally, to enhance teaching and learning from the textbook, the Instructor Companion Site provides instructors with book-specific lecture and class tools, such as PowerPoint® presentations, images, the instructor's manual, videos, and more, all available online via *www.cengage.com/login*. Lastly, Cengage Learning Testing Powered by Cognero is a flexible online system that allows the instructor to author, edit, and manage test bank content from multiple Cengage Learning solutions.

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1 THE CELL: A MICROCOSM OF LIFE

COMPONENTS OF CELLS
Plasma Membrane
Cytoplasmic Matrix
Mitochondrion
Nucleus
Endoplasmic Reticulum and Golgi Apparatus
Lysosomes and Peroxisomes
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Catalytic Proteins (Enzymes)
APOPTOSIS
BIOLOGICAL ENERGY
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The Role of High-Energy Phosphate in Energy Storage
Coupled Reactions in the Transfer of Energy
Reduction Potentials
SUMMARY
PERSPECTIVE
NUTRITIONAL GENOMICS: ANOTHER PERSPECTIVE ON FOOD
BY RUTH DEBUSK, PhD, RD

CELLS ARE THE VERY ESSENCE OF LIFE. **Cells** may be defined as the basic living, structural, and functional units of the human body. They vary greatly in size, chemical composition, and function, but each one is a remarkable miniaturization of human life. Cells move, grow, ingest "food," excrete wastes, react to their environment, and reproduce. This chapter provides a brief review of the basics of a cell, including cellular components, biological energy, and an overview of a cell's natural life span.

Cells of multicellular organisms are called **eukaryotic cells** (from the Greek *eu* meaning "true," and *karyon* meaning "nucleus"). Eukaryotic cells evolved from simpler, more primitive cells called **prokaryotic cells** (from the Greek meaning "before nucleus"). One distinguishing feature between the two cell types is that eukaryotic cells possess a defined nucleus, whereas prokaryotic cells do not. Also, eukaryotic cells are larger and much more complex structurally and functionally than their ancestors. Because this text addresses human metabolism and nutrition, all descriptions of cellular structure and function in this and subsequent chapters pertain to eukaryotic cells.

While specialization among cells is necessary for life, cells, in general, have certain basic similarities. All human cells have a **plasma membrane** and a nucleus (or have had a nucleus), and most contain an endoplasmic reticulum, Golgi apparatus, and mitochondria. For convenience of discussion, a "typical cell" is presented (Figure 1.1) to enable the identification of the various organelles and their functions, which characterize cellular life. Our discussion begins with the plasma membrane which forms the outer boundary of the cell, and then moves inward to examine the organelles found within the cell.

COMPONENTS OF CELLS

Plasma Membrane

The plasma membrane is a sheet-like structure that encapsulates and surrounds the cell, allowing it to exist as a distinct unit. The plasma membrane, like other membranes within the cell, has distinct structural characteristics and functions.

- Plasma membranes are asymmetrical, with different inside and outside "faces."
- Plasma membranes are not static, but are fluid structures.

Plasma membranes are composed primarily of proteins, cholesterol, and phospholipids. Phospholipids, shown in Figure 1.2, provide both a **hydrophobic** and a hydrophilic moiety that allows them to spontaneously form bimolecular sheets, called lipid bilayers, in aqueous environments like the human body.

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Figure 1.1 Three-dimensional depiction of a typical mammalian liver cell.
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It is this lipid bilayer that determines the structure of the plasma membrane. The fatty acid portion (hydrocarbon chain) of the phospholipids forms the hydrophobic (water-fearing) core of the membrane bilayer; it also inhibits many water-soluble compounds from passing into the cell and helps to retain water-soluble substances within the cell. The glycerol and phosphate-containing portions (polar head) of the phospholipid are hydrophilic (i.e., polar, water loving) and thus are oriented toward the cell's aqueous environments found both outside the cell and in the cell cytosol.

Another important membrane lipid is cholesterol (Figure 1.3). Cholesterol influences the fluidity and thus permeability of membranes, affecting what may pass into and out of the cell; membranes with higher levels of cholesterol are less fluid. Within the membrane, cholesterol's hydrocarbon side chain associates with the fatty acid/hydrocarbon chain portion of the phospholipids and cholesterol's hydroxyl groups are positioned close to the polar head groups of the phospholipids. Cholesterol's rigid planar steroid rings are positioned so as to interact with and stabilize the regions of the hydrocarbon chains closest to the polar head groups of the phospholipids. The rest of the hydrocarbon chain remains flexible and fluid.

Both integral and peripheral proteins are found interspersed with the plasma membrane's lipid bilayer (Figure 1.3). These proteins are responsible for several membrane functions including mediating information transfer (as **receptors**), transporting ions and molecules (as channels, carriers, gates, and pumps), acting as cell adhesion molecules, and speeding up metabolic activities (as **enzymes**). Integral proteins are attached and embedded in the membrane through hydrophobic interactions; they are often transmembrane, spanning the entire structure. Peripheral proteins, in contrast, are associated with membranes through ionic interactions and are located on or near the membrane surface. Peripheral proteins may be attached to integral membrane proteins either directly or through intermediate proteins. Many of these membrane proteins have either lipid or carbohydrate attachments.

Carbohydrates are present in plasma membranes as glycolipids and glycoproteins. While some carbohydrate is found in all membranes, most of the glycolipids and glycoproteins of the cell are associated with the plasma membrane. The carbohydrate moiety of the membrane glycoproteins and glycolipids provides asymmetry to the membrane because the oligosaccharide side chains are located exclusively on the membrane layer facing the cell's

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Figure 1.2 Lipid bilayer structure of biological membranes.

outer surface (and not toward the cytosol). In plasma membranes, these outer sugar residues form what is called the glycocalyx, the layer of carbohydrate on the cell's outer surface. On the membranes of the organelles within the cell, however, the oligosaccharides are directed inward. The plasma membrane glycoproteins may serve as the receptors for hormones, certain nutrients, and other substances that influence cellular function. Glycoproteins also may help regulate the intracellular communication necessary for cell growth and functions. Intracellular

Figure 1.3 Fluid model of cell membrane. Lipids and proteins are mobile and can move laterally in the membrane.

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communication occurs through pathways that convert information from one part of a cell to another in response to external stimuli. Generally, it involves the passage of chemical messengers from organelle to organelle or within the lipid bilayers of membranes. Intracellular communication is examined more closely in the "Receptors and Intracellular Signaling" section of this chapter.

Membranes are not structurally distinct from the aqueous compartments of the cell they surround. For example, the **cytosol** (or **cytoplasm**), which is the aqueous, gel-like, transparent substance, fills the cell and, together with a system of filaments, connects the various membranes of the cell. This interconnection creates a structure that makes it possible for a signal generated at one part of the cell to be transmitted quickly and efficiently to other regions of the cell.

Cytoplasmic Matrix

The cytoplasmic (or cytosolic) matrix consists of a system of filaments or fibers (referred to as the cytoskeleton) that is found within the cytosol (Figures 1.1 and 1.4). The cytoskeleton provides cells with:

- structural support, which defines the cell's shape and helps to maintain its function
- a framework for positioning the various organelles (such as microvilli, which are extensions of intestinal cells)
- a network to direct the movement of materials and organelles within the cells
- a means of independent locomotion for specialized cells (such as sperm, white blood cells, and fibroblasts)
- a pathway for intercellular communication among cellular components (vital for cell activation and survival)
- possible transfer of RNA and DNA [1].

The cytoskeleton is made up of three groups of fibers: **microtubules**, **intermediate filaments**, and **microfilaments**.

Microtubules, Intermediate Filaments and Microfilaments

Microtubules are hollow (with about a 24 nm outer diameter), relatively rigid tubular structures (Figure 1.4). They consist of primarily two proteins—α-tubulin and β-tubulin—which form heterodimers that polymerize end-to-end. Microtubules, once formed, can be further lengthened at one end by the addition of more dimers; the other end, however, may undergo disassembly. Microtubules interact with a number of intracellular components, including proteins. They provide mechanical support, like a platform or scaffold, to influence cell shape. They also provide a structure for the intracellular movement of organelles and the assembly of cellular components (such as spindle fibers for mitosis). Flagella and cilia also rely on microtubules for movement.

Intermediate filaments, about 10 nm in diameter, are a heterogeneous group of fibers that are dynamic, undergoing constant assembly and disassembly, controlled in part by phosphorylation and dephosphorylation. Intermediate filaments (Figure 1.4) provide mechanical strength to cells that are subjected to physical stress, such as neurons, muscle cells, and epithelial cells lining body cavities.

Microfilaments, the thinnest (about 4–6 nm in diameter) of the fibers making up the cytoskeleton, are long, linear, solid fibers made up of actin. Microfilaments, like the other fibers, polymerize and unpolymerize according to the needs of the cells. Microfilaments provide scaffolding or tracks for various cell functions. Microfilaments interact with microtubules to facilitate the movement of cellular organelles and vesicles, and their interactions with intermediate filaments are thought to enable communication from extracellular stimuli to organelles within the cytosol.

Structural Arrangement

The structural arrangement within the cell influences metabolic pathways. The fluid portion of the matrix contains small molecules such as glucose, amino acids, oxygen, and carbon dioxide. This aqueous part of the cell is in contact with the cytoskeleton over a very broad surface area, and enables enzymes that are associated with the polymeric lattice to be in close proximity to their substrate molecules in the aqueous portion. Furthermore, the enzymes that catalyze the reactions of many metabolic pathways are oriented sequentially so that the product of one reaction is released in close proximity to the next enzyme for which it is a substrate; this enhances the velocity of the overall metabolic pathway. Such an arrangement exists among the enzymes that participate in glycolysis. Some other metabolic pathways that occur in the cytoplasmic matrix and that might be similarly affected include the **hexose monophosphate shunt** (pentose phosphate pathway), glycogenesis, glycogenolysis, and fatty acid synthesis. The cytoplasmic matrix of eukaryotic cells contains a number of organelles, enclosed in bilayer membranes and described briefly in the following sections.

Mitochondrion

The **mitochondria** are the primary sites of oxygen use in cells and are responsible for most of the metabolic energy (ATP) produced in cells. All cells in the body, with the exception of the erythrocyte, possess mitochondria. The erythrocyte disposes of its mitochondria and nucleus during the maturation process and then must depend solely on energy produced through anaerobic mechanisms,

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Figure 1.4 The cytoskeleton (microtrabecular lattice) provides a structure for cell organelles, microvilli (as found in intestinal mucosa cells), and large molecules. The cytosol is shown at about 300,000 times its actual size and was derived from hundreds of images of cultured cells viewed in a high-voltage electron microscope.

Source: Adapted from Porter and Tucker, "The Ground Substance of the Cell," 1981, *Scientific American*. Used by permission of Nelson Prentiss.

primarily glycolysis. The mitochondria in different tissues vary according to the function of the tissue. In muscle, for example, the mitochondria are held tightly among the fibers of the contractile system. In the liver, however, the mitochondria have fewer restraints and move freely through the cytoplasmic matrix.

Mitochondrial Membrane

The mitochondrion consists of a matrix or interior space surrounded by a double membrane (Figures 1.5 and 1.6). The mitochondrial outer membrane is relatively porous, whereas the inner membrane is selectively permeable, serving as a barrier between the cytoplasmic matrix and the mitochondrial matrix. The inner membrane has many invaginations, called the cristae, which increase its surface area, and has all the components of the electron transport chain embedded within it.

The electron transport (respiratory) chain is central to the process of **oxidative phosphorylation**, the mechanism by which most cellular ATP is produced. The components of the electron transport chain carry electrons and hydrogens during the catalytic oxidation of nutrients by

Figure 1.5 The mitochondrion.

enzymes in the mitochondrial matrix. The details of this process are described more fully in Chapter 3. Briefly, the mitochondria carry out the flow of electrons through the electron transport chain. This electron flow is strongly exothermic, and the energy released is used in part for ATP synthesis, an endothermic process. Molecular oxygen is ultimately, but indirectly, the oxidizing agent in these

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Figure 1.6 Overview of a cross section of the mitochondria.

reactions. The function of the **electron transport chain** is to couple the energy released by nutrient oxidation to the formation of ATP. The chain components are precisely positioned within the inner mitochondrial membrane, an important feature of the mitochondria, because it brings the products released in the matrix into close proximity with molecular oxygen. Figure 1.6 shows the flow of major reactants into and out of the mitochondrion.

Mitochondrial Matrix

Among the metabolic enzyme systems functioning in the mitochondrial matrix are those that catalyze the reactions of the tricarboxylic cycle (TCA cycle; Chapter 3) and fatty acid oxidation (Chapter 5). Other enzymes are involved in the oxidative decarboxylation and carboxylation of pyruvate (Chapter 3) and in certain reactions of amino acid metabolism (Chapter 6).

Mitochondria are capable of both fission and fusion, depending on the needs of the cell. They reproduce by dividing in two. Although the nucleus contains most of the cell's deoxyribonucleic acid (DNA), the mitochondrial matrix contains a small amount of DNA and a few ribosomes, enabling limited synthesis of protein within the mitochondrion. Most mitochondrial enzymes are coded by nuclear DNA, synthesized on the rough endoplasmic reticulum (RER) in the cytosol, and then incorporated into existing mitochondria. The genes contained in mitochondrial DNA, unlike those in the nucleus, are inherited only from the mother and code primarily for proteins needed for normal mitochondrial function and for ATP production. Several diseases—such as cytochrome c oxidase deficiency (also called complex IV deficiency), Leigh syndrome, and Kearns-Sayre syndrome—result from mutations in mitochondrial genes.

Nucleus

The nucleus (see Figure 1.1) is the largest of the organelles within the cell. Because of its DNA content, the nucleus initiates and regulates most cellular activities. Surrounding the nucleus is the **nuclear envelope**, a dynamic structure composed of an inner and an outer membrane. The dynamic nature of these membranes makes communication possible between the nucleus and the cytoplasmic matrix and allows a continuous channel between the nucleus and the endoplasmic reticulum. At various intervals the two membranes of the nuclear envelope fuse, creating pores in the envelope. Clusters of proteins on the outer nuclear membrane serve as microtubule organization centers (MTOCs); these centers function to begin polymerizing and organizing the microtubules during mitosis. Within the nucleus, a matrix exists to facilitate nuclear functions.

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The nucleus (or nuclear matrix) contains substances such as minerals needed for nuclear function and molecules of DNA. DNA encodes the cell's genetic information plus all the enzymes needed for its duplication. DNA is found wrapped around proteins called histones, and organized into structures called chromatin. Long strands of DNA and histones are known as chromosomes. Also within the nucleus is the **nucleolus**, a nonmembranebound structure, containing ribosomal RNA (rRNA), proteins, and DNA; it is the site of rRNA transcription and processing, and of ribosome assembly/synthesis.

Encoded within the nuclear DNA are thousands of **genes** that direct the synthesis of proteins. Each gene codes for a single specific protein. The cell **genome** is the entire set of genetic information, that is, all of the DNA within the cell. Barring mutations that may arise in the DNA, daughter cells, produced from a parent cell by mitosis, possess the identical genomic makeup of the parent cell. The process of DNA replication enables the DNA to be precisely copied at the time of mitosis.

After the cell receives a signal that protein synthesis is needed, protein biosynthesis occurs in phases referred to as transcription, translation, and elongation (Figure 1.7). Each phase requires DNA activity, RNA activity, or both. These phases, together with replication, are reviewed briefly in this chapter, but the scope of this subject is large; interested readers should consult a current cell biology text or comprehensive biochemistry text for a more thorough description of protein biosynthesis.

Nucleic Acids

Nucleic acids (DNA and RNA) are macromolecules formed from repeating units called **nucleotides**, sometimes referred to as nucleotide bases or just bases. Structurally, they consist of a nitrogenous core (either purine or pyrimidine), a pentose sugar (ribose in RNA, deoxyribose in DNA), and phosphate. Five different nucleotides are contained in the structures of nucleic acids: adenylic acid and guanylic acid are purines, and cytidylic acid, uridylic acid, and thymidylic acid are pyrimidines. The nucleotides are more commonly referred to by their nitrogenous base core only—namely, adenine, guanine, cytosine, uracil, and thymine, respectively. For convenience, particularly in describing the sequence of the polymeric nucleotides in a nucleic acid, the single-letter abbreviations are most often used. Adenine (A), guanine (G), and cytosine (C) are common to both DNA and RNA, whereas uracil (U) is unique to RNA and thymine (T) is found only in DNA. When two

Figure 1.7 Steps of protein synthesis. (1) Signals that protein synthesis needs to occur. (2) Transcription: The DNA molecule (gene) synthesizes the corresponding mRNA. (3) Translation: The corresponding mRNA molecule binds to a ribosome and directs protein synthesis based on the codon for each amino acid and the appropriate tRNA.
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strands of nucleic acids interact with each other—as occurs in replication, transcription, and translation—bases in one strand pair specifically with bases in the second strand: A always pairs with T or U, and G pairs with C, in what is called **complementary base pairing**.

The nucleotides are connected by phosphates esterified to hydroxyl groups on the pentose—that is, deoxyribose or ribose—component of the nucleotide. The carbon atoms of the pentoses are assigned prime (′) numbers for identification. The phosphate group connects the 3′ carbon of one nucleotide with the 5′ carbon of the next nucleotide in the sequence. The 3′ carbon of the latter nucleotide in turn is connected to the 5′ carbon of the next nucleotide in the sequence, and so on. Therefore, nucleotides are attached to each other by 3′, 5′ diester bonds. The ends of a nucleic acid chain are called either the free 3′ end or the free 5′ end, meaning that the hydroxyl groups at those positions are not attached by phosphate to another nucleotide.

Cell Replication

Cell replication involves the synthesis of daughter DNA molecules that are identical to the parental DNA. At cell division, the cell must copy its genome with a high degree of fidelity. Each strand of the DNA molecule acts as a template for synthesizing a new strand (Figure 1.8). The DNA molecule consists of two large strands of nucleic acid that are intertwined to form a double helix. During cell division the two unravel, with each forming a template for synthesizing a new strand through complementary base pairing. Incoming nucleotide bases first pair with their complementary bases in the template and then are connected through phosphate diester bonds by the enzyme DNA polymerase. The end result of the **replication** process is two new DNA chains that join with the two chains from the parent molecule to produce two new DNA molecules. Each new DNA molecule is therefore identical in base sequence to the parent, and each new cell of a tissue consequently carries within its nucleus identical information to direct its functioning. The two strands in the DNA double helix are antiparallel, which means that the free 5′ end of one strand is connected to the free 3′ end of the other. With this process, a cell is able to copy or replicate its genes before it passes them on to the daughter cell. Although errors sometimes occur during replication, mechanisms exist that correct or repair mismatched or damaged DNA.

Transcription

Transcription is the process by which the genetic information (through the sequence of base pairs) in a single strand of DNA makes a specific sequence of bases in a messenger RNA (mRNA) chain (see Figure 1.7). A single strand of DNA can make many copies of the corresponding mRNA,

Figure 1.8 DNA replication.

which become multiple templates for the assembly of a specific protein. This process multiplies the information contained in the DNA to produce many corresponding protein molecules. Transcription may require **transcription factors**, discussed under the subsection "Control of Gene Expression."

Transcription proceeds continuously throughout the entire life cycle of the cell. In the process, various sections of the DNA molecule unravel, and one strand—called the **sense strand**—serves as the template for synthesizing mRNA. Sequences of DNA known as promoters allow genes to be turned "off" or "on" and can initiate transcription; this promoter is usually found near (upstream) of the gene. The genetic code (gene) of the DNA is transcribed into mRNA through complementary base pairing, as in DNA replication, except that the purine adenine (A) pairs with the pyrimidine uracil (U) instead of with thymine (T). Genes are composed of critically sequenced base pairs along the entire length of the DNA strand that is being transcribed. A gene, on average, is just over 1,000 base pairs in length, compared with the nearly 5 million (5×10^6) base pair length of typical chromosomal DNA chains. Although these figures provide a rough estimate of the number of genes per transcribed DNA chain, not all the base pairs of a gene are transcribed into functional mRNA.

Many genes for specific proteins are located on regions of the DNA nucleotide sequences that are not adjacent

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to each other. Those regions that are part of the gene but do not code for a protein product are called **introns** (intervening sequences), and have to be removed from the mRNA before it is translated into protein (see the "Translation" section of this chapter). Enzymes excise the introns from the newly formed mRNA, and the ends of the functional, active mRNA segments are spliced together in a process called post-transcriptional processing. The gene segments that get both transcribed and translated into the protein product are called **exons** (expressed sequences).

Translation

Translation is the process by which genetic information in an mRNA molecule is turned into the sequence of amino acids in the protein. After the mRNA is synthesized in the nucleus (see Figure 1.7), the mRNA is exported into the cytoplasmic matrix, where it is attached to ribosomal RNA (rRNA) of the ribosomes of the rough endoplasmic reticulum (RER) or to the free-standing polyribosomes (also called polysomes). On the ribosomes, the transcribed genetic code in the mRNA is used to bring amino acids into a specific sequence that produces the specified protein.

The genetic code for specifying the amino acid sequence of a protein resides in the mRNA in the form of three-base sequences called **codons**. Each codon codes for a single amino acid. Although a given amino acid may have several codons (e.g., the codons CUU, CUC, CUA, and CUG all code for the amino acid leucine), codons can code for only one amino acid. Each amino acid has one or more transfer RNAs (tRNAs), which deliver the amino acid to the mRNA for peptide synthesis. The three-base sequences of the tRNA attach to the codons by complementary base pairing.

Amino acids are first activated by ATP at their carboxyl end and then transferred to their specific tRNAs that bear the **anticodon** complementary to each amino acid's codon. For example, because codons that code for leucine are sequenced CUU, CUC, CUA, or CUG, the only tRNAs to which an activated leucine can be attached would need to have the anticodon sequence GAA, GAG, GAU, or GAC. The tRNAs then bring the amino acids to the mRNA situated at the protein synthesis site on the ribosomes. After the amino acids are positioned according to codon–anticodon association, peptide bonds are formed between the aligned amino acids in a process called **elongation** (see Figure 1.7). Elongation extends the polypeptide chain of the protein product by translation. Each incoming amino acid is connected to the end of the growing peptide chain with a free carboxyl group (C-terminal end) by formation of further peptide bonds. New amino acids are incorporated until all the codons (corresponding to one completed protein or polypeptide chain) of the mRNA have been translated. At this point, the process stops, signaled by a "nonsense" codon that does not code for any amino acid. The completed protein dissociates from the mRNA. After translation, the newly synthesized protein may require some chemical, structural, or spatial (three-dimensional) modification to attain its active form.

Post-translational modifications of proteins may involve, for example, the covalent addition of functional groups or the cleavage of a portion of the protein. Common modifications include phosphorylation as well as glycosylation, ubiquitination, methylation, and acetylation, among others. An example of protein modifications involving proteolytic cleavage is that needed to convert zymogens, such as those involved in protein digestion, to active enzymes.

Control of Gene Expression

Each cell in the body contains a complete set of genes. Only a portion of the genes are expressed in specialized cells of a given organ. The regulation of gene expression occurs primarily at three different levels. (1) Transcription-level control mechanisms determine if a particular gene can be transcribed. Transcriptional control is accomplished by large numbers of proteins (called transcriptional factors) that bind to the DNA at a site other than the one involved in serving as a template for the mRNA. These transcriptional factors can enhance, inhibit, or, in some cases, alter the frequency (number of times transcription occurs within a specified time span) of the gene's transcription. Several hormones, such as insulin, thyroid hormone, glucagon, and glucocorticoids, as well as nutrients, such as vitamins A and D, can alter the transcription of DNA by binding along with transcription-factor proteins to DNA. (2) Processing-level control mechanisms determine the path by which mRNA can be translated into a polypeptide. This mechanism of regulating gene expression is based on the splicing of RNA molecules, thus making it possible for one gene to code for two associated proteins. (3) Translation-level control mechanisms determine whether a particular mRNA is actually translated and, if so, how often and for how long. The translation-level control mechanism can involve the localization of the mRNA in a particular part of the cell or organ. It can also operate through interactions between specific mRNAs and various small RNA strands present within the cytosol. **MicroRNAs** (abbreviated miRNA) are small non-coding RNAs that silence gene expression by binding to mRNA to inhibit its translation and/or promote its degradation. For more detailed information on the control of gene expression and its relationship to disease, which is vastly more complex than has been presented here, the reader is referred to a recent textbook on molecular biology and biochemistry or cell biology.

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Endoplasmic Reticulum and Golgi Apparatus

The **endoplasmic reticulum (ER)** is an extensive network of membranous channels pervading throughout the cytosol and providing continuity among the nuclear envelope, the Golgi apparatus, and the plasma membrane (see Figure 1.1). This structure, therefore, is a mechanism for communication from the innermost part of the cell to its exterior. In the laboratory, however, the ER cannot be separated from the cell as an isolated entity; during mechanical homogenization, the structure is disrupted and reforms into small spherical particles called microsomes.

The ER is classified as either rough (granular) or smooth (agranular). The granularity or lack of granularity is determined by the presence or absence of ribosomes. Rough endoplasmic reticulum (RER), so named because it is studded with ribosomes, abounds in cells where protein synthesis is a primary function. Smooth endoplasmic reticulum (SER) is found in most cells; however, because it is the site of synthesis for a variety of lipids, it is more abundant in cells that synthesize steroid hormones (e.g., within the adrenal cortex and gonads) and in liver cells, which synthesize fat transport molecules (the lipoproteins). In skeletal muscle, the smooth endoplasmic reticulum is called **sarcoplasmic reticulum** and is the site of the calcium ion pump, a necessity for the contractile process.

Ribosomes associated with RER are composed of ribosomal RNA and structural protein. All proteins to be secreted (or excreted) from the cell or destined to be incorporated into an organelle membrane in the cell are synthesized on the RER. The clusters of ribosomes (i.e., polyribosomes or polysomes) that are freestanding in the cytosol are also the synthesis site for some proteins. All proteins synthesized in polyribosomes in the cytosol remain within the cytoplasmic matrix or are incorporated into an organelle.

Located on the RER of liver cells is a system of enzymes important in metabolizing many different drugs. This enzyme complex consists of a family of **cytochromes** called the P450 system that functions along with other enzymes. The P450 system is particularly active in oxidizing drugs, but because its action results in the simultaneous **oxidation** of other compounds as well, the system is collectively referred to as the mixed-function oxidase system. **Lipophilic** substances—such as steroid hormones and numerous drugs—can be made **hydrophilic** by oxidation, reduction, or hydrolysis, to enable their excretion in the bile or urine. This system is discussed further in Chapter 5.

The **Golgi apparatus** functions closely with the ER in trafficking and sorting proteins that are synthesized in the cell; it is particularly prominent in neurons and secretory cells. It consists of four to eight membrane-enclosed, flattened cisternae that are stacked in parallel (see Figure 1.1). The Golgi cisternae are often referred to as "stacks" because of this arrangement. Tubular networks are present at either end of the Golgi stacks.

- The *cis*-Golgi network is a compartment that accepts newly synthesized proteins coming from the ER.
- The *trans*-Golgi network is the exit site of the Golgi apparatus. It sorts proteins for delivery to their next destination.

Proteins destined for the Golgi apparatus form within the RER. Once they are transferred to the Golgi apparatus, additional molecules (such as carbohydrates or lipids) can be added to them there. The Golgi apparatus is the site for membrane differentiation and the development of surface specificity. For example, the polysaccharide moieties of mucopolysaccharides and of the membrane glycoproteins are synthesized and attached to the protein during its passage through the Golgi apparatus. Such an arrangement allows for the continual replacement of cellular membranes, including the plasma membrane.

The ER is a quality-control organelle in that it prevents proteins that have not achieved their normal tertiary or quaternary structure from reaching the cell surface. The ER can retrieve or retain proteins destined for residency within the ER, or it can target proteins for delivery to the *cis*-Golgi compartment. Retrieved or exported protein "cargo" is coated with protein complexes called coatomers, abbreviated COPs (coat proteins). Some coatomers are structurally similar to the clathrin coat of endocytic vesicles and are described later in this chapter. The choice of what is retrieved or retained by the ER and what is exported to the Golgi apparatus is mediated by signals that are inherent in the terminal amino acid sequences of the proteins in question. Certain amino acid sequences of cargo proteins are thought to interact specifically with certain coatomers.

The membrane-bound compartments of the ER and the Golgi apparatus are interconnected by transport vesicles, in which cargo proteins are moved from compartment to compartment. The vesicles leaving a compartment are formed by a budding and pinching off of the compartment membrane, and the vesicles then fuse with the membrane of the target compartment.

Secretion of products such as proteins from the cell can be either constitutive or regulated. If secretion follows a constitutive course, the secretion rate remains relatively constant, uninfluenced by external regulation. Regulated secretion, as the name implies, is affected by regulatory factors, and therefore its rate is changeable.

Among the more interesting areas of biomolecular research has been determining how newly synthesized proteins find their way from the ribosomes to their intended destinations. While proteins synthesized on the free polyribosomes remain within the cell to perform their specific structural, digestive, regulatory, or other functions, other proteins are destined elsewhere. At the time of synthesis, signal sequences direct proteins to

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their appropriate target compartment. These targeting sequences, located at the N-terminus of the protein, are generally cleaved (though not always) when the protein reaches its destination. Interaction between the signal sequences and specific receptors located on the various membranes permits the protein to enter its designated membrane or become incorporated into the designated organelle. It is believed that in at least some cases, diseases result not just from the synthesis of enzymes that are inactive or deficient, but also result from the synthesis of proteins that fail to reach their correct destination [2].

Lysosomes and Peroxisomes

Lysosomes and **peroxisomes** are cell organelles packed with enzymes. Whereas the lysosomes (see Figure 1.1) serve as the cell's digestive system, the peroxisomes perform some specific oxidative catabolic reactions. Lysosomes are found in all cells with the exception of red blood cells but in varying numbers. Approximately 36 enzymes capable of degrading substances such as proteins, polysaccharides, nucleic acids, and phospholipids are held within the confines of a single thick lysosomal membrane. The membrane surrounding these catabolic enzymes has the capacity for selective fusion with other vesicles so that **catabolism** (or degradation) may occur as necessary. Further information on the role of lysosomes in protein and cell turnover is provided in Chapter 6.

Peroxisomes are small intracellular enzyme-containing organelles surrounded by a single membrane. They are believed to originate by "budding" from the smooth endoplasmic reticulum. The peroxisomes are similar to the lysosomes; however, rather than having digestive action, the peroxisomal enzymes are catabolic oxidative enzymes. Very-long-chain fatty acids are oxidized in peroxisomes, while most other fatty acids are oxidized in the mitochondrial matrix. Peroxisomes are also the site for certain reactions of amino acid catabolism and for the oxidation of ethanol to acetaldehyde. Hydrogen peroxide H_2O_2 is often produced within peroxisomes; this peroxisomal segregation from other cell parts is helpful given the reactive and destructive nature of H_2O_2 to cell components. The presence of the enzyme catalase within peroxisomes is also helpful for H_2O_2 degradation into water and molecular oxygen.

SELECTED CELLULAR PROTEINS

Two roles of cellular proteins are discussed; these roles include **receptors**, that is, proteins that modify the cell's response to its environment, and **enzymes**, that is, proteins serving as catalysts for biochemical reactions within cells. The reader is directed to Chapter 6 for information on other roles of proteins in the body.

Receptors

Receptors are highly specific proteins located in the plasma membrane and facing the exterior of the cell. Bound to the outer surface of these specific proteins are oligosaccharide chains, which are believed to act as recognition markers. Membrane receptors act as attachment sites for specific external stimuli such as hormones, growth factors, antibodies, lipoproteins, and certain nutrients (examples are shown in Figures 1.9 and 1.10). These molecular stimuli, which bind specifically to receptors, are called **ligands**. Receptors are also located on the membranes of cell organelles; less is known about these receptors, but they appear to be glycoproteins necessary for correctly positioning newly synthesized cellular proteins.

Figure 1.9 An example of an internal chemical signal by a second messenger.

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1. Ligand binds with its receptor on the cell membrane.
2. Ligand and receptor move into a clathrin-coated pit.
3. Pit closes off and forms a clathrin-coated vesicle.
4. The vesicle forms an endosome.
5. Ligand can be used by the cell or undergo lysosomal degradation.
6. Receptor is recycled to the surface of the cell membrane.

Figure 1.10 Internalization of a stimulus into a cell via its receptor.

Although most receptor proteins are probably integral membrane proteins, some may be peripheral. In addition, receptor proteins can vary widely in their composition and mechanism of action. Although the composition and mechanism of action of many receptors have not yet been determined, at least three distinct types of receptors are known to exist and are listed and described hereafter:

- those that generate internal chemical signals
- those that function as ion channels
- those that internalize stimuli.

Receptors That Generate Internal Chemical Signals

Upon interaction between some receptors and ligands, an internal chemical signal is generated to affect internal cellular processes. The internal chemical signal most often produced by a stimulus–receptor interaction is 3′, 5′-cyclic adenosine monophosphate (cyclic adenosine monophosphate [AMP], or cAMP). It is formed from adenosine triphosphate (ATP) by the enzyme adenyl cyclase. Cyclic AMP is frequently referred to as the second messenger in the stimulation of target cells by hormones. Figure 1.9 presents a model for the ligand-binding action of receptors, which leads to production of the internal signal cAMP. As shown in the figure, the stimulated receptor reacts with guanosine triphosphate (GTP)–binding protein (G-protein), which activates adenyl cyclase, triggering production of cAMP from ATP. G-protein is a trimer with three subunits (designated α, β, and γ). The α-subunit binds with GDP or GTP and has GTPase activity. Attachment of a hormone to the receptor stimulates the exchange of GDP for GTP. The GTP binding causes the trimers to disassociate and the α unit to associate with an effector protein, adenyl cyclase. A single hormone-binding site can produce many cAMP molecules.

The mechanism of action of cAMP signaling within the cell is complex, but it can be viewed briefly as follows: cAMP is an activator of protein kinases. **Protein kinases** are enzymes that phosphorylate (add phosphate groups to) other enzymes and, in doing so, generally convert the enzymes from inactive forms into active forms. Protein kinases that can be activated by cAMP contain two subunits: one catalytic and one regulatory. In the inactive form of the kinase, the two subunits are bound in such a way that the catalytic portion of the molecule is inhibited sterically by the presence of the regulatory subunit. Phosphorylation of the enzyme by cAMP causes the subunits to dissociate, thereby freeing the catalytic subunit, which regains its full catalytic capacity. As protein kinases serve to phosphorylate proteins and generally activate them, phosphatases work in opposition in order to remove phosphate groups from proteins and inactivate them. Thus, together the protein kinases and phosphatases function to turn on and off enzymes.

Many intracellular chemical messengers are known other than those cited as examples in this section. Listed here, along with cAMP, are several additional examples:

- cyclic AMP (cAMP)
- cyclic GMP (cGMP)
- Ca^{2+}
- inositol triphosphate
- diacyl glycerol
- fructose-2,6-bisphosphate.

Receptors That Function as Ion Channels

Receptors can also act as ion channels. In some cases, the binding of the ligand to its receptor causes a voltage change, which then becomes the signal for a cellular response. Such is the case when the neurotransmitter acetylcholine is the stimulus. The receptor for acetylcholine appears to

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function as an ion channel in response to voltage change. Stimulation by acetylcholine signals the channels to open, allowing sodium (Na^+) ions to pass through an otherwise impermeable membrane.

Receptors That Internalize Stimuli

The internalization of a stimulus into a fibroblast by way of its receptor is illustrated in Figure 1.10. Receptors that perform in such a manner exist for a variety of biologically active molecules, including several hormones. Low-density lipoproteins (LDLs) are taken up by certain cells in much the same fashion (see Chapter 5), except that their receptors, rather than being mobile, are already clustered in coated pits. These pits, vesicles formed from the plasma membrane, are coated with several proteins, among which clathrin is primary. A coated pit containing the receptor with its ligand soon loses the clathrin coating and forms a smooth-walled vesicle. This vesicle delivers the ligand into the cell and then is recycled, along with the receptor, into the plasma membrane. If the endocytotic process is for scavenging, the ligand (perhaps a protein) is not used by the cell but instead undergoes lysosomal degradation, as shown in Figure 1.10 and exemplified by the endocytosis of LDL.

Receptors' Role in Homeostasis

The cells of every organ in the body have specialized receptors that respond to changes in external conditions. The reaction of a fibroblast to changes in blood glucose level is a good example of cellular adjustment to the existing environment that is made possible through receptor proteins. When blood glucose levels are low, the hormone epinephrine is released by the adrenal medulla. Epinephrine attaches to and activates its receptor protein on the fibroblast, thereby causing it to stimulate G-protein and adenyl cyclase, which catalyzes the formation of cAMP from ATP. Then cAMP initiates a series of enzyme phosphorylation modifications, as described earlier in this section, which ultimately generate glucose 1-phosphate for use by the fibroblast. In contrast, when blood glucose levels are elevated, the hormone insulin is secreted by the β-cells of the pancreas and reacts with receptors on the fibroblast membrane. Insulin facilitates glucose entry by increasing the number of cell membrane glucose receptors, which transport glucose in the cell. (Glucose transporters are covered in Chapter 3.)

Catalytic Proteins (Enzymes)

Enzymes, which are found in all cellular compartments, are catalysts that take part in a reaction but are not part of the final product of that reaction. Some enzymes function externally (such as within the digestive tract); examples include some digestive enzymes, such as isomaltase, lactase, sucrase, maltase, and some peptidases, which are located on the brush border membrane of the epithelial cells lining the small intestine. Other enzymes that are components of the cellular membranes and most enzymes associated with organelle membranes are found on the inner membrane surface. For example, the enzymes of the electron transport chain are located within the inner membrane of the mitochondria.

Enzymes have an "active site" where they bind with a substrate. The functional activity of some enzymes, however, depends not only on the enzyme's protein portion, but also on a nonprotein prosthetic group or coenzyme/cofactor. Many of the B-vitamins serve as coenzymes and several minerals—such as Mg, Zn, Cu, Mn, and Fe—serve as inorganic prosthetic groups (or cofactors) for enzymes.

An enzyme's active site possesses high specificity. This means that a substrate must "fit" perfectly into the specific contours of the enzyme's active site so that the reacting parts of the substrate are in close proximity to the reacting parts of the enzyme. The most common analogy used to describe this is a lock and key. The concept of interlocking pieces of a puzzle has also been used to convey that the substrate and enzyme must fit. The enzyme's specificity can come from the reactive groups of its amino acids as part of the amino acid sequence or primary structure. The specificity may also originate from the three-dimensional or tertiary structure of the enzyme. Mutations in genes that alter a protein's amino acid composition can result in changes in enzyme structure and/or its active site and thus affect its ability to bind to its substrate(s). Such defects can lead to inborn errors (genetic disorders) of metabolism such as phenylketonuria (PKU).

The velocity of an enzyme-catalyzed reaction (the number of molecules of substrate reacted on in a specified time) increases if all of the active sites on the enzyme are "filled" with substrate. As the concentration of the substrate increases, the number of molecules of substrate available to the enzyme increases. This increases the number of substrate molecules acted on by the enzyme-catalyzed reaction and is said to increase the rate of the reaction. However, this relationship applies only to a concentration of substrate that is less than the concentration that "saturates" the enzyme. At saturation levels of substrate, the enzyme functions at its maximum velocity (V_{max}), and the occurrence of a still higher concentration of substrate cannot increase the velocity further.

The velocity of a chemical reaction is defined by an equilibrium constant. For enzyme-catalyzed reactions this equilibrium constant is known as K_m, or the Michaelis constant. K_m is a useful parameter that aids in establishing how enzymes react in the living cell. K_m represents the concentration of a substrate that is found in an occurring reaction when the reaction is at one-half its maximum velocity. If an enzyme has a high K_m value, then an abundance of substrate must be present to raise the rate of reaction to half its maximum velocity; in other words,

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the enzyme has a low affinity for its substrate and it takes more substrate to react with the active site of the enzyme. An example of an enzyme with a high K_m is glucokinase, found in the liver cells. Because glucose can diffuse freely into the liver, the fact that glucokinase has a high K_m is very important to blood glucose regulation. If glucokinase had a low K_m/high affinity for glucose, too much glucose would be removed from the blood during periods of fasting. Glucokinase (with its high K_m but low affinity) can still convert excess glucose to glucose phosphate when the glucose load is high—for example, following a high-carbohydrate meal; however, the liver glucokinase does not function at its maximum velocity when glucose levels are in the normal range. The enzyme thus protects against high cellular concentrations of glucose.

The nature of enzyme catalysis can be described by the following reactions:

$$\text{Enzyme (E)} + \text{substrate (S)} \leftrightarrow \text{E—S complex}$$

(reversible reaction)

The substrate activated by combination with the enzyme is converted into an enzyme–product (E–P) complex through rearrangement of the substrate's ions and atoms:

$$\text{E–S} \leftrightarrow \text{E–P}$$

$$\text{E–P} \rightarrow \text{E} + \text{P}$$

The product is released, and the enzyme is free to react with more of the substrate.

Reversibility

Most biochemical reactions are reversible, meaning that the same enzyme catalyzes a reaction in both directions. The extent to which a reaction can proceed in a reverse direction depends on several factors, the most important of which are the relative concentrations of substrate (reactant) and product and the differences in energy content between reactant and product. In instances when a large disparity in either energy content or concentration exists between reactant and product, the reaction can proceed in only one direction. Such a reaction is unidirectional rather than reversible. This topic is discussed later in this chapter. In unidirectional reactions, the same enzyme cannot catalyze in both directions. Instead, a different enzyme is required to catalyze the reverse direction of the reaction. Comparing glycolysis (the oxidation of glucose) with gluconeogenesis (the synthesis of glucose) allows us to see how unidirectional reactions may be reversed by introducing a different enzyme.

Simultaneous reactions, catalyzed by various multienzyme systems or pathways, constitute cellular metabolism. Enzymes are compartmentalized within the cell and function in sequential chains. An example of a multienzyme system is the TCA cycle located in the mitochondrial matrix. Each sequential reaction is catalyzed by a different enzyme, and some reactions are reversible, whereas others are unidirectional. Although some reactions in almost any pathway are reversible, it is important to understand that removal of one of the products (by that product reacting to produce the next compound in the pathway) drives the reaction toward forming more of that product. Removing (or using) the product, then, becomes the driving force that causes reactions to proceed primarily in the desired direction.

Regulation

An important aspect of nutritional biochemistry is the regulation of metabolic pathways. Anabolic (synthetic) and catabolic (oxidative) reactions must be kept in a balance appropriate for life (and perhaps growth). Regulation primarily involves the adjustment of the catalytic activity of certain participating enzymes. This enzyme regulation occurs through three major mechanisms:

- covalent modification of enzymes (also referred to as post-translational modification)
- modulation of allosteric enzymes
- increase in enzyme concentration by induction (synthesis of more enzyme).

Covalent Modification With the first of these mechanisms, covalent modification, the enzyme is inactive until a post-translational modification is made. This is usually achieved by the addition or hydrolytic removal of phosphate groups to or from the enzyme, as previously discussed in the subsection "Receptors That Generate Internal Chemical Signals." One example of covalent modification of enzymes is the regulation of glycogenesis (synthesis of glycogen from glucose) and glycogenolysis (breakdown of glycogen to glucose) (see Chapter 3). Another covalent modification involves cleavage; for example, some enzymes (like those secreted into the digestive tract to digest proteins) are synthesized as inactive proenzymes (also called zymogens). To activate the proenzyme (make it a functional enzyme), a portion of the proenzyme is hydrolyzed.

Allosteric Enzyme Modulation A second regulatory mechanism is that exerted by certain unique enzymes called allosteric enzymes. The term *allosteric* refers to the fact that these enzymes possess an allosteric or specific "other" site besides the catalytic site. Specific compounds, called modulators, can bind to these allosteric sites and profoundly influence the activity of these regulatory enzymes. Modulators may be positive (i.e., causing an increase in enzyme activity), or they may exert a negative effect (i.e., inhibit activity). Modulating substances are believed to alter the activity of the allosteric enzyme by changing the conformation (three-dimensional structure) of the polypeptide chain or chains of the enzyme, thereby altering the binding of its catalytic site with the intended substrate. Negative modulators are

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often the end products of a sequence of reactions. As an end product accumulates above a certain critical concentration, it can inhibit, through an allosteric enzyme, its own further production.

An excellent example of an allosteric enzyme is phosphofructokinase in the glycolytic pathway. Glycolysis gives rise to pyruvate, which is decarboxylated and oxidized to acetyl-CoA, which enters the mitochondrion and is further oxidized by the TCA cycle by combining with oxaloacetate to form citrate. Citrate is a negative modulator of phosphofructokinase. Therefore, an accumulation of citrate in the cell matrix causes the glycolytic pathway to be inhibited by regulating phosphofructokinase. In contrast, an accumulation of AMP or adenosine diphosphate (ADP), which indicates that ATP is depleted, signals the need for additional energy in the cell in the form of ATP. AMP or ADP therefore modulates phosphofructokinase positively. The result is an active glycolytic pathway that ultimately leads to the formation of more ATP through the TCA cycle–electron transport chain connection.

Allosteric mechanisms of regulation are considered to be of one of two types. In one type, the K series, the K_m is affected, which alters the binding of the substrate to the enzyme. If the allosteric effect is positive, the enzyme can become "saturated" at a lower concentration. The other type of allosteric regulation, called the V series, increases the maximum velocity of the enzymatic reaction. If the allosteric effector is an inhibitor, the maximum velocity (V_m) of the reaction will be decreased.

Induction The third mechanism of enzyme regulation, *enzyme induction*, creates changes in the concentration of certain inducible enzymes by increasing enzyme synthesis. Inducible enzymes are adaptive, meaning that they are synthesized at rates dictated by cellular circumstances. In contrast, constitutive enzymes, which are synthesized at a relatively constant rate, are uninfluenced by external stimuli. Induction usually occurs through the action of certain hormones, such as the steroid hormones and the thyroid hormones, and is exerted through changes in the expression of genes encoding the enzymes. Dietary changes can elicit the induction of some enzymes necessary to cope with the changing nutrient load. This regulatory mechanism is relatively slow, however, compared to the first two mechanisms, which exert their effects in terms of seconds or minutes.

The reverse of induction is the blockage of enzyme synthesis by blocking the formation of the mRNA of specific enzymes. This regulation of translation is one of the means by which small molecules, reacting with cellular proteins, can exert their effect on enzyme concentration and the activity of metabolic pathways.

Specific examples of enzyme regulation are described in subsequent chapters addressing nutrient metabolism. It should be noted at this point, however, that *enzymes targeted for regulation essentially catalyze unidirectional reactions*. In every metabolic pathway, at least one reaction is essentially irreversible, exergonic, and enzyme limited. That is, the rate of the reaction is limited only by the activity of the enzyme catalyzing it. Such enzymes are frequently called the regulatory enzymes, capable of being stimulated or suppressed by one of the mechanisms described. Logically, an enzyme catalyzing a reaction reversibly at near equilibrium in the cell cannot be a regulatory enzyme because its up- or downregulation would affect its forward and reverse activities equally. This effect, in turn, would not accomplish the purpose of regulation, which is to stimulate the rate of the metabolic pathway in one direction to exceed the rate of the pathway in the reverse direction.

Examples of Enzyme Types

Enzymes participating in cellular reactions are located throughout the cell in both the cytoplasmic matrix and the various organelles. The location of specific enzymes depends on the site of the metabolic pathways or metabolic reactions in which those enzymes participate. Enzyme classification, therefore, is based on the type of reaction catalyzed by the various enzymes. Enzymes fall within six general classifications:

- **Oxidoreductases** (dehydrogenases, reductases, oxidases, peroxidases, hydroxylases, and oxygenases) are enzymes that catalyze all reactions in which one compound is oxidized and another is reduced. Examples of oxidoreductases are the enzymes found in the electron transport chain located on the inner membrane of the mitochondria. Other examples are the cytochrome P450 enzymes located on the ER of liver cells.
- **Transferases** are enzymes that catalyze reactions not involving oxidation and reduction in which a functional group is transferred from one substrate to another. Included in this group of enzymes are transketolase, transaldolase, transmethylase, and the transaminases. The transaminases (α-amino transferases), which figure so prominently in protein metabolism, are located primarily in the mitochondrial matrix.
- **Hydrolases** (esterases, amidases, peptidases, phosphatases, and glycosidases) are enzymes that catalyze cleavage of bonds between carbon atoms and some other kind of atom by adding water. Digestive enzymes fall within this classification, as do those enzymes contained within lysosomes.
- **Lyases** (decarboxylases, aldolases, synthetases, cleavage enzymes, deaminases, nucleotide cyclases, hydrases or hydratases, and dehydratases) are enzymes that catalyze cleavage of carbon-carbon, carbon-sulfur, and certain carbon-nitrogen bonds (peptide bonds excluded) without hydrolysis or oxidation-reduction. Citrate lyase, which frees acetyl-CoA for fatty acid synthesis in the

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cytosol, is a good example of an enzyme belonging to this classification.

- **Isomerases** (racemases, epimerases, and mutases) are enzymes that catalyze the interconversion of optical or geometric isomers. Phosphohexose isomerase, which converts glucose-6-phosphate to fructose-6-phosphate in glycolysis (occurring in the cytosol), exemplifies this particular class of enzyme.
- **Ligases** are enzymes that catalyze the formation of bonds between carbon and a variety of other atoms, including oxygen, sulfur, and nitrogen. Forming bonds catalyzed by ligases requires energy that usually is provided by hydrolysis of ATP. An example of a ligase is acetyl-CoA carboxylase, which initiates fatty acid synthesis in the cytosol. Through the action of acetyl-CoA carboxylase, a bicarbonate ion (HCO_3^-) is attached to acetyl-CoA to form malonyl-CoA, the initial compound formed in the synthesis of fatty acids.

Clinical Applications of Cellular Enzymes

Enzymes in the body are synthesized intracellularly, and most of them function within the cell in which they were formed. Variations in amino acid sequence are not uncommon among some enzymes that catalyze the same reaction but are found in different tissues (such as the liver, muscle, and heart); such enzymes may be referred to as isozymes (or isoenzymes or protein isomers). Once made, some enzymes are secreted in an inactive form and are rendered active in the extracellular fluids where they function. Those that function in the blood are called plasma-specific enzymes.

Diagnostic enzymology focuses on intracellular enzymes, which, because of a problem within the cell structure, escape from the cell and ultimately express their activity in the serum. By measuring the serum activity of these released enzymes, both the site and often the extent of the cellular damage may be determined. If the site of the damage is to be determined with reasonable accuracy, the enzyme being measured must exhibit a relatively high degree of organ or tissue specificity. For instance, lactate dehydrogenase (LDH) is an enzyme that is widely distributed among cells such as the heart, liver, skeletal muscle, lymph nodes, erythrocytes, and platelets. Elevated serum levels of LDH do not have diagnostic value until the enzyme is separated into its five different isozyme forms and each is measured individually. Each isozyme is organ specific. The amount of elevation of the isozyme from the heart is an indication of the extent of tissue damage following, for example, a heart attack.

Intracellular enzymes are normally retained within the cell where they are produced by the plasma membrane. The plasma membrane is metabolically active, and its integrity depends on the local environment. Any process, for example, that impairs the cell's use of nutrients can compromise the structural integrity of the plasma membrane. Membrane failure can also arise from mechanical disruption, such as would be caused by a viral attack on the cell. Damage to the plasma membrane is manifested as leakiness and eventual cell death, allowing an unimpeded passage of substances, including enzymes, from intracellular to extracellular compartments such as the blood.

Factors contributing to cellular damage and resulting in abnormal egress of cellular enzymes include, for example, hypoxia (inadequate oxygen supply), tissue necrosis and **ischemia** (impaired blood flow to a tissue or part of a tissue that in turn deprives affected cells of oxygen and nutrients), and damage from viral attack or organic chemicals such as alcohol and organophosphorus pesticide. Increased production of enzymes and other substances can also cause a spike in its serum concentration. Cancers affecting certain tissues can cause such increases. Substances that occur in body fluids as a result of malignant disease are called tumor markers. A tumor marker may be produced by the tumor itself or by the host, in response to a tumor. In addition to enzymes and isozymes, other forms of tumor markers include hormones, oncofetal protein antigens such as carcinoembryonic antigen (CEA), and products of oncogenes. **Oncogenes** are mutated genes that encode abnormal, mitosis-signaling proteins, which, in turn, can promote unregulated cell division.

Increases in blood serum concentrations of cellular enzymes can be indicators of even minor cellular damage because the intracellular concentration of enzymes is hundreds or thousands of times greater than in blood. However, not all intracellular enzymes are valuable in diagnosing damage to the cells in which they are contained. Several conditions must be met for the enzyme to be suitably diagnostic:

- The enzyme must have a sufficiently high degree of organ or tissue specificity.
- A steep concentration gradient of enzyme activity must exist between the interior and exterior of the cells under normal conditions. This makes small increases in serum activity detectible (assuming the laboratory assay is sensitive).
- The enzyme must function in the cytosol of the cell so that it leaks out whenever the plasma membrane suffers significant damage.
- The enzyme must be stable for a reasonable time period in the vascular compartment.

APOPTOSIS

Dying is said to be a normal part of living. So it is with the cell. Like every living thing, a cell has a well-defined life span, after which its structural and functional integrity

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diminishes and it is removed from the body. Many terms have been used to describe naturally occurring cell death. It is now most commonly referred to as *programmed cell death*, to distinguish it from pathological cell death, which is not part of the normal physiological process. The term describing programmed cell death is ***apoptosis***, a word borrowed from the Greek meaning to "fall out."

Cells are constantly turned over in the body. For instance, 10^{10} neutrophils (a type of white blood cell) die and are replaced each day [3]. As cells die, they are replaced by new cells that are continuously being formed through cell mitosis. However, both daughter cells formed in the mitotic process do not always enjoy the full life span of the parent. If they did, the number of cells, and consequently tissue mass, could increase inordinately. Therefore, one of the two cells produced by mitosis generally is programmed to die before its sister. In fact, most dying cells are already doomed at the time they are formed. Those targeted for death are usually smaller than their surviving sisters, and their degradation begins even before the mitosis generating them is complete. The processes of cell division and cell death must be carefully regulated to generate the proper number of cells during development. Once cells mature, the appropriate number of cells must be maintained.

Apoptotic cell death (and cell survival) is brought about by several mechanisms. An intracellular (or intrinsic) pathway can be triggered by several different stimuli such as irreparable DNA damage and hypoxia, among others. Upon stimulation, proapoptotic factors (such as Bax, Bad, Bid, Noxa, and PUMA) are released into the cytosol from the mitochondria secondary to increased outer mitochondrial membrane permeability. Activation of mitochondrial death signaling occurs via the release of cytochrome c (among other cytotoxic proteins) into the cytosol. The binding of cytochrome c to apoptotic protein activating factor (Apaf-1) with involvement from caspase-9 and ATP leads to the formation of a multiprotein complex called an apoptosome. The apoptosome facilitates the recruitment and activation of other selected **caspases** (proteases with cysteine at their active sites) including caspase-3 and caspase-7. While the exact sequence of events leading to cell death is unclear, it is thought to involve the production of reactive oxygen species among other substances that induce structural alterations to the cell and its components, resulting in its death.

The extracellular (extrinsic) pathway for apoptosis is triggered when specific ligands such as molecules that belong to the **tumor necrosis factor** (TNF) family of **cytokines** bind to cell surface death receptors and generate apoptotic signaling. TNFs act through a series of protein–protein interactions that ultimately activate several caspases including caspase-3 and caspase-7 to induce cell death.

Apoptosis can also be triggered by cytotoxic T-lymphocytes and natural killer (NK) cells. Natural killer cells, for example, release (from cytosolic granules) granzymes and a protein called perforin, which create pores in the membranes of cells targeted for destruction such as those that are stressed, infected, or malignant. Caspases also can be involved in this process.

The removal of a dead cell's contents occurs without any of its contents escaping into the extracellular fluid. Thus, apoptosis does not trigger autoimmunity. However, defects in the apoptotic process may increase susceptibility to autoimmune diseases. Studies are ongoing to determine if specific human autoimmune diseases are related to such defects.

In contrast to apoptosis, which is programmed and characterized by cell shrinkage followed by cell breakup, the cell death process of **oncosis** (from *onksos*, meaning "swelling") results from cell injury and is characterized by cellular swelling, along with swelling of the mitochondrial, nucleus and cytosol, and cytosol vacuolization.

Because cell death can be activated by specific genes, the expression of these genes must be tightly controlled to avoid inappropriate cell death. Interestingly, many of the proteins released in the process of apoptosis are found in the mitochondria or in its outer membrane space. Most have a specific role there and only when they are released into the cytosol do they have a role in apoptosis. The BCL-2 family is one key group of proteins involved in the regulation of the mitochondrial intrinsic apoptotic pathway by promoting or inhibiting mitochondrial outer membrane permeability. Examples of some antiapoptotic factors include Bc1-xL and Bcl-2, which protect the cell against apoptotic stimuli. Heat shock proteins also may attenuate apoptosis. Nutrients including vitamins A and D also exhibit roles in cell proliferation, differentiation, and growth, and sphingolipids are involved in survival along with cell growth, adhesion, and **motility**.

The study of how cell death can be controlled has important implications since the dysregulation of apoptosis is thought to be involved in the pathophysiology of numerous diseases. If cell death is prevented, then a transformed cell can continue to grow (rather than be destroyed) and promote oncogenesis (the formation of a tumor). Research in the area of apoptosis abounds; a few reviews [4–9] are listed for the interested reader.

BIOLOGICAL ENERGY

The previous sections of this chapter provide some descriptive insight into the makeup of a cell, how it reproduces, and how large and small molecules are synthesized within a cell or move in or out of a cell. All of these activities require energy. The cell obtains this energy from small

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molecules transformed (oxidized) to provide heat and chemical energy. The small molecules that are constantly required are supplied by the nutrients in food. The next section covers some basics of energy needs in the cell.

Most of the processes that sustain life involve energy. Some processes use energy, and others release it. The term *energy* conjures an image of physical "vim and vigor," the fast runner or the weightlifter straining to lift hundreds of pounds. This notion of energy is accurate insofar as the contraction of muscle fibers associated with mechanical work is an energy-demanding process, requiring adenosine triphosphate (ATP), the major storage form of molecular energy in the cell. Beyond the ATP required for physical exertion, the living body has other, equally important, requirements for energy, including:

- the biosynthetic (anabolic) systems by which substances can be formed from simpler precursors
- active transport systems by which compounds or ions can be moved across membranes against a concentration gradient
- the transfer of genetic information.

This section addresses the key role of energy transformation and heat production in using nutrients and sustaining life.

Energy Release and Consumption in Chemical Reactions

Energy used by the body is ultimately derived from the energy contained in the **macronutrients**—carbohydrate, fat, and protein (and alcohol). If this energy is released, it may simply be expressed as heat, as would occur in the combustion of flammable substances, or be preserved in the form of other chemical energy. Energy cannot be created or destroyed; it can only be transformed. Burning a molecule of glucose outside the body liberates heat, along with CO_2 and H_2O as products of combustion, as shown:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{heat}$$

The metabolism of glucose to the same CO_2 and H_2O within the cell is nearly identical to that of simple combustion. The difference is that in metabolic oxidation a significant portion of the released energy is salvaged as chemical energy in the form of new, high-energy bonds. These bonds represent a usable source of energy for driving energy-requiring processes. Such stored energy is generally contained in phosphate anhydride bonds, chiefly those of ATP (Figure 1.11). The analogy between the combustion and the metabolic oxidation of a typical nutrient (palmitic acid) is illustrated in Figure 1.12. The metabolic oxidation illustrated releases 59% of the heat produced by the combustion and conserves about 40% of the chemical energy.

Figure 1.11 Adenosine triphosphate (ATP).

Expressions of Energy

Units of Energy

The unit of energy used throughout this text is the calorie, abbreviated cal. In the expression of the higher caloric values encountered in nutrition, the unit kilocalories (kcal) is often used: 1kcal = 1,000 cal. The international scientific community and many scientific journals use another unit of energy, called the joule (J) or the kilojoule (kJ). Calories can easily be converted to joules by the factor 4.18:

$$1 \text{ cal} = 4.18 \text{ J, or } 1 \text{ kcal} = 4.18 \text{ kJ}$$

To help you become familiar with both terms, this text primarily uses *calories* or *kilocalories*, followed by the corresponding values in joules or kilojoules in parentheses; joules and kilojoules are sometimes used in scientific publications.

Free Energy

The potential energy inherent in the chemical bonds of nutrients is released if the molecules undergo oxidation either through combustion or through oxidation within the cell. This energy is defined as **free energy** if, on its release, it is capable of doing work at constant temperature and pressure—a condition that is met within the cell. In equations, G is used as an abbreviation for free energy and ΔG for the change in free energy.

CO_2 and H_2O are the products of the complete oxidation of organic molecules containing only carbon, hydrogen, and oxygen, and they have an inherent free energy. The energy released in the course of oxidation of the organic molecules is in the form of either heat or chemical energy. The products have less free energy than do the original reactants. Because energy is neither created nor lost during the reaction, the total energy remains constant. Thus, the difference between the free energy in the products and that in the reactants in a given chemical reaction is a useful parameter for estimating the tendency for that reaction to occur. This difference is symbolized as follows:

$$G_{\text{products}} - G_{\text{reactants}} = \Delta G \text{ of the reaction}$$

where G is free energy and Δ is a symbol signifying change.

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Figure 1.12 A comparison of the simple combustion and the metabolic oxidation of the fatty acid palmitate.

Exothermic and Endothermic Reactions

If the *G* value of the reactants is greater than the *G* value of the products, as in the case of the oxidation reaction, the reaction is said to be **exothermic**, or energy releasing, and the change in *G* (ΔG) is negative. In contrast, a positive ΔG indicates that the *G* value of the products is greater than that of the reactants, indicating that energy must be supplied to the system to convert the reactants into the higher-energy products. Such a reaction is called **endothermic**, or energy requiring.

Exothermic and endothermic reactions are sometimes referred to as downhill and uphill reactions, respectively, terms that help create an image of energy input and release. The free energy levels of reactants and products in a typical exothermic, or downhill, reaction can be likened to a boulder on a hillside that can occupy two positions, A and B, as illustrated in Figure 1.13. As the boulder descends to level B from level A, energy capable of doing work is liberated, and the change in free energy is a negative value. The reverse reaction, moving the boulder uphill to level A from level B, necessitates an input of energy, or an endothermic process, and the change is a positive value. The quantity of energy released in the downhill reaction is precisely the same as the quantity of energy required for the reverse (uphill) reaction—only the sign of ΔG changes.

Activation Energy

Although exothermic reactions are favored over endothermic reactions in that they require no external energy input, they do not occur spontaneously. If they did, no energy-producing nutrients or fuels would exist throughout the universe because they would all have

Figure 1.13 The uphill–downhill concept illustrating energy-releasing and energy-demanding processes.

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transformed spontaneously to their lower energy level. A certain amount of energy must be introduced into reactant molecules to activate them to their **transition state**, a higher energy level or barrier at which the exothermic conversion to products can indeed take place. The energy that must be imposed on the system to raise the reactants to their transition state is called the **activation energy**. Refer again to the boulder-and-hillside analogy in Figure 1.13. The boulder does not spontaneously descend until the required activation energy can dislodge it from its resting place to the brink of the slope.

Cellular Energy

The cell derives its energy from a series of chemical reactions, each of which exhibits a free energy change. The reactions occur sequentially as nutrients are systematically oxidized ultimately to CO_2 and H_2O. Nearly all the reactions in the cell are catalyzed by enzymes. Within a given catabolic pathway—for example, the oxidation of glucose to CO_2 and H_2O—some reactions may be energy consuming (have a $+\Delta G$ for the reaction). However, energy-releasing (those with a $-\Delta G$) reactions are favored, so the net energy transformation for the entire pathway has a $-\Delta G$ and is exothermic.

Reversibility of Chemical Reactions

Most cellular reactions are reversible, meaning that an enzyme (E) that can catalyze the conversion of hypothetical substance A into substance B can also catalyze the reverse reaction, as shown:

Using the A, B interconversion as an example, let us review the concept of reversibility of a chemical reaction. In the presence of the specific enzyme E, substance A is converted to substance B. Initially, the reaction is unidirectional because only A is present. However, because the enzyme is also capable of converting substance B to substance A, the reverse reaction becomes significant as the concentration of B increases. From the moment the reaction is initiated, the amount of A decreases, while the amount of B increases to the point at which the rate of the two reactions becomes equal. At that point, the concentrations of A and B no longer change, and the system is said to be in equilibrium. Enzymes are only catalysts and do not change the equilibrium of the reaction. This concept is discussed more fully later. Whether the A → B reaction or the B → A reaction is energetically favored is indicated by the relative concentrations of A and B at equilibrium.

The equilibrium between reactants and products can be defined in mathematical terms and is called the equilibrium constant (K_{eq}). K_{eq} is simply the ratio of the equilibrium concentration of product B to that of reactant A: $K_{eq} = [B]/[A]$. The [] signify the concentration. If the denominator ([A]) is very small, dividing it into a much larger number results in K_{eq} being large. [A] will be small if most of A (the reactant) is converted to the product B. In other words, K_{eq} increases in value when the concentration of A decreases and that of B increases. If K_{eq} has a value greater than 1, substance B is formed from substance A, whereas a value of K_{eq} less than 1 indicates that at equilibrium A will be formed from B. An equilibrium constant equal to 1 indicates that no bias exists for either reaction. The K_{eq} of a reaction can be used to calculate the standard free energy change of the reaction.

Standard Free Energy Change

To compare the energy released or consumed in different reactions, it is convenient to define the free energy at standard conditions. Standard conditions are defined precisely: a temperature of 25°C (298 K); a pressure of 1.0 atm (atmosphere); and the presence of both the reactants and the products at their standard concentrations, namely 1.0 mol/L. The standard free energy change ΔG^0 (the superscript zero designates standard conditions) for a chemical reaction is a constant for that particular reaction. The ΔG^0 is defined as the difference between the free energy content of the reactants and the free energy content of the products under standard conditions. Under such conditions, ΔG^0 is mathematically related to K_{eq} by the equation

$$\Delta G^0 = -2.3\ RT \log K_{eq}$$

where R is the gas constant (1.987 cal/mol) and T is the absolute temperature, 298 K in this case. The factors 2.3, R, and T are constants, and their product is equal to –2.3(1.987)(298), or –1,362 cal/mol. The equation therefore simplifies to

$$\Delta G^0 = -1{,}362 \log K_{eq}$$

This topic is important in understanding the energetics of metabolic pathways, but you should refer to a biochemistry textbook for additional information on this subject.

Equilibrium Constant and Standard Free Energy Change

The equilibrium constant of a reaction determines the sign and magnitude of the standard free energy change. For example, referring once again to the A → B reaction, the logarithm of a K_{eq} value greater than 1.0 will be positive, and because it is multiplied by a negative number, the sign of ΔG^0 will be negative. We have established that the reaction A → B is energetically favored if ΔG^0 is negative. Conversely, the log of a K_{eq} value less than 1.0 would be negative, and when multiplied by a negative number the sign of ΔG^0 would be positive. The ΔG^0 in this case indicates that the formation of A from B (B → A) is favored in the equilibrium.

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Standard pH

For most compartments in the body, the pH is near neutral; for biochemical reactions, a standard pH value of 7 is adopted by convention. For human nutrition, the standard free energy change of reactions is designated $\Delta G^{0\prime}$ This book uses this notation.

Nonstandard Physiological Conditions

Physiologically standard conditions do not often exist. The difference between standard conditions and nonstandard conditions can explain why a reaction having a positive $\Delta G^{0\prime}$ can proceed exothermically ($-\Delta G^0$) in the cell. For example, consider the reaction catalyzed by the enzyme triosephosphate isomerase (TPI) shown in Figure 1.14. This particular reaction occurs in the glycolytic pathway through which glucose is converted to pyruvate. (The chemical structures and the pathway are discussed in detail in Chapter 3.) In the glycolytic pathway, the enzyme aldolase produces 1 mol each of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G-3-P) from 1mol of fructose-1,6-bisphosphate. Let us focus on the reaction that TPI catalyzes, which is an isomerization between the two products of the aldolase reaction. As explained in Chapter 3, only the G-3-P is further degraded in the subsequent reactions of glycolysis. This circumstance results in a substantially lower concentration of the G-3-P metabolite than of DHAP.

For this reaction, two important conditions within the cell deviate from "standard conditions": namely, the temperature is the temperature of the body, ~37°C (310 K), and neither the G-3-P nor DHAP are at 1.0 mol/L concentrations. The value of $\Delta G^{0\prime}$ for the reaction DHAP (reactant) → G-3-P (product) is +1,830 cal/mol (+7,657 J/mol), indicating that under standard conditions the formation of DHAP is preferred over the formation of G-3-P. If we assume that the cellular concentration of DHAP is 50 times that of G-3-P because G-3-P is further metabolized, ΔG^0 for the reaction is calculated to be equal to –577 cal/mol (–2,414 J/mol). The negative ΔG^0 shows that the reaction to form G-3-P is favored, as shown, despite the positive ΔG^0 for this reaction.

The Role of High-Energy Phosphate in Energy Storage

The preceding section addressed the fundamental principle of free energy changes in chemical reactions and the fact that the cell obtains this chemical free energy through the catabolism of nutrient molecules. It also stated that this energy must somehow be used to drive the various energy-requiring processes and anabolic reactions so important in normal cell function. This section explains how ATP can be used as a universal source of energy to drive reactions. Examples of very-high-energy phosphate compounds are shown in Figure 1.15. Phosphoenolpyruvate and 1,3-bisphosphoglycerate are components of the oxidative pathway of glucose (Chapter 3), and creatine phosphate (also called phosphocreatine) is a storage form of high-energy phosphate available to replenish ATP in muscle. The hydrolysis of the phosphate anhydride bonds of ATP can liberate the stored chemical energy when needed. ATP thus can be thought of as an energy reservoir, serving as the major linking intermediate between energy-releasing and energy-demanding chemical reactions in the cell. In nearly all cases, the energy stored in ATP is released by the enzymatic hydrolysis of the anhydride bond connecting the β- and γ-phosphates in the molecule (see Figure 1.11). The products of this hydrolysis are adenosine diphosphate (ADP) and inorganic phosphate (P_i). In certain instances, the free phosphate group is transferred to various acceptors, a reaction that activates the acceptors to higher energy levels. The involvement of ATP as a link between the energy-releasing and energy-requiring cellular reactions and processes is summarized in Figure 1.16.

Coupled Reactions in the Transfer of Energy

Some reactions require energy, and others yield energy. The coupling of these reactions makes it possible for a pathway to continue. The oxidation of glucose in the glycolysis pathway demonstrates the importance of coupled reactions in metabolism. An understanding of how

Figure 1.14 Example of a shift in the equilibrium by changing from standard conditions to physiological conditions.

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Figure 1.15 Examples of very-high-energy phosphate compounds.

chemical energy is transformed from macronutrients (the carbohydrate, protein, fat, and alcohol in food) to storage forms (such as ATP), and how the stored energy is used to synthesize needed compounds for the body is fundamental to the study of human nutrition. These topics are covered in this section as well as throughout this book. The $\Delta G^{0\prime}$ value for the phosphate bond hydrolysis of ATP is intermediate between those of certain high-energy phosphate compounds and compounds that possess relatively low-energy phosphate esters. ATP's central position on the energy scale lets it serve as an intermediate

Figure 1.16 An illustration of how ATP is generated from the coupling of ADP and phosphate through the oxidative catabolism of nutrients and how it in turn is used for energy-requiring processes.

Figure 1.17 Examples of high-energy phosphate bonds being transferred.

carrier of phosphate groups. ADP can accept the phosphate groups from high-energy phosphate donor molecules and then, as ATP, transfer them to lower-energy receptor molecules. Two examples of this transfer are shown in Figure 1.17. By receiving the phosphate groups, the acceptor molecules become activated to a higher energy level, from which they can undergo subsequent reactions such as entering the glycolysis pathway. The end result is the transfer of chemical energy from donor molecules through ATP to receptor molecules. The second example is the release of a P_i group from creatine phosphate; this P_i joins with ADP forming ATP. Creatine phosphate serves as a ready reservoir to renew ATP levels quickly, particularly in muscle.

If a given quantity of energy is released in an exothermic reaction, the same amount of energy must be added to the system for that reaction to be driven in the reverse direction. For example, hydrolysis of the phosphate-ester bond of glucose-6-phosphate liberates 3,300 cal/mol (13.8 kJ/mol) of energy, and the reverse reaction, in

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Figure 1.18 Exothermic reactions.

which the phosphate is added to glucose to form glucose-6-phosphate, necessitates the input of 3,300 cal/mol (13.8 kJ/mol). These reactions can be expressed in terms of their standard free energy changes as shown in Figure 1.18. To phosphorylate glucose, the reaction must be coupled with the hydrolysis of ATP, which provides the necessary energy. The additional energy from the reaction is dissipated as heat.

The addition of phosphate to a molecule is called a phosphorylation reaction. It generally is accomplished by the enzymatic transfer of the terminal phosphate group of ATP to the molecule, rather than by the addition of free phosphate, as suggested in Figure 1.18. The reverse reaction is hypothetical, designed only to illustrate the energy requirement for phosphorylation of the glucose molecule. In fact, the enzymatic phosphorylation of glucose by ATP is the first reaction glucose undergoes upon entering the cell. This reaction promotes glucose to a higher energy level, from which it may be indirectly incorporated into glycogen as stored carbohydrate or systematically oxidized for energy. Phosphorylation therefore can be viewed as occurring in two reaction steps: (1) hydrolysis of ATP to ADP and phosphate and (2) addition of the phosphate to the substrate (glucose) molecule. A net energy change for the two reactions coupled together is shown in Figure 1.18. The net $\Delta G^{0\prime}$ for the coupled reaction is –4,000 cal/mol (16.7 kJ/mol).

The significance of these coupled reactions cannot be overstated. They show that even though energy is consumed in the endothermic formation of glucose-6-phosphate from glucose and phosphate, the energy released by the ATP hydrolysis is sufficient to force (or drive) the endothermic reaction that "costs" only 3,300 cal/mol. The coupled reactions result in 4,000 cal/mol (16.7 kJ/mol) left over. The reaction is catalyzed by the enzyme hexokinase or glucokinase, both of which hydrolyze the ATP and transfer the phosphate group to glucose. The enzyme brings the ATP and the glucose into close proximity, reducing the activation energy of the reactants and facilitating the phosphate group transfer. The overall reaction, which results in activating glucose at the expense of ATP, is energetically favorable, as evidenced by its high, negative standard free energy change.

Reduction Potentials

As we will see when we discuss the formation of ATP in Chapter 3, ATP is formed in the electron transport chain after the macronutrients are oxidized. To better understand these oxidations and reductions, you need to understand reduction potentials. The energy to synthesize ATP becomes available following a sequence of individual reduction-oxidation (redox) reactions along the electron transport chain, with each component having a characteristic ability to donate and accept electrons. The released energy is used in part to synthesize ATP from ADP and phosphate. The tendency of a compound to donate and to receive electrons is expressed in terms of its **standard reduction potential**, $E_{0\prime}$. The more negative the values of $E_{0\prime}$ are, the greater the ability of the compound to donate electrons, whereas increasingly positive values signify an increasing tendency to accept electrons. The reducing capacity of a compound (its tendency to donate H^+ and electrons) can be expressed by the $E_{0\prime}$ value of its half-reaction, also called the compound's electromotive potential.

Free energy changes accompany the transfer of electrons between electron donor–acceptor pairs of compounds and are related to the measurable electromotive force of the electron flow. Remember that *in electron transfer, an electron donor reduces the acceptor, and in the process the electron donor becomes oxidized. Consequently, the acceptor, as it is reduced, oxidizes the donor*. The quantity of energy released is directly proportional to the difference in the standard reduction potentials, $\Delta E_{0\prime}$, between the partners of the redox pair. The free energy of a redox reaction and the $\Delta E_{0\prime}$ of the interacting compounds are related by the expression

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$$\Delta G^{0'} = -n\mathrm{F}\Delta E_{0'}$$

where $\Delta G^{0'}$ is the standard free energy change in calories, n is the number of electrons transferred, and F is a constant called the Faraday (23,062 cal absolute volt equivalent).

An example of a reduction-oxidation reaction that occurs within the electron transport system is the transfer of **hydrogen atoms** and electrons from NADH through the flavin mononucleotide (FMN)–linked enzyme NADH dehydrogenase to oxidized coenzyme Q (CoQ). The half-reactions and $E_{0'}$ values for each of these reactions follow:

$$\mathrm{NADH + H^+ \rightarrow NAD^+ + 2H^+ + 2e^-}$$

$$E_{0'} = -0.32 \text{ volt}$$

$$\mathrm{CoQH_2 \rightarrow CoQ + 2H^+ + 2e^-}$$

$$E_{0'} = +0.04 \text{ volt}$$

Because the NAD^+ system has a relatively more negative $E_{0'}$ value than the CoQ system, NAD^+ has a greater reducing potential than the CoQ system because electrons tend to flow toward the system with the more positive $E_{0'}$. The reduction of CoQ by NADH therefore is predictable, and the coupled reaction, linked by the FMN of NADH dehydrogenase, can be written as follows:

Inserting this value for $\Delta E_0'$ into the energy equation gives

$$\Delta G^{0'} = -2(23{,}062)(0.36) = -16{,}604 \text{ cal/mol}$$

The amount of energy liberated from this single reduction-oxidation reaction within the electron transport chain therefore is more than enough to phosphorylate ADP to ATP, which, as you will recall, requires about 7,300 cal/mol (35.7 kJ).

SUMMARY

This brief journey through the cell—beginning with its outer surface, the plasma membrane, and moving into its innermost part, where the nucleus is located—provides a view of how this living entity functions. Characteristics of the cell that seem particularly notable are as follows:

- The flexibility of the plasma membrane in adjusting or reacting to its environment while protecting the cell as it monitors what may pass into or out of the cell. Prominent in the membrane's reaction to its environment are the receptor proteins, which are synthesized on the rough endoplasmic reticulum and moved through the Golgi apparatus to their intended site on the plasma membrane.
- The communication among the various components of the cell made possible through the cytosol, with its microtrabecular network, and also through the endoplasmic reticulum and Golgi apparatus. The networking is such that communications flow not only among components within the cell but also between the nucleus and the plasma membrane.
- The efficient division of labor among the cell components (organelles). Each component has its own specific functions to perform, with little overlap. Furthermore, much evidence is accumulating to support the concept of an "assembly line" not only in oxidative phosphorylation on the inner membrane of the mitochondrion but also in other metabolic pathways, wherever they occur.
- The superb management exercised by the nucleus to ensure that all the needed proteins are synthesized. The proteins needed as recognition markers, receptors, transport vehicles, and enzymes are available and located in the appropriate place in the cell as needed.
- The fact that, like all living things, cells must die a natural death. This programmed process is called apoptosis, a particularly attractive focus of current research.

Despite the efficiency of the cell, it is still not a totally self-sufficient unit. Its continued operation is contingent on receiving appropriate and sufficient nutrients. Nutrients needed include not only those that can be used to produce energy, ATP, but also those stored as chemical energy. Most of the stored chemical energy is needed to maintain normal body temperature (released as heat energy). About 40% of the stored energy is conserved in the form of high-energy phosphate bonds, principally ATP. The ATP can, in turn, activate various substrates by phosphorylation to higher energy levels from which they can undergo metabolism by specific enzymes. The exothermic hydrolysis of the ATP phosphate is sufficient to drive the endothermic phosphorylation, thereby completing the energy transfer from nutrient to metabolite. The oxidative pathways for the macronutrients (carbohydrate, fat, and protein) and alcohol provide a continuous flow of energy for maintaining heat and replenishing ATP. The cell also needs nutrients required as building blocks

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for structural macromolecules. In addition, the cell must have an adequate supply of the so-called regulatory nutrients (i.e., vitamins, minerals, and water).

With a view of the structure of the "typical cell," the division of labor among cellular component parts, and the location within the cell where many of the key metabolic reactions necessary to continue life take place, we can now consider in subsequent chapters how the cell receives its nourishment and how the nutrients are metabolized.

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Web Sites

www.genome.gov/10001772
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NUTRITIONAL GENOMICS: ANOTHER PERSPECTIVE ON FOOD BY RUTH DEBUSK, PhD, RD

Nutritional genomics is concerned with gene–environment interactions. This emerging discipline uses genetic technology to study the mechanisms by which genes and environmental factors communicate and the functional consequences of such interactions. A major focus of research is the influence of these interactions on human health. Among the anticipated successes that will flow from nutritional genomics research is the ability to identify effective approaches for the management and prevention of diet-related disease.

One fundamental biological principle underlying gene–environment interactions is critically important to the functional ability, and thereby health, of living organisms: The information contained within a gene, when translated into the amino acid sequence of a protein, is directly related to the functional capacity of the organism. For example, the gene *INS* encodes the information needed to make the protein hormone insulin. Once synthesized, insulin plays a key role in the entry of glucose into muscle cells, where it can supply cellular energy. In the absence of insulin or in the presence of an insulin protein whose function is impaired, glucose is not able to enter certain cells as needed and diabetes results.

In a second example, the enzyme 5,10-methylene tetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate, a coenzyme form of the B vitamin folate. This enzyme is encoded by the *MTHFR* gene. In individuals with a variation in this gene, the activity of the enzyme is impaired and the dietary requirement for folate is elevated as compared with the recommended Dietary Reference Intake level. By supplying higher levels of folate in the diet, food is able to "rescue" an individual from his or her genetic limitation in the *MTHFR* gene. Thus food, in addition to providing gustatory and social pleasure, is a powerful environmental factor in terms of communicating with the genetic material and influencing biological responses.

NUTRIGENETICS, NUTRIGENOMICS, AND NUTRITIONAL EPIGENETICS

Nutritional genomics encompasses the subdisciplines of nutrigenetics, nutrigenomics, and nutritional epigenetics. The previously mentioned *INS* and *MTHFR* examples describe the biological outcomes of a change in the deoxyribonucleic acid (DNA) (a "gene variant") and are examples of nutrigenetics. This subdiscipline is concerned with detecting gene variants within an individual, discovering their effect on function, and identifying which environmental factors interact with those variants to trigger dysfunction or disease. The expectation is that, as was seen with the *INS* and *MTHFR* gene variants, the presence of a variant potentially alters the individual's goodness-of-fit with his or her environment compared with someone who does not have the variant. This information can provide the clinician with clues as to the individual's genetic susceptibilities that increase the risk of disease and which foods and other environmental factors to avoid.

Examples abound of gene variants that convey a latent genetic susceptibility to developing a disease state that manifests only upon exposure to a specific food. Food allergies, intolerances, and sensitivities provide interesting examples. Immunoglobulin E (IgE)–mediated food allergy is one such example in which a genetic susceptibility lies dormant until triggered by the interaction with an offending food. With appropriate nutrigenetic testing, it is possible to know in advance that a person is at risk of potentially deadly anaphylaxis from certain foods. Nutrigenetics can also help to eliminate the trial-and-error aspects of food intolerances. Lactose intolerance is a case in point. Whether the production of lactase, the enzyme responsible for digesting the milk sugar lactose, persists beyond childhood is genetically determined and varies by population. For Caucasians of northern European descent, lactase persistence and lifelong lactose tolerance is the norm and results from a single nucleotide change in the lactase (*LCT*) gene [1]. It is presumed that other food intolerances are also genetically based and that their early detection will enable diets to be tailored to the individual's metabolic capabilities.

Celiac disease is similarly genetically determined but environmentally triggered, can manifest at any stage in the life cycle, and is characterized by inflammation and an immune response following ingestion of gluten from wheat, barley, and rye. This disorder is estimated to occur at a frequency of 1 in 133–200 individuals, depending on the study population [2– 5]. Of the gene variants associated with the development of celiac disease, changes in the *HLA-DQ2* and *HLA-DQ8* genes have been identified as being necessary but not sufficient for the development of the disorder [6]. Being able to detect these variants prenatally or at least early in the postnatal period prevents an infant from developing the disease. Failure to recognize celiac susceptibility and prevent its occurrence through lifelong adherence to a gluten-free diet can result in severe digestive tract inflammation, intestinal tract malignancies, malabsorption, and, ultimately, severe malnutrition.

Nutrigenomics is another subdiscipline of nutritional genomics. This subdiscipline is concerned with identifying environmental factors that have an effect on the expression of genes, identifying which genes respond to which environmental factors, defining the mechanisms involved, and determining useful health-related applications of these interactions. Nutrigenomics is of interest from a diet–disease perspective because it holds the promise of using food in a targeted fashion, beyond food's ability to supply the raw materials for cellular function. If, for example, an individual has a susceptibility to **chronic** inflammation, the clinician may recommend that he or she eat a diet that supplies sufficient omega-3 fatty acids to reduce the expression of genes that code for inflammatory cytokines, thereby blunting the inflammatory response.

The *GST* gene that encodes glutathione-S-tranferase, an enzyme that functions in the Phase II biotransformation of lipid-soluble toxins into water-soluble forms that can be excreted, exemplifies both nutrigenetics and nutrigenomics. Individuals whose genome includes a variant in the *GST* gene will be impaired in their ability to protect against toxins and their detrimental effects. The *GST* variant is an example of nutrigenetics, in that it illustrates the effect of having an impaired Phase II enzyme and its consequences to biotransformation. Impaired biotransformation can lead to disease for an individual regularly exposed to an environment with an elevated level of toxic chemicals.

The *GST* example also serves as an example of nutrigenomics. In humans there are two additional *GST* genes that encode similar enzymes that can compensate for the faulty gene. The expression of these additional *GST* genes can be switched on by glucosinolates, sulfur-containing metabolites formed from the digestion of cruciferous vegetables such as broccoli and other members of the cabbage family. Nutrigenomics researchers are interested in identifying environmental factors that can increase the expression of other genes that can circumvent the limitation caused by a particular gene variant, such as those seen with the faulty *GST* gene. In this case, food is the environmental factor and glucosinolates are the bioactive components within food that can communicate with the genetic material and influence gene expression. Similarly Suhr and colleagues [7] have identified several phytonutrients from food that confer chemoprotection by controlling gene expression. Additional discussion of the role of bioactives in influencing gene expression can be found in the "Bioactive Food Components" section.

A third subdiscipline of nutritional genomics is nutritional epigenetics, which represents yet another mechanism for regulating gene expression. Epigenetics is the study of changes in

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gene expression that do not involve changes in the nucleotide sequence of DNA. Instead, chemical "tags" that can affect gene expression are added (to the DNA or to the histone proteins associated with DNA). One common type of epigenetic regulation of gene expression involves opening and closing DNA to control its accessibility to being transcribed. DNA that is tightly compacted is not available to be transcribed and, thus, expressed. The addition and removal of acetyl groups from the histone proteins that aid DNA in condensing and decondensing is a common mechanism for controlling gene expression. A second mechanism, the addition and removal of methyl groups to cytosine-containing nucleotides within the DNA sequence, can similarly control gene expression. The presence of methyl groups typically silences gene expression. In both instances, the ultimate source of these tags—that is, the acetyl and methyl groups—is the diet. The end result is the regulation of gene expression, which makes nutritional epigenetics yet another mechanism for fine-tuning the control of this process. Nutritional epigenetics is particularly important during development, during cellular differentiation, and in maintaining the distinct pattern of gene expression that characterizes the myocyte in contrast to the hepatocyte, for example.

Furthermore, a cell's pattern of tags is heritable and can be passed to subsequent generations. From the work of Wolff and colleagues [8], Waterland and Jirtle [9], and Waterland [10], it is clear that diet plays a major role in epigenetic patterning. Although there are currently more questions than answers concerning epigenetics and its associated mechanisms and consequences, nutrition can be expected to factor prominently and will be an important determinant in the ability to reach one's genetic potential and optimal health [11,12].

Nutritional genomics is an emerging field and considerable research is needed to generate well-documented associations among genes, diseases, and food before this field will fully make its impact. Once the research foundation is in place, the expectation is that nutritional genomics will be the source of effective therapeutic approaches to diet-related disease.

GENETIC VARIATION AND FUNCTION

As described previously, faulty *INS* and *MTHFR* genes have functional consequences for the individual. These detrimental variations in the *INS* and *MTHFR* genes come about through changes (mutations) in the nucleotide sequence of the DNA over evolutionary time and are called gene variants. The vast majority of gene variants result from a change in one nucleotide subunit of DNA. When a change in a single nucleotide occurs frequently in a population, it is referred to as a "single-nucleotide polymorphism" or "SNP" (pronounced "snip").

It is estimated that SNPs comprise approximately 10% of the human genome. SNPs are the basis for the uniqueness of each individual, and some result in differences in observable traits, such as hair color, eye color, or stature. The majority influence metabolic processes critical to the workings of the trillions of cells that comprise the human body and provide its functional abilities. When a SNP in the DNA results in a change in the amino acid structure of the encoded protein such that the folding of the protein is altered in a way that negatively influences its function, the potential exists for dysfunction or disease to result. Although such changes are technically mutations (any change in the DNA sequence is a mutation), the term *gene variant* is typically used for those mutations whose impact on function is not sufficiently detrimental to cause disease by themselves. Such mutations are "silent" in their effect on function until they interact with one or more environmental factors. Thus it is not the existence of a change in the DNA but the impact of that change on function that is consequential.

Once a person's variants are known, a well-documented association has been demonstrated between the variant and a disease, and the mechanism by which the dysfunction is triggered has been identified, then developing a therapeutic strategy for countering the negative effect on function becomes possible. For example, the *VDR* gene codes for the vitamin D receptor that is needed for cells to absorb vitamin D. If one has a variant in the *VDR* gene that impairs the absorption of vitamin D, a therapeutic intervention might include increased exposure to sunlight, increased intake of vitamin D–containing foods, a vitamin D dietary supplement, or a combination of these approaches. Clinicians then have an effective approach for countering the genetic limitations of that individual to prevent or at least limit the severity of the dysfunction associated with particular gene variants.

Gene variants are detected using well-established genetic technology. Because each cell with a nucleus contains a full complement of the individual's genetic material, multiple sources of DNA samples exist, from white blood cells to secretions to swabs taken from the inside of the cheek. Swabbing the cheek is a noninvasive method that is increasingly used to obtain DNA for genetic testing. In the laboratory the DNA is extracted and amplified, and specific "probe" sequences with fluorescent dye attached are used to query whether a sample from an individual contains a particular gene variant. If it does, the fluorescent probe will bind to the sample DNA and can then be detected.

BIOACTIVE FOOD COMPONENTS

Bioactive food components were introduced in the discussion of nutrigenomics. Of keen interest to researchers are the mechanisms by which food influences gene expression. Lipophilic, small-molecular-weight molecules such as essential fatty acids, vitamin A, and steroid molecules are able to traverse the cellular and nuclear membranes. They subsequently interact with DNA by means of transcription factors, specialized proteins that bind to DNA in one region of the protein and in a second region are able to bind small-molecular-weight ligands. Many of these ligands originate with the diet and are capable of binding to one or more transcription factors and influencing gene expression. Expression may be activated or silenced fully or partially to meet the ever-changing needs of the cells.

Bioactives that are either too large or too hydrophilic to pass through the lipid bilayer of the cellular and nuclear membranes communicate with the cell by interacting with cell surface receptors. Binding to the receptors triggers **signal transduction**, a cascade of events that typically leads to the **translocation** of a transcription factor to the nucleus, where it can then bind DNA and turn gene expression on or off, as appropriate.

The identification and isolation of bioactive food components is an active area of study within nutrigenomics. Bioactives may be traditional nutrients, such as vitamins or essential fatty acids, or nontraditional nutrients, such as the phytonutrients epigallocatechin-3-O-gallate from green tea, lycopene from tomatoes, and resveratrol from purple grape juice. Bioactive food components may also be potential toxins that enter the food supply inadvertently. In addition to the example of glucosinolates in cruciferous vegetables discussed previously, another bioactive food component that has positive implications for many inflammatory disease states is derived from linolenic acid, an essential fatty acid of the omega-3 class. This bioactive can modulate the expression of genes that promote inflammation, such as the *PPARG* (peroxisome proliferator-activated receptor gamma), *IL1* (interleukin 1), *IL6* (interleukin 6), and *COX2* (cyclooxygenase-2) genes [13,14].

The communication between bioactive food components and the genetic material is an intricate web of events by which cells adjust to the state of their environment. As the fund of knowledge about which bioactives affect which genes and influence which functions accumulates, diet therapy is expected to become increasingly effective because it will become possible to select specific foods to target particular mechanisms. Furthermore, expect to see a movement away from general nutrient recommendations intended for the "average" person and toward recommendations personalized for the individual.

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2 THE DIGESTIVE SYSTEM: MECHANISM FOR NOURISHING THE BODY

THE STRUCTURES OF THE DIGESTIVE TRACT AND THE DIGESTIVE AND ABSORPTIVE PROCESSES

The Oral Cavity
The Esophagus
The Stomach
The Small Intestine
The Accessory Organs
The Absorptive Process
The Colon (Large Intestine)

COORDINATION AND REGULATION OF THE DIGESTIVE PROCESS

Neural Regulation
Regulatory Peptides

SUMMARY

PERSPECTIVE

THE NUTRITIONAL IMPACT OF ROUX-EN-Y GASTRIC BYPASS, A SURGICAL APPROACH FOR THE TREATMENT OF OBESITY

NUTRITION INCLUDES THE SCIENCE OF NOURISHMENT. Ingestion of foods and beverages provides the body with at least one, if not more, of the nutrients needed to nourish the body. The body needs six classes of nutrients: carbohydrate, lipid, protein, vitamins, minerals, and water. For the body to use the carbohydrate, lipid, protein, and some vitamins and minerals found in foods, the food must first be digested—in other words, the food first must be broken down mechanically and chemically. This process of digestion occurs in the digestive tract and, once complete, yields nutrients ready for absorption and use by the body.

THE STRUCTURES OF THE DIGESTIVE TRACT AND THE DIGESTIVE AND ABSORPTIVE PROCESSES

The digestive tract, approximately 16 feet in length, includes organs that comprise the gastrointestinal (GI) tract (also called the alimentary canal or gut) as well as three accessory organs. The main structures of the digestive tract include the oral cavity, esophagus, and stomach (collectively referred to as the upper digestive tract), and the small and large intestines (called the lower digestive tract). The accessory organs include the pancreas, liver, and gallbladder. The accessory organs provide or store secretions that ultimately are delivered to the lumen (interior passageway) of the digestive tract and aid in the digestive and absorptive processes. Figure 2.1 illustrates the digestive tract and accessory organs. Figure 2.2 provides a cross-sectional view of the gastrointestinal tract that shows the lumen and the four main tunics, or layers, of the gastrointestinal tract:

- the mucosa
- the submucosa
- the muscularis externa
- the serosa.

This first layer, the mucosa, is the innermost layer, and is made of three sublayers: the mucosal membrane, the lamina propria, and the muscularis mucosa. The mucosa acts as a membrane, consists of epithelial cells that line the lumen of the gastrointestinal tract, and is the inner surface layer that is in contact with the food (and its nutrients) that we eat. In the small intestine, this layer is arranged differently than in other sections of the digestive tract (as discussed under "Structural Aspects, Secretions, and the Digestive Processes of the Small Intestine"). Both exocrine and **endocrine** cells are found among

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Figure 2.1 The digestive tract and its accessory organs.
Source: Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

the epithelial cells of the mucosa. The exocrine cells secrete a variety of enzymes and juices into the lumen of the gastrointestinal tract, and the endocrine (also called enteroendocrine) cells secrete various hormones into the blood. The lamina propria, another sublayer, lies adjacent to the epithelium and consists of primarily connective tissue and lymphoid tissue. This lymphoid tissue contains a number of cells, especially macrophages and lymphocytes, which provide protection against microorganisms. The third sublayer of the mucosa, the muscularis mucosa, is made up of a thin layer of smooth muscle.

Next to the mucosa is the submucosa. The submucosa, the second tunic or layer, is made up of connective tissue, blood and lymphatic vessels, more lymphoid tissue, and a network of nerves called the submucosal plexus, or plexus of Meissner. This plexus (plexus means network) controls, in part, gastrointestinal secretions and local blood flow. The lymphoid tissue in the submucosa is similar to that found in the mucosa and protects the body against ingested foreign substances. The submucosa connects the first mucosal layer of the gastrointestinal tract to the muscularis externa, or third layer of the gastrointestinal tract.

The muscularis externa contains inner circular and outer longitudinal smooth muscles that surround (lie on top of) the submucosa and facilitate motility. This layer also includes the myenteric plexus, or plexus of Auerbach, which lies between the circular and the longitudinal muscles. This plexus controls the frequency and strength of contractions of the muscularis to regulate gastrointestinal motility.

The outermost layer, the serosa (sometimes called the adventitia) consists of relatively flat mesothelial cells that

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Figure 2.2 The sublayers of the small intestine.
Source: Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

produce small amounts of lubricating fluids. For many areas of the digestive tract, this layer is continuous with the peritoneum. The peritoneum is a membrane with two layers within the abdominal cavity. In the abdominal cavity, the visceral peritoneum surrounds the stomach and intestine, and the parietal peritoneum lines the pelvic cavity walls. These membranes are somewhat permeable and highly vascularized. Between the two membranes is the peritoneal cavity. The selective permeability and the rich blood supply of peritoneal membranes allow the peritoneal cavity to be used in dialysis, an ultrafiltration process used to treat kidney failure.

Immune system protection is located throughout the gastrointestinal tract (and called gut-associated lymphoid tissue or GALT), especially the mucosa and submucosa layers of the small intestine (and sometimes called mucosa-associated lymphoid tissue or MALT). Atrophy of these mucosa and submucosa layers can result in bacterial translocation from the intestine into the blood, leading to sepsis (infection). Within these layers of the digestive tract, immunoprotection is provided by leukocytes, especially T- and B-lymphocytes; plasma cells; natural killer (NK) cells; macrophages; microfold (M) cells; and dendritic cells, among others. Many of these cells are found in Peyer's patches, which are aggregates of lymphoid tissue, usually present in a single layer, in the mucosa and submucosa. The plasma cells produce secretory IgA, which binds antigens ingested with foods, inhibits the growth of pathogenic bacteria, and inhibits bacterial translocation. Tissue macrophages secrete cytokines, which exhibit a variety of immunoprotective effects to defend against foreign substances. The M-cells are antigen-presenting cells; these M-cells pass or transport foreign antigens to the

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Peyer's patches or lymphocytes, which in turn mount an immune response. After processing the foreign antigens, some of these lymphocytes are released from the Peyer's patches and enter circulation to augment the immune response. Dendritic cells, a type of macrophage, also are found in the gastrointestinal tract. Dendritic cells destroy foreign substances and then serve as antigen-presenting cells to stimulate lymphocyte activity and proliferation. The processing and presentation of antigens by antigen-presenting cells further triggers recognition of antigens by other parts of the immune system as "safe" or "harmful."

The digestive process begins in the oral cavity and proceeds sequentially through the esophagus, stomach, small intestine, and finally into the colon (large intestine). The next subsections of this chapter describe the structures and digestive processes that occur in each of these parts of the digestive tract. Other sections include information on the structures and roles of the pancreas, liver, and gallbladder, and the roles of a variety of enzymes. Table 2.1 provides an overview of some of the enzymes and **zymogens** (also referred to as proenzymes or inactive enzymes, which must be altered to function as an enzyme) that participate in digesting the nutrients in foods.

The Oral Cavity

The mouth and pharynx (or throat) constitute the oral cavity and provide the entryway to the digestive tract. On entering the mouth, food is chewed by the actions of the teeth and jaw muscles and is made ready for swallowing by mixing with secretions (saliva) released from the salivary glands. Three pairs of small, bilateral saliva-secreting

Table 2.1 Digestive Enzymes and Their Actions

Enzyme or Zymogen/Enzyme	Site of Secretion	Preferred Substrate(s)	Primary Site of Action
Salivary α-amylase	Mouth	α (1-4) bonds in starch, dextrins	Mouth, stomach
Lingual lipase	Mouth	Triacylglycerol	Mouth, stomach
Pepsinogen/pepsin	Stomach	Carboxyl end of phe, tyr, trp, met, leu, glu, asp*	Stomach
Gastric lipase	Stomach	Triacylglycerol (mostly medium chain)	Stomach
Trypsinogen/trypsin	Pancreas	Carboxyl end of lys, arg*	Small intestine
Chymotrypsinogen/chymotrypsin	Pancreas	Carboxyl end of phe, tyr, trp, met, asn, his*	Small intestine
Procarboxypeptidase/carboxypeptidase A	Pancreas	C-terminal neutral amino acids	Small intestine
Carboxypeptidase B	Pancreas	C-terminal basic amino acids	Small intestine
Proelastase/elastase	Pancreas	Fibrous connective tissue proteins—elastin	Small intestine
Collagenase	Pancreas	Collagen	Small intestine
Ribonuclease	Pancreas	Ribonucleic acids	Small intestine
Deoxyribonuclease	Pancreas	Deoxyribonucleic acids	Small intestine
Pancreatic α-amylase	Pancreas	α (1-4) bonds, in starch, maltotriose	Small intestine
Pancreatic lipase and colipase	Pancreas	Triacylglycerol	Small intestine
Phospholipase	Pancreas	Lecithin and other phospholipids	Small intestine
Cholesterol esterase	Pancreas	Cholesterol esters	Small intestine
Retinyl ester hydrolase	Pancreas	Retinyl esters	Small intestine
Amino peptidases	Small intestine	N-terminal amino acids	Small intestine
Dipeptidases	Small intestine	Dipeptides	Small intestine
Nucleotidase	Small intestine	Nucleotides	Small intestine
Nucleosidase	Small intestine	Nucleosides	Small intestine
Alkaline phosphatase	Small intestine	Organic phosphates	Small intestine
Monoglyceride lipase	Small intestine	Monoglycerides	Small intestine
Alpha dextrinase or isomaltase	Small intestine	α (1-6) bonds in dextrins, oligosaccharides	Small intestine
Glucoamylase, glucosidase, and sucrase	Small intestine	α (1-4) bonds in maltose, maltotriose	Small intestine
Trehalase	Small intestine	Trehalose	Small intestine
Disaccharidases	Small intestine		Small intestine
Sucrase		Sucrose	
Maltase		Maltose	
Lactase		Lactose	

* Amino acid abbreviations: phe, phenylalanine; tyr, tyrosine; trp, tryptophan; met, methionine; leu, leucine; glu, glutamic acid; asp, aspartic acid; lys, lysine; arg, arginine; asn, asparagine; and his, histidine.

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salivary glands—the parotid, the submandibular, and the sublingual—are distributed throughout the lining of the oral cavity, along the jaw from the base of the ear to the chin (Figure 2.3). Secretions (about 1–2 L/day) from these glands constitute saliva, which is made up of mostly water (99.5%) along with proteins (enzymes, mucus, antiviral/antibacterial proteins), electrolytes (sodium, potassium, chloride), and some solutes (urea, phosphates, bicarbonate). The water in saliva helps dissolve foods. The principal enzyme of saliva is salivary α–amylase (also called ptyalin; see Table 2.1). This enzyme hydrolyzes internal α (1-4) bonds within starch. A second digestive enzyme, **lingual lipase**, is produced by lingual serous glands on the tongue and in the back of the mouth. This enzyme hydrolyzes dietary triacylglycerols (triglycerides) primarily after food has been swallowed and is in the stomach. The enzyme's activity diminishes with age and is limited by the coalescing of the fats within the stomach. Lingual lipase activity is most helpful in infants, enhancing the digestion of triacylglycerols in milk. Mucus in the saliva lubricates food and coats and protects the oral mucosa. Some of the antibacterial and antiviral proteins in saliva include the antibody IgA (immunoglobulin A) and the enzyme lysozyme, which lyses (destroys) the cell walls of some bacteria. An R-protein in saliva functions in the stomach to enhance the absorption of vitamin B_{12}. Bicarbonate in saliva assists in neutralizing acids in consumed foods and acids produced by bacteria inhabiting the oral cavity. The pH of saliva is about 7.

Saliva is released into the oral cavity 24 hours per day. Basal, or resting, secretion rates (when we are not eating) are about 0.3–0.5 mL/minute, and with food consumption, saliva secretion rates usually increase to about 2 mL/minute. Insufficient saliva production results in xerostomia (dry mouth), and may occur with the use of some medications, cancer-associated radiation and chemotherapies, as well as disorders such as Parkinson's disease and Sjögren's syndrome, among others. Insufficient saliva production not only causes the mouth and throat to become dry, but also impairs swallowing and diminishes the cleansing of our teeth and gums from food residue, acids, and old epithelial cells that have been shed from the oral mucosa. Dental caries and gum disease result if preventative care is not taken. Saliva substitutes and stimulants to increase saliva production can be helpful for some with xerostomia.

Figure 2.3 Secretions of the oral cavity.

The Esophagus

From the mouth, food, now mixed with saliva and called a bolus, is passed through the pharynx into the esophagus. The esophagus is about 10 inches long and close to an inch (2 cm) in diameter (see Figure 2.1). The passage of the bolus of food from the oral cavity into the esophagus constitutes swallowing. Swallowing, which can be divided into several stages (voluntary, pharyngeal, and esophageal), is a **reflex** response initiated by a voluntary action and regulated by the swallowing center in the medulla of the brain. To swallow food, the esophageal sphincter relaxes, allowing the esophagus to open. Food then passes into the esophagus. Simultaneously, the larynx (part of the respiratory tract) moves upward, inducing the epiglottis to shift over the glottis. The closure of the glottis is important in keeping food from entering the trachea, which leads to the lungs. Once food is in the esophagus, the larynx shifts downward to allow the glottis to reopen.

When the bolus of food moves into and down the esophagus, both the striated (voluntary) muscle of the upper portion of the esophagus and the smooth (involuntary) muscle of the distal portion are stretched and stimulated by the **nervous system**. The result is peristalsis, a progressive wave-like motion that moves the bolus through the esophagus into the stomach in usually less than 10 seconds. While the swallowing of food triggers the primary peristaltic wave, secondary waves (through the activation of stretch receptors in the esophagus) also may be initiated if, for example, food gets lodged in the esophagus. Peristalsis occurs throughout the digestive tract from the esophagus to the colon and propels the contents in the lumen distally.

At the lower (distal) end of the esophagus, just above the juncture with the stomach, lies the gastroesophageal sphincter, also called the lower esophageal sphincter (Figure 2.4). Calling it a sphincter may be a misnomer because no consensus exists about whether this particular muscle area is sufficiently hypertrophied to constitute a true sphincter. Several sphincters or valves, which are circular muscles, are located throughout the digestive tract; these sphincters allow food to pass from one section of the gastrointestinal tract to another. On swallowing, the gastroesophageal

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sphincter pressure drops. This drop in gastroesophageal sphincter pressure relaxes (opens) the sphincter so that food may pass from the esophagus into the stomach.

Multiple mechanisms, including neural and hormonal, regulate gastroesophageal sphincter pressure. The musculature of the gastroesophageal sphincter has a tonic pressure that is normally higher than the intragastric pressure (the pressure within the stomach). This high tonic pressure keeps the sphincter closed. Keeping this sphincter closed is important because it prevents gastroesophageal reflux (the movement of substances from the stomach back into the esophagus).

Figure 2.4 Structure of the stomach including a gastric gland and its secretions.
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Selected Disorders of the Esophagus

A person experiencing gastroesophageal reflux feels a burning sensation (known as heartburn or pyrosis) in the midchest. The burning usually occurs after eating, and may last for several hours. Repeated episodes may be diagnosed as gastroesophageal reflux disease (abbreviated GERD), also called acid reflux disease. Because of the low (acidic) pH of gastric (stomach) juices and because the esophageal mucosa does not have the same protective layers as does the gastric mucosa, significant damage to the esophagus may occur with chronic acid reflux including edema (swelling); tissue erosion and ulceration; blood vessel (usually capillary) damage; spasms; and fibrotic tissue formation, which can cause a narrowing (stricture) within the esophagus. Additional symptoms may include a chronic cough, excessive belching, and/or a sour taste in the mouth. While medications to neutralize the acid and/or to reduce acid production are important to promote healing, some dietary changes can also help. To minimize reductions in sphincter pressure, high-fat foods as well as chocolate, nicotine, alcohol, and carminatives (volatile oil extracts of plants, most often oils of spearmint and peppermint) should be avoided. Substances that increase gastric acid production (such as alcohol, excessive calcium, and decaffeinated and caffeinated coffee and tea) also should be avoided. Because citrus products and other acidic foods or beverages, as well as spices such as red and black pepper, nutmeg, cloves, and chili powder, can directly irritate inflamed tissues, avoidance of these substances is also encouraged. Additional suggestions include: (1) Eating smaller (versus larger) meals and drinking fluids between meals (versus with meals), since large gastric volume may promote reflux; (2) Losing weight (if overweight or obese) and avoiding tight-fitting clothes, since these may directly increase gastric pressure; and (3) Avoiding lying down, lifting, or bending for at least 2 hours after eating, since such actions place gastric contents nearer to the sphincter and may promote reflux. A discussion of some of the medications used in the management of gastroesophageal reflux disease as well as ulcers is presented in the section "Selected Disorders of the Stomach." Surgical treatment of chronic acid reflux that has not responded to medications and dietary changes usually involves fundoplication, a procedure in which a portion of the stomach (the fundus) is wrapped around the sphincter (and thus tightens it).

The Stomach

Once the bolus of food has passed through the gastroesophageal sphincter, it enters the stomach, a J-shaped sac-like organ located on the left side of the abdomen under the diaphragm. The stomach extends from the gastroesophageal sphincter to the duodenum, the upper or proximal section of the small intestine. The stomach contains four main regions (shown in Figure 2.4):

- the cardia region lies below the gastroesophageal sphincter and receives the swallowed food (bolus) from the esophagus.
- the fundus lies adjacent or lateral to and above the cardia.
- the body, the large central region, serves primarily as the reservoir for swallowed food and is the main production site for gastric juice.
- the antrum or pyloric portion, the lower (distal) one-third of the stomach, provides strong peristalsis to grind and mix food with the gastric juices (which forms **chyme**, a thick, semiliquid mass of partially digested food) and to empty the chyme into the duodenum.

The stomach's circular, longitudinal, and oblique smooth muscles enable the mixing of the food with gastric juices, including its acid and enzymes. The volume of the stomach when empty (resting) is about 50 mL (~2 oz), but on being filled it can expand to accommodate from 1 L to approximately 1.5 L (~37–52 oz). When the stomach is empty, folds (called rugae) present in all but the antrum section are visible; however, as we eat and the stomach fills, the rugae disappear. Receptive relaxation allows gastric expansion with food intake with minimal impact on intragastric pressure unless food intake exceeds the stomach's volume capacity.

Gastric juices, which are produced in significant quantities by glands found within the gastric mucosa and submucosa, facilitate the digestion of nutrients within the chyme. These glands include:

- the cardiac glands, found in a narrow rim at the juncture of the esophagus and the stomach
- the oxyntic glands, found in the fundus and body
- the pyloric glands, located primarily in the antrum.

Several cell types, which secrete different substances, are found within gastric glands, as shown in Figure 2.4. Some of the cells and their secretions that are found in a gastric oxyntic gland include:

- neck (mucous) cells, which secrete mucus
- parietal (oxyntic) cells, which secrete hydrochloric acid and intrinsic factor
- chief (peptic) cells, which secrete pepsinogen and gastric lipase
- enteroendocrine cells, which secrete a variety of hormones.

Unlike the oxyntic glands, the cardiac glands contain no parietal cells and the pyloric glands contain no chief cells.

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The main constituents of gastric juice produced by the different cells of these gastric glands include water, electrolytes, hydrochloric acid, enzymes, mucus, and intrinsic factor. About 2 L (usual range 1–3 L) of this juice are secreted each day. The next section describes some of these constituents: hydrochloric acid, enzymes, and mucus. A discussion of intrinsic factor, which is found in gastric juice and needed for vitamin B_{12} absorption, is provided in Chapter 9.

Gastric Juice

Gastric juice contains an abundance of hydrochloric acid, which is secreted as separate hydrogen ions (H^+) and chloride ions (Cl^-) from parietal cells into the lumen of the stomach. The mechanism by which hydrochloric acid is secreted is shown and described in Figure 2.5.

The high concentration of hydrochloric acid in the gastric juice is responsible for its low pH, about 2. The pH value is the negative logarithm of the hydrogen ion concentration. The lower the pH is, the more acidic the solution is. Figure 2.6 shows the approximate pH values of body fluids and, for comparison, some other compounds and beverages. Notice that the pH of orange juice (and typically of all fruit juices) is higher than that of gastric juice. Thus, drinking such juices does not lower the gastric pH.

In addition to creating an acid environment, hydrochloric acid has several other functions in gastric juice, including:

- converting or activating the zymogen pepsinogen to form pepsin (needed for protein digestion)

1. Parietal cells actively secrete hydrogen (H^+) and chloride (Cl^-) by two different transport systems. A hydrogen (proton) potassium ATPase exchange system (H^+, K^+-ATPase), also referred to as a proton pump, secretes hydrogens (protons) into the lumen in exchange for potassium ions (K^+) with each ATP molecule hydrolyzed.
2. Following the active exchange, the potassium ions typically diffuse out of the parietal cells and back into the lumen.
3. The hydrogen arises, along with bicarbonate, from the dissociation of carbonic acid (H_2CO_3). The carbonic acid is generated within the parietal cell from carbonic anhydrase, an enzyme found in high concentrations within parietal cells, using water and carbon dioxide. The water and carbon dioxide are produced within the cell from normal metabolism; the carbon dioxide also may arise in the cell following diffusion from the plasma.
4. The chloride ions needed to form hydrochloric acid arise initially from the plasma from which they are transported by a secondary active transport system in exchange for bicarbonate into the parietal cells. This antiporter carries simultaneously the bicarbonate down its concentration gradient into the plasma and the chloride against its concentration gradient into the parietal cell.
5. From the parietal cells, the chloride ions then diffuse out via a chloride channel into the gastric lumen joining the hydrogen ions to generate hydrochloric acid.

Figure 2.5 Mechanism of HCl secretion.
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Figure 2.6 Approximate pHs of selected body fluids, compounds, and beverages.

- denaturing proteins (i.e., destructing or "uncoiling" the tertiary and secondary protein structures to expose the protein's interior peptide bonds so pepsin can perform its enzymatic functions)
- releasing various nutrients such as minerals from organic complexes so absorption can occur
- acting as a bactericide agent (needed to kill bacteria ingested along with food).

Three enzymes (see Table 2.1) are found in gastric juice. The enzyme pepsin is secreted into gastric juice initially as a zymogen called pepsinogen. Specifically, pepsinogen is secreted in granules into the gastric lumen by chief cells when they are stimulated by acetylcholine and/or acid. Pepsinogen is then converted (activated) to pepsin, an active enzyme, by hydrochloric acid or the presence of previously formed pepsin in the gastric lumen.

$$\text{Pepsinogen} \xrightarrow{\text{Acid or pepsin}} \text{Pepsin}$$

Pepsin functions as a protease, an enzyme that hydrolyzes proteins. Specifically, pepsin is an endopeptidase, meaning that it hydrolyzes interior peptide bonds within proteins. Optimal pepsin activity occurs at about pH 3.5. Another enzyme made by gastric chief cells is gastric lipase. Gastric lipase hydrolyzes fatty acids from glycerol's third carbon in triacylglycerols. This enzyme is thought to be responsible for up to about 20% of lipid digestion. The salivary α-amylase found in gastric juice originates from the salivary glands of the mouth. This enzyme, which hydrolyzes starch, retains some activity in the stomach until it is inactivated by the low pH of gastric juice. Additional information about pepsin and salivary α-amylase can be found in Chapters 6 and 3, respectively. Gastric lipase is discussed further in Chapter 5.

Gastric juice also contains mucus, which is secreted both by neck (mucous) cells in gastric glands and by mucosal epithelial cells; these epithelial cells also release bicarbonate (HCO_3^-). Mucus composition varies depending on its location in the digestive tract, but it generally consists of a network of different glycoproteins called **mucins**. Most mucins bind water and are gel-forming and thus provide lubrication and protection. In the stomach, mucus both coats the gastric contents as well as forms a layer about 2 mm thick on the gastric mucosal membrane to coat and protect it. Embedded within this gastric mucus layer is bicarbonate creating a local pH of about 6–7 versus the very acidic pH of about 2 in the gastric lumen. Production and release of mucus within the stomach is enhanced by **prostaglandins**, vagal nerve stimulation, acetylcholine, and various hormones. Substances that inhibit or diminish mucus secretion increase the risk for ulcer formation.

Regulation of Gastric Secretions

The regulation of gastric secretions can be divided into three phases based on events occurring before food reaches the stomach, once food is in the stomach, and after food has left the stomach. Multiple mechanisms, both neural and chemical, influence each of the three phases; some of the many hormones and peptides that are involved are shown in Figure 2.7 and are presented later in the chapter in Table 2.2.

In the cephalic (first) phase, eating or tasting food, as well as thinking about, seeing, and/or smelling food, stimulates gastric secretions. Vagal stimulation of primarily the submucosal plexus promotes the secretion of the neurotransmitter acetylcholine and enhances the release of the hormone gastrin from G cells. Acetylcholine and gastrin both trigger the release of the paracrine histamine by mast cells and enterochromaffin-like cells in the gastric glands. Each stimulates hydrochloric acid secretion by parietal cells—histamine binds to H_2 receptors, gastrin binds to gastrin receptors, and acetylcholine acts on muscarinic receptors on the parietal cells. Additionally, acetylcholine stimulates the chief cells, promoting enzyme release.

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Figure 2.7 The effects of selected gastrointestinal hormones/peptides on gastrointestinal tract secretions and motility.

The second, or gastric, phase occurs when ingested food reaches the stomach. Distension of the stomach (identified by stretch receptors in the stomach layers) along with the presence of protein and some other consumed substances, especially caffeine and alcohol, in the stomach enhance gastric secretions in this phase. The ability of proteins, primarily those that have been digested into small peptides and/or amino acids, to enhance gastric secretions occurs through multiple pathways including, for example, stimulating chemoreceptors that initiate submucosal plexus nerve activity; promoting gastrin release; and activating the parasympathetic nervous system, which further enhances vagal activity to the stomach.

The third, or intestinal, phase of gastric secretions occurs after food has left the stomach and has entered the duodenum. In this phase, a reduction in chyme volume in the stomach and a reduction in the pH of gastric juice (to < 2) trigger the release of somatostatin by D cells in the pancreas, antrum, and duodenum. Somatostatin, which acts in a paracrine fashion by entering gastric juice, diminishes parietal cell, G cell, and enterochromaffin-like cell secretions. Additionally, some of the factors that inhibit gastric emptying (as discussed in the next section) also inhibit the release of gastric secretions. These factors include the presence of hyperosmolar chyme and acidic chyme, as well as fat-containing chyme in the duodenum. Furthermore, chyme's presence in the duodenum both causes distension (eliciting responses from the submucosal plexus and myenteric plexus) and triggers the release of secretin and cholecystokinin. These actions reduce gastric secretions as well as reduce peristalsis in the antrum, and slow gastric emptying to "finish up" digestive actions in the stomach, but simultaneously these hormones also promote digestive processes within the small intestine. Other hormones that play lesser roles in diminishing gastric acid production include glucose-dependent insulinotrophic peptide and peptide YY, the paracrine glucagon-like peptides, and the neurocrine vasoactive intestinal polypeptide.

Regulation of Gastric Motility and Gastric Emptying

Peristalsis occurring in the stomach is strongest in the lower body and **antral** sections. The peristaltic waves propel the digestive contents through the stomach as well as through most of the other portions of the digestive tract. Additionally, in the antrum, retropulsion pushes the chyme back and forth between peristaltic contractions to help grind and liquify food particles. Another means of motility present in the stomach is a basic electrical rhythm that is initiated by the interstitial cells of Cajal (also referred to as pacemaker cells), found in the outer circular muscles (muscularis externa) near the myenteric plexus in the body of the stomach at the greater curvature. The pacemaker cells in the stomach generate wave-like signals (or slow-wave potentials) at a rate of about three per minute that move from the fundus toward the pyloric sphincter and help to coordinate peristalsis and other motor activity.

Gastric emptying is affected by factors both in the stomach and duodenum. In the antrum of the stomach,

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the strength of the peristaltic contractions is affected by the volume of the chyme, with gastric distension typically increasing gastric motility. In the duodenal bulb (the first few centimeters of the proximal duodenum), receptors are sensitive to distension/volume, as well as the **osmolarity**, nutrient content, and acidity of the chyme. Distension from an excessive volume of chyme in the duodenum reduces gastric emptying. Similarly, the presence in the duodenum of hypertonic/hyperosmolar (very concentrated) chyme, which occurs, for example, with increased gastric emptying and/or delayed nutrient (especially amino acid and/or glucose) absorption, slows gastric emptying. Dietary fat intake also has an inhibitory effect on gastric emptying, versus carbohydrate-rich and protein-rich foods; in fact, a high-fat meal may take up to 6 hours to digest versus typically less than 4 hours for a meal consisting of mostly carbohydrates and protein. The delay in gastric emptying is mediated primarily by the hormone cholecystokinin whose release is triggered by the presence of fat in the duodenum. Cholecystokinin primarily promotes **bile** secretion into the duodenum, enabling fat emulsification and digestion, but also inhibits gastric emptying. The presence of unneutralized acidic chyme in the duodenum stimulates the release of secretin that both slows gastric emptying of the acidic chyme into the duodenum and stimulates the release of pancreatic juice, which functions in part to neutralize the acid. In addition to cholecystokinin and secretin, the paracrine somatostatin, and to a lesser extent the hormones pancreatic polypeptide and peptide YY and the paracrine glucagon-like peptides, diminish gastric emptying.

Some other factors affecting gastric motility result from neural reflexes and involvement of the autonomic nervous system. Distension in the duodenum inhibits gastric emptying, as previously discussed; additionally, distension in the distal small intestine also impacts gastrointestinal tract motility. The nerve reflex known as the ileogastric reflex is elicited by distension in the ileum and results in diminished gastric emptying. This action allows more time for the contents of the ileum to be emptied before more chyme is released from the stomach into the duodenum. Finally, emotions such as fear, anger, and sadness, among others, inhibit or excite the digestive system's smooth muscles via the autonomic nervous system to affect gastric emptying and intestinal motility.

The secretions and contractions within the stomach promote physical disintegration of solid foods into liquid form and continue the digestive processes that began in the oral cavity. However, most nutrients from these digestive actions in the upper gastrointestinal tract are not yet ready to be absorbed into the body; the stomach absorbs only alcohol and small quantities of water and a few minerals including iodide and fluoride. Before most nutrient absorption can occur, additional digestive actions are needed within the small intestine. Complete liquefaction of chyme is not necessary for the stomach contents to empty into the duodenum (which occurs via the pyloric sphincter, found at the junction of the antrum and the duodenum). Particles as large as 3 mm in diameter (~1/8 inch) can be emptied from the stomach through the sphincter, but solid particles are usually emptied with fluids when they have been degraded to a diameter of about 2 mm or less. Approximately 1–5 mL (~up to 1 tsp) of chyme enters the duodenum about twice per minute. Gastric emptying following a meal usually takes between 1 and 4 hours; however, in those who are critically ill, gastric emptying may be delayed and can result in larger gastric residual volumes. These gastric residuals need to be monitored closely in hospitalized patients being fed into their stomach via a tube. Should the rate of tube feeding be greater than the rate of gastric emptying, vomiting (emesis) and aspiration may occur. Problems from delayed gastric emptying (called gastroparesis) also can occur in those who are not critically ill and/or being tube fed. Gastroparesis can occur with damage to the vagus nerve from diabetes and some neurological conditions. If untreated, gastroparesis may cause malnutrition and, in those with diabetes, difficulty controlling blood glucose concentrations.

Selected Disorders of the Stomach

Peptic ulcer disease (PUD), commonly referred to as ulcers, is characterized by the presence of ulcerations or erosions usually in the mucosa and submucosa layers of the stomach (antrum area), duodenum (first few centimeters), and/or lower esophagus. Perforations, however, can also occur, affecting all four layers of the digestive tract. Multiple factors promote the formation of ulcers. Zollinger-Ellison syndrome, from the presence of a gastrin-producing tumor, is a rare condition characterized by extremely copious secretion of gastrin into the blood. The hypergastrinemia (higher than normal blood gastrin concentrations) promotes excessive hydrochloric acid release into the stomach and the formation of numerous ulcers in the stomach and duodenum, and sometimes even the jejunum. A more common cause of ulcers is from the bacterium *Helicobacter (H.) pylori*, but any factor that disrupts the integrity of the mucosa (including normal defense and repair systems) can increase the likelihood of ulcer formation. Chronic ingestion of alcohol as well as the excessive use of aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, for example, both disrupt the normally tight junctions between gastric mucosal cells (that prevent acid penetration) and diminish the production of the bicarbonate and mucus (which form a protective barrier on the mucosal membrane of the gastrointestinal tract). The dietary recommendations and medications used to treat peptic ulcers are similar to those described for gastroesophageal reflux disease. (Some of the dietary changes have been previously addressed; see "Selected Disorders of the Esophagus.") A brief discussion

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of the mechanisms of action for two groups of frequently used medications in the treatment of ulcers and gastroesophageal reflux disease and their effects on nutrient utilization follows.

One group of medications used to treat these conditions is called H_2 receptor blockers—including Tagamet (cimetidine), Zantac (ranitidine), Pepcid (famotidine), and Axid (nizatidine). These medications function by binding to the H_2 receptors on the parietal cells. Consequently, when histamine is released, it cannot bind to these H_2 receptors (the drug blocks histamine's ability to bind), and acid release from the parietal cell is diminished. Another group of drugs, referred to as proton pump inhibitors—including Prilosec (omeprazole), Nexium (esomeprazole), Protonix (pantoprazole), Aciphex (rabeprazole), and Dexilant (dexlansoprazole)—works by binding to the ATPase/proton pump (see Figure 2.5) at the secretory surface of the parietal cell and thus directly inhibits hydrogen release. In comparison with other medications, the proton pump inhibitors are the most effective at inhibiting acid production. However, long-term use can cause bacterial overgrowth and can negatively impact the absorption of vitamin B_{12} and several minerals that tend to benefit from an acidic environment.

Recurrent ulcers that are not responsive to medications and diet changes as well as other conditions affecting the stomach, such as cancer, may necessitate the surgical removal (resection) of a portion of the stomach. Gastric restriction and resection procedures are also used for the treatment of obesity and are discussed further in the Perspective for this chapter. Removing a portion of the stomach negatively impacts normal digestive tract functions. One such complication is a condition called dumping syndrome. Dumping syndrome occurs after eating (from about 30 minutes to 3 hours) and results initially from hyperosmolar (concentrated) chyme getting released "too rapidly" into the duodenum. This "dumping" occurs because the size of the stomach, which is now considerably reduced, can no longer serve as a storage reservoir, produce its usual volume of digestive juices, or mix the ingested food with gastric juices to create a diluted, partially digested chyme mixture. The hyperosmolar (concentrated) chyme in the duodenum in turn causes fluid from the blood to be "pulled or drawn" quickly into the lumen of the duodenum to dilute its contents and create a more isotonic chyme. Such actions promote some of the symptoms of dumping syndrome, which include dizziness, weakness, tachycardia (rapid heartbeat), and hypotension (associated with the reduction in vascular fluid), as well as nausea, abdominal distension, and pain. Other symptoms may include gas, diarrhea, and abdominal pain from the fermentation of the undigested nutrients by bacteria in the intestines, and weakness, palpitations, and hypoglycemia (low blood glucose). The hypoglycemia results when there is excessive **insulin** secretion occurring secondary to the consumption of foods usually rich in simple sugars (monosaccharides and disaccharides), which get absorbed too quickly from the duodenum and into the blood. To help alleviate some of the nutritional complications of gastric resection, some of the several dietary modifications include eating foods slowly, limiting the intake of foods high in simple sugars, and limiting the consumption of fluids with meals (to lessen the gastric volume, which promotes rapid emptying). Medications that delay gastric emptying and reduce gut motility also may ameliorate some of the symptoms.

The Small Intestine

Once through the pyloric sphincter, chyme enters the small intestine. The small intestine (Figure 2.8), which represents the main site for both nutrient digestion and absorption, is composed of the duodenum (slightly less than 1 foot long with about a 2-inch diameter), the jejunum (slightly over 8 feet long), and the ileum (about 11½ feet long). Microscopy is generally needed to identify where one of these sections of the small intestine ends and the other begins. However, the Treitz ligament, a suspensory ligament, is found at about the site where the duodenum and jejunum meet. Furthermore, there is a slight size difference with the lumen of the jejunum (about 1¼–1½ inches) being generally slightly larger than that of the ileum (about 1–1¼ inches).

Structural Aspects, Secretions, and Digestive Processes of the Small Intestine

Although the structure of the small intestine consists of the same layers identified in Figure 2.2, the small intestine is structured with an enhanced surface area to absorb nutrients. The small intestine has a surface area of approximately 300 m^2, an area about equal to a 3-foot-wide sidewalk more than three football fields in length. Several structures, shown in Figure 2.9, that contribute to this enormous surface area include:

- large circular folds of the mucosa, called the folds of Kerckring, that protrude into the lumen of the small intestine
- finger-like projections, called villi, that project out into the lumen of the intestine and consist of hundreds of intestinal cells called **enterocytes** (these cells are also referred to as absorptive, epithelial, and/or mucosal cells) along with blood capillaries and a lacteal (lymphatic vessel) for transport of nutrients out of the enterocytes

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The small intestine is divided into three regions: the duodenum, jejunum, and ileum. The ileocecal sphincter regulates the flow of material from the ileum, the last segment of the small intestine, into the cecum, the first portion of the large intestine.

The duodenum receives secretions from the gallbladder via the common bile duct. The pancreas releases its secretions into the pancreatic duct, which eventually joins the common bile duct. The sphincter of Oddi regulates the flow of these secretions into the duodenum.

Figure 2.8 The small intestine.
Source: Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

- **microvilli**, hair-like extensions of the plasma membrane of the enterocytes that make up the villi. A square millimeter of cell surface is believed to have as many as 2×10^5 microvilli projections.

The microvilli (Figure 2.10) possess a surface coat, or glycocalyx, consisting of numerous fine filaments that extend almost perpendicular from the membrane to which it is attached out into the lumen. This enterocyte membrane bordering the lumen is referred to as the enterocyte's brush border (also called **apical**) membrane. Many of the digestive enzymes produced by the enterocytes are structurally glycoproteins, and the carbohydrate (glyco) portion of these glycoprotein enzymes make up part of the **glycocalyx**. These enzymes hydrolyze already partially digested nutrients, especially carbohydrates and protein. Some nutrients that are not completely digested on the brush border, however, may be further digested within the cytosol of the enterocytes.

Covering the brush border membrane is an area called the unstirred water (fluid) layer. That is, the unstirred water layer lies between the enterocyte's brush border membrane and the intestinal lumen. Its presence can affect lipid absorption. More detailed information on carbohydrate, fat, and protein digestion and absorption is provided in Chapters 3, 5, and 6, respectively.

Between the villi of the small intestine are small pits or pockets called the crypts of Lieberkühn (Figure 2.9). Stem cells in these crypts continuously undergo mitosis. The new cells migrate upward and out of the crypts toward the tips of the villi, and as they migrate, they differentiate into other cell types. Billions of old enterocytes, which die by apoptosis and are sloughed off daily into the intestinal lumen for excretion in the feces, are replaced by new enterocytes about every 3–5 days. Some of the other cells found in the crypts include Paneth cells that secrete both antimicrobial peptides (called defensins), lysozymes that can destroy bacterial cell walls, and goblet cells that secrete both small cysteine-rich proteins with antifungal activity and mucus, which adheres to the mucosa and acts as a protective barrier. Cells and glands in the crypts of Lieberkühn also secrete large volumes of intestinal juices into the lumen of the small intestine to facilitate nutrient digestion. Much of this fluid is reabsorbed.

Brunner's glands, located in the mucosa and submucosa of the first few centimeters of the duodenum (duodenal bulb), as well as exocrine cells of the pancreas, also release secretions into the small intestine. The secretions of the

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Figure 2.9 The structure of the small intestine.
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Brunner's glands are rich in mucus to coat (protect) the intestinal mucosa cells. The secretions of the pancreas are rich in bicarbonate to neutralize the acidic chyme (released by the stomach) and create a more alkaline environment, with a pH of approximately 8.2–9.3. This higher pH is also important for optimal enzyme activity within the intestine.

Regulation of Intestinal Motility and Secretions

Chyme is propelled through the small intestine by contractions (Figure 2.11) that are influenced by the nervous system as well as various hormones and peptides. For example, the neuropeptide vasoactive intestinal polypeptide promotes intestinal motility and secretions, while the paracrine glucagon-like peptides diminish intestinal motility.

Peristaltic waves, or progressive contractions (like those that are in the esophagus and stomach), direct the chyme distally from the duodenum toward the ileocecal sphincter. Segmentation contractions, standing contractions of intestinal circular smooth muscles, also occur as nutrients from food are being digested. The segmentation contractions are especially important for promoting a bidirectional flow of the chyme in the small

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Figure 2.10 Structure of the absorptive cell of the small intestine.

intestine, thus prolonging contact between the intestinal cells and the digested nutrients within the chyme for absorption to occur. The basal electrical rhythm generated from the interstitial cells of Cajal located throughout the muscularis externa layer of the small intestine induces the contractions, which occur at a frequency of about 11 or 12 contractions per minute in the duodenum and about 7 or 8 contractions per minute in the ileum.

Neural reflexes also affect motility during digestion. These reflexes, discussed in more detail in the section "Neural Regulation," generally help to coordinate motility and secretions between one section of the digestive tract and another. These actions, for example, may slow processes in one organ to allow actions in another organ to "catch up"; for example, slowing gastric secretions and/or gastric emptying if large volumes of chyme requiring digestion were present in the small intestine.

While the aforementioned processes regulate intestinal motility during meals (i.e., digestive period), another type of motor activity occurs largely in the small intestine between meals. The migrating motility or myoelectric complex (MMC), a series of weak contractions that occur in several phases, moves distally down the intestine at regular intervals between periods of digestion (i.e., between meals). The MMC helps to empty or "sweep out" the intestines as well as to prevent bacterial overgrowth. The hormone motilin, secreted by M cells of the stomach, small intestine, and colon during fasting (i.e., between meals), primarily stimulates the activity of this complex. Transit time within the small intestine ranges from about 3 to 5 hours.

The Accessory Organs

Three organs—the pancreas, liver, and gallbladder—facilitate the digestive and absorptive processes in the small intestine. The next section of this chapter describes each of these organs and its role in nutrient digestion, absorption, or both.

The Pancreas

The pancreas is a slender, elongated organ that ranges in length from about 6 to 9 inches. The pancreas is found behind the greater curvature of the stomach, lying between the stomach and the duodenum (Figures 2.1 and 2.12). The organ contains both endocrine and exocrine cells (Figure 2.12b).

The endocrine cells are found among the 1–2 million cells that make up the islets of Langerhans, located primarily in the tail region of the pancreas. While these cells comprise less than 5% of the gland's volume, they are responsible for the secretion of several important hormones. The A or α cells secrete glucagon. The B or β cells secrete insulin, and the D or δ cells secrete somatostatin. Yet, while these hormones exert enormous regulatory control, it is the exocrine cells of the pancreas that are more involved in the digestive processes with the production of pancreatic juice and enzymes.

The exocrine portion of the pancreas contains acinar secretory cells, which are arranged in a circular pattern and are attached to small ducts. Cells in the ducts produce the pancreas's alkaline-rich juice, while the acinar secretory cells produce and package into granules the

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Figure 2.11 Movement of chyme in the gastrointestinal tract.
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digestive enzymes that get released by **exocytosis** into the lumens of the small ducts. The small ducts within the pancreas coalesce to form the pancreatic duct of Wirsung, which runs the length of the pancreas and connects with the common bile duct at the ampulla of Vater to form a common channel (bile pancreatic duct). The bile pancreatic duct empties through the sphincter of Oddi (Figure 2.12a) into the duodenum. The enzyme-rich and alkaline-rich secretions from the pancreas are needed for the digestive processes within the small intestine.

Pancreatic Juice and Digestive Enzymes The pancreas releases up to about 2 L of its secretions daily into the duodenum. The juice contains mostly water, electrolytes (the cations sodium, potassium, and calcium and the anion chloride), and bicarbonate (as $NaHCO_3$). The bicarbonate neutralizes the acidic chyme released from the stomach into the duodenum and creates a more alkaline pH needed for enzyme activity within the intestinal lumen. The enzymes released by the acinar secretory cells, listed in Table 2.1, digest approximately half (50%) of all ingested carbohydrates, half (50%) of all proteins, and almost all (80–90%) of ingested fat. The **proteases**—enzymes that digest proteins—are typically released as zymogens and include trypsinogen, chymotrypsinogen, procarboxypeptidases, proelastase, and collagenase. Release in this inactive state is important because, if secreted in an active form, they could digest the proteins within the pancreatic cells in which they were formed. The zymogen trypsinogen is of particular significance in that once it has become activated in the duodenum, it then functions to activate several other zymogens (chymotrypsinogen, procarboxypeptidase, proelastase) and the enzyme phospholipase A_2 needed for fat digestion. A protein called trypsin inhibitor, also synthesized by the pancreas, protects the pancreas by binding to trypsin should it have been accidently activated within the pancreas. By binding to trypsin, the inhibitor prevents trypsin from activating other zymogens within the pancreas and causing pancreatitis (inflammation of the pancreas).

As a group, proteases hydrolyze peptide bonds within proteins, resulting in the production of smaller polypeptides or proteins that are shorter in length than the original polypeptide or protein (see Chapter 6 for additional information on protein digestion). Enzymes

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Figure 2.12 (a) The ducts of the gallbladder, liver, and pancreas. (b) Schematic representation of the exocrine and endocrine portions of the pancreas.
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that are released also participate in the digestion of starch (pancreatic α-amylase) and fats (pancreatic lipase, phospholipase A_2, and colipase), as discussed further in Chapters 3 and 5, respectively.

Regulation of Pancreatic Secretions The primary stimuli for the release of pancreatic juice are the hormones secretin and cholecystokinin. Secretin, produced by S cells in the proximal small intestine, is secreted into the blood primarily in response to the presence of unneutralized acidic chyme in the duodenum. Secretin stimulates pancreatic duct cells to secrete into the duodenum its bicarbonate-rich juices, which in turn neutralize the acidic chyme. Additionally, cholecystokinin is secreted by I cells of the proximal small intestine and enteric nerves in response to the presence of fat and partially digested proteins in the duodenum. Cholecystokinin acts on the acinar secretory cells to stimulate digestive enzyme release into the duodenum. Acetylcholine also functions to enhance enzyme release by acinar cells, while the hormone pancreatic polypeptide and the paracrine somatostatin act in reverse, that is, inhibiting pancreatic exocrine secretions.

Selected Disorders of the Pancreas Pancreatitis (the suffix *-itis* meaning "inflammation") occurs when zymogens become activated within the pancreas and digest pancreatic tissue and sometimes associated tissues including blood vessels and fat. The condition can occur with excessive alcohol consumption, hypertriglyceridemia (**serum** triglycerides in excess of usually about 1,000 mg/dL), blockage of the pancreatic duct (e.g., from gallstones), viral infections, and pancreatic injury, among others. Abdominal pain, usually in the upper left quadrant and that worsens with food intake, is a major symptom. A number of biochemical blood indices become altered with pancreatitis. Most notably, the enzymes pancreatic amylase and lipase, which are normally released into the duodenum, leak out of the damaged pancreas and become elevated in the blood (where they are not normally found). The nutritional management of individuals with especially **acute**, severe pancreatitis is quite involved (and beyond the scope of this book); however, a few nutritional implications of pancreatitis related to digestive functions are provided here. First, because the damaged pancreas cannot produce enzymes in sufficient quantities, the patient often requires the provision of nutrients that are already partially hydrolyzed (rather than intact) or supplements of pancreatic enzymes, especially lipase, to replace those not being released by the malfunctioning pancreas. In addition, because bicarbonate secretion from the pancreas is frequently diminished with pancreatitis, and because this bicarbonate is needed to neutralize acid from the stomach and increase the pH of intestinal juices for enzyme function, medications such as antacids are sometimes provided. Depending on the severity, the individual may also need to be fed through a tube placed into the jejunum and may require suctioning of his or her gastric contents to minimize stimulation of the pancreas.

The Liver

Another accessory organ to the gastrointestinal tract is the liver, pictured in Figures 2.1, 2.12, and 2.13. The liver, the largest single internal organ of the body, is made up of two lobes, the right lobe and the left lobe. These lobes in turn contain functional units called lobules. The lobules are made up of plates or sheets of hepatocytes (liver cells)

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Figure 2.13 The anatomy of the liver.
Source: Sherwood, *Human Physiology*, 9/e. © Cengage Learning.

(Figure 2.13). The plates of cells are arranged so that they radiate out from central veins. Thus, the liver has multiple plates of cells radiating from multiple central veins. The central veins direct blood from the liver into general circulation through the hepatic veins and then ultimately into the inferior vena cava. Blood passes between the plates of liver cells by way of sinusoids, which function like a channel and arise from branches of the hepatic artery and from the portal vein. The portal vein brings blood rich in nutrients from the digestive tract to the liver. Sinusoids allow blood from these two blood vessels (the portal vein and the hepatic artery) to mix and also enable uptake of nutrients through the endothelial cells that line the sinusoids. Sinusoids also contain macrophages called Kupffer's cells, which phagocytize bacteria and other foreign substances and thus serve to protect the body. Bile canaliculi lie between the hepatocytes in the hepatic plates. Following hepatocyte production of bile, the bile is secreted into the canaliculi, which then carry it to a duct at the periphery of the lobules. The hepatic ducts from the different lobules unite and join with the cystic duct from the gallbladder to form the common bile duct.

Bile Synthesis and Function The liver produces bile, a greenish-yellow fluid composed mainly of bile acids and salts but also cholesterol, phospholipids, and bile pigments dissolved in an alkaline solution. The bile acids are synthesized in the hepatocytes from cholesterol, which in a series of reactions is oxidized to generate chenodeoxycholic acid and cholic acid, the two principal or primary bile acids. These bile acids combine primarily with sodium, but also with potassium and calcium, to form bile salts. Once formed, these bile salts conjugate primarily (~75%) with the amino acid *gly*cine, forming *gly*cocholic acid and *gly*cochenodeoxycholic acid, and to a lesser extent (25%) with the amino acid *taur*ine, forming *taur*ocholic acid and *taur*ochenodeoxycholic acid. Conjugation of the bile with these amino acids improves their ability to form micelles.

In addition to bile salts, small amounts of cholesterol and phospholipids, especially lecithin, are found in

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bile, and make up what is referred to as the bile acid–dependent fraction of bile. In addition, bile contains water, bicarbonate, and bile pigments, mainly bilirubin and/or biliverdin (waste end products of hemoglobin degradation) that are conjugated with glucuronic acid. It is these bile pigments that give bile much of its color. This fraction of the bile is referred to as bile acid independent.

Bile acts like a detergent to emulsify fat, that is, to break down large fat globules into small (about 1 mm diameter) fat droplets. More specifically, the bile helps to absorb lipids by forming small (<10 nm) spherical, cylindrical, or disk-like complexes called micelles. Micelles, which can contain as many as 40 bile salt molecules, allow pancreatic lipase to better access and hydrolyze bonds within the triacylglycerols in the micelles. More thorough coverage of the function of bile is found in Chapter 5.

The Gallbladder

The gallbladder, a small organ with a capacity of approximately 40–50 mL (1.4–1.8 oz), is located on the surface of the liver (Figure 2.14). Bile that is made in the liver is concentrated in the gallbladder whose mucosa absorbs large quantities of water along with some electrolytes that were originally present in the bile. This concentration of the bile facilitates its storage given the small volume capacity of the gallbladder.

Cholecystokinin, secreted into the blood by I cells of the proximal small intestine in response to the presence of fat-ladden chyme in the duodenum, stimulates the gallbladder to contract and the sphincter of Oddi to relax. These actions allow the release of bile into the duodenum where it functions to emulsify fat. The closing of the sphincter of Oddi directs the bile back into the gallbladder.

Figure 2.14 Enterohepatic circulation of bile.
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Selected Disorders of the Gallbladder The presence of gallstones (cholelithiasis) in the gallbladder is fairly common, especially among older adults. Most stones are cholesterol based, although some consist primarily of pigments, usually bilirubin. Stones can form when bile remains sequestrated within the gallbladder and is infrequently released (gallbladder hypomotility occurring, for example, with the use of parenteral nutrition versus oral intake). More commonly, gallstones form when the bile becomes supersaturated with cholesterol. The presence in the bile of large amounts of cholesterol, which is not very soluble, causes the cholesterol to precipitate out of the solution and provides a crystalline-like structure or nuclei around or within which calcium, bilirubin, phospholipids, and other compounds deposit, to ultimately form a "stone." Other unknown factors also may promote nucleation, or this formation of a crystalline-like structure, upon which gallstones form. Gallstones, once produced, may reside silently in the gallbladder; irritate the organ, causing cholecystitis (inflammation of the gallbladder); or lodge in the common bile duct, blocking the flow of bile (choledocholithiasis) or the flow of pancreatic juice, causing pancreatitis. When gallstones block any of these ducts, surgical removal of the gallbladder (cholecystectomy) is often needed. However, in those without a gallbladder, the common bile duct generally remains intact, allowing bile release from the liver directly (without any storage in the gallbladder) into the duodenum. Individuals who have had a cholecystectomy often need to eat low-fat foods, as high-fat meals can promote abdominal pain and **steatorrhea**.

The Recirculation and Excretion of Bile The human body has a total bile pool of about 2.5–5.0 g. Greater than 90% of the bile salts secreted into the duodenum are reabsorbed primarily by active transport in the distal ileum. Small amounts of the bile may also be passively reabsorbed in the colon. Absorbed bile enters the portal vein and is transported, attached to the plasma protein albumin in the blood, back to the liver. Once in the liver, the reabsorbed bile is reconjugated to amino acids if necessary and secreted along with the newly synthesized bile into the duodenum during digestion. New bile acids typically are synthesized in amounts about equal to those lost in the feces. The circulation of bile, termed ***enterohepatic circulation***, is pictured in Figure 2.14. The pool of bile is thought to recycle at least twice per meal.

Some of the bile acids that are not reabsorbed in the small intestine may be deconjugated by bacteria (via bacterial bile salt hydrolase) to form secondary bile acids (Figure 2.15). Cholic acid is converted to the secondary bile acid deoxycholic acid. Chenodeoxycholic acid is converted to the secondary bile acid lithocholic acid, which, unlike deoxycholic acid, is typically excreted in the feces. It has been suggested that this bacterial enzymatic activity on bile salts may regulate in part both cholesterol metabolism and energy balance in the host. About 0.5 g of bile salts are lost daily in the feces.

Bile Circulation and Hypercholesterolemia Knowing how bile is recirculated and excreted helps in understanding the mechanisms by which various drug therapies and functional foods help in the treatment of high blood cholesterol concentrations (hypercholesterolemia). Medications—specifically, **resins** such as cholestyramine (Questran)—that bind bile in the gastrointestinal tract and enhance its fecal excretion from

Figure 2.15 The synthesis of secondary bile acids by intestinal bacteria.

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the body are used to treat hypercholesterolemia in some individuals. Additionally, plant (phyto-) stanols and sterols are added to some foods such as margarines, orange juice, and granola bars. These phytostanols and phytosterols (as well as some dietary fibers) bind bile and dietary and endogenous cholesterol in the gastrointestinal tract and enhance their fecal excretion from the body. The increased fecal excretion of the bile, decreased recirculation of the bile, and decreased absorption of cholesterol require the body to use cholesterol to synthesize new bile acids. The increased use of cholesterol to make more bile diminishes the body's cholesterol concentrations. Thus, the use of such medications and functional foods can lead to lower blood cholesterol concentrations and reduced risk of cardiovascular disease. Health claims on the labels of some of the products containing phytosterols state that "Plant sterols, eaten twice a day with food for a total of 1.3 g daily total, may reduce heart disease risk in a diet low in saturated fat and cholesterol." Daily consumption of plant sterols has been shown to decrease total and low-density lipoprotein (LDL) **plasma** cholesterol concentrations in people with normal or high blood lipid concentrations.

The Absorptive Process

Following all the actions of the various secretions and enzymes from the mouth, stomach, pancreas, and small intestine and with the help of bile from the liver and gallbladder, the now digested nutrients are ready to be absorbed, that is, to enter the cells of the gastrointestinal tract. The absorption of most nutrients begins in the duodenum and continues throughout the jejunum and ileum, as shown in Figure 2.16. Generally, most absorption occurs in the proximal (upper) portion of the small intestine, but some nutrients are absorbed primarily in the distal ileum, making all regions of the small intestine valuable to adequately nourishing the body.

Figure 2.16 Primary sites of nutrient absorption in the gastrointestinal tract.

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The digestion and absorption of nutrients within the small intestine are rapid, with most of the carbohydrate, protein, and fat being absorbed within a few hours after chyme has reached the small intestine. The presence of unabsorbed food in the ileum may increase the amount of time material remains in the small intestine and therefore may increase nutrient absorption. Transit time of unabsorbed substances in the small intestine is about 3–6 hours.

Nutrients are absorbed across the brush border membrane into enterocytes primarily by diffusion, facilitated diffusion, active transport, and/or, occasionally, **pinocytosis** or **endocytosis** (Figure 2.17). To be available for use in the body, transport across the enterocyte's basolateral (serosal) membrane is also needed. A few nutrients, however, gain entry into the body via a paracellular (between cells) route. Tight junctions between cells of the mucosa are found throughout the digestive tract to help regulate "what gets in" and "what does not get into" the body via this paracellular route. Membrane-spanning proteins and scaffolding proteins function together to alter these junctions and thus to regulate permeability. Scaffolding proteins link membrane proteins to the cell's cytoskeletal network. Membrane-spanning proteins traverse the paracellular space. Examples of membrane-spanning proteins include occludins and

Diffusion. Some substances, such as water and small lipid molecules, cross membranes freely. The concentration of substances that can diffuse across cell membranes tends to equalize on the two sides of the membrane, so that the substance moves from the higher concentration to the lower concentration; that is, it moves down a concentration gradient.

Facilitated diffusion. Other compounds cannot cross cell membranes without a specific carrier. The carrier may affect the permeability of the membrane in such a way that the substance is admitted, or it may shuttle the compound from one side of the membrane to the other. Facilitated diffusion, like simple diffusion, allows an equalization of the substance on both sides of the membrane. The figure illustrates the shuttle process:

1. Carrier loads particle on outside of cell.
2. Carrier releases particle on inside of cell.
3. Reversal of 1 and 2.

Active transport. Substances that need to be concentrated on one side of the cell membrane or the other require active transport, which involves energy expenditure. The energy is supplied by ATP, and Na^+ is usually involved in the active transport mechanism. The figure illustrates the unidirectional movement of a substance requiring active transport:

1. Carrier loads particle on outside of cell.
2. Carrier releases particle on inside of cell.
3. Carrier returns to outside to pick up another particle.

Pinocytosis. Some large molecules are moved into the cell via engulfment by the cell membrane. The figure illustrates the process:

1. Substance contacts cell membrane.
2. Membrane wraps around or engulfs the substance.
3. The sac formed separates from the membrane and moves into the cell.

Figure 2.17 Primary mechanisms for nutrient absorption.

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junction adhesion molecules as well as a group of proteins called claudins. Membrane-spanning proteins function to seal up or tighten the paracellular space and thus minimize entry into the body. Other membrane-spanning proteins, such as claudin-2, form ion-selective pores that open up the space to facilitate paracellular absorption for nutrients like calcium and magnesium. Changes in tight junctions (as occur with mutations in specific claudins) that increase intestinal permeability (and allow substances to enter the body that would not normally "get in") have been associated with intestinal inflammation and disease.

The mechanism of absorption for a nutrient depends on several factors: solubility (fat versus water) of the nutrient, the concentration or electrical gradient, and the size of the molecule to be absorbed. The absorption and transport of amino acids, peptides, monosaccharides, fatty acids, monoacylglycerols, and glycerol—that is, the end products of **macronutrient** digestion—are considered in depth in Chapters 3, 5, and 6. The digestion and mechanisms of absorption for each of the vitamins and minerals are described in detail in Chapters 9–14; the general sites of absorption are shown in Figure 2.16.

Unabsorbed intestinal contents are passed from the ileum (the terminal or most distal section of the small intestine) through the ileocecal sphincter into the colon. Some of these unabsorbed materials, however, serve as substrates for bacteria that inhabit the small intestine and colon. The ileocecal sphincter, in addition to controlling the passage of contents from the small intestine into the large intestine, helps to prevent the migration of bacteria from the large intestine back into the small intestine. Bacterial overgrowth in the small intestine may result in nutritional deficiencies not only from bacteria-induced mucosal cell destruction, but also from direct bacterial use of nutrients, bacterial destruction of the nutrients, and/or bacterial destruction of substances (such as bile) needed for nutrient absorption. Criterion for bacterial overgrowth is typically the presence of fecal microorganisms in the small intestine at a density of $> 10^5$ microbes/mL. Bacterial overgrowth in the small intestine induces deficiencies of nutrients, such as vitamin B_{12} and iron, which the bacteria use for their own growth. Additionally, the bacteria may induce deficiencies of thiamin and fat-soluble vitamins. Fat-soluble vitamin deficiencies occur with bacterial deconjugation of bile that is needed for fat and fat-soluble vitamin absorption. Thiamin can be destroyed in the small intestine from thiaminases released by the bacteria.

The Colon (Large Intestine)

Once through the ileocecal sphincter, materials move into the cecum, the right side of the colon, and then move sequentially through the ascending, transverse, descending, and sigmoid sections (Figure 2.18). The colon in its entirety is almost 5 feet long and is larger in diameter (about 3 inches) than the small intestine (about 1½ inches), thus explaining the terminology distinction (large versus small) between the two intestines.

Rather than being a part of the entire wall of the digestive tract, as it is in the upper digestive tract, the longitudinal muscle in the colon is gathered into three muscular bands or strips called teniae (also spelled taenia or teneae) coli that extend throughout most of the colon. The length of the teniae coli is smaller than that of the underlying circular muscles and mucosa, which causes the underlying layers to form pouches called haustra.

On initially entering the colon, the contents are still quite fluid. Contraction of the musculature of the large intestine is coordinated so as to mix the intestinal contents and to keep material in the proximal (ascending) colon a sufficient length of time for absorption of nutrients to occur. The proximal colonic mucosal cells typically absorb sodium, chloride, and water. About 90–95% of the water and sodium entering the colon each day is absorbed. Colonic absorption of sodium, which occurs by active transport and which enhances water absorption, is influenced by a number of factors, including hormones. Antidiuretic hormone (also called vasopressin) secreted from the pituitary gland, for example, decreases sodium absorption, whereas glucocorticoids like cortisol secreted

Figure 2.18 The colon.
Source: Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

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from the adrenal gland and mineralocorticoids such as aldosterone secreted from the adrenal gland increase sodium absorption in the colon. Further information on water and electrolyte absorption is found in Chapter 12.

Colonic Secretions and Motility and Their Regulation

Secretions into the lumen of the colon are few, but present. Goblet cells secrete mucus. Mucus acts as a lubricant for fecal matter and protects the colonic mucosal cells. The mucus, present in a double layer, lies between the colonic mucosal cells and the bacteria that reside in the colon and thus help reduce the likelihood of bacterial translocation. Bicarbonate is also secreted into the lumen in exchange for chloride, which is absorbed. Bicarbonate provides an alkaline environment that helps neutralize acids produced by colonic anaerobic bacteria.

Haustral contractions, characterized as oscillating contractions of the circular muscles, provide one form of motility within the large intestine. These contractions are regulated in part by the basic electrical rhythm of the colon's smooth muscle layer and occur at a rate of about two to six contractions per minute. Peristalsis provides minimal motility in the colon. Instead, more vigorous mass-action peristaltic-like contractions (i.e., contractions of large sections of smooth muscle within the colon) promote movement of material from one section of the colon to the next toward the rectum. Neural reflexes also affect motility. For example, the gastrocolic reflex, which occurs in response to gastrin and enteric nervous system activity, promotes contractions within the distal colon and rectum to promote defecation.

The end result of the passage of material through the colon, which usually takes about 12–72 hours, is that the unabsorbed materials are progressively dehydrated. Typically, the approximately 500 mL to 1 L of materials that enter the large intestine each day is reduced to about 150–200 g of defecated material. This fecal material is about 75% water and 25% solids. Fecal solids usually include sloughed gastrointestinal cells, digestive juice constituents, fiber, small amounts of unabsorbed fat and bile, and bacteria. The bacteria may account for about 30% of dry fecal weight.

Colonic Bacteria

The trillions of microorganisms (which can weigh up to 5 lbs) that are living in the gastrointestinal tract make up our gut microbiota (or **microflora**). These microorganisms include both gram-negative and gram-positive bacterial strains, representing over 1,000 species. Although intestinal bacterial counts in the large intestine have been reported to be as high as 10^{12} per gram of gastrointestinal tract contents, bacteria are found throughout the gastrointestinal tract. The mouth contains mostly anaerobic bacteria. The stomach contains few bacteria because of its low pH, but some more acid-resistant bacteria that are present include lactobacilli and streptococci. The proximal small intestine contains both aerobes and facultative anaerobes. Most bacteria found in the ileum and large intestine are anaerobes, including bacteroides, lactobacilli, and clostridia. Other examples of bacteria that inhabit the large intestine are bifidobacteria, methanogens, eubacteria, and streptococci. Anaerobic species are thought to outnumber aerobic species by at least 10-fold, but the exact composition of the microflora is affected by a variety of factors such as substrate availability, pH, medications, and diet, among others.

Bacteria gain nutrients for their own growth from undigested and/or unabsorbed food residues in the intestines. Enzymes synthesized by the bacteria but lacking in humans allow for the digestion of many nutrients to generate substrates for bacterial energy production and to attain, for example, carbon atoms necessary for bacterial maintenance and/or growth. Starch that has not undergone hydrolysis by pancreatic amylase, for example, may be used by gram-negative bacteroides and by gram-positive bifidobacteria or eubacteria. Mucins found in mucus secretions of the gastrointestinal tract may be broken down and used by bacteria such as bacteroides, bifidobacteria, and clostridia. Digestive enzymes themselves may even serve as substrates for bacteria such as bacteroides and clostridia. In addition, sugar alcohols such as sorbitol and xylitol; disaccharides such as lactose; and some fibers may be degraded by selected bacteria in the colon.

Many products are generated from the bacterial use of undigested and unabsorbed materials in the colon. Several B-vitamins as well as vitamin K are produced by bacteria in the colon and may be absorbed to varying degrees. Some particularly beneficial acids that are produced during carbohydrate **fermentation** (an anaerobic process by which bacteria break down substances, primarily carbohydrate and protein) by specific strains of bacteria include lactic acid and three **short-chain fatty acids**—acetic acid, butyric acid, and propionic acid. These short-chain fatty acids provide many benefits to the host, as shown in part in Figure 2.19, and more specifically listed hereafter.

- **Acidify the luminal environment**. The presence of short-chain fatty acids in the colon decreases the pH within the lumen of the colon. This more acidic environment has several positive effects. (1) With the more acidic pH, free bile acids become less soluble and the activity of bacterial 7 α dehydroxylase diminishes (optimal pH ~ 6–6.5) resulting in decreased conversion of primary bile acids to secondary (more harmful) bile acids. (2) With the lower pH, calcium, released with fiber degradation, binds to and promotes the excretion of bile acids (and thus prevents their conversion

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Figure 2.19 Some benefits from the presence of bacteria in the large intestine.

to secondary bile acids). (3) The lower pH favors the growth of beneficial lactobacilli and bifidobacteria and inhibits the growth of pH-sensitive pathogenic bacteria. (4) The acidic environment enhances the production of mucin, which forms part of the physical barrier overlying intestinal cells. This increased mucin content provides a greater physical barrier and decreases the likelihood of pathogenic bacterial colonization as well as bacterial translocation. (5) The low pH may improve the absorption of minerals released during fermentation.

- **Serve as signaling molecules** by interacting with receptors on enteroendocrine cells that mediate the synthesis of hormones and peptides and by effects on histone acetylation involved in gene expression.
- **Exhibit trophic effects**, specifically stimulating proliferation and growth and maintaining the integrity (preventing atrophy) of the colonic mucosal cells.
- **Improve colonic and splanchnic blood flow**. Short-chain fatty acids are thought to directly affect smooth muscle as well as to interact with the enteric nervous system. This improved blood flow enhances both the delivery of nutrients to the colon and the transport of nutrients from the colon to the liver. (Note that the term ***splanchnic*** generally refers to organs in the abdominal cavity such as the liver, spleen, stomach, and intestines.)
- **Increase water and sodium absorption** in the colon. The absorption of the short-chain fatty acids in turn stimulates water and sodium absorption into the mucosal cells of the colon.
- **Provide energy and serve as substrates for use within cells**. Over 95% of the short-chain fatty acids are absorbed and utilized by the body. Butyric acid serves as a major energy source for colonic mucosal cells. In fact, butyric acid is thought to supply colonic cells with over two-thirds of their energy needs. Absorbed propionic acid and acetic acid are transported via the portal vein to the liver. In the liver, propionic acid is largely metabolized along with small amounts of acetic acid. Much of the propionic acid is converted to succinyl-CoA, which may be used by the liver for glucose or energy production. Propionic acid also may alter cholesterol metabolism. Most of the acetic acid passes through the liver and is used by other tissues, including skeletal and cardiac muscle and the kidneys and brain. Acetic acid may be used for the synthesis of cholesterol and fatty acids. Short-chain fatty acids also may impact glycogenolysis and play a role in insulin release and/or sensitivity.

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- **May inhibit tumors**. ***In vitro*** studies suggest short-chain fatty acids promote apoptosis and promote the arrest of growth and differentiation in tumor cell lines.
- **Stimulate the immune system** by enhancing the production of macrophages, T-helper lymphocytes, leukocytes, antibodies, and cytokines and improving antibody response.

As can be gleaned from this list, the short-chain fatty acids generated by bacteria in the gastrointestinal tract play several important roles. The bacteria themselves also provide direct benefits and augment some of the benefits attained from the short-chain fatty acids. Some examples of healthful actions of bacteria include the ability to:

- **Enhance the host's immune defense system** by increasing secretory IgA production, tightening the mucosal barrier, enhancing cytokine responses, enhancing phagocytic activity, and producing antimicrobial substances such as bacteriocin.
- **Displace, exclude, or antagonize pathogenic bacteria from colonizing**, for example, by competing for attachment sites on the intestinal mucosa, by strengthening the mucosal barrier to normalize intestinal permeability and to prevent pathogenic bacterial translocation, and by producing substances like biosurfactants that reduce adhesion of pathogens to the mucosa.
- **Scavenge, sequester, transform, and/or promote the excretion of harmful/carcinogenic substances** such as bile acids, nitrosamines, heterocyclic amines, and mutagenic compounds. Moreover, some bacteria, such as *Lactobacillus acidophilus*, may be able to inhibit the production of carcinogenic compounds.
- **Enhance fecal bulk and dilute fecal contents** to minimize exposure with colonic mucosal cells.

Another possible role of the microbiota is in energy metabolism and thus regulation of body weight. Data are limited at present but some studies suggest products generated by colonic microbes may exert signals that influence brain activity, including effects on appetite regulation and energy metabolism.

A less desirable result of the presence of colonic bacteria is gas production, although swallowed air also contributes to this problem. Several different gases are generated by these bacteria, including methane (CH_4), hydrogen (H_2), hydrogen sulfide (H_2S), and carbon dioxide (CO_2). One estimate suggests that colonic bacterial fermentation of about 10 g of carbohydrate can generate several liters of hydrogen gas. While much of the hydrogen and other gases that are generated can be used by other bacteria in the colon, gases that are not used are excreted.

Measurement of hydrogen gas produced by bacteria is used as a basis to diagnose lactose intolerance, a condition in which the enzyme lactase is not made in sufficient quantities to digest the disaccharide lactose. Lactose intolerance is fairly common among adults, especially those of African American, Native American, and Asian heritage. When a person with lactose intolerance ingests the carbohydrate lactose (e.g., by drinking milk), the undigested lactose enters the colon and is **fermented** by colonic bacteria. These colonic bacteria, upon fermenting the lactose, produce more hydrogen gas than usual. Much of this hydrogen gas made by the bacteria is absorbed by the body and then exhaled in the breath. In fact, to diagnose lactose intolerance, a person may be asked to consume about 50 g of lactose and have their breath analyzed for hydrogen gas for the next several hours. Generally, if the person is lactose intolerant, hydrogen gas excretion in the breath increases for about 1–1½ hours after lactose is consumed. An absence of an increase in breath hydrogen gas concentrations suggests adequate lactose digestion. Symptoms of lactose intolerance include bloating, gas, and abdominal pain.

Other products are made as bacteria degrade amino acids in the colon. For example, bacterial degradation of the branched-chain amino acids generates the branched-chain fatty acids isobutyric acid and isovaleric acid. Deamination (removal of the amino group) of aromatic amino acids yields phenolic compounds. Amines such as histamine result from bacterial decarboxylation of amino acids such as histidine. Ammonia is generated by bacterial deamination of amino acids as well as by bacterial urease action on urea that has been secreted into the gastrointestinal tract from the blood. The ammonia can be absorbed by the colon and circulated to the liver, where it can be reused to synthesize urea or amino acids. About 25%, or 8 g, of the body's urea may be handled in this fashion. This process must be controlled in people with liver disease (cirrhosis) as high amounts of ammonia in the blood are thought to contribute to the development of hepatic encephalopathy (coma). Uric acid and creatinine may also be released into the digestive tract and metabolized by colonic bacteria.

Intestinal Conditions and Probiotics Imbalances in the number and composition of gut microbiota have been linked with a number of conditions like inflammatory bowel diseases (Crohn's disease and ulcerative colitis), colon cancer, rheumatoid arthritis, and diabetes, among others, and have prompted increased therapeutic use of **probiotics** (*pro* means "life" in Greek) and **prebiotics**. Probiotics are live microorganisms (i.e., active cultures of specific strains of bacteria) that when administered in adequate amounts confer health benefits to its hosts. Prebiotics (discussed in more detail in Chapter 4) are substances that are not digested by human digestive enzymes but confer health benefits to the host by acting as substrates for the growth and/or activity of one or more species of healthful bacteria in the colon.

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The most common probiotic bacteria are lactic acid bacteria, usually strains of Lactobacillus and Bifidobacterium genera. To be considered a probiotic, the product must contain 100 million live active bacteria per gram. At present, probiotics are mostly consumed as yogurt with live cultures and as fermented or cultured milk and milk products (such as buttermilk and kefir). In the United States, yogurt is often fermented by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and milk is usually fermented by *L. acidophilus* and *L. casei*. Other bacteria used to manufacture dairy products include *Leuconostoc esntheroides*, *L. mesenteroides*, and *Lactococcus lactis*. Other food sources of probiotics include miso, tempeh, and some soy beverages/products.

Consumption of probiotics has been shown to improve symptoms of irritable bowel syndrome and inflammatory bowel diseases as well as several types of diarrhea. To be effective, probiotics usually need to contain 1–10 billion colony-forming units (CFUs) per dose, with doses given once or twice daily or sometimes a few times per week. Tolerance is typically satisfactory; however, bacterial sepsis (infection) is possible, especially in those with impaired immune function (immunosuppression), intestinal tract dysfunction (characterized by increased gastrointestinal permeability or a defective barrier), or other chronic health conditions such as diabetes mellitus, cancer, abscesses, and organ transplant.

COORDINATION AND REGULATION OF THE DIGESTIVE PROCESS

The central nervous system, which comprises the brain and spinal cord, affects the body via efferent neurons. Efferent neurons to skeletal muscles make up the somatic division, and efferent neurons to the internal organs represent the autonomic division of the nervous system. The autonomic division can be divided into the sympathetic and the parasympathetic nervous systems.

Neural Regulation

The autonomic division communicates with the digestive organs directly, but it can also communicate with the digestive tract's own (local) nervous system. Generally, the sympathetic system decreases or slows down digestive tract motility and secretions, while the parasympathetic nervous system stimulates the digestive tract, promoting motility (such as peristalsis), gastrointestinal reflexes, and the secretion of hormones and enzymes. The parasympathetic system interacts with the digestive tract primarily through the vagus nerve.

The digestive system's local nervous system is known as the enteric nervous system or the intrinsic nerve plexuses and includes about 100 million neurons and their processes embedded in the layers of the gastrointestinal tract beginning in the esophagus and extending to the anus. The enteric nervous system consists of two neuronal networks or plexuses: the myenteric or Auerbach plexus and the submucosal or Meissner plexus. Sensory information is received by the enteric nervous system in part from different receptors within the gastrointestinal tract layers; these receptors monitor "local" conditions within the digestive organs. Mechanoreceptors detect distension or pressure in the gastrointestinal tract walls. Chemoreceptors monitor changes in chemical composition, and osmoreceptors detect changes in the osmolarity, such as that of chyme. Receipt of this sensory information by the enteric nervous system results in changes in the digestive tract's smooth muscle functions (affecting motility) and/or changes to specific cells and glands (affecting the release of enzymes and hormones). Neural reflexes also may result from the stimulation of these receptors, as discussed in the next paragraph. The myenteric plexus, which lies between the circular and longitudinal smooth muscles of the digestive tract, generally controls motility, and when this plexus is stimulated, gastrointestinal activity generally increases. The submucosal plexus typically controls the release of secretions and affects local blood flow. Some of the many neurotransmitters released by the enteric nervous system are acetylcholine, 5-hydroxytryptamine (serotonin), norepinephrine, gamma aminobutyric acid (GABA), vasoactive intestinal polypeptide, and nitric oxide.

Neural reflexes also occur within the digestive tract to effect changes in secretions, blood flow, and/or motility. For example, with the ileogastric reflex, gastric motility is inhibited when the ileum becomes distended. This action allows more time for the contents of the lower small intestine, the ileum, to be emptied before more chyme is released from the stomach into the upper small intestine. With the gastroileal reflex, ileal motility is stimulated when gastric motility and secretions increase. This neural reflex promotes overall motility within the stomach and small intestine. Other reflexes also affect the intestines. For example, with the colonoileal reflex, stimulation of receptors within the colon in turn inhibits the emptying of the contents from the ileum into the colon. Such actions slow down overall motility in these organs. Similar actions occur with the intestinointestinal reflex, which diminishes intestinal motility when a segment of the intestine is overdistended.

Regulatory Peptides

Factors influencing digestion and absorption are coordinated, in part, by a group of gastrointestinal tract molecules called regulatory peptides or, more specifically,

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gastrointestinal hormones and neuropeptides. More than 100 regulatory peptides are thought to affect gastrointestinal functions. These peptides are released by endocrine cells within the digestive tract or its accessory organs, by enteric nerves, or both. These enteroendocrine cells, which are often identified by letters (e.g., G cells, S cells, I cells, etc.), are found throughout the digestive system. Most of the regulatory peptides released by these cells work in an endocrine manner, being released into the blood in response to specific stimuli and traveling to region(s) of the digestive tract and/or its accessory organs to evoke changes. A few regulatory peptides, however, work in a paracrine manner, being released into the local area where they diffuse through extracellular spaces to evoke changes in target tissues.

Regulatory peptides affect a variety of digestive functions, such as gastrointestinal tract motility, cell growth, and the secretion of digestive enzymes, electrolytes, and water. Most, but not all, have multiple actions; some are strictly inhibitory or stimulatory, whereas some mediate both types of responses. Many of the functions of regulatory peptides have been addressed to varying degrees in the sections on regulation of gastric and intestinal secretions and motility. Table 2.2 summarizes some of the functions of a few of these peptides, while more detailed information is provided hereafter.

- Gastrin, secreted into the blood primarily by G cells in the antrum of the stomach and proximal small intestine, acts mainly in the stomach to stimulate the release of hydrochloric acid and pepsin, and to a lesser extent to stimulate gastric motility and emptying. Gastrin also stimulates the release of histamine, which further induces gastric acid release, and has trophic actions (stimulates cell growth) on gastric and intestinal mucosa. Gastrin release is stimulated mainly by gastric distension and the presence of protein digestion products in the stomach, as well as by the release of gastrin-releasing polypeptide by the vagus nerve. Gastrin secretion is inhibited by the presence of acid in the antrum and by the release of somatostatin.
- Cholecystokinin (CCK), secreted into the blood by I cells of the proximal small intestine and by enteric nerves in the distal ileum and colon, principally stimulates pancreatic acinar secretory cells to release digestive enzymes into the duodenum. It also has trophic actions on the pancreas and stimulates gallbladder contraction and the relaxation of the sphincter of Oddi to facilitate the release of bile into the duodenum. Lesser roles of cholecystokinin include decreasing gastric emptying and gastric acid secretion. Cholecystokinin release is stimulated by the presence of protein digestion products and fat in the duodenum, which is logical given the hormone's actions on the pancreas, but its release diminishes as nutrients are absorbed or moved into more distal sections of the digestive tract. In neurons in the brain, cholecystokinin is thought to influence the perception of appetite, among other processes.

Table 2.2 Selected Regulatory Hormones/Peptides of the Gastrointestinal Tract, Their Main Production Site(s), and Selected Digestive Tract Functions

Hormone/Peptide	Main Production Sites	Selected Function(s)
Gastrin	Stomach and small intestine	Stimulates gastric acid secretion Stimulates pepsinogen secretion
Cholecystokinin	Small intestine and enteric nerves	Stimulates gallbladder contraction Stimulates sphincter of Oddi relaxation Stimulates pancreatic enzyme secretion
Secretin	Small intestine	Stimulates pancreas juice secretion Diminishes gastric emptying Diminishes gastric acid secretion
Motilin	Stomach and intestines	Stimulates gastric and intestinal motility between meals
Glucose-dependent insulinotropic peptide	Small intestine	Stimulates insulin secretion May diminish gastric acid secretion
Peptide YY	Small and large intestines	Diminishes gastric acid secretion Diminishes gastric emptying
Somatostatin	Pancreas, stomach, and small intestine	Diminishes gastric acid secretion Diminishes gastric emptying Diminishes pancreatic enzyme secretions Inhibits gallbladder contraction
Glucagon-like peptides	Small and large intestines	Stimulates insulin secretion Reduces digestive tract motility Reduces gastric secretions
Pancreatic polypeptide	Pancreas	Decreases gastric emptying Reduces pancreatic exocrine secretions

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- Secretin is secreted into the blood by S cells in the proximal small intestine in response to the presence of unneutralized acidic chyme and the products of protein digestion in the duodenum. Secretin acts primarily on pancreatic duct cells, stimulating the release of pancreatic juice rich in bicarbonate. The presence of this bicarbonate in the duodenum in turn neutralizes the acidic chyme and serves as feedback control. Secretin also exhibits trophic action on the pancreas and decreases gastric acid secretion and gastric emptying.
- Peptide YY (PYY), secreted into the blood by L cells of the ileum and colon, decreases appetite as well as decreases gastric acid secretion and gastric emptying. Its release is stimulated by the presence of fat in the small intestine.
- Motilin, secreted by M cells in the stomach, small intestine, and colon, controls the MMC, promoting gastric emptying and stimulating motility in the intestines between meals. Its release is stimulated by acetylcholine and serotonin. Acetylcholine is released by nerves. Serotonin is released both from nerves and from enterochromaffin-like cells within the gastrointestinal tract.

Four paracrines affecting the digestive tract are somatostatin, histamine, glucagon-like peptides, and insulin-like growth factor-1.

- Somatostatin, synthesized by pancreatic δ (D) cells as well as cells in the antrum and duodenum, inhibits gastrin release, and thus inhibits gastric acid secretion, through effects on parietal and enterochromaffin-like cells. Somatostatin also suppresses the actions of gastrin, glucose-dependent insulinotropic peptide, secretin, vasoactive intestinal polypeptide, and motilin. Further actions include inhibition of gastric emptying, pancreatic exocrine secretions, and gallbladder contraction. Release of the somatostatin is promoted by a drop, below about 2, in the pH of gastric juice.
- Histamine, secreted by mast cells and enterochromaffin-like cells in the stomach, stimulates parietal cells to secrete hydrochloric acid. Histamine release is stimulated by both gastrin and acetylcholine.
- Glucagon-like peptides, secreted by L cells of the distal small intestine and colon and by the nervous system, primarily stimulate the pancreas to release insulin and inhibit glucagon secretion. The peptides also may decrease appetite and diminish gastric emptying, gastric secretions, and intestinal motility. Release of the peptides occurs with the presence of nutrients in the lumen of the small intestine.
- Insulin-like growth factor-1, also secreted by endocrine cells of the gastrointestinal tract, increases proliferation of the gastrointestinal tract. Its release is stimulated by the presence of nutrients in the digestive tract.

Of the following neurocrine peptides involved with digestive tract functions, vasoactive intestinal polypeptide has one of the larger roles. Vasoactive intestinal polypeptide (VIP) is present in gastrointestinal tract nerves and the central nervous system, and may also be present in the blood. The peptide is thought to stimulate intestinal and pancreatic secretions, relax intestinal smooth muscle including most gastrointestinal sphincters, and inhibit gastric acid secretion. Another neuropeptide called neurotensin is produced by both neurons and N cells of the small intestine (especially the ileum), but its exact physiological role in the digestive process at normal circulating concentrations is unclear. The peptide, however, is known to have multiple actions in the brain.

Two hormones exhibiting lesser direct effects on the digestive tract but impacting nutrient utilization include glucose-dependent insulinotropic peptide (previously called gastric inhibitory peptide) and amylin. Glucose-dependent insulinotropic peptide (GIP), a peptide produced by K cells of the duodenum and jejunum, primarily functions to stimulate insulin release by the pancreatic beta cells. The hormone also may inhibit gastric acid secretion. Amylin, a hormone that is cosecreted with the insulin by pancreatic beta cells, functions to inhibit glucagon secretion as well as gastric emptying. Insulin's role in promoting glucose uptake, along with the role of another pancreatic hormone glucagon, is discussed in detail in Chapter 3.

In addition to direct effects on the digestive tract and effects on nutrient utilization, other hormones affect appetite. While a discussion of appetite regulation is beyond the scope of this chapter, information of a few appetite-regulating hormones is presented here as well as in Chapter 8. **Ghrelin**, a peptide secreted primarily from endocrine cells of the stomach, acts on the hypothalamus to stimulate food intake. Plasma concentrations of ghrelin typically rise before eating (e.g., a fasting situation) and decrease immediately after eating, especially carbohydrates. Two other appetite-enhancing peptides include neuropeptide Y (NPY) and agouti-related protein (AGRP). **Leptin**, secreted mainly by white adipose tissue in proportion to fat stores, suppresses food intake. Leptin's activity occurs at least in part in conjunction with α-melanocyte-stimulating hormone (α-MSH), which stimulates MC_4 receptors, primarily in the hypothalamus. Another hormone suppressing food intake in conjunction with leptin is corticotropin-releasing hormone (CRH).

A review of these regulatory peptides clearly shows that these various mediators of the digestive processes work in concert to stimulate and inhibit food intake as needed and to coordinate the movement of digestive tract contents and the breakdown of the nutrients within the digestive tract.

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SUMMARY

Examining the various mechanisms in the gastrointestinal tract that allow food to be ingested, digested, and absorbed, and its residue to then be excreted reveals the complexity of the digestion and absorption processes. Normal digestion and absorption of nutrients depend not only on a healthy digestive tract but also on integration of the digestive system with the nervous, endocrine, and circulatory systems.

The many factors that influence digestion and absorption—including dispersion and mixing of ingested food, quantity and composition of gastrointestinal secretions, enterocyte integrity, the expanse of intestinal absorptive area, and the transit time of intestinal contents—must be coordinated so that the body can be nourished without disrupting the homeostasis of body fluids. Much of the coordination required is provided by regulatory peptides, some of which are provided by the nervous system as well as by the endocrine cells of the gastrointestinal tract.

Although the basic structure of the digestive tract—which consists of the mucosa, submucosa, muscularis externa, and serosa—remains the same throughout, structural modifications enable various segments of the gastrointestinal tract to perform more specific functions. Gastric glands that underlie the gastric mucosa secrete fluids and compounds necessary for the stomach's digestive functions. Other particularly noteworthy features are the villi and the microvilli, which dramatically increase the surface area exposed to the contents of the intestinal lumen. This enlarged surface area helps maximize absorption, not only of ingested nutrients but also of endogenous secretions released into the gastrointestinal tract.

Study of the digestive system makes abundantly clear the fact that a person's adequate nourishment, and therefore his or her health, depends in large measure on a normally functioning gastrointestinal tract. Particularly crucial to nourishment and health is a normally functioning small intestine because that is where the greatest amount of digestion and absorption occurs. Later chapters of this book expand on digestion and absorption of individual nutrients.

Web Site

www.nlm.nih.gov/research/visible/visible_human.html
The Visible Human Project

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THE NUTRITIONAL IMPACT OF ROUX-EN-Y GASTRIC BYPASS, A SURGICAL APPROACH FOR THE TREATMENT OF OBESITY

Obesity is a national epidemic in the United States and one of the most prevalent health conditions worldwide, with close to 2 billion people classified as either overweight or obese. While changes in lifestyle, including diet and physical activity, and, if needed, pharmacological intervention, are the preferred treatment approaches for obesity, obese individuals who meet selected criteria and who are at increased risk of obesity-related mortality may be candidates for bariatric surgery.

Bariatric surgical options can be classified as restrictive, malabsorptive, or both. Restrictive procedures, such as gastric banding and sleeve gastrectomy, reduce the size of the stomach by up to 85%, which thus limits gastric volume and food intake. The digestive tract, however, remains intact with these restrictive procedures. Malabsorptive procedures reduce nutrient absorption. Bariatric surgical procedures, such as biliopancreatic diversion with or without duodenal switch and Roux-en-Y gastric bypass (RYGB), are both restrictive and malabsorptive, reducing the size of the stomach as well as altering intestinal tract continuity. Specifically, with RYGB, the proximal and distal portions of the stomach are surgically separated and a small gastric pouch is created. A loop of the jejunum (referred to as the Roux limb) is attached to the gastric pouch with shorter Roux limbs resulting in greater post-op malabsorption. A biliopancreatic limb is attached to the Roux limb at a site distal to the anastomosis (attachment) of the stomach pouch and jejunum. A large section of the stomach and the duodenum are surgically stapled and bypassed (Figure 1).

RYGB is the most common bariatric procedure performed in the United States. Yet, it is not without complications, both medical and nutritional. Macronutrient and micronutrient deficiencies occur following RYGB. Some result from poor compliance to postsurgical nutritional treatment plans, while many others occur due to RYGB-induced modifications to the digestive tract. Some of the surgically induced alterations most impacting digestion and absorption include reducing the size of the stomach, shortening the length of the small intestine in contact with nutrients, and disrupting the normal continuity of the digestive system and its accessory organs (affecting bile release and pancreatic secretions). Additionally, bacterial overgrowth in the "bypassed section" of the small intestine can promote deficiencies of some nutrients. This perspective focuses on some of the most prevalent nutritional consequences associated with RYGB.

Of the macronutrients, protein deficiency occurs rather frequently. It typically results from inadequate protein intake, reduced gastric acid secretion (which normally facilitates protein denaturation and pepsinogen activation in the stomach to facilitate protein digestion), insufficient amino acid absorption (reduced absorptive surface), and extreme weight loss. For several weeks post-op, only very small amounts of foods, usually in liquid form, are permitted; such restrictions make ingestion of recommended amounts of nutrients, especially protein, challenging. Protein intakes of 1.1–1.5 g/kg per ideal body weight or in total amounts ranging from about 60 to 120 g daily are recommended for bariatric surgical patients. Additionally, supplemental leucine (which has been shown to promote protein synthesis) for protein-malnourished bariatric patients also has been recommended. Postsurgical monitoring should include regularly scheduled measurements of muscle strength and muscle mass, which are often negatively impacted with poor protein status. Some physical symptoms suggesting protein deficiency may include brittle hair and alopecia (hair loss), generalized edema (swelling), and asthenia (weakness).

Several vitamin deficiencies occur among bariatric surgical patients. Of the water-soluble vitamins, deficiencies of thiamin, vitamin B_{12}, and folate are common. Thiamin deficiency occurs with excessive or recurrent vomiting (emesis), which is often present, as well as from reductions in thiamin intake and absorption (normally from the proximal small intestine). Treatment of thiamin deficiency characterized by neurologic symptoms may require parenteral administration of the vitamin. In the absence of neurologic symptoms, oral thiamin supplementation in doses of about 50–100 mg/day is usually recommended to attain or maintain thiamin status.

Figure 1 The anatomy of a Roux-en-Y gastric bypass.

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Vitamin B_{12} deficiency results from several surgery-induced problems, especially insufficient intrinsic factor. Intrinsic factor is made by parietal cells in the stomach and binds to the vitamin in the duodenum so it can be absorbed in the ileum. However, with RYGB, secretions from gastric parietal cells are reduced, so little intrinsic factor is released. Additionally, hydrochloric acid in the stomach is needed to help release the vitamin from foods, but as with intrinsic factor, the amount of acid produced after RYGB is often not sufficient to facilitate this release. A third factor contributing to deficiency is inadequate intake. Finally, should bacterial overgrowth occur, the bacteria use the vitamin for their own growth needs and thus limit the vitamin's availability. Because most individuals have large stores of vitamin B_{12}, deficiency symptoms (i.e., neurological problems, cognitive dysfunction, and macrocytic anemia, among others) may not appear for some time. Treatment of a vitamin B_{12} deficiency generally requires injections of the vitamin, but because about 1–3% of vitamin B_{12} may be absorbed without intrinsic factor, oral ingestion of high doses of the vitamin (about 1,000–2,000 µg/day) or vitamin B_{12} nasal sprays can sometimes correct the deficiency.

Folate deficiency may result from inadequate dietary intake and/or from insufficient absorption of folate due to surgery-induced changes in the intestinal continuity. Oral, supplemental folate in amounts of 800–1,000 µg per day for several months is usually needed to treat the deficiency.

Fat malabsorption occurs in RYGP primarily if the common channel below the biliopancreatic and Roux limb anastomosis is too short (i.e., less than about 100 cm). Fat malabsorption in turn leads to malabsorption and deficiencies of the fat-soluble vitamins. Insufficient bile (which is no longer directed into the duodenum through the sphincter of Oddi) and the bypassing of much of the jejunum, where most fat-soluble vitamins are absorbed also contribute to the malabsorption. Vitamin D problems also occur with obesity because the greater amounts of subcutaneous fat that are present store more of the vitamin and don't release (mobilize) the vitamin into the blood as quickly when intake is insufficient. Of the fat-soluble vitamins, deficiencies of vitamins D and A are common, although physical signs of a vitamin D deficiency are not usually present. Low serum 25-hydroxyvitamin D concentrations, especially if coupled with high serum parathyroid hormone concentrations, suggest impaired vitamin D status. Treatment requires oral supplements of the vitamin in amounts ranging from about 3,000 IU (75 µg) to 10,000 IU (250 µg) (but sometimes higher doses) per day for several months or until serum 25-hydroxyvitamin D concentrations exceed 30 ng/mL. Vitamin A deficiency is typically characterized by low serum retinol and vision/ophthalmological problems. Symptomatic vitamin A deficiency is usually treated with oral supplements, providing 5,000–25,000 IU vitamin A per day, and may be needed for 6–12 months to correct the deficit. Short-term treatment with larger doses has also been used with severe vitamin A deficiency.

Of the minerals, calcium, iron, zinc, and copper deficiencies are commonly reported. Calcium is best absorbed from a slightly acidic environment in the proximal small intestine and requires adequate vitamin D status; however, these conditions do not exist following RYGB. General practice guidelines suggest up to 2 g of elemental calcium along with vitamin D supplements daily for those who have had RYGB.

Iron deficiency is one of the most well-studied and documented deficiencies in those who have had RYGB. Aforementioned reductions in acid production and rerouting of the proximal intestine represent surgical-induced changes contributing to the deficiency. Inflammation, which may be present with obesity, also diminishes intestinal iron absorption. Finally, iron intake is often poor because meat (a good source of iron) is frequently not tolerated. Deficiency is usually detected by evaluation of biochemical indices such as low serum ferritin, increased serum soluble transferrin receptors, low transferrin saturation, elevated total iron-binding capacity, low serum iron, and low mean cell volume (MCV). MCV, however, may be normal with the copresence of vitamin B_{12} and folate deficiencies, and ferritin concentrations may be elevated in the presence of inflammation. While treatment of deficiency often requires intravenously administered iron, oral doses (in amounts up to 300 mg) may be tried initially. Typically lower doses of iron taken orally a couple of times per day are better tolerated (less side effects) than higher doses taken less frequently. Ingestion of foods rich in vitamin C along with the iron supplements is normally recommended to facilitate iron absorption.

Zinc and copper deficiencies have also been documented in bariatric surgery patients. Poor dietary intake of foods rich in these trace minerals and reductions in gastric acid contribute to the deficiencies. Additionally, both nutrients, like calcium and iron, are better absorbed from a slightly acidic environment in the proximal small intestine. Classic symptoms of zinc deficiency include skin lesions, poor wound healing, and hair loss (alopecia). Plasma or blood cell zinc concentrations also decrease with deficiency, along with 24-hour urinary zinc excretion. Practice guidelines suggest oral supplementation providing about 10–40 mg elemental zinc per day to treat deficiency; prolonged intakes in amounts higher than 40 mg per day can induce copper deficiency or impair copper status. Providing 1–2 mg of elemental copper with such zinc supplementation is suggested to minimize this interaction. However, dosages of 2–5 mg (sometimes higher) of elemental copper per day (given in divided doses) for up to 3 months may be needed to correct copper deficiency and replenish stores. In some cases, intravenous infusion of copper may be initially needed prior to oral supplementation. Serum copper and ceruloplasmin concentrations, which are reduced with deficiency, can be used to assess copper. Copper deficiency is also characterized by neutropenia, thrombocytopenia, hypochromic anemia, decreased erythropoiesis, and neurologic dysfunction.

Bariatric surgery is an effective treatment for obesity and many of its comorbidities. Yet, as can be gleaned from this perspective, the RYGB procedure is not without nutritional consequences. This perspective has reviewed some of the more prevalent nutritional complications associated with RYGB. The articles at the end of this Perspective provide additional information on the complications associated with bariatric surgeries.

Suggested Readings

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3 CARBOHYDRATES

OVERVIEW OF STRUCTURAL FEATURES
SIMPLE CARBOHYDRATES
Monosaccharides
Disaccharides
COMPLEX CARBOHYDRATES
Oligosaccharides
Polysaccharides
DIGESTION
Digestion of Polysaccharides
Digestion of Disaccharides
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Glycolysis
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Gluconeogenesis
REGULATION OF METABOLISM
Allosteric Enzyme Modulation
Covalent Regulation
Genetic Regulation
Directional Shifts in Reversible Reactions
Metabolic Control of Glycolysis and Gluconeogenesis
SUMMARY
PERSPECTIVE
WHAT CARBOHYDRATES DO AMERICANS EAT?

THE MAJOR SOURCE OF ENERGY FUEL in the average human diet is carbohydrate, supplying half or more of the total caloric intake. Roughly half of dietary carbohydrate is in the form of polysaccharides such as starches and dextrins, derived largely from cereal grains and vegetables. The remaining half is supplied as simple sugars, the most abundant being sucrose, followed by lactose, maltose, glucose, and fructose.

OVERVIEW OF STRUCTURAL FEATURES

Carbohydrates are polyhydroxy aldehydes or ketones, or substances that produce these compounds when hydrolyzed. They are constructed from carbon, oxygen, and hydrogen atoms that occur in a proportion that approximates that of a "hydrate of carbon," CH_2O, accounting for the term *carbohydrate*. Carbohydrates comprise two major classes: simple carbohydrates and complex carbohydrates. Simple carbohydrates include monosaccharides and disaccharides. Complex carbohydrates include oligosaccharides containing 3–10 saccharide units and polysaccharides containing more than 10 units (Figure 3.1).

Simple Carbohydrates

- **Monosaccharides** are structurally the simplest form of carbohydrate in that they cannot be reduced in size to smaller carbohydrate units by hydrolysis. Monosaccharides are called simple sugars and are sometimes referred to as monosaccharide units or residues. The most abundant monosaccharide in nature—and certainly the most important nutritionally—is the six-carbon sugar glucose.
- **Disaccharides** consist of two monosaccharide units joined by covalent bonds. Within this group, sucrose, consisting of one glucose and one fructose residue, is nutritionally the most significant, furnishing approximately one-third of total dietary carbohydrate in an average Western diet.

Complex Carbohydrates

- **Oligosaccharides** consist of short chains of monosaccharide units that are also joined by covalent bonds. The number of units is designated by a prefix (*tri-*, *tetra-*, *penta-*, and so on), followed by the word *saccharide*. Some oligosaccharides known as the "flatulent sugars" are found naturally in legumes and grains, whereas other oligosaccharides called dextrins are often added

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Figure 3.1 Classification of carbohydrates.
Source: Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

to food and beverage products to improve their texture, appearance, and nutritional value.

- **Polysaccharides** are long chains of monosaccharide units that may number from several into the hundreds or even thousands. The major polysaccharides of interest in nutrition are glycogen, found in certain animal tissues, and starch and cellulose, both of plant origin. All of these polysaccharides consist of only glucose units.

SIMPLE CARBOHYDRATES

Monosaccharides

As monosaccharides occur in nature or arise as intermediate products in digestion, they contain from three to seven carbon atoms and accordingly are termed *trioses*, *tetroses*, *pentoses*, *hexoses*, and *heptoses*. They cannot be further broken down with mild hydrolytic conditions, only with strong chemical oxidizing agents. In addition to hydroxyl groups, these compounds possess a functional carbonyl group, C=O, that can be either an aldehyde or a ketone. Hence, they are further designated as *aldoses*, sugars having an aldehyde group, and *ketoses*, sugars possessing a ketone group. These two classifications together with the number of carbon atoms describe a particular monosaccharide. For example, a five-carbon sugar having a ketone group is a ketopentose; a six-carbon aldehyde-possessing sugar is an aldohexose, and so forth.

Stereoisomerism: Chiral Carbons

A brief discussion of stereoisomerism—the occurrence of a molecule in different spatial configurations—as it relates to carbohydrates is provided here because most biological systems are stereospecific. For a more extensive discussion, refer to a general biochemistry text.

Many organic substances, including carbohydrates, are optically active: If plane-polarized light is passed through a solution of the substances, the plane of light is rotated to the right (for dextrorotatory substances) or to the left (for levorotatory ones). The direction and extent of the rotation are characteristic of a particular compound and depend on the substance's concentration and temperature and the wavelength of the light. The right or left direction of light rotation is expressed as + (dextrorotatory) or – (levorotatory), and the number of angular degrees indicates the extent of rotation.

Optical activity is attributed to the presence of one or more asymmetrical or chiral carbon atoms in the molecule. **Chiral carbon** atoms have four different atoms or groups covalently attached to them. Aldoses with at least three carbon atoms and ketoses with at least four carbons have a chiral carbon atom. Because different groups are attached, it is not possible to move any two atoms or groups to other positions and rotate the new structure so that it can be superimposed on the original. Instead, when two of these molecules are side by side, repositioning groups in one creates a pair of molecules that are mirror images of each other. The molecules are said to be enantiomers, a special class within a broader family of compounds called **stereoisomers**. Diastereomers, another type of stereoisomers, are compounds having two or more chiral carbon atoms that have the same four groups attached but are not mirror images of each other.

If an asymmetrical substance rotates the plane of polarized light a certain number of degrees to the right, its enantiomer rotates the light the same number of degrees to the left. Enantiomers exist in D or L orientation, and if

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Figure 3.2 Structural formulas of the D and L configurations of glyceraldehyde.

a compound is structurally D, its enantiomer is L. The D or L designation does not predict the direction of rotation of plane-polarized light, but rather is simply a structural analogy to the reference compound glyceraldehyde. Glyceraldehyde's D and L forms are, by convention, drawn as shown in Figure 3.2. Note that in the D configuration the —OH on the chiral carbon points to the right, and in the L configuration, to the left. Remember, these forms are not superimposable.

Monosaccharides with more than three carbons have more than one chiral center. In such cases, the highest-numbered chiral carbon indicates whether the molecule is of the D or the L configuration. Monosaccharides of the D configuration are much more important nutritionally than their L **isomers** because D isomers exist as such in dietary carbohydrate and are metabolized specifically in that form. The reason for this specificity is that the enzymes involved in carbohydrate digestion and metabolism are stereospecific for D sugars, meaning that they react only with D sugars and are inactive toward L forms. The D and L forms of glucose and fructose are shown in Figure 3.3. Note that all of the —OH groups of the stereoisomers are flipped to the opposite side.

In Figure 3.3 the structures of glucose and fructose are shown as open-chain models, in which the carbonyl (aldehyde or ketone) functions are free. The monosaccharides generally do not exist in open-chain form, as explained later, but they are shown that way here to clarify the D-L concept and to illustrate the **anomeric carbon**, the carbon atom comprising the carbonyl function. Notice that the anomeric carbon is number 1 in the aldose (glucose) and number 2 in the ketose (fructose). This is due to the fact that the carbon bearing the most important functional group is given the lowest number possible.

Ring Structures

In solution, the monosaccharides do not exist in an open-chain form. They do not undergo reactions characteristic of true aldehydes and ketones. Instead, the molecules form a cyclic ring structure through a reaction between the carbonyl group and a hydroxyl group. If the cyclized sugar contains an aldehyde, it is called a hemiacetal; if the sugar contains a keto group, it is called a hemiketal. This formation of the cyclic structures forms an additional chiral carbon. Therefore, the participating groups within a monosaccharide are the aldehyde or ketone of the anomeric carbon atom and the alcohol group attached to the highest-numbered chiral carbon atom, as illustrated in Table 3.1 using the examples of D-glucose, D-galactose, and D-fructose. The formation of the hemiacetal or hemiketal produces a new chiral center at the anomeric carbon, designated by an asterisk in the structures in Table 3.1, and therefore the bond direction of the newly formed hydroxyl becomes significant. In the Fisher projections shown, the anomeric hydroxyls are arbitrarily positioned to the right, resulting in an alpha (α) configuration. If the anomeric hydroxyl were directed to the left, the structure would be in a beta (β) configuration. Cyclization to the hemiacetal or hemiketal can produce either the α- or the β-isomer. In aqueous solution, an equilibrium mixture of the α-, β-, and open isomers exists, with the concentration of the β form roughly twice that of the α form. In essence, the α-hemiacetal can change to the open structure and again form a ring with either the α or the β configuration.

Figure 3.3 Structural (open-chain) models of the D and L forms of the monosaccharides glucose and fructose.

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Table 3.1 Various Structural Representations among the Hexoses: Glucose, Galactose, and Fructose

Hexose	Fisher Projection	Cyclized Fisher Projection	Haworth	Simplified Haworth
α-D-glucose				
α-D-galactose				
β-D-fructose				

* Anomeric carbon.

Stereoisomerism among the monosaccharides, and also among other nutrients such as amino acids and lipids, has important metabolic implications because of the stereospecificity of certain metabolic enzymes. An interesting example of stereospecificity is the action of the digestive enzyme α-amylase, which hydrolyzes polyglucose molecules such as starches, in which the glucose units are connected through an α-linkage. Cellulose is also a polymer of glucose, but one in which the monomeric glucose residues are connected by β-linkages, and it is resistant to the α-amylase hydrolysis present in the human digestive system.

Haworth Models

The structures of the cyclized monosaccharides are more conveniently and accurately represented by Haworth models. In such models the carbons and oxygen comprising the five- or six-membered ring are depicted as lying in a horizontal plane, with the hydroxyl groups pointing down or up from the plane. Those groups directed to the right in the open-chain structure point down in the Haworth model, and those directed to the left point up. Table 3.1 shows the structural relationship of simple projection and Haworth formulas for the major naturally occurring hexoses: glucose, galactose, and fructose. Remember that in solution the cyclic monosaccharides open and close to form an equilibrium between the α and the β forms. Regardless of how the cyclic structure is written, the molecule exists in both forms in solution unless the anomeric carbon has formed a chemical bond and is no longer able to open and close. The different ways of drawing the structures are presented here because all are used in the nutrition literature. Chemists often portray the structures to show the true

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Figure 3.4 Structural formulas of the pentoses ribose and deoxyribose and of the alcohol ribitol.

bond angles. The structures can be shown in a boat configuration or a chair configuration. Additional information can be obtained from a biochemistry textbook.

Pentoses

Compared to the hexoses, pentose sugars furnish little dietary energy because relatively few are available in the diet. However, they are readily synthesized in the cell from hexose precursors and are incorporated into metabolically important compounds. The aldopentose ribose, for example, is a constituent of key nucleotides such as the adenosine phosphates—adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), cyclic adenosine monophosphate (cAMP), and the nicotinamide adenine dinucleotides (NAD^+, $NADP^+$). Ribose and its deoxygenated form, deoxyribose, are part of the structures of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), respectively. Ribitol, a reduction product of ribose, is a constituent of the vitamin riboflavin and of the flavin coenzymes: flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). The structural formulas of ribose, deoxyribose, and ribitol are depicted in Figure 3.4.

Amino and Acid Derivatives

Amino sugars, including glucosamine and galactosamine, occur in oligosaccharides and polysaccharides such as chitin and chrondroitin. The amino sugars have an amino group replacing the −OH on C2. Monosaccharides such as glucose can be enzymatically oxidized to glucuronic acid. Glucuronic acid is part of the glucuronic acid pathway (discussed later in this chapter) and is found in many glycoproteins, covered in Chapter 6.

Reducing Sugars

Monosaccharides that are cyclized into hemiacetals or hemiketals are sometimes called reducing sugars because they are capable of reducing other substances, such as the copper ion (from Cu^{2+} to Cu^+). This property is useful in identifying which end of a polysaccharide chain has the monosaccharide unit that can open and close. This role of reducing sugars is discussed in more detail in the section on polysaccharides.

Disaccharides

Disaccharides contain two monosaccharide units attached to one another through acetal bonds. Acetal bonds, also called glycosidic bonds because they occur in the special case of carbohydrate structures, are formed between a hydroxyl group of one monosaccharide unit and a hydroxyl group of a second monosaccharide, with the elimination of one molecule of water. The glycosidic bonds generally involve the hydroxyl group on the anomeric carbon of one member of the pair of monosaccharides and the hydroxyl group on carbon 4 or 6 of the second member. Furthermore, the glycosidic bond can be α or β in orientation, depending on whether the anomeric hydroxyl group was α or β before the glycosidic bond was formed and on the specificity of the enzymatic reaction catalyzing their formation. Specific glycosidic bonds therefore may be designated α(1-4), β(1-4), α(1-6), and so on. Disaccharides are major energy-supplying nutrients in the diet. The most common disaccharides in the diet are maltose, lactose, and sucrose (Figure 3.5).

Maltose

Maltose is formed primarily from the partial hydrolysis of starch and therefore is found in malt beverages such as beer and malt liquors. It consists of two glucose units linked through an α(1-4) glycosidic bond. The glucose unit on the right in Figure 3.5 is shown with the anomeric carbon in the β position (thus called β-maltose).

Lactose

Lactose is found naturally only in milk and milk products. It is composed of galactose linked by a β(1-4) glycosidic bond to glucose. The anomeric carbon of glucose is in the α position (Figure 3.5).

Sucrose

Sucrose (cane sugar, beet sugar) is the most widely distributed of the disaccharides and is the most commonly used natural sweetener. It is composed of glucose and fructose and is structurally unique in that its glycosidic bond involves the anomeric hydroxyl of both residues. The linkage is α with respect to the glucose residue and β with respect to the fructose residue (Figure 3.5). Because it has no free hemiacetal or hemiketal function, sucrose is not a reducing sugar.

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Figure 3.5 Common disaccharides.

Trehalose

Another disaccharide, trehalose, is found naturally in fungi (mushrooms) and in other foods of the plant kingdom. Trehalose is an α(1-1) linkage of two D-glucose molecules. It is a nonreducing sugar. Since trehalose is digested slowly, provokes a low glycemic response, and possesses different physical and chemical properties from other sugars, it has become an ingredient in processed foods in Japan and other countries. It has been granted Generally Recognized as Safe (GRAS) status by the U.S. Food and Drug Administration (FDA) [1,2].

COMPLEX CARBOHYDRATES

Oligosaccharides

Raffinose (a trisaccharide), stachyose (a tetrasaccharide), and verbascose (a pentasaccharide) are made up of glucose, galactose, and fructose and are found in beans, peas, bran, and whole grains. Human digestive enzymes do not hydrolyze them, but the bacteria within the intestine can digest them, producing gases that cause flatulence. Dextrins are a category of oligosaccharides composed entirely of glucose and used as an additive in foods, pharmaceuticals, and nutritional supplements. Dextrins are made from starch, which is hydrolyzed under controlled conditions to produce glucose chains of desired lengths. The shorter-chain dextrins (3–20 glucose units) are used most frequently for food and drug applications and will be listed on product labels as maltodextrin, corn syrup solids, or hydrolyzed corn starch. Note that commonly used dextrins will be categorized as either oligo- or polysaccharides, depending on the specific chain length.

Polysaccharides

The glycosidic bonding of monosaccharide residues may be repeated many times to form high-molecular-weight polymers called polysaccharides. If the structure is composed of a single type of monomeric unit, it is called a homopolysaccharide. If two or more different types of monosaccharides make up its structure, it is called a heteropolysaccharide. Both types exist in nature; however, homopolysaccharides are of far greater importance in nutrition because of their abundance in many natural foods. The polyglucoses starch and glycogen, for example, are the major storage forms of carbohydrate in plant and animal tissues, respectively. Polyglucoses range in molecular weight from a few thousand to 500,000.

The reducing property of a saccharide is useful in describing polysaccharide structure by enabling one end of a linear polysaccharide to be distinguished from the other. In a polyglucose chain, for example, the glucose residue at one end of the chain has a hemiacetal group because its anomeric carbon atom is not involved in acetal bonding to another glucose residue. The residue at the other end of the chain is not in hemiacetal form because it is attached by acetal bonding to the next residue in the chain. A linear polyglucose molecule therefore has a reducing end (the hemiacetal end) and a nonreducing end (at which no hemiacetal exists). This notation is useful in designating at which end of a polysaccharide certain enzymatic reactions occur.

Starch

The most common digestible polysaccharide in plants is starch. Its two forms, amylose and amylopectin, are both polymers of D-glucose. The amylose molecule is a linear, unbranched chain in which the glucose residues are attached solely through α(1-4) glycosidic bonds. In water, amylose chains adopt a helical conformation, as shown in Figure 3.6a. Amylopectin, on the other hand, is a branched-chain polymer, with branch points occurring through α(1-6) bonds, as illustrated in Figure 3.6b. Both amylose and amylopectin occur in cereal grains, potatoes, legumes, and other vegetables. Amylose contributes about 15–20%, and amylopectin 80–85%, of the total starch content of these foods.

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Figure 3.6 Structure of starches and glycogen.
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Glycogen

The major form of stored carbohydrate in animal tissues is glycogen, which is localized primarily in liver and skeletal muscle. Glycogen is similar to amylopectin, but more highly branched (Figure 3.6c). The glucose residues within glycogen serve as a readily available source of glucose. When dictated by the body's energy demands, glucose residues are sequentially removed enzymatically from the nonreducing ends of the glycogen chains and enter energy-releasing pathways of metabolism. This process, called **glycogenolysis**, is discussed later in this chapter. The high degree of branching in glycogen and amylopectin offers a distinct metabolic advantage because it presents a large number of nonreducing ends from which glucose residues can be cleaved.

Cellulose

Cellulose is the major component of cell walls in plants and, like the starches, a homopolysaccharide of glucose. It differs from the starches in that the glycosidic bonds connecting the residues are β(1-4), rendering the molecule resistant to the digestive enzyme α-amylase, which is stereospecific to favor α(1-4) linkages. Because cellulose is not digestible by mammalian digestive enzymes, it is defined as a dietary fiber and is not considered an energy source. However, colonic bacteria can digest it, resulting in several digestion products including short-chain fatty acids that provide energy to the body and play important roles in the gastrointestinal tract. A more extensive discussion of fiber and short-chain fatty acids is presented in Chapter 4.

DIGESTION

Polysaccharides and disaccharides are the most abundant carbohydrates in the food supply, although some free glucose and fructose are present in honey, certain fruits and vegetables, "invert" sugar used in confections, and high-fructose corn syrup. Before dietary carbohydrates can be used by the body's cells, they must first be absorbed from the gastrointestinal (GI) tract into the bloodstream, a process normally restricted to monosaccharides—the form of carbohydrates enterocytes can absorb. Poly-, tri-, and disaccharides therefore must be hydrolyzed. The hydrolytic enzymes involved are collectively called **glycosidases** or, alternatively, **carbohydrases**.

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Digestion of Polysaccharides

The digestion of polysaccharides (starches) starts in the mouth. The key enzyme is salivary α-amylase, a glycosidase that specifically hydrolyzes α(1-4) glycosidic linkages. The β(1-4) bonds of cellulose, the β(1-4) bonds of lactose, and the α(1-6) linkages that form branch points in the starch amylopectin are resistant to this enzyme. Given the short period of time that food is in the mouth before being swallowed, this phase of digestion produces few mono- or disaccharides. However, the salivary amylase action continues in the stomach until the gastric acid penetrates the food bolus and lowers the pH sufficiently to inactivate the enzyme.

The starches move into the duodenum and jejunum, where they are acted upon by pancreatic α-amylase. The presence of pancreatic bicarbonate in the duodenum elevates the pH to a level favorable for enzymatic function. The α-amylase hydrolyzes α(1-4) glycosidic bonds in both amylose and amylopectin to produce oligosaccharides (also called dextrins or limit dextrins), maltose, and maltotriose (Figure 3.7). The branched oligosaccharides, trisaccharide maltotriose, and maltose are further digested by specific enzymes in the brush border. α-amylase can further break down the oligosaccharides to maltose and maltotriose. The partially hydrolyzed amylopectin is not fully digested by α-amylase; this enzyme's action stops several residues short of the α(1-6) bonds, leaving a limit dextrin. These limit dextrins are acted on by α-dextrinase (also called isomaltase), which is attached to the brush border membrane. This enzyme contains two polypeptides with two active sites, one with specificity to α(1-4) linkages and the other with specificity to both α(1-4) and α(1-6) linkages. α-dextrinase is the only intestinal enzyme that will hydrolyze α(1-6) glycosidic bonds. Glucose is released from limit dextrins by the combined action of α-dextrinase and other brush border enzymes (Figure 3.7).

A portion of the starch of beans and certain vegetables and other resistant starches are not fully digested (see also Chapter 4). This is partially due to the accessibility of the food to the enzyme and partially related to naturally occurring amylase inhibitors in some foods. α-amylase inhibitors can be used to impede digestion of dietary starch and thus reduce nutrient absorption as a means to combat the overweight and obesity problem [3].

Digestion of Disaccharides

Virtually no digestion of disaccharides or small oligosaccharides occurs in the mouth, stomach, or lumen of the small intestine. Digestion takes place almost entirely within the microvilli (the brush border) of the upper small intestine via disaccharidase activity, and the resulting monosaccharides immediately enter the enterocytes with the facilitation of specific transporters (discussed later) (Figures 2.10 and 2.17). Among the enzymes located on the enterocytes are lactase, sucrase, maltase, and trehalase. Lactase catalyzes the cleavage of lactose to equimolar amounts of galactose and glucose. As was pointed out earlier, lactose has a β(1–4) linkage, and lactase is stereospecific for this β linkage. Lactase activity is high in infants, but in most mammals, including humans, it decreases a few years after weaning. This diminishing activity can lead to lactose malabsorption and intolerance. The frequency of lactose intolerance in human populations varies widely depending on geography, race, and ethnicity. The highest frequency is seen in Native Americans and in people of Asian, African, and Middle Eastern descent. The lowest frequency is seen in white individuals originating from northern European countries. Many lactose-free products are available for individuals with lactose intolerance. Additionally, lactase can be added directly to regular milk products to hydrolyze the lactose.

Sucrase hydrolyzes sucrose to yield one glucose and one fructose residue. Maltase hydrolyzes maltose to yield two glucose units. Trehalase is a brush border disaccharidase that hydrolyzes the α(1-1) glycosidic bonds of trehalose to yield two molecules of glucose.

In summary, nearly all dietary starches and disaccharides ultimately are hydrolyzed completely by specific glycosidases to their constituent monosaccharide units. Monosaccharides, together with small amounts of remaining disaccharides, can then be absorbed by the intestinal mucosal cells.

ABSORPTION, TRANSPORT, AND DISTRIBUTION

The wall of the small intestine is composed of absorptive enterocytes that line projections called villi that extend into the lumen. On the surface of the lumen side, the absorptive cells have hairlike projections called microvilli (the brush border). The anatomic advantage of the villi–microvilli structure (shown in Figures 2.9 and 2.10) is that it presents an enormous surface area to the intestinal contents, thereby facilitating absorption. The absorptive capacity of the human intestine has been estimated to amount to about 5,400 g/day for glucose and 4,800 g/day for fructose—a capacity that would never be reached in a normal diet. Digestion and absorption of carbohydrates are so efficient that nearly all monosaccharides are usually absorbed by the end of the jejunum.

Intestinal Absorption of Glucose and Galactose

After carbohydrate digestion, glucose and galactose are absorbed into the enterocyte by the same mechanisms involving both active transport and facilitated transport.

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Amylose: Salivary glands release salivary α-amylase, which hydrolyzes α(1-4) glycosidic bonds in amylose, forming dextrins.

Amylopectin: Salivary glands release salivary α-amylase, which hydrolyzes α(1-4) glycosidic bonds in amylopectin, forming dextrins.

A. Digestion of amylose and amylopectin in the mouth

Amylose: Acidity of gastric juice destroys the enzymatic activity of α-amylase. The dextrins pass unchanged into the small intestine.

Amylopectin: Acidity of gastric juice destroys the enzymatic activity of salivary α-amylase. The dextrins pass unchanged into the small intestine.

B. There is no digestion of amylose and amylopectin in the stomach

Amylose: The pancreas releases pancreatic α-amylase, which hydrolyzes α(1-4) glycosidic bonds, into the small intestine. Dextrins are broken down into maltose.

Amylopectin: The pancreas releases pancreatic α-amylase, which hydrolyzes α(1-4) glycosidic bonds to produce limit dextrins, maltotriose, isomaltose, and maltose. Hydrolysis stops four residues away from the α(1-6) bond.

C. Digestion of amylose and amylopectin in the small intestine

Amylose: Maltose is hydrolyzed by maltase, a brush border enzyme, forming free glucose.

Amylopectin: Maltose, maltotriose, and isomaltose are further hydrolyzed in the brush border by the enzyme maltase or α-dextrinase to glucose. α-dextrinase is the sole carbohydrase capable of hydrolysing α(1-6) glycosidic bonds.

D. Digestion of amylose and amylopectin on the brush border of the small intestine

Figure 3.7 Starch digestion.

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The relative contribution of active transport versus facilitated transport depends on the amount of carbohydrate consumed; facilitated transport participates to a greater extent following a large carbohydrate meal.

Active Transport

The active transport mechanism for glucose and galactose absorption into enterocytes requires energy as ATP and the involvement of a specific transporter protein, designated sodium-glucose transporter 1 (SGLT1) (Figure 3.8a). The SGLT1 is positioned on the intestinal lumen side of the enterocyte (in the brush border membrane) and simultaneously transports one molecule of glucose (or galactose) and two molecules of Na^+ in the same direction and is thus a symporter. A mutation in the *SGLT1* gene is associated with glucose-galactose malabsorption. The SGLT1 protein has two binding sites: One binds Na^+ and the other binds glucose. The glucose binding site is not available unless the transport protein has already bound Na^+. The attachment of Na^+ to the carrier increases the transport protein's affinity for glucose. Sodium is moving down a concentration gradient because the intracellular concentration of Na^+ is low. After Na^+ and glucose are transported into the enterocyte, they are released from SGLT1. As the intracellular concentration increases, glucose binds to another transporter in the basolateral membrane, designated glucose transporter type 2 (GLUT2). GLUT2 is a low-affinity, high-capacity transporter that facilitates the exit of glucose and other hexoses from the enterocyte into the underlying capillaries for delivery into the hepatic portal vein.

Na^+ that has entered the cell is "pumped" back out by the energy-requiring Na^+/K^+-ATPase located in the basolateral membrane. Na^+/K^+-ATPase works by first combining with ATP in the presence of Na^+ on the inner surface of the cell membrane. The enzyme then is phosphorylated by the breakdown of ATP to adenosine diphosphate (ADP) and consequently is able to move three Na^+ out of the enterocyte. On the outer surface of the cell membrane, the ATPase becomes dephosphorylated by hydrolysis in the presence of K^+ and then is able to return two K^+ into the cell. The term *pump* is used because the Na and K ions are both transported across the membrane against their concentration gradients. The overall process of glucose transport into the enterocyte via SGLT1 is considered *active* transport because of the involvement of Na^+/K^+-ATPase at the basolateral membrane. The activity of the Na^+/K^+-ATPase is responsible for most of the active transport in the body and is the major energy demand of the body at rest.

Facilitated Transport

Some glucose (and galactose) can be absorbed into the enterocyte independent of SGLT1 and thus without the input of energy. When glucose concentration in the

Figure 3.8 Transport of monosaccharides into enterocytes. (a) Active transport of glucose and galactose requiring ATP and Na^+. (b) Facilitated transport of glucose and galactose into the enterocyte by GLUT2 when the intestinal lumen glucose levels are high; glucose and galactose may also exit the cell with assistance from GLUT2. (c) Fructose entering the enterocyte via transport facilitated by GLUT5 and leaving the cell via transport facilitated by GLUT2.

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intestinal mucosa is high, such as after the ingestion of a large carbohydrate-containing meal, glucose is transported into the enterocyte by GLUT2 in the brush border membrane [4]. When large amounts of glucose enter the enterocyte, intracellular GLUT2 is translocated to the brush border membrane by the movement of the cytoskeleton and the contraction of myosin. After high-carbohydrate meals, more glucose is transported into the enterocyte by facilitated transport than by active transport via SGLT1.

Rising levels of blood glucose triggers insulin secretion, causing GLUT2 to be translocated from the brush border membrane back to intracellular vesicles. While GLUT2 is not directly dependent on insulin for facilitated transport, this indirect effect of insulin results in reduced intestinal glucose absorption when blood glucose levels are high. In insulin-resistant individuals or those with type 2 diabetes, GLUT2 is resistant to the effect of insulin, and the GLUT2 remains in the brush border membrane. The result is that glucose continues to be absorbed at a higher rate [4,5]. The role of insulin in metabolic regulation is discussed in detail later in this chapter and in Chapters 7 and 8.

Intestinal Absorption of Fructose

The primary mechanism for fructose transport into the enterocyte is via a specific facilitative transporter, GLUT5 (Figure 3.8c). GLUT5 has a high affinity for fructose and is not influenced by the presence of glucose [6]. Fructose absorption does not require energy (i.e., Na^+-ATP-dependent transport by SGLT1). The rate of uptake of fructose is much slower than that of both glucose and galactose but is increased when GLUT2 is present in the brush border membrane of the enterocyte, as discussed previously. As the intracellular concentration increases, fructose is transported from the enterocyte into the hepatic portal vein by GLUT2 in the basolateral membrane, the same transporter that moves glucose out of the cell. The facilitative transport process can proceed only down a concentration gradient. At typical dietary intakes, there is no fructose in the systemic circulation due to efficient removal by the liver, where it is phosphorylated and trapped in the hepatocytes. Although fructose is absorbed more slowly than glucose or galactose, which are actively absorbed, it is absorbed faster than sugar alcohols such as sorbitol and xylitol, which are absorbed purely by passive diffusion.

Many individuals (about 60%) cannot completely absorb fructose when consumed in large amounts, ranging from 20 to 50 g [7]. Those with limited absorption who ingest large amounts of fructose experience intestinal pain, gas, and diarrhea, symptomatic of malabsorption. This level of intake is readily achievable for individuals who consume 25–30 ounces of a carbonated beverage sweetened with either sugar or high-fructose corn syrup. It has been observed that the threshold of fructose that can be consumed before malabsorption symptoms develop increases in the presence of high levels of glucose (as is present in sucrose and high-fructose corn syrup); this may relate to the presence of GLUT2 in the brush border membrane [8]. The Perspective at the end of this chapter discusses the trends in carbohydrate intake over the past several decades and the major food sources that deliver the glucose and fructose to the enterocyte for absorption.

Post-Absorption Facilitated Transport

Following the intestinal absorption of glucose, galactose, and fructose, they enter the hepatic portal vein, where they are carried directly to the liver. Essentially all of the galactose and fructose is taken up by the liver through specific facilitated transporters and metabolized, whereas only 30–40% of glucose is taken up by the liver, with the majority passing through into the systemic circulation. This explains why glucose, but not galactose or fructose, is found in the peripheral blood and why the latter sugars are not directly subject to the strict hormonal regulation that is such an important part of glucose homeostasis. Galactose is largely converted glucose derivatives and stored as liver glycogen through pathways described later in this chapter. In contrast, the majority of fructose enters an alternative pathway and is catabolized for energy according to the liver's energy demand. If an occasional meal is high in fructose, and the liver's energy needs have been met, excess fructose is converted to triacylglycerol and transported out of the liver for distribution to muscle and adipose tissue. However, diets chronically high in fructose can cause **hyperlipidemia** and triacylglycerol accumulation in the liver.

Glucose is nutritionally the most abundant monosaccharide because it is the exclusive constituent of starch and also occurs in each of three major disaccharides (Figure 3.1). The portion of dietary glucose taken up by the liver can be used for energy, stored as glycogen, or returned to the blood during nonfed periods by pathways described in Chapter 7. The remainder of the glucose passes into the systemic blood supply and is then distributed among other tissues, such as muscle, kidneys, brain, and adipose tissue. Glucose enters the cells in these organs by facilitated transport. In skeletal muscle and adipose tissue the process is insulin dependent, whereas in the liver, kidneys, brain, **erythrocytes** (red blood cells), and other tissues it is insulin independent. Because of the nutritional importance of glucose, the facilitated transport process by which it enters the cells of certain organs and tissues warrants a closer look. The following section explores the process in greater detail.

Glucose Transporters

Glucose is effectively used by a wide variety of cell types under normal conditions, and its concentration in the blood must be precisely controlled. Glucose plays a central

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role in metabolism and cellular homeostasis. Most cells in the body are dependent upon a continuous supply of glucose to supply energy in the form of ATP. The signs and symptoms associated with diabetes mellitus are a graphic example of the consequences of a disturbance in glucose homeostasis.

The cellular uptake of glucose requires that it cross the plasma membrane of the cell. The highly polar glucose molecule cannot move across the cellular membrane by simple diffusion because it cannot pass through the nonpolar matrix of the lipid bilayer. For glucose to be used by cells, an efficient transport system for moving the molecule into and out of cells is essential. In certain cells, such as epithelial cells of the small intestine and renal tubule, glucose crosses the plasma membrane against a concentration gradient by active transport, pumped by an Na^+/K^+-ATPase symport system (SGLT1), as described previously. However, not all cells require an energy-dependent transporter for glucose uptake. A family of protein carriers functions in the facilitated transport of glucose and other monosaccharides, and is called glucose transporters, abbreviated GLUT.

A total of 14 glucose transport proteins have been identified, along with the genes that code for them. The genome project has aided in this identification because, considered collectively, all transport proteins share a structure in common and have similar sequences in the genes that code for them. About 28% of the amino acid sequences are common within the family of transport proteins. Each GLUT is an integral protein, penetrating and spanning the lipid bilayer of the plasma membrane. Twelve transmembrane α-helix segments are present in each of the transporters. Figure 3.9 shows a typical transporter, which is oriented so that hydrophilic regions of the protein chain protrude into the extracellular and cytoplasmic media, while the hydrophobic regions traverse the membrane, juxtaposed with the membrane's lipid matrix.

In its simplest form, a transporter protein:

- has a specific combining site for the molecule being transported
- undergoes a conformational change upon binding the molecule, allowing the molecule to be translocated to the other side of the membrane and released
- has the ability to reverse the conformational changes without the molecule being bound to the transporter so that the process can be repeated.

This section focuses on the family of glucose transport proteins (GLUTs). Of the 14 GLUT isoforms that have been identified, only those that have been well studied and shown to have a major role in glucose metabolism are summarized in Table 3.2, though all 14 are described briefly. All cells express at least one GLUT isoform on their plasma membrane. The different isoforms have distinct tissue distributions and biochemical properties, and they contribute to the precise disposal of glucose according to varying physiological conditions. Redundancy of GLUT proteins in cells throughout the body helps to ensure the uptake and use of glucose as a critical fuel source under a variety of physiological conditions. Major characteristics of each of the GLUTs are: [9]

- GLUT1 was the first GLUT identified and the most intensely studied. As the most ubiquitously expressed GLUT, GLUT1 is responsible for the basic supply of glucose to erythrocytes, endothelial cells of the brain, and most fetal tissue. It supplies the glucose to the developing central nervous system during embryogenesis.
- GLUT2 is a low-affinity, high-capacity transporter with predominant expression in the β-cells of the pancreas, liver, small intestine, and kidney. As discussed previously, GLUT2 is involved in the transport of glucose and fructose from enterocytes into the portal blood, and when the concentration of glucose in the intestinal

Figure 3.9 A model for the structural orientation of the glucose transporter.

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Table 3.2 Glucose Transporters (GLUT)

Transporter Protein	Substrates	Major Sites of Expression
GLUT1	Glucose, galactose, mannose, glucosamine	Erythrocytes, central nervous system, blood–brain barrier, placenta, fetal tissues in general
GLUT2	Glucose, galactose, fructose, mannose, glucosamine	Liver, β-cells of pancreas, kidney, small intestine
GLUT3	Glucose, galactose, mannose, xylose, dehydroascorbic acid	Brain (neurons), spermatozoa, placenta, preimplantation embryos
GLUT4 (insulin dependent)	Glucose, glucosamine, dehydroascorbic acid	Muscle, heart, brown and white adipocytes
GLUT5	Fructose, but not glucose	Intestine, kidney, brain, skeletal muscle, adipose tissue

lumen is high, it transports glucose and fructose into the enterocyte. The rate of transport is highly dependent upon the blood glucose concentration. In the pancreas, GLUT2 appears to be the sensitive indicator of blood glucose levels and is involved in the release of insulin from the β-cells. High insulin levels cause GLUT2 to leave the plasma membrane of the enterocyte and return to storage vesicles.

- GLUT3 is a high-affinity glucose transporter with predominant expression in those tissues that are highly dependent upon glucose, such as the brain and neurons. It is also expressed in cells and tissues that have a high requirement for glucose such as spermatozoa, the placenta, and preimplantation embryos. Some data suggest that a possible dysregulation of GLUT3 might lead to glucose deficits in the brain and thus to dyslexia in children.
- GLUT4 is the primary means by which insulin regulates the cellular uptake of glucose in muscle and adipose tissue. Other cells and tissues such as the liver, kidneys, erythrocytes, and brain do not express GLUT4 and therefore are not dependent upon insulin for glucose uptake. One of the actions of insulin is to cause the translocation of GLUT4 from GLUT4 storage vesicles (GSV; discussed in the next section).
- GLUT5 is specific for the transport of fructose and will not transport glucose. It is expressed primarily in the small intestine, but to a lesser degree in kidney, brain, skeletal muscle, and adipose tissue also.
- GLUT6 (which was formerly designated GLUT9) is expressed primarily in the brain, spleen, and peripheral leukocytes. It appears to transport hexoses only at higher concentrations.
- GLUT7 has been found in the small intestine and colon. It has a high affinity for glucose and fructose but does not bind galactose or xylose.
- GLUT8 (formerly GLUTXI) is expressed mainly in the testis with lower levels in the brain, adrenal gland, liver, spleen, brown adipose tissue, and lung. This GLUT has been studied mostly in animal models.
- GLUT9 is primarily detected in the liver and kidney, with lower levels found in the small intestine, placenta, lung, and leukocytes. The metabolic importance of GLUT9 is not fully understood. In addition to a high affinity for glucose and fructose, GLUT9's primary function appears to be the transport of uric acid.
- GLUT10 is present in the heart, lung, brain, liver, skeletal muscle, pancreas, placenta, and kidney. Its physiological role in humans is under investigation and is not fully understood at this time.
- GLUT11 has been cloned based on genomic information and is expressed in three isoforms. Its physiological role in humans has not been identified.
- GLUT12 has been identified in the skeletal muscle, heart, small intestine, and prostrate. The amount of GLUT12 found in the cellular membrane is increased by insulin (similar to GLUT4) in the normal individual, but not under conditions of obesity and type 2 diabetes that cause resistant insulin receptors. GLUT12 does not appear to be stored in storage vesicles and does not undergo cycling between storage vesicles and the membrane like GLUT4 does. The affinity of GLUT12 for glucose is unknown at this time, but it does transfer glucose, so there is some degree of affinity.
- GLUT13 is highly expressed in certain regions of the brain and to a lower extent in adipose tissue and the kidney. This GLUT has no carbohydrate transport activity. It appears to transport hydrogen ions (protons; H^+) and inositol. Its physiological role is not completely understood at this time.
- GLUT14 is similar (if not identical) to GLUT3 and possibly serves as a backup for that GLUT. It is expressed in the testis.

The current knowledge of GLUTs and their physiological actions has been acquired using molecular biology techniques. The genome project identified genes that had a high degree of similarity to the GLUTs, providing a reason and the tools to look for them in various tissues. The protein molecules were then cloned. Another technique that has been used, called the knockout mouse, blocks the expression of the specific gene under study. With this technique it is possible to determine what effect the absence of the GLUT has on the animal, and hence to learn more of its function.

Role of Insulin

Insulin and GLUT4 play extremely important roles in the uptake of glucose in muscle and adipose tissue, especially following a carbohydrate-rich meal. The sequence of events involving insulin and GLUT4

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are critical to normalizing blood glucose and thus preventing **hyperglycemia**. When blood glucose levels are elevated, insulin is released by the β-cells of the pancreas into the bloodstream, where it circulates and binds with specific insulin receptors on cell membranes. Insulin binding causes GLUT4 to translocate to the cell surface, but binding also results in other important cellular responses as depicted in Figure 3.10.

GLUT4 is an insulin-responsive transporter that is synthesized on the ribosomes of the rough endoplasmic reticulum and then transferred to the Golgi apparatus, where it is packaged into GLUT4 storage vesicles (GSVs). Binding of insulin to its receptor causes the GSV to translocate to the cell membrane. Key to the ability of insulin to bind to the receptor site on the cell membranes of skeletal muscle, cardiac muscle, or adipose tissue cells are the activation of phosphatidylinositol-3-kinase and the cascading reactions that follow. This activity is discussed more fully in Chapter 7. The net result of insulin's effects on the cell membrane is to cause translocation of GLUT4 to the cell membrane; this process can be described simply as follows:

1. The biosynthesis of GLUT4 and its storage in GSVs are stimulated.
2. The GSVs are transported to the cell membrane by elements of the cytoskeleton including the microtubules and actin.
3. An interaction between GSVs and the plasma membrane occurs, mediated by a tethering complex; this is a step called tethering.
4. The GSVs dock with the plasma membrane in preparation for fusion.
5. The lipid bilayers of the GSVs and plasma membrane fuse.
6. Endocytosis—the GLUT4 becomes part of the plasma membrane and is available for transporting glucose into the cell.

In the presence of insulin, GLUT4 cycles continuously through the endosomal system. In insulin-resistant states or at low insulin levels, the GLUT4 stays in the GSVs and its presence in the cell membrane is reduced. Interestingly, exercise causes similar translocation of GLUT4 from the GSVs to the cell membrane, as well as increased GLUT4 expression [10].

Glucose Entry into Interstitial Fluid

The endothelial tissue of which blood vessel walls are constructed is freely permeable to glucose. Some tissues, most notably the brain, possess an additional layer of epithelial tissue between the blood vessel and the cells of the brain. Unlike the endothelium, epithelial layers are not readily permeable to many substrates, and the passage of metabolites, such as glucose, through them requires

Figure 3.10 Insulin signaling pathways and the translocation of GLUT4. Abbreviations: GRB2, growth factor receptor binding protein-2; IRS1, insulin receptor substrate 1; PI3K, phosphatidylinositol-3-kinase; PIP_3, phosphatidylinositol-3,4,5-trisphosphate; PDK1, PIP_3-dependent kinase 1; PKB, protein kinase B (also called Akt).
Source: Adapted from Augstin R., *Life*, 2010;62:315–33, Figure 3B.

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active transport or facilitated diffusion. For this reason, the epithelium is called the blood–tissue barrier of the body. Among the blood–tissue barriers studied—including those of the brain, cerebrospinal fluid, retina, testes, and placenta—GLUT1 appears to be the prime isoform for cross-barrier glucose transport, though other GLUTs appear to be involved (see Table 3.2).

Maintenance of Blood Glucose Concentration

Maintenance of normal blood glucose concentration is an important homeostatic function and is a major function of the small intestine, liver, kidneys, skeletal muscle, and adipose tissue. Regulation is the net effect of the organs' metabolic processes that remove glucose from or return glucose to the blood. These pathways, which are examined in detail in the section "Integrated Metabolism in Tissues," are hormonally influenced, primarily by the antagonistic pancreatic hormones insulin and **glucagon** and to a lesser extent by the glucocorticoid hormones of the adrenal cortex. The rise in blood glucose following the ingestion of carbohydrate, for example, triggers the release of insulin while reducing the secretion of glucagon. Insulin is the main hormone that lowers blood glucose levels and is the primary anabolic hormone. Insulin stimulates the cellular uptake of glucose, amino acids, and lipid, which leads to their conversion to storage forms in muscle and adipose tissue. The storage form for glucose, glycogen, is synthesized through the process called **glycogenesis**. Glucagon, the primary catabolic hormone that has opposite effects of insulin, increases the breakdown of liver glycogen by a process called glycogenolysis. (Glucagon also increases the breakdown of lipid stored in adipose tissue and inhibits the synthesis of proteins, as discussed in later chapters.) Additional mechanisms to increase blood glucose levels include an increase in the secretion of glucocorticoid hormones, primarily cortisol. Glucocorticoids cause increased activity of hepatic **gluconeogenesis**, a process of glucose synthesis described in detail in a later section of this chapter.

GLYCEMIC RESPONSE TO CARBOHYDRATES

The rate at which glucose is absorbed from the intestinal tract appears to be an important parameter in controlling the homeostasis of blood glucose, insulin release, obesity, and possibly weight loss, and has led to the concepts of glycemic index (GI) and glycemic load (GL). Persistently elevated blood glucose and insulin levels are also linked with obesity and the development of chronic diseases. The role of these factors in the development of insulin resistance and type 2 diabetes is covered in Chapters 7 and 8.

Glycemic Index and Glycemic Load

The **glycemic index** is an alternative way to classify dietary carbohydrates. It has been suggested that the glycemic index (GI) and **glycemic load** (GL) offer a means to examine the relative risks of diets designed to prevent coronary heart disease (CHD) and obesity.

The effect that carbohydrate-containing foods have on blood glucose concentrations is called the glycemic response to the food. Some foods that are rapidly digested and absorbed (high-GI foods) cause a rapid rise in blood glucose levels that, because of the effect of insulin released in response, can subsequently lead to a rapid fall even below the fasting level. Other foods cause a slower and more extended rise with a lower peak level of glucose and insulin and a gradual fall (low-GI foods). The glycemic index concept was developed to provide a numerical value to represent the effect of a particular food on blood glucose levels. It provides a quantitative comparison between foods. The glycemic index is defined as the increase in blood glucose level above the baseline level (fasting level) during a 2-hour period following the consumption of a defined amount of carbohydrate (usually 50 g) compared with the same amount of carbohydrate in a reference food. A related quantitative measure, the glycemic load, considers both the quantity and the quality of the carbohydrate in a food. The glycemic load equals the glycemic index times the grams of carbohydrate in a typical portion of the food. A food's GI and GL can be quite different; for example, the carbohydrate in carrots has a high GI score, but the GL for carrots is low because a half-cup serving of carrots contains only 6.13 g of carbohydrate. The higher the GL, the greater the expected elevation in blood glucose and the insulinogenic effect of the food.

Some studies of GI values have used glucose as the reference food, while others used white bread. The reference food is assigned a score of 100. In practice, the glycemic index is measured by determining the elevation of blood glucose for 2 hours following ingestion and plotting the values against time. The area under the curve for the test food is divided by the area under the curve for the reference food, and the result is multiplied by 100 (Figure 3.11). If glucose is used as the reference food and assigned a glycemic index of 100, white bread has a GI of about 71. When white bread is used as the reference, some foods will have a glycemic index of greater than 100.

There are many potential criticisms of the use of GI and GL for labeling purposes, foremost the wide variation of GI values for apparently similar foods and between laboratories. Factors that may cause this variation include the amount of carbohydrate in the meal, composition of the meal (particularly fiber, protein, and fat), previous meal composition, physical activity level of the subjects, choice of the reference food, and glucose tolerance of the subjects [11,12]. The variations observed could also

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Calculation of Glycemic Index

1. The elevation in blood glucose level above the baseline following consumption of a high-glycemic index food or 50 g of glucose in a reference food (glucose or white bread). The glycemic index of the reference food is by definition equal to 100 (graph a).

2. The elevation of blood glucose levels above the baseline following the intake of 50 g of glucose in a low-glycemic index food (graph b).

3. The glycemic index is calculated by dividing the area under the curve for the test food by the area under the curve for the reference food and multiplying the result by 100.

Figure 3.11 Blood glucose changes following carbohydrate intake (glycemic index).
Source: Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

Table 3.3 Glycemic Index of Common Foods with White Bread and Glucose Used as the Reference Food

Food Tested	Glycemic Index	
	White Bread = 100	Glucose = 100
White bread[1]	100	71
Baked russet potato[1]	107.7	76.5
Instant mashed potatoes[1]	123.5	87.7
Boiled red potato (hot)[1]	125.9	89.4
Boiled red potato (cold)[1]	79.2	56.2
Bran muffin[2]	85	60
Coca Cola[2]	90	63
Apple juice, unsweetened[2]	57	40
Tomato juice[2]	54	38
Bagel[2]	103	72
Whole-meal rye bread[2]	89	62
Rye-kernel bread[2] (pumpernickel)	58	41
Whole-wheat bread[2]	74	52
All-Bran cereal[2]	54	38
Cheerios[2]	106	74
Corn Flakes[2]	116	81
Raisin Bran[2]	87	61
Sweet corn[2]	86	60
Couscous[2]	81	61
Rice[2]	73	51
Brown rice[2]	72	50
Ice cream[2]	89	62
Soy milk[2]	63	44
Raw apple[2]	57	40
Banana[2]	73	51
Orange[2]	69	48
Raw pineapple[2]	94	66
Baked beans[2]	57	40
Dried beans[2]	52	36
Kidney beans[2]	33	23
Lentils[2]	40	28
Spaghetti, durum wheat (boiled)[2]	91	64
Spaghetti, whole meal (boiled)[2]	32	46
Sucrose[2]	83	58

[1] Source for data: Fernandes G, Velangi A, Wolever TM. Glycemic index of potatoes commonly consumed in North America. J Am Diet Assoc. 2005; 105:557–62.
[2] Source for data: Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. Am J ClinNutr. 2002; 76:5-76.

reflect real differences among samples of the same food due to factors such as its food form, ripeness, location of growth, and variety. For example, the glycemic index for a baked russet potato is 76.5 and for an instant mashed potato is 87.7 (using glucose as the reference food) [13]. Even the temperature of the food can make a difference: A boiled red potato eaten hot (with the starch gelatinized) has a glycemic index of 89.4, but the same potato eaten cooler (with the starch back to a crystalline structure) has a glycemic index of 56.2 (Table 3.3).

Glycemic index and glycemic load have proven useful in evaluating the risk of developing chronic disease and obesity. Long-term consumption of a diet with a relatively high GL is associated with an increased risk of obesity,

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type 2 diabetes, and cardiovascular diseases [14–16]. The literature suggests that the longer and higher the elevation of blood glucose and insulin, the greater the risk of developing chronic diseases and obesity.

Many published tables provide the glycemic index for different foods. The most complete is an international table [17]. Selected examples from this publication have been reproduced in Table 3.3 along with the glycemic index of potatoes. Remember that the food products differ in different regions of the world. The glycemic indices listed in Table 3.3 are intended to be used to show trends, not to prepare diets.

Figure 3.12 Integrated overview of carbohydrate metabolic pathways.

INTEGRATED METABOLISM IN TISSUES

The metabolic fate of the monosaccharides, especially glucose, depends to a great extent on the body's energy needs. This section covers the individual pathways of carbohydrate metabolism. The following section addresses the ways metabolism is regulated, including covalent modifications, allosteric mechanisms, substrate-level regulation, induction, post-translational modification, and translocation. Several terms used in carbohydrate metabolism sound and appear to be similar but are in fact quite different. The metabolic pathways of carbohydrate metabolism are listed below:

- **Glycogenesis**: The synthesis of glycogen
- **Glycogenolysis**: The breakdown of glycogen
- **Glycolysis**: The oxidation of glucose to pyruvate
- **Gluconeogenesis**: The synthesis of glucose from noncarbohydrate sources
- **Pentose phosphate pathway (hexose monophosphate shunt)**: The production of five-carbon monosaccharides (pentoses) and nicotinamide adenine dinucleotide phosphate (NADPH)
- **Tricarboxylic acid (TCA) cycle**: The oxidation of acetyl-CoA to yield CO_2 and high-energy electrons.

An integrated overview of these pathways is given in Figure 3.12. The metabolism of glycogen is covered first, followed by the energy-producing pathways (glycolysis and the TCA cycle). A detailed review of the pathways' intermediary metabolites and sites of regulation is provided in the sections that follow. The detailed pathways with the names of the chemicals and their structures are shown in the later figures. These are followed with a discussion of the individual reactions and additional comments that are particularly significant from a nutritional standpoint. It is important to recognize that under physiological conditions, many of these molecules exist as conjugated bases and are named accordingly (e.g., *pyruvate* instead of pyruvic acid, *lactate* instead of lactic acid). Because of the central role of glucose in carbohydrate nutrition, its metabolic fate is featured here. The entry of fructose and galactose into the metabolic pathways is introduced later in the discussion.

Glycogenesis

The term *glycogenesis* refers to the pathway by which glucose ultimately is converted into its storage form glycogen—a process vital to ensuring a reserve of quick energy. The major sites of glycogen synthesis and storage are the liver and skeletal muscle, while a small amount of glycogen is found in the kidneys and heart, among other tissues. Glycogen accounts for as much as 7% of the weight of the liver, particularly following a high-carbohydrate meal. Liver glycogen can be broken down to glucose and reenter the bloodstream. Therefore, it plays an important role in maintaining blood glucose homeostasis. The other major site of glycogen storage is skeletal muscle. In human skeletal muscle, glycogen generally accounts for a little less than 1% of the weight of the tissue. Although the concentration of glycogen in the liver is greater, muscle stores account for most of the body's glycogen because the muscle makes up a much greater portion of the body's weight. The liver can store approximately 100 g of glycogen, whereas muscle can store about 500 g. The glycogen stores in muscle are an energy source within that muscle fiber and cannot directly contribute to blood glucose levels. (Muscle lacks the enzyme that converts the phosphorylated glucose back to free glucose.) We discuss how metabolic products of glucose can return to the liver and be converted to glucose.

The initial part of the glycogenic pathway is illustrated in Figure 3.13. Glucose is first phosphorylated upon entering the cell, producing glucose-6-phosphate. In muscle and other nonhepatic cells, the enzyme catalyzing this phosphate transfer from ATP is hexokinase, a mixture of hexokinase isozymes type 1 and 2. The properties of

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Figure 3.13 (a) Reactions of glycogenesis, by which the formation of glycogen from glucose occurs. (b) The primer function of glycogenin. The glucosyl transferase activity of glycogenin catalyzes the attachment to itself of from two to seven glucose residues transferred from UDP-glucose. The letter *n* represents an unspecified number of UDP-glucose molecules.

this enzyme are shown in Table 3.4. Muscle hexokinase is an allosteric enzyme that is negatively modulated by the product of the reaction, glucose-6-phosphate. This means that when the muscle cell has adequate glucose-6-phosphate, the entry of additional glucose into the cell is slowed. Muscle hexokinase has a low K_m, which means it can function at maximum velocity when blood glucose levels are at normal (fasting) levels.

Glucose **phosphorylation** in the liver is catalyzed primarily by a hexokinase isozyme called glucokinase (sometimes called hexokinase 4). Although the reaction product, glucose-6-phosphate, is the same as in other tissues, interesting differences distinguish glucokinase from hexokinase (Table 3.4). For example, muscle hexokinase is allosterically inhibited by glucose-6-phosphate, whereas liver glucokinase is not. This characteristic allows excess glucose entering the liver cell to be phosphorylated quickly and encourages glucose entry when blood glucose levels are elevated. Also, glucokinase has a much higher K_m than hexokinase, meaning that it can convert glucose to its phosphate form at a higher velocity should the blood concentration of glucose rise significantly, particularly after a carbohydrate-rich meal.

Table 3.4 Properties of Hexokinase and Glucokinase

Hexokinase (Types 1 and 2)	Glucokinase (Hexokinase Type 4)
Located in muscle, brain, and adipose tissue	Located in liver and pancreas
Allosterically inhibited by glucose-6-P (its product)	Not inhibited by glucose-6-P
Low K_m; function at maximum velocity at fasting blood glucose concentrations	High K_m; functions at maximum velocity only when glucose levels are high (such as following a high-carbohydrate meal)
Not induced by insulin in normal individuals	Induced by insulin in normal individuals
Not induced by insulin in insulin-resistant individuals	Not induced by insulin in insulin-resistant individuals

Glycogenesis is initiated by the presence of glucose-6-phosphate. The phosphorylation of glucose as it enters the liver cell keeps the level of free glucose low, which

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enhances the entry of glucose into the liver cell due to the concentration gradient between the blood and the liver cell interior. Therefore, the liver has the capacity to reduce blood glucose concentration when it becomes high. Remember, the liver is not dependent upon insulin for glucose transport into the cell, but glucokinase is inducible by insulin. Insulin blood levels are increased by elevated blood glucose levels. Glucokinase activity is below normal in people with type 1 diabetes mellitus because they have very low insulin levels, and the glucokinase is therefore not induced. In type 2 diabetes the glucokinase is not induced by the insulin-resistant membrane receptors. In either case, the low glucokinase activity contributes to the liver cell's inability to rapidly take up and metabolize glucose, which results even though GLUT2 of the liver is not regulated by insulin.

Newly synthesized glucose via gluconeogenesis provides another source of glucose-6-phosphate that can be used for glycogen synthesis in the liver, even when there is an abundance of glucose following a carbohydrate-rich meal. As discussed in detail later in this chapter, gluconeogenesis produces glucose-6-phosphate from noncarbohydrate sources including lactate, a by-product of glycolysis in red blood cells and muscle. While it may seem paradoxical for both gluconeogenesis and glycogenesis to function simultaneously, gluconeogenesis provides about one-third of the glucose-6-phosphate used for glycogen synthesis in the liver.

The next step in glycogenesis is the transfer of phosphate from carbon 6 of the glucose molecule to carbon 1 in a reaction catalyzed by the enzyme phosphoglucomutase (Figure 3.13). Nucleoside triphosphates other than ATP sometimes function as activating substances in intermediary metabolism. In the next reaction of glycogenesis, energy derived from the hydrolysis of the α-β-phosphate anhydride bond of uridine triphosphate (UTP to UMP) allows the resulting uridine monophosphate to be coupled to the glucose-1-phosphate to form uridine diphosphate-glucose (UDP-glucose). Glucose is incorporated into glycogen as UDP-glucose. The reaction is catalyzed by glycogen synthase and requires some preformed glycogen as a primer, to which the incoming glucose units can be attached. The initial glycogen is formed by binding a glucose residue to a tyrosine residue of a protein called glycogenin. In this case, glycogenin acts as the primer. Additional glucose residues are attached by glycogen synthase to form chains of up to eight units. The role of glycogenin in glycogenesis has been reviewed [18]. In muscle the protein remains in the core of the glycogen molecule, but in the liver more glycogen molecules than glycogenin molecules are present, so the glycogen must separate from the protein. Glycogen synthase exists in an active (dephosphorylated) form and a less active (phosphorylated) form. Insulin facilitates glycogen synthesis by stimulating the dephosphorylation of glycogen synthase. The glycogen synthase reaction is the primary target of insulin's stimulatory effect on glycogenesis.

Figure 3.14 Formation of glycogen branches by the branching enzyme.

When six or seven glucose molecules are added to the glycogen chain, the branching enzyme transfers them to a C—6—OH group (Figure 3.14). Glycogen synthase cannot form the α(1-6) bonds of the branch points. This action is left to the amylo(1-4 →1-6)-transglycosylase or branching enzyme, which transfers a seven-residue oligosaccharide segment from the end of the main glycogen chain to carbon number 6 hydroxyl groups. Branching within the glycogen molecule is important because it increases the molecule's solubility and compactness. Branching also

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makes available many nonreducing ends of chains from which glucose residues can be cleaved rapidly and used for energy, in the process known as glycogenolysis and described in the following section. The overall pathway of glycogenesis, like most synthetic pathways, consumes energy because an ATP and a UTP are consumed for each molecule of glucose introduced.

Glycogenolysis

The potential energy of glycogen is contained within the glucose residues that make up its structure. In accordance with the body's energy demands, the residues can be systematically cleaved one at a time from the nonreducing ends of the glycogen branches and routed through energy-releasing pathways. The breakdown of glycogen into individual glucose units, in the form of glucose-1-phosphate, is called glycogenolysis and is catalyzed by the enzyme glycogen phosphorylase. The steps involved in glycogenolysis are shown in Figure 3.15.

Although glycogen phosphorylase cleaves α(1-4) glycosidic bonds, it cannot hydrolyze α(1-6) bonds. Phosphorylase acts repetitively along linear portions of the glycogen molecule until it reaches a point four glucose residues away from an α(1-6) branch point. Here the degradation process stops, resuming only after an enzyme called the debranching enzyme cleaves the α(1-6) bond at the branch point.

At times of heightened glycogenolytic activity, the formation of increased amounts of glucose-1-phosphate shifts the phosphoglucomutase reaction toward production of the 6-phosphate isomer. In the liver (and kidneys), glucose-6-phosphate can enter into the oxidative pathway for glucose (glycolysis) or become free glucose. The conversion of glucose-6-phosphate to free glucose requires the action of glucose-6-phosphatase. This enzyme is not expressed in muscle cells or adipocytes. Therefore, free glucose can be formed only from liver or kidney glycogen and transported through the bloodstream to other tissues for oxidation.

Like its counterpart glycogenesis, glycogenolysis is highly regulated. Its catalyzing enzyme, phosphorylase, is regulated by both covalent and allosteric mechanisms. The regulation is different for the phosphorylation isozymes in muscle than in liver. The muscle and liver isozymes fulfill different physiological purposes: In muscle, the glucose is released from glycogen to provide glucose for energy within the cell, whereas in the liver the glucose is released to provide blood glucose. As phosphorylase is activated for glycogen phosphorylation, glycogen synthase is inhibited.

Glycogenolysis Regulation

Covalent Regulation Covalent regulation of phosphorylase is enhanced by glucagon and the catecholamines, epinephrine and norepinephrine. These hormones cause a covalent modification of phosphorylase by converting it to an active form through the second messenger cAMP, which regulates the phosphorylation site of the enzymes involved, as discussed in Chapter 1. These hormones bind to a receptor on the cell membrane that causes adenyl cyclase to be activated to produce cAMP. The cAMP causes inactive phosphorylase kinase to become active by phosphorylating it. The active phosphorylase kinase plus ATP converts inactive

Figure 3.15 The reactions of glycogenolysis, by which glucose residues are sequentially removed from the nonreducing ends of glycogen segments.

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Figure 3.16 An overview of the regulation of glycogen phosphorylase. It is positively regulated covalently by cAMP and positively allosterically regulated by AMP. It is negatively regulated by ATP and glucose-6-P, which cause shifts in the equilibrium between the inactive and active ("b") forms.

(nonphosphorylated) phosphorylase b to active (phosphorylated) phosphorylase a. The phosphorylated phosphorylase is less sensitive to the allosteric activation discussed later in this chapter. Phosphorylase a can be converted back to the inactive form, phosphorylase b, by phosphoprotein phosphatase 1 (PP-1). A Nobel Prize was awarded for elucidating this pathway (Figure 3.16).

Allosteric Activation The allosteric activation of phosphorylase b is carried out by AMP to convert it to the active phosphorylase a. When energy levels are low, cellular ATP has been hydrolyzed to AMP, more energy is needed, and the phosphorylase a releases glucose-1-phosphate. The AMP binds to an allosteric site on phosphorylase b, which increases the binding of the glycogen (see Figure 1.10). This allosteric site can also bind ATP, which is an allosteric inhibitor of the enzyme. Glucose-6-phosphate and caffeine are also allosteric inhibitors of the enzyme.

Muscle Phosphorylase The muscle and liver phosphorylase are isozymes. The muscle enzyme releases glucose-1-phosphate, which can be converted to glucose-6-phosphate that enters into the glycolysis pathway to provide energy for the cell. Muscle phosphorylase is more sensitive to intracellular ligands such as AMP for activation. The muscle enzyme is inhibited by metabolites, ATP, glucose-6-phosphate, and glucose. During times of stress the hormones epinephrine and norepinephrine stimulate cAMP synthesis and along with PP-1 covalently modify phosphorylase to the active form. Nervous stimulation and Ca^{2+} ions have the same effect.

Liver Phosphorylase Liver phosphorylase is less sensitive to intracellular ligands. It shows a weak increase in activity in the presence of AMP (10–20%) and is insensitive to inhibition by ATP or glucose-6-phosphate. Liver phosphorylase is regulated by hormonal controls such as glucagon.

Glycolysis

Glycolysis is the pathway by which glucose is degraded into two 3-carbon units, pyruvate. From pyruvate, the metabolic course depends largely on the availability of reducing units in the cytosol, which is dependent upon the availability of oxygen within the cell. Glycolysis can function under either aerobic or anaerobic conditions. Under anaerobic conditions or in a situation without sufficient reducing equivalents due to either the lack of oxygen or high cellular metabolism, pyruvate is converted to lactate. Under otherwise normal conditions, the conversion to lactate occurs mainly in times of strenuous exercise when the demand for oxygen by the working muscles exceeds that which is available. Lactate produced under anaerobic conditions can also diffuse from the muscle to the bloodstream and be carried to the liver for conversion to glucose. Under these anaerobic conditions, glycolysis releases a small amount of usable energy that can help sustain the muscles even in a state of oxygen debt. Providing this energy is the major function of the anaerobic pathway of glucose to lactate. Anaerobic glycolysis is the sole source of energy for erythrocytes because these cells do not contain mitochondria. Both the brain and gastrointestinal tract also produce much of their energy from glycolysis.

Under aerobic conditions, pyruvate can be transported into the mitochondria and participate in the TCA cycle, in which it becomes completely oxidized to CO_2 and H_2O. Complete oxidation is accompanied by the release of relatively large amounts of energy, much of which is captured in ATP molecules by the mechanism of oxidative phosphorylation. The glycolytic enzymes function within the cytosol of the cell, but the enzymes catalyzing the TCA cycle reactions are located within the mitochondrion. Therefore, pyruvate must enter the mitochondrion for complete oxidation. Glycolysis followed by TCA cycle activity (aerobic catabolism of glucose) demands an ample

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supply of oxygen, a condition that generally is met in normal, resting mammalian cells. In a normal, aerobic situation, complete oxidation of pyruvate generally occurs, with only a small amount of lactate being formed. The primary importance of glycolysis in energy metabolism, therefore, is in providing the initial sequence of reactions (to pyruvate) necessary for the complete oxidation of glucose by the TCA cycle, which supplies relatively large quantities of ATP.

Nearly all cells conduct glycolysis, but most of the energy derived from dietary carbohydrates is stored and/or utilized by the liver, muscle, and adipose tissue, which together constitute a major portion of total body mass. The brain is an extravagant consumer of carbohydrate energy, but lacks the ability to store it. In cells that lack mitochondria, such as erythrocytes, the pathway of glycolysis is the sole provider of ATP by the mechanism of substrate-level phosphorylation of ADP, discussed later in this chapter. The pathway of glycolysis, under both aerobic and anaerobic conditions, is summarized in Figure 3.17. While the figure illustrates how each monosaccharide enters the glycolytic pathway, including glucose from glycogen breakdown, each possibility may not be present in every cell. Following are comments on selected reactions (the numbers correspond to the numbers in Figure 3.17).

1. The hexokinase/glucokinase reaction consumes 1 mol ATP/mol glucose. The properties of these enzymes were covered in Table 3.4. Glucokinase is present in the liver and pancreas. Hexokinase is located in muscle, adipose tissue, and the brain. As discussed earlier, the hexokinase in muscle has a low K_m, which means it can function at maximum velocity at normal blood glucose levels. When the muscle cell accumulates glucose-6-phosphate, the hexokinase is inhibited. Liver glucokinase, in contrast, has a high K_m, which means it requires a high concentration of glucose in blood to function at maximum velocity. The liver does not remove large quantities of glucose from blood unless blood glucose is elevated. Glucokinase in the liver is induced by insulin. Phosphorylating glucose serves to "prime" the glycolytic pathway by trapping glucose in the cell and energizing the molecule for subsequent reactions.

2. Phosphoglucose isomerase (also called glucose phosphate isomerase) catalyzes movement of the carbonyl group from the first carbon (glucose) to the second carbon (fructose). This is an interconversion of isomers—glucose-6-phosphate to fructose-6-phosphate—and is reversible.

3. Phosphofructokinase catalyzes the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate using an ATP. The term *bis* means that the two phosphates are on different carbons. The phosphofructokinase reaction is an important regulatory site. This irreversible step commits the cell to metabolize glucose rather than converting it to another sugar or storing it as glycogen. Phosphofructokinase is negatively modulated (by allosteric mechanisms) by ATP and citrate (a product of the TCA cycle and an indication that energy needs are met). The inhibition by ATP is reversed by AMP, an indication that the cell needs more energy. There is a relationship among the levels of ATP, ADP, and AMP. They are interconverted by the reaction:

$$\text{ADP} + \text{ADP} \leftrightarrow \text{ATP} + \text{AMP}$$

This reaction is catalyzed by adenylate kinase. When the reaction reaches equilibrium the quantity of ADP is about 10% of that of ATP, and AMP levels are less than 1% of those of ATP. Small changes in ATP are amplified in changes in AMP. The regulation of phosphofructokinase reactions is modulated by the relative amounts of ATP and AMP.

Phosphofructokinase is also regulated by fructose-2,6-bisphosphate, which is a potent allosteric activator that increases the affinity of the enzyme for its substrate, fructose-6-phosphate. Levels of fructose-2,6-bisphosphate are controlled by the enzyme phosphofructokinase-2. This enzyme is induced by glucagon and is different from the phosphofructokinase in the glycolytic pathway. Other activities of this enzyme are discussed in the gluconeogenesis section of this chapter.

4. Fructose bisphosphate aldolase (or simply aldolase) cleaves fructose-1,6-bisphosphate, a hexose, into two triose phosphates, glyceraldehyde-3-phosphate (G-3-P) and dihydroxyacetone phosphate (DHAP). The remaining steps in glycolysis involve three-carbon units rather than six-carbon units.

5. The isomers G-3-P and DHAP are interconverted by the enzyme triose phosphate isomerase. In an isolated system, the equilibrium favors DHAP formation. In the cellular environment, however, it is shifted completely toward producing G-3-P because this metabolite is continuously removed from the equilibrium by the subsequent reaction 6.

6. In this reaction, G-3-P is oxidized to a carboxylic acid, while inorganic phosphate is incorporated as a carboxylic phosphoric anhydride bond (a high-energy compound). The enzyme is glyceraldehyde-3-phosphate dehydrogenase, which uses NAD^+ as its hydrogen-accepting cosubstrate. Under aerobic conditions, the NADH formed is reoxidized to NAD^+ by O_2 through the electron transport chain in the mitochondria, as explained in the next section. The reason why O_2 is not necessary to sustain the reaction of converting G-3-P to 1,3-bisphosphoglycerate is that under anaerobic conditions the NAD^+ consumed is restored by a subsequent reaction converting pyruvate to lactate (see reaction 11).

7. This reaction, catalyzed by phosphoglycerate kinase, exemplifies a substrate-level phosphorylation of ADP. A more detailed review of substrate-level phosphorylation, by which ATP is formed from ADP by the transfer of a phosphate from a high-energy donor molecule, is covered

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Figure 3.17 Glycolysis, indicating the mode of entry of glucose, fructose, glycogen, and galactose into glycolysis. Hydroxyl groups on ring structures are indicated by a line pointing above or below the ring.

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in the "**Substrate-Level Phosphorylation**" section. Two moles of ATP are synthesized because glucose (a hexose) yields two trioses. This reaction replaces the two ATPs used to prime glycolysis. Under conditions of high ATP (which means low ADP) the reaction can be reversed.

8 Phosphoglycerate mutase catalyzes the transfer of the phosphate group from the number 3 carbon to the number 2 carbon of the glycerate molecule.

9 Dehydration of 2-phosphoglycerate by the enzyme enolase introduces a double bond that imparts high energy to the phosphate bond.

10 Phosphoenolpyruvate (PEP) donates its phosphate group to ADP in a reaction catalyzed by pyruvate kinase to yield pyruvate. This is the second site of substrate-level phosphorylation of ADP in the glycolytic pathway to make two ATPs. Two ATPs were used to prime glycolysis, two were produced in reaction 7, and two were produced in this reaction, for a net gain of two ATPs to this point. The hexose has now been split into two 3-carbon units. Phosphoenolpyruvate kinase is a highly regulated enzyme. It is activated allosterically by AMP and fructose-1,6-bisphosphate and inhibited by ATP, acetyl-CoA, and alanine. In the liver it is also regulated covalently by glucagon through the cAMP mechanism discussed earlier, which transfers a phosphoryl group from ATP. The phosphorylated enzyme is more sensitive to inhibition by ATP.

11 The lactate dehydrogenase reaction reduces pyruvate to lactate while oxidizing NADH to NAD^+. The NAD^+ formed in the reaction can replace the NAD^+ consumed earlier under anaerobic conditions. This reaction is most active in situations of oxygen debt, as occurs in prolonged muscular activity. Under aerobic conditions, pyruvate enters the mitochondrion for complete oxidation via TCA cycle. A third important option available to pyruvate is its conversion to the amino acid alanine by amino transferase, a reaction by which pyruvate acquires an amino group from the amino acid glutamate (Chapter 6). The alternate pathways for pyruvate, together with the fact that pyruvate is also the product of the catabolism of various amino acids, makes pyruvate an important link between protein (amino acid) and carbohydrate metabolism.

12 This reaction occurs in muscle, kidneys, and other nonhepatic tissues where fructose is phosphorylated by hexokinase to form fructose-6-phosphate. This is a relatively unimportant reaction because the liver clears nearly all of the dietary fructose when first absorbed from the gastrointestinal tract. The hexokinase reaction is slow and occurs only in the presence of high levels of blood fructose, a situation that is rarely encountered in humans.

13 Fructokinase is abundant in the liver and catalyzes the conversion of fructose to fructose-1-phosphate. This reaction is important when fructose is consumed because nearly all dietary fructose enters the hepatocyte on the first pass. Following a carbohydrate-rich meal, fructose is committed to glycolysis rather than being converted to glucose and stored as glycogen. Because fructose-1-phosphate enters glycolysis at reaction 4—and because reaction 3 is irreversible and stimulated by insulin following a meal—fructose in the liver follows a one-way trip to becoming pyruvate (and possibly lactate).

14–15 Like glucose and fructose, galactose is first phosphorylated (by galactokinase) to form galactose-1-phosphate, which occurs primarily in the liver when first absorbed from the gastrointestinal tract. The galactose-1-phosphate is converted to UDP-galactose, which is converted to UDP-glucose by subsequent reactions. The result of these reactions is the production of glucose-1-phosphate. When dietary galactose is accompanied by comparatively large amounts of glucose, the glucose-1-phosphate from galactose is driven mostly toward glycogenesis as the flow of glucose-1-phosphate from glucose pushes the reaction toward glycogenesis.

16 This is the point where glucose-6-phosphate enters into a pathway called the pentose phosphate pathway (hexose monophosphate shunt), which is discussed later in this chapter.

17 Glycogenesis is the conversion of glucose-1-phosphate into glycogen and occurs primarily in the liver and skeletal muscle. Glycogenesis is stimulated by insulin following a carbohydrate-rich meal (see Chapter 7).

18 Glycogenolysis is the hydrolysis of glycogen into individual glucose-1-phosphate units. In skeletal muscle, the liberated glucose-1-phosphate enters glycolysis for energy utilization. In the liver, glucose-1-phosphate can enter glycolysis or be converted to free glucose for release into the system circulation.

The Tricarboxylic Acid Cycle

The tricarboxylic acid (TCA) cycle, also called the **Krebs cycle** or the citric acid cycle, is central to energy metabolism in the body. Under aerobic conditions, the TCA cycle is the final pathway by which fuel molecules—carbohydrates, fatty acids, and amino acids—are completely oxidized to CO_2 so that their energy is released and transferred to ATP molecules. Greater than 90% of food energy captured and used in the human body involves the TCA cycle in conjunction with oxidative phosphorylation (see Figure 1.7). The enzymes of the TCA cycle are located in the mitochondrial matrix and work in concert with "energy carriers" that shuttle high-energy electrons released by the TCA cycle to the electron transport chain located in the inner mitochondrial membrane. These so-called energy carriers, NADH and $FADH_2$, are formed by

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reduction (accept electrons) when fuel molecules are oxidized (lose electrons). In this way, the main function of the TCA cycle is to generate high-energy electrons that power the synthesis of ATP via oxidative phosphorylation. The TCA cycle is shown in Figure 3.18.

The primary molecule entering the TCA cycle is acetyl-CoA. Therefore, fuel molecules must first be transported into mitochondria and converted to acetyl-CoA for complete oxidation. In the case of carbohydrate, glycolysis in the cytosol produces pyruvate, which is transported into mitochondria and converted acetyl-CoA (discussed in the next section). Fatty acids and amino acids are also transported into mitochondria and converted to acetyl-CoA (discussed in later chapters).

Conversion of Pyruvate to Acetyl-CoA

Conversion of pyruvate to acetyl-CoA is accomplished in the mitochondrial matrix by a multienzyme complex called the pyruvate dehydrogenase complex (PDC). This multienzyme system is made up of three enzymes: pyruvate dehydrogenase, dihydrolipoamide acetyltransferase, and dihydrolipoamide dehydrogenase. Several cofactors are required for the reaction, including coenzyme A (CoA), thiamin pyrophosphate, Mg^{2+}, NAD^+, FAD, and lipoic acid. Four vitamins, therefore, are necessary for the activity of the complex: pantothenic acid (a component of CoA), thiamin, niacin, and riboflavin. The role of these vitamins and others as precursors of coenzymes is discussed in Chapter 9. The net effect of the complex is decarboxylation, producing CO_2, and dehydrogenation of pyruvate, with NAD^+ serving as the terminal acceptor of a hydride ion (one proton and two electrons). The active sites of the three enzymes are packed closely together, which allows the passing of the product of one reaction to the next enzyme. This reaction yields energy because the oxidation of NADH produces ATP by oxidative phosphorylation. The reaction is regulated allosterically: negatively by ATP, acetyl-CoA, and NADH, and positively by NAD^+ and ADP. The PDC is also regulated covalently: a Mg^{2+}-dependent enzyme, pyruvate dehydrogenase kinase, phosphorylates the complex when NADH and acetyl-CoA levels rise. Reactivation of the PDC occurs by the enzyme pyruvate dehydrogenase phosphatase, which removes the phosphate. Insulin and Ca^{2+} ions activate the kinase to activate the PDC.

Release of High-Energy Electrons

The condensation of acetyl-CoA with oxaloacetate initiates the TCA cycle reactions. Note that the TCA cycle itself does not directly generate much ATP. Rather, it generates high-energy electrons that are transferred to NAD^+ and FAD, thus yielding NADH and FADH, respectively. Because the reactions are cyclic, oxaloacetate is regenerated after one trip through the cycle, so a relatively small number of oxaloacetate molecules can generate large amounts of NADH and FADH as acetyl-CoA continually feeds into the cycle. Following are comments on the individual reactions (Figure 3.18):

1. The formation of citrate from oxaloacetate and acetyl-CoA is catalyzed by the enzyme citrate synthase. The reaction is regulated negatively by NADH and succinyl-CoA.
2. The isomerization of citrate to isocitrate involves *cis* aconitate as an intermediate. The isomerization, catalyzed by aconitase, results in the repositioning of the —OH group onto an adjacent carbon.
3. Catalyzed by the enzyme isocitrate dehydrogenase, this is the first of four dehydrogenation reactions within the cycle. The main products are NADH, CO_2, and α-ketoglutarate. The reaction is positively modulated by ADP and negatively modulated by ATP and NADH.
4. Decarboxylation and dehydrogenation of α-ketoglutarate is mechanistically identical to the pyruvate dehydrogenase complex reaction in its multienzyme–multicofactor requirement. The main products are NADH, CO_2, and succinyl-CoA.
5. Succinyl-CoA contains a high-energy thioester bond that is hydrolyzed by the enzyme succinyl-CoA synthetase (also called succinyl thiokinase) and that releases sufficient energy to drive the phosphorylation of guanosine diphosphate (GDP) by inorganic phosphate. The resulting guanosine triphosphate (GTP) can transfer its phosphate to ADP to make ATP in a reaction catalyzed by the enzyme nucleoside diphosphate kinase. This reaction is another example of ATP production through substrate-level phosphorylation.
6. The succinate dehydrogenase reaction yields fumarate and uses FAD instead of NAD^+ as a proton and electron acceptor. Succinate dehydrogenase is bound in the inner membrane of the mitochondria. Other TCA enzymes are found in the mitochondrial matrix.
7. Fumarase incorporates the elements of H_2O across the double bond of fumarate to form malate.
8. The conversion of malate to oxaloacetate completes the cycle. NAD^+ acts as the proton and electron acceptor in this dehydrogenation reaction, which is catalyzed by malate dehydrogenase.

Oxaloacetate and Tricarboxylic Acid Cycle Intermediates

To keep the TCA cycle functioning, oxaloacetate and/or other TCA cycle intermediates that can form oxaloacetate must be replenished in the cycle. Oxaloacetate, fumarate, succinyl-CoA, and α-ketoglutarate can all be formed from certain amino acids, but the single most important mechanism for ensuring an ample supply of oxaloacetate is the reaction that forms oxaloacetate (four carbons) directly

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1. Acetyl-CoA adds two carbons to oxaloacetate to start the cycle.
2. Isomerization takes place by removing H_2O and then adding it back.
3. A CO_2 is lost and a NADH is produced.
4. Another CO_2 is lost and another NADH is produced.
5. A substrate-level phosphorylation.
6. FAD^+ is reduced to form $FADH_2$.
7. Add H_2O across the double bond.
8. Third NADH produced in the TCA cycle. One $FADH_2$ and one NADH produced in the conversion of pyruvate to acetyl-CoA.

Figure 3.18 The tricarboxylic acid (TCA) cycle.

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Figure 3.19 The reaction by which oxaloacetate is formed directly from pyruvate.

from pyruvate (three carbons) by the addition of CO_2. This reaction, shown in Figure 3.19, is catalyzed by pyruvate carboxylase. The "uphill" incorporation of CO_2 is accomplished at the expense of ATP, and the reaction requires the participation of biotin (see Chapter 9). The conversion of pyruvate to oxaloacetate is called an **anaplerotic** (replenishing) process because of its role in restoring oxaloacetate to the cycle. Interestingly, pyruvate carboxylase is regulated positively by acetyl-CoA, thereby ensuring oxaloacetate formation in response to increasing levels of acetyl-CoA.

NADH from Glycolysis: The Shuttle Systems

NADH produced in the cytosol during glycolysis (the glyceraldehyde-3-phosphate dehydrogenase reaction) is unable to directly participate in oxidative phosphorylation because the inner mitochondrial membrane is impermeable to NADH. Under anaerobic conditions, NADH in the cytosol is used in the lactate dehydrogenase reduction of pyruvate to lactate, thereby becoming reoxidized to NAD^+ without involving oxygen. In this manner, NAD^+ is restored to sustain the glyceraldehyde-3-phosphate dehydrogenase reaction, allowing the production of lactate to continue in the absence of oxygen.

When the supply of oxygen is adequate to allow total oxidation of incoming glucose, the production of pyruvate and NADH from glycolysis is accelerated and lactate is not formed. In this situation, the reducing equivalents of NADH (the protons and electrons) are transported from the cytosol to the mitochondrial matrix by two separate shuttle systems. These shuttle systems are specific to certain tissues. The glycerol-3-phosphate shuttle functions in the brain and skeletal muscle, whereas the more active malate–aspartate shuttle functions in the liver, kidney, and heart.

Glycerol-3-Phosphate Shuttle System NADH in the cytosol transfers its reducing equivalents to dihydroxyacetone phosphate, forming glycerol-3-phosphate that freely diffuses across the outer mitochondrial membrane. The reaction is catalyzed by the cytosolic isoform of glycerol-3-phosphate dehydrogenase. The reducing equivalents of glycerol-3-phosphate are then transferred to FAD that is associated with a membrane-bound isoform of glycerol-3-phosphate dehydrogenase located on the outer face of the inner mitochondrial membrane. Finally, the

Figure 3.20 Glycerol-3-phosphate shuttle.

resulting $FADH_2$ transfers its electrons directly to the electron transport chain, producing 1.5 ATPs per mole of NADH (Figure 3.20). This shuttle is not reversible.

Malate–Aspartate Shuttle System The most active shuttle compound, malate, is freely permeable to the inner mitochondrial membrane. Oxaloacetate from the cytosol is reduced by the NADH to form malate and NAD^+. The malate is oxidized by the enzyme malate dehydrogenase to oxaloacetate in the matrix of mitochondria, producing NADH that enters the electron transport chain and generates 2.5 ATPs per mole of NADH. The oxaloacetate undergoes transamination by aspartate amino transferase to form aspartate, which is freely permeable to the inner membrane and can move back out into the cytosol. The effect is that reducing equivalents of NADH are transferred into mitochondria, even though the inner mitochondrial membrane is impermeable to NADH itself (Figure 3.21). This shuttle is reversible.

Formation of ATP

The majority of energy-requiring reactions in the body depend on ATP as a cosubstrate to furnish the energy that drives the reaction. Thus, ATP acts as the main energy currency and must be continually synthesized from the energy provided by macronutrients. In the case of carbohydrates, glycolysis, the TCA cycle, and the electron transport chain work together to synthesize ATP from the complete oxidation of the starting substrate (glucose, fructose, and galactose). The anaerobic steps that occur in the cytosol (glycolysis) are able to synthesis a small number of ATP, whereas the majority of ATP are synthesized in the

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Figure 3.21 Malate–aspartate shuttle.

mitochondria by oxidative phosphorylation. The large ionic gradient created by the release of high-energy electrons and protons via the TCA cycle is what powers the synthesis of ATP in oxidative phosphorylation. In contrast, some ATP are synthesized by direct phosphorylation involving high-energy phosphate donors, referred to as substrate-level phosphorylation.

Substrate-Level Phosphorylation

Two reactions in glycolysis and one reaction in the TCA cycle produce ATP by substrate-level phosphorylation. Phosphorylation of ADP to form ATP is accomplished by phosphate donors having more energy than the amount needed $\Delta G^0 = +7{,}300$ cal/mol or $+35.7$ kJ/mol) for the reaction.

Table 3.5 lists the standard free energy of hydrolysis of selected phosphate-containing compounds in both kcal and kJ. Phosphorylated molecules have a wide range of free energies of hydrolysis of their phosphate groups. Many of them release less energy than ATP, but some release more. Figure 1.15 gives the structures of phosphoenolpyruvate, 1,3-bisphosphoglycerate, and phosphocreatine, three compounds that have more free energy than ATP and are capable of phosphorylating ADP. The ΔG^0 of hydrolysis of the compounds, listed in Table 3.5, is called the phosphate group transfer potential and is a measure of the compounds' capacities to donate phosphate groups to other substances. The more negative the transfer potential, the more potent the phosphate-donating power. Therefore, a compound that releases more energy on hydrolysis of its phosphate can transfer that phosphate to an acceptor molecule having a relatively more positive transfer potential. For this transfer to occur in actuality, however, there must be a specific enzyme to catalyze the transfer. A phosphate

Table 3.5 Free Energy of Hydrolysis (Phosphate Group Transfer Potential) of Some Phosphorylated Compounds

Compound	ΔG^0(cal)	ΔG^0(kJ)
Phosphoenolpyruvate	–14,800	–62.2
1,3-diphosphoglycerate	–11,800	–49.6
Phosphocreatine	–10,300	–43.3
ATP	–7,300	–35.7
Glucose-1-phosphate	–5,000	–21.0
Adenosine monophosphate (AMP)	–3,400	–9.2
Glucose-6-phosphate	–3,300	–13.9

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Figure 3.22 (a) Example of high-energy phosphate bond being transferred from high-energy compound phosphocreatine to form ATP. (b) The transfer of the high-energy phosphate bond to a compound that becomes activated, allowing it to enter into the glycolytic pathway.

group can be enzymatically transferred from ATP to glucose, a transfer that can be predicted from Table 3.5. It can also be predicted from Table 3.5 that compounds with a more negative phosphate group transfer potential than ATP can transfer phosphate to ADP, forming ATP. This kind of reaction does, in fact, occur in the hexokinase/glucokinase reactions. The phosphorylation of ADP by phosphocreatine represents an important mode for ATP formation in muscle, and the reaction exemplifies a substrate-level phosphorylation (Figure 3.22).

Biological Oxidation and the Electron Transport Chain

The majority of ATP synthesized in mitochondria begins with the oxidation of fuel molecules and the release of electrons and protons by the TCA cycle. The electrons and protons are delivered (by NADH and $FADH_2$) to the inner mitochondrial membrane where the electrons are passed through a series of intermediate compounds and ultimately to molecular oxygen, which becomes reduced to H_2O in the process. The compounds participating in this sequential reduction-oxidation constitute the electron transport chain, also known as the respiratory chain because the electron transfer is linked to the uptake of O_2, which is made available to the tissues by respiration. The energy provided by the electron flow allows the protons to be translocated from the mitochondrial matrix to the space between the inner and outer membranes, which creates an energy gradient that powers the phosphorylation of ADP to form ATP. The term *oxidative phosphorylation* is a descriptive blend of simultaneous processes involving electron transport, translocation of protons, oxidation of a metabolite by oxygen, and the phosphorylation of ADP to make ATP.

Cellular oxidation of a compound can occur by several different reactions: the addition of oxygen, the removal of electrons, and the removal of protons and electrons together (as hydrogen atoms or hydride ions). All of these reactions are catalyzed by enzymes collectively termed *oxidoreductases*. Among these, the **dehydrogenases** remove protons and electrons from nutrient metabolites and are particularly important in energy transformation. The protons and electrons removed from metabolites by dehydrogenases generally produce NADH or $FADH_2$, which are either already in or shuttled into the mitochondria and move along the electron transport chain.

After oxidation of substrate molecules by a dehydrogenase enzyme, the protons and electrons are transferred to a cosubstrate, such as the vitamin-derived nicotinamide adenine dinucleotide (NAD^+) or flavin adenine dinucleotide (FAD). The structures of both the oxidized and reduced forms of these cosubstrates are shown in Figures 3.23 and 3.24. After accepting protons and electrons from reactions of the TCA cycle, NADH

* P added on this —OH group for NADP.

Figure 3.23 Nicotinamide adenine dinucleotide (NAD^+) and its reduced form (NADH).

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Figure 3.24 Flavin adenine dinucleotide (FAD) and its reduced form ($FADH_2$). R = ribitol phosphate + AMP.

and $FADH_2$ move to the inner mitochondrial membrane to initiate the electron transport chain. The sequential arrangement of reactions in the electron transport chain is shown in Figure 3.25. Dashed lines outline the four complexes. Either NADH or $FADH_2$ is the initial electron donor for the electron transport chain.

Anatomical Site for the Electron Transport Chain

The structure of the mitochondrion is illustrated in Figures 1.6 and 1.7. Refer to Chapter 1 for a description of the outer membrane, which is permeable to most molecules smaller than 10 kilodaltons, and the inner membrane, which has very limited permeability. Remember that the enzymes of the TCA cycle, except for succinyl-CoA synthetase and those involved in fatty acid oxidation (discussed in Chapter 5), are located in the matrix of the mitochondria. The translocation of protons (H^+) from within the matrix to the intermembrane space (the space between the cristae and outer membrane) provides much of the energy that drives the phosphorylation of ADP to make ATP. The electron transport chain starts with NADH or $FADH_2$, whether it is shuttled in from the cytosol, as discussed previously, or produced within the mitochondria.

Components of the Electron Transport Chain and Oxidative Phosphorylation

Glycolysis produces cytoplasmic NADH and $FADH_2$, and their shuttling into the mitochondria has already been discussed (see Figures 3.20 and 3.21). Figure 3.26 presents a highly simplified overview of the electron transport chain. As indicated, the reactions actually take place in four distinct complexes of associated proteins and enzymes, which can be isolated and purified. Complex I, NADH-coenzyme Q (CoQ) reductase, accepts electrons from NADH that resulted from glycolysis, the TCA cycle, and fatty acid oxidation. Complex II, succinate CoQ dehydrogenase, includes the membrane-bound succinate dehydrogenase that is part of the TCA cycle. Both Complex I and II produce $CoQH_2$. $CoQH_2$ is the substrate for Complex III, CoQ–cytochrome c reductase. Complex IV is cytochrome oxidase. It is responsible for reducing molecular oxygen to form H_2O. The complexes work independently and are connected by mobile acceptors of electrons,

Figure 3.25 The sequential arrangement of the components of the electron transport chain, showing its division into four complexes, I, II, III, and IV. Coenzyme Q (ubiquinone) is shared by complexes I, II, and III. Cyt c is shared by complexes III and IV.

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Figure 3.26 Schematic of electron transport modules connecting through coenzyme Q.

CoQ and cytochrome c. Each complex is discussed briefly here. For a more detailed explanation, consult a general biochemistry textbook.

Complex I: NADH–Coenzyme Q Oxidoreductase Complex I—also known as NADH dehydrogenase—transfers a pair of electrons from NADH to CoQ. The structures of the oxidized and reduced forms of CoQ are shown in Figure 3.27. Complex I is made of many polypeptide chains, a molecule of FMN, and several Fe-S clusters, along with additional iron molecules. The iron molecules bind with the sulfur-containing amino acid cysteine. The iron transfers one electron at a time, cycling between Fe^{2+} and Fe^{3+}. CoQ is a highly hydrophobic compound and it diffuses freely in the hydrophobic core of the inner membrane. The result of the multistep reaction is the transfer of electrons and protons from NADH to CoQ to form first CoQ hydroquinone and then $CoQH_2$ and actively transfer protons from the matrix side of the inner mitochondrial membrane to the intermembrane space. The importance of the buildup of protons in the intermembrane space is discussed in the following sections. The oxidation of NADH through the electron transport chain results in the synthesis of approximately 2.5 ATP molecules.

Figure 3.27 Oxidized and reduced forms of coenzyme Q, or ubiquinone. The subscript *n* indicates the number of isoprenoid units in the side chain (most commonly 10). A one-electron transfer results in the formation of a semiquinone with only one of the quinone groups reduced.

Complex II: Succinate Dehydrogenase Complex II is the succinate dehydrogenase enzyme, which is the only TCA cycle enzyme that is an integral part of the inner mitochondrial membrane. Beside the succinate dehydrogenase, Complex II contains a FAD protein and Fe-S clusters (similar to those discussed previously). When succinate is converted to fumarate in the TCA cycle, FAD is reduced to $FADH_2$. The $FADH_2$ is oxidized with one electron transfer through the Fe-S centers to reduce CoQ to $CoQH_2$. Unlike in Complex I, the protons released from $FADH_2$ are not transported to the intermembrane space. The oxidation of $FADH_2$ through the electron transport chain results in the formation of approximately 1.5 molecules of ATP.

Complex III: Coenzyme Q–Cytochrome c Oxidoreductase Reduced CoQ passes its electrons to cytochrome c in the third complex of the electron transport chain in a pathway known as the Q cycle. The complex contains three different cytochromes and Fe-S protein. The cytochromes contain heme molecules with an iron molecule in the center. The iron in the center of the cytochromes is oxidized and reduced as electrons flow through. Electrons pass through the Q cycle in two phases. In the first phase a $CoQH_2$ (**ubiquinol**) passes one electron to form the semiquinone (one of the two hydroquinones oxidized), then another electron and proton are transferred to the semiquinone to produce oxidized CoQ (quinone), releasing four protons to the intermembrane space. The electrons are then transferred to cytochrome c_1 (and associated cytochromes), and CoQ picks up two protons from the matrix, resulting in the reduction of a CoQ to $CoQH_2$. This means that two turns of the CoQ cycle result in the oxidation of $2CoQH_2$ to CoQ, the release of $4H^+$ in the intermembrane space, and the reduction of one CoQ to $CoQH_2$. Like CoQ, cytochrome c is a mobile carrier. This characteristic means that cytochrome c is able to migrate along the membrane. Cytochrome c associates loosely with the inner mitochondrial membrane on the matrix side of the membrane.

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It can then pass its electrons on to cytochrome c oxidase in Complex IV, which is discussed next.

Complex IV: Cytochrome c Oxidase Complex IV is called cytochrome c oxidase. It accepts electrons from cytochrome c and catalyzes a four-electron reduction of oxygen to form water. This reaction is the final one in the oxidation of the energy-providing nutrients (carbohydrate, fat, protein, and alcohol) to produce usable chemical energy in the form of ATP. The structure of cytochrome c oxidase is known; it is made up of multiple subunits. Some of the subunits are encoded from nuclear DNA and some from mitochondrial DNA. These latter proteins contain iron and copper. These metal ions cycle between their oxidized (Fe^{3+}, Cu^{2+}) and reduced (Fe^{2+}, Cu^{1+}) states. Cytochrome c oxidase also contains two cytochromes, cytochrome a and cytochrome a_3, which contain different heme moieties. Protons are transported to the intermembrane space.

Electron transport can carry on without phosphorylation, but the phosphorylation of ADP to form ATP (discussed in the next section) is dependent upon electron transport, with the transport terminating as molecular oxygen is reduced to H_2O. A schematic of the inner mitochondrial membrane showing the four complexes of the electron transport chain is shown in Figure 3.28. The free energy change at various sites within the electron transport chain is shown in Table 3.6.

Table 3.6 Free Energy Changes at Various Sites within the Electron Transport Chain Showing Phosphorylation Sites

Reaction	$\Delta G^{\circ\prime}$ (cal/mol)	ADP Phosphorylation Site?
$NAD^+ \longrightarrow FMN$	–922	No
$FMN \longrightarrow CoQ$	–15,682	Yes
$CoQ \longrightarrow$ cyt b	–1,380	No
Cyt b $\longrightarrow$ cyt c1	–7,380	Yes
Cyt c1 $\longrightarrow$ cyt c	–922	No
Cyt c $\longrightarrow$ cyt a	–1,845	No
Cyt a $\longrightarrow$ $\frac{1}{2}O_2$	–24,450	Yes

Phosphorylation of ADP to Form ATP

The intimate association of energy release with oxidation is exemplified by the oxidation of glucose to CO_2 plus water and energy, discussed earlier in this chapter. Glycolysis occurs in the cytosol; the TCA cycle, electron transport, proton translocation, and oxidative phosphorylation occur in the mitochondria. It has already been established that the complete oxidation of 1 glucose yields either 30 or 32 ATPs. The complete biological oxidation of 1 mol of glucose yields approximately 700 kcal (or 2,937 kJ). The standard free energy for the hydrolysis of ATP that has been used throughout this chapter is 7.3 kcal (30.5 kJ). However, standard conditions are at a concentration of 1 mol/L, whereas the concentration of ATP within the cell is closer

Figure 3.28 An illustration of oxidative phosphorylation coupled with ATP synthase. Energy from electron transport pumps protons into the intermembrane space from the matrix against a concentration gradient. The protons move back into the matrix through channels in the F_0F_1 ATP-synthase aggregate.

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to 1–5 mmol/L. The free energy of hydrolysis at this concentration is closer to 12 kcal (50 kJ). The free energies of other compounds with high phosphate transport potential such as phosphoenolpyruvate, 1,3-bisphosphoglycerate, and phosphocreatine are also increased proportionally. It is more straightforward to use standard free energy in talking about these reactions. However, to determine the energy efficiency of the biological oxidation of glucose, the free energy of ATP under biological conditions must be considered. In living cells, 32 mol of ATP capture 384 kcal (32 × 12). The efficiency is therefore 384/700 × 100 or about 54% [19]. The remaining energy is released as heat. This is an efficient process as engines go.

The previous discussion on electron transport focused on the translocation of protons (H^+) from the matrix to the intermembrane space. This translocation is vital to the phosphorylation of ADP to form ATP. The translocation of protons requires energy but in return creates a pool of potential energy. The generally accepted mechanism for the synthesis of ATP was first proposed by Peter Mitchell in 1961. He proposed that the energy stored in the difference in the concentration of H^+ between the matrix of the mitochondria and the intermembrane space was the driving force for coupled ATP formation. This proposal was called the chemiosmotic hypothesis. We examine its main points to support our understanding of the coupling of phosphorylation with the electron transport chain. A recent review presents current research about proton translocation [20].

Translocation of H^+ To determine if the pH gradient and electrical charge difference are sufficient to provide the energy for ATP synthesis, we must examine the number of H^+ translocated at each complex. Direct measurements have been difficult and disagreement exists among exerts, but the consensus is that for every 2 electrons that pass through Complex I (NADH dehydrogenase) and Complex III, 4 H^+ are translocated by each complex, for a total of 8. For Complex IV an additional 2 H^+ are translocated by each pair of electrons passing through the complex. No protons are translocated in Complex II. This means that for every NADH oxidized to water, a total of 10 protons are translocated from the matrix to the intermembrane space. The electrical charge across the inner membrane changes because of the positively charged protons in the intermembrane space, a difference estimated to be approximately 0.18 volts. It is also assumed that the pH difference between the mitochondrial matrix and the inner membrane is one unit. Using these assumptions, the free energy available is –94.49 kcal/mol (–23.3 kJ/mol). This is the potential free energy available to move protons back into the matrix of the mitochondria and at the same time couple phosphorylation of ADP to ATP with electron transport. Paul Boyer and John Walker shared the 1997 Nobel Prize for chemistry for their work on ATP synthase. A review of Paul Boyer's research on ATP synthase sums up several decades of work [21].

ATP Synthase Figure 3.28 illustrates electron transport, proton translocation, and oxidative phosphorylation. The disparity in both the proton concentration and electrical charge on either side of the inner membrane of the mitochondria has already been discussed. It is this proton gradient that provides the energy for ATP synthesis that occurs with the aid of ATP synthase. ATP synthase is made up of two main components, F_0 and F_1, each with multiple subunits. F_0 is fixed in the membrane and F_1 sticks out of the membrane into the mitochondrial matrix. Respiratory stalks extend from the cristae. If these stalks are removed, electron transport can proceed, but phosphorylation of ADP does not occur. Some of the subunits of F_1 are capable of rotating and have sites that bind ATP, ADP, and P_i. They also contain channels that allow proton movement through the membrane. For each pair of electrons traversing Complex IV, the rotating subunits of F_1 can complete one rotation and produce three ATPs. At the same time, protons from the intermembrane space are moved back into the matrix from the intermembrane space. The number of protons moved back depends on the number of subunits in the rotating stalk (this can vary between 10 and 15), resulting in 3–5 protons per ATP formed moving back into the matrix. The return flow of protons furnishes the energy necessary for the synthesis of ATP from ADP and P_i.

ATP is synthesized in mitochondria but must be moved to the cytosol to supply energy for the cell. There is an ATP-ADP translocase that shuttles ATP out of the mitochondria and ADP in. With the shuttle, the equivalent of one proton is moved from the cytosol to the mitochondrial matrix. Since the synthesis of one of ATP involves the movement of three protons from the cytosol to the matrix, with the translocase activity about four protons total are moved back into the matrix.

ATPs Produced by Complete Glucose Oxidation

The complete oxidation of glucose to CO_2 and H_2O can be shown by this equation:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O + \text{energy}$$

Complete oxidation is achieved by the combined reaction sequences of the glycolytic and TCA cycle pathways. The energy-conserving steps yield a net of two ATPs by substrate-level reactions in the glycolytic pathway and two ATPs (or one ATP and one GTP) by substrate-level reactions in the TCA cycle. In addition, there are three NADH and one $FADH_2$ produced from each acetyl-CoA that goes through the TCA cycle. Two acetyl-CoAs are produced from each molecule of glucose, which releases two molecules of CO_2 and two NADH. In summary, one molecule of glucose produces:

- 6 molecules of CO_2 (released)
- 4 ATP
- 10 NADH
- 2 $FADH_2$.

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The NADH and $FADH_2$ are in the matrix of the mitochondria and are oxidized by the electron transport chain and oxidative phosphorylation to ultimately produce ATP. By convention, it has been assumed in the past that three ATPs are formed by oxidative phosphorylation from NADH, and two ATPs are formed from $FADH_2$. As previously discussed, the actual number of ATPs formed from NADH is closer to 2.5; for $FADH_2$, it is 1.5. If the integers (3/2) are used for the number of ATPs produced from NADH/$FADH_2$, a total of 38 mol of ATP are formed. If we accept the 2.5/1.5 ratio, 32 mol of ATP are produced from each mol of glucose. Oxidative phosphorylation is only active under aerobic conditions. Under anaerobic conditions, only two ATPs are produced from each glucose at substrate level.

The actual number of ATPs formed aerobically from glucose varies because of the two different shuttle mechanisms that transport the electrons from NADH produced by the glycolytic pathway into the mitochondria. One mechanism, the glycerol-3-phosphate shuttle system, transfers the electrons to $FADH_2$ and therefore yields only 1.5 ATPs. The other shuttle system, the malate–aspartate shuttle, transfers the electrons to NADH inside the mitochondria and yields 2.5 ATPs.

The conversion of the chemical energy of carbohydrates to form ATP is an integral part of carbohydrate metabolism. The next sections cover other aspects of carbohydrate metabolism. Comprehensive reviews of electron transport, proton translocation, and oxidative phosphorylation are available to the interested reader [22–24].

The Pentose Phosphate Pathway (Hexose Monophosphate Shunt)

The pentose phosphate pathway (also called the hexose monophosphate shunt) is one of the pathways that is available to glucose in the cytosol and is shown in Figure 3.29. It generates important intermediates not produced in other pathways. The pentose phosphate pathway has two important products:

- pentose phosphates, necessary for the synthesis of the nucleic acids found in DNA and RNA and for other nucleotides (see Figure 3.4)
- the reduced cosubstrate NADPH, used for important metabolic functions, including the biosynthesis of fatty acids (Chapter 5), the maintenance of reducing substrates in red blood cells necessary to ensure the functional integrity of the cells, and drug metabolism in the liver.

The cells of some tissues have a high demand for NADPH, particularly those that are active in the synthesis of fatty acids, such as cells of the mammary gland, adipose tissue, adrenal cortex, and liver. These tissues predictably engage the entire pentose phosphate pathway, recycling pentose phosphates back to glucose-6-phosphate to repeat the cycle and ensure an ample supply of NADPH. The pathway reactions that include the dehydrogenase reactions and therefore the formation of NADPH from $NADP^+$ are called the oxidative reactions of the pathway. This segment of the pathway is illustrated on the left in Figure 3.29. The pentose phosphate pathway also synthesizes three-, four-, five-, six-, and seven-carbon sugars.

This pathway begins by oxidizing glucose-6-phosphate in two consecutive dehydrogenase reactions catalyzed by glucose-6-phosphate dehydrogenase (G-6-PD) and 6-phosphogluconate dehydrogenase (6-PGD). Both reactions require $NADP^+$ as cosubstrate, accounting for the formation of NADPH as a reduction product. The first reaction (G-6-PD) is irreversible and highly regulated. It is strongly inhibited by the cosubstrate NADPH and fatty acid CoAs. Pentose phosphate formation is achieved by the decarboxylation of 6-phosphogluconate to form the pentose phosphate, ribulose 5-phosphate, which in turn is isomerized to its aldose isomer, ribose 5-phosphate.

Pentose phosphates can subsequently be "recycled" back to hexose phosphates through the transketolase and transaldolase reactions illustrated in Figure 3.29. This recycling of pentose phosphates to hexose phosphates therefore does not produce pentoses, but it does ensure generous production of NADPH as the cycle repeats.

The re-formation of glucose-6-phosphate from the pentose phosphates, through reactions catalyzed by transketolase, transaldolase, and hexose phosphate isomerase, is called the nonoxidative reactions of the pathway and is shown on the right in Figure 3.29. Transketolase and transaldolase enzymes catalyze complex reactions in which three-, four-, five-, six-, and seven-carbon phosphate sugars are interconverted. These reactions are detailed in most comprehensive biochemistry texts.

The reversibility of the transketolase and transaldolase reactions allows hexose phosphates to be converted directly into pentose phosphates, bypassing the oxidative reactions. Therefore, cells that undergo a more rapid rate of replication and that consequently have a greater need for pentose phosphates for nucleic acid synthesis can produce these products in this manner.

The pathway's activity is low in skeletal muscle because of the limited demand for NADPH (fatty acid synthesis) in this tissue and also because of muscle's reliance on glucose and fatty acids for energy metabolism. Glucose-6-phosphate can be used for either glycolysis or for the pentose phosphate pathway. The choice is made based upon the cell's needs for energy (by assessing the ATP/ADP ratio) or for biosynthesis (by assessing the $NADP^+$/NADPH ratio). The level of NADPH is generally much higher than that of $NADP^+$.

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Figure 3.29 The pentose phosphate pathway (hexose monophosphate shunt), showing the oxidative stage (left side of diagram) and the nonoxidative stage (right side of diagram). Abbreviations: G-6-PD, glucose-6-phosphate dehydrogenase; 6-PGD, 6-phosphogluconate dehydrogenase.

Gluconeogenesis

Glucose is an essential nutrient for most cells. The brain and other tissues of the central nervous system (CNS) and red blood cells are particularly dependent upon glucose as a nutrient. When dietary intake of carbohydrate is reduced and blood glucose concentration declines, hormones including glucagon trigger accelerated glucose synthesis from noncarbohydrate sources in a process called gluconeogenesis. Lactate, glycerol (a product of triacylglycerol hydrolysis), and certain amino acids represent important noncarbohydrate sources. The liver is the major site of this activity, although under certain circumstances, such as prolonged starvation, the kidneys become increasingly important in gluconeogenesis. Most of the glucose formed by the liver and the kidneys is released into the blood to maintain blood glucose levels.

Many steps in gluconeogenesis are the reverse of glycolysis. Gluconeogenesis synthesizes glucose and

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consumes ATP and NAD^+ rather than producing ATP and NADH. Most of the cytoplasmic enzymes involved in glycolysis, which is the conversion of glucose to pyruvate, catalyze their reactions reversibly and therefore provide the means for also converting pyruvate to glucose. When the cell is oxidizing glucose for energy, however, it does not need to make glucose from gluconeogenesis. Both glycolysis and gluconeogenesis must be regulated, and it is the nonreversible reactions that are regulated. Three reactions in the glycolytic sequence are highly exergonic, highly regulated, and *not* reversible: those catalyzed by the enzymes glucokinase (hexokinase), phosphofructokinase, and pyruvate kinase (sites 1, 3, and 10 in Figure 3.17). All of these reactions involve ATP and are unidirectional by virtue of the high, negative free energy change of the reactions. Therefore, the process of gluconeogenesis requires that these reactions be either bypassed or circumvented by other enzyme systems. The presence or absence of these enzymes determines whether a certain organ or tissue is capable of conducting gluconeogenesis. As shown in Figure 3.30, the glucokinase and phosphofructokinase reactions can be bypassed by specific phosphatases (glucose-6-phosphatase and fructose-1,6-bisphosphatase, respectively) that remove phosphate groups by hydrolysis.

The bypass of the pyruvate kinase reaction involves the formation of oxaloacetate as an intermediate. Mitochondrial pyruvate can be converted to oxaloacetate by pyruvate carboxylase, a reaction that was discussed earlier as an *anaplerotic* process. Oxaloacetate, in turn, can be decarboxylated and phosphorylated to phosphoenolpyruvate (PEP) by PEP carboxykinase, thereby completing the bypass of the pyruvate kinase reaction. However, the PEP carboxykinase reaction is a cytosolic reaction and therefore oxaloacetate must leave the mitochondrion to be acted upon by the enzyme. Because the mitochondrial membrane is impermeable to oxaloacetate, it must first be converted to either malate (by malate dehydrogenase) or aspartate (by transamination

Figure 3.30 The principal regulatory mechanisms in glycolysis and gluconeogenesis. Nonreversible reactions of glycolysis and gluconeogenesis showing regulated steps. Inhibitors are indicated by minus signs and activators by plus signs.
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with glutamate; see Chapter 6), both of which freely traverse the mitochondrial membrane. This mechanism is similar to the malate–aspartate shuttle previously discussed. In the cytosol, the malate or aspartate can be converted back to oxaloacetate by malate dehydrogenase or aspartate aminotransferase (glutamate oxaloacetate transaminase), respectively.

Noncarbohydrate Sources

Amino Acid Utilization The conversion of pyruvate to oxaloacetate in the initial steps of gluconeogenesis allows for the carbon skeletons of various amino acids to enter the gluconeogenic pathway. Such amino acids accordingly are called glucogenic. Glucogenic amino acids can be catabolized to pyruvate or oxaloacetate when metabolic conditions favor glucose synthesis. Furthermore, since some amino acids can convert to various TCA cycle intermediates—and because the intermediates can leave the mitochondrion in the form of malate or aspartate—utilization of TCA cycle intermediates represents another way that amino acids can be converted to glucose. Reactions showing the entry of noncarbohydrate substances into the gluconeogenic system are shown in Figure 3.31.

Lactate Utilization Lactate is produced by red blood cells continuously and by skeletal muscle during strenuous physical exertion. The majority of lactate produced is released into the blood, where it travels to the liver for conversion to glucose via gluconeogenesis. The newly made glucose can, in turn, be released into the blood. Recall that muscle cells lack glucose-6-phosphatase and cannot produce free glucose from noncarbohydrate sources. Thus, the liver is able

Figure 3.31 The reactions of gluconeogenesis, showing the bypass of the unidirectional pyruvate kinase reaction and the entry into the pathway of noncarbohydrate substances such as glycerol, lactate, and amino acids. Abbreviations: G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FBP, fructose-1,6-bisphosphate; DHAP, dihydroxyacetone phosphate; G3P, glyceraldehyde-3-phosphate; BPG, 1,3-bisphosphoglycerate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate.
Source: Garrett & Grisham, *Biochemistry*, 4th Edition. © Cengage Learning.

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to prevent the accumulation of lactate while replenishing blood glucose. This is an important relationship between muscle and liver, especially during strenuous (anaerobic) physical activity when blood glucose is being used, at least in part, to fuel muscle by glycolysis that produces lactate. The ability of the liver to convert muscle-derived lactate to glucose, and for muscle to take up that glucose and use it in glycolysis, constitutes the **Cori cycle**.

Glycerol Utilization The hydrolysis of triacylglycerols stored in adipose tissue produces fatty acids and glycerol (discussed further in Chapter 5). Fatty acids are a rich source of energy that provides fuel for muscle and other tissues by being catabolized to acetyl-CoA for entry into the TCA cycle (see Figure 3.18). The remaining glycerol molecule is released from adipose tissue into the blood, where is travels to the liver for conversion to glucose via gluconeogenesis.

Fatty acids, in contrast with glycerol and other noncarbohydrate sources, cannot be used to make glucose because humans lack the necessary enzymes to convert acetyl-CoA to pyruvate or any intermediate along the gluconeogenic pathway. It is conceivable that after fatty acid–derived acetyl-CoA enters the TCA cycle, diverting oxaloacetate or other TCA-cycle intermediates into gluconeogenesis would reflect an indirect contribution of fatty acids to glucose synthesis. This scenario does occur to a very limited extent, which explains why trace amounts of carbon atoms from fatty acids are found in newly synthesized glucose. However, such a pathway is insignificant and unsustainable because depletion of oxaloacetate prevents the TCA cycle from continuing. Another interesting scenario by which fatty acids might provide carbon skeletons for gluconeogenesis is based on computer modeling of all possible enzyme systems and pathways present in humans [25]. The model suggests that when fatty acids are used for ketone body synthesis during carbohydrate deficit (see Chapter 5), the by-product acetone can be used to make pyruvate, which can enter the gluconeogenic pathway. While theoretically possible, additional metabolic research is needed to confirm whether this pathway is a quantitatively important source of glucose.

REGULATION OF METABOLISM

This section focuses on the general mechanisms used for regulation and then describes the regulation of glycolysis and gluconeogenesis as a more detailed example. The regulatory mechanisms for the other pathways are similar. The TCA cycle is the most prolific producer of ATP through oxidative phosphorylation and uses acetyl-CoA produced from glucose, fatty acids, and certain amino acids. The regulation of these pathways is covered in the appropriate chapters.

The purpose of regulation of glycolysis and gluconeogenesis is to maintain homeostasis. The reactions of metabolism are altered to meet the nutritional and biochemical demands of the body. An excellent example of metabolic regulation is the reciprocal regulation of the glycolysis (catabolic) pathways and the gluconeogenic (anabolic) pathways. The glycolytic conversion of glucose to pyruvate liberates energy, whereas the reversal of the process from pyruvate to glucose (gluconeogenesis) consumes energy. The pyruvate kinase bypass in itself is energetically expensive, considering that 1 mol of ATP and 1 mol of GTP must be expended in converting intramitochondrial pyruvate to extramitochondrial PEP (Figure 3.31). It follows that among the factors that regulate the glycolysis/gluconeogenesis activity ratio is the body's need for energy. In a broad sense, regulation is achieved by four mechanisms:

- negative or positive modulation of allosteric enzymes by effector compounds
- hormonal activation by covalent modification or induction of specific enzymes
- directional shifts in reversible reactions by changes in reactant or product concentrations
- translocation of enzymes within the cell (covered in Chapter 1).

The concept of enzyme regulation was covered in Chapter 1, but a brief discussion of the principles is included here, with the regulation of carbohydrate metabolism in mind. This is followed by a more detailed examination of the regulation of glycolysis and gluconeogenesis.

Allosteric Enzyme Modulation

Allosteric mechanisms can stimulate or suppress the enzymatic activity of a pathway. An allosteric, or regulatory, enzyme is said to be positively or negatively modulated. Modulators, which are usually compounds within the pathway, generally act by altering the conformational structure of the allosteric enzyme. Allosteric enzymes catalyze unidirectional, or nonreversible, reactions. The modulators of the enzymes of the unidirectional reactions must either stimulate or suppress a reaction in one direction only. General examples of allosteric modulators are presented in the following sections.

AMP, ADP, and ATP as Allosteric Modulators

An indication of the energy status of a cell and an important regulatory factor in energy metabolism is the ratio of the cellular concentrations of ADP (or AMP) to ATP. The usual breakdown product of ATP is ADP, but as ADP increases in concentration, some of it becomes enzymatically converted to AMP (to produce an ATP). Therefore, ADP and/or AMP accumulation can signify an excessive use of ATP and its depletion.

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AMP, ADP, and ATP all act as modulators of certain allosteric enzymes, but the effect of AMP or ADP opposes that of ATP. For example, if ATP is abundant and ADP is scarce, additional energy is not needed. Energy-releasing (ATP-producing) pathways are negatively modulated, reducing the production of additional ATP. The reverse is also true; an increase in AMP (or ADP) concentration conversely signifies a depletion of ATP and the need to produce more of this energy source. In such a case, AMP or ADP can positively modulate allosteric enzymes of the energy-releasing pathways.

Two examples of positive modulation by AMP are its ability to cause a shift from the inactive form of phosphorylase b to an active form in glycogenolysis and the activation of phosphofructokinase in the glycolytic pathway, discussed in the next paragraph. Increased levels of AMP are accompanied by an enhanced activity of either of these reactions that encourages glucose catabolism. The resulting shift in metabolic direction, as signaled by the AMP buildup, causes the release of energy as glucose is metabolized and helps restore depleted ATP stores.

Phosphofructokinase is modulated positively by AMP and ADP and negatively by ATP. As the store of ATP increases, slowing of the glycolytic pathway is called for. Phosphofructokinase is an extremely important rate-controlling allosteric enzyme and is modulated by a variety of substances. Its regulatory function has already been described in Chapter 1.

Other regulatory enzymes in carbohydrate metabolism that are modulated by ATP—all negatively—are pyruvate dehydrogenase complex, citrate synthase, and isocitrate dehydrogenase. Pyruvate dehydrogenase complex is positively modulated by AMP, and citrate synthase and isocitrate dehydrogenase are positively modulated by ADP.

Regulatory Effect of NADH/NAD$^+$ and NADPH/NADP$^+$

Another example of allosteric mechanisms is the ratio of NADH to NAD$^+$. NADH and NAD$^+$ can regulate their own formation through negative modulation. NADH is a product of glycolysis. Its buildup would indicate the pathway is not needed to produce additional ATP. If NAD$^+$ accumulates, the oxidative step in glycolysis would be favored. In the fasted state, the liver typically has a high NAD$^+$/NADH ratio (about 700, meaning that the level of NADH is low) and it produces more glucose than it needs through gluconeogenesis, releasing the glucose into the blood. In contrast, muscle will be actively catabolizing glucose, and its NAD$^+$/NADH ratio will be lower and will favor lactate production. Dehydrogenase reactions, which involve the interconversion of the reduced and oxidized forms of the cosubstrate, are reversible. If metabolic conditions cause either NADH or NAD$^+$ to accumulate, the equilibrium is shifted to return the ratio to normal. Pyruvate dehydrogenase complex is positively modulated by NAD$^+$, whereas pyruvate kinase, citrate synthase, and α-ketoglutarate dehydrogenase are negatively modulated by NADH.

Whether the pentose phosphate pathway makes pentoses or NADPH is dependent upon the level of NADPH and NADP$^+$. Glucose-6-phosphate dehydrogenase is inhibited by high levels of NADPH and acetyl-CoA, which would indicate that demands for lipid biosynthesis are met. If the NADPH levels drop, the pathway can produce ribose. If the cell has more ribose than needed, the pathway follows the reaction on the right side of Figure 3.29 and makes more glucose and more NADPH.

Covalent Regulation

Covalent modification is another mechanism of enzyme (resulting in pathway) regulation. This involves the binding or unbinding of a group by a covalent bond and is one of the mechanisms by which hormones can exert their action. Examples include the covalent regulation of glycogen synthase and glycogen phosphorylase, enzymes discussed in the sections on glycogenesis and glycogenolysis, respectively.

Phosphorylation inactivates glycogen synthase, whereas dephosphorylation activates it. In contrast, phosphorylation activates glycogen phosphorylase, and dephosphorylation inactivates it. These actions can be controlled by the actions of glucagon and epinephrine. Both hormones function by the phosphorylation of pathway enzymes through the second messenger cAMP.

Genetic Regulation

Another important example of enzyme regulation is through genetic control. The abundance of an enzyme can be either induced or suppressed. Such a change might arise through a prolonged shift in the dietary intake of certain nutrients. Induction stimulates transcription of new messenger RNA, programmed to produce the enzyme.

Specific hormones can influence (induce or suppress) the expression of a gene. One of the actions of certain hormones such as cortisol is to stimulate protein breakdown and decrease protein synthesis in skeletal muscle. In the liver, cortisol stimulates glycogen synthesis and gluconeogenesis by increasing the expression of several genes that encode for enzymes of the gluconeogenic pathway.

Directional Shifts in Reversible Reactions

Another control mechanism for pathways is based on enzyme kinetics, the concentration of the reactants and products in the cell. Most enzymes catalyze reactions reversibly,

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and the preferred direction in which a reversible reaction is proceeding at a particular moment is largely dependent upon the relative concentration of each reactant and product. An increasing concentration of one of the reactants drives or forces the reaction toward forming the other.

This concept is exemplified by the phosphoglucomutase reaction, which interconverts glucose-6-phosphate and glucose-1-phosphate and which functions in the pathways of glycogenesis and glycogenolysis (see Figures 3.13 and 3.15). At times of heightened glycogenolytic activity (rapid breakdown of glycogen), glucose-1-phosphate concentration rises sharply, driving the reaction toward the formation of glucose-6-phosphate. With the body at rest, gluconeogenesis and glycogenesis are accelerated, increasing the concentration of glucose-6-phosphate. This increase in turn shifts the phosphoglucomutase reaction toward the formation of glucose-1-phosphate and ultimately glycogen.

Metabolic Control of Glycolysis and Gluconeogenesis

Most enzymatic reactions are reversible, depending on their free energy. Yet certainly in a given cell and generally in the cells of a particular organ the pathways are going in only one direction at a given time. The previous sections reviewed the different methods the body uses for controlling metabolic pathways. Glycolysis and gluconeogenesis provide examples of these control mechanisms in action. Figure 3.30 shows the reactions in both pathways that are under metabolic control by the mechanisms discussed, with the regulation of glycolysis on the left and that of gluconeogenesis on the right. The modulators that are activators are indicated by a plus sign, and those that are inhibitors by a minus sign.

The end result of gluconeogenesis is the formation of glucose, the molecule with which glycolysis begins. It is also true that the end product of glycolysis is pyruvate, and pyruvate is the first reactant of gluconeogenesis. As was pointed out earlier, however, gluconeogenesis is not simply the reversal of glycolysis. These two pathways are controlled reciprocally. Which of the two pathways is active at a given time depends on the energy status of the cell. In glycolysis there are three regulated enzymes, all of which catalyze exergonic reactions: hexokinase (glucokinase), phosphofructokinase, and pyruvate kinase. These three reactions are replaced in the gluconeogenic pathway with those catalyzed by glucose-6-phosphatase; fructose-1,6-bisphosphatase; and the pyruvate carboxylase-PEP-carboxylase pair. The control of these reactions is considered for each pathway. The fate of pyruvate is strongly dependent upon acetyl-CoA levels. Acetyl-CoA inhibits the glycolytic enzyme pyruvate kinase allosterically and activates pyruvate carboxylase. This latter enzyme is found only in the mitochondria and is part of the gluconeogenic pathway that transfers mitochondrial pyruvate to PEP. If the TCA cycle is not active (adequate cellular ATP), the pyruvate is converted to glucose via gluconeogenesis.

In gluconeogenesis, glucose-6-phosphatase is controlled by the level of substrate. Because the K_m for this enzyme is much higher than the level of glucose-6-phosphate that is normally present, the reaction proceeds very slowly unless a high concentration of this substrate accumulates. A buildup of glucose-6-phosphate is needed to activate the gluconeogenesis pathway.

Another control point for gluconeogenesis is the enzyme fructose-1,6-bisphosphatase, which is allosterically inhibited by AMP and activated by citrate. The effects of AMP and citrate on this enzyme are the opposite in glycolysis. When AMP levels are low (which means ATP is adequate) the gluconeogenesis pathway is active and glycolysis is reduced. Another allosteric regulator of fructose-1,6-bisphosphatase is fructose-2,6-bisphosphate. The levels of fructose-2,6-bisphosphate are controlled by the enzyme phosphofructokinase-2 (PFK-2). This enzyme is different than the phosphofructokinase of the glycolytic pathway. Fructose-6-phosphate (the substrate of phosphofructokinase of glycolysis) activates PFK-2, which would inhibit gluconeogenesis.

Another means of control for these two pathways is the level of enzymes. In glycolysis, glucokinase, phosphofructokinase, and pyruvate kinase are inducible enzymes, meaning that their concentrations can rise and fall in response to molecular signals such as a sustained change in the concentration of a certain metabolite. In the gluconeogenic pathway glucose-6-phosphatase, fructose bisphosphatase, PEP carboxykinase, and pyruvate carboxylase are inducible. The other enzymes of both pathways are constitutive (Chapter 1), meaning that their rate of synthesis is constant. Glucagon and glucocorticoid hormones are known to stimulate gluconeogenesis by inducing the key gluconeogenic enzymes to form, and insulin may stimulate glycolysis by inducing increased synthesis of key glycolytic enzymes.

The interrelationship among pathways of carbohydrate metabolism is exemplified by the regulation of blood glucose concentration. The integration of the pathways, a topic of Chapter 7, is best understood after metabolism of lipids and amino acids has been discussed (Chapters 5 and 6). Largely through the opposing effects of insulin and glucagon, the fasting serum glucose level normally is maintained within the approximate range of 80–100 mg/dL (4.5–5.5 mmol/L). Whenever blood glucose levels are excessive or sustained at high levels because insulin is insufficient, other insulin-independent pathways of carbohydrate metabolism for lowering blood glucose become increasingly active. Such insulin-independent pathways are indicated in Figure 3.32. The overactivity of these pathways in certain tissues is believed to be partly responsible for the clinical manifestations of type 1 diabetes mellitus.

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Figure 3.32 Insulin-independent and -dependent pathways of glucose metabolism.

SUMMARY

This chapter has dealt with a subject of vital importance in nutrition: the release and conversion of the energy contained within nutrient molecules into ATP energy usable by the body. It examines an important food source of that energy, carbohydrates. The major sources of dietary carbohydrate are the starches and the disaccharides. In the course of digestion, these are hydrolyzed by specific glycosidases to their component monosaccharides, which are absorbed into the intestine cell by active and facilitative transport. Practically all fructose and galactose is transported to the liver to be metabolized. Some glucose is transported to the liver, while the majority of glucose is transported into cells of various tissues, passing through the cells' outer membrane by facilitative transport by way of transporters. Different tissues use different GLUTs that are part of the family of glucose transporters. The GLUT4 that transports glucose into muscle and adipose tissue is stimulated by insulin. Insulin translocates the preformed GLUT4 from intracellular vesicles to the cell membrane. In the cells, monosaccharides are first phosphorylated at the expense of ATP and then can follow any of several integrated pathways of metabolism. In muscle, brain, and adipose tissue, glucose is phosphorylated by hexokinase (types 1 and 2). In the liver, glucose is phosphorylated by an isoenzyme of hexokinase called glucokinase; fructose is phosphorylated mainly by fructokinase; and galactose is phosphorylated by galactokinase.

During times of energy excess, cellular glucose and certain metabolites can be converted to glycogen, primarily in liver and skeletal muscle. Liver glycogen is mostly made from dietary and circulating glucose, while about one-third of the glucose-6-phosphate converted to glycogen is derived from gluconeogenesis (lactate, pyruvate, and TCA cycle intermediates). When energy is needed cellular glucose can be routed through the energy-releasing pathways of glycolysis and the TCA cycle for ATP production. Glycolytic reactions convert glucose (or glucose residues from glycogen) to pyruvate. From pyruvate, either an aerobic course (complete oxidation in the TCA cycle) or an anaerobic course (to lactate) can be followed. Nearly all the energy formed by the oxidation of carbohydrates to CO_2 and H_2O is released via the TCA cycle, as reduced coenzymes are oxidized by mitochondrial electron transport. On complete oxidation, approximately 40% of this energy is retained in the high-energy phosphate bonds of ATP. The remaining energy supplies heat to the body.

Noncarbohydrate substances derived from the other major nutrients—including lactate from red blood cells and muscle, glycerol from triacylglycerols, and certain amino acids—can be converted to glucose or glycogen by the pathways of gluconeogenesis. The basic carbon skeleton of fatty acids (metabolized to acetyl-CoA units) cannot be converted to a net synthesis of glucose, but some of the carbons from fatty acids find their way into the carbohydrate molecule due to small amounts of TCA cycle intermediates being used in gluconeogenesis. In gluconeogenesis, the reactions are basically the reversible reactions of glycolysis, shifted toward glucose synthesis in accordance with reduced energy demand by the body. Three kinase reactions occurring in glycolysis are not reversible, however, requiring the involvement of different enzymes and pathways to circumvent those reactions in the process of gluconeogenesis. Muscle glycogen provides a source of glucose for energy only for muscle fibers in which it is stored because muscle lacks the enzyme glucose-6-phosphatase, which forms free glucose from glucose-6-phosphate. Glucose-6-phosphatase is active in the liver, however, which means that the liver can release free glucose from its glycogen stores into the circulation for maintaining blood glucose and for use by other tissues. The Cori cycle describes the liver's uptake and gluconeogenic conversion of muscle-produced lactate to glucose.

A metabolic pathway is regulated according to the body's need for energy or for maintaining homeostatic cellular concentrations of certain metabolites. Regulation is exerted mainly through hormones, through substrate

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concentrations (which can affect the velocity of enzyme reactions), and through allosteric enzymes that can be modulated negatively or positively by certain pathway products.

In Chapters 5 and 6, we see that fatty acids and the carbon skeleton of various amino acids also are ultimately oxidized through the TCA cycle. The amino acids that do become TCA cycle intermediates, however, may not be completely oxidized to CO_2 and H_2O, but instead may leave the cycle to be converted to glucose or glycogen (by gluconeogenesis) should dietary intake of carbohydrate be low. The glycerol portion of triacylglycerols enters the glycolytic pathway at the level of dihydroxyacetone phosphate, from which point it can be oxidized for energy or used to synthesize glucose or glycogen. The fatty acids from triacylglycerols enter the TCA cycle as acetyl-CoA, which is oxidized to CO_2 and H_2O but cannot contribute carbon for the net synthesis of glucose. This topic is considered further in Chapter 5.

These examples of the entrance of noncarbohydrate substances into the pathways discussed in this chapter are cited here to remind the reader that these pathways are not singularly committed to carbohydrate metabolism. Rather, they must be thought of as common ground for the interconversion and oxidation of fats and proteins as well as carbohydrate. Maintaining this broad perspective will be essential when we move on to Chapters 5 and 6, which examine the metabolism of lipids and proteins, respectively.

Much of the energy needs of the body are met by the production and utilization of ATP. ATP can be generated by two distinct mechanisms:

1. the transfer of a phosphate group from compounds with a very-high-energy phosphate transfer potential to ADP, a process called substrate-level phosphorylation
2. the TCA cycle and oxidative phosphorylation, by which high-energy electrons derived from food molecules are passed through the electron transport chain in mitochondria, creating an energy gradient used to phosphorylate ADP to form ATP.

Oxidative phosphorylation is the major route for ATP production. Electron flow in the electron transport chain is from reduced cosubstrates to molecular oxygen. Molecular oxygen becomes the ultimate oxidizing agent and becomes H_2O in the process. The downhill flow of electrons and proton translocation generate sufficient energy to affect oxidative phosphorylation at multiple sites along the chain. The energy from this process that is not conserved as chemical energy (ATP) is given off as heat. About 60% of the energy assumes the form of heat.

Carbohydrate metabolism, including the energy-releasing, systematic oxidation of glucose to CO_2 and H_2O, exemplifies reactions of substrate-level and oxidative phosphorylation. Similar energy transfer happens with the fatty acid and amino acid pathways whenever a dehydration reaction occurs.

The pentose phosphate pathway generates important intermediates not produced in other pathways of the body, such as pentose phosphates for RNA and DNA synthesis and NADPH, which is used in the synthesis of fatty acids and in drug metabolism.

This chapter provides examples of the regulation of metabolism, an important topic in nutrition. This topic is revisited several times in Chapters 7 and 8. Understanding the integration of metabolism and the control of energy balance is important. Much of the effects of exercise, disease, weight loss, and weight gain can be explained with these principles.

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www.medscape.com
WebMD. Provides specialty information and education for physicians and other health professionals.

www.cdc.gov
Centers for Disease Control and Prevention

www.ama-assn.org
American Medical Association

http://vcell.ndsu.edu/animations/
A series of "Virtual Cell" animations demonstrating electron transport chain, ATP synthesis, insulin signaling, and other biological processes funded by the National Science Foundation. Other animations are applicable to other chapters and are a good resource.

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WHAT CARBOHYDRATES DO AMERICANS EAT?

Although a seemingly simple question, measuring the food and nutrients we eat is a difficult task. Several methods, based primarily on self-reported data, have been used to directly assess the amount of food consumed by individuals [1]. The accuracy of such methods depends entirely on the ability of subjects to know the foods they are eating; to know portion size and record the amount of each food; to record every food and beverage consumed; and to be truthful. In view of these requirements, it is easy to see why direct methods frequently result in underreporting of intake, particularly by subjects with elevated body mass index [2–5]. The National Health and Nutrition Examination Surveys (NHANES), funded and managed by the Centers for Disease Control and Prevention, have been ongoing since the 1960s and represent the most widely used dataset for estimating food intake using direct assessment [6].

A different approach to estimating food intake is to measure the amount of food available for human consumption in the United States. The total amount available of each food category is then divided by the total population for each year and expressed on a per capita basis. The U.S. Department of Agriculture has been reporting such data since 1909, which is useful for determining food consumption trends because they are a proxy for actual food intake. Food availability data—sometimes called food "disappearance" because the data reflect available food that "disappears" into the food marketing system—may overestimate actual intake by individuals due to inclusion of nonedible food portions and food lost through waste and spoilage in the home and marketing system. Consequently, the USDA provides loss-adjusted food availability data to more closely reflect actual intake [7].

Documenting food intake by direct or indirect methods is just the first step in learning what nutrients we consume. Converting food intake into nutrient intake requires knowledge about the chemical composition of every food consumed. The Agricultural Research Service of the USDA maintains the most comprehensive system for collecting and disseminating food composition data. Information contained in the *USDA National Nutrient Database for Standard Reference* provides the basis for nearly all public and commercial nutrient databases and food composition tables used in the United States and several foreign countries [8]. The Nutrient Database contains information for approximately 150 components of food, including essential and nonessential nutrients, for over 8,600 foods. The database is constantly being updated and expanded as new information becomes available. The data comes from academic research, the food industry, government laboratories, and independent food-testing laboratories. Values in the database may also be based on calculations using appropriate algorithms, factors, or recipes.

Valuable information regarding food and nutrient intake can be obtained by combining the food availability and nutrient composition data. Each database is freely accessible and can be downloaded for combining, although the USDA has already done much of the work for us. Spreadsheets containing the combined data, called the *Nutrient Content of the U.S. Food Supply*, are also available for downloading [9]. With this arsenal of data, one can choose to examine the type and amount of food consumed, their nutrient composition, or the amount of nutrients consumed by major food groups.

CARBOHYDRATES IN THE FOOD SUPPLY

Examining the USDA data for the years 1970 to 2010 reveals many things. First, carbohydrates are the most abundant macronutrient (by weight) in the food supply and contribute most of the total energy in the American diet (as shown in Figure 1). Second, during this 40-year period, carbohydrate availability increased about 10% [10]. This period of time is significant because obesity prevalence in children and adults increased in parallel [11]. It is tempting to blame the increase in carbohydrate intake for the increase in obesity prevalence, but one should be cautious in assuming a direct causal relationship on the basis of correlations alone without further research.

A third observation gleaned from the USDA data for the year 2010 is that most of the carbohydrate was provided by grain products (42%) and sugar and sweeteners (35%). The remaining contributors of carbohydrate were comparatively

Figure 1 Per capita availability of macronutrients from all food sources in the U.S. food supply.

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minor and included vegetables (7%), fruits (6%), and dairy products (6%). Moreover, since 1970 the carbohydrate contributed by grain products increased 24%, whereas the contribution from sugar and sweeteners as a group increased only about 1% [9]. Many consumers may be confused by this outcome because the facts contradict the avalanche of misinformation in the lay press and on social media claiming that intake of sugar and sweeteners has skyrocketed—a conclusion that is clearly not supported by data.

Confusion may also stem from the widespread misunderstanding of sugar and sweeteners in the food supply. As illustrated in Figure 2, sugar (sucrose) was the primary sweetening agent used in 1970. High-fructose corn syrups (HFCS) were introduced after 1970 as a sugar alternative because of lower cost and desirable functional properties. Two major types of HFCS are used by food manufacturers, HFCS-42 and HFCS-55. The saccharide composition of HFCS-42 is 42% fructose, 52% glucose, and 6% other saccharides, whereas the saccharide composition of HFCS-55 is 55% fructose, 41% glucose, and 4% other saccharides. Food manufacturers generally use HFCS-42 as a sweetening agent in dry products such as cereals and baked goods. HFCS-55 is used mainly in beverages such as fruit juices and soft drinks. When present together in the U.S. food supply, the two HFCS contribute about equal proportions of fructose and glucose, which is identical to the saccharide composition of sugar (50% fructose, 50% glucose). So while it is true HFCS have significantly increased in the food supply since 1970, the availability of sugar and sweeteners combined has changed very little, due to the replacement of sugar with HFCS. Consumers can be easily misled when they hear only the HFCS story.

GLUCOSE VERSUS FRUCTOSE

All digestible carbohydrates in the food supply, irrespective of the food source, must be broken down to their monosaccharide units for absorption into the body. Nature provides foods that, when digested, yield mostly glucose and fructose (and, to a lesser extent, galactose if dairy products are consumed). Starch yields exclusively glucose; sugar and HFCS yield equal amounts of glucose and fructose; dairy products containing lactose yield glucose and galactose; and some foods provide glucose and fructose as monosaccharides. In view of the heightened awareness of fructose as a potential contributor to obesity-related diseases [12], it is useful to express the USDA food availability data in terms of the component monosaccharides resulting from carbohydrate digestion. Making such a calculation, as shown in Figure 3, allows us to examine the amounts of glucose, fructose, and galactose available for absorption from all digestible carbohydrates consumed.

Figure 3 can be interpreted as the "carbohydrate" line from Figure 1, broken down into its monosaccharide units. Viewing the data this way clearly shows glucose, not fructose, is the most abundant saccharide provided by food carbohydrates. Recall that the major food source of carbohydrates is grain products that contribute only glucose when starch is digested. The second most abundant food source of carbohydrates, sugars and sweeteners, contributes equal amounts of glucose and fructose, while fruits and vegetables contribute smaller amounts of both glucose and fructose. Thus, every food category that contains carbohydrate contributes glucose, resulting in four times more glucose than fructose in the food supply.

Another important observation from Figure 3 is the change that occurred in monosaccharide availability between 1970 and 2010. The overall trend in glucose availability increased 13%, whereas the overall trend in fructose availability did not change during the 40-year period [10]. Once again, these facts contradict information found in the lay press and on social media that incorrectly emphasize an increase in fructose when the spotlight should be focused on the significant increase in glucose.

Conclusion

Measuring food and nutrient intake of Americans can be accomplished by indirect methods using the USDA food availability and nutrient composition databases. This approach offers the advantage of examining trends over time (decades), and it avoids the difficulties of surveying individuals directly. Using the loss-adjusted USDA data also allows us to determine the food sources of all major nutrients and the amounts available on a per capita basis. In this Perspective, examination of the food sources and amounts of carbohydrate in the U.S. food supply reveals the following:

- Carbohydrates are the most abundant macronutrient in the food supply and provide the majority of dietary energy
- Grain products are the primary source of dietary carbohydrate, followed by sugar and sweeteners
- Between 1970 and 2010, the availability of carbohydrate from all food sources increased 10%
- Between 1970 and 2010, the availability of carbohydrate from grain products increased 24%
- Between 1970 and 2010, the availability of carbohydrate from sugar and sweeteners increased 1%
- The sugar and sweeteners category has not significantly increased because HFCS have merely replaced sugar
- Upon digestion, food carbohydrates yield four times more glucose than fructose
- Between 1970 and 2010, the availability of glucose from all food sources increased 13%
- Between 1970 and 2010, the availability of fructose from all food sources did not change.

Conclusions from these findings are best made when the entire picture is considered. Misinterpretations can easily be made when only a portion of the findings are used. For example, the use of HFCS has significantly increased since 1970,

Figure 2 Per capita availability of carbohydrates from sugar and high-fructose corn syrups (HFCS) in the U.S. food supply.

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Figure 3 Per capita availability of monosaccharides from digestible carbohydrates in the U.S. food supply.

leading some to conclude that HFCS (and the fructose they contribute) are the cause of obesity and metabolic diseases. However, when one considers the entire picture, the increased use of HFCS has mirrored the decline in sugar usage, resulting in virtually no change in the amount of saccharides contributed by the combined sugar and sweeteners group. Also, the glucose-to-fructose ratio in sugar and the HFCS together is approximately the same and has not changed since 1970, so fructose availability has remained relatively unchanged.

Perhaps the most important conclusion from the USDA data should focus on glucose as the major saccharide contributed by carbohydrates in the U.S. food supply. Significantly more glucose compared to fructose is available for intestinal absorption as a result of eating a typical American diet. Furthermore, the overall trend in glucose availability has increased since 1970, due mainly to increased availability of grain products. When addressing the dietary factors that contribute to obesity, it is logical to focus attention on all carbohydrates, the main energy source from food, to control total energy intake. The USDA data indicate that glucose from starch in grain products is the major carbohydrate that contributes to total energy intake.

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4 FIBER

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FIBER not only enhances the health of the gastrointestinal tract but fiber-rich foods play key roles in the prevention and management of several diseases. The 2015 Dietary Guidelines Advisory Committee for the Dietary Guidelines for Americans has labeled fiber as a "nutrient of public health concern," a designation based on findings that fiber intakes are low among most Americans and that fiber is important for health [1]. The varied health benefits of fiber are related to the fact that fiber is not a single entity or even a group of chemically related compounds, but instead consists of multiple different components with distinctive characteristics. This chapter addresses definitions, chemistries, properties, sources, health benefits, allowed health claims, food labels, and recommended intake of fiber.

DEFINITIONS

With the publication of the 2002 Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Protein, and Amino Acids by the National Academy of Sciences Food and Nutrition Board, definitions for dietary, functional, and total fiber were established. **Dietary fiber** refers to nondigestible (by human digestive enzymes) carbohydrates and lignin that are intact and intrinsic in plants [2]. Dietary fibers, listed later in Figure 4.2, include cellulose, hemicellulose, pectins, lignin, gums, β-glucans, fructans, and resistant starches [2]. **Functional fiber** consists of isolated, extracted, or manufactured nondigestible carbohydrates that have been shown to have beneficial physiological effects in humans [2]; they are usually added to foods as well as found in supplements. All dietary fibers are functional fibers with the exceptions of hemicellulose, fructans, and lignin (the Food and Nutrition Board stated that fructans and lignin require additional studies showing beneficial physiological effects in humans to be classified as functional fibers) [2]. Psyllium, a mucilage, is considered a functional fiber [2]. Chitin and chitosan, polydextrose and polyols, and resistant dextrins require additional studies showing positive physiological effects in humans to be considered functional fibers [2]. The term **total fiber** refers to dietary fiber present within the food plus functional fiber that has been added to the food.

In 2009, a branch of the World Health Organization adopted another definition of dietary fiber: Carbohydrate polymers with 10 or more monomeric units (i.e., monosaccharides), which are not hydrolyzed by human digestive enzymes and (a) are in foods (intrinsic and intact), or (b) have been extracted from food and have physiological benefits to health, or (c) are synthetic or modified and have physiological benefits to health [3]. Not included under this definition are oligosaccharides with degrees of polymerization between 3 and 9 (i.e., oligosaccharides containing chains of three to nine

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monosaccharides) such as some fructooligosaccharides and galactooligosaccharides [3]. The term *fiber* that appears on food labels reflects dietary fiber, but includes oligosaccharides [4].

FIBER AND PLANTS

Fiber is found in plant foods. Figure 4.1 shows the anatomy of a wheat plant. The endosperm of the plant contains mostly starch along with small quantities of fiber (mainly cellulose, hemicellulose, and resistant starch). The germ layer is rich primarily in some vitamins, minerals, and essential fatty acids, but also contains small amounts of fiber (mainly cellulose, lignin, and fructans). It is the bran component of cereals that contains the most fiber (over 95%). The outer bran layer of cereals consists of primary and secondary cell walls. These walls are fiber-rich, containing strands of cellulose arranged within a matrix of other fibers, especially hemicellulose and pectins, but also lesser amounts of fructans, resistant starch, and β-glucans. Other substances such as suberin (consisting of various phenolic compounds, long-chain alcohols, and polymeric esters of fatty acids), cutin (also made of polymeric esters of fatty acids that is secreted onto the plant surface), and waxes (complex hydrophobic, hydrocarbon compounds that coat the plant's external surfaces) are also components of the cell wall but do not contribute to the fiber content. Additional fibers may also be found within plants, but these vary with the plant species, the part of the plant (leaf, root, or stem), and the plant's maturity.

Whole-grain cereals and grain products provide cellulose, hemicellulose, lignin, some gums, β-glucans, some galactooligosaccharides (mainly raffinose and stachyose), and some fructans. Of the cereals, rye and barley typically contain more fiber than other grains. Fruits and vegetables provide almost equal quantities (~30%) of

Figure 4.1 The partial anatomy of a wheat plant.

Figure 4.2 Dietary fibers and some of their selected properties.

cellulose and pectin as well as some hemicellulose and, in selected fruits, some fructans and lesser amounts of other fibers. Legumes are also fiber-rich, containing cellulose, hemicellulose, pectins, gums, galactooligosaccharides, and resistant starches, among others. This next section reviews the chemistry and characteristics of fibers. Figure 4.2 shows these fibers and selected characteristics of the fibers; these characteristics and their impact on physiological processes and health are discussed in later sections of the chapter.

CHEMISTRY AND CHARACTERISTICS OF FIBER

Cellulose

Cellulose (Figure 4.3a), a dietary fiber and functional fiber, is a long, linear **polymer** (a high-molecular-weight substance made up of a repeating chain) of up to 10,000 β (1-4)–linked glucose units. Hydrogen bonding between sugar residues in adjacent, parallel-running cellulose chains imparts a three-dimensional structure to cellulose. Being a large, linear, neutrally charged molecule, cellulose is water insoluble, although it can be modified chemically (e.g., carboxymethyl cellulose, methylcellulose, and hydroxypropyl methylcellulose) for use as a food additive and this modified form may be more water soluble and a little more fermentable by colonic bacteria than naturally occurring cellulose. Cellulose that is found naturally in foods is not typically degraded by colonic bacteria. This nonfermentable characteristic of cellulose helps promote laxation. Examples of some cellulose-rich foods include whole grains, bran, legumes, peas, nuts, root vegetables, vegetables of the cabbage family, seeds (mainly the outer covering), and apples. Purified, powdered cellulose (usually isolated from wood) and modified cellulose are added to foods, for example, as a thickening or texturing agent or to prevent caking or syneresis (leakage of liquid). Some examples of foods to which cellulose or a modified form of cellulose is added include breads, cake mixes, sauces, sandwich spreads, dips, frozen meat products (e.g., chicken nuggets), and fruit juice mixes.

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(a) Cellulose

(b) Hemicellulose (major component sugars)

Backbone chain

D-xylose

D-mannose

D-galactose

Side chains

L-arabinose

4-O-methyl-D-glucuronic acid

D-galactose

(c) Pectin

(d) Phenols in lignin

Trans-coniferyl

Trans-sinapyl

Trans-p-coumaryl

(e) Gum arabic

X: L-rhamnopyranose or L-arabinofuranose
GALP: galactopyranose
GA: glucuronic acid

(f) β-glucan (from oats)

Figure 4.3 Chemical structures of dietary fibers and some functional fibers.

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(g) Inulin

(h) Fructooligosaccharide

(i) Raffinose

(j) Stachyose

(k) Verbascose

Figure 4.3 *(Continued)*

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Hemicellulose

Hemicellulose, another dietary fiber, consists of a heterogeneous group of polysaccharides. These polysaccharides vary among plants and within a plant depending on location. One example of a hemicellulose structure is β (1-4)–linked D-xylopyranose units with branches of 4-O-methyl D-glucopyranose uronic acids linked by α (1-2) bonds or with branches of L-arabinofuranosyl units linked by α (1-3) bonds. Hemicelluloses contain both hexoses and pentoses in their backbone and branched side chains. The β (1-4)–linked sugars in the backbone, which form a basis for hemicellulose classification, usually include the pentose xylose and hexoses such as mannose and galactose, while sugars such as arabinose, glucuronic acid, and galactose, among others, are found in the side chains. Some of these sugars are shown in Figure 4.3b. The sugars in the side chains confer important characteristics on the hemicellulose. For example, hemicelluloses that contain acids in their side chains are slightly charged and more water soluble, while other hemicelluloses are water insoluble. Similarly, fermentability of the hemicelluloses by intestinal bacteria is also influenced by these sugars and their positions. For example, hexose and uronic acid components of hemicellulose are more accessible to bacterial enzymes and thus more fermentable than are the other hemicellulose sugars. Foods that are relatively high in hemicellulose include whole grains as well as nuts, legumes, and some vegetables and fruits.

Pectins

Pectins, a dietary and functional fiber, represent another family of heterogeneous polysaccharides found in plant cell walls, intercellular regions of plants, and in the outer skin and rind of some fruits and vegetables. Galacturonic acid is a primary constituent of pectin's backbone and is found as an unbranched chain of α (1-4)–linked D-galacturonic acid units, as shown in Figure 4.3c. Chains of pentoses (xylose and arabinose) and hexoses (galactose, rhamnose, and fucose) are attached to pectin's backbone. Rich sources of pectins include many fruits—apples, berries, apricots, cherries, grapes, and citrus fruits—as well as legumes, nuts, and some vegetables. In some fruits, pectin is broken down as the fruit ripens and becomes softer. Commercially, pectins are usually extracted from citrus peel or apples and may be added to products, such as fruit strips, fruit juices, and icing, among others. In jellies and jams, pectin is used to promote gelling. Pectin is added to some enteral nutrition products used for tube feeding to provide a source of fiber in the diet. Pectins are water soluble and have a high ion-binding potential. In the digestive tract, pectins form viscous gels and are almost completely fermented by bacteria in the colon.

Lignin

Lignin is a highly branched polymer of phenol units (versus sugars) with strong intramolecular bonding. The primary phenols that compose lignin include trans-coniferyl, trans-sinapyl, and trans-p-coumaryl, shown in Figure 4.3d. Lignin provides structural support in plant cell walls. It is found in the bran layer of cereals and in the stems and seeds of fruits and vegetables. Lignin is insoluble in water, has hydrophobic binding capacity, and is generally not fermented by colonic bacteria. Lignin is a dietary fiber and may serve as a functional fiber. Foods high in lignin include wheat, rye, mature root vegetables such as carrots, flaxseed, and fruits with edible seeds such as many berries.

Gums

Gums, also called *hydrocolloids*, are secreted at the site of plant injury by specialized secretory cells and can be exuded from plants (i.e., forced out of plant tissues). Gums that originate as tree **exudates** include gum arabic, gum karaya, and gum ghatti; gum tragacanth is a shrub exudate. Gums are often highly branched and are composed of a variety of sugars and sugar derivatives. Gum arabic, shown in Figure 4.3e,for example, contains a main galactose backbone joined by β (1-3) linkages and β (1-6) linkages with side chains of galactose, arabinose, rhamnose, glucuronic acid, or methylglucuronic acid. The nonreducing ends terminate with a rhamnopyrosyl unit. Of the tree exudates, gum arabic is most commonly used as a food additive to promote gelling, thickening, and stabilizing. It is found in candies such as caramels, gumdrops, and toffees, as well as in other assorted products.

Guar gum and locust bean gum (also called carob gum) are made from the ground endosperm of guar seeds and locust bean seeds, respectively. These water-soluble gums consist mostly of galactomannans, which contain a mannose backbone in 1-4 linkages and in a 2:1 or 4:1 ratio with galactose present in the side chains. Guar galactomannans have more branches than locust bean galactomannans. Both guar gum and locust bean gum are added as a thickening agent and water-binding agent (among other roles) to products such as bakery goods, sauces, dairy products, ice creams, dips, and salad dressings. Gums are also found naturally in foods such as oatmeal, barley, and legumes. Gums are dietary and functional fibers. They are water soluble, fermentable by colonic bacteria, and some (like guar gum) form viscous gels.

β-Glucans

β-glucans (Figure 4.3f) are homopolymers of glucose units, but are smaller in size and contain different linkages than cellulose. Oat β-glucan consists of a chain of glucoses joined mostly in β (1-4) linkages but also some β (1-3) linkages. β-glucans are water soluble, highly fermentable by colonic

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bacteria, and form viscous gels within the digestive tract. β-glucans are found in relatively high amounts in two grains, oats (oat bran, rolled oats, and whole oat flour) and barley (whole grain and dry milled). β-glucans extracted from cereals are used commercially as a functional fiber because of their effectiveness in reducing serum cholesterol and moderating blood glucose concentrations. The Food and Drug Administration permits a health claim for β-glucans describing reductions in serum LDL cholesterol resulting from the daily consumption of ≥ 3 g of β-glucans from oats [5].

Fructans

Fructans, sometimes called *polyfructose*, include inulin, oligofructose, and fructooligosaccharides. Fructans are chemically composed of fructose units in chains of varying length. Inulin consists of a β (2-1)–linked fructose chain that contains from 2 to about 60 units (usually at least 10), with a glucose molecule at the end of the fructose chain linked by an α (1-2) bond (Figure 4.3g). Oligofructose is similar in structure to inulin but generally contains less than 10 fructose units. Inulin and oligofructose are dietary fibers. Fructooligosaccharides are a functional fiber formed from the partial hydrolysis of inulin or synthesized from sucrose by adding fructose; they typically contain about two to four or five fructose units, and may or may not contain an end glucose molecule (Figure 4.3h). Fructans, especially fructooligosaccharides and oligofructose, are water soluble and highly fermentable by colonic bacteria, but do not form viscous gels in the digestive tract. Fructooligosaccharides and inulin also function as prebiotics, promoting the growth of healthful bifidobacteria.

Fructans (mainly inulin) are found naturally in some plants. The most common food sources of inulin include chicory, asparagus, leeks, onions, garlic, Jerusalem artichoke, tomatoes, and bananas; fresh artichoke, for example, contains about 5.8 g per 100 g, and minced dried onion flakes provide 4 g per 100 g [6]. Wheat, barley, and rye also contain some fructans. Fructans are also added to some foods. Oligofructose is commonly used, for example, in cereals, fruit preparations for yogurt, dairy products, and frozen desserts. Inulin is used to replace fat in fillings, table spreads, dairy products, dressings, and frozen desserts, to name a few examples. Both inulin and fructooligosaccharides are found in supplements (such as fiber gummy supplements), and fructooligosaccharides are also added to foods. Americans are thought to consume up to about 4 g of fructans each day from foods.

Resistant Starch

Resistant starch (RS) is starch that cannot be or is not easily enzymatically digested. There are four main types (numbered 1 to 4) of resistant starch. RS_1 is starch that is physically inaccessible to digestion due to its location within a section of the plant's structure. Food sources of RS_1 include whole or partially milled grains and seeds. Resistant starch–designated RS_2 represents starch that resists digestion because it is tightly packaged inside of granules within foods. The tight packaging is associated with the linear structure of amylose (a component of starch along with amylopectin), and is especially prevalent in some "raw or uncooked" plant foods such as unripe (green) bananas, potatoes, and some legumes and maize. The heating of foods with these starches, however, gelatinizes the starch and increases its ability to be digested. Both RS_1 and RS_2 are dietary fibers.

Another resistant starch, RS_3, is called retrograde starch or amylose. It is formed with moist-heat cooking and then cooling of starch that has gelatinized. This cooking and then cooling alters the starch to make it more resistant to digestion. Examples of foods rich in RS_3 include cooked and cooled potatoes, rice, pasta, bread, and some corn. Lastly, RS_4 results from chemical modifications of starch (usually isolated from corn). Examples of modifications include the formation of starch esters or cross-bonded starches, which retard the ability of the starch to swell during cooking and thus keep it in a more granular form that resists digestion. This type of resistant starch is found in some corn-based products. RS_3 and RS_4 are functional fibers, and both may be partially fermented by colonic bacteria. RS_3 may also stimulate the growth of healthful bacteria in the colon, and may improve the glycemic response following carbohydrate ingestion. Americans are thought to consume up to about 10 g of resistant starch daily. Consumption of up to 20 g of resistant starch has been recommended to obtain health benefits.

Mucilages (Psyllium)

Mucilages are plant polysaccharides with a structure similar to gums. Mucilages are found in the seeds of a variety of plants, including flax and psyllium, among others. Psyllium, from the husk of psyllium seeds (also called plantago or fleas seed), contains several polysaccharides, including arabinoxylan, which has a xylose backbone and arabinose side chains. Psyllium is fairly soluble in water, containing about 70–80% water-soluble polysaccharides and 20–30% water-insoluble polysaccharides. Products to which psyllium has been added have high water-binding capacities and form viscous gels in the digestive tract. Psyllium is added to Metamucil® for its laxative properties as well as other products to promote reductions in serum lipids. The Food and Drug Administration permits a health claim for psyllium with consumption of 10.2 g (providing 7 g of viscous fiber) resulting in significant reductions in serum LDL cholesterol [5]. Foods containing psyllium that bear a health claim are required to state on the label that the

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food should be eaten with at least a full glass of liquid and that choking may result if the product is not ingested with enough liquid [5]. In addition, the label should state that the food should not be eaten if a person has difficulty swallowing [5].

Polydextrose and Polyols

Polydextrose is a polysaccharide consisting of glucose and sorbitol units that have been polymerized at high temperatures and under a partial vacuum. Polydextrose, available commercially, is added to foods as a bulking agent or as a sugar substitute. Polyols are hydrogenated carbohydrates or sugar alcohols and are used commercially to replace sugars in some foods; they do not, however, raise blood glucose concentrations to the same extent as sucrose and some other naturally occurring sugars. Examples of polyols include polyglycitol, sorbitol, xylitol, maltitol, mannitol, and isomalt. Polyglycitol and malitol, for example, are found in syrups; others are found in mints and gums. Polyols are also found naturally in some fruits like apples, watermelon, plums, peaches, and pears, to name a few. Polyols absorb water in the colon and contribute to laxation. Both polyols and polydextrose can be partially fermented by colonic bacteria and may enhance the growth of healthful bacteria. The Food and Nutrition Board of the National Academy of Sciences with the 2002 publication of dietary reference intakes for fiber designated polydextrose and polyols as functional fibers pending the results of additional studies showing physiological effects in humans [2].

Resistant Dextrins

Resistant dextrins, also called *resistant maltodextrins*, are generated by heating and enzymatically treating (with amylase) starch, usually cornstarch or wheat starch. Resistant dextrins chemically consist of glucose polymers containing a variety of glucosidic bonds. The resistant dextrin wheat dextrin is added to foods as well as found as a dietary supplement. Wheat dextrin is water soluble and fermentable by colonic bacteria; it also has been shown to enhance the growth of healthful bacteria in the colon. With the 2002 publication of Dietary Reference Intakes for fiber, the Food and Nutrition Board of the National Academy of Sciences designated resistant dextrins as functional fibers pending the results of additional studies showing physiological effects in humans [2].

Chitin and Chitosan

Chitin is a straight-chain polymer containing β (1-4)–linked glucose units, similar in structure to cellulose, but with an N-acetyl amino group substituted for the hydroxyl group at carbon 2 of glucose. Chitin is a component of the exoskeleton of insects and is found in the shells of crabs, shrimp, and lobsters.

Table 4.1 Food Sources of Fiber

Type of Fiber	Examples of Food Sources
Cellulose	All plant foods, especially wheat bran, legumes, nuts, peas, root vegetables (such as carrots), vegetables of the cabbage family, celery, broccoli, coverings of seeds, and apples
Hemicellulose	Whole grains, especially bran, nuts, and legumes
Lignin	Whole grains, especially wheat bran, mature root vegetables (such as carrots), fruits with edible seeds (such as strawberries), and broccoli (especially the stalk)
Pectins	Citrus fruits, strawberries, apples, raspberries, legumes, nuts, some vegetables (such as carrots), and oat products
Gums	Oatmeal, barley, and legumes
β-glucans	Oat products and barley
Resistant starches	RS_1: partially milled grains and seeds; RS_2: unripe (green) bananas, legumes, raw potato, and high-amylose corn; RS_3: rice, pasta, cold cooked potatoes, and high-amylose corn
Fructans	Chicory, asparagus, onion, garlic, artichoke, tomatoes, bananas, rye, and barley
Chitosan, chitin	Shells of crab, shrimp, and lobster

Chitosan is a deacetylated form of chitin. Both chitin and chitosan have high molecular weights, are insoluble in water, and can adsorb (interact or complex with) dietary lipids, primarily unesterified cholesterol and phospholipids, and promote their excretion in the feces. Modified forms of chitin and chitosan have been designed for nutraceutical and functional food applications. The Food and Nutrition Board of the National Academy of Sciences designated chitin and chitosan as functional fibers pending the results of additional studies showing physiological effects in humans. Table 4.1 lists some food sources of fiber.

SELECTED PROPERTIES OF FIBER AND THEIR PHYSIOLOGICAL IMPACT

The physiological effects and ultimately the health benefits of fiber vary based on certain characteristics of fiber, most notably viscosity and fermentability, but also to a lesser extent based on solubility and chain length (longer versus shorter chain). Shorter-chain fibers include fructooligosacharides (see section on fructans) and galactooligosaccharides (also called galactans). This latter group includes sugars such as raffinose, stachyose, and verbascose. Raffinose is a trisaccharide of fructose, glucose, and galactose (Figure 4.3i). Stachyose is a tetrasaccharide of fructose, glucose, and galactose to which another galactose is linked (Figure 4.3j). Verbascose is an oligosaccharide containing fructose, glucose, and three galactose molecules (Figure 4.3k). Galactooligosaccharides are found naturally in human milk and in peas (field peas, chickpeas, and green peas), lentils, and beans (such as soy,

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mung, lima, snap, northern, and navy, among others). Galactooligosaccharides, like fructooligosaccharides, are not digestible by human digestive enzymes, but are highly soluble and fermentable by colonic bacteria.

In contrast to the shorter-chain galacto- and fructooligosaccharides, the longer-chain fibers vary in degree of solubility and fermentability, and thus are sometimes subdivided into four groups: (1) soluble and highly fermentable, (2) intermediately soluble and fermentable, (3) insoluble and slowly fermentable, and (4) insoluble and nonfermentable. This section discusses the solubility, viscosity, and fermentability of fibers. However, as you read about these characteristics and their effects on the physiological processes, remember that we eat foods containing a mixture of dietary fiber, not foods with just cellulose, hemicellulose, pectins, gums, and so forth. Thus, the described effects on the body processes are more variable and are not as straightforward as presented in this chapter.

Solubility in Water

One approach to classifying fiber that has been used for decades is based on fiber's solubility or insolubility. Water-soluble fibers are those that dissolve in hot water, whereas insoluble fibers do not dissolve in hot water. Shorter-chain water-soluble fibers include both fructooligosaccharides and galactooligosaccharides. Longer-chain water-soluble fibers include pectins, gums (mainly guar), inulin, and resistant starches, as well as the resistant dextrin wheat dextrin. Fibers that are intermediately soluble include psyllium, β-glucans, and some hemicelluloses and pectins. Foods typically rich in water-soluble fibers include legumes, oats, barley, rye, chia, flaxseeds, most fruits (especially berries, bananas, apples, pears, plums, and prunes), some vegetables (carrots, broccoli, artichokes, and onions), and cooked and cooled pasta, rice, and potatoes.

Insoluble fibers include mainly cellulose, lignin, and some hemicelluloses, and to a lesser extent some pectins, some resistant starches, chitosan, and chitin. Examples of foods rich in insoluble fiber include whole-grain products, bran, legumes, nuts, seeds, some vegetables (such as cauliflower, zucchini, celery, and green beans), and some fruits. Generally, vegetables and most grain products contain more insoluble fibers than soluble fibers. Fruits tend to be higher in soluble fibers, which are found in the fruit's pulp and skin; the skin of fruit, however, also provides some insoluble fibers.

This solubility/insolubility approach to classifying fibers, which has been used as a basis for some observed biomarkers and health outcomes, is now considered, because of inconsistent findings, to be of less significance. For example, soluble fibers were generally accepted to delay gastric emptying, increase intestinal transit time (slower movement), and decrease nutrient absorption. These effects in turn positively impact blood glucose and lipid concentrations. In contrast, insoluble fibers were generally accepted to decrease (speed up) intestinal transit time and increase fecal weight to positively impact laxation. However, it is now known that not all soluble fibers alter nutrient absorption, and that insoluble fibers have varied effects on fecal weight. With these observations, the focus has shifted away from classifications based on solubility/insolubility and more toward viscosity and gel formation.

Viscosity and Gel Formation

Viscosity is related to fiber's ability both to bind or hold water (think of fiber as a dry sponge that hydrates or soaks up water and digestive juices as it moves through the digestive tract) and to form a gel (think of freshly made Jello® as it is starting to "set") within the digestive tract. Most fibers are strongly hydrophilic (water loving) with water-holding capacity influenced by chemical structure, particle size, pH, and electrolyte concentration. But, while most fibers hold water to some extent, not all fibers form a viscous gel when interacting with fluids within the digestive tract. It is this viscosity property of fiber, as well as another property, fermentability, that is most associated with health benefits as discussed in the next sections of this chapter.

Viscous gel-forming fibers include mainly pectins, β-glucans, mucilages (e.g., psyllium), and gums (mainly guar gum). These fibers, upon absorbing in some cases up to several times their weight in water, produce a viscous, gelatinous mass within the digestive tract. Ingesting foods rich in these gel-forming fibers is associated with:

- increased gastric distension, delayed gastric emptying, and longer intestinal transit time, which slows down the digestive process and may increase satiety (feelings of fullness)
- reduced nutrient digestion as the viscous gel traps nutrients (especially glucose and lipids) and interferes with their ability to interact with the digestive enzymes
- reduced micelle formation as the viscous gel traps bile (needed for micelle formation) and reduces lipid absorption and enterohepatic recirculation of bile
- decreased convective movement of nutrients (especially amino acid and fatty acids) within the intestinal lumen. Convective currents, induced by peristaltic movements, bring nutrients from the lumen to the intestinal cell's brush border membrane for absorption
- decreased nutrient (especially glucose and lipids) diffusion rates through a thickened, unstirred water layer that has become viscous and more "resistant" to nutrient movement (needed for absorption).

It is through these various actions that viscous, gel-forming fibers reduce the absorption of glucose and lipids such as cholesterol. Additionally, bile acids get

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trapped within the viscous gel to limit micelle formation that is needed for fat absorption; this further contributes to favorable effects on blood lipids. Other effects of gel formation on nutrients, such as on the bioavailability of carotenoids, vary with the food matrix and the fiber.

Fermentability

Fiber reaches the colon undigested by human digestive enzymes. Colonic bacteria then ferment (degrade to varying degrees) this undigested mass. Fibers that are not typically fermented include principally the water-insoluble fibers—cellulose and lignin, along with some hemicelluloses and some resistant starches like RS_1. Fibers that are not fermented are beneficial in promoting laxation and thus treating constipation primarily by increasing fecal bulk or volume, also called stool bulk or weight. The role of nonfermentable fibers in laxation is discussed further in the section on "Health Benefits of Fiber."

The fermentation of fibers occurs mainly in the proximal (upper) colon—that is, by the cecum and in the ascending region of the colon—and diminishes as the undigested mass moves through the transverse and descending sections of the colon. The shorter-chain fibers, fructooligosaccharides and galactooligosaccharides, are rapidly and almost completely fermented by bacteria in the colon. The longer-chain fermentable fibers (that are also soluble) include pectins, inulin, resistant starch, and gums (guar). Fermentable fibers that are of intermediate solubility include β-glucans and psyllium. Some insoluble fibers that are more slowly fermentable include some lignin and hemicelluloses. Additionally, the resistant dextrin wheat dextrin and polydextrose are fermentable.

Fermentation of fiber by colonic bacteria provides energy and substances for microbial growth as well as products such as short-chain fatty acids (discussed in Chapter 2) that may be used by the human host. The amount of energy realized to the host depends mostly upon the amount and type of fiber that is ingested and the short-chain fatty acids that are produced, but is usually estimated at about 1.5–2.5 kcal/g. While fermentable fibers do not contribute substantially to fecal bulk (as do the nonfermentable fibers), fermentable fibers increase fecal bacteria mass, and the increased bacteria mass in the feces in turn attracts water to enhance stool size. Some fermentable fibers also function in the colon as prebiotics.

Prebiotics are substances that are not digested by human digestive enzymes but provide health benefits to the host by acting as substrates for the growth and/or activity of one or more species of healthful bacteria in the colon. For a fiber, or other substance, to be considered as a prebiotic, three criteria must be satisfied. First, the ingredient must be able to resist digestion by human enzymes and absorption. Second, the ingredient must serve as a substrate for fermentation by intestinal microorganisms belonging to the human microbiota. Third, the ingredient must selectively stimulate the growth and/or activity of health-promoting intestinal bacteria. The main bacterial species associated with health and well-being in humans are those of the *Bifidobacterium* and *Lactobacillus* genera. The benefits from ingesting prebiotics relate most directly to their stimulation of the growth and/or activity of bacteria in the colon and to the bacteria-generated short-chain fatty acids from fiber fermentation. Food ingredients meeting the criteria for prebiotics include fructans, lactulose (at sublaxative doses), and galactooligosaccharides. Several other fibers (such as wheat dextrins, aracia gum, and polydextrose) also have been shown to stimulate the growth of various species of healthful bacteria or have been shown to provide health benefits through the production of short-chain fatty acids. The amounts of the various prebiotics that need to be ingested to promote desirable effects vary. Similarly, the side effects from prebiotic use (which may include excessive gas, abdominal bloating, cramping, and osmotic diarrhea) also vary with the amount and type of fiber consumed. Generally, shorter-chain fibers such as the oligosaccharides produce side effects at lower intakes than longer-chain fibers. The use of prebiotics has been shown to be helpful in the prevention of some types of diarrhea. This next section of the chapter addresses some of the health benefits and proposed mechanisms of action of fiber. The benefits of the presence of healthful bacteria in the colon and from the short-chain fatty acids that are generated from colonic bacteria are discussed in Chapter 2. Figure 4.4 reviews some of the physiological effects on the digestive tract from the consumption of fiber.

HEALTH BENEFITS OF FIBER

Several systematic reviews and meta-analyses have been conducted examining relationships between fiber intake and/or the intake of foods rich in fiber (most commonly whole grains, fruits, and vegetables) and specific diseases. Positive outcomes are reported, especially for cardiovascular disease but also for health, with inverse relationships between dietary fiber intake and overall mortality shown in both men and women. The roles of fiber in four areas—cardiovascular disease; diabetes; appetite, satiety, and weight control; and selected gastrointestinal disorders—are reviewed briefly hereafter.

Cardiovascular Disease

Studies examining fiber intake consistently report that ingestion of diets high in fiber is associated with a reduced risk of death from cardiovascular disease. Consistent evidence has also been reported for inverse relationships

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Figure 4.4 Selected gastrointestinal responses to fiber ingestion.

between intake of fruits and vegetables (primarily greater than five servings per day) and heart attack and stroke, and between intake of whole grains and heart disease.

Studies focusing on the effects of fiber or diets rich in fiber on heart disease risk factors, usually serum cholesterol concentrations, have also typically been favorable. Lower serum total and LDL cholesterol concentrations (and in some studies lower serum triglyceride concentrations) have been demonstrated with ingestion of several viscous gel-forming fibers, especially pectins, β-glucans, psyllium, and guar gum, but also to lesser degrees with ingestion of other fibers including resistant dextrins, methylcellulose, inulin, and fructooligosaccharides. The most well-studied cholesterol-lowering high-fiber foods/fibers are β-glucan from barley and oats, as well as psyllium. In fact, each of these has been studied sufficiently to have health claims.

Quantities of fiber needed to lower serum lipid concentrations vary; effective LDL-cholesterol lowering quantities for pectin range from about 12 to 24 g, for guar gum about 9–30 g, for barley β-glucan and methylcellulose about 5 g, and for psyllium and oat β-glucan about 6 g [7]. Ingestion of 60 g wheat dextrin, a resistant dextrin, also has been shown to reduce serum total cholesterol concentrations. To consume from foods the amount of fiber necessary to lower serum lipids, one would need to ingest, for example, about 6–10 servings per day of soluble fiber–rich fruits and vegetables, or about 2–3 servings per day of legumes or oat- or barley-based cereals. It is important to also note that consumption of plant foods provides phytosterols and phytostanols, which in amounts ranging from about 1.6 to 3 g/day has been shown to decrease total and LDL serum cholesterol concentrations.

Several modes of action are thought to promote fiber's hypercholesterolemic effects. These actions include reductions in cholesterol absorption and bile reabsorption and subsequent changes in hepatic cholesterol metabolism and clearance of lipoproteins. Basically, viscous gel-forming fibers trap bile acids and cholesterol within a gelatinous mass to limit micelle formation and absorption and thus enhance their excretion in the feces. The decrease in dietary cholesterol absorption and the decrease in bile acids returned to the liver (enterohepatic recirculation) necessitate the use of cholesterol that is in the body for synthesis of new bile acids. Additionally, changes in cholesterol metabolism and in lipoprotein clearance from the blood (associated with the upregulation of LDL receptors) result to also lower serum cholesterol concentrations. Moreover, cholesterol synthesis appears to be inhibited by fiber-induced shifts in bile acid production from cholic acid toward chenodeoxycholic acid and by propionic acid (the mechanisms are not known); these changes then positively impact serum cholesterol concentrations. Finally, reductions in hepatic triglycerides and fatty acid synthesis also may be contributing to the observed effects.

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Diets rich in high-fiber foods and the consumption of functional fibers like psyllium have also been associated with both lower systolic and diastolic blood pressure readings and with reductions in blood pressure among those with hypertension, another risk factor for heart disease. The 2015 Dietary Guidelines Advisory Committee concluded that there was consistent and strong evidence that dietary patterns that are lower in saturated fat, cholesterol, and sodium, and richer in *fiber*, potassium, and unsaturated fats are beneficial for reducing cardiovascular disease [1].

Diabetes Mellitus

Inverse associations between dietary fiber intake (as well as high intakes of fruits, vegetables, and complex carbohydrates) and risk of developing type 2 diabetes have been demonstrated in several studies. Consumption of diets high in fiber has also been generally associated with improved glycemic control (also referred to as blunting the glycemic response) in individuals with diabetes and prediabetes. Specifically, the ingestion of fiber supplements or foods rich in viscous gel-forming fibers improves glycemic control largely through reduced rates of glucose absorption and insulin secretion. Reductions in insulin secretion are thought to result at least in part both from slower glucose absorption into the blood as well as from altered secretion of gastrointestinal tract regulatory peptides such as glucagon-like peptides and glucose-dependent insulinotropic peptide, which influence glucagon and insulin secretion and gastrointestinal tract motility. Changes in glycogen catabolism and the resulting release of glucose into the blood also may be influenced by short-chain fatty acids that are produced with fiber fermentation in the colon. Improvements in glycemic control are usually observed with fiber intakes of at least 30 g per day, although fiber supplementation in doses of at least 20 g may also be beneficial. The 2015 Dietary Guidelines Advisory Committee states that there is moderate evidence that dietary patterns higher in fruits, vegetables, and whole grains (*note these are fiber-rich foods*) and lower in red and processed meats, high-fat dairy, refined grains, and sweets/sugar-sweetened beverages reduce the risk of developing type 2 diabetes [1].

Appetite and/or Satiety and Weight Control

Fiber-rich foods, versus low-fiber foods, tend to have a lower energy density and a higher volume, which can promote satiety. Satiety also may result from ingestion of foods containing viscous gel-forming fibers due to fiber-induced delays in gastric emptying and/or alterations in the release of digestive tract hormones known to modulate appetite such as ghrelin, glucagon like peptide-1, peptide YY, and cholecystokinin. Additionally, however, the consumption of nonviscous fibers such as galactooligosaccharides has been shown to reduce appetite; such actions have been attributed to changes in metabolism, gut microbiota, and gastrointestinal tract peptides. As expected from the diversity of polysaccharides, fiber's effects on satiety and appetite vary with the type, amount, and form (supplement or food) of fiber consumed, among other factors. And, while some studies have reported reduced energy intakes and weight loss on high-fiber diets, others have not. The 2015 Dietary Guidelines Advisory Committee does not mention fiber in relation to body weight; but states that there is moderate evidence that dietary patterns higher in fruits, vegetables, and whole grains (i.e., high-fiber foods), that include seafood and legumes, that are moderate in dairy products (low-fat and nonfat) and alcohol; that are lower in red and processed meats, and that are low in sugar-sweetened beverages and refined grains are associated with favorable outcomes related to healthy body weight (including lower body mass index, waist circumferences, or percent body fat) or risk of obesity [1].

Gastrointestinal Disorders

Fiber intake has been linked with several gastrointestinal conditions. Three disorders, constipation, diverticular disease, and colon cancer, that have been associated with low fiber consumption, and one condition, irritable bowel syndrome, that has been linked with the consumption of specific fibers are discussed.

Constipation is characterized by long transit time, difficult stool expulsion, low stool output, and incomplete rectal emptying. Increasing consumption of fiber through supplements or fiber-rich foods can improve constipation. While all fibers are beneficial, nonfermentable or less fermentable fibers (such as psyllium, cellulose, inulin, etc.) tend to increase fecal bulk to a greater extent than more fermentable fibers. Fecal bulk is especially affected by water-holding capacity and particle size. Nonfermentable fibers, such as those found in wheat bran, are highly effective in laxation because they can absorb several times their weight of water, thereby increasing fecal volume. This larger fecal volume decreases intraluminal pressure, and provides for a greater frequency of defecation (i.e., reduced/quicker intestinal transit time). Particle size also plays a role, with, for example, larger or coarser bran having the ability to hold more water (than smaller or finer bran) and thus providing greater fecal volume. Several products on the market designed to help individuals with constipation contain fiber. Fiberall® and Metamucil®, for example, contain psyllium. Benefiber® contains wheat dextrin, and FiberChoice® contains inulin. Orafti® contains fructooligosaccharides, and Citrucel® contains

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methylcellulose. Increasing fiber intake to at least 20 g per day is generally recommended to help treat constipation.

Another gastrointestinal tract disorder that has been linked to diets low in fiber is diverticular disease, which is characterized by the presence of diverticula in the colon. Diverticula, protruding or bulging pouches of the wall of the colon, are thought to form when the colon's wall weakens. This weakening is theorized to result in chronic constipation associated with low fecal bulk and straining to pass hard fecal matter. (The straining increases the pressure inside the colon and weakens its walls.) When fecal matter becomes trapped in the diverticula, the pouches become inflamed (called diverticulitis) and the person experiences pain and sometimes fever, diarrhea, gastrointestinal bleeding, and infection. Diets high in fibers that increase stool weight, as discussed in the preceding section on constipation, reduce straining and the likelihood of fecal matter becoming trapped in the diverticula. However, whether a high-fiber diet reduces the likelihood of formation of new diverticula once the condition has developed is unclear [8]. Recommendations for fiber intake for those with diverticular disease are the same as those recommended for all Americans, about 20–35 g per day.

Epidemiologic studies, meta-analyses, and prospective studies have shown that diets high in fruits, vegetables, and whole grains are linked with lower risk of colon cancer. Fiber intake also has been linked with a lower risk of colorectal cancer as well as some other cancers (breast, esophageal, etc.) in some, but not all, studies. Yet, the mechanism and whether it is fiber and/or another constituent in high-fiber foods contributing to the link are not clear. Many mechanisms have been suggested to explain how fiber may be preventive against colon cancer, including (1) adsorbing and promoting the excretion of primary bile acids, thereby decreasing their free concentration and availability for conversion to more harmful (carcinogenic) secondary bile acids; (2) adsorbing procarcinogens and carcinogens and/or diluting intestinal contents to minimize carcinogenic compound interactions with colonic mucosal cells; and (3) reducing colonic transit time, which in turn decreases the time during which toxins can be synthesized and in which they are in contact with the colonic mucosa cells. Other indirect effects of fiber also have been speculated as helpful to reduce colon cancer risk. For example, the short-chain fatty acids that are generated during fiber fermentation in the colon decrease the pH within the lumen of the colon; this acidification in turn confers several benefits to the host, as described in Chapter 2. Unfortunately, intervention study results have not been positive. For example, the Polyp Prevention Trial, which examined the effect of a high-fiber, low-fat diet on the recurrence of adenoma (a marker of colorectal cancer), failed to show benefits. Researchers agree that additional studies evaluating the appropriateness of different biomarkers, fiber types, form, and dosages, among other factors, on the incidence of colon cancer are needed. At present, the American Cancer Society, as well as several other organizations charged with improving health, recommends eating fiber-rich foods from several food groups, especially fruits, vegetables, and whole grains, to reduce the risk of cancer. The 2015 Dietary Guidelines Advisory Committee does not single out fiber, but states that there is moderate evidence for an inverse relationship between dietary patterns that are higher in fruits, vegetables, legumes, whole grains (i.e., fiber-rich foods), lean meats/seafood, and low-fat dairy; moderate in alcohol; and low in red and/or processed meats, saturated fat, and sodas/sweets relative to other dietary patterns and risk of colon/rectal cancer [1].

Whereas the aforementioned health conditions have been linked with inadequate fiber intake, irritable bowel syndrome is a condition that has been associated with the ingestion of selected fibers, including some that act as prebiotics. The classic symptoms of this condition—bloating, gas (flatulence), abdominal cramping, and diarrhea or constipation or a mixed bowel pattern—often occur after eating. Causative agents triggering these symptoms in susceptible individuals typically include several short-chain, highly fermentable fibers including fructooligosaccharides and galactooligosaccharides, as well as polyols. In addition to these fibers, the monosaccharide fructose and the disaccharide lactose (along with wheat bran) also may trigger symptoms. These substances have been coined FODMAP—*f*ermentable, *o*ligo-, *d*i-, *m*onosaccharides, and *p*olyols—and restriction of foods rich in these carbohydrates is purported to help alleviate symptoms of irritable bowel syndrome.

The low-FODMAP diet consists of an extensive list of "foods to avoid." For example, to minimize fructose consumption, one must limit foods with added high-fructose corn syrup, which include many beverages along with sauces and condiments (barbecue sauce, ketchup, syrups, etc.), along with foods naturally rich in fructose such as agave, honey, and many fruits. Fructans (which must be limited) are found in many vegetables. To avoid galactooligosaccharides, one must minimize intakes of most legumes and peas. Lactose is found primarily in dairy products. Polyols are found in chewing gums and mints as well as some fruits. Variable benefits from these dietary restrictions on observed symptoms have been reported, but long-term efficacy data for the low-FODMAP diet are needed. Similarly, the effectiveness of fiber in treating irritable bowel syndrome symptoms has been examined in systematic reviews with generally mixed results. Of the various fibers, psyllium appears to be relatively helpful in improving some symptoms, especially in those with constipation versus those with diarrhea.

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FOOD LABELS AND HEALTH CLAIMS

Nutrient recommendations for fiber, as well as for other nutrients, are found on the Nutrition Facts panel on food labels. The recommendation for fiber provided on food panel labels is 25 g of dietary fiber for a 2,000-kcal diet. Some food labels also provide information on quantities of soluble and insoluble fibers in the product. For example, the label on a box of cereal might show that a serving (1 cup) provides 7 g of dietary fiber, with 6 g listed as insoluble and 1 g listed as soluble.

Based on the total amount of dietary fiber provided by a serving of the food, food labels may state that the food is an "excellent" or "good" source of fiber. Foods claiming to be an "excellent source of fiber" by the manufacturer must provide at least 20% of recommendations in a serving—that is, 0.20 × 25 g, or 5 g of fiber. Foods may be considered a "good source of fiber" if they provide 10% of recommendations or 2.5 g of fiber/serving.

The Food and Drug Administration (FDA) has approved several fiber-related health claims [5]. The claims typically focus on consumption of fiber-rich foods such as fruits, vegetables, and whole grains coupled with consumption of a low-fat diet, as shown below.

- Diets low in fat and rich in high-fiber foods (or rich in fruits and vegetables) may reduce the risk of certain cancers.
- Diets low in saturated fat (or low in fat) and rich in soluble fiber (or rich in whole oats and psyllium seed husk) may reduce the risk of heart disease.
- Diets low in total fat, saturated fat, and cholesterol and rich in whole grains and other plant foods may help reduce the risk of heart disease.

RECOMMENDED FIBER INTAKE

Recommendations for increasing the amount of fiber in the U.S. diet have come from several government agencies and private organizations, each with a concern for improving the health of Americans. The Dietary Guidelines suggest that Americans ingest 14 g of fiber per 1,000 kcal. In 2002, the National Academy of Sciences Food and Nutrition Board established Dietary Reference Intakes, specifically Adequate Intakes, for fiber. These recommendations, shown in Table 4.2, were established based on amounts of fiber shown to protect against heart disease [2]. Unfortunately, most Americans fail to meet recommendations, with intakes often reaching only about 18 g of fiber per day [2].

Table 4.3 shows the dietary fiber content of selected foods. General estimates of fiber intake can be calculated assuming each serving of fruits, vegetables, and whole grains provides 2 g of dietary fiber and each serving of legumes contributing 5 g of dietary fiber. To complete the estimation, fiber from any consumed fiber supplements and from the ingestion of any high-fiber cereals or other products should be added to the total.

Table 4.2 Recommended Fiber Intakes [2]

Population Group	Age (years)	Total Fiber (g)
Men	19–50	38
	≥ 51	31
Women	19–50	25
	≥ 51	21
Children	1–3	19
	4–8	25
Girls	9–18	26
Boys	9–13	31
	14–18	38

The MyPlate guidelines from the U.S. Department of Agriculture do not provide recommendations on individual nutrient intakes like fiber, but instead focus on consuming foods from within and across food groups to meet nutrient needs. MyPlate suggests that adults consume at least 2 cups of fruits, 2½ cups of vegetables, and a minimum of 3 oz of whole grains per day, with additional recommendations to include at least 1½ cups of beans and peas weekly [9]; exact recommended amounts vary with a person's gender, age, and total energy needs.

No Tolerable Upper Intake Level for dietary fiber or functional fiber has been established [2]. Tolerance to fiber intake varies from person to person, and problems associated with the use of supplements vary with the type and dose of fiber ingested. Generally, supplements containing fibers that are very rapidly fermented are associated with more undesirable side effects than those that are more slowly or not fermented. The most common complaints with fiber "over" consumption include abdominal discomfort, bloating, gas, and altered stool output; however, gastrointestinal tract tolerance generally improves over time.

Reduced absorption of some minerals also has been purported as an adverse effect of ingesting too much dietary fiber. And, while this may be a problem in individuals consuming fiber in quantities well in excess of recommended amounts, it is not likely that healthy adults consuming recommended amounts of fiber will develop mineral deficiencies. The proposed "problem" is thought to occur because of the adsorption of some divalent minerals (including calcium, magnesium, zinc, and iron) to some fibers (like those containing uronic acid, such as hemicellulose, pectins, and gums, as well as with lignin, which has both carboxyl and hydroxyl groups).

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Table 4.3 Dietary Fiber Content of Selected Foods* [10,11]

Food Group	Soluble Fiber (g / 100 g)	Insoluble Fiber (g / 100 g)	Total
Fruits (raw)			
Apple with skin	0.70	2.00	2.70
Banana	0.58	1.21	1.79
Grapes	0.24	0.36	0.60
Mango	0.69	1.08	1.76
Orange	1.37	0.99	2.35
Peach with skin	1.31	1.54	2.85
Pear with skin	0.92	2.25	3.16
Pineapple	0.04	1.42	1.46
Plum with skin	1.12	1.76	2.88
Strawberries	0.60	1.70	2.30
Watermelon	0.13	0.27	0.40
Legumes/Beans (cooked)			
Black			8.7
Kidney	1.36	5.77	7.13
Lima	1.02	4.21	5.23
Navy			10.5
Pinto	0.99	5.66	6.65
Nuts			
Almonds			12.3
Cashews			3.2
Pecans			9.6
Peanuts			8.1
Walnuts			6.7

Food Group	Soluble Fiber (g / 100 g)	Insoluble Fiber (g / 100 g)	Total
Vegetables (cooked)			
Asparagus			2.0
Broccoli	1.85	2.81	4.66
Carrots	1.58	2.29	3.87
Cauliflower	0.70	3.50	4.20
Corn			2.0
Lettuce (raw)			1.3
Mushrooms			2.4
Potato baked			
With skin	0.61	1.70	2.31
Boiled, no skin	0.99	1.06	2.05
Grain and Grain Products			
Rice			
White			0.3
Brown			1.8
Couscous			2.8
Bread			
White			2.4
Whole grain			6.8
Crackers (wheat)			10.6
Cereals (cold)			
All Bran®			29.3
Raisin Bran®			11.1
Corn Flakes®			2.5
Cheerios®			10

* Soluble and insoluble fiber contents provided when available.

Yet, countering these possible effects are studies that show that fermentation of these fibers and the resulting acidic environment enhance the release of minerals from fiber and promote mineral absorption from the colon. Maillard products are also mentioned in the scientific literature as having mineral binding potential. These products contain enzyme-resistant linkages between the amino group of amino acids, especially lysine, and the carbonyl group of reducing sugars, which have formed during cooking, particularly in baking and frying foods. Yet, as with fiber ingestion, mineral deficiencies are not thought to be likely from the ingestion of Maillard products.

SUMMARY

The physiological effects of fiber in the gastrointestinal tract are as varied as the number of fiber components and their physiochemical properties. Two important characteristics related to health are viscosity/gel formation and fermentability. These characteristics not only impact digestive tract function and health but also affect risk factors for disease, especially heart disease and diabetes. To obtain fiber through the diet, food sources of fiber need to be varied, ideally within and across all plant-based food groups including whole-grain cereals and cereal products, legumes, nuts, seeds, fruits, and vegetables.

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THE FLAVONOIDS: ROLES IN HEALTH AND DISEASE PREVENTION

Chapter 4 described fiber and some of its characteristics that make it important in the diet. However, other substances in plant foods are also of significance. These substances are known as **phytochemicals**, a group of compounds that are biologically active in the body. Of the thousands of phytochemicals, polyphenolic phytochemicals (also referred to as polyphenols, meaning they contain more than one phenol unit), make up the largest group.

The polyphenols include more than 8,000 compounds, and can be divided into a variety of classes. One of the largest of these classes is the flavonoids, which include a group of over 4,000 plant metabolites. This Perspective reviews some of the more ubiquitous flavonoids in foods and their potential roles in maintaining health and preventing disease.

FLAVONOIDS

The flavonoids are organic, bioactive, polyphenolic secondary metabolites that occur in small quantities in a wide variety of plants (especially fruits, vegetables, nuts, seeds, herbs, spices, and tea). The flavonoids of dietary significance can be divided, based on functional groups attached to the common flavone backbone, into six subclasses—flavonols, flavanols, flavones, flavanones, anthocyanins, and isoflavones. The flavone and flavonols are subclasses, however, and are sometimes grouped together and referred to as 4-oxoflavonoids. Table 1 provides a list of these flavonoid subclasses along with major food sources.

Flavonols

The flavonol subclass includes two main compounds—quercetin and kaempferol, but also myricetin and isorhamnectin. These flavonols are widely found in foods (Table 1). Quercetin is among the more well studied of the flavonoids, and exhibits several biological actions helpful in the prevention of cardiovascular disease and some of its risk factors like hypertension. Kaempferol and myricetin also have some antihypertensive and antiathlerosclerotic properties.

Flavanols

Flavanols, also called flavan-3-ols, are another subclass of flavonoids, and can be further categorized based on chemical structure. Monomer forms are called catechins, and condensed or polymerized forms are called proanthocyanidins or tannins. Some of the food sources containing these flavanols are listed in Table 1. Catechins may help reduce the risk of hypertension and cardiovascular disease. Of the proanthocyanidins in foods, procyanidin is one of the most common, and studies suggest it may be beneficial in preventing heart disease and cancer. Flavanol intake has been estimated at 50–100 mg/day in the United States [1].

Flavones and Flavanones

Another category of flavonoids are the flavones, which include luteolin and apigenin. Only a few foods, listed in Table 1, have been identified as good sources of flavones. In comparison with the other flavonoids, not as much research has been conducted on these phytochemicals.

The flavanones also consist of just a few compounds, primarily narigenin, hesperetin, and eriodictyol, and are found mostly in citrus fruits and their juices. A glass of fruit juice is thought to provide 40–140 mg of flavanone glycosides [2]. Both hesperetin and its glycoside (meaning attached to a sugar) form hesperidin are found in relatively high amounts in oranges, and exhibit several biological properties that are thought to aid in the prevention of both cardiovascular disease and cancer. Naringenin also demonstrates activities that are anti-inflammatory and antiatherogenic [3].

Anthocyanins

Anthocyanins are pigments found mostly in the skin of plants, and thus provide color (usually red, blue, or purple) to many fruits and vegetables. Major food sources include blueberries, strawberries, raspberries, red grapes, and blackberries, among others listed in Table 1. A 100 g serving of berries can provide up to 500 mg of anthocyanins [2].

Anthocyanins are found free (unattached) as well as attached to sugars (anthocyanidin glycosides) or acyl groups in foods. Of the dozens of anthocyanidins, the six most commonly found include cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin. Consumption of anthocyanins and/or foods rich in these flavonoids has been suggested to benefit the heart, eyes (vision), and nerves, and may also be protective against cancer and diabetes [4].

Isoflavones

A final category of flavonoids is the isoflavones; the two main isoflavones are genistein and daidzein. They are found mostly in soybeans and soy products, as presented in Table 1. Isoflavones, along with lignans (found in seeds, whole grains, nuts, and some fruits and vegetables) and coumestans (found in broccoli and sprouts) are phytoestrogens; they are structurally similar to estrogen in that the phenol ring can bind to estrogen receptors on some body tissues. Soy products have been marketed for use by women during perimenopause to help alleviate some of the side effects of diminished natural estrogen in the body.

Table 1 Flavonoid Subclasses, Common Phytochemicals, and Their Sources

Flavonoid Subclass	Common Phytochemicals	Main Sources
Flavonols	Quercetin, kaempferol, myricetin, and isorhamnetin	Onions, tea, olives, kale, leafy lettuce, cranberries, tomatoes, cherries, apples, applesauce, turnip greens, endive, ginkgo biloba, chili peppers, chives, and celery
Flavanols	Catechins, epicatechin, and epigallo-catechin-3-gallate	Green tea, pears, grapes, wine, berries, apples, applesauce, apple juice, cocoa and cocoa products
Derived tannins	Theaflavins, theorubigins, and theabrownins	Fermented teas (black and oolong)
Condensed tannins/ proanthocyanidins	Procyanidins, prodelphinidins, and propelargonidin	Cocoa, cocoa products, stone fruits, grapes, wine, strawberries, cranberries, legumes, cinnamon, beer, and barley
Flavones	Apigenin and luteolin	Parsley, thyme, celery, celery seed, oregano, and hot peppers
Flavanones	Hesperetin, naringenin, and eriodictyol	Citrus fruits and juices
Anthocyanins	Cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin	Berries, cherries, bananas, plums, oranges, grapes, and red wine
Isoflavones	Genistein, daidzein, equol, and Glycitein	Legumes, especially soybeans and soy foods—soynuts, soy milk, tofu, miso, soy sauce, and edamame

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Table 2 Phytochemicals and Their Sources

Phytochemical Class	Common Phytochemicals	Sources
Carotenoids	β-carotene, α-carotene, lutein, and lycopene	Tomatoes, pumpkins, squash, carrots, watermelon, papayas, guavas
Terpenes	Limonene and carvone	Citrus fruits, cherries, ginkgo biloba
Organosulphides	Diallyl sulphide, allyl methyl sulphide, and S-allylcysteine	Garlic, onions, leeks, cruciferous vegetables (broccoli, cabbage, Brussels sprouts, mustard, watercress)
Phenolic acids	Hydroxycinnamic acids: caffeic acid, ferulic acid, chlorogenic acid, and neochlorogenic curcumin	Coffee, blueberries, cherries, pears, apples, oranges, grapefruit, tomatoes, kiwi, plums, white potatoes
Phenolic acids	Hydroxybenzoic acids: ellagic and gallic acids	Grapes, grape juice, red wine, tea, raspberries, strawberries, nuts
Lignans	Secoisolariciresinol and matairesinol	Berries, flaxseeds, sesame seeds, legumes, nuts, broccoli, cabbage, kale, rye bread
Saponins	Panaxadiol and panaxatriol	Alfalfa sprouts, potatoes, tomatoes, ginseng
Phytosterols	β-sitosterol, campesterol, and stigmasterol	Vegetable oils (soy, rapeseed, corn, sunflower)
Glucosinolates	Glucobrassicin, gluconapin, sinigrin, and glucoiberin	Cruciferous vegetables (see organosulphides)
Isothiocyanates	Allylisothiocyanates and indoles	Cruciferous vegetables (see organosulphides)

Other Phytochemicals

These flavonoids are among the thousands of phytochemicals found in foods. Some additional classes and examples of phytochemicals within each class, along with some food sources are listed in Table 2. Within each of these classes are phytochemicals with a wide range of biological actions that also are thought to help protect against disease and maintain health.

Although Tables 1 and 2 provide examples of foods containing different phytochemicals, note that most plant foods contain multiple phytochemicals. Tomatoes, for example, may contain as many as 10,000 different phytochemicals, and tea provides several flavonoids, flavonols, flavanols, and proanthocyanidins, along with other phytochemicals. Phytochemical contents further vary based on the plant species, its stage of ripeness, and the methods used for storing and processing the plant as well as with the climate or environmental conditions in which the plant was grown.

An Overview of Flavonoid Digestion, Absorption, and Metabolism

Most phytochemicals are found in foods in a variety of forms, and these forms influence the digestion and the rate and extent of absorption of the phytochemical. Polyphenols in foods may exist free (unattached) or in some cases as a glycoside conjugate (also called a glycone). The names of the conjugated and unconjugated forms differ slightly; for example, the flavanone hesperidin is conjugated to sugar, and its free/unconjugated form is known as hesperetin.

In some cases, the glycoside forms of the flavonoids must be digested to aglycones (unconjugated forms) before being absorbed. Other phytochemicals do not require extensive digestion, and may be more directly absorbed from the small intestine (and to a small extent the stomach). Glycosylated quercetin, for example, may be absorbed directly or hydrolyzed first by β-glycosidase. Many other digestive enzymes in the small intestine also assist in the cleavage of sugars (and other functional groups) bound to the flavonoids to enable absorption. The method of absorption of most flavonoids is thought to involve carriers; however, the absorptive processes have not been clearly elucidated.

Some flavonoids are neither digested nor absorbed in the upper digestive tract, but instead undergo degradation by colonic microflora. The bacteria hydrolyze the glycosides, (as well as other attached functional groups such as glucuronides, sulfates, amides, lactones, etc.) generating metabolites that may be absorbed or that exert effects on the body from within the colon. Lignans, for example, are metabolized by colonic bacteria to the metabolites enterodiol and enterolactone, which are then absorbed. These enterolignans exhibit weak estrogenic activity and/or antiestrogenic effects upon binding to estrogen receptors on various body tissues. Bacteria in the colon also utilize anthocyanin glycosides, deglycosylating them to aglycones, which are then further degraded. The extent of absorption of the products generated from the actions of the bacteria is not well established.

Once absorbed, most flavonoid metabolites are conjugated in the cells of the small intestine and then enter portal blood for transport to the liver. Some metabolites, however, efflux from the enterocyte back into the lumen of the small intestine via adenosine-binding cassette (ABC) transporters. Those flavonoid metabolites that enter portal blood are taken up largely by the liver where they undergo further metabolism, especially conjugation with methyl or sulfate groups, or glucuronic acid. These conjugated metabolites are then released into systemic circulation bound to plasma proteins like albumin. The amount of the metabolites present in the plasma varies considerably with the type of flavonoid consumed, the food source, and the amount ingested; little is known about the metabolism of all the different polyphenols in the body, and thus about what metabolites are present in the plasma after consumption of a specific polyphenol.

FLAVONOIDS AND HEALTH AND DISEASE PREVENTION

Diets rich in plant foods (whole grains, legumes, nuts, seeds, vegetables, and fruits) are typically associated with reductions in the risk of various diseases or conditions, especially cardiovascular disease, but also to a lesser extent some cancers, neurodegenerative conditions, and osteoporotic fractures, among others. Diets rich in plant foods, as we now know, are also rich in flavonoids and other phytochemicals. Dietary flavonoid intakes and/or specific flavonoids have been shown in some studies to be beneficial in disease prevention, primarily reducing cardiovascular disease risk and mortality, nonfatal events, and all-cause mortality [5–8].

Flavonoids exhibit a broad spectrum of biological activities that affect a variety of metabolic processes that may be related to the development of diseases. Several flavonoids provide cardioprotective effects with antioxidant and anti-inflammatory functions, vasodilatory effects (blood vessel relaxation), antiplatelet adhesion, and anticoagulant effects. Quercetin, a well-studied flavonol, for example, exhibits direct antioxidant functions (scavenging free radicals), activates signaling pathways, inhibits inflammation, and promotes vascular relaxation [3-9]. Kaempferol also has antihypertensive actions via enhancing endothelium vasorelaxation and protecting against endothelial damage [3-9]. Myricetin, another flavonol, also demonstrates antiplatelet, antihypertensive, and antiatherosclerotic properties. Catechins (monomeric flavanols) are also anti-inflammatory, and some isoflavones exhibit cholesterol-lowering effects that may be protective against heart disease.

It is a variety of actions of several flavonoids that are also thought to help in the prevention of some cancers. Some of these actions include antioxidant and anti-inflammatory functions, antiangiogenesis actions, and antiproliferative and apoptotic effects on tumor cells. The catechins (flavanols), for example, target signaling pathways to inhibit the growth of some cancers and promote apoptosis [5,9-11]. The flavonol quercetin exhibits direct antioxidant functions (scavenging free radicals), has apoptotic effects, and activates signaling pathways, which may be beneficial in cancer prevention [3,5,9-11]. The flavonol myricetin also demonstrates properties that may reduce the development of some cancers. Additionally, the isoflavone genestein, lignans, glucosinolates, isothiocyanates, terpenes, and some phenolic acids such

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as hydroxycinnamic acid have been shown to inhibit tumor formation and/or proliferation.

Inflammation is thought to contribute to the development of some neurodegenerative conditions. Flavonoids are thought to suppress neuroinflammation, as well as target signaling pathways and enhance cerebrovascular blood flow to improve cognitive function [10-12]. Diets high in flavonoids are thus thought to reduce age-associated cognitive impairments and/or cognitive decline.

Many of the demonstrated actions of flavonoids have been studied *in vitro*, in cultured cells, or in isolated tissues using specific glycosides or aglycone forms of the various phytochemicals. The forms of the polyphenolic phytochemicals used in the studies, however, have not been consistently the same as the forms in which the polyphenolic phytochemicals are found in the body. Moreover, the amounts or concentrations of the phytochemicals used in the studies have often been much higher than the amounts of the phytochemicals found naturally in the body. Differences in the metabolism of the thousands of phytochemicals in the body also complicate the interpretation of research studies and the ability to make recommendations. While more prospective studies are being conducted in humans, the results of such studies are typically mixed. To date, study findings most support the role of flavonoids in reducing the risk of cardiovascular diseases and/or its risk factors; however, more prospective, randomized controlled trials are needed to further examine the effectiveness of flavonoids in the prevention of neurodegenerative conditions and diseases such as cancers and diabetes. See *Suggested Readings* for more information on phytochemicals, including specific mechanisms by which the various flavonoids and other phytochemicals are thought to function.

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5 LIPIDS

STRUCTURE AND BIOLOGICAL IMPORTANCE
Fatty Acids
Triacylglycerols (Triglycerides)
Phospholipids
Sphingolipids
Sterols
DIETARY SOURCES
Recommended Intakes
DIGESTION
Triacylglycerol Digestion
Phospholipid Digestion
Cholesterol Ester Digestion
ABSORPTION
Fatty Acid, Monoacylglycerol, and Lysophospholipid Absorption
Cholesterol Absorption
Lipid Release into Circulation
TRANSPORT AND STORAGE
Lipoprotein Structure
Lipoprotein Metabolism
LIPIDS, LIPOPROTEINS, AND CARDIOVASCULAR DISEASE RISK
Cholesterol
Saturated and Unsaturated Fatty Acids
Trans Fatty Acids
Lipoprotein(a)
Apolipoprotein E
INTEGRATED METABOLISM IN TISSUES
Catabolism of Triacylglycerols and Fatty Acids
Formation of Ketone Bodies
Synthesis of Fatty Acids
Synthesis of Triacylglycerols and Phospholipids
Synthesis, Catabolism, and Whole-Body Balance of Cholesterol
REGULATION OF LIPID METABOLISM
Fatty Acids
Cholesterol
BROWN FAT THERMOGENESIS
ETHYL ALCOHOL: METABOLISM AND BIOCHEMICAL IMPACT
The Alcohol Dehydrogenase (ADH) Pathway
The Microsomal Ethanol Oxidizing System (MEOS)
The Catalase System
Alcoholism: Biochemical and Metabolic Alterations
Alcohol in Moderation: The Brighter Side
SUMMARY
PERSPECTIVE
THE ROLE OF LIPOPROTEINS AND INFLAMMATION IN ATHEROSCLEROSIS

THE PROPERTY THAT SETS LIPIDS apart from other major nutrients is their solubility in organic solvents such as ether, chloroform, and acetone. If lipids are defined according to this property, as is generally the case, many diverse molecules fit the criteria and are thus considered lipids. Unlike carbohydrates and proteins, classifying lipids on the basis of solubility covers a broad range of molecules with diverse structural and functional properties. Such biological diversity is a benefit to plants and animals due to the many roles lipids play. As body fat, lipids serve as a depot of stored energy, provide protection to internal organs, and insulate against heat loss. Lipids also form the basis of cellular membranes, steroid hormones, bile acids, **eicosanoids**, and other signaling molecules. The roles of the fat-soluble vitamins are discussed in Chapter 10.

The diversity of lipids poses a challenge in creating a classification system beyond their solubility property. A traditional way of classifying lipids is based on how many products result from hydrolysis: "simple" lipids are those yielding two types of products on hydrolysis, whereas "complex" lipids yield three or more products. An alternative way of classifying lipids is based on the products of synthesis. In this system, lipids are defined as molecules arising from two distinct pathways that produce fatty acids (and their derivatives) or **sterols** (and their derivatives). Neither system is entirely adequate for the study of nutrition in which emphasis is placed on the structure and function of lipids. Consequently, the lipids discussed in this chapter are limited to those most relevant to human nutrition and are organized by their structural and functional similarities:

- Fatty acids
- Triacylglycerols, diacylglycerols, and monoacylglycerols
- **Phospholipids**
- **Sphingolipids**
- Sterols (cholesterol, bile acids, and phytosterols).

This chapter also describes lipoproteins—complexes of lipids and proteins—that allow lipids to be transported in the aqueous environment of the blood. Finally, this chapter discusses the metabolism of ethyl alcohol. Although not a lipid, ethyl alcohol is a common dietary component and is catabolized similarly to lipids.

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STRUCTURE AND BIOLOGICAL IMPORTANCE

Fatty Acids

As a class, fatty acids are the simplest of the lipids. They are composed of a hydrocarbon chain with a methyl group at one end and a carboxylic acid group at the other. Therefore, fatty acids have a polar, hydrophilic end and a nonpolar, hydrophobic end that is insoluble in water (Figure 5.1). Fatty acids exist alone or as components of the more complex lipids, discussed in later sections. They are of vital importance as an energy nutrient, furnishing most of the calories derived from dietary fat.

The lengths of the hydrocarbon chains of fatty acids found in foods and body tissues vary from 4 to about 24 carbon atoms, although the most common fatty acids in nature are 18 carbons. The fatty acids may be saturated (SFA), monounsaturated (MUFA, possessing 1 carbon-carbon double bond), or polyunsaturated (PUFA, having 2 or more carbon-carbon double bonds). PUFAs of nutritional interest may have as many as 6 double bonds. Where a carbon-carbon double bond exists, there is an opportunity for either *cis* or *trans* geometric isomerism that significantly affects the molecular configuration and functionality of the molecule. The *cis* isomer results in folding and bending of the molecule into a U-like orientation, whereas the *trans* form has the effect of extending the molecule into a linear shape similar to that of saturated fatty acids. The more carbon-carbon *cis* double bonds occurring within a chain, the more pronounced is the bending effect. The degree of bending plays an important role in the structure and function of cell membranes. The structures in Figure 5.1 illustrate saturation and unsaturation in an 18-carbon fatty acid and show how *cis* or *trans* isomerization affects the molecular configuration.

Most naturally occurring unsaturated fatty acids are of the *cis* configuration, although the *trans* form does appear in some natural plant oils, in dairy products, and lamb

Figure 5.1 Structures of selected fatty acids.

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and beef fat as a result of biohydrogenation by ruminant bacteria. *Trans* fatty acids may also be commercially produced as a result of partial hydrogenation. *Trans* fatty acids resulting from biohydrogenation contain the *trans* double bond mostly at the Δ^{11} carbon, whereas commercial hydrogenation produces a normal distribution of *trans* isomers with the peak around the Δ^{10} isomer. Partial hydrogenation, a process commonly used in making frying oils and commercial food products, is designed to solidify vegetable oils at room temperature. Double bonds of *cis* orientation that are not reduced in the process undergo an isomeric rearrangement to the *trans* form, which is energetically more stable. The hydrogenation of the fatty acids (as part of triacylglycerols) changes the melting point, giving the product a higher degree of plasticity (spreadability) and hardness so that it remains solid at room temperature, which are desirable to both the consumer and the food manufacturer. Partially hydrogenated oils have often been used as frying oils to enhance their stability at frying temperatures. Higher frying temperatures reduce the uptake of fat during cooking. Due to health concerns, the availability of *trans* fatty acids from partially hydrogenated oils in the U.S. food supply has significantly declined in recent years and is currently estimated to be 1.3 g per person per day [1]. The role of *trans* fatty acids in the etiology of cardiovascular disease (CVD) is discussed in the section "Lipids, Lipoproteins, and Cardiovascular Disease Risk" in this chapter.

Fatty Acid Nomenclature

Two systems of notation have been developed to provide a shorthand way to indicate the chemical structure of a fatty acid. Both systems are used regularly and will be used interchangeably in the text for different purposes.

The delta (Δ) system of notation has been established to denote the chain length of the fatty acids and the number and position of any double bonds that may be present. For example, the notation 18:2 $\Delta^{9,12}$ describes linoleic acid. The first number, 18 in this case, represents the number of carbon atoms; the number following the colon refers to the total number of double bonds present; and the superscript numbers following the delta symbol designate the carbon atoms at which the double bonds begin, counting from the carboxyl end of the fatty acid.

A second commonly used system of notation locates the position of double bonds on carbon atoms counted from the methyl, or omega (ω), end of the hydrocarbon chain. For instance, the notation for linoleic acid would be 18:2 ω-6. Substitution of the omega symbol with the letter *n* has been popularized. Using this designation, the notation for linoleic acid would be expressed as 18:2 n-6. In this system, the total number of carbon atoms in the chain is given by the first number, the number of double bonds is given by the number following the colon, and the location (carbon atom number) of the first double bond counting from the methyl end is given by the number following ω- or n-. This system of notation takes into account the fact that double bonds in a fatty acid are always positioned so that they are separated by three carbons. Thus, if you know the total number of double bonds and the location of the first relative to either the methyl or carboxylic end, you can determine the locations of the remaining double bonds.

Figure 5.2 demonstrates the designation of linoleic acid using each of the two systems: 18:2 $\Delta^{9,12}$ (delta) or 18:2 ω-6 or 18:2 n-6 (omega). The fatty acid α-linolenic acid, which contains three double bonds, is identified as 18:3 $\Delta^{9,12,15}$ or 18:3 ω-3 or 18:3 n-3.

Table 5.1 lists some naturally occurring fatty acids and their dietary sources. For unsaturated fatty acids, the table shows the Δ and ω system designations, and commonly used abbreviations. The list includes only those fatty acids with chain lengths of 14 or more carbon atoms because these fatty acids are most important both nutritionally and functionally. For example, palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2) together

Figure 5.2 The structure of linoleic acid, showing the two systems for nomenclature.

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Table 5.1 Some Naturally Occurring Fatty Acids

Notation	Common Name	Formula	Source*
Saturated Fatty Acids			
14:0	Myristic acid	$CH_3-(CH_2)_{12}-COOH$	Coconut and palm kernel oil, fish oils
16:0	Palmitic acid	$CH_3-(CH_2)_{14}-COOH$	All animal and plant fats, notably palm oil
18:0	Stearic acid	$CH_3-(CH_2)_{16}-COOH$	All animal and plant fats, notably cocoa butter
20:0	Arachidic acid	$CH_3-(CH_2)_{18}-COOH$	Peanut oil, wild-caught salmon oil
24:0	Lignoceric acid	$CH_3-(CH_2)_{22}-COOH$	Peanut oil
Unsaturated Fatty Acids			
16:1 Δ^9 (n-7)	Palmitoleic acid	$CH_3-(CH_2)_5-CH=CH-(CH_2)_7-COOH$	Fish oils, poultry fat
18:1 Δ^9 (n-9)	Oleic acid	$CH_3-(CH_2)_7-CH=CH-(CH_2)_7-COOH$	All animal and plant fats
18:2 $\Delta^{9,12}$ (n-6)	Linoleic acid	$CH_3-(CH_2)_4-CH=CH-CH_2-CH=CH-(CH_2)_7-COOH$	Most plant oils, poultry fat
18:3 $\Delta^{9,12,15}$ (n-3)	α-linolenic acid	$CH_3-(CH_2-CH=CH)_3-(CH_2)_7-COOH$	Linseed (flax), soybean, and canola oils
20:4 $\Delta^{5,8,11,14}$ (n-6)	Arachidonic acid	$CH_3-(CH_2)_3-(CH_2-CH=CH)_4-(CH_2)_3-COOH$	Fish oils
20:5 $\Delta^{5,8,11,14,17}$ (n-3)	Eicosapentaenoic acid	$CH_3-(CH_2-CH=CH)_5-(CH_2)_3-COOH$	Marine algae and fish that consume the algae
22:6 $\Delta^{4,7,10,13,16,19}$ (n-3)	Docosahexaenoic acid	$CH_3-(CH_2-CH=CH)_6-(CH_2)_2-COOH$	Marine algae and fish that consume the algae

* Fats and oils in the food supply contain many types of fatty acids of varying proportions. The sources listed here indicate foods that are comparatively enriched in the specific fatty acid.

account for about 90% of the fatty acids in the average U.S. diet. However, shorter-chain fatty acids do occur in nature and are present in the food supply. Butyric acid (4:0) and lauric acid (12:0), for instance, are abundant in milk fat and coconut oil, respectively.

Most fatty acids have an even number of carbon atoms. The reason for this will be evident in the discussion of fatty acid synthesis. Odd-number-carbon fatty acids occur naturally to some extent in some food sources. For example, certain fish, such as menhaden, mullet, and tuna, as well as the bacterium *Euglena gracilis*, contain comparatively high concentrations of odd-numbered-carbon fatty acids.

Essential Fatty Acids

If fat is entirely excluded from the diet of humans, a condition develops that is characterized by retarded growth, dermatitis, kidney lesions, and early death. Studies have shown that eating certain unsaturated fatty acids is effective in curing the conditions related to the lack of these fatty acids. Two unsaturated fatty acids cannot be synthesized in the body and must be acquired in the diet from plant foods. The two essential fatty acids are linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3). They are essential because humans lack enzymes called Δ^{12} and Δ^{15} desaturases, which incorporate double bonds at these positions. These enzymes are found only in plants. Humans are incapable of forming double bonds beyond the Δ^9 carbon in the chain. If a $\Delta^{9,12}$ fatty acid is obtained from the diet, however, additional double bonds can be incorporated at Δ^6 (**desaturation**). Fatty acid chains can also be elongated by the enzymatic addition of two carbon atoms at the carboxylic acid end of the chain. These reactions are discussed further in the "Synthesis of Fatty Acids" section of this chapter.

In mammalian cells, linoleic acid can be converted to arachidonic acid (20:4 n-6) via the so-called "omega-6 pathway." The intermediates in the desaturation and **elongation** pathway are:

linoleic acid (18:2 n-6)
↓
γ−linolenic acid (18:3 n-6)
↓
eicosatriaenoic acid (20:3 n-6)
↓
arachidonic acid (20:4 n-6)

In a similar manner, α-linolenic acid can be converted to eicosapentaenoic acid (20:5 n-3) via the "omega-3 pathway." Both arachidonic and eicosapentaenoic acid are metabolically significant because they are precursors of eicosanoids, important signaling molecules discussed later in this chapter. Linseed (flax) oil is particularly rich in α-linolenic acid, whereas fish oils are good sources of eicosapentaenoic acid and docosahexaenoic acid (22:6 n-3). The fatty acid composition of common fats and oils is given in Table 5.2. It is interesting to note that fatty acid composition in wild-caught salmon is different than farm-raised salmon, due to differences in the diets that these fish consume. Farm-raised salmon are often fed plant sources of protein and fat (corn or soybean meal) that influences their fatty acid composition.

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Table 5.2 Fatty Acid Composition of Fats and Oils

	SFA									MUFA			PUFA					
	Butyric 4:0	Caproic 6:0	Caprylic 8:0	Capric 10:0	Lauric 12:0	Myristic 14:0	Palmitic 16:0	Stearic 18:0	Arachidic 20:0	Palmitoleic 16:1 n-9	Oleic 18:1 n-9	Gadoleic 20:1 n-9	Linoleic 18:2 n-6	Arachadonic 20:4 n-6	α-Linolenic 18:3 n-3	Eicosapentaenoic 20:5 n-3	Docosapentaenoic 22:5 n-3	Docosahexaenoic 22:6 n-3
Plant Oils										% of Total Fat								
Canola Oil							4.3	2.1	0.7	0.2	61.7		18.6		9.1			
Cocoa Butter						0.1	25.4	33.2		0.2	32.6		2.8		0.1			
Coconut Oil		0.8	8.2	5.6	43.7	16.8	8.4	2.5			5.9		1.7					
Corn Oil						0.1	10.6	1.8	0.4	0.1	27.3	0.1	53.2		1.2			
Cottonseed Oil						0.8	22.7	2.3	0.1	0.8	17.0		51.5		0.2			
Flax (Linseed) Oil						0.1	5.1	3.4		0.1	18.3		14.2		53.4			
Olive Oil							11.3	2.0	0.4	1.3	71.3	0.3	9.8		0.8			
Palm Oil					0.1	1.0	43.5	4.3		0.3	36.6	0.1	9.1		0.2			
Palm Kernel Oil		0.2	3.3	3.7	47.0	16.4	8.1	2.8			11.4		1.6					
Peanut Oil						0.1	9.5	2.2	1.4	0.1	44.8	1.3	32.0					
Safflower Oil						0.1	4.3	2.3	0.3	0.1	12.0	0.1	77.7		0.4			
Soybean Oil						0.1	10.6	4.0	0.3	0.1	23.2		53.7		7.6			
Sunflower Oil						0.1	7.0	4.5	0.4	0.1	18.7	0.1	67.5		0.8			
Animal Fats																		
Beef Tallow					0.9	3.7	24.9	18.9	0.1	4.2	36.0	0.3	3.1		0.6			
Chicken Fat					0.1	0.9	21.6	6.0	0.1	5.7	37.3	1.1	19.5		1.0			
Lard (Pork Fat)				0.1	0.2	1.3	23.8	13.5	0.1	2.7	41.2	1.0	10.2		0.1			
Milk (Butter) Fat	3.2	1.9	1.1	2.5	2.8	10.0	26.2	12.1	0.1	2.2	25.0		2.2		1.5			
Fish Oils																		
Herring Oil					0.2	7.2	11.7	0.8		9.6	12.0	13.6	1.1	0.3	0.8	6.3	0.6	4.2
Mackerel Oil						4.9	15.3	3.0		5.2	16.4	7.5	1.6	1.3	1.1	6.5	1.5	10.1
Menhaden Oil						8.0	15.1	3.8		10.5	14.5	1.3	2.2	1.2	1.5	13.2	4.9	8.6
Salmon Oil (wild)						2.1	10.0	3.3		4.0	21.3	3.5	2.7	4.2	4.7	5.1	4.5	17.6
Salmon Oil (farmed)						4.1	14.0	3.7	0.2	5.9	20.2	2.0	6.7	0.7	1.1	6.4	2.9	8.2

Source: U.S. Department of Agriculture, National Nutrient Database for Standard Reference, Release 28.

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n-6 versus n-3 Fatty Acids

It is estimated that our human ancestors consumed foods that provided equal amounts of n-6 and n-3 fatty acids. Today, the intake of n-3 fatty acids is quite low and overwhelmed by n-6 fatty acids in the diet, with linoleic acid providing 80–90% of all PUFA. This is due to the widespread use of plant oils, such as soybean oil, in the production of manufactured food products and in foodservice frying oils, coupled with the relatively low intake of fish and other n-3 fatty acid sources in Western diets. Assessing the metabolic impact of dietary n-6 and n-3 fatty acids is important in the field of nutrition. The disproportionate amounts of n-6 and n-3 fatty acids can have metabolic consequences that are discussed further in the "Synthesis of Fatty Acids" section of this chapter.

Triacylglycerols (Triglycerides)

Most adipose tissue is composed of triacylglycerols, which represent a highly concentrated form of stored energy. (*Triacylglycerols* is the currently accepted name that has replaced the older name *triglycerides*.) Triacylglycerols also account for nearly 95% of dietary fat. Structurally, they are composed of a trihydroxyalcohol, glycerol, to which three fatty acids are attached by ester bonds, as shown in Figure 5.3; the formation of each of these ester bonds liberates a water molecule. The fatty acids may be all the same (a simple triacylglycerol) or different (a mixed triacylglycerol). The fatty acids in triacylglycerols can be all saturated, all monounsaturated, all polyunsaturated, or any combination. Triacylglycerols exist as fats (solid) or oils (liquid) at room temperature, depending on the nature of the component fatty acids. Triacylglycerols that contain a relatively high proportion of short-chain fatty acids or unsaturated fatty acids tend to be liquid oils at room temperature, whereas those made up of saturated fatty acids of longer chain length have higher melting points and thus exist as solid fats.

Carbons 1 and 3 of glycerol are not the same when viewed in a three-dimensional model. Also, when different fatty acids are attached to the first and third carbons of glycerol, the second carbon becomes asymmetric. (See Chapter 3 for a discussion of stereoisomerism.) Enzymes of the body are able to distinguish between the three carbons of glycerol and are generally quite specific. This specificity is important in digesting and synthesizing triacylglycerols, as is discussed later in this chapter.

The specific glycerol hydroxyl group to which a certain fatty acid is attached is indicated by a numbering system for the three glycerol carbons, in much the same way as glyceraldehyde is numbered (see Figure 3.2). This system is complicated somewhat by the fact that the central carbon of the glycerol is asymmetrical when different fatty acids are esterified at the two end carbon atoms and therefore may exist in either the D or the L form. A system of nomenclature called stereospecific numbering (*sn*) has been adopted in which the glycerol is presented as shown in Figure 5.3, with the C-2 hydroxyl group oriented to the left (L) and the carbons numbered 1 through 3 beginning at the top.

Acylglycerols may be composed of glycerol esterified to a single fatty acid (a monoacylglycerol) or to two fatty acids (a diacylglycerol), with the fatty acids attached to any of the three carbons of glycerol. Though present in the body only in small amounts, the mono- and diacylglycerols are important intermediates in some metabolic reactions and may form the basis of other lipid classes. They are also used in processed foods, where they function as emulsifying agents.

Figure 5.3 Linkage of fatty acids to glycerol to form a triacylglycerol. Chain length of fatty acid is (n + 2).
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When triacylglycerols in adipose tissue are used for energy, the fatty acids are cleaved from glycerol by lipases and released from the cell in free (nonesterified) form. The free fatty acids bind to serum albumin and are transported to various tissues for oxidation via the tricarboxylic acid cycle (TCA cycle).

Phospholipids

Phospholipids, as the name implies, are phosphate-containing lipids that form the structural basis of all cell membranes, including the membranes of organelles within the cell (see Figures 1.2 and 1.3). Because of their **amphipathic** properties, phospholipids are also critical components of plasma lipoproteins in which phospholipids, triacylglycerols, and other lipids form stable complexes, thus allowing them to be transported in the blood.

Glycerol forms the structural backbone of phospholipids. Fatty acids are esterified to the hydroxyl groups at the *sn*-1 and *sn*-2 positions of glycerol; a phosphate group is esterified at the *sn*-3 position and, in turn, a polar "head group" is esterified to the phosphate. A phospholipid molecule lacking the head group is known as phosphatidic acid (Figure 5.4). The conventional numbering of the glycerol carbon atoms is the same as that for triacylglycerols, provided the glycerol is written in the L configuration so that the *sn*-2 fatty acid constituent is directed to the left, as shown in Figure 5.4. The fatty acid portion of the molecule is hydrophobic, while the phosphate and the polar head group are hydrophilic, thus giving phospholipids their amphipathic property. Phospholipids generally have a saturated fatty acid at *sn*-1 and an unsaturated fatty acid at *sn*-2, although many fatty acid combinations are possible, resulting in a broad range of distinct phospholipids. Despite this fact, phospholipids are named according to the specific head group rather than their fatty acids. Common head group molecules are choline, ethanolamine, serine, and inositol, each possessing an alcohol group through which esterification to the phosphate takes place (Figure 5.4). The compounds are named as the phosphatidyl derivatives of the alcohols, as indicated in the figure. The most common phospholipid in mammal tissues is phosphatidylcholine, making up about half of the phospholipids in cell membranes, followed by phosphatidylethanolamine in terms of abundance. Food grade phosphatidylcholine (called lecithin) is produced commercially from egg yolks and soybeans and used as an emulsifier in the production of foods that contain both fat and water, such as margarine and chocolate. Phosphatidylserine and phosphatidylinositol are also found in cell membranes, but they serve important functions beyond membrane structure. Phosphatidylserine participates in apoptosis by attracting phagocytes during cellular degradation. Phosphatidylinositol participates in several cell functions, as described in the next section.

Figure 5.4 Typical structure of phospholipids.
Source: Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

Figure 5.5 Structure of diphosphatidylglycerol (cardiolipin).

Diphosphatidylglycerol is another phospholipid found in several tissues of the body. It is also called cardiolipin and was originally identified within heart muscle (Figure 5.5). The structure of cardiolipin can be viewed as two phospholipid molecules that share a common head

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group of glycerol. The overall structure therefore contains three glycerol molecules and four fatty acids. Cardiolipin is located exclusively in the inner membrane of mitochondria and attaches cytochrome c to the membrane.

The main structure of phospholipids described thus far involves ester bonds between the glycerol backbone and the fatty acids at the *sn*-1 and *sn*-2 positions. In some cases that linkage is an ether bond (—C—O—C—) or vinyl ether bond (—C—O—C=C—), resulting in the so-called "ether phospholipids." Platelet-activating factor is perhaps the most studied ether phospholipid, having a fatty acid ether bond at *sn*-1, an acetate ester bond at *sn*-2, and phospho-choline as the head group at *sn*-3. Platelet-activating factor is an important signaling molecule that participates in several metabolic events including inflammation, platelet aggregation, and neural functions. Another well-known ether phospholipid is plasmalogen, which has a fatty acid vinyl ether bond at *sn*-1, a fatty acid ester bond at *sn*-2, and phospho-choline, ethanolamine, or serine as the head group at *sn*-3. Plasmalogens are found in many tissues, most notably the heart and brain. Choline plasmalogen constitutes up to 40% of all phospholipids in the heart, while ethanolamine plasmalogen makes up about 20% of phospholipids in the brain and is concentrated mostly in the myelin sheath.

Biological Roles of Phospholipids

Phospholipids play several important roles in the body. Phospholipids are more polar than the triacylglycerols and sterols, and therefore tend to attract water molecules. Because of their amphipathic nature, phospholipids are found on the surface of blood-borne lipoprotein particles, thereby stabilizing the particles in the aqueous medium. Furthermore, phospholipids are important components of cell and organelle membranes, where they form bilayers (see Chapter 1) and serve as a selective barrier for the passage of water-soluble and fat-soluble materials across the membrane. In addition to lending structural support to the membrane, they serve as a source of physiologically active compounds. We discuss later how arachidonate can be released on demand from membrane-bound phosphatidylcholine and phosphatidylinositol when it is needed for synthesis of eicosanoids.

Phosphatidylinositol plays a specific role in anchoring membrane proteins when the proteins are covalently attached to lipids. Phosphatidylinositols anchor a wide variety of surface antigens and other surface enzymes in eukaryotic cells. In addition, certain hydrolytic products of phosphatidylinositol are active in intracellular signaling and act as second messengers in hormone function. An example of this role is the mechanism of action of insulin (discussed in Chapter 3). Phosphatidylinositol in the plasma membrane can be doubly phosphorylated by ATP, forming phosphatidylinositol-4,5-bisphosphate. Stimulation of the cell by certain hormones, such as insulin, activates a specific phospholipase C, which produces inositol-1,4,5-trisphosphate and diacylglycerol. Both of these products function as second messengers in cell signaling. Inositol-1,4,5-trisphosphate causes the release of Ca^{2+} held within membrane-bound compartments of the cell, triggering the activation of a variety of Ca^{2+}-dependent enzymes and hormonal responses. Diacylglycerol binds to and activates an enzyme, protein kinase C, which transfers phosphate groups to several cytosolic proteins, thereby altering their enzymatic activities. This dual-signal hypothesis of phosphatidylinositol hydrolysis is represented in Figure 5.6 [2].

Figure 5.6 Inositol dual signaling system.

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Sphingolipids

Sphingolipids are found in the plasma membrane of all cells, although their concentration is highest in cells of the central nervous system. Unlike the lipid classes discussed thus far, sphingolipids are built on the amino alcohol sphingosine rather than glycerol as the structural backbone (Figure 5.7). All sphingolipids have a fatty acid attached to the amino group (R^1 in the figure). The simplest sphingolipid is **ceramide**, in which the terminal hydroxyl has no other group attached. The other sphingolipids build on ceramide, with substituent molecules attached to the terminal hydroxyl group (R^2 in the figure). **Sphingomyelin** is formed when phosphocholine is added to ceramide. (Due to the presence of phosphate, sphingomyelin can also be considered a phospholipid, although it makes more sense to classify it primarily as a sphingolipid because of its structural similarity to other sphingolipids.) Sphingomyelin is particularly abundant in the myelin sheath of nerve tissues and thus important for nervous system function.

Cerebrosides are formed when a single sugar molecule, either galactose or glucose, attaches to the terminal hydroxyl group of ceramide. Galactocerebrosides are abundant in the myelin sheath of nerves and in brain tissue, particularly the white matter, whereas glucocerebrosides are found mainly in spleen and red blood cells. Cerebrosides are located on the plasma membranes where they serve a protective role, acting as an insulator and facilitator in the proper conduction of nervous impulses. **Gangliosides** resemble cerebrosides, except they have multiple sugar units linked to the terminal hydroxyl group of ceramide. In addition, gangliosides have a negatively charged sialic acid molecule attached to the oligosaccharide chain. Gangliosides are located on the outer surface of plasma membranes mainly in nerve tissue where they function as markers in cellular recognition and as receptors for certain hormones and toxins including the cholera toxin.

Sterols

Sterols are structurally quite different than the other lipid classes. They are characterized by having a four-ring steroid nucleus and at least one hydroxyl group, hence the name sterol (steroid alcohol). This section describes three categories of sterols important to human nutrition: cholesterol, bile acids, and phytosterols.

Cholesterol

Cholesterol is the most common sterol in humans. It can exist in free form, or the hydroxyl group at C-3 can be esterified with a fatty acid. The structure of cholesterol is shown in Figure 5.8, along with the numbering system for the carbons in the steroid nucleus and the side chain. Cholesterol is an important constituent of plasma membranes along with phospholipids due to its amphipathic nature. In free form, the hydroxyl group of cholesterol interacts with the phospholipid head group so that the hydrophobic side chain of cholesterol is oriented in parallel with the fatty acids of phospholipids (see Figure 1.3). Cholesterol constitutes nearly 25% of the lipids in plasma membranes of some nerve cells, but may be absent in intracellular membranes. Cells can regulate the amount of cholesterol in membranes by esterifying "excess" cholesterol with a fatty acid and storing the cholesterol esters in vesicles within the cytosol. When unesterified (free) cholesterol is needed, the cholesterol esters are hydrolyzed and free cholesterol is transported to the membrane.

Cholesterol serves as the precursor for many important steroids in the body, including the bile acids; steroid sex hormones such as estrogens, androgens, and progesterone; the adrenocortical hormones; and vitamin D (cholecalciferol). The major derivatives of cholesterol are shown in Figure 5.9. These steroids differ structurally from one another in the arrangement of double bonds in the ring system, the presence of carbonyl or hydroxyl groups, and the nature of the side chain at C-17. All of

Sphingolipid	R^1	R^2
Ceramide	Fatty acid	H
Sphingomyelin	Fatty acid	Phosphocholine
Cerebroside	Fatty acid	Galactose or glucose
Ganglioside	Fatty acid	Oligosaccharide

Figure 5.7 Structure of sphingolipids.

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Figure 5.8 Structure of a sterol, cholesterol, and a cholesterol ester.
Source: Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

these structural modifications are mediated by enzymes that function as dehydrogenases, isomerases, hydroxylases, or desmolases. Desmolases remove or shorten the length of side chains on the steroid nucleus.

Bile Acids and Bile Salts

As discussed in Chapter 2, bile acids and bile salts are critical components of bile that act as detergents in the small intestine to emulsify dietary lipids for digestion and absorption. The liver synthesizes two bile acids, cholic acid and chenodeoxycholic acid, each of which is conjugated with either glycine or taurine, resulting in four different primary bile salts (Figure 5.10). After the newly formed bile salts enter the small intestine via bile secretion, they are subject to dehydroxylation by bacteria, thus producing secondary bile salts. All of the bile salts can be reabsorbed into the enterohepatic circulation and returned to the liver. In this way, secondary bile salts,

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Figure 5.9 The formation of physiologically important steroids from cholesterol. Only representative compounds from each category of steroid are shown.

while not directly synthesized by the liver, are present in gallbladder bile.

Phytosterols

Plant cell membranes contain structural sterols in a manner similar to cholesterol in animal cells. These phytosterols are structurally similar to cholesterol, with only slight differences in the side chain (refer to Figure 5.8). Some phytosterols are actually stanols—meaning, the double bond between carbons 5 and 6 is eliminated by saturating the molecule with hydrogen atoms. Strictly speaking, stanols are chemically different than sterols, but they are often counted together under the heading of phytosterols. Stanols constitute about 5–10% of total phytosterols present in nature. The hydroxyl group at C-3 of phytosterols can be esterified with a fatty acid.

Phytosterols are found throughout the food supply. Plant oils, legumes, nuts, and seeds have relatively high concentrations of phytosterols, while fruits and vegetables have low concentrations. Although cereal grains have only modest concentrations, humans consume large amounts of grain products, making them a quantitatively important source of phytosterols. Intake of total phytosterols from natural food sources is about 200–300 mg/day, with Asian and vegetarian diets providing significantly more [3]. The manufacture of foods and supplements enriched with phytosterols has increased in recent years because of their cholesterol-lowering properties. Phytosterol intake of 2 g/day results in blood cholesterol reductions of 10% or more. Approval to make health claims about phytosterols on food and supplement products has been granted by the U.S. Food and Drug Administration, the European Foods Safety Authority, and Health Canada. Because of their similarity to cholesterol, phytosterols have the ability to displace cholesterol from micelles that form during digestion, reducing the amount of cholesterol available for intestinal absorption.

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Figure 5.10 The formation of glycocholate, taurocholate, glycochenodeoxycholate, and taurochenodeoxycholate conjugated bile acids.

DIETARY SOURCES

Triacylglycerols—fats and oils—are ubiquitous in the food supply. They are found naturally in both plant and animal foods. They are also present in prepared and manufactured foods. Foods prepared in restaurants often contain high-fat ingredients and are cooked in oil; grocery stores are abundant with prepared and packaged foods containing fat; and many consumers use fats and oils when cooking at home. In order to track the amount and type of fat consumed in the United States, the Agricultural Research Service of the U.S. Department of Agriculture documents each year the major food groups that contribute fat to the food supply [4]. As indicated in Figure 5.11, the primary source of fat is the "Cooking Oils" category, which represents all uses of edible oils (mostly plant derived) in the United States, including those used in the food industry for the manufacture of food products; cooking and frying oils used by restaurants and other foodservice institutions; and salad and cooking oils used directly by consumers. The "Shortening" category represents solid fats (mostly plant derived) that are used for similar purposes as "Cooking Oils". Figure 5.11 further shows that the food categories of animal origin contribute significant amounts of fat in the food supply. Note that "Butter" has been separated from "Dairy Products" to emphasize its individual contribution to overall fat intake. The "Other" category includes fat contributed by fruits, vegetables, fish, and specialty oils.

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Figure 5.11 Dietary fat contribution from major food groups.
Source: U.S. Department of Agriculture, Nutrient Content of the U.S. Food Supply, 2010.

The proportion of SFA, MUFA, and PUFA comprising the fat of each food group is also illustrated in Figure 5.11. The majority of SFA is contributed by cooking oils, red meats, and dairy products. Although the relative amount of SFA in cooking oils is lower than red meats and dairy products, the widespread use of cooking oils means they provide about 20% of all SFA in the food supply. Cooking oils (mostly plant derived) supply nearly all of the PUFA consumed in the United States, of which 80–90% is linoleic acid. Cooking oils, shortening, and red meats provide most of the MUFA, of which > 90% is oleic acid. Knowing the fatty acid composition of common fats and oils (Table 5.2) can help guide health care professionals and consumers in making well-informed decisions about dietary fats.

Although the information in Figure 5.11 shows only the major food groups, it is helpful to know the main contributors of fat when seeking to modify one's fat intake. For example, if reduction in total fat intake is desired, focusing on all foods made with or cooked in "cooking oils" would be a good starting point. Reduction in red meats, dairy products, and foods made with shortening would also be advisable. If reducing SFA is the goal, the obvious targets are red meats and dairy products because of their relatively high proportion of SFA. However, products containing cooking oils and shortening should not be overlooked, particularly plant-derived solid fats that have relatively high proportions of SFA, such as palm kernel, palm, and coconut oils. Table 5.3 provides the fat content of foods commonly consumed in the United States. In updating the 2015 Dietary Guidelines for Americans, the Advisory Committee emphasized that "strong and consistent evidence from [randomized controlled trials] shows that replacing SFA with unsaturated fats, especially PUFA, significantly reduces total and LDL cholesterol . . . and reduces the risk of [cardiovascular disease] events and coronary mortality" [5]. These relationships are discussed later in the chapter.

Table 5.3 Fat Content of Common Foods

		SFA	MUFA	n-6 PUFA	n-3 PUFA
		grams			
Bean burrito	1 each	4.3	2.3	3.5	0.6
Beef, top sirloin, broiled	3 oz	3.1	3.4	0.3	0.0
Beef, ribeye, broiled	3 oz	4.9	5.1	0.4	< 0.1
Butter	1 Tbsp	7.3	3.0	0.4	< 0.1
Cheddar cheese	1 oz	5.3	2.6	0.3	< 0.1
Chicken breast, with skin, fried	1 med	4.9	7.6	4.0	0.2
Chicken breast, without skin, baked	1 med	0.9	1.1	0.6	< 0.1
Coconut "milk," regular	1 cup	42.7	2.1	0.5	0.0
Corn dog	1 each	2.7	3.7	2.4	0.2
Dressing, olive oil and vinegar	2 Tbsp	1.9	9.9	1.3	< 0.1
Dressing, ranch	2 Tbsp	2.1	2.8	6.7	1.1
Eggs, hard-boiled	2 large	1.6	2.0	0.6	< 0.1
Fish, Atlantic mackerel, baked	3 oz	3.5	6.0	0.2	3.4
Fish, salmon, baked	3 oz	1.4	2.3	0.3	1.8
Fish, whitefish, breaded, fried	3 oz	3.1	2.7	6.2	0.4
Ham, sliced	3 oz	2.5	3.7	0.6	< 0.1
M&Ms candies, 1.7 oz	1 pkg	6.3	2.5	0.4	< 0.1
Nuts, almonds	1 oz	1.2	9.4	3.7	< 0.1
Nuts, peanuts	1 oz	2.2	7.4	2.8	< 0.1
Nuts, walnuts	1 oz	1.7	2.5	10.8	2.6
Popcorn, microwave, regular	1 bag	12.7	0.3	3.6	0.0
Popcorn, microwave, light	1 bag	1.2	3.6	2.9	0.2

Source: USDA, Nutrient Database for Standard Reference, Release 28.

The intake of *trans* fatty acid (1.3 g/day) is relatively minor, although they may be found in shortening, cooking oils, red meats (beef), dairy products, butter, margarine, and tallow. As mentioned earlier in the chapter, partially hydrogenated oils have been used extensively as frying oils

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and as an ingredient in manufactured products because of their superior functional properties that include greater shelf-life and stability at high frying temperatures. However, their use has significantly declined because of health concerns about *trans* fatty acids. Federal labeling requirements for packaged foods, laws restricting their use, and grassroots efforts have contributed to the decreased use of partially hydrogenated oils. Food labeling regulations currently require products to specify the amount of *trans* fatty acids per serving. Food processors want to be able to label their products as having "zero *trans* fat." One strategy to accomplish this is to use a blend of natural oils containing a chain length and unsaturation level that provide the desired properties without any hydrogenation. For example, margarines that once contained hydrogenated oils may now contain a blend of soybean oil (liquid at room temperature) with palm and/or palm kernel oil (solid at room temperature). Note that labeling regulations permit a food containing less than 0.5 g of *trans* fat per serving to be labeled as providing zero. Consuming several servings of foods labeled as containing zero *trans* fat can result in consuming more than the recommended level.

Recommended Intakes

Recommendations regarding dietary fat and fatty acids have historically come from several governmental and nongovernmental (nonprofit) organizations, including the American Heart Association, the Institute of Medicine (IOM), the U.S. Department of Agriculture, and the U.S. Department of Health and Human Services. These organizations work together in order to provide a cohesive message when making recommendations to the public.

The Food and Nutrition Board of the IOM has not established a Recommended Dietary Allowance (RDA) value for total fat intake. Adequate Intake (AI) levels have been established for infants, but not for adults or children over the age of 12 months (see inside front cover of the book). Rather than focusing on total fat, current recommendations and guidelines focus on specific fatty acids due to their individual effects associated with the prevention or promotion of disease.

AIs are established for the essential fatty acids, linoleic (18:2 n-6) and α-linolenic acid (18:3 n-3), at levels that prevent deficiency symptoms. The AI for α-linolenic acid is also set at levels believed to provide overall health benefits associated with the consumption of n-3 fatty acids (discussed later in the chapter). Table 5.2 shows flax (linseed) oil as having the highest percentage of α-linolenic acid among the common plant oils, followed by canola and soybean oils. α-Linolenic acid serves as a precursor for the highly unsaturated n-3 fatty acids (EPA and DHA), although the conversion efficiency of α-linolenic to EPA and DHA acid is very low in humans. Therefore, consumption of EPA and DHA (present in fatty fish) can avert deficiencies associated with low α-linolenic acid.

Trans fatty acids appear to provide no specific health benefits beyond providing energy. Therefore, no RDA or AI has been set. While it may seem tempting to establish an Upper Limit (UL) for *trans* fatty acids, they are unavoidable in the food supply and restricting intake of "natural" foods containing *trans* fatty acids, such as dairy products and meats, could have the unintended effect of creating deficiencies in other essential nutrients, notably high-quality protein and vitamins D and B_{12}. Nevertheless, it is recommended that *trans* fatty acid consumption be as low as possible while consuming a nutritionally adequate diet.

The Dietary Guidelines for Americans recommend limiting SFA intake to 10% of total calories and that unsaturated fatty acids should be the primary source of dietary fat. Whether SFA are replaced by MUFA or PUFA depends on the dietary strategy employed, but in either case will likely result in health benefits. When SFA are replaced with PUFA, it is recommended that n-3 PUFA be selected to minimize the metabolic effects of "too much" n-6 linoleic acid. With regard to MUFA, much attention has focused on the so-called Mediterranean diet. Defining this diet has been challenging, but its general characteristics include high levels of MUFA intake (largely from olive oil) and significantly lower PUFA intake compared to the United States [6]. The Mediterranean diet also includes relatively high amounts of fiber and protein.

DIGESTION

Dietary lipids are hydrophobic and therefore pose a special problem to digestive enzymes. Like all proteins, digestive enzymes are hydrophilic and normally function in an aqueous environment. The dietary lipid targeted for digestion is emulsified by an efficient process, mediated mainly by bile salts. This emulsification greatly increases the surface area of the dietary lipid, consequently increasing the accessibility of the fat to digestive enzymes.

Triacylglycerols, phospholipids, cholesterol, and phytosterols provide the lipid component of the typical Western diet. Of these, triacylglycerols, commonly called fats or triglycerides, are by far the major contributor. The National Health and Nutrition Examination Survey (NHANES) for the years 2011–2012 found that males 20 years and over consume an average of 94 g/day and females 20 years and over consume an average of 67 g/day [7]. The intake of cholesterol is significantly less, estimated to be 338 and 229 mg/day for the same groups, respectively [7]. Phytosterol intake is not tracked by NHANES, but, as mentioned earlier, it is about 200–300 mg/day, similar to cholesterol intake. Also not tracked by NHANES is phospholipid

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intake, although it is estimated to be about 2–3 g/day. Digestive enzymes involved in breaking down dietary lipids in the gastrointestinal (GI) tract are esterases that cleave the fatty acid ester bonds within triacylglycerols (lipases), phospholipids (phospholipases), cholesterol esters (cholesterol esterase), and phytosterol esters (also cholesterol esterase).

Triacylglycerol Digestion

Most dietary triacylglycerol digestion is completed in the lumen of the small intestine, although the process actually begins in the mouth and stomach with lingual lipase released by the serous gland, which lies beneath the tongue, and gastric lipase produced by the chief cells of the stomach. Basal secretion of these lipases apparently occurs continuously but can be stimulated by neural sympathetic agonists, high dietary fat, and sucking and swallowing. These lipases account for limited triacylglycerol digestion (10–30%) that occurs in the stomach. The lipase activity is made possible by the enzymes' particularly high stability at the low pH of the gastric juices. Gastric lipase readily penetrates milk fat globules without substrate stabilization by bile salts, a feature that makes it particularly important for fat digestion in the suckling infant, whose pancreatic function may not be fully developed. Both lingual and gastric lipases act preferentially on triacylglycerols containing medium- and short-chain fatty acids. They preferentially hydrolyze fatty acids at the *sn*-3 position, releasing a fatty acid and 1,2-diacylglycerols as products. This specificity again is advantageous for the suckling infant because in milk, triacylglycerols' short- and medium-chain fatty acids are usually esterified at the *sn*-3 position [8]. Short- and medium-chain fatty acids are metabolized more directly than are long-chain fatty acids—that is, they can be absorbed into the blood and transported directly to the liver via the hepatic portal vein, as discussed later in this chapter. Commercially available high-energy formulas for preterm infants, which are rich in triacylglycerols containing short- and medium-chain fatty acids esterified at the *sn*-3 position, are designed to take advantage of the lipases' specificity. These products supply ample energy to the premature infant in a small volume [8].

For dietary triacylglycerols to be hydrolyzed by lingual and gastric lipases in the stomach, some degree of emulsification must occur to expose a sufficient surface area of the substrate. Muscle contractions of the stomach and the squirting of the fat through a partially opened pyloric sphincter produce shear forces sufficient for emulsification. Also, potential emulsifiers in the acid milieu of the stomach include complex polysaccharides, phospholipids, and peptic digests of dietary proteins. The presence of undigested lipid in the stomach delays the rate at which the stomach contents empty, presumably by way of hormones of the enterogastrone family such as secretin, which inhibits gastric motility. Dietary fats therefore have a "high satiety value."

The partially hydrolyzed lipid emulsion leaves the stomach and enters the duodenum as small lipid droplets. Further emulsification takes place because as mechanical shearing continues, it is complemented by bile salts that are released from the gallbladder as a result of stimulation by the hormone cholecystokinin (CCK). The small intestine has the capacity to digest a large quantity of triacylglycerols: up to 600 g with 95% efficiency [9]. Significant hydrolysis and absorption, especially of the long-chain fatty acids, require less acidity, appropriate lipases, more effective emulsifying agents (bile salts), and specialized absorptive cells. These conditions are provided in the lumen of the upper small intestine.

The pancreas simultaneously releases pancreatic lipase and bicarbonate, elevating the pH to a level suitable for pancreatic lipase activity. In combination with bile salts, the triacylglycerol breakdown products (free fatty acids and mono- and diacylglycerols) are themselves excellent emulsifying agents due to their amphipathic properties. Such molecules tend to arrange themselves on the surface of small fat particles, with their hydrophobic regions pointed inward and their hydrophilic regions turned outward toward the water phase. This chemical action, together with the help of peristaltic agitation, converts the fat into small droplets with a greatly increased surface area. The small droplets then can be readily acted upon by pancreatic lipase.

The action of pancreatic lipase on ingested triacylglycerols results in a complex mixture of diacylglycerols, monoacylglycerols, and free fatty acids. Its specificity is primarily toward *sn*-1-linked fatty acids and secondarily to *sn*-3 bonds. Therefore, the digestive action of pancreatic lipase progresses from triacylglycerols → 2,3-diacylglycerols and 1,2-diacylglycerols → 2-monoacylglycerols. Only a small percentage of the triacylglycerols is hydrolyzed totally to free glycerol. The complete hydrolysis of triacylglycerols that does occur probably follows the isomerization of the 2-monoacylglycerol to 1-monoacylglycerol, which is then hydrolyzed. Thus, the action of pancreatic lipase produces mostly 2-monoacylglycerols and free fatty acids that gradually shrink the size of the small fat droplet, finally resulting in bile salt–stabilized micelles. An overview of triacylglycerol digestion is summarized in Table 5.4.

An inhibitor of gastric and pancreatic lipase, orlistat, has been developed to reduce the absorption of dietary triacylglycerols. It is marketed both as Xenical, a prescription-only product, and Alli, an over-the-counter product. The rationale for use is that when the hydrolysis of triacylglycerols is restricted, less dietary fat will be absorbed, resulting in decreased caloric intake. Xenical inhibits the absorption about 30%, equivalent to a reduction of about 200 kcal from fat per day. As one might

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Table 5.4 Overview of Triacylglycerol Digestion

Location	Major Events	Required Enzyme or Secretion	Details
Mouth	Triacylglycerol → *Minor amount of digestion* → Triacylglycerols, diacylglycerols, and fatty acids	Lingual lipase produced in the salivary glands	
Stomach	*Additional digestion* → Triacylglycerol, diacylglycerol, and fatty acids	Gastric lipase produced in the stomach	
Small intestine	*Phase I: Emulsification* → Emulsified triacylglycerols, diacylglycerols, and fatty acid micelles → *Phase II: Enzymatic digestion* → Monoacylglycerols and fatty acids	Bile; no lipase Pancreatic lipase produced in the pancreas	

expect, the most frequently reported side effects of orlistat are gastrointestinal discomfort, fecal incontinence, and steatorrhea (presence of fat in feces).

The Role of Colipase

Pancreatic lipase activation is complex, requiring the participation of the protein colipase, calcium ions, and bile salts. Colipase is formed by the hydrolytic activation by trypsin of procolipase, also of pancreatic origin. It contains approximately 100 amino acid residues and possesses distinctly hydrophobic regions that are believed to act as lipid-binding sites. Colipase has been shown to associate strongly with pancreatic lipase and therefore may act as an anchor, or linking point, for attachment of the enzyme to the bile salt–stabilized fat droplet.

Phospholipid Digestion

Phospholipids are hydrolyzed by a specific esterase, phospholipase A_2, made and secreted by the pancreas. Recall from Chapter 2 that, in addition to dietary sources of phospholipid, the bile releases significant amounts of phospholipid (specifically phosphatidylcholine) into the small intestine, perhaps five times more than the diet provides. Both dietary and biliary phospholipid is subject to hydrolysis by phospholipase A_2, which targets the fatty acid at the *sn*-2 position of glycerol. The products of hydrolysis are lysophospholipid and a free fatty acid. These products, together with the products of triacylglycerol digestion and bile salts, incorporate into the resulting micelles for transport to the intestinal cell. Micelles that contain hydrolyzed lipids are negatively charged and have a much smaller diameter (~5 nm) than the unhydrolyzed precursor particles, allowing them access to the intramicrovillus spaces (50–100 nm) of the intestinal membrane.

Cholesterol Ester Digestion

Some of the cholesterol present in food is esterified with a fatty acid. About 10% of the cholesterol in egg yolks is esterified, whereas about 50% in meat and poultry is esterified.

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Figure 5.12 Summary of digestion and absorption of dietary lipids.
Abbreviations: TAG, triacylglycerol; C, cholesterol; CE, cholesterol ester; PL, phospholipid; DAG, diacylglycerol; MAG, monoacylglycerol; FA, fatty acid; and α-GP, α-glycerolphosphate.

Cholesterol esters cannot be absorbed and therefore must be hydrolyzed to free cholesterol and free fatty acid to be incorporated into micelles for delivery to intestinal cells. Hydrolysis is achieved by cholesterol esterase, made and secreted by the pancreas. Free cholesterol from the diet (and from bile) requires no digestion and can directly incorporate in micelles. Cholesterol esterase also hydrolyzes phytosterol esters consumed in the diet. As previously mentioned, free phytosterols can displace cholesterol from the micelle, resulting in less cholesterol being available for absorption. A summary of the digestion of lipids is shown in Figure 5.12.

ABSORPTION

Micelles contain the final digestion products from lipid hydrolysis, including free long-chain fatty acids, 2-monoacylglycerols, lysophospholipids, free cholesterol, and phytosterols, as well as fat-soluble vitamins. Stabilized by the polar bile salts, the micellar particles are sufficiently water soluble to penetrate the unstirred water layer that bathes the enterocytes of the small intestine. Micelles are small enough to interact with the microvilli at the brush border, whereupon their lipid contents move into the enterocytes. The term *absorption* refers to an overall process that includes the transport of digested lipids from the intestinal lumen across the brush border membrane; the reassembly of those lipids by esterification; and finally the release of the lipids into the circulation.

Fatty Acid, Monoacylglycerol, and Lysophospholipid Absorption

The mechanism for moving fatty acids, monoacylglycerols, and lysophospholipids across the brush border membrane is not fully understood, although two general mechanisms

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have been suggested involving a protein-independent diffusion model and a protein-dependent model. Diffusion across the brush border membrane occurs when the concentration in the intestinal lumen exceeds that of the cell. Diffusion is made possible because of the similar amphipathic nature of both digestion products and membrane lipids, which allows the fatty acids, monoacylglycerols, and lysophospholipids to associate with the membrane lipids as they pass into the cell interior. Protein-dependent transport appears to involve transporters that are lipid specific.

An important protein transporter of fatty acids in enterocytes is called CD36, located on the brush border. CD36 is expressed in a variety of cells throughout the body where it serves as a binding site for other molecules in addition to fatty acids [10]. CD36 also transports monoacylglycerols into the cell, but whether it transports lysophospholipids is unknown. Well-defined transport proteins for lysophospholipids are known to exist in yeast, although the presence of similar proteins in mammalian cells, while assumed to exist, have not been reported in the scientific literature.

Other proteins implicated in fatty acid uptake into enterocytes are a family of proteins called the fatty acid transport proteins (FATP), particularly FATP4. However, unlike CD36, which functions on the brush border membrane, FATP4 resides primarily in intracellular membranes where it facilitates the attachment of coenzyme A to fatty acids already in the cell, thereby priming the fatty acids for synthesis of triacylglycerols, phospholipids, or cholesterol esters. Note that dietary lipids require digestion for transport across the brush border membrane, but in order to move them out of the enterocyte, the lipids need to be reassembled into triacylglycerols, phospholipids, or cholesterol esters. Consequently, FATP4 promotes the absorption of fatty acids by facilitating the flow of lipids through the enterocyte.

In the enterocyte the products of lipid digestion (free fatty acids, monoacylglycerols, and lysophospholipids) move to the endoplasmic reticulum where they are re-esterified. There appears to be specific transport proteins, called fatty acid binding proteins (FABP), that carry them in the aqueous cytosol. FABP were first discovered to carry fatty acids (hence the name), but have since been reported to transport lysophospholipids and monoacylglycerols [10]. Once in the endoplasmic reticulum, acyltransferases transfer the fatty acid–CoA molecules onto the monoacylglycerol and lysophospholipid to produce triacylglycerol and phospholipid, respectively. Note that triacylglycerols can also be synthesized from α-glycerophosphate in the enterocytes. This metabolite can be formed either from the phosphorylation of free glycerol or from reduction of dihydroxyacetone phosphate, an intermediate in the pathway of glycolysis (see Figure 3.17).

Cholesterol Absorption

Cholesterol that enters the small intestine comes from two sources: the diet and bile. As previously mentioned, dietary intake of cholesterol is about 300 mg/day, whereas the bile contributes 800–1,400 mg/day. Because the majority of cholesterol available for absorption is of hepatic origin, the efficiency of absorption can affect how much cholesterol is retained in the body. Cholesterol not absorbed is excreted in the feces. Given that no oxidative pathway for cholesterol exists in humans, fecal excretion represents the primary catabolic route in which whole-body cholesterol homeostasis in maintained. Therefore, the efficiency of cholesterol absorption is a critical point of regulation and the target of drug and dietary therapies that block absorption and promote the removal of cholesterol from the body [11].

Cholesterol in the intestine must incorporate into micelles for delivery to the enterocyte. Uptake by the cell is mediated by a brush border protein called Niemann-Pick C1 like 1 (NPC1L1). Once inside the cell, cholesterol is carried through the cytosol by sterol carrier proteins. Cholesterol may incorporate into enterocyte membranes, although the majority is esterified in preparation for transport out of the cell as a component of large lipid-protein aggregates called **chylomicrons**. Cholesterol esterification is catalyzed by acyl-CoA:cholesterol acyltransferase 2 (ACAT2), which is required for chylomicron formation to occur.

Phytosterols are also transported into the intestinal cell by NPC1L1. Despite the ability of NPC1L1 to transport both cholesterol and phytosterols, essentially no phytosterols incorporate into chylomicrons or enter the circulation. This is due to the presence of two additional proteins—members of the ATP-binding cassette (ABC) transporter family called ABCG5 and ABCG8—that reside adjacent to NPC1L1 in the brush border membrane. The role of ABCG5 and ABCG8 is to redirect phytosterols back into the intestinal lumen immediately after being taken into the cell. ABCG5 and ABCG8 also redirect some cholesterol back into the intestinal lumen, so that the overall efficiency of cholesterol absorption is about 50–60% [11]. A rare autosomal recessive disorder called sitosterolemia can occur as a result of mutations in either ABCG5 or ABCG8, causing hyperabsorption of cholesterol and phytosterols.

Strategies to block cholesterol absorption date back to the 1950s when patients with elevated blood cholesterol were given a commercial preparation of phytosterols suspended in fruit-flavored syrup (marketed as *Cytellin*). Phytosterols are known to displace cholesterol from micelles and compete for binding to NCP1L1. The product had limited success and was largely replaced by powerful prescription drugs, including ezetimibe, which directly inhibits NPC1L1, resulting in significant reductions (about 18%) in blood cholesterol levels. For patients who cannot tolerate prescription drugs, foods and supplements enriched with phytosterols are increasingly available and effective at reducing blood cholesterol concentration by 10% or more [3].

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Lipid Release into Circulation

For dietary lipids to be fully absorbed into the circulation, they must first be packaged in a form that allows for transport in the aqueous bloodstream. Lipids that are re-esterified in the endoplasmic reticulum of the enterocytes are assembled into large lipid-protein aggregate structures called chylomicrons [12]. The formation of chylomicrons occurs in direct response to eating a fat-containing meal; therefore, the proportions of the various lipids in chylomicrons reflect that of the diet. Chylomicrons are spherical particles containing mostly triacylglycerols and some cholesterol esters in the core (due to their hydrophobicity), with amphipathic phospholipids, free cholesterol, and protein on the surface. The main protein added to the particle surface is called **apolipoprotein** B-48 (apoB-48) that helps stabilize the triacylglycerol-rich chylomicron in the aqueous environment of the circulation. Chylomicrons are released from the enterocytes by exocytosis into the lymphatic system, where they travel a few inches via the thoracic duct to the left subclavian vein, at which point they enter the systemic blood circulation. The metabolic advantage of first entering the lymphatic system is to bypass the liver, an organ that would have catabolized the chylomicrons if they had entered the hepatic portal vein. This short detour around the liver allows chylomicrons to deliver their triacylglycerol cargo to other tissues such as muscle and adipose tissue (discussed in detail in the next section).

Medium-chain fatty acids (those containing 6–12 carbon atoms), if present in the diet, have the ability to pass from the enterocyte directly into the portal blood, where they bind to albumin and are transported directly to the liver. Most of the medium-chain fatty acids escape esterification in the enterocyte and enter the portal blood as free fatty acids. The different fates of the long- and medium-chain fatty acids result from the specificity of the acyl-CoA synthetase enzymes for long-chain fatty acids. Triacylglycerols containing medium-chain fatty acids are used clinically to treat patients with intestinal disorders because the medium-chain fatty acids can be absorbed directly to the portal blood without the need for chylomicron formation. Key features of intestinal digestion and absorption of lipids are depicted in Figure 5.12.

TRANSPORT AND STORAGE

Lipids are transported in the blood as components of highly organized lipid-protein complexes (or particles) called **lipoproteins**. Chylomicrons, as mentioned in the previous section, are a class of lipoproteins. The other classes are very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Each lipoprotein class participates in transport systems that can be defined as *exogenous (dietary) lipid transport, endogenous lipid transport*, and *reverse cholesterol transport*. In this section, the structure of lipoproteins is first described, followed by a discussion on the lipid transport systems and the central role of the liver.

Lipoprotein Structure

All lipoproteins share similar structural features in which hydrophobic, nonpolar "neutral" lipids (triacylglycerols and cholesterol esters) reside in the spherical core, surrounded by a monolayer of amphipathic phospholipids and free cholesterol that partitions the neutral lipid from the aqueous environment. Added to the surface are proteins (called apolipoproteins or apoproteins) that impart structural stability and functionality by serving as enzyme activators or ligands for cell receptors. The arrangement of the lipid and protein components of a typical lipoprotein is represented in Figure 5.13. The illustration depicts the apoproteins as being either peripheral (residing mostly on the external

Figure 5.13 Generalized structure of a plasma lipoprotein.

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Table 5.5 Apolipoproteins of Human Plasma Lipoproteins

Apolipoprotein	Lipoprotein(s)	Molecular Mass (Da)	Additional Remarks
apoA-1	HDL, chylomicrons	28,000	Activator of lecithin: cholesterol acyltransferase (LCAT); ligand for HDL receptor
apoA-2	HDL, chylomicrons	17,000	Structure is two identical monomers joined by a disulfide bridge
apoA-4	Secreted with chylomicrons but transfers to HDL	46,000	Associated with the formation of triacylglycerol-rich lipoproteins; function unknown
apoB-100	LDL, VLDL, IDL	550,000	Synthesized in liver; ligand for LDL receptor
apoB-48	Chylomicrons, chylomicron remnants	260,000	Synthesized in intestine
apoC-1	VLDL, HDL, chylomicrons	7,600	Possible activator of LCAT
apoC-2	VLDL, HDL, chylomicrons	8,916	Activator of extrahepatic lipoprotein lipase
apoC-3	VLDL, HDL, chylomicrons	8,750	Several polymorphic forms depending on content of sialic acids
apoD	Subfraction of HDL	20,000	Function unknown
apoE	VLDL, HDL, chylomicrons, chylomicron remnants	34,000	Ligand for chylomicron remnant receptor

surface of the lipoprotein) or integral (having multiple regions that span the phospholipid monolayer). The apoproteins are abbreviated "apo" and are identified using letters and numbers. Each lipoprotein class will have a complement of apoproteins that is characteristic of that class; for example, chylomicrons are defined by having apoB-48, apoA-1, apoC-2, apoE, and so on. A partial listing of the apoproteins—together with their molecular weight, the lipoprotein class with which they are associated, and their postulated physiological function—is found in Table 5.5.

Figure 5.14 Lipid and protein of lipoprotein classes.
Source: Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

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In addition to the apoprotein composition, each lipoprotein class has its own characteristic lipid composition, physical properties, and metabolic function. Initially, lipoproteins were separated from serum by electrophoresis and therefore were named based on their movement in an electrical gradient. Later, they were separated by centrifugation and were named based on their density. These names persist even though other methods are often used for their separation. Lipoproteins with higher proportions of lipid have a lower density. The largest and least dense lipoproteins are the chylomicrons, having a high lipid:protein ratio. The smallest and most dense are HDL, having a low lipid:protein ratio. The relative percentage of lipids and protein in each lipoprotein class is shown in Figure 5.14.

Lipoprotein Metabolism

The main function of lipoproteins is to transport lipids in the blood. Each lipoprotein class is specialized with regard to which lipids are transported, where the lipids are delivered, and the lipoprotein's metabolic fate after the job is completed. The *exogenous lipid transport* system involves chylomicrons and refers to the transport of dietary lipids, primarily triacylglycerols, from the intestine to peripheral tissues for storage or energy utilization. This system operates only after a fat-containing meal. Chylomicrons disappear after all of the dietary triacylglycerols are delivered to target tissues. The *endogenous lipid transport* system involves VLDL, IDL, and LDL and refers to the transport of triacylglycerols from the liver to peripheral tissues for storage or energy utilization. This system operates continuously to maintain proper balance of fatty acids and triacylglycerols that accumulate in the liver during normal metabolism. *Reverse cholesterol transport* involves HDL and refers to the ability of HDL to pick up excess cholesterol from peripheral tissues and deliver it to the liver for excretion from the body (via bile) or conversion to other important molecules (see Figure 5.9).

Exogenous Lipid Transport

Immediately after a fat-containing meal, the exogenous (dietary) lipids are packaged into chylomicrons within the enterocyte and distributed to peripheral tissues, mainly muscle and adipose tissue. When chylomicrons are released from the enterocyte, they contain mostly triacylglycerols, reflecting the abundance of triacylglycerols in the diet. They also contain apoB-48 and apoA-1. The apoB-48 protein made by the intestine is related to apoB-100 (made by the liver), in that both arise from the same gene, although the intestinal cell contains a stop codon that results in a truncated protein that is 48% of the sequence of apoB-100. After chylomicrons enter the blood, they acquire more apoproteins (mainly apoE and apoC-2) from HDL as the lipoproteins interact in the circulation. The exogenous lipid transport system and chylomicron metabolism are illustrated in Figure 5.15.

Figure 5.15 Exogenous lipid transport.
Abbreviations: TAG, triacylglycerol; MAG, monoacylglycerols; PL, phospholipid; HL, hepatic lipase; LRP, LDL receptor-related protein; C, cholesterol and cholesterol esters.
Source: Modified from *Harpers Illustrated Biochemistry*, Figure 25-3, page 221, by R. K. Murray, D. K. Rodwell, and W. Victor, 27th edition (Lange Medical Books/McGraw-Hill 2006).

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Chylomicrons enter the bloodstream at a relatively slow rate, which prevents excessive increases in blood triacylglycerol levels. Entry of chylomicrons into the blood can continue for up to 14 hours after consumption of a large meal rich in fat. Blood triacylglycerol concentration usually peaks 30 minutes to 3 hours after a meal and returns to near normal within 5–6 hours. These times can vary, however, depending on the stomach emptying time, which in turn depends on the size and composition of the meal. The presence of triacylglycerol-laden chylomicrons accounts for the turbidity (milky appearance) of postprandial plasma and can interfere with clinical readings when "fasting triglyceride" values are desired. Twelve hours of fasting is usually required to obtain true readings that are devoid of chylomicrons.

Circulating chylomicrons interact with tissues that express the enzyme lipoprotein lipase, primarily skeletal muscle, heart muscle, and adipose tissue (but not liver). This interaction occurs due to the presence of apoC-2, which activates the enzyme. Chylomicrons dock on the cell surface where lipoprotein lipase hydrolyzes the triacylglycerols, producing free fatty acids and 2-monoacylglycerols that are quickly taken up into the cells. As the chylomicron becomes depleted of its core triacylglycerols, the lipoprotein structure shrinks in size, yet retains the other lipids. About 80% of the chylomicron triacylglycerols are delivered to target tissues in this manner. Once depleted of its triacylglycerols, the chylomicron "remnant" particles separate from the cell surface and reenter the circulation. The **chylomicron remnants** may donate some of its apoproteins to HDL. The chylomicron remnants travel to the liver where a specific receptor recognizes their apoE component, enabling the uptake of the entire particle into hepatocytes. The receptor is called LDL receptor–related protein 1 (LRP1). A second receptor, syndecan-1, has recently been identified that also binds chylomicron remnants and removes them from the circulation [13]. In addition, lipoproteins that have apoE, such as chylomicron remnants, can bind to the LDL receptor (discussed in the next section) and be cleared from the circulation. Hepatic lipase is a key enzyme that hydrolyzes the triacylglycerols and phospholipids of the chylomicron remnants as they enter the hepatocyte.

The interaction of chylomicrons with adipocytes and subsequent lipid metabolism in the fed state is presented in Figure 5.16. Adipocytes are the major storage site

1. Glucose is metabolized to make acetyl-CoA, which can be converted to fatty acids.
2. Lipoprotein lipase acts on TAG in chylomicrons (CHYLM) causing free fatty acids (FFA) and MAG to enter the adipocyte.
3. Lipoprotein lipase acts on VLDL so FFA and MAG enter the cell.
4. The pathways favor energy storage as TAG. Insulin stimulates lipogenesis by promoting entry of glucose into the cell and by inhibiting the hormone-sensitive lipase that hydrolyzes the stored TAG to FFA and glycerol.

Figure 5.16 Lipid metabolism in the adipose cell following a meal.
Abbreviations: CHYLM, chylomicron; DAG, diacylglycerol; MAG, monoacylglycerol; TAG, triacylglycerol; and FFA, free fatty acid.

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for triacylglycerol and the most likely target of chylomicrons following a fat-containing meal. Usually the amount of fat consumed by an individual in a single meal exceeds the immediate energy demands of tissues. Therefore most dietary triacylglycerol must be stored, at least temporarily, until needed when energy demand exceeds energy intake. Triacylglycerol is in a continuous state of turnover in adipocytes; that is, constant lipolysis (hydrolysis during energy needs) is countered by constant re-esterification to form triacylglycerols (storage during energy excess). These two processes are not simply forward and reverse directions of the same reactions but are different pathways involving different enzymes and substrates. In the fed state, metabolic pathways in adipocytes favor triacylglycerol synthesis, a process strongly influenced by insulin. Insulin increases the uptake of free fatty acids and monoacylglycerols in adipocytes by stimulating lipoprotein lipase. Insulin also accelerates the entry of glucose into adipocytes and its conversion to fatty acids. Glycolysis in adipocytes provides a source of glycerol-3-phosphate for re-esterification with the fatty acids to form triacylglycerols. Absorbed monoacylglycerols also furnish the glycerol backbone for re-esterification. Insulin further exerts its lipogenic action on adipose by strongly inhibiting hormone-sensitive lipase, which hydrolyzes stored triacylglycerols, thus favoring triacylglycerol synthesis.

Endogenous Lipid Transport

The endogenous lipid transport system begins and ends with the liver. In brief, hepatic triacylglycerols are packaged in VLDL and delivered to peripheral tissues in a manner similar to chylomicrons. After delivery, the leftover particles, referred to as LDL, are depleted of triacylglycerol but relatively enriched in cholesterol. As remnant particles, LDL are removed from the circulation for catabolism by specific receptors on the plasma membrane of cells, primarily hepatocytes. If the LDL receptors are in short supply, LDL can accumulate in the blood, causing the concentration of LDL-associated cholesterol to rise. The health implications of elevated LDL cholesterol concentration are discussed later in this chapter.

The synthesis and role of VLDL are discussed first. The liver has a limited capacity to store triacylglycerols and must continually move them out for transport to peripheral tissues where they can be stored or used for energy. The liver's ability to synthesize and secrete triacylglycerols in VLDL helps to maintain the balance of energy-containing nutrients throughout the body. The liver is capable of synthesizing new fatty acids and triacylglycerols from nonlipid precursors such as glucose, fructose, and amino acids. It can also utilize "preformed" lipids delivered to it as chylomicron remnants, LDL, and HDL. A third source of lipid for VLDL synthesis comes from free fatty acids bound to serum albumin that are taken up by the liver. The free fatty acids may be of dietary origin (absorbed directly into the portal blood) or from adipose tissue (released into the systemic circulation during lipolysis). Figure 5.17 depicts the interrelationships among the pathways of lipid, carbohydrate, and protein metabolism in the liver, illustrating how lipids from remnant particle uptake, albumin-bound free fatty acids, and nonlipid precursors can be converted to triacylglycerols and secreted as VLDL into the systemic circulation.

Glucose, fructose, and amino acids that enter the liver from the hepatic portal vein can be converted to fatty acids and incorporated into VLDL if in excess and other demands for these molecules are met. Excess glucose and fructose not used for energy via the TCA cycle results in an accumulation of acetyl-CoA, which can be used to synthesize fatty acids (see Chapter 3). The glycerol needed for triacylglycerols is made from triose phosphates such as glycerol-3-phosphate. Amino acids can serve as precursors for fatty acids because they can be metabolically converted to acetyl-CoA or pyruvate. The synthesis of fatty acids, triacylglycerols, and phospholipids is described in detail later in this chapter.

In addition to triacylglycerols, the liver processes phospholipids, cholesterol, and cholesterol esters. Phospholipids from chylomicron remnants can incorporate into cell membranes or be used in the assembly of VLDL. Cholesterol and cholesterol esters from chylomicron remnants may be used in several ways:

- converted to bile salts and secreted in the bile
- secreted directly into the bile as free cholesterol
- incorporated into cellular membranes as free cholesterol
- incorporated into VLDL and released into the blood.

VLDL are assembled in the liver from endogenous triacylglycerols in much the same way as chylomicrons are assembled in the enterocytes from dietary triacylglycerols. The lipids are carried to the endoplasmic reticulum, assembled into VLDL with its complement of apoproteins, and secreted from the cell by exocytosis. The main structural apoprotein on VLDL is apoB-100; one molecule of apoB-100 is associated with each VLDL particle. Because of its large size, the apoB-100 protein encircles the VLDL particle with several regions that anchor within the phospholipid monolayer. Newly secreted VLDL also contain apoC-1 and apoE. Circulating VLDL acquire apoC-2 and additional apoE

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1. Dietary nutrients enter the liver through the portal vein. Glucose can be converted to glycogen or enter glycolysis.
2. Amino acids enter the amino acid pool and some are metabolized to produce pyruvate and oxaloacetate.
3. Serum FFA, bound to albumin, enter the fatty pool and are TAG.
4. CR enter the hepatocyte by endocytosis, and are taken up by a lysosome. FFA, MAG, and C are released. The lipids are reformed to TAG and CE and packaged.
5. TAG, C, and PL are packaged with apolipoproteins and enter the circulation as VLDL.
6. VLDL deliver triacylglycerols to muscle and adipose tissue.

Figure 5.17 Metabolism in the liver following a fatty meal.
Abbreviations: CR, chylomicron remnant; ALB, albumin; FFA, free fatty acid; MAG, monoacylglycerol; DAG, diacylglycerol; C, cholesterol; CE, cholesterol ester; TAG, triacylglycerol.

from HDL. The main features of the endogenous lipid transport system are depicted in Figure 5.18.

By virtue of apoC-2 on its surface, VLDL bind to and interact with lipoprotein lipase on adipose and muscle cells in a manner similar to the binding and hydrolysis of triacylglycerols in chylomicrons. Within the muscle cell, the free fatty acids and monoacylglycerols from VLDL are primarily oxidized for energy, with only limited amounts resynthesized for storage as triacylglycerols. Endurance-trained muscle, however, does contain some triacylglycerol deposits. In adipose tissue, in contrast, the absorbed fatty acids are largely used to resynthesize triacylglycerols for storage. As the triacylglycerols are removed from VLDL, a smaller transient IDL particle is formed. A few IDL particles may separate from the cell and return to the circulation; however, most remain attached and the removal of triacylglycerols continues until a triacylglycerol-depleted LDL particle remains. As LDL particles shrink in size, they lose all of their apoproteins except apoB-100. Several events can determine the size of LDL, their interaction with lipoprotein lipase, and other lipoproteins with the intravascular space where exchange of lipids can occur. Clinical studies have indicated that small dense LDL are more atherogenic than larger LDL, which emphasizes the importance of having a more thorough analysis conducted on LDL subfractions in individuals who are at risk for cardiovascular disease. The LDL particles separate from the cell and enter the circulation with a significantly different lipid profile compared to VLDL. Whereas VLDL are rich in triacylglycerols, LDL are composed of the remaining lipids that were secreted by the liver in VLDL. The relative percentage of phospholipids, free cholesterol, and cholesterol esters in LDL are greater than VLDL (see Figure 5.14), making LDL the primary carrier of cholesterol in the bloodstream of most people. Furthermore, apoB-100 is the only remaining apoprotein on LDL (one molecule per LDL particle). It is imperative that LDL, as the major carrier of cholesterol, be removed from the blood to prevent the accumulation of LDL cholesterol.

Clearance of LDL from blood is accomplished by a cell surface receptor—the LDL receptor—that recognizes apoB-100 and binds LDL particles for uptake into

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Figure 5.18 Endogenous lipid transport.
Abbreviations: B-100, apolipoprotein B-100; E, apolipoprotein E; TAG, triacylglycerol; C, cholesterol and cholesterol esters; and PL, phospholipid.
Source: Modified from *Harpers Illustrated Biochemistry*, Figure 25-4, page 222, by R. K. Murray, D. K. Rodwell, and W. Victor, 27th edition (Lange Medical Books/McGraw-Hill 2006).

the cell [14]. ApoE also binds to the LDL receptor, so lipoproteins expressing apoE also have the potential to be cleared from the circulation via the LDL receptor. LDL binds to the LDL receptors on cell membranes with high affinity and specificity. The LDL receptors located on hepatocytes are particularly important, as they remove 70–80% of LDL from the circulation. Membrane-bound LDL is then internalized by endocytosis. The interaction between the receptors and apoB-100 is the key to the cell's internalization of the LDL. Figure 5.19 depicts the fate of the LDL particle following its binding to the membrane receptor. The internalized LDL particle is carried to lysosomes, and the receptor is released and returns to the surface of the cell. In the lysosome the apoprotein and cholesterol ester components are hydrolyzed by lysosomal enzymes into amino acids, free fatty acids, and free cholesterol. The influx of free cholesterol exerts the following regulatory functions:

- The rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), is suppressed through decreased transcription of the reductase gene and the concomitant increased degradation of the enzyme.
- The enzyme regulating cholesterol esterification, acyl-CoA:cholesterol acyltransferase (ACAT), is activated, thus promoting cholesterol ester storage.
- The synthesis of LDL receptors is suppressed through decreased transcription of the receptor gene, thereby preventing further entry of LDL into the cell.

The LDL receptor has been extensively studied since elevated serum LDL-cholesterol concentration is a known risk factor for cardiovascular disease (CVD). Factors that influence the number of receptors on the cell surface impact LDL-cholesterol concentration. Dietary components are known to strongly influence the number of LDL receptors: saturated and *trans* fatty acids decrease receptors, whereas soluble fiber and phytosterols increase receptors. In addition, obesity reduces the number of LDL receptors; therefore, obese individuals are less responsive to dietary interventions that normally improve serum cholesterol profiles [15]. Genetic studies have also identified naturally occurring mutations that result in abnormal LDL receptors that can cause dramatically elevated cholesterol levels, termed *familial hypercholesterolemia*. More recently, a hepatic protein called PCSK9 was discovered that binds to LDL receptors and disrupts the recycling mechanism that returns the receptors to the cell surface after internalization. Interestingly, people with a mutation in PCSK9 that disables its function have low LDL cholesterol concentration and have lower risk of developing CVD [14].

Reverse Cholesterol Transport

Reverse cholesterol transport refers to the ability of circulating HDL to pick up excess cholesterol from peripheral tissues and deliver it to the liver for excretion from the body via bile, as either free cholesterol or bile acids. While every cell in the body can synthesize cholesterol, mammals lack the oxidative enzymes necessary to degrade cholesterol. Therefore, the transport of cholesterol from

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Figure 5.19 Sequential steps in endocytosis of LDL leading to synthesis and storage of cholesterol ester.
Source: M. Brown, J. Goldstein, "Receptor mediated endocytosis: insights from the lipoprotein receptor system." © 1986 The Noble Foundation. Used by courtesy of The Samuel Roberts Noble Foundation, Ardmore, OK.

peripheral cells to the liver for excretion is a critically important pathway for maintaining cholesterol homeostasis. Conversion of cholesterol to regulatory molecules (hormones) also occurs in the liver and other tissues, but this is quantitatively small compared to the amount excreted through the bile. The role of HDL in reverse cholesterol transport is shown in Figure 5.20.

The process by which HDL collects cholesterol from peripheral tissues and transports it to the liver involves multiple cell surface receptors, intravascular enzymes, and transfer of lipids among circulating lipoproteins. One constant throughout the entire process is HDL's main apoprotein, apoA-1. Unlike chylomicrons and VLDL, which are assembled into complete lipoproteins within the cell, HDL arise entirely within the intravascular space starting with lipid-free apoA-1. Molecules of apoA-1 are produced and secreted into the circulation by the liver and small intestine; apoA-1 released from chylomicrons and VLDL during triacylglycerol hydrolysis may also be used to create HDL. Nascent HDL are made when the lipid-free apoA-1 binds to the liver ABCA1 receptor and acquires phospholipids and free cholesterol from the hepatocyte. Nascent HDL are discoidal in shape due to the ability of the amphipathic lipids to form a bilayer. Additional phospholipids and cholesterol are acquired when nascent HDL interact with ABCA1 and another receptor, SR-B1, located in peripheral tissues such as muscle, adipose, and macrophages within coronary arteries. The ability of nascent HDL to accept cholesterol from macrophages benefits the cardiovascular system by reducing the amount of deposited cholesterol in the vascular endothelium, thus decreasing the risk of CVD (discussed in detail in the next section).

As nascent HDL acquire phospholipids and cholesterol, they also acquire an intravascular enzyme called lecithin:cholesterol acyltransferase (LCAT). This enzyme forms cholesterol esters by catalyzing the transfer of fatty acids (usually polyunsaturated) from the *sn*-2 position of phosphatidylcholine to free cholesterol within the HDL particle. Because the resulting cholesterol esters are nonpolar, they migrate to the core of the particle, forming mature HDL. The small spherical HDL can further interact with peripheral tissues as the apoA-1 binds to SR-B1 and yet another receptor, ABCG1. (Mature HDL bind to ABCG1, but not ABCA1. Both nascent and mature HDL bind to SR-B1.) Further binding to cell receptors and the continued action of LCAT causes HDL to grow in size. The accumulated cholesterol esters in HDL can be transferred to other lipoproteins through the action of cholesterol ester transfer protein (CETP). By distributing cholesterol esters to VLDL and LDL, cholesterol ester transfer protein helps to reduce the size of HDL so that interaction with cell surface receptors is optimized, thus increasing HDL's ability to accept more cholesterol.

The final step in reverse cholesterol transport is the binding of HDL to SR-B1 receptors on the surface of hepatocytes. Two actions are possible: the cholesterol esters may be selectively deposited in the liver cells and the depleted HDL returned to the circulation, or the entire HDL particle may be internalized and degraded. Intracellular degradation of HDL occurs in lysosomes in a manner similar to the degradation of LDL (see Figure 5.18). The cholesterol esters are hydrolyzed by cholesterol ester hydrolase, and the free cholesterol can be secreted directly into bile or converted to bile salts and

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1. Lipid-free apoA-1 is secreted by the liver and intestine. It is also released from chylomicrons and VLDL during TAG hydrolysis.
2. ApoA-1 acquires PL and C from interaction with liver ABCA1, resulting in nascent HDL particles.
3. Nascent HDL acquire additional PL and C via ABCA1 and additional C via SR-B1 in peripheral tissues.
4. The enzyme LCAT, carried on HDL particles, esterifies C to CE that migrate to the particle core.
5. The now spherical mature HDL continue to acquire PL and C via ABCG1 and C via SR-B1 in peripheral tissues.
6. LCAT continues to esterify C to CE, forming larger HDL.
7. Some CE are transferred to VLDL and LDL, mediated by CETP.
8. Liver SR-B1 binds HDL. CE may be selectively removed, or the HDL particle may be internalized and degraded.

Figure 5.20 Reverse cholesterol transport.

secreted (see Figure 5.10). This process is the major route by which cholesterol is eliminated from the body. The efficiency with which HDL accept and transport cholesterol is reflected in the distribution of HDL particle sizes that exist in the circulation. As large HDL represent the final stage just prior to delivery to the liver, a high proportion of large HDL are associated with decreased risk of CVD. A preponderance of small HDL, it is hypothesized, reflects inefficiencies in the ability of HDL to gather cholesterol esters for delivery to the liver. Small HDL are positively associated with CVD [16].

Additional functions of HDL have recently been suggested [17]. These include a role as an anti-inflammatory regulator through interactions with the vascular endothelium and circulating inflammatory cells. Some evidence supports the idea that HDL is an integral component of innate immunity. HDL has also been shown to have antiapoptotic functions for a number of cell types, including vascular endothelial and smooth muscle cells, some leukocytes, pancreatic β cells, cardiomyocytes, and bone-forming cells. Further research will reveal its biological importance in these areas [17].

LIPIDS, LIPOPROTEINS, AND CARDIOVASCULAR DISEASE RISK

Atherosclerosis is a degenerative disease of the vascular endothelium. The principal players in the atherogenic process are cells of the immune system, which cause a pro-inflammatory environment, and lipids, primarily cholesterol and cholesterol esters. An early response to arterial endothelial cell injury is an increased adherence of monocytes and T lymphocytes to the area of the injury. Cytokines, protein products of the monocytes and lymphocytes, mediate the atherogenic process by chemotactically attracting phagocytic cells to the area. Additional exposure to a high level of circulating LDL and the deposition and oxidative modification of cholesterol esters further promote the inflammatory process. The process is marked

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by the uptake of LDL by phagocytic cells that become engorged with lipid, called foam cells. Phagocytic uptake is accelerated if the apoB-100 component of the LDL has been oxidized. The lipid-filled foam cells may then infiltrate the endothelium and develop into a fatty plaque. As lipid continues to accumulate within the plaque, the lumen of the blood vessel is progressively occluded. Atherosclerosis was once considered to be a disease caused exclusively by dyslipidemia; however, atherosclerosis is now considered to be a disease of both dyslipidemia and immune-system–induced inflammation. The Perspective at the end of this chapter discusses in detail the role of lipoproteins and inflammation in atherosclerosis development.

Ever since plaque was found to be composed chiefly of lipids, an enormous research effort has been underway to investigate the possible link between dietary lipids and the development of atherosclerosis. The presumed existence of such a link has come to be known as the lipid hypothesis, which maintains that dietary lipid intake can alter blood lipid levels, which in turn initiate or exacerbate atherogenesis. The next section contains a brief account of the involvement of certain dietary lipids, and of genetically acquired apolipoproteins, in atherogenesis.

Cholesterol

At center stage in the lipid hypothesis controversy is cholesterol. The effects of dietary interventions designed to improve serum lipid profiles are often measured by the extent to which the interventions raise or lower serum cholesterol. This reasoning is justified in that cholesterol is a major component of atherogenic fatty plaque, and many studies have linked CVD risk to chronically elevated serum cholesterol levels. Receiving the greatest attention, however, is not so much the change in total cholesterol concentration but how the cholesterol is distributed between its two major transport lipoproteins, LDL and HDL. Because cholesterol is commonly and conveniently quantified in clinical laboratories, assays can be used to establish LDL:HDL ratios by measuring the amount of cholesterol in each of the lipoprotein classes. Assayed cholesterol associated with LDL is designated LDL-C by laboratory analysts, and cholesterol transported in HDL is designated HDL-C.

Because maintaining relatively low serum levels of LDL and relatively high levels of HDL (a low LDL:HDL ratio) is desirable, the concept of "good" and "bad" cholesterol emerged. The "good" form is the cholesterol associated with HDL, and the "bad" form is the cholesterol transported as LDL. It is important to understand, however, that cholesterol itself is not good or bad; rather, it serves as a proxy for the relative concentrations of LDL and HDL, ratios of which can indeed be good or bad. LDL:HDL ratios are, in fact, determined more reliably by measurements other than cholesterol content. For example, immunological methods for quantifying apoB (the major LDL apoprotein) and apoA-1 (the primary HDL apoprotein) are now widely used. Ratios of apoA to apoB then serve as an indicator of CVD risk, with risk decreasing as the ratio decreases [18].

ApoB in the nonfasted serum includes both apoB-48, made in the enterocyte, and apoB-100, made in the hepatocyte; the majority is apoB-100 and is found in VLDL, IDL, and LDL. The total moles of apoB-100 present in the serum indicate the number of potentially atherogenic particles.

ApoA-1 is the major apolipoprotein in the HDL particles that are part of the reverse cholesterol transport system. HDL particles are antiatherogenic. They also have anti-inflammatory and antioxidant properties. Measurements of apoB and apoA should be made in fasting samples so that "contamination" of apoB-48 (chylomicrons) is avoided.

The impact of dietary cholesterol on serum cholesterol levels has been a controversial topic for many years. While there is definitive evidence that elevated LDL-C (and high LDL:HDL ratio) increases CVD risk, a link between dietary cholesterol and serum cholesterol has never been firmly established. The controversy started in 1968 when the American Heart Association announced a recommendation to limit cholesterol intake to less than 300 mg/day, focusing specifically on eggs (no more than three egg yolks per week) because of their high cholesterol content. Despite having weak evidence for making such a recommendation, and having no clear rationale for choosing 300 mg/day as the benchmark, the recommendation created a fear of dietary cholesterol that has persisted for nearly 50 years [19]. The preponderance of research, however, clearly indicates that dietary cholesterol has little or no impact on serum cholesterol. This is because compensatory mechanisms are engaged when cholesterol is consumed, such as increased biliary cholesterol excretion and the downregulation of cholesterol synthesis (discussed in the "Synthesis of Cholesterol" section later in this chapter). The American Heart Association and the 2015 Dietary Guidelines for Americans no longer recommend a restriction on cholesterol intake.

Saturated and Unsaturated Fatty Acids

Extensive research has examined the effects of ingestion of dietary fats containing primarily saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), or *trans* fatty acids on total serum cholesterol or LDL-C levels as end points. The findings from older research studies generally led to the conclusions that SFA are hypercholesterolemic, PUFA are hypocholesterolemic, and MUFA are neutral (neither increasing nor lowering serum cholesterol). A comprehensive review of the scientific literature linking diet and

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chronic disease was published in 1989 by the National Research Council and provides an excellent historical perspective [20].

It is still thought to be important to reduce SFA intake, but it also matters what is used to replace SFA in the diet. When 1% of energy from SFA is replaced with PUFA, the LDL-C is reduced and is likely to produce a 2–3% reduction in the incidence of coronary heart disease [21]. Insufficient evidence exists to judge the effects of replacing SFA with MUFA. Furthermore, replacing SFA with carbohydrate produces no benefits, and may even be associated with moderately higher risk of CHD. The potential risk of CVD is actually more complicated than what is implied by correlations to total serum cholesterol or LDL-C. It involves a combination of genetics, dietary factors, level of obesity, exercise, and other lifestyle determinants. The cholesterolemic response to individual fatty acids, even those within a single fatty acid class, is heterogeneous. This heterogeneity is particularly noticeable among the long-chain SFA. Strong evidence indicates that lauric (12:0), myristic (14:0), and palmitic (16:0) acids are all hypercholesterolemic, specifically raising LDL-C. On the other hand, stearic acid (18:0) reduces levels of total cholesterol and LDL-C when compared to other long-chain SFA and appears more neutral in its effect. Therefore, stearic acid should not be grouped with other SFA with respect to LDL-C effects. Oleic acid (18:1) and linoleic acid (18:2 n-6) are hypocholesterolemic compared to 12:0, 14:0, and 16:0 fatty acids, with linoleic acid being the more potent of the two, independently lowering total and LDL cholesterol [22].

Trans Fatty Acids

The reason for the concern about dietary *trans* fatty acids is primarily because of their effects on serum lipids. Dietary *trans* fatty acids may be more unfavorable than SFA because not only do *trans* fatty acids raise LDL-C, but they lower HDL-C. *Trans* fatty acids also appear to correlate more strongly with CVD mortality than SFA [23]. However, some caution is needed when interpreting such data because in nearly all clinical studies, the metabolic effects of *trans* fatty acids have been compared to other dietary fatty acids on a gram-per-gram basis, which misrepresents their actual proportions in the food supply. According to the U.S. Department of Agriculture food availability database, the per capita intake of SFA, MUFA, and PUFA is approximately 35, 45, and 26 g/day, respectively [4]. The per capita intake of *trans* fatty acids is significantly less at 1.3 g/day [1]. When expressed as a percentage of total energy consumed, the contribution of *trans* fatty acids is only 0.5% of total energy, whereas SFA, MUFA, and PUFA contribute 13%, 17%, and 10% of energy, respectively. Therefore, studies that examine the isocaloric substitution of fatty acids (often at levels of 2% of energy or more) overestimate the impact of *trans* fatty acids, given their low abundance in the food supply. Interpretation of results is also complicated by the uncertainty of knowing whether experimental outcomes were due to inclusion of *trans* fatty acids or the removal of displaced fatty acids. Despite these experimental shortcomings, the American Heart Association and the Dietary Guidelines for Americans recommend that *trans* fatty acid intake be as low as possible (zero intake, though advisable, is not considered practical because of the small amount of natural *trans* fat in the food supply).

Lipoprotein(a)

Lipoprotein(a) [Lp(a)] is composed of a low-density lipoprotein (LDL) particle containing apoB-100 and a covalently linked glycoprotein called apolipoprotein(a). The physiological function of the Lp(a) particle has not been identified, although it is associated with increased risk of CVD. Unlike other lipoprotein classes, the serum concentration of Lp(a) is genetically determined. It exhibits a very broad and skewed distribution in the population and is not influenced by dietary or other environmental factors. Apolipoprotein(a) has a strong structural homology (similar amino acid sequence) with plasminogen. Plasminogen is the inactive precursor of the enzyme plasmin, which dissolves blood clots by its hydrolytic action on fibrin. Apolipoprotein(a) has several genetic isoforms that vary in size. The smaller molecular weight isoforms appear to be more pathogenic [24].

Apolipoprotein E

Among the apolipoproteins already discussed in this chapter, apoE deserves special mention because of its multiple roles in lipid metabolism, neurobiology, and cellular function. There are three isoforms of apoE in humans: apoE2, apoE3, and apoE4. One of the isoforms, apoE4, has been associated with CVD and Alzheimer's disease.

A single individual inherits one apoE allele from each parent, thus various homozygous and heterozygous combinations are possible. Allele frequencies show nonrandom global distribution, with the frequency of apoE4 increasing as one moves north from the equator. The apoE4 frequency is higher in people of northern European descent (about 25%) and lower in Mediterranean and Asian populations (< 10%). It has been known for many years that individuals of northern European origin have an increased risk for CVD, but only part of this increased risk is considered to be due to the dysfunction in LDL and lipid metabolism. The isoforms of apoE display preferences for specific lipoprotein classes, with apoE4 having a preference for larger triacylglycerol-rich chylomicrons and VLDL. Consequently, apoE4 can remain associated with LDL particles during VLDL catabolism. The presence

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of apoE4 on LDL can increase LDL's affinity for receptors on macrophages present in atherosclerotic plaque. ApoE itself has been shown to increase oxidative stress and inflammation. While apoE is made mostly by the liver, it is also made by the brain, kidney, spleen, adipose tissue, and macrophages. Macrophage-derived apoE is abundant in atherosclerotic plaques, where it influences platelet aggregation, macrophage cholesterol efflux, expression of adhesion molecules, and inhibition of smooth muscle proliferation and migration [25]. See the Perspective at the end of this chapter for more about the role of inflammation in atherosclerosis.

There are also several neurological consequences of apoE4. For example, it has been shown to be a risk factor in early onset of Alzheimer's disease, poorer outcomes following traumatic brain injury, and postoperative cognitive dysfunction. The mechanism for its association with these diseases is not fully understood, but it is thought to be related to the increased oxidative stress and pro-inflammatory properties of apoE4 compared to the other isoforms of apoE. It is interesting that CVD risk is greater in smokers with apoE4 than nonsmokers. Some of the association of apoE4 with diseases is still controversial and must await additional research for confirmation [25].

INTEGRATED METABOLISM IN TISSUES

Catabolism of Triacylglycerols and Fatty Acids

Triacylglycerols stored in adipose tissue represent a major energy reserve. During times of energy need such as engaging in exercise, consuming low-calorie diets, or simply sleeping through the night, the stored triacylglycerols are mobilized by lipase-catalyzed hydrolysis and released into the circulation as free fatty acids. Only adipocytes have the ability to release free fatty acids into the bloodstream. The free fatty acids bind to albumin for transport to most energy-requiring cells in the body (except red blood cells), where they are oxidized via the TCA cycle for ATP production. In this way, adipose tissue is constantly taking up and releasing fatty acids throughout the day to meet constant energy needs in the face of sporadic energy consumption.

The complete hydrolysis of triacylglycerols yields glycerol and three fatty acids. In the body, this hydrolysis occurs largely through the coordinated activity of three lipases: adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL). The three enzymes each hydrolyze one fatty acid from the glycerol backbone in sequence. ATGL preferentially hydrolyzes fatty acids at the *sn*-2 position (ATGL can also hydrolyze at the *sn*-1 position to a lesser extent). Next, HSL hydrolyzes the fatty acid at the *sn*-3 position and MGL targets the remaining fatty acid at the *sn*-1 position (or *sn*-2, depending on the initial action of ATGL). Regulation of triacylglycerol hydrolysis within the adipocyte is significantly more complicated than once thought. A cascade of events leading to enzyme activation (by phosphorylation) is controlled by the hormone epinephrine, considered a master regulator of lipolysis [26]. The following events occur in the main regulatory pathway:

- Epinephrine binds to cell surface adrenergic receptors ($\beta_{1,2}$-AR).
- Activated receptors interact with adenylyl cyclase.
- Activated adenylyl cyclase converts intracellular ATP to cAMP.
- Increased intracellular cAMP activates protein kinase A.
- Activated protein kinase A phosphorylates HSL, causing HSL translocation to the lipid droplet surface.
- Activated protein kinase A also phosphorylates the protein perilipin 1 on the lipid droplet surface, promoting the release of another protein (comparative gene identification-58), which stimulates ATGL.

In addition to epinephrine, lipolysis is stimulated by natriuretic peptides. The cardiac hormones atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) increase in the circulation during exercise and are important stimulating factors of lipolysis. The following events occur in the signaling pathway mediated by natriuretic peptides:

- ANP and BNP bind to type-A natriuretic peptide receptors.
- Activated receptors interact with guanylyl cyclase.
- Activated guanylyl cyclase converts intracellular GTP to cGMP.
- Increased intracellular cGMP activates protein kinase G.
- Activated protein kinase G phosphorylates HSL and perilipin 1, causing their activation in a manner similar to protein kinase A.

In addition to epinephrine and natriuretic peptides, other factors can act as regulators either directly by receptor-mediated signaling or indirectly by affecting the lipolytic cascade. These factors include adrenocorticotrophic hormone, thyroid-stimulating hormone, growth hormone, tumor necrosis factor α, and glucocorticoids [26].

After the complete hydrolysis of triacylglycerols, the liberated free fatty acids are secreted by the adipocyte and bind to albumin in the circulation for transport to energy-requiring tissues. The remaining glycerol cannot be metabolized by adipose tissue and is secreted into the

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circulation. The glycerol can be used for energy by the liver and by certain other tissues that have the enzyme glycerokinase, which converts glycerol to glycerol phosphate. Glycerol phosphate can enter the glycolytic pathway at the level of dihydroxyacetone phosphate, from which point either energy oxidation or gluconeogenesis can occur (review Figure 3.17).

Fatty acids are a rich source of energy; on an equal-weight basis they surpass carbohydrates in this property. This occurs because fatty acids exist in a more reduced state than that of carbohydrate and therefore undergo a greater extent of oxidation en route to CO_2 and H_2O. Many tissues are capable of oxidizing fatty acids by way of a mechanism called β-oxidation, described later in this chapter. When the fatty acid enters the cell, it is first activated by coenzyme A to acyl-CoA, in an energy-requiring reaction catalyzed by cytosolic fatty acyl-CoA synthetase (Figure 5.21). The reaction consumes two high-energy phosphate bonds to yield AMP. This is equivalent to using two ATPs. The pyrophosphate that is produced is quickly hydrolyzed, which ensures that the reaction is irreversible.

Mitochondrial Transfer of Acyl-CoA

The oxidation of fatty acids occurs primarily within the mitochondrion and produces energy through oxidative phosphorylation (see Chapter 3). Peroxisomes can also oxidize fatty acids, but this pathway does not provide energy for the cell. Short-chain fatty acids can pass directly into the mitochondrial matrix and form acyl-CoA derivatives in the matrix. Long-chain fatty acids and their CoA derivatives are incapable of crossing the inner mitochondrial membrane (but can cross the permeable outer membrane), so a membrane transport system is necessary. The carrier molecule for this system is carnitine (see Chapter 6), which can be synthesized in humans from lysine and methionine, and is found in high concentration in muscle. The activated fatty acid (acyl-CoA) is joined covalently to carnitine at the cytosolic side of the outer mitochondrial membrane by the transferase enzyme carnitine acyltransferase I (CAT I). Carnitine:acylcarnitine transferase moves the acyl-carnitine across the inner membrane; then a second transferase, carnitine acyltransferase II (CAT II), located on the inner face of the inner membrane, releases the acyl-carnitine to form acyl-CoA and carnitine (Figure 5.22).

Figure 5.21 Activation of fatty acid by coenzyme A.

β-Oxidation of Fatty Acids

The oxidation of the activated fatty acid in the mitochondrion occurs through a cyclic degradative pathway by which two-carbon units in the form of acetyl-CoA are cleaved one acetyl-CoA at a time from the carboxyl end. The reactions of β-oxidation are summarized in Figure 5.23, using palmitic acid (16:0) as the example. The activated palmitoyl-CoA is acted upon by the enzyme acyl-CoA dehydrogenase to produce a double bond between the α- and β-carbons. There are four such dehydrogenases, each specific to a range of chain lengths. The enzymes specific for longer chain lengths are bound to the inner membrane and those for shorter chain lengths are free in the matrix. The unsaturated acyl-CoA adds the elements of water in a stereospecific way across the double bond to form a β-hydroxyacyl-CoA with the aid of the enzyme enoyl-CoA hydratase, sometimes called crotonase. The β-hydroxy group is then oxidized to a ketone by the NAD^+-requiring enzyme β-hydroxyacyl-CoA dehydrogenase, producing a NADH that can enter the electron transport chain to produce about 2.5 ATPs. The β-ketoacyl-CoA is cleaved by β-ketothiolase, resulting in the insertion of another CoA and cleavage at the β-carbon. The products of this reaction are acetyl-CoA and a saturated CoA-activated fatty acid that has two fewer carbons than the original fatty acid. The acetyl-CoA enters the TCA cycle for further oxidation and the activated fatty acid with two

Figure 5.22 Membrane transport system for transporting fatty acyl-CoA across the inner mitochondrial membrane.

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1. The formation of a double bond between the α-and β-carbons is catalyzed by acyl-CoA dehydrogenase. There are four such dehydrogenases, each specific to a range of chain lengths.
2. The unsaturated acyl-CoA adds the elements of water in a stereospecific way. The reaction is catalyzed by enoyl-CoA hydratase, sometimes called crotonase.
3. The β-hydroxy group is oxidized to the ketone by the NAD^+–requiring enzyme β-hydroxyacyl-CoA dehydrogenase.
4. The β-ketoacyl-CoA is cleaved by acyltransferase (also called thiolase), resulting in the insertion of CoA and the cleavage at the β-carbon. The products of this reaction are acetyl-CoA and a saturated CoA-activated fatty acid having two fewer carbons than the original fatty acid.
5. This entire sequence of reactions is repeated, with two carbons being removed with each cycle.
6. A $FADH_2$ is oxidized by the electron transport system and produces 1.5 ATPs.
7. A NADH is produced and is oxidized by the electron transport system to produce about 2.5 ATPs.
8. Each acetyl-CoA is further oxidized by the TCA cycle to produce 10 ATPs.

Figure 5.23 The mitochondrial β-oxidation of an activated fatty acid using palmitate as an example.

fewer carbons continues the β-oxidation cycle, losing two carbons with each turn.

Energy Yield in Fatty Acid Oxidation

The complete β-oxidation of one 16-carbon palmitic acid molecule requires seven rotations of the cycle and produces eight acetyl-CoA, seven $FADH_2$, and seven NADH molecules. The $FADH_2$ and NADH directly enter the electron transport chain and yield on average 1.5 ATP/mole $FADH_2$ and 2.5 ATP/mole NADH by oxidative phosphorylation. The acetyl-CoAs are oxidized to CO_2 and H_2O in the TCA cycle (and oxidative phosphorylation), with an average yield of 10 ATP/mole acetyl-CoA (see Chapter 3). Using the example of palmitate (16 carbons), we can summarize the yield of ATP as follows:

7 $FADH_2$	$7 \times 1.5 = 10.5$
7 NADH	$7 \times 2.5 = 17.5$
8 acetyl-CoA	$8 \times 10 = 80$
Total ATPs produced	108
2 ATPs for activation	−2
Net ATPs	106

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Figure 5.24 Sequential β-oxidation of oleic acid, showing the location of the double bond using the Δ nomenclature system. Numbers above the carbons represent the original carbon numbers of oleic acid.

As nearly one-half of dietary and body fatty acids are unsaturated, they provide a considerable portion of lipid-derived energy. They are catabolized by β-oxidation in the mitochondrion in nearly the same way as their saturated counterparts, except that one fewer fatty acyl-CoA dehydrogenase reaction is required for each double bond present. This is because the double bond introduced into the saturated fatty acid by the reaction occurs naturally in unsaturated fatty acids. However, the specificity of the enoyl-CoA hydratase reaction requires that the double bond be between the second and third carbon for the hydration to take place, and the preexisting double bond may not occupy the Δ^2 position. For example, after three cycles of β-carbon oxidation, the position of the double bond in what was originally a Δ^9 monounsaturated fatty acid will occupy a Δ^3 position. Figure 5.24 shows 18:1 Δ^9 undergoing three cycles of β-oxidation. At the end of the third cycle, the fatty acid is a Δ^3 fatty acid. The presence of a specific enoyl-CoA-isomerase then shifts the double bond from a *cis* Δ^3 to *trans* Δ^2, allowing the subsequent reactions to proceed. The oxidation of an unsaturated fatty acid results in somewhat less energy production than oxidation of a saturated fatty acid of the same chain length because for each double bond present, the $FADH_2$- producing fatty acyl-CoA dehydrogenase reaction is bypassed, resulting in 1.5 fewer ATPs per cycle.

Although most fatty acids metabolized are composed of an even number of carbon atoms, small amounts of fatty acids having an odd number of carbon atoms are also used for energy. β-oxidation occurs as described above, with the liberation of acetyl-CoA until a residual three-carbon propionyl-CoA remains. The subsequent oxidation of propionyl-CoA requires reactions that use the vitamins biotin and B_{12} in a coenzymatic role (Figure 5.25). Because the succinyl-CoA formed in the course of these reactions can be converted into glucose, the odd-number carbon fatty acids are uniquely glucogenic among all the fatty acids.

Formation of Ketone Bodies

Normally, the concentration of the ketone bodies in the blood is very low, but will increase in situations of accelerated fatty acid oxidation that occurs during body fat reduction (consuming low-energy, low-carbohydrate diets) or in uncontrolled type 1 diabetes. Under such conditions, an abundance of free fatty acids is released by adipocytes into the circulation, which exceeds the ability of tissues to oxidize them. Furthermore, glucose-requiring tissues, including the brain and red blood cells, cannot use fatty acids for energy and their need for alternative fuels increases. Fortunately, the liver is able to handle excess free fatty acids by converting them to the so-called **ketone bodies** in a process called **ketogenesis**. Following β-oxidation, the liver converts the excess acetyl-CoA to acetoacetate, β-hydroxybutyrate, and acetone. Acetoacetate and β-hydroxybutyrate are not oxidized further in the liver but instead are transported by the blood to peripheral tissues, where they can be converted back to acetyl-CoA

Figure 5.25 Oxidation of propionyl-CoA.

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and oxidized through the TCA cycle. Acetone is a minor player and arises in the blood by spontaneous decarboxylation of acetoacetate. The steps in ketone body formation, which occurs only in the mitochondria, are shown in Figure 5.26. The reversibility of the β-hydroxybutyrate dehydrogenase reaction, together with enzymes present in extrahepatic tissues that convert acetoacetate to acetyl-CoA (shown by the broken arrows in Figure 5.26), reveals how the ketone bodies can serve as a source of fuel in these tissues. Ketone body formation is actually an "overflow" pathway for acetyl-CoA use, providing another way for the liver to distribute fuel to peripheral cells.

Fat loss strategies often include consumption of low-carbohydrate and/or low-energy diets. Mild increases in ketone bodies, called **ketosis**, is to be expected during fat loss and usually poses no harm. However, in uncontrolled diabetes mellitus, in starvation, or with prolonged consumption of a very-low-carbohydrate diet, ketone bodies can rise to dangerous levels that lower the pH of the blood, resulting in **ketoacidosis** (sometimes called diabetic ketoacidosis in someone with diabetes). Recall from Chapter 3 that for the TCA cycle to function, the supply of four-carbon molecules must be adequate. These TCA cycle intermediates are formed mainly from pyruvate (formed during glycolysis). When the supply of carbohydrate is inadequate, there is insufficient glucose for glycolysis to occur at a normal rate and less pyruvate is produced. Thus the pool of oxaloacetate (made from pyruvate), with which the acetyl-CoA normally combines for oxidation in the TCA cycle, is reduced. As carbohydrate use by cells diminishes, oxidation of fatty acids accelerates to provide substrate (acetyl-CoA) for the TCA cycle. This shift to fat catabolism, coupled with reduced oxaloacetate availability, results in an accumulation of acetyl-CoA. As would be expected, an increase in ketone body formation follows. On one hand, the liver's ability to deliver ketone bodies to peripheral tissues such as the brain and muscle is an important mechanism for providing fuel in periods of prolonged energy deficit. On the other hand, untreated ketoacidosis can lead to low blood pressure, dehydration, coma, and death.

Figure 5.26 Steps in hepatic ketone body formation.

Synthesis of Fatty Acids

Except for the essential fatty acids linoleic acid and α-linolenic acid, most human cells are capable of synthesizing fatty acids from acetyl-CoA. The major sites of synthesis are the liver, lungs, adipose tissue, lactating mammary glands, brain, and kidneys. The initial reaction, a carboxylation reaction, occurs in the cytosol and is catalyzed by acetyl-CoA carboxylase; the role of biotin, which serves as a coenzyme for the carboxylase, is discussed in Chapter 9. ATP furnishes the energy needed to attach the new carboxyl group to acetyl-CoA (Figure 5.27).

Nearly all acetyl-CoA needed for fatty acid synthesis is produced in the mitochondrial matrix. It is formed there from the oxidation of pyruvate, which may arise from the oxidation of glucose and fructose (and possibly fatty acids), and from the degradation of the carbon skeletons of some amino acids (see Chapter 6). Some acetyl-CoA is formed in the cytosol directly from amino acid catabolism. The synthesis of fatty acids occurs in the cytosol, but acetyl-CoA produced within the mitochondrial matrix is unable to exit through the mitochondrial membrane. The major mechanism for the transfer of acetyl-CoA to the cytosol is its reaction with oxaloacetate to form citrate, which can pass through the mitochondrial membranes. In the cytosol, citrate lyase converts the citrate back to oxaloacetate and acetyl-CoA. This reaction, shown here, is essentially the reversal of the citrate synthetase reaction of the TCA cycle, except that it requires expenditure of ATP.

The enzymes involved in fatty acid synthesis are arranged in a complex called the fatty acid synthase system, which is found in the cytosol. Key components of this complex are the acyl carrier protein (ACP) and the condensing enzyme, both of which possess free sulfhydryl (—SH) groups to which the acetyl-CoA and malonyl-CoA building blocks attach. ACP is structurally similar to CoA (see Figure 9.19). Both possess a 4'-phosphopantetheine component (pantothenic acid coupled through β-alanine to thioethanolamine) and phosphate. The thioethanolamine contributes the free —SH group to the complex.

Figure 5.27 Formation of malonyl-CoA from acetyl-CoA and CO_2 (carboxylation reaction).

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Figure 5.28 "Loading" of sulfhydryl groups into the fatty acid synthase system.

The free —SH of the condensing enzyme is contributed by the amino acid cysteine.

Before the actual steps in the elongation of the fatty acid chain can begin, the two —SH groups must be "loaded" correctly with malonyl and acetyl groups. Acetyl-CoA is transferred to ACP, with the loss of CoA, to form acetyl-ACP. The acetyl group is then transferred again to the —SH of the condensing enzyme, leaving available the ACP—SH, to which malonyl-CoA attaches, again with the loss of CoA. This loading of the complex can be represented as in Figure 5.28. The extension of the fatty acid chain then proceeds through the following sequential steps, which are also shown schematically in Figure 5.29 along with the enzymes and cofactors catalyzing their actions. The enzymes catalyzing these reactions are also part of the fatty acid synthase complex, along with ACP and condensing enzyme.

The first step is the coupling of the carbonyl carbon of the acetyl group to the C-2 of malonyl-ACP with the elimination of the malonyl carboxyl group as CO_2. The β-ketone is then reduced, with NADPH serving as hydrogen donor. (The NADPH is generated by the pentose phosphate pathway in the cytosol, as discussed in Chapter 3.) The resulting alcohol is dehydrated, yielding a double bond. The double bond is reduced to butyryl-ACP, again with NADPH acting as reducing agent. The butyryl group is transferred to the condensing enzyme, exposing the ACP sulfhydryl site, which accepts a second molecule of malonyl-CoA. A second condensation reaction takes place, coupling the butyryl group on the condensing enzyme to C-2 of the malonyl-ACP. The six-carbon chain is then reduced and transferred to condensing enzyme in a repetition of steps 2 through 5. A third molecule of malonyl-CoA attaches at ACP—SH, and so forth. The completed fatty acid chain is hydrolyzed from the ACP without transfer to the condensing enzyme. The normal product of the fatty acid synthase system is palmitate, 16:0. It can in turn be lengthened by fatty acid **elongation** systems to stearic acid, 18:0, and even longer saturated fatty acids. Elongation occurs by the addition of two-carbon units at the carboxylic acid end of the chain. Furthermore, by **desaturation** reactions, palmitate and stearate can be converted to their corresponding Δ^9 monounsaturated fatty acids, palmitoleic acid (16:1) and oleic acid (18:1), respectively. Fatty acid desaturation reactions are catalyzed by enzymes referred to as mixed-function oxidases, so called because two different substrates are oxidized: the fatty acid (by removal of hydrogen atoms to form the new double bond) and NADPH. Oxygen is the terminal hydrogen and electron acceptor to form H_2O.

Essential Fatty Acids

Recall that human cells cannot introduce additional double bonds beyond the Δ^9 carbon because they lack enzymes called Δ^{12} and Δ^{15} desaturases. That is why linoleic acid (18:2 $\Delta^{9,12}$) and α-linolenic acid (ALA) (18:3 $\Delta^{9,12,15}$) are essential fatty acids and must be obtained from the diet (plant sources). The Western diet is replete with n-6 linoleic acid and once it is consumed, longer, more highly unsaturated fatty acids can be formed from it by a combination of elongation and desaturation reactions. When n-3 α-linolenic acid is consumed, it is also subject to elongation and desaturated in parallel reactions. Figure 5.30 outlines the synthesis of various PUFAs from linoleic acid and α-linolenic acid, listing their chemical and common names. The biologically active compounds derived from these PUFAs are also shown, along with the enzymes involved. These compounds include eicosanoids—prostaglandins, prostacyclins, **thromboxanes**, and **leukotrienes**—produced from both n-6 and n-3 pathways. Note that the eicosanoids derived from each pathway are different and generally have opposing biological effects, as discussed below. Resolvins and neuroproctectins come from docosahexaenoic acid (DHA) [27].

Elongation and desaturation of the essential fatty acids occurs in the smooth ER. Linoleic acid undergoes a desaturation by the enzyme delta-6-desaturase (d-6-d) to form γ-linolenic acid (18:3 n-6). The next step is an elongation catalyzed by the enzyme elongase (ELG) to form dihomo-γ-linolenic acid (20:3 n-6). Arachidonic acid (20:4 n-6) is then formed by a second desaturation. ALA (n-3) undergoes comparable reactions to form eicosapentaenoic acid (EPA; 20:5 n-3). Since the n-6 and n-3 fatty acids follow the same pathway with the same enzymes, they compete, and an excess of one family causes a significant change in the conversion of the other family [28]. The eicosanoids (both n-6 and n-3) are esterified with the glycerol backbone to form phospholipids or triacylglycerols and incorporate into membranes. Arachidonic acid is predominant in membranes, so n-6 synthesis predominates. Arachidonic acid and EPA go

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Figure 5.29 The steps in the synthesis of fatty acid. CE (condensing enzyme) and ACP (acyl carrier protein) are members of a complex of enzymes referred to as the fatty acid synthase system.

through further elongation and desaturations in the smooth ER to form tetracosapentoenoic acid (24:5 n-6) and tetracosahexaenoic acid (24:6 n-3). These fatty acids are transferred to the peroxisome, where they undergo β-oxidation to form docosapentaenoic acid (22:5 n-6) and docosahexaenoic acid (DHA; 22:6 n-3).

Humans of all ages require linoleic and α-linolenic acid (or their derivatives) in the diet for normal growth and cellular metabolism. The n-6 and n-3 fatty acids are metabolized by the same series of desaturases and elongases to longer-chain polyunsaturated fatty acids as described previously. Deficiency symptoms for the n-6 series that have

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Figure 5.30 PUFA biosynthesis. The IUPAC names (all-*cis*) and the common names (in square brackets) with abbreviations are reported. ELG indicates elongase, while d-6-d and d-5-d indicate delta desaturases. In plants, a n-3 desaturase (n-3-d) converts LA to ALA. Mammals convert LA and ALA to long-chain fatty acids using a series of desaturation and elongation reactions in the ER. However, the synthesis of DHA from 24:6 n-3 and osbond acid (22:5 n-6) from 24:5 n-6 requires the synthesis of 24:6 n-3 and 24:5 n-6 in the ER, and their passage into the peroxisome, where they undergo one cycle of beta-oxidation to produce DHA and osbond acid, which move back to the ER (red arrows). Formation of resolvins and protectins from DHA is also shown.

Source: Russo, G.L., Dietary n-6 and n-3 polyunsaturated fatty acids: From biochemistry to clinical implication in cardiovascular prevention. Biochemical Pharmacology, 77; 2009:937–46. Reprinted by permission.

been identified in adults and children include poor growth and skin abnormalities (dry or scaly skin, raised bumps, and hair loss). Deficiency symptoms for the n-3 series include neurological and visual abnormalities. Infants have been observed to have similar neurological abnormalities when maintained on a regimen that was lacking in n-3 α-linolenic acid. Human milk contains more of the essential fatty acids (though the level varies), as well as the elongated derivatives EPA and DHA, than do most infant formulas. There is evidence that n-3 essential fatty acids are necessary for neural tissue and retinal photoreceptor membranes. Both term and preterm infants can convert n-3 essential fatty acids to the long-chain polyunsaturated fatty acids, but whether they can convert them at an adequate rate to meet their needs is unclear. Essential n-3 fatty acid deficiency appears to be more common among preterm infants than term infants. Infant formulas containing n-3 EPA and n-6 arachidonic acid are now available.

Eicosanoids: Fatty Acid Derivatives of Physiological Significance

As long-chain arachidonic acid, α-linolenic acid, EPA, and DHA are synthesized, they incorporate into phospholipids (and triacylglycerols) and thus become an integral part

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of cell membranes. The higher the degree of unsaturation among the fatty acids within a membrane, the greater the fluidity of that membrane. The membrane's fluidity is an important determinant for the hormone-receptor binding sites. For example, it has been hypothesized that insulin resistance might be associated with the development of cells with a rigid membrane, which limits the expression of insulin receptors and reduces their number [29].

When eicosanoids are synthesized, the polyunsaturated fatty acid precursors are mobilized from the phospholipids or triacylglycerols by phospholipase A2. Further reactions can then produce the biologically active eicosanoids, as shown in Figure 5.30. Note that n-6 arachidonic acid produces the eicosanoids in what is called the 2 and 4 series, while EPA produces eicosanoids in the 1 and 3 series. Cyclo-oxygenases and lipoxygenases convert AA to the prostaglandin-2-, thromboxane-2-, and leukotriene-4-series; various hydroperoxy- and hydroxyl-eicosatetraenoic acid (HPETE and HETE) derivatives; and lipoxin A_4. EPA is metabolized to the eicosanoids of the prostaglandin-3-, leukotriene-5-, and thromboxane-3-series. DHA can be metabolized to other biologically active compounds that include resolvins, docosatrienes, and neuroprotectins.

Opposing Effects of n-6 and n-3 Fatty Acid–Derived Eicosanoids

Table 5.6 highlights the effects of individual messengers derived from n-6 arachidonic acid (AA) and n-3 EPA and DHA. Note that most of the arachidonic acid–derived messengers are pro-inflammatory or show other disease-propagating effects, whereas n-3 derivatives oppose these effects. Mediators of the n-6 prostaglandin family are pro-arrhythmic, while the messengers derived from n-3 EPA and DHA are antiarrhythmic, anti-inflammatory, or vasodilators. Thromboxanes (TXB_2) produced from n-6 AA activate platelets (which promote blood clots) and cause vasoconstriction (which raises blood pressure); in opposition, the n-3 thromboxane (TXB_3) inhibits platelets and causes vasodilation. Similarly, whereas n-6 leukotriene (LTB_4) from arachidonic acid is pro-inflammatory and leads to the production of inflammatory cytokines, the corresponding n-3 leukotriene (LTB_5) from EPA and DHA is anti-inflammatory and actually blocks the biosynthesis of the inflammatory leukotriene derived from arachidonic acid. Other anti-inflammatory derivatives of EPA include resolvins (RV1 or RVD) and nuclear receptors (NF).

The cardioprotective effects of n-3 fatty acids (especially EPA and DHA) are the basis of the recommendation for

Table 5.6 n-3 and n-6 Fatty Acid–Derived Messengers and Their Physiological Effects

Messenger Classes	Arachidonic Acid (n-6)–Derived Messengers	Physiological Effects	EPA- and DHA (n-3)–Derived Messengers	Physiological Effects
Prostaglandins	PGD_2		PGD_3	
	PGE_2	Pro-arrhythmic	PGE_3	Anti-arrhythmic
	PGF_2		PGF_3	
	PGI_2	Pro-arrhythmic	PGI_3	Anti-arrhythmic
Thromboxanes	TXA_2	Platelet activator	TXA_3	Platelet inhibitor
	TXB_2	Vasoconstriction	TXB_3	Vasodilation
Leukotrienes	LTA_4		LTA_5	
	LTB_4	Pro-inflammatory	LTB_5	Anti-inflammatory
	LTC_4		LTC_5	
	LTE_4		LTD_5	
	LTD_4		LTE_5	
Epoxyeclosatrienoic derivatives	5,6-EET			
	8,9-EET			
	11,12-EET	Pro-inflammatory		
	14,15-EET			
Hydroxyleicosatetraenoic derivatives	5-HETE			
	12-HETE			
	15-HETE			
Lipoxins	LXA_4			
Resolvins			RVE1	Anti-inflammatory
			RVD	Anti-inflammatory
Neuroprotectin			NPD1	Anti-inflammatory

Source: Based on data from Heird W., Lapillonne A., The role of essential fatty acids in development. Annu Rev Nutr. 2005;25:549–71.

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the increased consumption of fish, particularly deep-water fish such as herring, salmon, and tuna [28]. Benefits have been shown most consistently when meals featuring fish are consumed at least twice per week rather than fish oil supplements. n-3 fatty acids also benefit the nervous system, where DHA is concentrated and appears to function in photoreceptors and synaptic membranes. DHA thus plays roles in vision, neuroprotection, successful aging, and memory in addition to its anti-inflammatory and inflammation-resolving properties as compared to n-6 PUFAs. A recent review covers the **signal-lipidomics** of DHA [29]; this broad research area encompasses cellular and molecular signaling pathways regulated by DHA, its bioactive derivatives, and its receptor-mediated actions.

Impact of Diet on Fatty Acid Synthesis

Following a carbohydrate-rich meal, *de novo* fatty acid synthesis (lipogenesis) increases mainly in the liver, but also in many other tissues. In the fed state, the amount of carbohydrate consumed usually exceeds immediate energy needs, so the excess is first stored as glycogen in liver and muscle (see Chapter 3). When glycogen stores reach capacity, the remaining carbohydrates are converted to fatty acids and triacylglycerols. Some tissues such as cardiac muscle, skeletal muscle, and adipose tissue can store triacylglycerols at the site of lipogenesis. In contrast, the liver packages newly synthesized triacylglycerols in VLDL and releases them into the circulation for delivery to tissues that express lipoprotein lipase, including the adipose tissue, cardiac muscle, skeletal muscle, lactating mammary gland, brain, kidney, and, to lesser extent, lung and spleen tissue.

The main carbohydrates that reach the liver after a meal are glucose (from starch and simple sugars) and fructose (from simple sugars). The presence of glucose and insulin in the systemic circulation stimulates the lipogenic enzymes. The regulatory role of fructose is less certain, but it may stimulate lipogenesis by insulin-independent mechanisms [30]. Nevertheless, both dietary glucose and fructose not immediately used for energy in the liver can be converted to fatty acids and triacylglycerols, then transported out in VLDL. Prolonged intake of diets excessively high in glucose and fructose can lead to triacylglycerol accumulation in adipose tissue and in the liver, resulting in nonalcoholic fatty liver disease (NAFLD), although the dietary level of sugars that constitutes "excess" is a matter of debate. Whether fructose alone affects hepatic lipogenesis and NAFLD independently of excessive energy intake remains uncertain [30].

Synthesis of Triacylglycerols and Phospholipids

The synthesis of triacylglycerols and phospholipids share common precursors and are considered together in this section. The precursors are CoA-activated fatty acids and glycerol-3-phosphate, the latter produced either from the reduction of dihydroxyacetone phosphate or from the phosphorylation of glycerol. These and subsequent reactions of the pathways are shown in Figure 5.31, which depicts two pathways for phosphatidylcholine synthesis from diacylglycerol. The *de novo* pathway of phosphatidylcholine synthesis is the major route. However, the importance of the salvage pathway increases when a deficiency of the essential amino acid methionine exists. Triacylglycerols synthesized in the liver are assembled into VLDL and secreted into the circulation for delivery to tissues expressing lipoprotein lipase (mainly adipose tissue and cardiac and skeletal muscle). Triacylglycerols synthesized in extrahepatic tissues can be stored at the site of lipogenesis.

Synthesis, Catabolism, and Whole-Body Balance of Cholesterol

Unlike the triacylglycerols and fatty acids, cholesterol is not an energy-containing nutrient, nor is it required in the diet since all cells can synthesize it. Another unusual feature

Figure 5.31 A schematic summary of the synthesis of triacylglycerols and lecithin showing that precursors are shared. In lecithin formation, three moles of activated methionine (S-adenosylmethionine) introduce three methyl groups in the *de novo* pathway, and choline is introduced as CDP (cytidine diphosphate)–choline in the so-called salvage pathway.

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of cholesterol is that no degradative (oxidative) enzymes exist in mammals, so cholesterol catabolism depends on its conversion to other molecules and its elimination from the body through biliary excretion. Its four-ring core structure remains intact in the course of its catabolism. The concept of *whole-body cholesterol balance* was developed many years ago as a useful way to describe cholesterol homeostasis in which "input" includes synthesis and dietary intake, whereas "output" includes biliary excretion as free cholesterol or bile salts (after hepatic conversion from cholesterol). While cholesterol can also be converted to hormones and 7-dehydrocholesterol (which can then be used to synthesize vitamin D) (see Figure 5.9), these pathways are quantitatively small compared to biliary output and are not usually considered in models of whole-body cholesterol balance. The discussion in this section begins with the liver and its ability to mediate cholesterol output through bile, followed by cholesterol synthesis and the coordination of events necessary to maintain cholesterol homeostasis.

The liver is the central organ that mediates the elimination of cholesterol from the body. Cholesterol, primarily in the form of its ester, is delivered to the liver as a component of chylomicron remnants, LDL, and HDL. The cholesterol ester that is destined for excretion is either hydrolyzed by cholesterol esterase to its free form, which is secreted directly into the bile canaliculi, or it is first converted into bile salts before entering the bile. As shown in Figure 5.10, conversion of cholesterol to bile salts involves addition of hydroxyl groups to the ring structure and conjugation of the side chain with glycine or taurine. The effect of these reactions is to enhance the water solubility of the sterol, facilitating its secretion into bile. Up to 10 times more bile salts than cholesterol is present in bile, although the proportions can change depending on many factors that include a person's diet and interaction of bile salts with intestinal microbiota [31].

Recall that bile salts and cholesterol can be reabsorbed and returned to the liver as part of the enterohepatic circulation. Their absorption efficiency can influence their rate of fecal excretion and thereby affect whole-body cholesterol balance. Bile salts returning to the liver from the intestine repress the formation of an enzyme that catalyzes the rate-limiting step in their conversion from cholesterol. If the bile salts are prevented from returning to the liver, the activity of this enzyme increases, stimulating the conversion of cholesterol to bile acids and leading to their excretion. The removal of bile salts is, in fact, a therapeutic treatment for hypercholesterolemia that employs an unabsorbable, cationic resin (cholestyramine) to bind bile salts in the intestinal lumen and prevent them from returning to the liver. Preventing the reabsorption of cholesterol is also a strategy for treating hypercholesterolemia, which can be achieved by drugs (ezetimibe) and diet (phytosterols), both strategies having been discussed earlier in this chapter. Recall that cholesterol present in the intestinal lumen can come from both the liver and diet, although the majority is from the liver. Consequently, blocking cholesterol absorption can be an effective treatment for hypercholesterolemia even in people (vegans) who consume no animal products.

Changes in biliary excretion of bile salts and cholesterol are compensated for by changes in the rate of whole-body cholesterol synthesis. Nearly all tissues in the body are capable of synthesizing cholesterol from acetyl-CoA. The liver accounts for about 20% of endogenous cholesterol synthesis. Among the extrahepatic tissues, which are responsible for all other cholesterol synthesis, the intestine is probably the most active. Endogenous synthesis accounts for most (and perhaps all, in vegans) of cholesterol "input" and can quickly adjust in response to changes in cholesterol and bile salt absorption, the efficiency of lipoprotein cholesterol transport, and the cholesterol needs of cells throughout the body.

Figure 5.32 An overview of the pathway of cholesterol biosynthesis in the hepatocyte indicating the negative regulatory effect of cholesterol on the HMG-CoA reductase reaction.

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At least 26 steps are known to be involved in the formation of cholesterol from acetyl-CoA. The individual steps are not provided here, but the synthesis of cholesterol can be thought of as occurring in three stages:

1. a cytosolic sequence by which 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) is formed from 3 mol of acetyl-CoA
2. the conversion of HMG-CoA to squalene, including the important rate-limiting step of cholesterol synthesis, in which HMG-CoA is reduced to mevalonic acid by HMG-CoA reductase
3. the formation of cholesterol from squalene.

The rate of cholesterol synthesis is controlled in each cell by a negative feedback regulation of the HMG-CoA reductase reaction. A family of drugs called statins are HMG-CoA inhibitors and are widely used to block endogenous cholesterol synthesis, which forces cells (particularly in the liver) to increase the number of LDL receptors to recruit needed cholesterol from the circulation. The net effect is a significant reduction in serum LDL cholesterol concentration. A brief scheme of hepatic cholesterol synthesis and its regulation is shown in Figure 5.32.

REGULATION OF LIPID METABOLISM

Fatty Acids

The regulation of fatty acid synthesis and oxidation is closely linked to carbohydrate status—synthesis increases when adequate carbohydrates are consumed, whereas oxidation increases in carbohydrate deficit. Fatty acids formed in the cytosol of lipogenic cells can either be converted into triacylglycerols and phospholipids or be transported via carnitine into the mitochondrion for oxidation. The enzyme carnitine acyltransferase I, which catalyzes the transfer of fatty acyl groups to carnitine (see Figure 5.22), is specifically inhibited by malonyl-CoA. Recall that malonyl-CoA is the first intermediate in the synthesis of fatty acids. Therefore, it is logical that an increase in the concentration of malonyl-CoA would promote fatty acid synthesis while inhibiting fatty acid oxidation. Malonyl-CoA concentration increases whenever a person is well supplied with carbohydrate. Excess glucose that cannot be oxidized through the glycolytic pathway or stored as glycogen is converted to fatty acids then stored as triacylglycerols.

Blood glucose levels also affect lipolysis and fatty acid oxidation. Hyperglycemia triggers the release of insulin, which promotes glucose transport into adipocytes for conversion to fatty acids. Insulin also inhibits lipolysis in adipocytes by antagonizing the effects of hormones that stimulate lipases, particularly hormone-sensitive lipase (HSL) as discussed earlier in this chapter. The extent to which adipocytes convert glucose to fatty acids has been a matter of debate. While the lipogenic enzymes are highly expressed in human adipocytes, methods used to measure the conversion rates are inadequate and likely underestimate the true contribution. Hypoglycemia, on the other hand, results in a reduced intracellular supply of glucose, thereby suppressing lipogenesis. Furthermore, the low level of insulin accompanying the hypoglycemic state would favor lipolysis, with a flow of free fatty acids into the bloodstream. Low blood glucose (and therefore low intracellular levels) also stimulates fatty acid oxidation. In this case, accelerated oxidation of fatty acids follows the reduction in TCA cycle activity, which in turn results from inadequate oxaloacetate availability.

An important allosteric enzyme involved in the regulation of fatty acid synthesis is acetyl-CoA carboxylase, which forms malonyl-CoA from acetyl-CoA (see Figure 5.27). This enzyme is positively stimulated by citrate in the cytosol, but in its absence is barely active. Recall that citrate is part of the shuttle for moving acetyl-CoA from the mitochondria (a major site of production) to the cytosol, where fatty acids are synthesized. Citrate is continuously produced in the mitochondrion as a TCA cycle intermediate, but its concentration in the cytosol is normally low. When mitochondrial citrate concentration increases, it can escape to the cytosol by way of a transport protein called the citrate carrier (CIC) [32]. In the cytosol citrate acts as a positive allosteric signal to acetyl-CoA carboxylase, thereby increasing the rate of formation of malonyl-CoA, resulting in lipogenesis. Recent studies have reported that diets rich in PUFA, but not SFA or MUFA, inhibit citrate transport into the cytosol by decreasing transcription and translation of CIC, with subsequent decreases in acetyl-CoA carboxylase and fatty acid synthase activity. These results suggest an interesting mechanism whereby PUFA-rich diets may protect against hepatic fat accumulation and possibly serve as treatment for NAFLD [32].

Acetyl-CoA carboxylase can be modulated negatively by palmitoyl-CoA, which is the end product of fatty acid synthesis. This situation would most likely arise when free fatty acid concentrations increase as a result of insufficient glycerol-3-phosphate, with which fatty acids must combine to form triacylglycerols (see Figure 5.31). Deficient glycerol-3-phosphate levels would likely stem from inadequate carbohydrate availability. In such a situation, regulation would logically favor fatty acid oxidation rather than synthesis.

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Cholesterol

The liver is the central organ responsible for maintaining cholesterol homeostasis. Cholesterol metabolism in hepatocytes is unique among the body's cells because of its ability to (1) synthesize cholesterol; (2) accept cholesterol from the circulation via lipoprotein receptors; (3) store excess cholesterol as cholesterol esters; (4) package cholesterol esters into VLDL and secrete them into the circulation; (5) convert cholesterol to bile salts; and (6) release bile salts and free cholesterol via bile into the small intestine for excretion from the body. The combined results of these coordinated events help to regulate the serum concentration of LDL-C and HDL-C, as well as whole-body cholesterol balance.

The size of the intracellular cholesterol pool is key to the regulation of each of the metabolic pathways that contribute to cholesterol homeostasis. The intracellular cholesterol pool exists primarily as free cholesterol within membranes of the endoplasmic reticulum (ER) where its presence can influence the activity of regulatory proteins (enzymes, receptors, transports, etc.). When the intracellular pool increases, regulatory events are engaged that normalize the level of free cholesterol in the cell. First, cholesterol synthesis is inhibited by downregulation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (see Figure 5.32). Second, LDL receptor production is decreased and they are translocated away from the cell surface to prevent further uptake of LDL from the circulation (Step 4 in Figure 5.19 is disrupted). Third, cholesterol esterification is accelerated by upregulation of acyl-CoA:cholesterol acyltransferase (ACAT). The cholesterol esters may be store in cytosol vesicles or they may be secreted by the cell as a component of VLDL. Fourth, bile acid synthesis is increased by upregulation of the rate-limiting enzyme cholesterol-7α-hydroxylase (CYP7A1). All of the enzymes mentioned above reside in the ER and are therefore controlled directly by the regulatory pool of cholesterol. As in the case of LDL receptors, they are synthesized in the ER and are subject to the same direct control.

In contrast, as the intracellular cholesterol pool decreases, the opposite regulatory events occur. HMG-CoA reductase is upregulated to synthesize more cholesterol. LDL receptor production and translocation to the cell surface increases to recruit more LDL-C from the circulation. Cholesterol esters stored in cytosolic vesicles are hydrolyzed by cholesterol ester hydrolase (and possibly hormone-sensitive lipase) to yield more free cholesterol. CYP7A1 is downregulated and fewer bile acids are synthesized, although this does negatively affect bile function because of the relatively large amount of bile salts produced throughout the day.

BROWN FAT THERMOGENESIS

Brown adipose tissue (or brown fat) is metabolically active, and greater abundance in the neck area of adults is associated with lower adiposity. In contrast to energy-storing white fat, energy-burning brown fat contributes to increasing energy expenditure and insulin sensitivity. Brown fat obtains its name from its high degree of vascularity and the abundant mitochondria present in its adipocytes. Recall that the mitochondria are pigmented, owing to the cytochromes and perhaps other oxidative pigments associated with electron transport. Not only do brown fat cells contain larger numbers of mitochondria than white fat cells do, but the mitochondria are also structurally different and contain uncoupling protein 1 (UCP1) to promote **thermogenesis** (heat production) at the expense of producing ATP. Brown fat is also derived from a different embryological origin than white fat [33].

Brown fat mitochondria have special H^+ pores in their inner membranes, formed by integral uncoupling protein 1 (UCP1). UCP1 is a translocator of protons, which allows the external H^+ pumped out of the mitochondrial matrix by electron transport to flow back into the matrix and thus become unavailable to drive F_0F_1 ATP synthase, the site of phosphorylation. Remember that it is the H^+ gradient that causes the conformational changes that result in the phosphorylation of ADP to produce ATP. Figure 5.33 illustrates how the proposed mechanism of brown fat thermogenesis relates to the chemiosmotic theory of oxidative phosphorylation. Membrane pores of brown fat mitochondria allow the cycling of protons, which lowers the proton concentration in the inner membrane space and results in heat generation rather than ATP production. This cycling appears to be regulated by the 32,000-dalton UCP1.

Three types of external stimuli trigger thermogenesis: (1) ingestion of food, (2) prolonged exposure to cold temperature, and (3) exercise through the muscle-derived hormones. The first two events stimulate the tissue via sympathetic innervation via the hormone norepinephrine. The sympathetic signal has a stimulatory and hypertrophic effect on brown adipose tissue. This effect enhances expression of the UCP1 in the inner membrane of the mitochondrion and accelerates synthesis of lipoprotein lipase and glucose transporters to make more fatty acids and glucose available to meet the higher metabolic demand. Theoretically, then, weight reduction should accompany a higher activity of brown fat and, indeed, an association between obesity and deficient brown fat cell function has been established [33]. Obese individuals seem to have less brown fat than nonobese individuals. Better understanding of the brown adipose tissue's activity may increase the potential for enhancing energy expenditure by increasing its quantity or activity through new drugs.

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Figure 5.33 A schematic of the inner membrane of a mitochondrion from brown adipose tissue showing uncoupling protein 1 (UCP1), the components of the electron transport chain, and ATP synthase. H^+ are translocated to the intermembrane space by electron transport and flow back into the mitochondrial matrix by ATP synthase and UCP 1 when it is activated. UCP1 is activated by β-adrenergic stimulation, cold stress, and free fatty acids. It is inhibited by free purine nucleotides (not complexed with Mg) such as ADP, ATP, GDP, and GTP.

ETHYL ALCOHOL: METABOLISM AND BIOCHEMICAL IMPACT

Ethyl alcohol (ethanol) is neither a carbohydrate nor a lipid. Though empirically ethanol's structure (CH_3-CH_2-OH) most closely resembles a carbohydrate, its metabolism most closely resembles fatty acid catabolism. We have chosen to review it in this chapter for several reasons. First, it is a common dietary component, being consumed in the form of alcoholic beverages such as beer, wine, and distilled spirits. Second, the pathways that oxidize ethyl alcohol also oxidize (or detoxify) other exogenous substances in the body. Although ethanol has been part of the human diet for centuries, it provides no metabolic benefits (other than energy) and is considered "empty calories." Furthermore, prolonged consumption of excessive amounts can lead to health problems, most notably liver damage. Each gram of ethanol yields 7 kcal, and ethanol may account for up to 10% of the total energy intake of moderate consumers and up to 50% for alcoholics. Because of its widespread consumption and relatively high caloric potency, it commands attention in a nutrition textbook.

Ethanol is readily absorbed into the blood through the entire gastrointestinal tract. It is transported unaltered in the circulation and then oxidatively degraded in tissues, primarily the liver. Ethanol is first oxidized to acetaldehyde and then to acetate, which can enter the circulation. In most tissues, the acetate subsequently is converted to acetyl-CoA and oxidized via the TCA cycle. As depicted in Figure 5.34, at least three enzyme systems are capable of ethanol oxidation:

- the alcohol dehydrogenase (ADH) system
- the microsomal ethanol oxidizing system (MEOS)
- the catalase system.

Of these, the catalase system is the least active, probably accounting for <2% of ***in vivo*** ethanol oxidation. While most ingested ethanol is oxidized by the hepatic and, to some extent, the gastric MEOS and ADH system, it can also be metabolized in nonhepatic tissues that do not contain ADH, such as the brain, by the MEOS and catalase system [34].

The Alcohol Dehydrogenase (ADH) Pathway

ADH is a soluble enzyme functioning in the cytosol of hepatocytes. It is an ordinary NAD^+-requiring dehydrogenase and can oxidize ethanol to acetaldehyde. The acetaldehyde is then transported to the mitochondria and further oxidized to acetate in a reaction catalyzed by acetaldehyde dehydrogenase (ALDH2). The NADH formed

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1. The majority of ethanol is oxidized by ADH in the cytosol. Reduced NADH, a byproduct of the reaction, accumulates when excessive ethanol is consumed.
2. The microsomal ethanol oxidizing system (MEOS) accounts for up to 20% of ethanol oxidation. Located in the ER, the CYP2E1 enzyme is a cytochrome P450 mixed-function oxidase. NADPH is concurrently oxidized in the reaction.
3. The catalase system in peroxisomes plays a minor role in total ethanol oxidation. The reaction requires H_2O_2.
4. The initial product of ethanol oxidation, acetaldehyde, is transported to mitochondria and further oxidized to acetate by ALDH2. Reduced NADH accumulates in mitochondria when excessive ethanol is consumed.
5. The final product of ethanol oxidation, acetate, will be metabolized to acetyl-CoA and used for energy via the TCA cycle or for fatty acid synthesis.

Figure 5.34 Ethanol oxidation in hepatocytes.

by ADH can be oxidized by mitochondrial electron transport by way of the NADH shuttle systems (see Chapter 3), thereby giving rise to ATP formation by oxidative phosphorylation. The K_m of ADH for ethanol is approximately 1 millimolar, or about 5 mg/dL. (K_m is reviewed in Chapter 1, in the section dealing with enzymes.) This means that at this cellular concentration of ethanol, ADH is functioning at half its maximum velocity. At concentrations three or four times the K_m, the enzyme is saturated with the ethanol substrate and is catalyzing at its maximum rate. Concentrations of ethanol in the cell more than four times the K_m level cannot be completely oxidized by ADH.

Because ethanol is an exogenous dietary ingredient, there is no "normal" concentration of ethanol in the cells or the bloodstream. The so-called toxic level of blood ethanol, however, is considered to be in the range of 50–80 mg/dL and is defined by its pharmacological actions. The high lipid solubility of ethanol allows it to passively enter cells with ease. If its cellular concentration reaches a level even one-third or one-fourth of that of the blood at toxic levels, ADH becomes saturated by the substrate and will be functioning at its maximum velocity. The excess, or "spillover," ethanol then must be metabolized by alternate systems, the most important being the MEOS. The depletion of NAD^+ brought about by the high level of activity of ADH can also force the shift to the MEOS, which does not require NAD^+ for its oxidative reactions. The depletion of NAD^+ increases the NADH:NAD^+ ratio and impairs NAD^+-requiring reactions such as the TCA cycle, gluconeogenesis, and fatty acid oxidation. The buildup of acetyl-CoA encourages fatty acid synthesis and TAG accumulation in the liver, as discussed later in the "High NADH:NAD^+ Ratio" section.

ADH is also active in gastric mucosal cells. Interestingly, there appears to be a significant gender difference in the level of its activity in these cells. Young (premenopausal) females develop higher blood alcohol levels than male counterparts with equal consumption and consequently display a lower tolerance for alcohol and are at greater risk of toxic effects in the liver. The lower level of ADH activity in the female gastric mucosa is believed to account for this observation [35].

The Microsomal Ethanol Oxidizing System (MEOS)

The MEOS is able to oxidize a wide variety of compounds in addition to ethanol, including fatty acids, **aromatic** hydrocarbons, steroids, and barbiturate drugs. The oxidation is mediated by a cytochrome P450 enzyme, CYP2E1, and involves a system of electron transport, similar to the mitochondrial electron transport system described in detail in Chapter 3. Because the MEOS is microsomal (isolated ER) and associated with the smooth endoplasmic reticulum, it is sometimes referred to as the microsomal electron transport system. Another distinction of the system is its requirement for a special cytochrome called cytochrome P450, which acts as an intermediate electron carrier. Cytochrome P450 is not a single compound but rather exists as a family of structurally related cytochromes, the members of which share the property of absorbing light that has a wavelength of 450 nm.

Ethanol oxidation by the MEOS involves several linked reduction–oxidation reactions that result in the simultaneous oxidation of NADPH and the reduction of molecular oxygen to H_2O (Figure 5.34). Because two substrates (ethanol and NADPH) are oxidized

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concurrently, the enzymes involved are commonly called mixed-function oxidases. Both oxygen atoms are reduced to H_2O, and therefore two H_2O molecules are formed in the reactions. Acting as intermediate carriers of electrons from NADPH to oxygen are FAD, FMN, and a cytochrome P450 system (not shown in the figure).

An important feature of the MEOS is that certain of its enzymes, including the cytochrome P450 units, are inducible by ethanol—particularly at higher concentrations of ethanol. With increased synthesis of these enzymes, the hepatocytes can metabolize ethanol much more effectively, thereby establishing a state of metabolic tolerance. Compared with a normal (nondrinking or light-drinking) subject, an individual in a state of metabolic tolerance to ethanol can ingest larger quantities of the substance before showing the effects of intoxication. When enzyme induction occurs, however, it can also accelerate the metabolism of other substances metabolized by the microsomal system. In other words, tolerance to ethanol induced by heavy drinking can render a person tolerant to other substances as well.

The Catalase System

The enzyme catalase is located in peroxisomes and, in the presence of hydrogen peroxide (H_2O_2), is capable of oxidizing ethanol to acetaldehyde. In view of the high capacity of hepatic ADH to oxidize ethanol, the catalase system is considered a minor pathway except in tissues, such as the brain, which lack ADH. Chronic alcohol consumption may increase H_2O_2 in the liver and thus increase catalase activity.

Alcoholism: Biochemical and Metabolic Alterations

Excessive consumption of ethanol can lead to alcoholism, defined by the National Council on Alcoholism as consumption that is capable of producing pathological changes. Alcoholism is a serious socioeconomic and health problem, exemplified by the fact that in the United States alcohol is the third leading preventable cause of death [36]. The well-known consequences of alcoholism—fatty liver, hepatic disease (cirrhosis), lactic acidemia, and metabolic tolerance—can be explained by the manner in which ethanol is metabolized. Basically, the consequences of excessive alcohol intake are explainable by the metabolic effects of (1) acetaldehyde toxicity, (2) high $NADH:NAD^+$ ratio, (3) substrate competition, and (4) induced metabolic tolerance.

Acetaldehyde Toxicity

All pathways of ethanol oxidation produce acetaldehyde, which is believed to exert direct adverse effects on metabolic systems. For example, acetaldehyde is able to attach covalently to proteins, forming protein adducts. Should the adduct involve an enzyme, the activity of that enzyme could be impaired. Acetaldehyde has also been shown to impede the formation of microtubules in liver cells and to cause the development of perivenular fibrosis, either of which is believed to initiate the events leading to cirrhosis. These and other possible adverse effects of acetaldehyde have been reviewed [37].

High $NADH:NAD^+$ Ratio

The oxidation of ethanol increases the concentration of NADH at the expense of NAD^+, thereby elevating the $NADH:NAD^+$ ratio. This occurs because both ADH in the cytosol and ALDH2 in the mitochondria use NAD^+ as a cosubstrate. NADH is an important regulator of certain dehydrogenase reactions. The rise in concentration of NADH represents an overproduction of reducing equivalents, which in turn acts as a signal for a metabolic shift toward reduction—namely, hydrogenation. Such a shift can account for the fatty liver (through the anabolic activity producing fatty acids) and lactic acidemia (high blood-lactate levels resulting from increased reduction of pyruvate to lactic acid) that often accompany alcoholism. For example, lactic acidemia can be attributed in part to the direct effect of NADH in shifting the lactate dehydrogenase (LDH) reaction toward the formation of lactate. The reaction, which follows, is driven to the right by the high concentration of NADH:

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xleftrightarrow{LDH} \text{Lactate} + \text{NAD}^+$$

Lipids accumulate in most tissues in which ethanol is metabolized, resulting in fatty liver, fatty myocardium, fatty renal tubules, and so on. The mechanism appears to involve both increased lipid synthesis and decreased lipid removal and can be explained in part by the increased $NADH:NAD^+$ ratio. As NADH accumulates, it slows dehydrogenase reactions of the TCA cycle, such as the isocitrate dehydrogenase and α-ketoglutarate dehydrogenase reactions, thereby slowing the overall activity of the cycle. This results in an accumulation of citrate, which positively regulates acetyl-CoA carboxylase. Acetyl-CoA carboxylase, which converts acetyl-CoA into malonyl-CoA by the attachment of a carboxyl group, is the key regulatory enzyme for the synthesis of fatty acids from acetyl-CoA. The high $NADH:NAD^+$ ratio therefore directs metabolism away from TCA cycle oxidation and toward fatty acid synthesis.

Also contributing to the lipogenic effect of alcoholism is the effect of NADH on the glycerophosphate dehydrogenase (GPDH) reaction. This reaction, shown in Figure 5.35, favors the reduction of dihydroxyacetone phosphate (DHAP) to glycerol-3-phosphate if NADH concentration is high. Glycerol-3-phosphate provides the glycerol component in the synthesis of triacylglycerols.

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Figure 5.35 The reduction of dihydroxyacetone phosphate (DHAP) to glycerol-3-P, producing NAD^+ from NADH.

Figure 5.36 The reversible reaction of glutamate and NAD^+ forming α-ketoglutarate, NADH, and ammonia, catalyzed by glutamate dehydrogenase (GluDH).
Source: Based on data from Heird W., Lapillonne A., The role of essential fatty acids in development. Annu Rev Nutr. 2005;25:549–71.

Therefore, a high NADH:NAD^+ ratio stimulates the synthesis of both the fatty acids and the glycerol components of triacylglycerols, contributing to the cellular fat accumulation that develops in alcoholism.

A rise in NADH concentration also affects the glutamate dehydrogenase (GluDH) reaction (Figure 5.36), resulting in impaired gluconeogenesis. The GluDH reaction is extremely important in gluconeogenesis because of the role it plays in the conversion of amino acids to their carbon skeletons by transamination and in the release of their amino groups as NH_3. A shift in the reaction toward glutamate because of the elevated NADH depletes the availability of α-ketoglutarate, which is the major acceptor of amino groups in the transamination of amino acids.

Substrate Competition

A well-established nutritional problem associated with excessive alcohol metabolism is a deficiency of vitamin A. Two aspects of ethanol interference with normal metabolism probably can account for this problem. One is the effect of ethanol on retinol dehydrogenase, the cytosolic enzyme that converts retinol to retinal. Retinal is required for the synthesis of photo pigments used in vision. Retinol dehydrogenase is thought to be identical to ADH, and therefore ethanol competitively inhibits the hepatic conversion of retinol to retinal. In addition to this substrate competition effect, ethanol may interfere with retinol metabolism through induced metabolic tolerance.

Induced Metabolic Tolerance

As explained earlier, ethanol can induce enzymes of the MEOS, causing an increased rate of metabolism of substrates oxidized by this system. Retinol, like ethanol, spills over into the MEOS when ADH is saturated and NAD^+ stores are low because of heavy ingestion of ethanol. Ethanol induction of retinol-metabolizing enzymes, most notably CYP2E1, can then occur. Although induction accelerates the hepatic oxidation of retinol, the oxidation product is not retinal but other polar, inert products of oxidation. The hepatic depletion of retinol can therefore be attributed to its accelerated metabolism, which is secondary to ethanol induction of a metabolizing enzyme. In effect, the alcoholic subject becomes tolerant to vitamin A, necessitating a higher dietary intake of the vitamin to maintain normal hepatocyte concentrations.

Alcohol in Moderation: The Brighter Side

Alcohol is a nutritional "Jekyll and Hyde," and which face it flaunts is clearly a function of the extent to which it is consumed. We focused earlier on the effects of alcohol at high intake levels and the negative impact of alcoholism on metabolism and nutrition. Epidemiological data from worldwide studies consistently demonstrate there is an inverse association between moderate alcohol or wine intake and coronary heart disease (CHD). Positive effects of alcohol or wine intake on oxidative stress, insulin insensitivity, diabetes mellitus, and inflammation have been shown [38].

Ethanol is known to elevate the level of high-density lipoproteins (HDL) in serum and to lower the amount of serum lipoprotein(a) [39]. Both effects favor a decrease in cardiovascular disease risk. In addition to the epidemiological data, prospective studies have shown that alcohol intake correlates with serum HDL-C levels to explain 50–70% of the inverse association of alcohol with risk of CHD [40]. The suggested mechanism is HDL's ability to protect against the deposition of arterial fatty plaque (atherogenesis), whereas high levels of lipoprotein(a) appear to promote it. The effects of these and other lipoproteins on atherogenesis were discussed earlier in this chapter. Dementia shares several risk factors with cardiovascular disease. A U-shaped relationship of alcohol intake with risk of dementia has been reported, indicating a beneficial effect of moderate consumption.

Among the three classes of alcoholic beverages—wine, beer, and distilled spirits—the strongest correlations are with the amount of ethanol consumed and not the individual types of beverages. The common belief is that distilled spirits and beer have about equal benefits and wine has somewhat more, but scientific data that substantiates that belief has not been reported. Wine, particularly red wine, contains many substances with antioxidant properties. Most of the antioxidant chemicals in grapes are found in the skin and seeds. The skin is included in the production of red wine. The polyphenols and a variety of other antioxidants that can reduce reactive oxygen species (ROS) are present. ROS contribute to the oxidative stress that causes inflammation and contributes to cardiovascular disease [41].

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SUMMARY

The hydrophobic character of lipids makes them unique among the major nutrients, requiring special handling in the body's aqueous milieu. Ingested fat must be finely dispersed in the intestinal lumen to present a sufficiently large surface area for enzymatic digestion to occur. In the bloodstream, reassembled lipids must be associated with proteins to form lipoproteins, thus ensuring their solubility in that environment while undergoing transport. The major sites for the formation of lipoproteins are the intestine, which produces them from diet-derived lipids, and the liver, which forms lipoproteins from endogenous lipids. Central to the processes of lipid transport and storage is adipose tissue, which accumulates fat as triacylglycerols when the intake of energy-producing nutrients is greater than the body's caloric needs. When energy demands so dictate, fatty acids are released from storage and transported to other tissues for oxidation. The mobilization follows the adipocyte's response to specific hormonal signals that stimulate the activity of the intracellular lipases.

Fatty acids are a rich source of energy. Their mitochondrial oxidation furnishes large amounts of acetyl-CoA for TCA cycle catabolism, and in situations of low carbohydrate intake or use, as occurs in starvation or diabetes, the rate of fatty acid oxidation increases significantly with concomitant acetyl-CoA accumulation. This causes an increase in the level of ketone bodies, organic acids that can be deleterious through their disturbance of acid-base balance but that also are beneficial as sources of fuel to tissues such as the muscle and brain in periods of starvation.

Although the lipids are thought of first and foremost as energy sources, some can be identified with intriguing hormone-like functions ranging from blood pressure alteration and platelet aggregation to enhancement of immunological surveillance. These potent bioactive substances are the eicosanoids (prostaglandins, thromboxanes, and leukotrienes), all of which are derived from the fatty acids arachidonic acid (n-6), eicosatetraenoic acid (n-3), and docosahexaenoic acid (n-3).

Dietary lipids have been implicated in atherogenesis, the process leading to development of the degenerative cardiovascular disease. Major considerations in preventing and controlling atherosclerosis have been the concentration of cholesterol in the blood and the relative hypo- or hypercholesterolemic effect of certain diets. Saturated and *trans* fatty acids are generally believed to be hypercholesterolemic, whereas polyunsaturated *cis* fatty acids tend to lower serum cholesterol. Monounsaturated *cis* fatty acids, representing the most abundant fatty acids in the food supply, have neutral effects on serum cholesterol.

Fatty acids can be synthesized by cytosolic enzyme systems when energy provided by carbohydrate is adequate. The synthesis begins with simple precursors such as acetyl-CoA and can be triggered by hormonal signals or by elevated levels of citrate, which acts as a regulatory substance. Blood glucose concentration also acts as a sensitive regulator of lipogenesis, which is stimulated when a hyperglycemic state exists.

Ethanol is catabolized ultimately to acetyl-CoA, furnishing energy through its TCA cycle oxidation. The nutritional complexities of alcohol abuse were discussed.

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These articles discuss the political and health implications of banning trans fat from the food supply. Also discussed is the extent to which such bans lower trans fat consumption.

Web Sites

fnic.nal.usda.gov/diet-and-disease

Food and Nutrition Information Center of the National Agricultural Library (U.S. Department of Agriculture).

www.heart.org

American Heart Association. Provides dietary recommendations for reducing risk of cardiovascular diseases.

www.eatright.org

Academy of Nutrition and Dietetics. Provides dietary and exercise recommendations for all age groups.

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THE ROLE OF LIPOPROTEINS AND INFLAMMATION IN ATHEROSCLEROSIS

Atherosclerosis is a disease of the arteries in which lipid-filled plaques develop within the artery wall, causing the arterial lumen to narrow (stenosis), thereby restricting blood flow and the supply of oxygen (ischemia). Atherosclerotic plaque is the root cause of coronary heart disease (also called ischemic heart disease), ischemic stroke, and peripheral artery disease. These conditions belong to the larger category of cardiovascular diseases (CVD), along with all other nonatherosclerotic diseases of the cardiovascular system. Atherosclerotic diseases account for nearly 20% of all deaths in the United States, second only to cancer [1].

Atherosclerosis is a chronic inflammatory disease as well as a disorder of lipid metabolism [2]. Chapter 5 covered the role played by disorders in lipid and lipoprotein metabolism in the development of atherosclerosis and CVD. There are still many aspects of atherogenesis we do not fully understand, but considerable insight has been gained beyond the classical view of a couple of decades ago. The classical view postulated an ever-increasing plaque with a concomitant decrease in size of the arterial lumen, much like the accumulation of rust in a water pipe, caused by alterations in lipid metabolism [3]. It is now recognized that plaque development is caused by the combination of inflammation and dyslipidemia. Under normal circumstances, the components of blood—red cells, white cells, and platelets—flow past the inner lining without adhering to it. Fatty streaks are prevalent but asymptomatic in young people; they may at some point cause symptoms or just disappear. So what happens to start the process that causes a plaque to form, and what triggers a plaque to produce a blood clot (thrombosis)? Just how is inflammation involved? This Perspective explores the current understanding of atherosclerosis.

Inflammation is just one part of the body's innate immune response to tissue damage or a foreign object. Inflammation is a nonspecific response to injury in which phagocytic cells, neutrophils and macrophages, play an important role. The inflammation response is similar regardless of the cause of the damage. Typically, when there is an injury, the local macrophages release cytokines, which are chemicals released by leukocytes that function as mediators for the inflammation response [4].

Larger arteries are considered to be elastic—they contain the protein elastin—and do not restrict flow to any great extent. The medium- to small-size arteries, often called arterioles, make up most of the arterial bed that restricts blood flow and increases blood pressure. Of these, smaller coronary arteries of the heart are a special example. Unlike peripheral arteries, coronary arteries fill when the heart is in diastole (relaxed) and empty during systole (contracted). If a coronary artery contains an atherosclerotic plaque and develops a clot, a myocardial infarct occurs and can cause death of the cardiac muscle [5]. These smaller arteries do not contain the elastin protein but do contain more smooth muscle. Their walls are made up of three layers: the inner layer or tunica intima, the middle layer or tunica media, and the outer layer or adventia. This outer layer contains connective tissue, nerve endings, mast cells, fibroblasts, and micro-vessels. The innermost layer, the intima, includes a monolayer of endothelial cells that are in contact with the blood plus a small layer of smooth muscle cells under the endothelial cells. The middle layer also contains smooth muscle cells along with a variety of other cell types.

When these arteries are subjected to any of several negative stimuli associated with risk factors for CVD such as hypertension, dyslipidemia, or pro-inflammatory mediators, the endothelial cells produce proteins on their outer matrix that act as an adhering factor. The platelets, the first blood component to interact with the endothelial cells, further change the cell surface, allowing leukocytes (white blood cells) to begin to attach to the endothelial cells. The first cells to attach are the macrophages, which release cytokines, which in turn call in additional leukocytes. The most common type of leukocyte, and the most numerous among the leukocytes adhering to the endothelial cells, is the monocyte. The monocytes are directed into the intima by pro-inflammatory effectors such as cytokines or tumor necrosis factor (TNF). The monocytes differentiate into macrophages and take up the cholesterol-rich LDL particles by endocytosis. When they have engulfed sufficient lipid they are called foam cells because of their microscopic appearance. The cytokines and TNF attract additional monocytes into the intima and media. Other leukocytes such as T-cells and mast cells also accumulate in the media. As the **atheroma** progress, additional smooth muscle cells from the media are attracted into the intima and then proliferate in response to platelet-derived growth factors. The smooth muscle cells produce the extracellular proteins collagen and elastin, forming a fibrous cap that covers the plaque [5].

The plaque can enlarge and thus narrow the lumen of the artery so that it begins to impede the blood flow; it may or may not progress to clot formation. The balance between inflammation and anti-inflammatory activity controls the progression of the atherosclerosis [2]. The thrombus (clot) is most likely to form when the fibrous cap is physically disrupted, which can be caused by several mechanisms [5]. The immune cells that have been attracted to the plaque, including activated macrophages, T-cells, and mast cells, produce a variety of molecules that can destabilize the plaque. These include inflammatory cytokines, activated oxygen species, proteases, coagulation factors, and vasoactive molecules [2]. Some of the smooth muscle cells and macrophages die, leaving lipid droplets and crystals of cholesterol that enhance the plaque's lipid core. The polyunsaturated fatty acids and cholesterol can be oxidized by the reactive oxygen species, producing epoxy fatty acids or oxysterols. The macrophages also produce pro-coagulant factors that make the lipid core more thrombogenic.

Known risk factors for CVD can initiate the pathogenic pathway. For instance, hypertension can increase arterial wall tension, leading to a disturbed repair process and aneurysm formation. Also, angiotensin is a major pressor hormone that alters the endothelial membrane and can be the first factor to initiate the leukocyte adherence. Cigarette smoking and diabetes likewise alter endothelial function, which increases the risk for plaque development, but the mechanism is unknown. Once the process begins with leukocyte adherence the cascade of events described previously follows, and plaque development is the result. However, if LDL levels are reduced, the plaque formation is retarded.

Recent research demonstrates that inflammation plays a key role in atherogenesis within small- to mid-size arteries, including coronary arteries. Immune cells and pro-inflammatory effectors are prominent in the early formation of the atherosclerotic plaque. The plaque would not be such a problem if it were not for its tendency to rupture and the subsequent clot formation. The known CVD risk factors also alter the expression of certain pro-inflammatory genes. Certain areas in the genome are associated with myocardial infarction [2].

HDL plays an important role in modifying plaque formation and development. HDL particles are involved in reverse cholesterol transport, as described in the previous chapter. HDL particles also have anti-inflammatory actions. The cholesterol ester transfer protein (CETP) facilitates the exchange of cholesterol esters in HDL for triacylglycerol in the LDL particles, which removes some of the oxidized lipid. The anti-inflammatory properties of HDL reverse some of the inflammation that drives the deposition of new lipid.

In summary, atherosclerosis was once thought to be due primarily to dyslipidemia, but it is now known that inflammation plays a major role in its development. With this increased

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understanding of the role of inflammation in atherosclerosis, the prevention and treatment of CVD can go beyond managing dyslipidemia to focus on reducing inflammation as well.

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6 PROTEIN

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SUMMARY
PERSPECTIVE
STRESS AND INFLAMMATION: IMPACT ON PROTEIN

THE IMPORTANCE OF PROTEIN IN NUTRITION and health cannot be overemphasized. It is quite appropriate that the Greek word chosen as a name for this nutrient is *proteos*, meaning "primary" or "taking first place." Proteins are found throughout the body, with over 40% of body protein found in skeletal muscle, over 25% found in body organs, and the rest found mostly in the skin and blood. Proteins are essential nutritionally because of their constituent amino acids, which the body must have to synthesize its own variety of proteins and nitrogen-containing molecules that make life possible. Each body protein is unique in the characteristics and sequence pattern of the amino acids that comprise its structure. This chapter focuses first on classifications of amino acids. Next, sources and digestion of protein to provide amino acids to the body are reviewed, along with how the amino acids are subsequently absorbed and metabolized in cells. The body's use of amino acids to make proteins and nitrogen-containing nonprotein compounds as well as the functional roles of these proteins and compounds are also presented. Lastly, changes in the body's protein (lean) mass with aging are covered, as are recommended intakes of protein, protein quality, assessment of protein and amino acid needs, and protein deficiency.

AMINO ACID CLASSIFICATION

Amino acids may be classified in a variety of ways, including by structure, net charge, polarity, and essentiality. This section addresses each of these four classifications.

Structure

Structurally, amino acids have a central carbon (C), at least one amino group ($—NH_2$), at least one carboxy (acid) group ($—COOH$), and a side chain (R group) that makes each amino acid unique. The generic amino acid may be represented as follows:

$$H_2N—\underset{\underset{R}{|}}{CH}—COOH$$

However, depending on the pH of the environment, the amino and carboxy groups can accept or donate H^+, and thus the amino group may be represented as $^+NH_3$ and the carboxy group as COO^-. The distinctive characteristics of the side chains of the amino acids that make up a polypeptide bestow on a protein its structure and influence its functional role in the body. These same distinctive characteristics determine whether certain amino acids can be synthesized in the body or must be ingested. Furthermore, these characteristics program the

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various amino acids for their specific metabolic pathways in the body. The differences among the side chains of the amino acids commonly found in body proteins are shown in Table 6.1. This division of amino acids based on structural similarities is one approach used to classify amino acids; dividing amino acids based on the presence or absence of a net charge is another.

Net Electrical Charge

Table 6.1 shows the structures of amino acids as they exist in an aqueous solution at the physiological pH, approximately 6–8, of the human body. Amino acids in an aqueous solution are ionized. The term ***zwitterion***, or dipolar ion, is applied to amino acids with no carboxy or

Table 6.1 Structural Classification of Amino Acids

1. *With aliphatic/nonpolar side chains*

Glycine (Gly)

Alanine (Ala)

Valine (Val)

Leucine (Leu)

Isoleucine (Ile)

2. *With side chains containing hydroxylic (OH) groups**

Serine (Ser)

Threonine (Thr)

3. *With side chains containing sulfur atoms*

Cysteine (Cys)

Methionine (Met)

4. *With side chains containing acidic groups or their amides*

Aspartic acid (Asp)

Glutamic acid (Glu)

Asparagine (Asn)

Glutamine (Gln)

5. *With side chains containing basic groups*

Arginine (Arg)

Lysine (Lys)

Histidine (His)

6. *With side chains containing aromatic ring*

Phenylalanine (Phe)

Tyrosine (Tyr)

Tryptophan (Trp)

* Although tyrosine contains a hydroxyl group, it is classified as an amino acid containing an aromatic ring (see Group 6).

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Table 6.1 (*Continued*)

7. *Imino acids*
Proline (Pro)

8. *Amino acids formed posttranslationally*
Cystine (Cys-S-S-Cys)

Hydroxylysine (Hyl)

Hydroxyproline (Hyp)

3-methylhistidine (3-meHis)

amino groups in their side chain to generate an additional charge to the molecule. Zwitterions have no net electrical charge because their side chains are not charged, and the one positive and one negative charge from the amino and carboxy groups, respectively, in their base structure cancel each other out. Amino acids with no net charge, also called neutral amino acids (and listed in Table 6.2A), do not migrate substantially if placed in an electric field.

Two groups of amino acids (Table 6.2B) exhibit a net charge. Because of the presence of an additional carboxy group in the side chain, the dicarboxylic (also called acidic) amino acids aspartic acid and glutamic acid exhibit a net negative charge at pH 7; these forms of the amino acids are called aspartate and glutamate. Dicarboxylic amino acids or proteins with a high content of dicarboxylic amino acids migrate toward the anode if placed in an electric field. In contrast, because of the presence of an additional amino group in the side chain, the basic (also called dibasic) amino acids (lysine, arginine, histidine) exhibit a net positive charge at pH 7. These amino acids or proteins rich in them will migrate toward the cathode if placed in an electrical field.

Polarity

The tendency of an amino acid to interact with water at physiological pH—that is, its polarity—represents another means of classifying amino acids. Polarity depends on the side chain or R group of the amino acid. Amino acids are classified as polar or nonpolar, although they can have varying levels of polarity. Polar charged amino acids include both the dicarboxylic (aspartic acid and glutamic acid) and basic (lysine, arginine, histidine) amino acids, as shown in the second column of Table 6.2C. Polar charged amino acids interact with aqueous environments, can form salt bridges, and can interact with electrolytes/minerals such as potassium, chloride, and phosphate.

The neutral amino acids interact with water to different degrees and can be divided into polar, nonpolar, and relatively nonpolar categories, as listed in the first, third, and last columns of Table 6.2C. The side chains of polar neutral amino acids (first column of Table 6.2C) contain functional groups—such as the hydroxyl group for serine and threonine, the sulfur atom for cysteine, and the amide group for asparagine and glutamine—that can interact through hydrogen bonds with water (the aqueous environment of cells). Polar amino acids are generally found on the surfaces of proteins. If not found on the surface, they are oriented inward and function at a protein's (such as an enzyme's) binding site.

In contrast, the amino acids listed in the third column of Table 6.2C contain side chains that do not interact with water and are categorized as nonpolar or hydrophobic (water fearing). Hydrophobicity tends to increase as the length of the side chain increases (i.e., the more carbons found in the side chain). The term *alipathic* is often used when discussing nonpolar amino acids; amino acids that are alipathic contain carbons and hydrogens in their side chains but no functional groups like hydroxyl groups.

As the last column in Table 6.2C reveals, the aromatic amino acids are considered relatively nonpolar. Tyrosine, for example, because of its hydroxyl group on the phenyl ring, can to a limited extent form hydrogen bonds with water—hence the term *relatively nonpolar*. Because they do not interact with water, the nonpolar (and often the relatively nonpolar) amino acids are typically found tightly coiled in proteins or compacted (e.g., attracted by van der

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Table 6.2A Neutral Amino Acids (one-letter codes)

Alanine (A)	Glycine (G)	Phenylalanine (F)	Trytophan (W)
Asparagine (N)	Isoleucine (I)	Proline (P)	Tyrosine (Y)
Cysteine (C)	Leucine (L)	Serine (S)	Valine (V)
Glutamine (Q)	Methionine (M)	Threonine (T)	

Table 6.2B Amino Acids (one-letter code) Exhibiting a Net Charge

Negatively Charged Amino Acids	Positively Charged Amino Acids
Aspartic acid (D)	Arginine (R)
Glutamic acid (E)	Histidine (H)
	Lysine (K)

Table 6.2C Polar and Nonpolar Amino Acids

Polar Neutral Amino Acids	Polar Charged Amino Acids	Nonpolar Neutral Amino Acids	Relatively Nonpolar Amino Acids
Asparagine	Arginine	Alanine	Phenylalanine
Cysteine	Lysine	Glycine	Tryptophan
Glutamine	Histidine	Isoleucine	Tyrosine
Serine	Glutamate	Leucine	
Threonine	Aspartate	Methionine	
		Proline	
		Valine	

Waal forces) and oriented toward or within the central region or core portion of proteins.

Essentiality

While amino acids can be classified based on structure or properties such as net charge and polarity, in 1957 Rose [1] categorized the amino acids found in proteins as nutritionally essential (indispensable) or nutritionally nonessential (dispensable). In the context of nutrition, the term *essential* or *indispensable* means that the body cannot make the nutrient (in this case the amino acid), and that it must be supplied by the diet. Back in the 1950s, only eight amino acids—leucine, isoleucine, valine, lysine, tryptophan, threonine, methionine, and phenylalanine—were considered essential for adult humans. Histidine was later added as an essential amino acid. Table 6.2D lists the essential amino acids.

Identifying amino acids strictly as nonessential/dispensable or essential/indispensable is an inflexible classification, however, that allows no gradations, even in decidedly different or changing physiological circumstances. Therefore, a third category exists: conditionally or acquired indispensable amino acids. A dispensable amino acid may become indispensable if, for example, there are rate limitations to its synthesis such as may occur if precursor availability is limited. Similarly,

Table 6.2D Essential/Indispensable Amino Acids

Phenylalanine	Methionine	Isoleucine
Valine	Tryptophan	Leucine
Threonine	Histidine	Lysine

Table 6.2E Conditionally Indispensable Amino Acids and Their Precursors

Amino Acid	Precursor(s)
Tyrosine	Phenylalanine
Cysteine	Methionine, serine
Proline	Glutamate
Arginine	Glutamine or glutamate, aspartate
Glutamine	Glutamate, ammonia

if an organ does not function properly then amino acid metabolism may not proceed normally. For example, neonates born prematurely often have immature organ function. Immature liver function or liver disease (cirrhosis) impairs both phenylalanine and methionine metabolism, which occurs primarily in the liver. Consequently, the amino acids tyrosine and cysteine, normally synthesized via phenylalanine and methionine catabolism, respectively, become indispensable until normal organ function is established. Inborn errors of amino acid metabolism, which result from inherited mutations in enzymes needed for amino acid use, represent another situation in which dispensable amino acids may become indispensable. Phenylalanine hydroxylase, the enzyme that converts phenylalanine to tyrosine, exhibits little to no activity in people with inborn error of metabolism known as classic phenylketonuria (PKU). Without adequate hydroxylase activity, tyrosine is not synthesized in the body and must be provided completely by the diet; in other words, tyrosine is indispensable for those with PKU. With trauma, endogenous arginine synthesis is typically not sufficient, and thus it is considered conditionally essential in conditions of trauma. Some examples of conditionally indispensable amino acids are listed in Table 6.2E, along with their usual amino acid precursors.

SOURCES OF AMINO ACIDS

Amino acids are derived from protein. Both dietary (exogenous) and endogenous proteins provide the body with amino acids. Dietary sources of protein include:

- animal products such as meat, poultry, fish, eggs, and dairy (with the exception of butter, sour cream, and cream cheese)
- plant products such as grains, grain products, legumes (including lentils, beans, and peas), nuts, seeds, and vegetables.

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Adult men and women (age 20–70 years) in the United States ingest about 98 g and 68 g of protein per day, respectively, and adults over 70 years ingest about 66 g per day [2]. Protein intake is typically greatest (over 60%) at the evening meal and smallest at the morning meal [2].

Following ingestion, exogenous proteins serve as sources of the essential amino acids, nonessential amino acids, and additional nitrogen needed to synthesize more nonessential amino acids, nitrogen-containing compounds , and protein in the body. The differences between animal and plant proteins are discussed in the section of this chapter on protein quality and protein synthesis.

Endogenous proteins presented to the digestive tract represent another source of amino acids and nitrogen. Endogenous proteins include:

- desquamated mucosal cells
- digestive enzymes and glycoproteins.

The digestive enzymes and glycoproteins are derived from secretions of the salivary glands, stomach, intestine, liver, and pancreas. The digestive tract's mucosal cells contain a variety of proteins (such as apoproteins, structural proteins, and cytosolic enzymes) that when sloughed into the gastrointestinal (GI) tract are degraded. Most of these endogenous proteins, which typically total 70 g or more per day, are digested and provide amino acids that are available for absorption. Digestion of these proteins and the absorption of subsequently generated amino acids are crucial for protein nutriture.

DIGESTION

This next section of the chapter addresses protein digestion within the gastrointestinal tract organs (Figure 6.1 and Table 6.3) and emphasizes the major enzymes responsible for protein digestion. Chapter 2 provides detailed information on the digestive tract, its organs, and digestive processes. As no appreciable digestion of protein occurs in the mouth or esophagus, this discussion focuses first on the stomach.

Stomach

The digestion of protein begins in the stomach with the action of hydrochloric acid (HCl), which is found in gastric juice. The hydrochloric acid content of the gastric juice results in a gastric pH less than 3 and enables denaturation

Figure 6.1 An overview of protein digestion.
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Table 6.3 Major Enzymes Responsible for Protein Digestion

Zymogen	Enzyme or Activator	Enzyme	Site of Activity	Substrate (peptide bonds adjacent to)	End Product(s)
Pepsinogen	HCl or pepsin →	Pepsin	Stomach	Most amino acids, including aromatic, dicarboxylic, leu, met	Peptides
Trypsinogen	Enteropeptidase or Trypsin →	Trypsin	Intestine	Basic amino acids	Smaller peptides, some free amino acids
Chymotrypsinogen	Trypsin →	Chymotrypsin	Intestine	Aromatic amino acids, met, asn, his	Smaller peptides, some free amino acids
Procarboxypeptidases	Trypsin →	Carboxypeptidase	Intestine		
		A		C-terminal neutral amino acids	Free amino acids
		B		C-terminal basic amino acids	Free amino acids
		Aminopeptidases	Intestine	N-terminal amino acids	Free amino acids

(disruption) of the quaternary, tertiary, and secondary structures of protein (shown later in Figures 6.17, 6.18, 6.19). Denaturants such as hydrochloric acid break apart hydrogen and electrostatic bonds to unfold or uncoil the protein; however, peptide bonds are not affected by the hydrochloric acid. Hydrochloric acid does, however, begin pepsin activation from pepsinogen, which is secreted as a zymogen (inactive enzyme) by gastric chief cells. Pepsin, once formed, is catalytic against pepsinogen as well as other proteins.

$$\text{Pepsinogen} \xrightarrow{\text{HCl or pepsin}} \text{Pepsin}$$

Pepsin functions as an **endopeptidase** (meaning that it hydrolyzes interior peptide bonds within proteins or polypeptides) at a pH < ~3.5. Specifically, pepsin attacks peptide bonds adjacent to the carboxy end of a relatively wide variety of amino acids (i.e., pepsin has low specificity), including leucine; methionine; the aromatic amino acids (phenylalanine, tyrosine, and tryptophan); and the dicarboxylic amino acids (glutamate and aspartate).

The end products of gastric protein digestion include primarily large polypeptides, along with some oligopeptides (short chains of amino acid peptide bonded to each other) and free amino acids. These end products are emptied in an acidic chyme through the pyloric sphincter into the duodenum (the proximal or upper part of the small intestine) for further digestion.

Small Intestine

The chyme–nutrient mixture that is delivered into the duodenum further stimulates the release of regulatory hormones and peptides such as secretin and cholecystokinin; these regulatory hormones and peptides facilitate further digestion of proteins and polypeptides. For example, secretin and cholecystokinin are carried by the blood to the pancreas, where selected pancreatic cells are stimulated to secrete zymogens and pancreatic juice containing bicarbonate, electrolytes, and water. In addition to pancreatic juice, the Brunner's glands of the small intestine release mucus-rich secretions needed for digestion.

Zymogens secreted by the pancreas into the intestine and further responsible for protein and polypeptide digestion include:

- trypsinogen
- chymotrypsinogen
- procarboxypeptidases A and B
- proelastase.

Within the small intestine, these zymogens are chemically altered to be converted into their respective active enzymes capable of protein hydrolysis. The activation of trypsinogen by enteropeptidase is important since the formation of trypsin facilitates activation of other zymogens. Yet, while trypsinogen activation is important in the intestine, extensive damage would occur should trypsinogen become active within the pancreas. To prevent this, the pancreas produces a compound called trypsin inhibitor.

$$\text{Trypsinogen} \xrightarrow{\text{Enteropeptidase}} \text{Trypsin}$$

Enteropeptidase (an endopeptidase formerly known as enterokinase), which is secreted from the enterocyte in response to cholecystokinin and secretin, converts trypsinogen to trypsin. Once trypsin is formed, it can convert (like enteropeptidase but to a lesser extent) other trypsinogen molecules and other zymogens to their respective active **proteolytic** enzymes (proteases).

$$\text{Trypsinogen} \xrightarrow{\text{Trypsin}} \text{Trypsin}$$

Trypsin, for example, activates the zymogen chymotrypsinogen, as shown here:

$$\text{Chymotrypsinogen} \xrightarrow{\text{Trypsin}} \text{Chymotrypsin}$$

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Trypsin and chymotrypsin are both endopeptidases. Trypsin is specific for peptide bonds at the carboxy end of basic amino acids (lysine and arginine). Excess trypsin also acts by negative feedback to inhibit trypsinogen synthesis by pancreatic cells, thereby regulating pancreatic zymogen secretion. Chymotrypsin is specific for peptide bonds at the carboxy end of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and for peptide bonds adjacent to methionine, asparagine, and histidine.

Procarboxypeptidases are also converted to carboxypeptidases by trypsin and function as **exopeptidases**.

$$\text{Procarboxypeptidases} \xrightarrow{\text{Trypsin}} \text{Carboxypeptidases}$$

These exopeptidases attack peptide bonds at the carboxy (C)–terminal end of polypeptides to release free amino acids. Carboxypeptidases are zinc dependent, specifically requiring zinc at the enzyme's active site. Carboxypeptidase A hydrolyzes peptides with C-terminal aromatic or aliphatic (nonpolar) neutral amino acids. Carboxypeptidase B cleaves basic amino acids from the C-terminal end to generate free basic amino acids as end products.

Elastase, once activated by trypsin, functions as an endopeptidase to hydrolyze bonds adjacent to aliphatic amino acids, including those in elastin. Elastin is a component of connective tissues and is found especially in meats.

Several peptidases are produced by enterocytes, including those in the ileum, enabling peptide digestion and amino acid absorption to occur in the distal small intestine. Some of these intestinal peptidases include the following:

- Aminopeptidases, which vary in specificity, cleave amino acids from the amino/(N)-terminal end of oligopeptides.
- **Dipeptidyl aminopeptidases**, some of which are magnesium dependent, hydrolyze dipeptides.
- Tripeptidases, which are specific for selected amino acids, hydrolyze tripeptides to yield a dipeptide and a free amino acid.

Not all tripeptides, however, undergo additional digestion to produce free amino acids at the brush border of enterocytes. Triglycine and proline-containing peptides tend to be absorbed intact and hydrolyzed within the enterocyte. The digestive process is influenced to some extent by the free amino acid end products, which can sometimes inhibit the activity of brush border peptidases (a process called end product inhibition) to diminish digestion.

Protein digestion yields two main end products: peptides (principally dipeptides and tripeptides) and free amino acids. To be used by the body, these end products must next be absorbed.

ABSORPTION

Absorption is the process by which the end products of digestion are transported from the lumen of the gastrointestinal tract, most often the small intestine, into the body. To get into the blood for transport to tissues, amino acids must cross two intestinal membranes, the brush border (also called apical) membrane and the basolateral (also called serosal) membrane. Of the 100+ g of amino acids present each day in the small intestine, the equivalent of about 10 g is not absorbed (and excreted and assayed as fecal nitrogen). This section of the chapter addresses transporters found in cell membranes that carry the end products of protein digestion into and out of cells.

Intestinal Cell Absorption

Amino Acid Absorption across the Intestinal Brush Border Membrane

Amino acid absorption occurs along the entire small intestine, but most amino acids are absorbed in the duodenum and jejunum. The absorption of amino acids into enterocytes requires carriers (integral membrane proteins); however, paracellular absorption—that is, absorption via passage through the tight junctions of enterocytes or transcellular endocytosis—can occur in occasional situations in which large volumes of hypotonic fluids containing some amino acids have been ingested.

Transport systems for amino acids have been traditionally designated using a lettering system, with a further distinction that uppercase letters be used for sodium dependence and lowercase letters for sodium independence; however, not all systems (e.g., the L, which is sodium independent) follow this rule. Moreover, with most of the systems now cloned, amino acid transporters have been reclassified and characterized in further detail. Table 6.4 lists some of the transport systems responsible for carrying amino acids across the brush border membrane into the intestinal cell and some examples of amino acids that are carried by each of these transport systems. The older nomenclature has been retained in this text because it facilitates associations between the transporter names and the amino acids carried by the transporters.

The transporters vary in mechanism of action. Some amino acid transporters, such as y^+, are passive and function as uniporters; they bind to one amino acid and transport

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Table 6.4 Some Systems Transporting Amino Acids across the Intestinal Cell Brush Border Membrane

Amino Acid Transport Systems	Sodium Required	Some Amino Acids Carried
L	No	Leucine, other neutral amino acids
B^o	Yes	Most neutral amino acids
IMINO	No	Proline, hydroxyproline
PAT (imino acid)	No	Proline, glycine, alanine, β-alanine
y^+	No	Basic amino acids—arginine, lysine, histidine
X^-_{AG}	Yes	Acidic amino acids—aspartate, glutamate
$B^{o,+}$	Yes	Most neutral and basic amino acids, β-alanine
$b^{o,+}$	No	Arginine, lysine, ornithine, cysteine
ASC	Yes	Alanine, serine, cysteine, threonine, glutamine
N	Yes	Glutamine, asparagine, histidine
X^-_c	No	Glutamate, cystine

it into the cell with its concentration gradient. Symporters simultaneously carry two substances into the cell. For example, basic amino acids may be carried into the cell along with neutral amino acids via the y^+L system. Lastly, some amino acids enter cells via an antiport mechanism in which two substances move across the enterocyte membrane in opposite directions. The movement of one substance across the enterocyte membrane and down its concentration gradient provides the means for the transport of a second substance against its gradient in the opposite direction. For example, with the X^-_{AG} antiport transporter, glutamate, H^+, and three Na^+ enter the cell in exchange for one K^+, and with the N system, glutamine and Na^+ enter the cell in exchange for H^+. Transport of leucine and the other branched-chain amino acids isoleucine and valine into some cells (such as muscle) may involve the bidirectional transport of glutamine. Most amino acids are thought to be transported across the enterocyte brush border membrane by sodium-dependent transporters, as shown and described in Figure 6.2.

The affinity (K_m) of a carrier for an amino acid is influenced both by the hydrocarbon mass of the amino acid's side chain and by the net electrical charge of the amino acid. As the hydrocarbon mass of the side chain increases, affinity increases [3]. Thus, the branched-chain amino acids typically are absorbed faster than smaller amino acids. Neutral amino acids also tend to be absorbed at higher rates than basic or acidic amino acids. Essential (indispensable) amino acids are absorbed faster than nonessential (dispensable) amino acids, with methionine, leucine, isoleucine, and valine being the

Figure 6.2 Sodium (Na^+)–dependent transport of an amino acid into a cell.

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most rapidly absorbed [3]. The most slowly absorbed amino acids are the two acidic amino acids, glutamate and aspartate, both of which are nonessential [3]. Changes in *de novo* synthesis of specific amino acid carriers help to ensure adequate capacity.

However, ingesting large quantities of one amino acid or a particular group of amino acids that use the same carrier system may create, depending on the amount ingested, competition among the amino acids for absorption. The result may be that the amino acid present in highest concentration is absorbed but also may impair the absorption of the other, less concentrated amino acids carried by that same system. Thus, amino acid supplements may result in impaired or imbalanced amino acid absorption. Moreover, absorption of peptides (discussed in the next section) is more rapid than absorption of an equivalent mixture of free amino acids. These differences in absorption impact protein synthesis as discussed in the "Protein Synthesis" section of this chapter. And, in those with intestinal resection or damage, ingestion of protein-containing foods (versus free amino acids) may improve growth of remaining enterocytes and enhance peptide transporter expression [4].

Peptide Absorption across the Intestinal Brush Border Membrane

Peptide (primarily dipeptide and tripeptide) transport across the brush border membrane of the enterocyte is accomplished by a transport system different from those that transport amino acids. The transport system designated PEPT1 appears to transport all di- and tripeptides. This transport of peptides across the brush border membrane using PEPT1 is associated with the comovement of protons (H^+) and thus depolarization of the brush border membrane. An area of low pH lying adjacent to the brush border surface of the enterocyte provides the driving force for the H^+ gradient. Thus, as shown in Figure 6.3, as the dipeptide or tripeptide is transported into the enterocyte, an H^+ ion also enters the enterocyte. The transport of the H^+ into the enterocyte results in an intracellular acidification. The H^+ ions are pumped back out into the lumen in exchange for Na^+ ions. A Na^+/K^+-ATPase allows for Na^+ extrusion at the basolateral membrane to maintain the gradient.

1 Peptides are transported into the intestinal cell along with H^+.

2 The H^+ are pumped back into the intestinal lumen in exchange for Na^+.

3 A Na^+, K^+–ATPase pumps Na^+ out of the cell in exchange for K^+ across the basolateral membrane.

Figure 6.3 Peptide transport. Peptides are transported across the brush border membrane of the intestinal epithelial mucosal cell.

Although peptides, like amino acids, compete with one another for transporters, peptide transport appears to occur more rapidly than amino acid transport and is thought to represent the primary means by which most amino acids enter the intestinal cell. In other words, the majority of amino acids are absorbed as peptides. Peptides, once within the enterocytes, are generally hydrolyzed by cytosolic peptidases to generate free intracellular amino acids. Intact peptides, however, can be found occasionally in circulation, and this is thought to result from entry of peptides into the body via paracellular (also called intercellular) routes. With illnesses, especially those affecting the intestines (such as inflammatory bowel diseases or celiac disease), the gastrointestinal tract can become more permeable, thus increasing the likelihood of peptides appearing intact in the blood.

Peptides found in the blood can be hydrolyzed by peptidases or proteases in the plasma or at the cell membrane (especially in the liver, kidneys, and muscle). Intracellular hydrolysis of the peptide, if absorbed intact, may also occur in the cytosol or in various organelles. Peptide transport into renal tubular cells is influenced by the net charge of the amino acid at the amino (N-) and the carboxy (C-) terminals. Peptides containing either basic or acidic amino acids at either the N- or C-terminal have lower affinity for transport than peptides with neutral side chains at these positions. The ability to administer peptides directly into the blood as in parenteral nutrition is of nutritional significance since some amino acids (e.g., tyrosine, cysteine, and glutamine) are insoluble or unstable in their free form. Administration of the peptide form, since it can be used by tissues, thus allows nutrients to be provided in situations in which traditional free amino acid parenteral mixtures are ineffective.

Amino Acid Absorption across the Intestinal Basolateral Membrane

For amino acids to enter the blood and be used by other body tissues, the amino acids must be transported across the basolateral (serosal) membrane of the

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Table 6.5 Some Systems Transporting Amino Acids across the Intestinal Cell Basolateral Membrane

Amino Acid Transport System	Sodium Required	Some Amino Acids Carried
L	No	Leucine, other neutral amino acids except proline
T	No	Phenylalanine, tyrosine, tryptophan
X_c^-	No	Glutamate, cystine
A	Yes	Alanine, glycine, serine, proline, cysteine, methionine
y^+L	Yes	Lysine, arginine, histidine, glutamine, methionine, leucine, alanine, cysteine

enterocyte and into interstitial fluid, where they enter the blood through capillaries of the villi for transport into the portal vein leading to the liver. The carriers found in the enterocyte's basolateral membrane are generally sodium independent and similar to those found in other cell membranes (see Table 6.5).

Defects in Amino Acid Absorption

The significance of the amino acid transporters becomes extremely apparent when genetic mutations prevent the synthesis of functional transporters. Lysinuric protein intolerance, for example, results from defects in the basic amino acid transporters in the intestine, liver, and kidneys. The defects cause poor absorption of lysine, arginine, and ornithine, and thus low plasma concentrations and availability of these amino acids for protein synthesis and for urea cycle activity. Symptoms of the disorder include hyperammonemia, growth retardation, muscle weakness, hepatomegaly, and hypotonia, among other problems. Nutrition support involves a protein-restricted diet to help minimize the hyperammonemia and supplements of citrulline to help improve arginine and ornithine production.

Similarly, the critical role of transporters is illustrated by another condition called **Hartnup disease**, an autosomal recessive genetic disorder that affects absorption (likely via the B transport system) of tryptophan and other neutral amino acids into intestinal and kidney cells. Hartnup disease causes malabsorption of tryptophan along with some other amino acids. Niacin deficiency can occur because of insufficient availability of tryptophan, which serves as a precursor of niacin (see Chapter 9). Mutations in another carrier result in intestinal cell methionine malabsorption that is characterized by large quantities of methionine along with branched-chain amino acids in the feces. Intestinal tryptophan malabsorption, due to a suspected defect in the gene coding for the T system in the basolateral membrane, is characterized by blue diaper syndrome along with failure to thrive, nephrocalcinosis, and hypercalcemia. Increased fecal tryptophan concentrations are found along with indoles generated by bacterial tryptophan degradation. The indoles, following colon cell absorption and transport to the liver, are converted to indoxylsulfate. The indoxylsulfate is then excreted from the body via the kidneys and feces. However, when indoxylsulfate is exposed to air (as with changing the diaper), it oxidizes to a blue (indigo) color, hence the name blue diaper syndrome.

Extraintestinal Cell Absorption

After amino acids are transported out of the enterocyte, they enter portal blood and are transported to tissues. Uptake of the amino acids into liver cells (hepatocytes), as well as cells of the kidneys and other organs, occurs by some carrier systems similar to those found in the intestinal cell membranes. The sodium-dependent N system is especially prominent in the periportal cells of the liver and functions as an antiporter to take up glutamine and sodium in exchange for H^+. The process occurs in reverse in the perivenous hepatic cells; glutamine is released in exchange for H^+. Hormones and cytokines, such as interleukin-1 and tumor necrosis factor α, influence amino acid transport. System A in hepatocytes, for example, is induced by glucagon and provides amino acid substrates for gluconeogenesis. System GLY is sodium dependent and specific for glycine; two sodium ions are transported for each glycine. The γ-glutamyl cycle is thought to be involved in amino acid transport through membranes of renal tubular cells, erythrocytes, and perhaps neurons. In the γ-glutamyl cycle, glutathione acts as a carrier of neutral amino acids into cells. The cycle is depicted in detail in Figure 6.4. Briefly, glutathione reacts with γ-glutamyl transpeptidase located in cell membranes, forming a γ-glutamyl enzyme complex, which binds a neutral amino acid at the cell surface and transports it into the cytosol for use.

AMINO ACID CATABOLISM

The liver is the primary site for the uptake of most amino acids following ingestion of a protein-containing meal. The liver is thought to monitor the absorbed amino acids and to adjust the rate of their metabolism (including catabolism or breakdown of amino acids, and anabolism or use of amino acids for synthesis) according to the needs of the body. In this section of

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1. Glutathione reacts with γ-glutamyl transpeptidase to form a γ-glutamyl enzyme complex.
2. The glutamate portion of glutathione remains attached to the enzyme complex while cysteinyl-glycine (Cys-Gly) is released and an amino acid binds to the glutamate enzyme complex. The cysteinyl-glycine is eventually cleaved into its constituent amino acids by a cytosolic peptidase.
3. γ-glutamyl cyclotransferase cleaves the peptide bond between the amino acid and the γ-carbon of the glutamate enzyme complex.
4. The free amino acid can be used within the cell.
5. 5-oxoproline generated from step 3 is used to reform glutamate and via several steps glutathione (step 1).

Figure 6.4 The γ-glutamyl cycle for transport of amino acids.

the chapter, an overview of amino acid catabolism is presented first. The specific catabolism of individual amino acids, along with some other uses of individual amino acids, is reviewed next. Later sections discuss the anabolic uses of amino acids for the synthesis of proteins and nitrogen-containing compounds, along with other roles of amino acids affecting protein utilization.

Catabolism of amino acids occurs to varying degrees in different tissues both during fasting periods and immediately after eating (the postprandial period). Liver cells have a high capacity for the uptake and catabolism of amino acids. In fact, after a meal, the liver takes up about 50–65% of amino acids from portal blood. The liver is the main site for the catabolism of the indispensable amino acids, with the exception of the branched-chain amino acids, which tend to be utilized to a greater extent by muscle and other organs such as the heart. Within the liver, the periportal hepatocytes catabolize most amino acids with the exception of glutamate and aspartate, which are metabolized to a greater extent by perivenous hepatocytes. The liver derives up to 50% of its energy (ATP) from amino acid oxidation; the energy generated may in turn be used for gluconeogenesis or urea synthesis, among other needs, depending on the body's state of nutriture. This section on amino acid catabolism focuses on the reactions that occur as amino acids are broken down in cells, including first the transamination and/or deamination of amino acids and then the disposal of ammonia. It next discusses the uses of the carbon skeleton of amino acids, as well as some other uses of amino acids.

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Transamination of Amino Acids

Frequently (but not always), the first step in amino acid catabolism is the transfer or removal of an amino acid's amino group. The process occurs by transamination and/or deamination. Transamination reactions involve the transfer of an amino group from one amino acid to an α-keto acid (also referred to as an amino acid carbon skeleton). The carbon skeleton/α-keto acid that gains the amino group becomes an amino acid, and the amino acid that loses its amino group becomes an α-keto acid. A generic transamination reaction can be written as:

$$\text{amino acid}_1 + \alpha\text{-keto acid}_2 \longleftrightarrow \alpha\text{-keto acid}_1 + \text{amino acid}_2$$

These reactions are important for the synthesis of many of the body's dispensable amino acids.

Transamination reactions are catalyzed by enzymes called aminotransferases/transaminases. These enzymes typically require vitamin B_6 in its coenzyme form, pyridoxal phosphate (PLP). Some examples of aminotransferases include tyrosine aminotransferase, branched-chain aminotransferases, alanine aminotransferase (ALT; formerly called glutamate pyruvate transaminase and abbreviated GPT), and aspartate aminotransferase (AST; formerly called glutamate oxaloacetate transaminase and abbreviated GOT). These last two aminotransferases (ALT and AST) are among the body's most active and involve three key amino acids and α-keto acids—alanine and its α-keto acid pyruvate, glutamate and its α-keto acid α-ketoglutarate, and aspartate and its α-keto acid oxaloacetate—as shown and described in Figure 6.5. Specifically, ALT transfers amino groups from alanine to an α-keto acid (e.g., α-ketoglutarate), forming pyruvate and another amino acid (e.g., glutamate), respectively. Similarly, AST transfers amino groups from aspartate to an α-keto acid (e.g., α-ketoglutarate), yielding oxaloacetate and another amino acid (e.g., glutamate), respectively. These reactions are reversible, and because glutamate and α-ketoglutarate readily transfer and/or accept amino groups, these compounds play central roles in amino acid metabolism.

Aminotransferases are found in varying concentrations in different tissues. For example, AST is found in higher concentrations in the heart than in the liver, muscle, and other tissues. In contrast, ALT is found in higher concentrations in the liver than in the heart but is also found in moderate amounts in the kidneys and small amounts in other tissues. Normal serum concentrations of these enzymes are low; however, with injury or disease to an organ, serum enzyme concentrations rise and can serve as an indicator of organ damage. For example, with liver damage, higher than normal blood concentrations of AST and ALT as well as other enzymes such as alkaline phosphatase and lactate dehydrogenase normally found in the liver are observed. With heart damage (as may occur with a heart attack), enzymes that are normally found in the heart, such as AST, "leak" out into the blood and serve as indicators of heart cell damage.

Interestingly, α-keto acids are sometimes used nutritionally. In kidney failure, nitrogenous compounds that are normally excreted in the urine accumulate in the blood. The provision of α-keto acids of some of the essential amino acids to someone with kidney failure allows some of the body's excess nitrogen to be used to aminate the α-keto acids. This results in the lowering of blood nitrogen concentrations while also providing the individual with essential nutrients. Three amino acids (lysine, histidine, and threonine), however, cannot undergo transamination to any appreciable extent; thus, these amino acids cannot be given effectively as α-keto acids.

Figure 6.5 Transamination reactions.

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Deamination of Amino Acids

In contrast to transamination reactions, **deamination** reactions involve only the removal of an amino group from an amino acid, with no transfer of the amino group to another compound. The amino group is released as ammonia, but at the body's physiological pH, ammonia is typically converted (in a reversible reaction) to the ammonium ion. Some amino acids that are more commonly deaminated include glutamate, histidine, serine, glycine, and threonine; however, many of these same amino acids also can be transaminated. The enzymes carrying out the deamination reactions are generally lyases, dehydratases, or dehydrogenases and produce an α-keto acid and ammonia or an ammonium ion. Figure 6.6 shows the deamination of the amino acid threonine by threonine dehydratase (which deaminates and dehydrates threonine) to form α-ketobutyrate and ammonia. The next section addresses the disposal of ammonia by the body.

Disposal of Ammonia

The ammonia (or ammonium ions) generated by deamination reactions is not the only source of ammonia found in the body. Major sources of ammonia and/or ammonium ions in the body include:

- formation in the body from chemical reactions such as deamination
- generation by the deamidation of the amide groups from glutamine and asparagine
- ingestion and absorption from foods (e.g, cheeses, processed meats)
- generation by the bacterial lysis of urea and amino acids in the gastrointestinal tract and subsequent absorption into the body.

Typically three enzymes—glutamate dehydrogenase, glutamine synthetase, and carbamoyl phosphate synthetase I (as part of the urea cycle)—assist in the removal of the ammonia/ammonium ions from body cells. These enzymes are found in high concentrations in the liver but are also found in other organs.

*The enzyme is called *dehydratase* rather than *deaminase* because the reaction proceeds by loss of elements of water. In the deamination, the amino group from the amino acid is removed. Vitamin B_6 as PLP is required by the enzyme.

Figure 6.6 The deamination of the amino acid threonine. In the deamination, the amino group from the amino acid is removed.

Glutamate and Glutamine Synthesis

Glutamate dehydrogenase readily uses an ammonia or ammonium ion ($^+NH_4$) and α-ketoglutarate to make the amino acid glutamate, as shown here:

$$^+NH_4 + \alpha\text{-ketoglutarate} + NADPH \longleftrightarrow \text{Glutamate} + NADP^+ + H_2O$$

The glutamate generated in this reversible reaction can then release the ammonia or ammonium ion for the synthesis of either urea or a dispensable amino acid.

Glutamine synthetase can also use ammonia or an ammonium ion for the amidation of glutamate's gamma carboxy group to form glutamine in an ATP-dependent reaction that also requires magnesium or manganese as follows:

$$^+NH_4 + \text{Glutamate} + ATP \longrightarrow \text{Glutamine} + ADP + P_i$$

Glutamine's functions, including its role in ammonia transport, are discussed further in this chapter in the "Interorgan Flow of Amino Acids and Organ-Specific Metabolism" section.

While the liver's perivenous cells and other body tissues readily synthesize glutamate and glutamine from ammonia or ammonium ions, the periportal hepatocytes are active in ureagenesis using (from portal blood) ammonia that was ingested in foods or obtained from bacterial synthesis in the intestine. These same periportal cells are responsible for almost all amino acid catabolism, so ammonia and ammonium ions generated during amino acid degradative reactions also can be immediately used for urea synthesis.

The Urea Cycle

The urea cycle, discovered by Sir Hans Krebs, functions in the liver and is extremely important for the removal of ammonia (and ammonium ions) from the body. Figure 6.7 reviews key compounds of the urea cycle and shows its relationship with amino acids and the tricarboxylic acid (TCA), also known as the Krebs, cycle. The five reactions of the urea cycle are also presented hereafter:

1. Ammonia (NH_3) (or an ammonium ion) combines with CO_2 (which can also be present as HCO_3^-) to form carbamoyl phosphate in a reaction catalyzed by mitochondrial carbamoyl phosphate synthetase I (CPSI) and using 2 mol of ATP and Mg^{2+}. N-acetyl-glutamate (NAG), made in the liver and intestine, is required as an allosteric activator to allow ATP binding.
2. Carbamoyl phosphate reacts with ornithine in the mitochondria to form citrulline using the enzyme ornithine transcarbamoylase (OTC). Citrulline in turn inhibits OTC activity.

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3. Aspartate reacts with citrulline once it has been transported into the cytosol to form argininosuccinate in a reaction catalyzed by argininosuccinate synthetase. This reaction is the rate-limiting step of the cycle. ATP (two high-energy bonds) and Mg^{2+} are required for the reaction. Argininosuccinate, arginine, and AMP + PP_i inhibit the enzyme.

4. Argininosuccinate is cleaved by argininosuccinase in the cytosol to form fumarate and arginine. Both fumarate and arginine inhibit argininosuccinase activity. Argininosuccinase is found in a variety of tissues throughout the body, especially the liver and kidneys. High concentrations of arginine increase the synthesis of N-acetylglutamate (NAG), which is needed in the reaction for the synthesis of carbamoyl phosphate in the mitochondria.

5. Urea is formed and ornithine is re-formed from the cleavage of arginine by arginase, a manganese-requiring hepatic enzyme. Arginase activity is inhibited by both ornithine and lysine, and may become rate limiting under conditions that limit manganese availability or that alter its affinity for manganese [5].

Overall, the urea cycle uses four high-energy bonds. The urea molecule derives one nitrogen from ammonia, a second nitrogen from aspartate, and its carbon from CO_2 (or as HCO_3^-). Once formed, urea typically travels in the blood to the kidneys for excretion in the urine; however, up to about 25% of urea may be secreted from the blood into the intestinal lumen, where it may be degraded by bacteria to yield ammonia.

Activities of urea cycle enzymes fluctuate with diet and hormone concentrations. For example, with low-protein diets or acidosis, urea synthesis diminishes and urinary urea nitrogen excretion decreases significantly. Thus, substrate availability results in short-term changes in the rate of ureagenesis. In the healthy individual with a normal protein intake, blood urea nitrogen (BUN) concentrations range from about 8–20 mg/dL, and urinary urea nitrogen represents about 80% of total urinary nitrogen.

1. Ammonia (NH_3) combines with CO_2 to form carbamoyl phosphate in a reaction catalyzed by mitochondrial carbamoyl phosphate synthetase I. N-acetyl-glutamate (NAG) allosterically activates the enzyme to allow ATP binding.

2. Carbamoyl phosphate reacts with ornithine using ornithine transcarbamoylase to form citrulline.

3. Aspartate reacts with citrulline once it has been transported into the cytosol. This step is catalyzed by argininosuccinate synthetase. ATP (two high-energy bonds) and Mg^{2+} are required for the reaction, and argininosuccinate is formed.

4. Argininosuccinate is cleaved by argininosuccinase in the cytosol to form fumarate and arginine.

5. Urea is formed and ornithine is re-formed from the cleavage of arginine by arginase, a manganese-requiring hepatic enzyme.

Figure 6.7 Interrelationships of amino acids and the urea and TCA/Krebs cycles in the liver.

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Glucocorticoids and glucagon, which promote amino acid degradation, typically increase mRNA for the urea cycle enzymes.

Several defects (genetic mutations) have been identified in urea cycle enzymes. Urea cycle enzyme defects typically result in high levels of blood ammonia. Ammonia in high concentrations in the blood is toxic, causing initially lethargy but ultimately coma and possibly death. Treatment of urea cycle disorders necessitates a protein-restricted diet, and depending on the enzyme that is defective, supplementation of citrulline and/or arginine (among other nutrients) may be prescribed. Urea synthesis is also diminished and blood ammonia concentrations increased in those with advanced liver disease. The elevated blood ammonia concentrations observed in liver disease are thought to contribute to hepatic encephalopathy, characterized in part by brain dysfunction including coma. One aspect of medical treatment for encephalopathy involves decreasing blood ammonia concentrations. Drugs such as lactulose are given to acidify the gastrointestinal tract contents and promote the diffusion of the ammonia out of the blood and into the gastrointestinal tract. Furthermore, antibiotics are prescribed that promote the destruction of intestinal tract bacteria that generate ammonia.

Carbon Skeleton/α-Keto Acid Uses

As outlined in Figure 6.8, once an amino group has been removed from an amino acid, the remaining part is called a carbon skeleton or α-keto acid.

$$\text{Amino acid} \longrightarrow NH_2 + \text{carbon skeleton/}\alpha\text{-keto acid}$$

Carbon skeletons of amino acids can be further metabolized with the potential for multiple uses in the cell, depending on the original amino acid from which they were derived and the body's physiological and nutritional state. An amino acid's carbon skeleton, for example, depending on the particular amino acid, can be used to produce energy, glucose, ketone bodies, cholesterol, and fatty acids.

Energy Production

The complete oxidation of amino acids generates energy, along with water, CO_2/HCO_3^-, and ammonia/ammonium ions. Amino acids are used for energy production when the diet is inadequate in energy. The oxidation of selected amino acids is presented in later subsections of this chapter.

Glucose Production

The production of glucose from a noncarbohydrate source such as amino acids is known as gluconeogenesis. Gluconeogenesis occurs primarily in the liver but also in the kidneys and small intestine; it is discussed in detail in Chapter 3. The carbon skeletons of several amino acids can be used to synthesize glucose. For example, oxaloacetate (the carbon skeleton of aspartate) and pyruvate (the carbon skeleton of alanine) may be used to produce glucose in body cells. In addition, the carbon skeleton of asparagine can be converted into oxaloacetate, and the carbon skeletons of glycine, serine, cysteine, tryptophan, and threonine can be converted into pyruvate for glucose production. Valine and methionine are also glucogenic, yielding succinyl-CoA. Thus, to be considered a glucogenic amino acid, catabolism of the amino acid must yield pyruvate or intermediates of the TCA cycle.

Figure 6.8 Possible fates of amino acids upon catabolism.

Ketone Body Production

For an amino acid to be considered ketogenic, the catabolism of the amino acid must generate acetyl-CoA or acetoacetate, which are used for the formation of ketone bodies (also referred to as ketones). Some amino acids are both glucogenic and ketogenic. Phenylalanine and tyrosine, for example, can be degraded to form fumarate (an intermediate of the TCA cycle), which can be used to form glucose, but also acetoacetate, which can be used to synthesize ketone bodies. Isoleucine is partially glucogenic, generating succinyl-CoA, but also ketogenic, yielding acetyl-CoA as well upon its catabolism. Threonine is partially glucogenic, yielding succinyl-CoA or pyruvate depending on its pathway of degradation, and partially ketogenic when degraded by another pathway to acetyl-CoA. Tryptophan is also considered partially ketogenic and partially glucogenic. Tryptophan yields acetyl-CoA as well as pyruvate upon catabolism. Leucine and lysine are the only totally ketogenic amino acids and upon catabolism generate acetyl-CoA. Figure 6.9 shows the general fates of amino acid carbon skeletons with respect to key intermediates of metabolism.

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*Physiological contribution unclear.

Figure 6.9 The fate of amino acid carbon skeletons. Ketogenic: Lysine and leucine. Partially ketogenic and glucogenic: Phenylalanine, isoleucine, threonine, tryptophan, and tyrosine. Glucogenic: alanine, glycine, cysteine, aspartate, asparagine, glutamate, glutamine, arginine, methionine, valine, histidine, and proline.

The conversion of amino acids to glucose is accelerated by a high blood glucagon-to-insulin ratio and by high blood cortisol concentrations. Glucagon concentrations are generally elevated in the blood when blood glucose concentrations are low, as may occur in between meals or with a fast in which liver glycogen stores have been depleted. Blood glucagon (and in some cases cortisol along with epinephrine) is also elevated in the presence of infection or trauma/injury and in certain disease states such as untreated diabetes mellitus and liver disease, to name a few.

Cholesterol Production

The oxidation of several amino acids, including isoleucine, leucine, lysine, tryptophan, and threonine, yields acetyl-CoA, which can be metabolized to produce cholesterol (Figure 6.9). Leucine, however, is also the only amino acid whose catabolism directly generates β-hydroxy β-methylglutaryl-CoA, an intermediate (shown later in Figure 6.36) in cholesterol synthesis. Moreover, leucine oxidation produces another metabolite, β-hydroxy β-methylbutyrate (HMB), which appears to promote *de novo* cholesterol synthesis in muscle, enabling cell growth and function. This topic is further discussed in the "Skeletal Muscle" section and more specifically in the "Isoleucine, Leucine, and Valine Catabolism" section. Cholesterol synthesis is discussed in detail in Chapter 5.

Fatty Acid Production

In times of excess energy and protein intakes coupled with adequate carbohydrate intake, the carbon skeleton of amino acids may be used to synthesize fatty acids. Leucine, for example, is used to synthesize fatty acids in adipose tissue. Fatty acid synthesis is discussed in detail in Chapter 5.

Hepatic Catabolism and Uses of Aromatic Amino Acids

The details of the metabolism of selected amino acids and the formation of TCA cycle and non-TCA cycle intermediates are discussed in the following sections. The catabolism of the amino acids is categorized primarily according to their structural classification, although also by net charge for the basic amino acids. The aromatic amino acids are discussed first, followed by the sulfur-containing amino acids, the branched-chain amino acids, the basic amino acids, and lastly other selected amino acids.

Amino acid catabolism occurs in most body tissues, although the liver plays a primary role for some amino acids (such as the aromatic and sulfur-containing amino acids) more than for others. For example, in advanced liver disease, the inability of the liver to take up and catabolize certain amino acids is evidenced by the increased plasma concentrations of both the aromatic amino acids—phenylalanine, tyrosine, and tryptophan—and the sulfur-containing amino acids methionine and cysteine.

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Phenylalanine and Tyrosine

As shown in Figures 6.9 and 6.10, phenylalanine and tyrosine are partially glucogenic because they are degraded to fumarate. In addition, phenylalanine and tyrosine are catabolized to acetoacetate and thus are partially ketogenic.

- The first step in the degradation of phenylalanine (Figure 6.10) is specific to the liver and the kidneys. Phenylalanine is converted to tyrosine by the enzyme phenylalanine hydroxylase, also called a monooxygenase. This enzyme is iron dependent, and tetrahydrobiopterin functions as a cosubstrate in the reaction. Enzyme activity is regulated by phosphorylation/dephosphorylation, with glucagon promoting phosphorylation and enzyme activity. Insulin has the opposite effect.
- Tyrosine degradation (Figure 6.10) begins with transamination by a vitamin B_6 (as PLP)–dependent tyrosine aminotransferase to yield p-hydroxyphenylpyruvate. Higher tyrosine concentrations as well as high cortisol levels promote increased tyrosine aminotransferase activity. The compound p-hydroxyphenylpyruvate, once formed, is then decarboxylated by a dioxygenase to generate homogentisate. Homogentisate dioxidase converts homogentisate to maleylacetoacetate, which is then isomerized to fumarylacetoacetate. A hydrolase converts fumarylacetoacetate into fumarate (a TCA cycle intermediate) and acetoacetate, which may be further metabolized to acetyl-CoA for energy, fatty acid, or ketone body production.

Tyrosine also can be used to synthesize other compounds. Some of these uses are mentioned hereafter and shown in Figure 6.10 as well as later in Figure 6.16.

- In neurons and the adrenal medulla, tyrosine is used for the synthesis of neurotransmitters and hormones, respectively. The initial reaction involves tyrosine hydroxylase (also called monooxygenase), an iron-dependent enzyme that hydroxylates tyrosine to generate

Figure 6.10 Phenylalanine and tyrosine metabolism.

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3,4-dihydroxyphenylalanine (L-dopa). Subsequent reactions utilizing L-dopa yield the catecholamines (dopamine, norepinephrine, and epinephrine). The catecholamines function as neurotransmitters in the nervous system; however, in circulation they (especially epinephrine) function as hormones and have major effects on nutrient metabolism.

- In melanocytes in the skin, eye, and hair cells, tyrosine is converted through multiple reactions into melanin. The reactions occur within melanosomes, membrane-bound organelles found in the melanocytes. Melanin is a pigment that gives color to skin, eyes, and hair.
- In the thyroid gland, tyrosine is taken up and used with iodine to synthesize thyroid hormones. These reactions are discussed in the section on iodine in Chapter 13.

Disorders of Phenylalanine and Tyrosine Metabolism

Several inborn errors have been identified in phenylalanine and tyrosine metabolism (Figure 6.10). Phenylketonuria (PKU), an autosomal recessive genetic disorder, occurs when the activity of phenylalanine hydroxylase, which converts phenylalanine to tyrosine, is defective. It is one of the most prevalent disorders of amino acid metabolism, with an incidence of about 1 in 10,000 in the United States. This enzymatic defect results in a buildup of phenylalanine and phenylalanine metabolites (phenyllactate, phenylpyruvate, and phenylacetate) in the blood and other body fluids. In addition, because phenylalanine's conversion to tyrosine is impaired, blood tyrosine concentrations diminish. If untreated, PKU causes neurologic problems such as seizures and hyperactivity, among other problems. The disorder is treated with a phenylalanine-restricted diet, which means that ingestion of natural protein-containing foods is extremely limited, and tyrosine must be added to the diet because it cannot be made in the body. In addition, labels on products that contain aspartame (brand name Equal®) must have a warning indicating that the product contains phenylalanine and thus its use must be restricted by those with PKU.

Impaired activity of the enzyme tyrosine aminotransferase, which converts tyrosine to p-hydroxyphenylpyruvate, results in the inborn error of metabolism known as tyrosinemia type II. Tyrosinemia type II, with a worldwide incidence of about 1 in 250,000, is characterized by high plasma tyrosine concentrations, skin and eye lesions, and impaired mental development. Treatment requires a diet restricted in both phenylalanine and tyrosine.

Another genetic disorder involving tyrosine degradation is alkaptonuria. The condition is not common worldwide (affecting 1 in 250,000 to 1 million); however, in Slovakia it affects about 1 in 19,000. Homogentisate dioxygenase, which is defective in alkaptonuria, normally converts homogentisic acid to maleylacetoacetate. Alkaptonuria is characterized by high concentrations of homogentisic acid in body tissues and fluids including urine. When homogentisic acid oxidizes, it turns a dark color, thus making the urine appear black when exposed to air. People with alkaptonuria often experience joint problems (such as arthritis) as the homogentisic acid accumulates in connective tissues. Dietary treatment is not usually prescribed.

Tryptophan

Another aromatic amino acid metabolized principally by the liver is tryptophan (Figure 6.11). Tryptophan is partially glucogenic because it is catabolized to form pyruvate; it is also partially ketogenic, forming acetyl-CoA as shown in Figure 6.9.

- The first step in tryptophan catabolism is catalyzed by the heme-iron-dependent tryptophan dioxygenase and produces N-formylkynurenine. Tryptophan dioxygenase is induced by glucagon as well as cortisol.
- Further catabolism of N-formylkynurenine yields formate and kynurenine. Kynurenine is metabolized by a monooxygenase to 3-hydroxykynurenine. Kynureninase, a vitamin B_6 (PLP)–dependent enzyme, converts 3-hydroxykynurenine to 3-hydroxyanthranilate and alanine. This alanine can be transaminated to form pyruvate, hence the glucogenic nature of tryptophan.
- Further catabolism of 3-hydroxyanthranilate forms 2-amino 3-carboxymuconic 6-semialdehyde. This compound is further metabolized to produce many additional compounds, including picolinate (a possible binding ligand for minerals), and 2-aminomuconic 6-semialdehyde, which is further metabolized in several reactions to acetyl-CoA (a ketogenic intermediate).

Tryptophan also has other fates in the body, as shown in Figure 6.11 and later in Figure 6.16. For example:

- Tryptophan metabolism through N-formylkynurenine generates the B vitamin niacin as nicotinamide as well as its coenzyme form nicotinamide adenine dinucleotide phosphate (NADP). Deficient protein intake and tryptophan malabsorption limit niacin synthesis in the body.
- Tryptophan is also used for the synthesis of serotonin (5-hydroxytryptamine) and melatonin (N-acetyl 5-methoxyserotonin). Melatonin is made primarily in the pineal gland, which lies in the center of the brain. Melatonin synthesis and release correspond with darkness; the hormone is thought to be involved mainly with the regulation of circadian rhythms and sleep. Supplement use has been helpful for some people with jet lag. Serotonin functions throughout the body including the gastrointestinal tract. It promotes vasoconstriction and smooth muscle contraction. It also functions as a neurotransmitter.

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Figure 6.11 Tryptophan metabolism.

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Disorders of Tryptophan Metabolism Inherited disorders also occur in tryptophan degradation. One disorder, α-ketoadipic aciduria, results from the defective activity of α-ketoadipic dehydrogenase, which converts α-ketoadipate to glutaryl-CoA. With this disorder, lysine, tryptophan, α-aminoadipate, α-ketoadipate, and α-hydroxyadipate build up in the blood and other body fluids. Affected infants become hypotonic, acidotic, and experience seizures and motor and developmental problems. A second autosomal recessive condition, glutaric aciduria (or acidemia) type 1, results from the defective activity of the riboflavin (as FAD)-dependent enzyme glutaryl-CoA dehydrogenase, which converts glutaryl-CoA to glutaconyl-CoA. As with α-ketoadipicaciduria, the enzyme glutaryl-CoA dehydrogenase is critical to the catabolism of tryptophan (Figure 6.11) as well as lysine (shown later in Figure 6.13). In glutaric aciduria type 1, glutaryl-CoA builds up and is converted to glutaric acid, which also accumulates in body fluids. Over time, affected infants develop acidosis, **ataxia**, seizures, and macrocephaly, among other problems. Treatment of both conditions requires a diet restricted in both lysine and tryptophan (because the reactions are common in both tryptophan and lysine degradation). For some individuals with glutaric aciduria type 1, riboflavin supplements have been shown to enhance residual glutaryl-CoA dehydrogenase activity.

Hepatic Catabolism and Uses of Sulfur (S)–Containing Amino Acids

The catabolism of methionine, an S-containing essential amino acid, occurs to a large extent in the liver and generates another S-containing nonessential amino acid, cysteine.

Methionine

Methionine is a glucogenic amino acid with its oxidation generating the TCA cycle intermediate succinyl-CoA (Figure 6.9). Methionine metabolism, shown in Figure 6.12, is briefly described here, and some of its uses are depicted later in Figure 6.16.

- The first step in methionine catabolism is the conversion of methionine to S-adenosyl methionine (SAM) by methionine adenosyl transferase (present in high concentrations in the liver) in an ATP-requiring reaction. SAM, the body's principal methyl donor, has many functions.
  - SAM provides methyl groups for the synthesis of nonprotein nitrogen-containing compounds, including carnitine and creatine.
  - SAM's methyl groups are needed for the synthesis of hormones such as epinephrine and melatonin.
  - SAM is needed for the metabolism of arsenic.
  - SAM's methyl groups are needed to maintain myelin. Inadequate methylation of myelin disrupts nerve cell functions, and ultimately may lead to neuropathy and ataxia, among other problems. Myelin, which is made from proteins and various lipids, surrounds and insulates the axon of a neuron, enabling faster conduction of nerve impulses.
  - SAM's methyl groups are used to methylate DNA and histone proteins and thus affect gene expression.
  - SAM affects membrane fluidity by providing methyl groups for the methylation of phospholipids in cell membranes.
  - SAM may be decarboxylated to form S-adenosyl methylthiopropylamine, an intermediate in the synthesis of the polyamines—putrescine, spermidine, and spermine. Polyamines are important in cell division and growth.
- The removal or donation of the methyl group from SAM yields the compound S-adenosyl homocysteine (SAH). SAH is converted to homocysteine and adenosine by S-adenosyl homocysteine hydrolase in a reversible reaction that favors SAH synthesis. SAH or a low SAM-to-SAH ratio diminishes methylation reactions with SAH, competing with SAM for the active site on the methyltransferase.
- Homocysteine, once formed, can be converted back to methionine either in a betaine-dependent reaction or in a vitamin B_{12}–and folate-dependent reaction.
  - Betaine, obtained from the diet or generated in the liver from choline oxidation, provides a methyl group that is transferred to homocysteine by the hepatic enzyme betaine homocysteine methyltransferase. With the loss of the methyl group, betaine becomes dimethylglycine. Dimethylglycine can be further demethylated to generate glycine.
  - The vitamin B_{12}– and folate-dependent methylation reaction to form methionine relies on methionine synthase (also called homocysteine methyltransferase), which requires cobalamin (vitamin B_{12}) as a coenzyme. Cobalamin receives the methyl group that is needed from 5-methyl THF, a derivative of folate. Failure to form methionine due to deficiencies in folate or vitamin B_{12} can lead to elevated plasma homocysteine concentrations, which have been shown to interfere with collagen cross-linking in bone and increase fracture risk. High plasma homocysteine concentrations are also a risk factor for heart disease and stroke.
- To be further metabolized, homocysteine reacts with the amino acid serine, forming cystathionine through the action of cystathionine (β) synthase. The enzyme requires vitamin B_6 in its PLP coenzyme form.

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1 Defects in this enzyme result in hypermethioninemia.

2 Defects in this enzyme result in homocystinuria.

3 Defects in this enzyme result in propionic acidemia.

4 Defects in this enzyme result in methylmalonic acidemia.

†SAM concentrations affect further methionine metabolism with high concentrations stimulating cystathionine synthase, which converts homocysteine to cystathionine. SAM also inhibits methylene tetrahydrofolate reductase activity, which forms the 5-methyl THF (also called N5-methyl THF and a form of folate) needed to regenerate methionine from homocysteine. Thus, SAM (when present in higher concentrations) facilitates the degradation of methionine.

*Common intermediate in the degradation of threonine, methionine, and isoleucine.

**Common intermediate in the degradation of threonine, methionine, isoleucine, and valine.

Figure 6.12 Methionine and cysteine metabolism.

A deficiency of vitamin B_6, like folate and vitamin B_{12}, can lead to elevated plasma homocysteine concentrations.

- Further catabolism of cystathionine requires cystathionine β lyase, another vitamin B_6 (as PLP)–dependent enzyme, and forms the dispensable amino acid cysteine. Also generated in the reaction is α-ketobutyrate, which is further decarboxylated to propionyl-CoA. The conversion of homocysteine to cysteine by cystathionine synthase and cystathionine lyase is sometimes called the transulfuration pathway. These reactions occur in the liver, but also in the kidneys, intestine, and pancreas.
- Propionyl-CoA (made from α-ketobutyrate) is next converted to D-methylmalonyl-CoA by the biotin-dependent enzyme propionyl-CoA carboxylase. D-methylmalonyl-CoA is then converted to L-methylmalonyl-CoA by a

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racemase. Then L-methyl-malonyl-CoA is converted by methylmalonyl-CoA mutase, a vitamin B_{12}–dependent enzyme, to the TCA cycle intermediate succinyl-CoA. See Figure 6.12.

Disorders of Methionine Metabolism Genetic defects in methionine adenosyl transferase, the enzyme that converts methionine to S-adenosyl methionine (SAM), result in hypermethioninemia. This condition's high blood methionine concentrations necessitate treatment with a diet restricted in methionine but increased in cysteine.

The genetic disorder homocystinuria results from defects in cystathionine β synthase, which converts homocysteine to cystathionine. The condition, which affects about 1 in 200,000 to 300,000 people worldwide, but about 1 in 65,000 in Ireland, is characterized by high blood homocysteine and methionine and low blood cysteine concentrations. The high plasma homocysteine concentrations can promote blood clot (thrombi) formation as well as increase the risk for heart disease. Other selected manifestations include skeletal problems including osteoporosis, ocular changes, and mental retardation. Treatment requires a diet low in methionine (and thus low intakes of protein-containing foods), added cysteine, and in some cases supplements of betaine and folate.

The genetic disorder propionic acidemia (with an incidence of about 1 in 35,000 to 70,000 but as many as 1 in 1,000 in Greenland and 1 in 2,000 to 3,000 in Saudi Arabia) results from defects in propionyl-CoA carboxylase, a biotin-dependent enzyme. Another disorder caused by genetic errors in the same pathway is methylmalonic acidemia, which results from impaired methylmalonyl-CoA mutase activity and affects about 1 in 48,000. Propionic acidemia is characterized by the accumulation of propionic acid in body fluids, and in methylmalonic acidemia, both propionic and methylmalonic acids (as well as other compounds such as methylcitrate, 3-hydroxy propionate, and tiglic acid) accumulate in body fluids. Infants exhibit excessive vomiting, ketoacidosis, hypertonia, failure to thrive, and respiratory difficulties, among other problems. Because propionyl-CoA and thus methylmalonyl-CoA are generated from not only methionine, as shown in Figure 6.12, but also from the degradation of both threonine (shown later in Figure 6.15) and isoleucine, and because the degradation of valine produces methylmalonyl-CoA (see later Figure 6.36), treatment of both conditions requires restriction of these amino acids. In addition, odd-number chain fatty acids and polyunsaturated fatty acids (in excessive amounts) generate propionyl-CoA and thus foods containing large amounts of these fatty acids must be restricted. In some cases, biotin supplements may improve the activity of propionyl-CoA carboxylase, but a restricted diet is still typically needed. Similarly, vitamin B_{12} supplements can sometimes improve methylmalonyl-CoA mutase activity in those with methylmalonic acidemia.

Cysteine

Cysteine is a nonessential amino acid. Hepatic concentrations of free cysteine appear to be tightly controlled. Cysteine is used like other amino acids for protein synthesis. It is also used to synthesize glutathione (discussed further in later sections of the chapter).

- Cysteine is metabolized by cysteine dioxygenase to cysteine sulfinate, which is used to produce the amino acid taurine (Figure 6.12). Taurine, a β-amino sulfonic acid, is made in the liver from cysteine but concentrated in muscle and the central nervous system; it is also found in smaller amounts in the heart, liver, and kidneys, among other tissues. Although taurine is not involved in protein synthesis, it is important in the retina, where it maintains membrane stability and photoreceptor cell function through its antioxidant abilities (such as scavenging peroxidative, e.g., oxychloride, products). Taurine also serves in the liver and intestine as a bile salt, taurocholate, and in the central nervous system as an inhibitory neurotransmitter.
- Cysteine degradation (Figure 6.12) yields pyruvate and sulfite. Sulfite is converted by sulfite oxidase (an iron- and molybdenum-dependent enzyme) to sulfate, which can be excreted in the urine or used to synthesize sulfolipids and sulfoproteins.

Hepatic Catabolism and Uses of Branched-Chain Amino Acids

The liver plays only a minor role in the initial catabolism of the three branched-chain amino acids—isoleucine, leucine, and valine. Transaminase activity needed to remove the amino groups is minimal in the liver, although hepatic transferases increase in response to glucocorticoids (cortisol), as may occur with infection, burns, trauma, or prolonged fasting. Thus, under normal circumstances, the branched-chain amino acids typically remain in circulation and are taken up and transaminated primarily by the skeletal muscle, but also by the heart, kidneys, diaphragm, and adipose tissue, if needed. Alpha-keto acids of the branched-chain amino acids, generated from branched-chain amino acid transamination, may be used within the tissues or released into circulation. The liver, among other organs, can further catabolize these α-keto acids. Additional information on branched-chain amino acid metabolism is found under "Skeletal Muscle" in the "Interorgan 'Flow' of Amino Acids and Organ-Specific Metabolism" section of this chapter.

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Hepatic Catabolism and Uses of Basic Amino Acids

Lysine

The catabolism of lysine, a totally ketogenic amino acid, generates acetyl-CoA, as shown in Figures 6.13 and 6.9. Lysine and tryptophan degradation share a common intermediate, α-ketoadipate, and thus share some common reactions. Lysine has another important use. After being methylated using SAM (made from methionine), lysine is used in the synthesis of carnitine (shown later in Figure 6.23), which is needed for fatty acid oxidation.

Disorders of Lysine Metabolism Defects in lysine degradation by glutaryl-CoA dehydrogenase and α-ketoadipate dehydrogenase result in glutaric aciduria type 1 and α-ketoadipic aciduria, respectively, as discussed in the "Disorders of Tryptophan Metabolism" section and shown in Figure 6.11.

Arginine

Arginine is metabolized mostly in the liver and kidneys but also in the intestine, lungs, and leukocytes. It is a glucogenic amino acid, as its catabolism generates the TCA cycle intermediate α-ketoglutarate (Figure 6.9). Arginine, however, also has several other uses, as shown in Figure 6.14 and later in Figure 6.16.

- In the liver, arginine is primarily degraded as part of the urea cycle to form urea and ornithine. Ornithine may be decarboxylated to form polyamines (putrescine, spermine, and spermidine), or it may be transaminated by ornithine aminotransferase to form, through a series of reactions, the amino acid proline. Polyamines are thought to function in cell signaling, growth, and proliferation.

Figure 6.13 Lysine metabolism.

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- In the kidneys, arginine is used with glycine to produce guanidinoacetate; guanidinoacetate is then released into the blood and travels to the liver, where it is converted into creatine. It is the kidneys that are also the primary site of arginine synthesis from citrulline, which was produced in the intestinal cells.
- Agmatine, made in the brain and central nervous system, among other organs, from the decarboxylation of arginine, is involved in neuromodulary functions.
- In endothelial cells, cerebellar neurons, neutrophils, and splanchnic tissues, arginine is used for nitric oxide production in a reaction catalyzed by nitric oxide synthase. Arginase activity influences arginine availability, with higher enzyme activity limiting arginine availability for nitric oxide synthesis. Asymmetric dimethylarginine (ADMA) also serves to inhibit nitric oxide production. Nitric oxide is involved in a variety of physiological processes including regulation of blood pressure (relaxation of vascular smooth muscle) and intestinal motility, inhibition of platelet aggregation, and macrophage function. Endothelium-derived nitric oxide produced in the heart helps maintain cardiovascular function. Nitric oxide also can combine with glutathione to form glutathionylated nitric oxide, a compound known to stimulate the glutathionylation of proteins. This posttranslational modification is thought to affect the function and stability of many cellular proteins. In critical illness such as trauma, the ratio of arginine to ADMA typically favors ADMA and has been associated with increased mortality and severity of heart failure. Supplemental arginine, provided enterally or parenterally, typically benefits trauma patients and is associated with improved wound healing.

Histidine

Histidine degradation is also shown in Figure 6.14. It is a gluconeogenic amino acid yielding α-ketoglutarate (Figure 6.9). Histidine also has several other uses, as depicted later in Figure 6.16.

- It may combine with β-alanine to generate carnosine, which is discussed in more detail in the section of this chapter on nitrogen-containing nonprotein compounds.
- Through a vitamin B_6 (as PLP)–dependent decarboxylation reaction, the amine histamine can be formed from histidine. Histamine functions in the brain as a neurotransmitter. In the gastrointestinal tract, it has many roles including the stimulation of gastric secretions

Figure 6.14 Arginine, proline, histidine, and glutamate metabolism.

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such as hydrochloric acid. In mast cells (found throughout the body in locations such as within the nose and mouth, blood vessels, and internal body surfaces) and in white blood cells (especially basophils), histamine exhibits immunologic roles. It stimulates constriction of bronchial smooth muscle and causes dilation or increased permeability of capillaries to facilitate white blood cell infiltration and **phagocytosis** of foreign antigens. This increased permeability may result in flushing (redness) of the skin and in the escaping of fluid into tissues, causing a runny nose and watery eyes.

- Another interesting fate of histidine relates to its post-translational modification. Specifically, histidine, in some body proteins like actin in muscle, becomes methylated and when the protein is broken down, the methylated histidine, called 3-methylhistidine (see Table 6.1), is released but cannot be reused to synthesize another protein. Because it cannot be reused, the 3-methylhistidine is excreted in the urine and is often used as an index of muscle catabolism. This topic is also discussed in the "Skeletal Muscle" section later in this chapter.

Hepatic Catabolism and Uses of Other Selected Amino Acids

Threonine

Threonine can be metabolized (as shown in Figure 6.15) by three different pathways and consequently is both glucogenic and ketogenic (Figure 6.9).

- One of the more commonly used pathways of degradation is through cytosolic threonine dehydratase to generate α-ketobutyrate, which is further catabolized to propionyl-CoA, then to D-methylmalonyl-CoA, L-methylmalonyl-CoA, and ultimately succinyl-CoA. These latter steps in the catabolism are shared with methionine and isoleucine, and in part with valine.
- Alternately, threonine may be degraded by mitochondrial threonine dehydrogenase to form aminoacetone, which is converted to methylglyoxal and then pyruvate. This pathway is thought to be used if cytosolic threonine concentrations are relatively high.
- In a third pathway, the mitochondrial threonine cleavage complex (composed of a dehydrogenase and a ligase) converts threonine to glycine and acetylaldehyde; acetylaldehyde is further metabolized to acetate and then to acetyl-CoA in an ATP- and CoA-dependent reaction. Glycine is discussed in the next section.

Threonine is found in fairly high concentrations relative to other amino acids in the glycoproteins of mucus. Consequently, in situations of intestinal inflammation characterized by excess mucus production, threonine needs are thought to be elevated.

Disorders of Threonine Metabolism Defects in two steps of threonine metabolism from propionyl-CoA to succinyl-CoA can lead to propionic acidemia and methylmalonic acidemia, as shown in Figure 6.15 and previously discussed in the "Disorders of Methionine Metabolism" section.

Figure 6.15 Threonine, glycine, and serine metabolism.

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Glycine and Serine

Glycine and serine can be produced from one another in a reversible reaction that requires folate as tetrahydrofolate (THF) and 5,10 methylene THF (also called N5 N10 methylene THF (Figure 6.15)). The reaction, which is catalyzed by the vitamin B_6 (as PLP)–dependent enzyme serine hydroxymethyltransferase, occurs in the liver and kidneys. Serine, if not needed for glycine production, can be deaminated/dehydrated to form pyruvate. Glycine, if not needed for serine production, can be catabolized via the glycine cleavage system to carbon dioxide and an ammonium ion in a folate (as THF)– and niacin (as NAD^+)–dependent reaction. Glycine is also used for the synthesis of glutathione, creatine, porphyrins, and the bile salt glycocholate. Serine is used for the synthesis of ethanolamine and choline for phospholipids.

Disorders of Glycine Metabolism Impaired glycine catabolism, secondary to mitochondrial glycine cleavage system defects, results in nonketotic hyperglycinemia. Infants with this autosomal recessive condition exhibit seizures, neurologic deterioration, flaccidity, and lethargy, among other problems. Blood and other body fluids contain increased glycine concentrations. Treatment requires a protein-restricted diet.

Figure 6.16 provides a summary of uses of some amino acids. The next section of the chapter further elaborates on anabolic uses of amino acids in the body.

Figure 6.16 A summary of the uses of selected amino acids for the synthesis of nitrogen-containing compounds and selected biogenic amines, hormones, and neuromodulators.

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PROTEIN SYNTHESIS

Anabolism, including protein synthesis, increases in tissues following the ingestion of food, which provides the amino acids and energy necessary to build body proteins. Each cellular protein exhibits a specific and characteristic rate of synthesis that is influenced by multiple, interdependent but converging actions. On a cellular level, some amino acids, for example, may directly affect gene expression through interactions with amino acid–responsive elements in the promoter regions of genes. Translation within cells is affected by the amount and stability of mRNA, the ribosome number (amount of ribosomal RNA, or rRNA), the activity of the ribosomes (rapidity of translation, or peptide formation), the presence of amino acids in the appropriate concentrations to attach to the tRNA (referred to as charged tRNA), and the hormonal environment, which in turn can be influenced by nutrients. On a more systemic level, increased plasma amino acids, increased blood flow, and hormone release, especially insulin, generally enhance muscle protein synthesis, which occurs for several hours following food ingestion [6,7]. Physical activity (exercise) further enhances protein synthesis in muscle [8].

Slow versus Fast Proteins

The form and nature of the ingested dietary protein influence its digestion and absorption, the rate of appearance of the amino acids in the plasma, and the subsequent utilization of the amino acids within cells. For example, amino acids released following ingestion and digestion of "fast proteins," which include whey protein, soy protein, amino acid mixtures, and protein hydrolysates (e.g., usually partially hydrolyzed whey protein), appear to be utilized differently in the body when compared with amino acids released following ingestion and digestion of "slow proteins" such as casein. Ingestion of fast proteins better stimulates muscle protein and whole-body protein synthesis than slow proteins both at rest and following resistance exercise. Ingestion of foods providing slow proteins, however, is important as the lower and more prolonged plasma amino acid concentrations that result from ingestion of these proteins help reduce protein breakdown. Thus, consuming foods (such as milk/dairy products) that provide a combination of "fast" and "slow" proteins (whey and casein, respectively) enable protein synthesis while reducing protein degradation.

Plant versus Animal Proteins

Plant-based protein sources are typically limiting in one or more essential amino acids, most often methionine and/or lysine, and have lower digestibility when compared with animal-based protein sources. (See the later sections of the chapter on "Protein Quality" and "Protein and Amino Acid Needs.") Animal-based protein sources may also be higher in leucine (helpful in promoting muscle protein synthesis as discussed later in this section) than plant-based protein sources [9]. These dietary differences can affect muscle protein synthesis, with lower synthesis observed when plant-based diets are consumed versus animal-based diets. Postprandial concentrations of amino acids that do not contain enough of all the essential amino acids (as may occur with consumption of solely plant-based dietary proteins) are thought to reduce amino acid availability to tissues for protein synthesis, and stimulate hepatic oxidation of amino acids and ureagenesis [9]. Higher consumption of animal-based protein foods has been associated with greater gains in lean body mass in individuals participating in resistance/strength-training exercises, and with better maintenance of muscle mass in individuals not engaged in resistance training [9]. Ingestion of higher amounts of plant foods at each meal or supplementation of plant foods with selected amino acids or some animal-based protein foods at meals may better promote anabolism [9].

Hormonal Effects

Hormones play a major role in amino acid utilization for protein synthesis. During prolonged periods in which food is not eaten, such as during the overnight hours or a more prolonged fast, protein synthesis still occurs but at a much lower rate (than occurs postprandially), and protein degradation predominates. The degradative processes are stimulated by epinephrine and cortisol release and by the higher glucagon-to-insulin ratio in the blood. This higher glucagon-to-insulin ratio diminishes insulin's ability to inhibit protein degradation and diminishes the overall rate of protein synthesis. Yet, while skeletal muscle experiences more extensive protein degradation and limited protein synthesis during postabsorptive periods, the glucagon-to-insulin ratio favoring glucagon stimulates the hepatic synthesis of some proteins, such as enzymes for gluconeogenesis and ureagenesis. Higher blood cortisol concentrations (which typically rise with depletion of hepatic glycogen stores occurring with fasting or with injury, sepsis, burns, etc.) further promote muscle protein catabolism and hepatic use of the amino acids for gluconeogenesis and ureagenesis.

In contrast to the general catabolic nature of glucagon, epinephrine, and cortisol, other hormones, such as insulin, are anabolic. Insulin, for example, which is secreted in response to a rise in incretins (glucose-dependent insulinotropic peptide and glucagon-like peptide 1 released in response to glucose in the digestive tract), a rise in blood glucose, and a rise in some blood amino acid concentrations (as occurs with food

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consumption), generally increases protein synthesis and decreases protein degradation. This effect occurs to a greater extent if both carbohydrate- and protein-containing foods are coingested versus ingestion of either carbohydrate or protein alone.

Insulin, upon binding to its receptors in cell membranes, exhibits multiple actions to promote protein synthesis. Insulin, for example, generally stimulates the transcellular movement of amino acid transporters to the cell membrane for use in amino acid uptake and increases the overall activity of amino acid transporters, including systems A, ASC, and N in the liver, muscle, and other tissues. Insulin antagonizes the activation of some enzymes responsible for amino acid oxidation (degradation); the phosphorylation and thus activation of phenylalanine hydroxylase (which degrades phenylalanine), for instance, is inhibited by insulin. Thus, insulin promotes the uptake of the amino acids into the tissues and inhibits the enzymes that are responsible for the degradation of the amino acids. Such actions of insulin facilitate the use of the amino acids for protein synthesis. Insulin, however, along with the amino acid leucine (which by itself acts as an insulin secretogogue [i.e., it stimulates insulin secretion]), also plays other roles in stimulating protein synthesis. While a discussion of all the mechanisms of insulin's actions upon binding to insulin receptors on cell membranes is beyond the scope of this section, some of insulin's intracellular effects result in the stimulation of mammalian target of rapamycin (mTOR).

Amino Acids, Intracellular Signaling, and mTOR

mTOR is a large protein kinase complex that functions as part of a signaling pathway, primarily stimulating and mediating the effects of insulin and leucine (and perhaps other molecules), on protein synthesis through different multiprotein complexes. While the mechanisms are not clear, mTOR is thought to "sense" the amino acid supply (along with information provided independently by insulin and leucine, among other factors) and to serve as a signal transduction pathway to promote protein synthesis and overall anabolism. mTOR consists of two units: complex 1 (mTORC1) and 2 (mTORC2); each complex is further made up of several components. Leucine (both in the plasma and intracellularly, and perhaps a metabolite of leucine, β-hydroxy-β-methylbutyrate abbreviated HMB), insulin, and insulin-like growth factor 1 signal mTORC1, resulting in activation of key enzymes and regulatory components required for mRNA translation and protein synthesis. mTORC1 signal activation occurs within about 30 minutes of meal consumption, and maximum protein synthesis occurs at about 1–1½ hours after eating and then declines [10].

Amino acids also affect two phases, initiation and elongation, of mRNA translation for protein synthesis. Initiation of translation involves numerous eukaryotic initiation factors (eIFs), which are needed for the delivery of Met-tRNAi^{MET} (the initiation transfer RNA) to the 40S ribosomal subunit and for the formation of a preinitiation complex. One of the eIFs needed for the binding of Met-tRNAi^{MET} to the ribosomal subunit is eIF2, whose activity is enhanced by amino acid availability and diminished with amino acid depletion within the cells. Additionally in initiation, the binding of the mRNA to the preinitiation complex relies on several additional eIFs. Amino acids, especially leucine, and insulin through interactions with mTOR increase the phosphorylation of binding protein(s) that in turn enhance some of the eIFs actions directed at mRNA-preinitiation complex binding. These effects in turn stimulate protein synthesis.

Another example of the role of leucine is in the elongation phase of translation. In elongation, translocation of the ribosomes along the mRNA must occur following the addition of an amino acid to the growing peptide. Eukaryotic elongation factor 2 (eEF2), which mediates the translocation, is regulated by a kinase whose activity appears to be affected by leucine, insulin, and mTORC1 functions.

Leucine and insulin are also thought to enhance mTOR activity to activate S6K1, which then acts on a target protein that regulates the translation of specific mRNAs. The specific mRNAs affected by S6K1 code for proteins needed for the translation of ribosomal proteins. Thus, the production of the synthetic machinery for translation is influenced by both insulin and leucine. Protein synthesis is also influenced by changes in cell volume resulting from cellular uptake of specific amino acids. These effects are thought to be mediated through intracellular signaling pathways that likely also involve mTOR.

Protein Intake, Distribution and Quantity at Meals

Muscle protein synthesis over a 24-hour period appears to be greater with consistent consumption of protein (in sufficient quantities) at each meal versus disproportionate or unequal ingestion of protein throughout the day [9–14]. Most Americans ingest higher quantities of protein at the evening meal versus other meals, especially breakfast [2]. Recommended amounts of protein per meal to maximize muscle protein synthesis vary and differ for younger versus older adults. These recommendations are discussed further in the section "Recommended Protein and Amino Acid Intakes."

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PROTEIN STRUCTURE AND ORGANIZATION

Proteins begin to fold and take "shape" as they are synthesized on the ribosomes. The shape or structure and organization of proteins are designated as primary, secondary, tertiary, and quaternary, although not all proteins have this fourth level (see Figures 6.17–6.20). The primary structure of a protein represents the amino acid sequence of the protein. The secondary structure is the coiling, folding, and/or bending of the protein. Various interactions between and among amino acids within the proteins contribute to the overall levels of organization. For example, hydrogen bonds between amino acids contribute to the secondary structures of proteins. Hydrogen (H) bonds are weak electrical attractions that can occur between hydrogen atoms and negatively charged atoms such as oxygen or nitrogen. Electrostatic attractions, also called ionic attractions or salt bonds, occur between oppositely charged side chains of amino acids, such as lysine and glutamate, to impact the secondary structure. In addition, hydrophobic interactions occur between the side chains of nonpolar amino acids. These interactions can generate particular structures, such as the α-helix and β-pleated sheet; the presence of these structures provides important attributes, including added stability, strength, and rigidity, to proteins. The α-helix and β-pleated sheet are particularly abundant in proteins with structural roles, such as collagen, elastin, and keratin.

The overall or total three-dimensional configuration of the protein is referred to as the tertiary structure. For example, the tertiary structures of globular proteins, which are named for their spherical shape, generally contain multiple α-helices and β-pleated sheets. Myoglobin, calmodulin, and many enzymes and serum proteins are globular proteins. The quaternary structure results from two or more polypeptide chains interacting. Some of the same interactions contributing to the secondary structure also contribute to these additional levels of organization and include the clustering of hydrophobic amino acids toward the center of the protein and the electrostatic attraction of oppositely charged amino acids. In addition to the weak noncovalent hydrogen, electrostatic, and hydrophobic bonding, strong covalent bonding (involving electron sharing) may occur. One of the more commonly formed covalent bonds occurs between cysteine residues where the —SH groups are oxidized to form disulfide bridges (—S—S—), as shown in Figure 6.19b. Together, the interactions among the amino acid side chains determine the protein's overall shape and, therefore, influence the protein's function in the body.

Figure 6.17 The primary structure of a protein.
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(a) α-Helix—a cylindrical shape formed by a coiling of the polypeptide chain on itself.

(b) β-Pleated sheet—the polypeptide chain is fully stretched out with side chains positioned either up or down. The stretched polypeptide may fold back on itself with segments packed together.

(c) Random coil—an unstable structure formed due to the presence of certain amino acids whose side chains interfere with one another.

Figure 6.18 Secondary structure of proteins.
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FUNCTIONAL ROLES OF PROTEINS AND NITROGEN-CONTAINING NONPROTEIN COMPOUNDS

The molecular architecture and activity of living cells depend largely on proteins, which make up over half of the solid content of cells and which show great variability in size, shape, and physical properties. Their physiological roles also are quite variable, and because of this variability, categorizing proteins according to their functions can be helpful in the study of human metabolism.

Catalysts

Enzymes are protein molecules (generally designated by the suffix *-ase*) that act as catalysts; they change the rate of reactions occurring in the body. Enzymes are necessary for sustaining life and are found in the body both intracellularly and extracellularly (e.g., in the blood).

Enzymes are constructed so that they combine selectively with other molecules (called substrates) in the cell. The active site on the enzyme (a small region usually in a crevice of the enzyme) is where the enzyme and substrate bind and the product is generated. Some enzymes, however, require a cofactor or coenzyme to carry out the reaction. Minerals such as zinc, iron, and copper function as cofactors for some enzymes. *Metalloprotein* is the name typically used for proteins to which minerals are complexed. Some, but not all, metalloproteins have enzymatic activity. B vitamins serve as coenzymes for many enzymes. *Flavoprotein* is the term generally used for protein enzymes bound to flavin mononucleotide (FMN) or flavin adenine nucleotide (FAD), coenzyme forms of the B vitamin riboflavin. Most human physiological processes require enzymes to promote chemical changes that could not otherwise occur. Some examples of different types of enzymes include dehydrogenases, which remove or transfer hydrogens; kinases, which add phosphate groups; and isomerases, which transfer atoms within a molecule. Some examples of physiological processes that depend on enzyme function include digestion, energy production, blood coagulation,

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Figure 6.19 (a) The tertiary structure of the protein α-lactalbumin. (b) Examples of interactions found in tertiary structures.
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Figure 6.20 Quaternary structure of the hemoglobin protein. Quaternary proteins are characterized by interactions between two or more (usually two or four) polypeptide chains. The aggregated form is called an oligomer. The polypeptide chains (called subunits) making up the oligomer are held together by hydrogen bonds and electrostatic salt bridges.
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and excitation and contraction of neuromuscular tissue. The section titled "Catalytic Proteins" in Chapter 1 provides further information on enzymes.

Messengers

Some proteins are hormones. **Hormones** act as chemical messengers in the body. They are synthesized and secreted by endocrine tissue (glands) and transported in the blood to target tissues or organs, where they bind to protein receptors on membranes. Hormones generally regulate metabolic processes, for example, by promoting enzyme synthesis or affecting enzyme activity.

Whereas some hormones are derived from cholesterol and classified as steroid hormones, others are derived from one or more amino acids. The amino acid tyrosine, for example, is used along with the mineral iodine to synthesize the thyroid hormones. Tyrosine is also used to synthesize the catecholamines, including dopamine, norepinephrine, and epinephrine. The hormone melatonin is derived in the brain from the amino acid tryptophan. Other hormones are made up of one or more polypeptide chains. Insulin, for example, consists of two polypeptide chains linked by a disulfide bridge. Glucagon, parathyroid hormone, and calcitonin each consist of a single polypeptide chain. Many other peptide hormones, such as adrenocorticotropic hormone (ACTH), somatotropin (growth hormone), and vasopressin (also known as antidiuretic hormone, or ADH), have important roles in human metabolism and nutrition. These hormones are discussed throughout this chapter and the book.

Structural Elements

Several proteins have structural roles in the body. Two groups of structural proteins include:

- contractile proteins
- fibrous proteins.

The two main contractile proteins, actin and myosin, are found in cardiac, skeletal, and smooth muscles. Skeletal muscle is found throughout the body and is under voluntary control. Contraction is calcium induced and involves not only actin and myosin, but also troponin and tropomyosin. Smooth muscle is found in many tissues including, for example, blood vessels, the lungs, the uterus, and the gastrointestinal tract. Smooth muscle is under involuntary control and contracts in response to calcium-induced phosphorylation of the structural protein myosin.

Fibrous proteins, which tend to be somewhat linear in shape, include collagen, elastin, and keratin and are found in bone, teeth, skin, tendons, cartilage, blood vessels, hair, and nails. Collagen is a group of well-studied proteins. Each type of collagen is made of three polypeptide (tropocollagen) chains that are cross-linked for strength. These chains, rather than forming specific secondary structures (α-helices or β-pleated sheets, discussed in the protein structure section), form a helical arrangement. The amino acid composition of the chains is rich in the amino acids glycine and proline. In addition, collagen contains two hydroxylated amino acids—hydroxylysine and hydroxyproline—that are not found in other proteins. Collagen polypeptides are also attached to carbohydrate chains and thus are considered to be glycoproteins. Other structural proteins, such as elastin, are associated with proteoglycans. Both glycoproteins and proteoglycans are conjugated proteins and are discussed further in the "Other Roles" section. Elastin, rich in glycine and alanine, is a component of connective tissues, especially blood vessels, ligaments, and the dermis. Keratins, a group of proteins typically found in the cytoskeletal structure of some cells (especially hair and some epithelial cells), help maintain cell integrity and stability. Keratin molecules interact, forming strong bundles referred to as intermediate filaments. Keratin's high cysteine content allows for the formation of strong disulfide bridges between keratin molecules.

Buffers

Proteins, because of their constituent amino acids, can serve as buffers in the body and thus help to regulate acid-base balance. A **buffer** is a compound that ameliorates a change in pH that would otherwise occur in response to the addition of alkali or acid to a solution. The pH of the blood and other body tissues must be maintained within an appropriate range. Blood pH ranges from about 7.35 to 7.45, whereas cellular pH levels are often more acidic. For example, the pH of red blood cells is about 7.2, and that of muscle cells is about 6.9. The H^+ concentration within cells is buffered by both the phosphate system and the amino acids in proteins. The protein hemoglobin, for example, functions as a buffer in red blood cells. In the plasma and extracellular fluid, proteins and the bicarbonate system serve as buffers. The buffering ability of proteins can be illustrated by the reaction H^++ protein ↔ Hprotein and is shown in Figure 6.21.

Fluid Balancers

In addition to acid-base balance, proteins influence fluid balance through their presence in the blood and in cells. More specifically, proteins help attract and keep water inside a particular area and contribute to **osmotic pressure**. Diminished blood/plasma concentrations of proteins result in a decrease in plasma osmotic pressure. When protein concentrations in the blood are less dense than normal, fluid "leaks" out of the blood and into interstitial spaces and causes swelling (and if severe even pitting edema). Restoring adequate concentrations of protein in the blood (e.g., by infusing the protein albumin intravenously) and within cells where proteins are also found (e.g., by providing sufficient

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Figure 6.21 The role of the amino acid in pH balance.
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dietary protein and energy to enable protein synthesis) promotes diffusion of water from the interstitial space back into the blood and back within cells.

Immunoprotectors

Immunoprotection is provided to the body in part by a group of proteins called **immunoproteins**, also called immunoglobulins (Ig) or antibodies (Ab). These immunoproteins, of which there are five major classes (IgG, IgA, IgM, IgE, and IgD), are Y-shaped proteins made of four polypeptide chains (two small chains called light [L] chains and two large chains called heavy [H] chains). The immunoglobulins are produced by plasma cells derived from B-lymphocytes, a type of white blood cell. Immunoglobulins function by binding to antigens—which typically consist of foreign substances, such as bacteria or viruses that have entered the body. By complexing with antigens, immunoglobulins create immunoprotein–antigen complexes that can be recognized and destroyed through reactions with either complement proteins or cytokines. The complement proteins (approximately 20) are produced primarily in the liver and circulate in the blood and extracellular fluid. Cytokines are produced by white blood cells such as T-helper (CD4) cells and macrophages. In addition, white blood cells such as macrophages and neutrophils also destroy foreign antigens through the process of phagocytosis.

Transporters

Transport proteins are a diverse group of functional proteins that provide a means of carrying substances, such as vitamins, minerals, and other nutrients, within the blood, into cells, out of cells, or within cells. In the blood, for example, the protein hemoglobin, found in red blood cells, transports oxygen and carbon dioxide. In cell membranes, some membrane-spanning proteins provide pores or channels through which substances such as sodium, potassium, chloride, and calcium may gain entry. Some of these pores may be gated open or closed in response to alterations in membrane potential (voltage gated) and/or the presence of a ligand. Ligands from outside the cell—such as hormones or neurotransmitters—or ligands from within the cytosol—such as calcium, cAMP, among others—initiate changes to affect gate function. Other proteins act as carriers of which there are several types. Uniporters carry only one substance across cell membranes; for example, some amino acid transporters are uniporters or symporters. Symporters carry more than one substance simultaneously across the cell membrane; in the intestinal brush border a glucose transporter carries both glucose and sodium into cells. Antiporters, another type of cell membrane protein transporter, function by exchanging one substance for another.

Of the hundreds of proteins in the blood, several serve as transporters. The concentration of total protein in human plasma typically totals up to about 7.5 g/dL. Lipoproteins, for example, transport cholesterol and triacylglycerol in the blood; the proteins in the lipoproteins are actually a group of about 10 different apoproteins that both enable lipid transport and direct the lipoproteins to cells for use. An apoprotein is a protein that is complexed or part of a molecule that also contains a nonprotein portion.

A few transport proteins of clinical significance include albumin, transthyretin, and retinol-binding protein. Albumin, the most abundant of the plasma proteins, transports nutrients such as tryptophan, fatty acids, and vitamin B_6;

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some minerals including zinc, calcium, and small amounts of copper; and some drugs. The protein is synthesized by the liver and released into the blood; changes in osmotic pressure and osmolality as well as inflammatory mediators affect its rate of synthesis. A healthy person makes about 9–12 g of albumin per day [15]. Albumin is often used in the absence of inflammation to assess an individual's protein status, specifically visceral (internal organ) protein status. Because of albumin's relatively long half-life (~14–18 days), however, it is not as good or as sensitive an indicator of visceral protein status as some of the other plasma proteins. The half-life is the time it takes for 50% of the amount of a protein such as albumin (or nonprotein compound) to be degraded.

Two other proteins synthesized by the liver and released into plasma are transthyretin (also called prealbumin) and retinol-binding protein. Retinol-binding protein, as its name implies, transports retinol (a form of vitamin A) but also thyroid hormone. Transthyretin and retinol-binding protein, like albumin, are also used as biochemical indicators of visceral protein status in the absence of inflammation. Because transthyretin and retinol-binding protein have relatively shorter half-lives (~2 days and 12 hours, respectively) than albumin, they are more sensitive indicators of changes in visceral protein status. The concentrations of albumin, prealbumin, and retinol-binding protein diminish in the blood over varying time periods (depending on their half-life) in people, for example, who have ingested inadequate dietary protein. Typically, plasma concentrations of albumin < 3.5 g/dL, prealbumin (transthyretin) < 18 mg/dL, and retinol-binding protein < 2.1 mg/dL suggest inadequate visceral protein status. A diet high in energy (kcal) and protein is needed to promote improvements (assuming the liver is healthy) in status.

Some transport proteins found in the blood are classified according to size and charge using the procedure protein electrophoresis. Globulins, a heterogeneous group of proteins with diverse transport functions, are identified using this method. Most globulins are synthesized in the liver, with the exception of the gamma globulins (antibodies), which are made mostly by plasma cells (mature B-lymphocytes). The four classes of globulins are listed here, along with some examples of proteins comprising each class and their transport roles:

- α1-globulins: glycoproteins and high-density lipoproteins (for lipid transport)
- α2-globulins: glycoproteins, haptoglobin (for free hemoglobin transport), ceruloplasmin (for copper transport and oxidase activity), prothrombin (for blood coagulation), and very-low-density lipoproteins (for lipid transport)
- β-globulins: transferrin (for iron and other mineral transport) and low-density lipoproteins (for lipid transport)
- γ-globulins: immunoglobulins or antibodies (for immunoprotection).

Some hospital laboratories report serum albumin concentrations relative to globulin concentrations. Normally, albumin concentrations exceed globulin concentrations slightly.

Acute-Phase Responders

Another heterogeneous group of proteins, called acute-phase or positive acute-phase reactant proteins, are made in the liver in response especially to acute (sudden), critical illnesses such as infection (sepsis) and injury/trauma. The body's reaction to such situations is referred to as an inflammatory response, and the magnitude of the response varies with the severity of the illness/situation. Some examples of these acute-phase proteins are C-reactive protein, fibronectin, orosomucoid (also called α1-acid glycoprotein), haptoglobin, serum amyloid A, α2-macroglobulin, ceruloplasmin, and metallothionein. Collectively, these proteins perform a variety of functions that protect the body, such as stimulating the immune system, promoting wound healing, and chelating and removing free iron from circulation to prevent its use by bacteria for growth. C-reactive protein is used clinically to evaluate inflammation in patients, and its concentration rises dramatically in the blood within a few hours of infection-induced inflammation. Diminishing plasma concentrations of C-reactive protein suggest the possibility of a less catabolic state.

The body generates a group of proteins called stress or heat shock proteins (abbreviated *hsp*). Heat shock proteins also function in cytoprotection and cell survival primarily by facilitating protein folding or refolding, trafficking, repair, assembly, and degradation. Heat shock proteins are categorized into families based on molecular weight (e.g., hsp 60, hsp 70, hsp 90). Some are synthesized constitutively while others are synthesized in response to stress, including heat stress and oxidative stress such as with exercise and other physical activity in warm environments. The amino acid glutamine also appears to enhance the expression of some heat shock proteins during critical illness (oxidative stress). More specifically, glutamine is thought to be necessary for the activation of specific transcription factors required for heat shock protein synthesis. The Perspective at the end of this chapter provides some additional information about the body's response to stress.

Other Roles

Proteins carry out many additional roles in the body. For example, some serve to transmit signals into and out of the cell. Between adjacent cells, proteins function in cell adhesion. A group of claudin proteins function in intestinal barrier regulation, with some claudins forming charge-selective pore channels, some enabling paracellular absorption, and others functioning to keep cell junctions tightly closed. The protein opsin is important for vision (as discussed under the section on vitamin A in Chapter 10).

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Table 6.6 Sources of Nitrogen for Some Nitrogen-Containing Nonprotein Compounds

Nitrogen-Containing Nonprotein Compound	Constituent Amino Acids
Glutathione	Cysteine, glycine, glutamate
Carnitine	Lysine, methionine
Creatine	Arginine, glycine, methionine
Carnosine	Histidine, β-alanine
Choline	Serine

Proteins also serve as receptors on cell membranes and can function in storage roles. For example, some minerals such as copper, iron, and zinc are stored in body tissues bound to proteins; these proteins are often called metalloproteins.

Many proteins in the body are conjugated proteins—that is, proteins that are joined to nonprotein components. Glycoproteins, one type of conjugated protein, represent a huge group of proteins with multiple functions. **Glycoproteins** consist of a protein covalently bound to a carbohydrate component. The carbohydrate in glycoproteins generally includes short chains of glucose, galactose, mannose, fucose, N-acetylglucosamine, N-acetylgalactosamine, and acetylneuraminic (sialic) acid at the terminal end of the oligosaccharide chain. The carbohydrate portion of the glycoprotein can make up as much as 85% of the glycoprotein's weight. The carbohydrate component is bound typically through an N-glycosidic linkage with asparagine's amide group in its side chain or through an O-glycosidic linkage with the hydroxy group in serine's or threonine's side chain. Glycoproteins are found in the blood, on the outer surface of plasma membranes, and in association with the extracellular matrix (which surrounds and supports some body cells). In the extracellular matrix of bone, for example, glycoproteins play structural roles. Mucus, which is found in body secretions, is rich in glycoproteins. Mucus both lubricates and protects epithelial cells in the body. Some of the body's hormones (such as thyrotropin) and blood proteins (such as transthyretin and immunoglobulins) are glycoproteins.

Another group of conjugated proteins is the proteoglycans, which are found in every tissue of the body. Most proteoglycans are associated with the extracellular matrix but also with other structural matrix components, where they form cross-links to enhance strength and resilience and to modulate adhesion and communication between cells and between the extracellular matrix and cells. **Proteoglycans** are macromolecules consisting of a core protein covalently conjugated, typically by O-glycosidic or N-glycosylamine linkages, to one or more **glycosaminoglycans**. Glycosaminoglycans consist of long chains of repeating disaccharides, comprise up to 95% of the weight of the proteoglycan, and are the main site of the proteoglycan that interacts with cell surface proteins or extracellular matrix proteins. Examples of glycosaminoglycans include hyaluronic acid (found in high concentrations in cartilage), chondroitin sulfate (found in high concentrations in bone and cartilage), keratan sulfate and dermatan sulfate (found in the cornea of the eye), and heparan sulfate (found in high concentrations in plasma cell membranes).

Nitrogen-Containing Nonprotein Compounds

In addition to their use in the synthesis of body proteins, amino acids are used to synthesize nitrogen-containing compounds that are not proteins but nonetheless play important roles in the body. This next section addresses the roles of some nutritionally significant nitrogen-containing nonprotein compounds (Table 6.6). Not included in this review, however, are a number of biogenic amines, neurotransmitters, and neuropeptides that are synthesized from amino acids in many glands, tissues, and organs throughout the body. A discussion of these compounds is found in this chapter in the "Brain and Accessory Tissues" section. Some of the compounds also are mentioned in sections that discuss the metabolism of amino acids.

Glutathione

Glutathione (Figure 6.22) is a tripeptide synthesized from three amino acids—glycine, cysteine, and glutamate—in most body cells. Its synthesis occurs in two steps, both ATP dependent. First, the γ carboxy group of glutamate is attached to the amino group of cysteine by γ glutamyl cysteine synthetase to form a peptidic γ linkage. In the second step, glutathione synthetase creates a peptide bond between the amino group of glycine and the carboxy group of cysteine to produce glutathione. The availability of cysteine appears to be the major factor influencing glutathione synthesis, although synthesis can be affected by administration of any of the precursor substrates or via activity of glutathione peroxidase and reductase, which catalyze other glutathione-dependent reactions.

Glutathione is referred to as a thiol because it contains a sulfhydryl (—SH) group in its reduced form (designated GSH). Glutathione can also be found in cells in its oxidized form (designated GSSG) and attached to proteins (up to about 15%). Normally, the ratio of GSH to GSSG in cells is >10 to 1; the GSH-to-GSSG ratio represents an indicator of the cell's redox state. In fact, the ratio of GSH to GSSG is thought to be the most important regulator of the cellular redox potential.

Figure 6.22 The structure of glutathione in its reduced form (GSH).

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Glutathione is found in the cytosol of most cells, but small amounts also are found within cell organelles and in the plasma. Glutathione has several functions. It is a major antioxidant with the ability to scavenge free radicals ($O_2^{\bullet}$ and $OH^{\bullet}$), thereby protecting critical cell components including SH-containing proteins against oxidation. With the enzyme glutathione peroxidase, glutathione protects cells by reacting with hydrogen peroxides (H_2O_2) and lipid hydroperoxides (LOOHs) before they can cause damage. Glutathione also transports amino acids as part of the γ-glutamyl cycle (Figure 6.4) in some tissues. It participates in the synthesis of leukotriene (LT) C4, which mediates the body's response to inflammation. Glutathione is also involved in the conversion of prostaglandin H2 to prostaglandins D2 and E2 by endoperoxide isomerase. Glutathione can conjugate with nitric oxide to form S-nitrosoglutathione.

Glutathione synthesis is sensitive to protein intake and pathological conditions. Hepatic, intestinal, and systemic GSH concentrations decline with poor protein intake as well as during inflammation and disease; this decline negatively impacts the body, necessitating strategies to enhance or at least maintain GSH concentrations. Glutathione is discussed further in the section on selenium in Chapter 13.

Carnitine

Carnitine, another nitrogen-containing compound, is made (Figure 6.23) from the amino acid lysine that has been methylated; the methyl groups are derived from S-adenosyl methionine (SAM), which is made in the body from the oxidation of the amino acid methionine. Following lysine methylation, trimethyllysine undergoes hydroxylation at the 3 position to form 3-OH trimethyllysine. Hydroxytrimethyllysine is further metabolized to generate γ-butyrobetaine and

1 Ascorbate functions as a reducing agent in two reactions. In both reactions for carnitine synthesis, the vitamin is needed to reduce the iron atom that has been oxidized (Fe^{3+}) in the reaction back to its reduced (Fe^{2+}) state.

Figure 6.23 Carnitine synthesis.

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subsequently carnitine. Iron, vitamin B_6 (as PLP), vitamin C, and niacin (as NAD^+) are needed for carnitine synthesis.

In addition to being synthesized in the liver and kidneys, carnitine is found in foods, especially meats such as beef and pork. In these foods, carnitine may be free or bound (as acylcarnitine) to long- or short-chain fatty acid esters. Carnitine from food or supplements is absorbed in the proximal small intestine by sodium-dependent active transport and passive diffusion; diffusion typically predominates with ingestion of supplements providing 0.5–6 g [16]. Approximately 54–87% of carnitine intake is absorbed. Intestinal absorption of carnitine is thought to be saturated with intakes of about 2 g [17]. Muscle represents the primary carnitine pool, although no carnitine is made there. Intramuscular concentrations of carnitine are generally 50 times greater than usual plasma concentrations. Carnitine homeostasis is maintained principally by the kidneys, with >90% of filtered carnitine and acylcarnitine being reabsorbed.

Carnitine, found in most body tissues, is needed for the transport of fatty acids, especially long-chain fatty acids, across the inner mitochondrial membrane for oxidation. The inner mitochondrial membrane is impermeable to long-chain (10 or more) fatty acyl-coenzyme (Co) As. This role of carnitine is discussed in more detail in Chapter 5. Carnitine is also needed for ketone catabolism for energy. Carnitine also forms acylcarnitines from short-chain acyl-CoAs. These acylcarnitines may serve to buffer the free CoA pool.

Carnitine deficiency, though rare, results in impaired energy metabolism. Carnitine supplementation increases plasma and muscle carnitine concentrations, and has been beneficial for some people with specific cardiac problems and diabetes. Supplementation with carnitine does not, however, "burn fat" as suggested in some advertisements.

Creatine

Creatine, a key component of the energy compound creatine phosphate, also called phosphocreatine, can be obtained from foods (primarily meat and fish) or synthesized from three amino acids in the body. Creatine synthesis, which is shown in Figure 6.24, begins first in the kidneys and requires arginine and glycine. The second step occurs in the liver and involves the methylation of guanidinoacetate using SAM (S-adenosyl methionine).

Once synthesized, creatine is released into the blood for transport to tissues. About 95% of creatine is in muscle, with the remaining 5% in organs such as the kidneys and brain. In tissues, creatine is found both in free form as creatine and in its phosphorylated form. The phosphorylation of creatine to form phosphocreatine is shown here:

Figure 6.24 Creatine synthesis and use.

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Phosphocreatine functions as a "storehouse for high-energy phosphate." In fact, over half of the creatine in muscle at rest is in the form of phosphocreatine. Phosphocreatine replenishes ATP in a muscle that is rapidly contracting. Remember, muscle contraction requires energy. This energy is obtained with the hydrolysis of ATP. However, the ATP in muscle can suffice for only a fraction of a second. Phosphocreatine, stored in the muscle and possessing a higher phosphate group transfer potential than ATP, can transfer a phosphoryl group to ADP, thereby forming ATP or assisting in ATP regeneration, providing energy for muscular activity. Creatine kinase, also called creatine phosphokinase (abbreviated CK or CPK), catalyzes the phosphate transfer in active muscle, as shown here.

Creatine kinase is made up of different subunits in different tissues. For example, in the heart, creatine kinase is made up of two subunits designated M and B. (The brain and muscle also have creatine kinase, but in these tissues the enzyme is made up of the BB and MM subunits, respectively.) Damage to the heart, as with a heart attack, causes the enzyme to "leak" out of the heart and reach elevated concentrations in the blood. Thus, an elevation in CK-MB in the blood along with other indicators is used to diagnose a heart attack. Similarly, damage to skeletal muscle, as may occur with trauma, results in elevations of CK-MM in the blood.

The availability of phosphocreatine and its use by muscle are thought to delay the breakdown of muscle glycogen stores, which upon further catabolism can also be used by muscle for energy. Creatine and creatine phosphate do not remain indefinitely in muscle; rather, both slowly but spontaneously cyclize (as shown in Figure 6.24) because of nonreversible, nonenzymatic dehydration. This cyclization of creatine and phosphocreatine forms creatinine. Once formed, creatinine leaves the muscle, passes across the glomerulus of the kidneys, and is excreted like other nitrogenous waste products (e.g., urea, ammonia, uric acid) in the urine. Creatinine clearance is sometimes used as a means of estimating kidney function. The urinary excretion of creatinine is used as an indicator of existing muscle mass, as discussed later in the section under "Skeletal Muscle" titled "Indicators of Muscle Mass and Muscle/Protein Catabolism." Not all creatinine, however, gets excreted in the urine. Small amounts may be secreted into the gut and, like urea, metabolized by intestinal bacteria. The effects of creatine supplementation on athletic performance are discussed in the Perspective for Chapter 7.

Figure 6.25 Carnosine.

Carnosine

Carnosine (also called β-alanyl histidine; Figure 6.25) is made from the amino acid histidine and β-alanine in an energy-dependent reaction catalyzed by carnosine synthetase. In the body, carnosine is synthesized and found largely in the cytosol of skeletal and cardiac muscle, but also in the brain, kidneys, and stomach. Related compounds include a methylated form of carnosine known as anserine (β-alanyl methylhistidine) and homocarnosine (γ-aminobutyryl histidine), among others. Carnosine is also found in foods, primarily meats, and may be digested into histidine and β-alanine in the intestine or possibly absorbed intact by peptide transporters. While not all of the functions of carnosine have been identified, some studies have shown that carnosine acts as both a buffer and an antioxidant within muscle cells; it may also reduce calcium needs for muscle contractility. The use of β-alanine supplements (about 3–6 g per day) increases muscle carnosine concentrations; the effects of supplementation on athletic performance are discussed in the Perspective for Chapter 7.

Choline

Choline (Figure 6.26) is made in the body primarily in the liver through the methylation (involving S-adenosyl methionine, or SAM) of the phospholipid phosphatidylethanolamine when linked with the catabolism of phosphatidylcholine. The formation of phosphatidylserine from phosphatidylcholine involving the replacement of choline with serine by phosphatidylserine synthase 1 also releases choline for other use in the body.

In foods, choline is found free (unattached) in small amounts but is more commonly found in foods bound as part of phosphatidylcholine (also called lecithin) and sphingomyelin, among other forms. Foods rich in lecithin include eggs, meats (especially liver and other organ meats), shrimp, cod, salmon, wheat germ, and legumes such as soybeans and peanuts. Lecithin is also added to many foods as an emulsifier. Intake is estimated at about 6–10 g/day [18]. Pancreatic enzymes hydrolyze some choline from its bound forms. Free choline is absorbed in the small intestine by diffusion and carrier-mediated uptake and transported via the blood to tissues. Choline existing as phosphatidylcholine and sphingomyelin are incorporated into chylomicrons for transport to tissues. The liver and kidneys store choline to a limited extent.

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Figure 6.26 Choline.

Choline is a nitrogen-containing compound that is also often presented and/or discussed with the B vitamins, although it is not defined as a vitamin. It has several functions. Most choline is used to synthesize phosphatidylcholine and sphingomyelin, major components of cell membranes. Phosphatidylcholine also functions in intracellular signaling and in the secretion of very-low-density lipoproteins from the liver. Sphingomyelin is a component of myelin that functions as a sheath around nerves and is important in nerve conduction. Choline is also used in the formation of platelet aggregating factor and for the neurotransmitter acetylcholine. To be converted to acetylcholine, free choline crosses the blood–brain barrier and enters cerebral cells from the plasma through a specific choline transport system. Within the presynaptic terminal of the neuron, acetylcholine is formed by the action of choline acetyltransferase as follows:

$$\text{Choline} + \text{acetyl-CoA} \longrightarrow \text{Acetylcholine} + \text{CoA}$$

The acetyl-CoA needed for the reaction is thought to arise from glucose metabolism by neural glycolysis and the action of the pyruvate dehydrogenase complex. Concentrations of choline in cholinergic neurons typically are below the K_m of choline acetyltransferase; thus, the enzyme normally is not saturated. Choline from acetylcholine can be reused following synaptic transmission; the enzyme acetylcholinesterase hydrolyzes the neurotransmitter. Phospholipases can also liberate choline from lecithin and sphingomyelin as needed.

Choline is oxidized in the liver and kidneys (see Figure 6.12). In the liver, choline oxidation generates betaine, which functions as a methyl donor in the generation of methionine from homocysteine. Further metabolism of betaine (also called trimethylglycine) generates dimethyl glycine (also called sarcosine), and subsequently glycine; the reactions require folate as tetrahydrofolate and generate another folate derivative, 5,10-methylene tetrahydrofolate. These reactions are shown in the section of Chapter 9 on folate (specifically, the amino acid metabolism of serine and glycine).

Experimental diets devoid of choline can decrease plasma choline and phosphatidylcholine concentrations. In some cases, insufficient dietary choline intakes promote muscle damage and the development of a fatty liver accompanied by altered liver enzymes and some hepatic necrosis. Low intakes of both choline and betaine have been associated with inflammation. Because *de novo* synthesis does not consistently meet the body's needs for choline, the Food and Nutrition Board has suggested an Adequate Intake of 425 mg and 550 mg of choline daily for adult females and males, respectively [18]. Such intakes are easily obtained through dietary consumption of animal products and foods containing fats. A Tolerable Upper Intake Level of 3.5 g of choline daily also has been set [18]. The **Tolerable Upper Intake Level** represents the highest level of daily intake that is likely to pose no risks of adverse health effects to most people in the general population [18]. Adverse effects associated with ingestion of large doses of choline include excessive sweating, salivation, vomiting, and a fishy body odor. Intakes of 7.5 g of choline have caused small hypotensive effects [18].

Purine and Pyrimidine Bases

Nitrogenous bases, along with a five-carbon sugar and phosphoric acid, are needed for the synthesis of two nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), in the body. It is amino acids that provide the source for the nitrogen in these bases. The nitrogenous bases can be divided into two categories: pyrimidines and purines. The pyrimidines are six-membered rings containing nitrogen atoms in positions 1 and 3. The pyrimidine bases include uracil, cytosine, and thymidine. Deoxycytidine and thymidine (also called deoxythymidine) are found in DNA. Cytidine and uridine are present in RNA. The purines are made up of two fused rings with nitrogen atoms in positions 1, 3, 7, and 9. The purine bases include adenine and guanine and are found in DNA as deoxyadenosine and deoxyguanosine and in RNA as adenosine and guanosine. A brief review of purine and pyrimidine synthesis and degradation follows.

The synthesis of the nitrogen-containing bases used to make nucleic acids and nucleotides occurs for the most part *de novo* in the liver. The individual steps in pyrimidine synthesis are shown in Figure 6.27. First, synthesis of the pyrimidines uracil, cytosine, and thymine (or in nucleotide form UTP, CTP, and TTP, respectively) is initiated by the formation of carbamoyl phosphate from the amino acid glutamine, CO_2, and ATP. The enzyme carbamoyl phosphate synthetase II catalyzes this reaction in the cytosol and is distinct from carbamoyl phosphate synthetase I, which is needed in the initial step of urea synthesis and is found in the mitochondria. Second, carbamoyl phosphate reacts with the amino acid aspartate to form N-carbamoyl aspartate. Aspartate transcarbamoylase catalyzes the reaction, which is the committed step in pyrimidine biosynthesis. Following several additional reactions, detailed in Figure 6.27, uridine monophosphate (UMP) is synthesized. Defects in the activity of either OMP decarboxylase used to make UMP (reaction 6 in Figure 6.27) or orotate phosphoribosyl transferase (reaction 5 in Figure 6.27) result in the genetic disorder orotic aciduria. This condition is characterized by megaloblastic anemia, leukopenia, retarded growth, and the excretion of large amounts of orotic acid in the urine.

The interconversions among the pyrimidine nucleoside triphosphates are shown in Figure 6.28 and are discussed

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Figure 6.27 The initial reactions of pyrimidine synthesis.

next. Once uridine monophosphate (UMP) is formed, it may react with other nucleoside di- and triphosphates. UMP can be converted to uridine diphosphate (UDP) utilizing ATP. UDP can be converted to uridine triphosphate (UTP) also using ATP, and UTP can be converted to cytosine triphosphate (CTP) using ATP and an amino group from glutamine. Alternately, UDP can be reduced to deoxy(d)UDP by ribonucleotide reductase; this reaction requires riboflavin as $FADH_2$ and the protein thioredoxin. DeoxyUDP can then be converted to dUMP. The formation of deoxythymidine (also called thymidine) monophosphate (dTMP or TMP) from dUMP is catalyzed by thymidylate synthetase; the reaction requires folate as 5,10 methylene tetrahydrofolate and forms another folate derivative dihydrofolate (DHF). Dihydrofolate reductase is needed to convert DHF to tetrahydrofolate, which is then converted to 5,10 methylene tetrahydrofolate and thus allows for dTMP synthesis. DeoxyTMP can be phosphorylated to form deoxythymidine diphosphate (dTDP) and then phosphorylated again to produce deoxythymidine triphosphate (dTTP, or abbreviated TTP). Thus, through these reactions CTP, (d)TTP, and UTP have been generated and can be used for the synthesis of DNA and RNA. The pyrimidine ring structure and its sources of carbon and nitrogen atoms along with the structures of the pyrimidine bases are shown in Figure 6.29. CTP is also used in phospholipid synthesis, and UTP is used to form activated intermediates in the metabolism of various sugars. Drugs used to treat cancer often target key enzymes needed for the synthesis of purines or pyrimidines, which are needed by both healthy and cancer cells to grow and multiply. The drug methotrexate, for example, inhibits dihydrofolate reductase activity and thereby decreases dTMP (and thus TTP) formation. Rapidly dividing cells such as cancer cells are more susceptible to the effects of these drugs.

The purine bases adenine and guanine (Figure 6.29) are synthesized *de novo* as nucleoside monophosphates by sequential addition of carbons and nitrogens to ribose-5-phosphate that has originated from the hexose monophosphate shunt. As shown in Figure 6.30, in the initial reaction, ribose 5-phosphate reacts with ATP to form 5-phosphoribosyl 1-pyrophosphate (PRPP). Glutamine then donates a nitrogen to form 5-phosphoribosylamine. This step represents the committed step in purine nucleotide synthesis. Next in a series of reactions, nitrogen and carbon atoms from glycine are added, formylation occurs by tetrahydrofolate, another nitrogen atom is donated by the amide group of glutamine, and ring closure occurs. Another set of reactions involving the addition of carbons from carbon dioxide and from 10-formyl THF (from folate) and a nitrogen from aspartate occurs. The net result of all of these reactions is the formation of

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1. UMP reacts with ATP to generate uridine diphosphate (UDP).
2. UDP is then converted to uridine triphosphate (UTP).
3. UTP is used with the amino acid glutamine (Gln) to make cytosine triphosphate (CTP).
4. UDP can be reduced using NADPH + H^+ in a reaction that also involves riboflavin and thioredoxin to form deoxyuridine diphosphate (dUDP).
5. dUDP can be converted to deoxyuridine monophosphate (dUMP).
6. dUMP can be converted to deoxythymidine monophosphate (also referred to as thymidine monophosphate and abbreviated dTMP or TMP, respectively) by the enzyme thymidylate synthetase. Folate as 5,10 methylene tetrahydrofolate (THF) provides a one-carbon unit to convert dUMP to dTMP. The dihydrofolate (DHF) that is formed must be converted back to THF for the cycle to continue. This reaction is catalyzed by DHF reductase, which is the target for the anti-cancer drug methotrexate.
7. dTMP can be phosphorylated using ATP to form deoxythymidine diphosphate (dTDP).
8. dTDP can be phosphorylated using ATP to form deoxythymidine triphosphate (dTTP), which is needed for DNA synthesis.

Figure 6.28 The formation of the pyrimidine nucleoside triphosphates UTP, CTP, and TTP for DNA and RNA synthesis.

a purine ring. The ring (Figure 6.29) is thus derived from components of several amino acids, including glutamine, glycine, and aspartate, as well as from folate and CO_2.

The formation of purine nucleoside triphosphates for DNA and RNA synthesis is shown in Figure 6.31. Inosine monophosphate (IMP) is used to synthesize adenosine monophosphate (AMP) and guanosine monophosphate (GMP). AMP and GMP are phosphorylated to ADP and GDP, respectively, by ATP. The deoxyribotides are formed at the diphosphate level by converting ribose to deoxyribose, thereby producing dADP and dGDP. ADP can be phosphorylated to ATP by oxidative phosphorylation; the remaining nucleotides are phosphorylated to their triphosphate form by ATP.

Purine nucleotides can also be synthesized by the salvage pathway, which requires much less energy than *de novo* synthesis. In the salvage pathway, the purine base adenine reacts with 5-phosphoribosyl 1-pyrophosphate (PRPP) to form AMP + PP_i in a reaction catalyzed by adenine phosphoribosyl transferase. The purine guanine can also react with PRPP to form GMP + PP_i. Hypoxanthine can react with PRPP to form IMP + PP_i. These last two reactions are catalyzed by hypoxanthine–guanine phosphoribosyl transferase. Defects in this enzyme cause the disorder Lesch-Nylan syndrome, a genetic X-linked condition characterized most notably by self-mutilation, such as the biting off of one's fingers, and premature death. Other symptoms include mental retardation and the accumulation of hypoxanthine, phosphoribosyl pyrophosphate, and uric acid in body fluids.

Degradation of pyrimidines involves the sequential hydrolysis of the nucleoside triphosphates to mononucleotides, nucleosides, and, finally, free bases. This process can be accomplished in most cells by lysosomal enzymes. During catabolism of pyrimidines, the ring is opened with the production of CO_2 and ammonia from the carbamoyl portion of the molecule. The ammonia can be converted into urea and excreted. Malonyl-CoA and methylmalonyl-CoA, produced from the remainder of the ring, follow their normal metabolic pathways, thus requiring no special excretion route.

Purines (GMP and AMP) are progressively oxidized for degradation primarily in the liver, yielding xanthine, which is converted to uric acid for excretion (Figure 6.32).

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Figure 6.29 The pyrimidine and purine ring structures and the pyrimidine and purine bases. Cytosine, adenine, and guanine are found in both DNA and RNA. Thymine is found in DNA and uracil only in RNA.

Figure 6.30 Synthesis of inosine monophosphate (IMP), which is used to synthesize other purine nucleotides.

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Figure 6.31 The formation of purines and nucleoside triphosphates needed for DNA and RNA synthesis.

1. AMP is deaminated to produce IMP.
2. IMP and GMP are dephosphorylated, generating inosine and guanosine, respectively.
3. A ribose is removed from the inosine and guanosine to form hypoxanthine and guanine, respectively.
4. Guanine is deaminated to form xanthine.
5. Hypoxanthine is converted to xanthine.
6. Xanthine is converted to uric acid, which is excreted in the urine.

Figure 6.32 The degradation of the purines AMP and GMP generates uric acid.

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Xanthine oxidoreductase, a molybdenum- and iron-dependent flavoenzyme, converts hypoxanthine (generated from AMP) to xanthine and also converts xanthine (made from both AMP and GMP) to uric acid. The oxidase form of the enzyme uses molecular oxygen and generates hydrogen peroxide, while the dehydrogenase form uses NAD^+ and forms $NADH + H^+$. The uric acid that is produced is normally excreted in the urine, although up to 200 mg also may be secreted into the digestive tract. In the disorder gout and in renal failure, uric acid accumulates in the body, causing painful joints among other problems. Allopurinol is one of several drugs used to treat gout; it works by binding to the enzyme to prevent its interaction with xanthine and hypoxanthine and thus diminish uric acid production. The oxidase (rather than the dehydrogenase) form of the enzyme predominates in several body tissues under conditions of oxygen deprivation (as with a heart attack). A problem in this situation is that when oxygen delivery relieves this deprivation, hydrogen peroxide and free radical production both increase and may further damage the injured tissues. Research involving introduction of enzymes and antioxidant nutrients to help minimize tissue damage with reoxygenation is ongoing.

INTERORGAN "FLOW" OF AMINO ACIDS AND ORGAN-SPECIFIC METABOLISM

While tissues and organs use amino acids to synthesize proteins and some nitrogen-containing compounds, the metabolism of the amino acids varies to some extent among the different organs. In many instances, the products generated from amino acid metabolism in one organ may be needed by another organ, creating a dependence between organs. This interdependence begins with the intestinal cells, which are the first cells of the body to receive dietary amino acids. The first part of this section covers amino acid metabolism by intestinal cells, followed by a discussion of amino acids in the plasma and then the specific roles that glutamine and alanine play among body tissues. Lastly, specific uses of amino acids by other selected tissues and organs such as skeletal muscle, the kidneys, and the brain are presented.

Intestinal Cell Amino Acid Metabolism

Intestinal cells use amino acids for energy production as well as for the synthesis of proteins and nitrogen-containing compounds. Some of the uses of amino acids in enterocytes include:

- structural proteins
- nucleotides
- apoproteins necessary for lipoprotein (chylomicron) formation
- new digestive enzymes
- hormones
- nitrogen-containing compounds.

Amino acids may be totally or partially metabolized within intestinal cells. It is estimated that the intestine (which represents about 3–6% of the body weight) uses 30–40% and splanchnic tissues use up to 50% of some of the essential amino acids absorbed from the diet [19]. Uses in the intestinal cells of individual amino acids vary. For example, the intestines appear to use up to about 90% of glutamate that is absorbed from the diet [19]. The next five subsections discuss the metabolism of glutamine, glutamate, aspartate, arginine, and methionine in intestinal cells. Figure 6.33 provides a partial overview of intestinal cell amino acid metabolism.

Intestinal Glutamine Metabolism

Glutamine serves several roles in the intestines. It is degraded extensively by intestinal cells, providing a primary source of energy. It has also been shown to have trophic (growth) effects, stimulating gastrointestinal mucosa cell proliferation. Consequently, glutamine helps to prevent both atrophy of gut mucosa and bacterial translocation. In addition, glutamine has been shown to enhance the synthesis of heat shock proteins. It is also needed in large quantities along with threonine for the synthesis of mucins found in gastrointestinal tract mucus secretions. These roles of glutamine in the gastrointestinal tract have prompted several companies to enrich enteral and parenteral (intravenous) nutrition products with glutamine. When glutamine is provided through tube feedings, over 50% of glutamine is extracted by the splanchnic (visceral) bed. It is estimated that the human gastrointestinal tract uses up to 10 g of glutamine per day, and that the cells of the immune system use over 10 g per day. In addition to dietary glutamine, much of the body's glutamine that is produced by the skeletal muscles (and to lesser extents by the lungs, brain, heart, and adipose tissue) is released and taken up, mostly by the intestinal cells.

Glutamine not used for energy production within the intestine also may be partially catabolized to generate ammonia and glutamate. The ammonia enters the portal blood for uptake by the liver or may be used within the intestinal cell for carbamoyl phosphate synthesis. The glutamate thus formed is discussed next.

Intestinal Glutamate Metabolism

In the intestinal cell, glutamate arises directly from the diet or from glutamine metabolism. It is often transaminated with pyruvate to form α-ketoglutarate and alanine (Figure 6.33); the alanine typically enters portal blood for transport to the liver. Glutamate not used for alanine synthesis is often

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Figure 6.33 A partial overview of amino acid metabolism in the intestinal cell.

used with glycine and cysteine to make glutathione, or it may be used to synthesize proline, as shown here:

The majority of proline synthesis is thought to occur through intestinal cell glutamate metabolism. Proline is then released into portal blood for delivery to the liver. Lastly, glutamate may be used along with aspartate to synthesize ornithine, which in turn may be released into portal blood or can be used to make citrulline (Figure 6.33). Thus, very little glutamate leaves the intestinal cell as glutamate and enters portal blood.

Intestinal Aspartate Metabolism

In addition to metabolism of glutamine and glutamate, metabolism of aspartate from the diet generally occurs within intestinal cells. Aspartate most often undergoes transamination to generate oxaloacetate; aspartate's amino group in turn is used to synthesize ornithine. Very little aspartate (like glutamate) leaves the intestinal cells as aspartate and is found in portal blood.

Intestinal Arginine Metabolism

Arginine is also used by intestinal cells. Up to 40% of dietary arginine is oxidized in enterocytes, yielding citrulline and urea [20]. Carbamoyl phosphate is synthesized in intestinal cells by the action of carbamoyl phosphate synthetase I using ammonia (NH_3), carbon dioxide (CO_2) or bicarbonate (HCO_3^-), and 2ATP, as shown in Figure 6.33 and here:

$$NH_3 + HCO_3^- + 2ATP \longrightarrow \text{Carbamoyl phosphate} + 2ADP + P_i$$

The carbamoyl phosphate in turn is used along with ornithine to synthesize citrulline in a reaction catalyzed by ornithine transcarbamoylase, as follows:

$$\text{Carbamoyl phosphate} + \text{Ornithine} \longrightarrow \text{Citrulline}$$

Citrulline that is made in the enterocytes is released into blood and then typically taken up, mostly by the kidneys, which use it for arginine synthesis (this pathway is referred to as the intestinal–renal axis of arginine synthesis and is not thought to be affected by dietary arginine ingestion). The liver may also take up the citrulline as needed for the urea cycle. Because of the role of the intestine in citrulline synthesis and the need for citrulline in arginine synthesis, arginine production can be impaired in individuals with intestinal injury. In such a situation, arginine becomes a conditionally essential amino acid and either arginine or citrulline must be supplemented in the diet.

Intestinal Methionine (and Cysteine) Metabolism

Methionine also is metabolized by intestinal cells. Studies suggest that up to 52% of methionine intake is metabolized in the gut [5]. Cysteine, generated from methionine or obtained directly from the diet, is used in the intestinal cells to make glutathione. Alternately, cysteine is metabolized

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primarily (70–90%) to taurine, and to a lesser extent (10–30%) to pyruvate and sulfite [5]. These reactions can be reviewed in Figure 6.12.

Amino Acids in the Plasma

After ingestion of a protein-containing meal, amino acid concentrations typically rise in the plasma for several hours, then return to basal concentrations. In basal situations and between meals, plasma amino acid concentrations are relatively stable and are species specific; however, absolute concentrations of specific amino acids in the plasma vary from person to person.

Amino acids circulating in the plasma and found within cells arise from digestion and absorption of dietary (exogenous) protein as well as from the breakdown of existing body (endogenous) tissues. These endogenous amino acids intermingle with exogenous amino acids to form a "pool" totaling about 150 g. The pool includes amino acids in the plasma as well as amino acids in the cytosol of body cells. Reuse of endogenous amino acids is thought to represent the primary source of amino acids for protein synthesis. Despite differences in protein intake and in degradation rates of tissue proteins, the pattern of the amino acids in the amino acid pool appears to remain relatively constant, although the pattern is quite different from that found in body proteins.

The total amount of the essential amino acids found in the pool is less than that of the nonessential amino acids. The essential amino acids found in greatest concentrations are lysine and threonine. Of the nonessential amino acids, those found in greatest concentrations are alanine, glutamate, aspartate, and glutamine. In fact, up to 80 g of glutamine can be found in the body's amino acid pool.

Amino acids within the pool, regardless of source, are taken up by tissues and metabolized in response to various stimuli such as hormones and physiological state. Tissues extract amino acids for energy production or for the synthesis of nonessential amino acids, protein, nitrogen-containing nonprotein compounds, biogenic amines, neurotransmitters, neuropeptides, hormones, glucose, fatty acids, or ketones, depending on the nutritional status and hormonal environment.

Glutamine and the Muscle, Intestine, Liver, and Kidneys

Glutamine has several major roles in the body, one of which is in ammonia transport. Whereas ammonia arising in the liver from amino acid reactions is typically shuttled into the urea cycle, this is not true in other tissues. In extrahepatic tissues, especially muscle but also the lungs, heart, brain, and adipose, glutamine synthetase catalyzes the utilization of ammonia or ammonium ions with glutamate in an ATP-dependent reaction to form glutamine. It is estimated that the body produces 40–80 g glutamine per day. Ammonia is typically generated in these cells by amino acid deamination and deamidation. In muscle it also forms from AMP deamination; AMP is generated in the muscle with ATP degradation as occurs rapidly with exercise. Glutamate is formed in muscle and other cells from the transamination of the branched-chain amino acids with α-ketoglutarate to form branched-chain α-keto acids and glutamate, respectively. As shown in Figure 6.34, ammonia generated from AMP deamination combines with the glutamate to produce glutamine.

The glutamine that is formed in the muscle is released into the blood and transported for use by other tissues. Whereas the cells of the gastrointestinal tract as well as the immune system (such as lymphocytes, monocytes, and macrophages) rely on glutamine catabolism for energy production, glutamine in the liver and kidneys is utilized differently. In the absorptive state (or with **alkalosis**), liver glutaminase activity increases, yielding ammonia for the urea cycle. In an acidotic state, the use of glutamine for the urea cycle diminishes, and the liver releases glutamine into the blood for transport to and uptake by the kidneys for use in acid-base balance. In the renal tubular cells, glutamine is catabolized by glutaminase to yield ammonia and glutamate. The glutamate may be further catabolized by glutamate dehydrogenase to yield α-ketoglutarate plus another ammonia. Ammonia reacts with H^+ to form an ammonium ion in the lumen of the kidney tubule; the ammonium ion is then excreted in the urine. Renal glutaminase activity and ammonia excretion increase with acidosis and decrease with alkalosis.

Glutamine use by cells increases dramatically with hypercatabolic conditions such as infection and trauma. In these conditions muscle glutamine release increases but cannot meet other cellular demands. Thus, glutamine "stores" can become depleted and some cell functions may become impaired. Remember, glutamine plays several roles that are especially critical with illness/injury. To briefly review, glutamine is used extensively by immune system cells. Glutamine promotes proliferation of these cells and glutamine metabolites are used directly by these cells, for example, for purine and pyrimidine synthesis. Purines and pyrimidines are required in large quantities by activated lymphocytes and macrophages. Expression of cell surface activation markers and production of cytokines such as interferon and tumor necrosis factor α by lymphocytes and lymphokine-activated killer cell activity also depend on glutamine. Furthermore, phagocytes require adequate glutamine availability. Glutamine also promotes the synthesis of heat shock/stress proteins, which help protect body cells. Glutamine prevents atrophy of the intestine, protects against intestinal bacterial translocation, and serves as the major substrate for energy production for intestinal cells. Finally, glutamine, along with alanine, uptake into cells

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promotes increases in cell volume with possible associated regulatory roles in intermediary metabolism. Glutamine supplementation, about 20–25 g/day, typically normalizes plasma glutamine concentrations and improves outcomes in critically ill patients. Administration of glutamine as a dipeptide (alanyl-glutamine or glycyl-glutamine) is needed in either an intravenous or enteral solution because the amino acid is not stable in aqueous solutions used in feeding. Dipeptidases on the surface epithelium of blood vessels are thought to hydrolyze the dipeptide so that the glutamine is available for use.

Alanine and the Liver and Muscle

In addition to glutamine, the amino acid alanine is also important in the intertissue (between tissues) transfer of amino groups generated from amino acid catabolism. As discussed in the previous section, transamination reactions in muscle generate glutamate, which is used, especially in a fed state/after eating, to synthesize glutamine for release into the blood. In several situations (between meals, with excessive glucose needs, with illness characterized by increased release of epinephrine and cortisol, or in situations such as fasting marked by low hepatic glycogen stores and a glucagon-to-insulin ratio favoring glucagon), glutamate typically transfers its amino group to pyruvate, generated from glucose oxidation via glycolysis, to form α-ketoglutarate and alanine, respectively. Once made, the alanine is released from the muscle into the blood for travel to the liver. Within the liver, alanine undergoes transamination back to pyruvate, which is then used to remake glucose. The glutamate that is generated with transamination can undergo deamination to provide ammonia for urea synthesis. These reactions are known

Figure 6.34 Some pathways of glutamine generation in muscle.

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1. Alanine is formed in muscle cells from transamination with glutamate (generated from leucine transamination) and from pyruvate (generated from glucose oxidation via glycolysis).
2. Alanine travels in the blood to the liver.
3. In the liver, alanine is transaminated with α-ketoglutarate to form pyruvate.
4. Pyruvate can be converted back to glucose in a series of reactions.
5. The glucose is released from the liver into the blood for uptake by tissues such as muscle, which use glucose for energy.
6. The glutamate formed in the liver can be deaminated to release ammonia; the ammonia is used in the liver for urea production.

Figure 6.35 The alanine–glucose cycle: alanine generation in muscle, and glucose generation in the liver.

as the glucose–alanine or alanine–glucose cycle and are shown in Figure 6.35. The glucose that is generated from the alanine is subsequently released into the blood, where it is available to be taken up and used by muscle. Muscle cells use the glucose through glycolysis and generate pyruvate. The formed pyruvate is again available for transamination to re-form alanine. This alanine–glucose cycle serves to transport nitrogen to the liver for conversion to urea while also allowing needed substrates to be regenerated.

Skeletal Muscle Use of Amino Acids

About 40% of the body's protein is found in muscle, and skeletal muscle mass represents about 43% of the body's mass. Uptake of amino acids by the skeletal muscles readily occurs following ingestion of food, especially a mixed meal rich in protein. Exercise further encourages amino acid uptake by muscles (see "Exercise and Nutrition" section in Chapter 7). After eating, skeletal muscles exhibit net protein synthesis (i.e., protein synthesis is greater than protein degradation).

In a postabsorptive state such as between meals or in a fasting situation, the reverse is true. Protein degradation predominates over synthesis, and amino acids may be released into the blood for use by other tissues. While alanine is released in the greatest concentration, other amino acids (including phenylalanine, methionine, lysine, arginine, histidine, tyrosine, proline, tryptophan, threonine, and glycine) are released in lesser quantities.

Muscle protein degradation is also associated with exercise. Cortisol, secreted by the adrenal glands, in response to exercise-induced stress, promotes, in part, this muscle and amino acid catabolism (see "Hormonal Regulation of Metabolism" and "Exercise and Nutrition" sections in Chapter 7).

Like other tissues, muscles preferentially catabolize some amino acids more than others; six amino acids (aspartate, asparagine, glutamate, leucine, isoleucine,

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and valine) appear to be catabolized to greater extents in the skeletal muscle than other tissues. This use of amino acids by muscle as well as leucine's role in promoting protein synthesis has prompted the consumption of branched-chain amino acid supplements by some athletes. The catabolism of the branched-chain amino acids (isoleucine, leucine, valine) is discussed in the following subsection and is shown in Figure 6.36.

Isoleucine, Leucine, and Valine Catabolism

Muscle, as well as the heart, kidneys, diaphragm, adipose tissue, and other organs (except, for the most part, the liver), possesses branched-chain aminotransferases, located in both the cytosol and mitochondria and responsible for the transamination of all three branched-chain amino acids. Following transamination, the α-keto acids of the branched-chain amino acids either remain within

*Branched-chain α-keto acid dehydrogenase (BCKAD), requiring thiamin as TDP/TPP, niacin as NADH, and Mg^{2+} and CoA from pantothenate.

**Common intermediate in the catabolism of methionine, threonine, isoleucine, and valine.

1. Defect in this enzyme complex causes maple syrup urine disease.
2. Defect in this enzyme results in propionic acidemia.
3. Defect in this enzyme results in methylmalonic acidemia.

Figure 6.36 Branched-chain amino acid metabolism.

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muscle or may be transported (bound to albumin) in the blood to other tissues (including the liver) for use.

Further catabolism of the branched-chain α-keto acids occurs by decarboxylation in an irreversible reaction catalyzed by the branched-chain α-keto acid dehydrogenase (BCKAD) complex. BCKAD is a large multienzyme complex made up of three subunits: E1α, E1β, and E2. This enzyme complex is found in the mitochondria of many tissues, including liver, muscle, heart, kidneys, intestine, and brain. It is highly regulated through phosphorylation (inactivation) and dephosphorylation (activation) mechanisms involving kinase and phosphatase proteins that act on the E1α subunit and act through end-product inhibition. This enzyme complex operates in a fashion similar to the pyruvate dehydrogenase complex (see Chapter 3) in that it requires thiamin in its coenzyme form TDP, niacin as NADH, and Mg^{2+} and CoA from pantothenic acid.

The details of the oxidation of the three branched-chain amino acids are shown in Figure 6.36. As with other amino acids, the complete oxidation of branched-chain amino acids yields products that are glucogenic and/or ketogenic. Valine oxidation yields succinyl-CoA, and is thus considered glucogenic. The end products of isoleucine catabolism are succinyl-CoA and acetyl-CoA, which are glucogenic and ketogenic, respectively. The complete oxidation of leucine results in acetyl-CoA and acetoacetate formation; acetoacetate may be further metabolized to form acetyl-CoA. Leucine is thus totally ketogenic.

Other common intermediates are formed during branched-chain amino acid oxidation. Isoleucine, for example, generates propionyl-CoA, which is a common intermediate in the degradative pathways of methionine and threonine. Valine catabolism generates methylmalonyl-CoA, a common intermediate in the degradative pathways of methionine, threonine, and isoleucine.

Leucine's metabolism also generates β-hydroxy β-methylbutyrate (HMB) (Figure 6.36). HMB is important for the production of β-hydroxy β-methylglutaryl (HMG)-CoA, a precursor for *de novo* cholesterol synthesis in the muscle. It appears that with some illnesses and with muscle damage, HMG-CoA concentrations may be inadequate to support cholesterol synthesis. Supplementation with HMB, usually as calcium HMB monohydrate (about 3 g per day given in three 1-g doses), provides cells with a source of HMG-CoA to maintain cholesterol synthesis and thus cell function. In addition, HMB appears to attenuate both muscle proteolysis and depression of muscle protein synthesis to improve muscle mass. Atrophy of muscle with muscle damage or secondary to conditions such as cancer, sepsis, and acquired immune deficiency syndrome (AIDS), among others, is due primarily to the activity of the ubiquitin-proteasome pathway (see the "Catabolism of Tissue Proteins" section); HMB appears to inhibit this pathway as well as to stimulate, along with leucine, protein synthesis through mTOR. HMB's effects have been demonstrated in healthy individuals as well as in those with conditions typically associated with muscle loss such as cancer and AIDS.

Leucine is one of the few amino acids that is completely oxidized in the muscle for energy. Leucine is oxidized in a manner similar to fatty acids, and its oxidation results in the production of 1 mol of acetyl-CoA and 1 mol of acetoacetate. Complete oxidation of leucine generates more ATP molecules on a molar basis than complete oxidation of glucose. Leucine appears to be preferentially oxidized during fasting situations. During fasting, leucine concentrations rise in the blood and muscle, and the capacity of the muscle to degrade leucine increases concurrently. This rise in capacity supplies the muscle with the equivalent of 3 mol of acetyl-CoA per molecule of leucine oxidized; the acetyl-CoA produces energy for the muscle while simultaneously inhibiting the oxidation of pyruvate, which is derived from glucose oxidation via glycolysis. Pyruvate is then transaminated to alanine and transported via the blood to the liver (see the previous section "Alanine and the Liver and Muscle").

Disorders of Isoleucine, Leucine, and Valine Metabolism

Maple syrup urine disease (MSUD) results from genetic mutations in BCKAD complex activity. The condition affects about 1 in 225,000 individuals worldwide, but in the Mennonite population in the United States it impacts about 1 in 150. MSUD, if untreated, results in an accumulation of the branched-chain amino acids and their alpha ketoacids in the blood and body fluids. The condition is characterized by acidosis, vomiting, lethargy, and frequently coma and death. High plasma leucine concentrations (versus high plasma isoleucine and valine) are more neurotoxic, and thus one aspect of management involves maintaining plasma concentrations of especially leucine but also isoleucine and valine in the normal range. A diet restricted in leucine, isoleucine, and valine intakes is required; large doses of thiamin are also tried to see if supplementation enhances residual BCKDC activity (remember thiamin is a coenzyme for the BCKDC).

Defects in some enzymes required for leucine degradation also have been documented (see Figure 6.36). Defects in isovaleryl-CoA dehydrogenase result in isovaleric acidemia. Although fairly rare, it is one of the more prevalent disorders of leucine metabolism, affecting about 1 in 250,000 worldwide but about 1 in 62,000 in Germany. Defects in β-methyl crotonyl-CoA carboxylase cause β-methyl crotonylglycinuria. Impaired activity of β-methylglutaconyl-CoA hydratase causes β-methyl-glutaconic aciduria and altered activity of β-hydroxyl β-methylglutaryl (HMG)-CoA lyase causes β-hydroxyl β-methylglutaric aciduria. Each of these disorders results in the production and accumulation of numerous acids and other compounds in body fluids,

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causing acidosis, dehydration, neurological problems, seizures, coma, and mental retardation, among other problems. A leucine-restricted diet is typically prescribed for these conditions. In some cases, to prevent the accumulation of toxic compounds, supplements of carnitine and glycine may be useful. Dietary fat restriction is also needed for those with HMG-CoA lyase deficiency.

Defective propionyl-CoA carboxylase and methylmalonyl-CoA mutase activities result in propionic acidemia and methylmalonic acidemia, respectively. These enzymes in addition to affecting valine and isoleucine oxidation are also common to methionine and threonine catabolism. Refer back to the section "Disorders of Methionine Metabolism."

Indicators of Muscle Mass and Muscle/Protein Catabolism

While muscle proteolysis generates amino acids that are released into the plasma for circulation to and use by other tissues, changes in plasma amino acid concentrations do not reflect changes in muscle mass. Instead, two previously mentioned compounds, creatinine and 3-methylhistidine, are used as indicators of existing muscle mass and muscle degradation, respectively. Urinary creatinine excretion is used to assess muscle mass because creatinine is the degradation product of creatine, which constitutes a fairly standard proportion of muscle (approximately 0.3–0.5% of muscle mass by weight). Urinary creatinine excretion reflects about 1.7% of the total creatine pool per day and is expressed per 24 hours, as a coefficient based on weight or height; however, because of variation in muscle creatine content, urinary creatinine is not always an accurate indicator of muscle mass.

The urinary excretion of 3-methylhistidine is used as an indicator of muscle catabolism (degradation). As mentioned under the section on histidine in "Hepatic Catabolism and Uses of Basic Amino Acids," the amino acid histidine is found in high concentrations as 3-methylhistidine in the muscle protein actin. Because 3-methylhistidine cannot be reused for protein synthesis following protein degradation and is excreted in the urine, its urinary excretion can be measured and serves as an indicator of muscle breakdown. A drawback to its use, though, is that actin is not found only in muscle but appears to occur in other body tissues, including the intestine and platelets, which have high turnover rates. Thus, urinary 3-methylhistidine excretion also may represent an index of protein breakdown for many nonmuscle tissues in the body.

Amino Acid Metabolism in the Kidneys

The kidneys preferentially take up and metabolize a number of amino acids and nitrogen-containing compounds (Figure 6.37). The kidneys' roles include:

- glutamine catabolism for acid-base balance
- glycine catabolism for acid-base balance
- serine synthesis from glycine
- arginine and glycine use to form guanidinoacetate for creatine synthesis
- glutathione catabolism
- arginine synthesis from citrulline
- tyrosine synthesis from phenylalanine
- histidine generation from carnosine degradation.

In fact, the kidneys are considered to be the major site in the body for arginine, histidine, serine, and perhaps tyrosine production [21].

Glutamine uptake by the kidneys has been estimated at 7–10 g per day [21] but uptake increases dramatically with acidosis, whereas glutamine uptake by the intestine, liver, and other organs diminishes. Especially in acidotic conditions, glutamine and then glutamate are deamidated and deaminated, respectively, in the kidneys, resulting in two ammonias. In the kidney's tubular lumen, the ammonias combine with H^+ ions and form ammonium ions, which are excreted in the urine. H^+ ions enter the tubular lumen in exchange for Na^+. In the lumen, the H^+ ions may also react with bicarbonate (HCO_3^-) to form water and carbon dioxide and with dibasic phosphate (HPO_4^{2-}) to form monobasic phosphate $(H_2PO_4^-)$. Glycine utilization by the kidneys under acidotic conditions is similar to glutamine utilization; glycine is degraded, forming ammonia and carbon dioxide. The ammonia then enters into the tubular lumen, where it reacts with H^+ ions, forming ammonium ions that are excreted in the urine. The loss of the H^+ from the body serves to increase blood pH from an acidotic state to a value ideally within the normal range of about 7.35–7.45.

Under healthy (nonacidotic) conditions, glycine is used by the kidneys (proximal tubule) for the synthesis of the amino acid serine. The kidneys also use glycine along with arginine for the synthesis of guanidinoacetate; this compound then travels to the liver, where it is used to generate creatine. The kidneys are thought to take up about 1.5 g of glycine per day [21]. Glycine, however, is also generated from glutathione catabolism in the proximal tubules of the kidneys.

Most arginine that is made in the body for tissue use is made in the kidneys from citrulline that was generated in the intestines and has been extracted from the blood; remember, the arginine made in the liver is immediately degraded to form urea and is thus not available to body tissues. It is estimated that the kidneys extract about 1.5 g of citrulline per day from the blood and release about 2–4 g of arginine daily [21].

Phenylalanine catabolism to tyrosine in the kidneys also has been demonstrated. It is estimated that the kidneys take up about 0.5–1 g of phenylalanine from the blood

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Figure 6.37 Amino acid metabolism in selected organs.

each day and releases about 1 g of tyrosine [21]. In addition to phenylalanine degradation, carnosine is oxidized by the kidneys, releasing histidine for use by other body tissues.

The kidneys also can generate glucose for the body. The kidneys, like the liver and to some extent like the small intestine, have the enzymes necessary for gluconeogenesis. See Chapter 3 for a detailed description of these reactions.

The role of the kidneys in nitrogen metabolism cannot be overemphasized. The organ is responsible for ridding the body of nitrogenous wastes that would otherwise accumulate in the blood plasma. Kidney glomeruli act as filters of blood plasma, and all the constituents in plasma, with the exception of plasma proteins, move into the filtrate. Essential nutrients such as sodium, amino acids, and glucose are actively reabsorbed as the filtrate moves through the tubules. Many other substances are not actively reabsorbed and must either move along an electrical gradient or move osmotically with water to enter the tubular cells. The amount of these substances that enters the tubular cells, then, depends on how much water moves into the cells and how permeable the cells are to the specific substances. The cell membranes are relatively impermeable to urea and uric acid, and are particularly impermeable to creatinine, little to none of which is typically reabsorbed.

Nitrogenous wastes found in the urine are listed in Table 6.7. About 80% of nitrogen is lost in the urine as urea under normal conditions. In acidotic conditions, urinary urea nitrogen losses decrease and urinary excretion of

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Table 6.7 Nitrogen-Containing Waste Products Excreted in the Urine

Compound	Approximate Amount Excreted/Day g/day	mmol/N
Urea	5–20	162–650
Creatinine	0.6–1.8	16–50
Uric acid	0.2–1.0	4–20
Ammonia	0.4–1.5	22–83

ammonium ions rises. In addition to urea and ammonia, usual nitrogenous wastes found in the urine include creatinine and uric acid, with lesser or trace amounts of creatine (<100 mg/day), protein (<100 mg/day), amino acids (<700 mg/day), and hippuric acid (<100 mg/day). Hippuric acid results from the conjugation of the amino acid glycine and benzoic acid, which is generated mostly in the liver from the catabolism of aromatic compounds. Because the benzoic acid is not water soluble, it must be conjugated for excretion. Trace amounts of other nitrogen-containing compounds such as porphobilinogen and metabolites of tryptophan also may be present in the urine. In addition to urinary nitrogen losses, nitrogen may be lost in the feces and sweat, and with the loss of hair and skin cells. These losses are referred to as insensible nitrogen losses.

Brain and Accessory Tissues and Amino Acids

The brain has a high capacity for the active transport of amino acids. In fact, the brain has transport systems for neutral, basic, and acidic amino acids. The transporters for some of the amino acids are almost fully saturated at normal plasma concentrations; this is especially true of the transporters for the large neutral amino acids like the branched-chain and the aromatic amino acids, which can compete with each other for the common carriers. The effects of this competition become especially apparent in conditions in which the blood concentrations of any of the branched-chain or aromatic amino acids become elevated. For example, in untreated PKU, elevations in blood phenylalanine result in increased uptake of this amino acid by the brain. In untreated MSUD, elevations in blood leucine, isoleucine, and valine result in the increased uptake of these amino acids (at the expense of the aromatic amino acids) into the brain. Moreover, in liver disease, the concentrations of the aromatic amino acids exceed those of the branched-chain amino acids and cause increased uptake of the aromatic amino acids by the brain. The elevations of amino acids in the brain alter brain function, causing a variety of neurologic problems such as impaired brain development and altered behavior and mental function, among other manifestations.

While it is clear that conditions like liver disease and inborn errors of amino acid metabolism can alter the brain's uptake of selected amino acids and cause neurological and behavioral changes, such effects have not been demonstrated consistently in healthy individuals who attempt to alter behavior by altering dietary intakes of nutrients. For example, ingestion of carbohydrate (without ingestion of protein) has been shown to increase the brain's uptake of tryptophan and raise serotonin concentrations but does not always result in the expected behavioral effects (such as feeling calm and relaxed) of elevated serotonin. In other words, varying nutrient intakes, including carbohydrate and protein, by eating selected foods is thought to have little effect on the brain's serotonergic function.

Neurotransmitters and Biogenic Amines

Neurotransmitters are compounds generated in the body that transmit signals from a neuron to a target cell across a synapse. Neurotransmitters are stored or packaged in the nerve axon terminal as vesicles or granules until stimuli arrive to effect their release into the synaptic cleft and allow for their binding to receptors on the postsynaptic side of the synapse. Neurotransmitter action on the cell membranes typically elicits an action or electrical potential. Several amino acids act directly as neurotransmitters, including:

- glycine, which acts primarily in the spinal cord as an inhibitory neurotransmitter
- taurine, which is thought to function as an inhibitory neurotransmitter
- aspartate, which is derived chiefly from glutamate through aspartate aminotransferase activity common in neural tissue, and is thought to act as an excitatory neurotransmitter in the central nervous system
- glutamate, which acts primarily in the brain and spinal cord as an excitatory neurotransmitter. Glutamate also can be decarboxylated in a vitamin B_6-(PLP)–dependent reaction to produce the neurotransmitter γ-amino butyric acid (GABA) (Figures 6.37 and 6.38). GABA functions in the brain as an inhibitory neurotransmitter.

Amino acids that are excitatory stimulate receptors on postsynaptic membranes; this stimulation in turn propagates the nerve impulse. In contrast, amino acids that are inhibitory retard the postsynaptic neuron from propagating nerve impulses.

Figure 6.38 GABA synthesis from the amino acid glutamate.

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Figure 6.39 Serotonin synthesis (from tryptophan) and degradation.

Many amino acids are catabolized within the brain and nervous system to generate biogenic amines. These biogenic amines also may function as neurotransmitters. Some of these amino acids and the amines they produce include:

- Tryptophan, which is used to synthesize serotonin (Figures 6.37 and 6.39). Serotonin functions as an excitatory neurotransmitter (biogenic amine) in the central nervous system and in circulation as a potent vasoconstrictor and stimulator of smooth muscle contraction. Serotonin affects sleep, mood, and appetite, as well as memory and learning (e.g., cognitive functions).
- Tyrosine, which is used in sympathetic neurons to make catechol derivatives, collectively called catecholamines (dopamine, norepinephrine, and epinephrine; Figures 6.37 and 6.40). In the brain and neurons, the catecholamines function as neurotransmitters. Dopamine affects a variety of behaviors as well as coordination of movement. Norepinephrine plays roles in alertness and sleep. Epinephrine is found in low concentrations in the brain; however, in circulation, it functions as a hormone with major (primarily catabolic) effects on nutrient metabolism.
- Histidine, which is decarboxylated to generate histamine (shown previously in Figure 6.14). The neurotransmitter histamine mediates attention and alertness, among other possible roles.

Once neurotransmitters and biogenic amines have exerted their actions, the fastest mechanism for their inactivation is uptake by adjacent cells or synaptic terminals. Enzymes responsible for catecholamines and serotonin degradation include monoamine oxidase and aldehyde dehydrogenase; catechol-O-methyltransferase (which is found in the liver, kidneys, and smooth muscle but not the neurons) also can methylate the catecholamines to effect their slower degradation after transport via the blood. The well-known interaction between medications known as monoamine oxidase inhibitors and foods high in amines such as tyramine is discussed in the Chapter 13 Perspective on nutrient–drug interactions. Other neurotransmitters are degraded by other pathways; histamine, for example, is catabolized by diamine oxidase.

Neuropeptides

Neuropeptides (also referred to as neuroactive peptides) are small protein-like compounds that are similar to neurotransmitters but have more diverse effects. They are derived from amino acids but are not necessarily biogenic amines. The central nervous system abounds in neuropeptides;

Figure 6.40 The structures of the catecholamines. The synthesis of these compounds is shown in Figure 6.10.

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in fact, many of the same peptides that were discussed in Chapter 2 in association with the intestinal tract are also found associated with the central nervous system.

Neuropeptides perform a variety of functions. Some peptides act as hormone-releasing factors; ACTH, for instance, is involved with cortisol release. Some, such as somatotropin or growth hormone, have endocrine effects. Others, such as the enkephalins, have modulatory actions on transmitter functions, mood, or behavior. The enkephalins and endorphins, though similar to natural opiates, possess a wide range of functions, including affecting pain sensation, blood pressure, body temperature, body movement, hormone secretion, feeding, and modulation of learning ability. Some additional examples of neuropeptides include alpha melanocyte-stimulating hormone, neuropeptide Y, agouti-related peptide, ghrelin, and neurotensin, to name a few.

The neurosecretory cells of the hypothalamus are foremost in the secretion of the neuropeptides. Those that have hormone action move out of the axons of the nerve cells into the pituitary, from which they are secreted. This linkage between the nervous system and the pituitary is of great significance in the overall control of metabolism because the pituitary gland is primary in coordinating the various endocrine glands scattered throughout the body.

Neuropeptides are expressed and released by neurons. Because the nucleus and ribosomes in neurons are found in the cell body and dendrites, the neuropeptides, once made, must travel to the end of the axon to be stored in vesicles for future release. The neuropeptides are typically stored as inactive precursor polypeptides, which must be cleaved to generate an active neuropeptide, as shown here:

Following synthesis of the active neuropeptide, it is released by exocytosis to perform its function at the membrane. After performing its function, the neuropeptide is hydrolyzed to its constituent amino acids.

Other Metabolic Roles of Amino Acids

While amino acids play important roles as neuropeptides, biogenic amines, and neurotransmitters in the brain and nervous system, they also serve other important functions. Glutamate is of significance as a means of ridding the brain of ammonia. Little glutamate is transported into the brain from the blood; rather, glucose that has been transported into the brain is metabolized to α-ketoglutarate, which can be converted to glutamate through reductive amination. Whenever excessive ammonia is present in the brain, glutamine is formed through the action of glutamine synthetase, which is highly active in neural tissues. A glutamate–glutamine cycle in the brain is thought to function as follows. Neurons take up glutamine and convert it to glutamate using glutaminase. The glutamate is released into the synapse (extracellular fluid) and then is taken up by astrocytes. Astrocytes convert the glutamate back to glutamine. The glutamine is then released. It can be reused by the neuron, but it is also freely diffusible and can move easily into the blood or cerebrospinal fluid, thereby allowing the removal of 2 mol of toxic ammonia from the brain. Any condition that causes an unusual elevation of blood ammonia (such as hepatic encephalopathy associated with advanced liver disease) can interfere with normal brain function, and treatment usually employs several strategies to reduce the blood ammonia concentration.

Leucine, as well as the other branched-chain amino acids, also provides the brain with nitrogen (amino groups). Astrocytes are thought to initially take up leucine and, using aminotransferases, remove its amino group for synthesis of glutamate and glutamine. Leucine's α-keto acid (α-ketoisocaproate), which is synthesized when the amino group is removed, is then taken up by neurons and reaminated to leucine using glutamate. Leucine and the other branched-chain amino acids are thought to provide 30–50% of the amino groups used by the brain for glutamate synthesis [22].

CATABOLISM OF TISSUE PROTEINS AND PROTEIN TURNOVER

Protein synthesis and protein degradation (i.e., protein turnover) are under independent controls but together account for about 10–25% of resting energy expenditure [23]. Rates of synthesis can be high, as with protein accretion during growth. Alternately, protein degradation can predominate, as during illness/injury. Rates of protein turnover also vary among the body tissues, as is evidenced in the more rapid turnover of visceral protein as compared with skeletal muscle. Yet, because of its mass, muscle accounts for about 25–35% of all protein turnover in the body. Total body protein turnover represents about 1–2% of body protein each day, and may total over 300 g.

The degradation of proteins yields amino acids that are mostly reused by body tissues. Proteins are degraded within cells primarily by the action of proteases, which are compartmentalized in the cytosol in lysosomes and proteasomes. The contributions of the lysosomes and proteasomes in proteolysis vary depending on the tissue and the physiological status. Nonetheless, the constant degradation of proteins is of prime importance because it ensures a flux of amino acids through the cytosol that can be used for cellular growth and/or maintenance.

Amino acid concentrations within cells serve as sensors and modulate intracellular signaling pathways. While mTOR integrates signals from insulin and leucine,

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among other molecules, to enhance protein synthesis, inhibition of mTORC1 along with the presence of other compounds/events stimulates protein degradation. While the mechanisms are unclear, cellular protein degradation increases when cellular quantities of amino acids are limited or imbalanced. These situations alter the binding of transcription factors to promoter regions on selected genes and inhibit the production of proteins involved in protein synthesis. Insufficient amino acid concentrations also trigger (through unclear means) **autophagy**, in which large sections of the cell's cytosol, including organelles and other constituents, are engulfed into vesicles and then degraded by lysosomes. The next section provides information on the two main pathways responsible for protein degradation within cells as well as additional information on factors that influence protein degradation.

Lysosomal Degradation (also called the Autophagic Lysosome Pathway)

Lysosomes are cell organelles (about 0.2–0.5 m in diameter) that act like a cellular garbage digestive system to degrade proteins, nucleic acids, lipids, and carbohydrates, among other compounds. Lysosomes are found in all mammalian cells, with the exception of red blood cells, but in varying numbers. For example, skeletal muscles contain few lysosomes, whereas hepatocytes are particularly rich in lysosomes.

Lysosomes acquire proteins (and other substances) for degradation from extracellular sites, from the cell membrane, and from intracellular sites. Endocytosis is responsible for the internalization of most extracellular proteins as well as integral membrane proteins. The endocytosed proteins are sequestered in endosomes, which then fuse with lysosomes. The lysosomes contain a variety of digestive enzymes, including protein-digesting endopeptidases and exopeptidases known as cathepsins. Examples of **cathepsins** (designated by letters), which vary in specificity, include B, H, and L (cysteine proteases) and cathepsin D (an aspartate protease). These lysosomal enzymes are active only at an acidic pH, which is achieved by a proton pump in the lysosomal membrane. This pump actively transports protons (H^+) from the cytosol into the lysosome to lower the lysosomal pH. Once activated, the proteases and other enzymes digest cellular proteins and components. No energy is needed for lysosomal digestion. The amino acids released from lysosomal proteolysis can be reused by the cell for protein synthesis or degraded based on cellular needs.

Autophagy ("self-eating") also occurs within cells and involves the degradation of cytosolic cell constituents by the lysosomal pathway. There are three main types: macroautophagy, microautophagy, and chaperone-mediated autophagy. The most studied is macroautophagy, which involves sequestration of portions of the cytosol into vesicles called autophagosomes and subsequent fusion of the autophagosomes with lysosomes. Following acidification of the fused autophagosome-lysosome environment by proton pumps, proteins are digested by the lysosomal proteases.

Autophagy is thought to be responsible for the degradation of longer-lived intracellular proteins as well as cellular organelles and macromolecules. Autophagy is induced under some pathological conditions and under conditions of nutrient deprivation. For example, autophagy in liver cells is enhanced by glucagon and suppressed by insulin, amino acids, and mTORC1; mTORC1 suppresses autophagy through phosphorylation of specific proteins. The high glucagon-to-insulin ratio is consistent with conditions in which cells are nutritionally (amino acid) deprived. In addition, in liver cells, cellular uptake and accumulation of three amino acids—leucine, phenylalanine, and tyrosine—as well as increases in cell volume associated with the sodium-dependent cellular uptake of alanine, glutamine, and proline inhibit autophagy. The exact mechanisms by which this inhibition occurs are not known.

Proteasomal Degradation (also called the Ubiquitin Proteasomal Pathway)

In addition to lysosome-mediated cellular protein degradation, proteasomes (protease systems or complexes) also degrade proteins. Proteasomes are large, oligomeric structures with a central cavity where degradation occurs. They are found in both the cytosol and nucleus of cells in two forms: 20S and 26S. The 20S proteasome consists of several subunits, of which three are responsible for protein degradation, specifically cleaving peptide bonds on the carboxy sides of acidic, basic, and hydrophobic amino acids. The 20S proteasome can be found bound by a 19S regulatory complex or cap to form a 26S proteasome, the form responsible for energy- and ubiquitination-dependent roles.

Ubiquitination is an ATP-dependent process by which proteins that are to be degraded are ligated to **ubiquitin**, a 76-amino acid polypeptide (Figure 6.41). The attachment of ubiquitin marks a protein for degradation; however, before the ubiquitin can be linked to a protein, it must first be activated by the enzyme E1. This activated ubiquitin is transferred to E2, ubiquitin-conjugating enzymes. Next, the carboxy end of ubiquitin is ligated by E3 to the protein substrate that is ultimately to be degraded. E3 has two distinct sites that interact with a targeted protein's N-terminal amino acid. Proteins with N-terminal basic or large hydrophobic amino acids such as lysine, arginine,

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1 The attachment of activated ubiquitin by E1, a subunit of the ubiquitin enzyme system, which hydrolyzes ATP to form a thiol ester with the carboxy end of ubiquitin. This activated ubiquitin is transferred to another enzyme protein, E2, referred to as ubiquitin-conjugating enzymes.

2 The carboxy end of ubiquitin is ligated by E3 to the protein substrate that is ultimately to be degraded. E3 has two distinct sites that interact with a targeted protein's N-terminal amino acid.

Figure 6.41 Proteasomal degradation of a protein.
Source: Adapted from "The ubiquitin-proteasome proteolysis pathway: potential for target of disease intervention" by Breen, H.B. and Espat, N.J., Journal of Parenteral and Enteral Nutrition 2004; 28:272–77. Copyright © 2004 by Sage Publications. Reprinted by permission of SAGE Publications.

histidine, leucine, isoleucine, asparagine, glutamine, tryptophan, phenylalanine, and tyrosine are typically susceptible to degradation by the proteasomal system. One or more (typically five) ubiquitin proteins bind to a protein substrate; at least four ubiquitin proteins appear to be needed to ensure recognition and degradation by the 26S proteasome. ATP is required to unfold the tertiary and secondary structures of the proteins. Once ubiquitins are ligated to the protein to be degraded and the protein structure permits, proteases present as part of the proteasome degrade the ubiquitinated proteins in a series of reactions. Following proteolysis, ubiquitin is released for reuse, and the amino acids from the degraded protein can be reused.

The proteasomal system accounts for the majority of proteolysis in skeletal muscle. However, in muscle and perhaps other tissues (including neurons and the brain), another group of proteases, called **calpains**, also may initially participate. Calpains are calcium-activated proteases and are designated as 1, micro- or μ-calpain, and 2, milli- or m-calpain, with the nomenclature indicative of the different levels of calcium needed for activation. In muscle the calpain proteases appear to work in sequence with the proteasomal system, whereby the calpain proteases initiate the degradation of damaged/oxidized myofibrillar proteins. The released myofilaments are then ligated to ubiquitin for further degradation.

Proteasomal degradation is thought to be responsible for the degradation of abnormal, damaged, denatured, or mislocated proteins and of regulatory proteins that typically have short half-lives (often less than 30 minutes). Some peptides generated from proteasomal digestion of abnormal, mutant, or foreign proteins have been shown to bind to components of the body's immune system for antigen presentation; this process helps the body to further mount an immune response, enabling swift destruction of bacteria and viruses that have entered the body. Proteasomal degradation also has been shown to increase during starvation as well as in pathological conditions such as sepsis, cancer, and trauma. Cytokines are thought to be involved (in part) with its activation. A metabolite of leucine, β-hydroxy β-methylbutyrate (HMB), however, appears to be able to attenuate protein degradation by the proteasomal pathway, possibly by phosphorylating (inactivating) kinases that are involved in the expression of the proteasome. Regulation of this system is not completely understood.

CHANGES IN BODY MASS WITH AGE

The reference numbers shown in Table 6.8, first developed in the 1970s, provide information on body composition, including muscle mass, based on average physical dimensions from measurements of thousands of people who participated in various surveys. These numbers are a frame of reference with which to examine gender differences in body composition; they are not

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Table 6.8 Body Composition of Reference Man and Woman

Reference Man	Reference Woman
Age: 20–24 yr	Age: 20–24 yr
Height: 68.5 in (174 cm)	Height: 64.5 in (164 cm)
Weight: 154 lb (70 kg)	Weight: 125 lb (56.8 kg)
Total fat: 23.1 lb (10.5 kg) (15.0% body weight)	Total fat: 33.8 lb (15.4 kg) (27.0% body weight)
Storage fat: 18.5 lb (8.4 kg) (12.0% body weight)	Storage fat: 18.8 lb (8.5 kg) (15.0% body weight)
Essential fat: 4.6 lb (2.1 kg) (3.0% body weight)	Essential fat: 15.0 lb (6.8 kg) (12.0% body weight)
Muscle: 69 lb (31.4 kg) (44.7% body weight)	Muscle: 45 lb (20.5 kg) (36.0% body weight)
Bone: 23 lb (10.4 kg) (14.9% body weight)	Bone: 15 lb (6.8 kg) (12.0% body weight)
Remainder: 38.9 lb (17.7 kg) (25. 3% body weight)	Remainder: 31.2 lb (14.2 kg) (25.0% body weight)

Sources: Adapted from Behnke A.R., Wilmore J.H., *Evaluation and Regulation of Body Build and Composition*. Englewood Cliffs, NJ: Prentice Hall, 1974; and Katch F.I., McArdle W.D., *Introduction to Nutrition, Exercise, and Health*, 4th ed., Philadelphia: Lea & Febiger, 1993, p. 235.

representative of an "ideal" body composition. As seen in Table 6.8, the reference man weighs 29.26 lb (13.3 kg) more and is 4 inches (~10 cm) taller than the reference woman (nonpregnant). Muscle accounts for 44.7% and body fat 15% (with 3% essential fat) of body weight in the male, whereas muscle is only 36.0% and body fat is 27% (12% essential fat) of body weight in the female. Essential fat is fat associated with bone marrow, the central nervous system, viscera (internal organs), and cell membranes, and in females, essential fat also includes fat in mammary glands and the pelvic region.

The difference in body composition is influenced not only by gender but also by other factors, including age, race, heredity, and stature. The influence of gender on body composition appears to exist from birth but becomes dramatically evident at puberty and continues throughout life. In both sexes, serum testosterone levels rise during adolescence; however, the increase is much greater in boys, whose testosterone values approach 10 times those of girls. As a result of this higher testosterone production and a growth spurt of longer duration, boys gain considerably more lean body mass than girls. Increased estrogen and progesterone concentrations and a shorter growth spurt duration contribute to greater gains in fat mass in females versus males during adolescence. The female achieves maximum lean body mass by about age 18 years, whereas the male continues accretion of lean body mass until about age 20 years. Such differences in lean body mass are largely responsible for the gender difference in nutrient requirements.

After 25 years of age, weight gain usually results from body fat accretion. As an example, healthy young (about age 25 years) men may average 20% body fat, while 55-year-old healthy men more likely average 30% body fat, and those who are 75 years old average 35% body fat. More marked increases occur in females than in males. In addition to the fat gains, lean body mass decreases with aging. Adults who maintain their weight may still gain fat and lose muscle. The decline in muscle mass occurs predominantly after age 50 years at a rate of about 1–2% per year. Skeletal muscle loss may be (in part) due to decreased physical activity and altered protein metabolism; the latter is thought to result from diminished anabolic hormone concentrations. A further effect of the decreased muscle mass with aging is a decrease in total body water, which is greater in females than in males. More specifically, extracellular fluid volume remains virtually unchanged, but interstitial fluid decreases and plasma volume increases. Atrophy of organs as well as loss of bone mass also occurs with aging. The loss of bone mass is discussed in the Perspective at the end of Chapter 11.

Sarcopenia (*sarx* referring to "flesh" in Greek and *penia* meaning "loss" or "low") is an age-associated condition in which there is a progressive, gradual loss of muscle mass and consequently decreased function and strength; it typically begins after the age of about 50 years. Specifically, skeletal muscle fiber numbers are diminished and the cross-sectional area of the remaining muscle fibers is reduced. Impaired muscle function results in frailty, diminished physical abilities (even for activities of daily living), and increased risk of falls. These changes in physical function are not explained solely based on the loss of muscle mass. The reductions in muscle mass (along with organ mass that occurs with aging), however, contribute to further changes such as decreases in metabolic rate.

Causes of sarcopenia are not completely understood but are likely multifactoral. Age-related loss of alpha motor neuron input to muscle is thought to be one major cause of the condition; this innervation is vital to muscle mass maintenance and strength. Age-associated oxidative damage in muscle also contributes, as it results in atrophy and loss of muscle fibers and muscle function. Moreover, with aging, the oxidized proteins generated in muscle may not be completely removed; this accumulation of "oxidized debris" diminishes muscle function and strength. Another cause of sarcopenia is likely the diminished concentrations of estrogen and testosterone, which have anabolic effects on muscle. Chronic low-grade inflammation associated with increased production of inflammatory cytokines (e.g., interleukins [IL] 1 and 6, tumor necrosis factor α) causes further catabolic effects. Altered insulin and growth hormone concentrations, which influence protein synthesis and degradation, also play roles in sarcopenia, as do reduced protein and nutrient intakes and physical inactivity that can occur with aging. Anabolic resistance may occur secondary to reduced metabolic responses

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to insulin, amino acids, and/or resistance exercise [10]. While prevention of sarcopenia may not be possible, the effects are thought to be reduced with consumption of adequate energy and protein (perhaps distributed evenly among meals and supplemented with essential amino acids) in combination with routine (about two to three times per week) resistance (strength) training [10,11,24].

PROTEIN QUALITY AND PROTEIN AND AMINO ACID NEEDS

The quality of a protein depends to some extent upon its digestibility but primarily on its indispensable amino acid composition—both the specific amounts and the proportions of these amino acids. Protein-containing foods can be divided into two categories:

- high-quality or complete proteins
- low-quality or incomplete proteins.

A **complete protein** contains all the indispensable amino acids in the approximate amounts needed by humans. Sources of complete proteins are mostly foods of animal origin such as milk, yogurt, cheese, eggs, meat, fish, and poultry. An exception is gelatin, which is of animal origin but lacks the indispensable amino acid tryptophan.

Incomplete proteins, or low-quality proteins, are derived from plant foods such as legumes, nuts, seeds, vegetables, and cereals/grains. Most plant foods tend to have too little of one or more indispensable amino acids. The exceptions are soy protein, quinoa, and chia seeds, which are of plant origin but are complete proteins. Vegetables may be low in methionine, and nuts and seeds may be low in lysine and threonine. The term ***limiting amino acid*** is used to describe the indispensable amino acid that is present in the lowest quantity in the food. Listed in Table 6.9 are examples of incomplete protein–containing foods and their limiting amino acid(s). Unless carefully planned, a diet containing only low-quality proteins may result in inadequate availability of selected indispensable amino acids and may reduce the body's ability to synthesize its own body proteins. The body cannot make a protein if an amino acid is missing.

Table 6.9 Incomplete Protein–Containing Foods and Their Limiting Amino Acids

Food Source of Incomplete Protein	Possible Limiting Amino Acid(s)
Wheat, rice, corn, other grains	Lysine, threonine (sometimes), and tryptophan (sometimes)
Vegetables	Methionine
Legumes, peas, lentils	Methionine (sulfur-containing amino acids)
Nuts and seeds	Lysine and threonine

To ensure that the body receives all the indispensable amino acids, certain protein-containing foods can be ingested together or combined so that their amino acid patterns become complementary. This practice or strategy is called mutual supplementation. For example, legumes, with their high content of lysine but low content of sulfur-containing amino acids, complement the grains, which are more than adequate in methionine and cysteine but limited in lysine. The lacto-ova vegetarian should have no problem with protein adequacy because when milk and eggs are combined—even in small amounts—with plant foods, the indispensable amino acids are supplied in adequate amounts. One exception is the combination of milk with legumes. Although milk contains more methionine and cysteine per gram of protein than do the legumes, it still fails to meet the standard of the ideal pattern for the sulfur-containing amino acids. Most, but not all, agree that the complementary proteins should be consumed at the same meal.

The digestibility of proteins is also important for amino acid use. The digestibility of a protein is a measure of the amounts of amino acids that are absorbed following ingestion of the given protein. Animal proteins have been found to be about 90–99% digestible, whereas plant proteins are about 70–90% digestible. Meat and cheese, for example, have a digestibility of 95%, and eggs are 97% digestible. Cooked split peas are about 70% digestible, and tofu is about 90% digestible. Both the digestibility of a protein and its amino acid content affect protein quality.

Evaluation of Protein Quality

Several methods are available to determine the protein quality of foods. A few of these methods are discussed in this section.

Protein Digestibility Corrected Amino Acid Score

The protein digestibility corrected amino acid score (PDCAAS) is a commonly used indicator of protein quality. In fact, foods intended for individuals over 1 year of age or with health claims must use the PDCAAS method to provide information on the product's food label. This method involves comparing the amount of the limiting amino acid for a test protein to the amount of the same amino acid in 1 g of a reference protein (usually egg or milk). This value is then multiplied by the test protein's digestibility, with the final PDCAA value truncated to 1.0 (or 100%). The formula is shown below.

$$\text{PDCAAS (\%)} = \frac{\text{Amount (mg) of limiting amino acid in 1 g of test protein}}{\text{Amount (mg) of same amino acid in 1 g of reference protein}} \times \text{True digestibility (\%)}$$

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The digestibility of the protein is determined by examining fecal nitrogen. This fecal measurement has been criticized because it includes not only nitrogen from the test protein but also from endogenous proteins such as from digestive secretions and cells. Moreover, bacterial fermentation of some proteins in the colon also overestimates digestibility and suggests a greater delivery of dietary amino acids to the body. Examples of foods with a PDCAAS of 100 include milk protein (casein), egg white, ground beef, and tuna, along with some other animal products. Soybean protein has a PDCAAS of 94, grain scores range from about 40 to 56, and the scores for various lentils, peas, and legumes range from <50 to about 70.

An alternate approach to this method involves a comparison of the amino acid composition of a test protein with an amino acid reference *pattern* (as opposed to reference protein). The reference pattern that has been selected for use for all people (except infants) is the amino acid requirements of preschool children age 1–3 years. The requirements of preschool children, which include needs for growth and development, are higher for each amino acid than are those of adults (who are not undergoing growth and development) and thus are considered to meet or exceed the needs of older people. The scoring pattern, expressed as (mg amino acid)/(g protein), is calculated by dividing the requirements of individual indispensable amino acids (in mg) for children by the protein requirements (in g). The scoring pattern for foods intended for children age 1 year or older and for older age groups is shown in Table 6.10, along with the recommended amino acid scoring pattern for infant formulas and foods, which is based on the amino acid composition of human milk [25]. Table 6.10 also shows the whole-egg pattern.

Digestible Indispensable Amino Acid Score

The digestible indispensable amino acid score (DIAAS) represents a newer method to assess protein quality. DIAAS considers the amount, profile, and digestibility of the indispensable amino acids in protein-containing foods. Specifically, as shown in the formula below, it compares the amount of a digestible indispensable amino acid in 1 g of a dietary test protein to the amount of the same indispensable amino acid in 1 g of the reference protein.

$$\text{DIAAS} = \frac{\text{mg of indispensable amino acid in 1g of a dietary test protein} \times \text{amino acid digestibility}}{\text{mg of the same indispensable amino acid in 1g of the reference protein}} \times 100$$

For regulatory purposes, two scoring patterns have been recommended as the reference protein: for infants the amino acid composition of human milk and for other age groups the pattern for young children age 6 months to 3 years (see Table 6.11). The ratio is best calculated for each indispensable amino acid, with the lowest value used as an indicator of the protein's dietary quality.

Unlike the PDCAAS, this method assesses the digestibility of the amino acids in the ileum versus the colon. Given amino acids are primarily absorbed in the upper small intestine and not in the colon, the measurement of ileal digestibility more accurately predicts utilization by humans and eliminates the impact of protein utilization by colonic microbiota. The DIAAS also allows for a ranking of different protein sources based on their ability to meet indispensable amino acid requirements. Since it does not truncate scores for individual foods as does the PDCAAS method, the technique allows distinction among protein sources that the PDCAAS method identified as having the same values. For example, many dairy protein sources and dairy components used in nutritional products (such as whey protein isolate) have scores greater than 100%. Scores that are over 100% mean that the protein source is high quality and that the protein is relatively high in a particular indispensable amino acid; this "extra" amino acid content could then complement another protein source that is lower in that particular indispensable amino acid.

Table 6.10 Amino Acid Scoring/Reference Patterns and Whole-Egg Pattern [20]

Amino Acid	Infants (mg/g protein)	Children and Adults (mg/g protein)	Whole Egg (mg/g protein)
Histidine	23	18	22
Isoleucine	57	25	54
Leucine	101	55	86
Lysine	69	51	70
Methionine + cysteine	38	25	57
Phenylalanine + tyrosine	87	47	93
Threonine	47	27	47
Tryptophan	18	7	17
Valine	56	32	66

Table 6.11 Amino Acid Scoring/Reference Patterns Used for Regulatory Purposes*

Amino Acid	Infants (mg/g protein human milk)	Children and Adults (mg/g protein)
Histidine	21	20
Isoleucine	55	32
Leucine	96	66
Lysine	69	57
Methionine + cysteine	33	27
Phenylalanine + tyrosine	94	52
Threonine	44	31
Tryptophan	17	8.5
Valine	55	43

* Report of an FAO Expert Consultation. Diet protein quality evaluation in human nutrition. FAO Food and Nutrition paper #92. 2011. Available at http://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf

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Protein Efficiency Ratio

The protein efficiency ratio (PER) represents body weight gained on a test protein divided by the grams of protein consumed. This method of assessing protein quality is used by food manufacturers for infant formulas and baby foods and is reported on the product's food label. To calculate the PER of proteins, young growing animals are typically placed on a standard diet with about 10% (by weight) of the diet as test protein. Weight gain is measured for a specific time period and compared to the amount of the protein consumed. The PER for the protein is then calculated using the following formula:

$$\text{PER} = \frac{\text{Gain in body weight (g)}}{\text{Grams of protein consumed}}$$

To illustrate, the PER for casein (a protein found in milk) is 2.5; thus, rats gain 2.5 g of weight for every 1 g of casein consumed. However, a food with a PER of 5 does not have double the protein quality of casein, with a PER of 2.5. Furthermore, although the PER allows determination of which proteins promote weight gain (per gram of protein ingested) in growing animals, no distinction is made regarding the composition (fat or muscle/organ) of the weight gain.

In addition to protein digestibility corrected amino acid score and protein efficiency ratio, which are used for nutrition labeling purposes, other methods—chemical or amino acid score, biological value, and net protein utilization—may be used to determine protein quality. A discussion of these methods follows.

Chemical or Amino Acid Score

The chemical score (also called the amino acid score) involves determination of the amino acid composition of a test protein. This procedure is done in a chemical laboratory using either an amino acid analyzer or high-performance liquid chromatography techniques. Only the indispensable amino acid content of the test protein is determined. The value is then compared with that of the reference protein, for example, the amino acid pattern of egg protein (considered to have a score of 100). The amino acid/chemical score of a food protein can be calculated as follows:

$$\text{Score of test protein} = \frac{\text{Indispensable amino acid in food protein (mg/g protein)}}{\text{Content of same amino acid in reference protein (mg/g protein)}}$$

The amino acid with the lowest score on a percentage basis in relation to the reference protein (egg) becomes the first limiting amino acid, the one with the next lowest score is the second limiting amino acid, and so on. For example, if after testing all amino acids, lysine were found to be present in the lowest amount relative to the reference protein (e.g., 85%), the test protein's chemical score would be 85. The amino acid present in the lowest amount is the limiting amino acid and determines the amino acid or chemical score for the protein. Table 6.10 gives the amino acid pattern in whole egg. Comparison of the quality of different food proteins against the standard of whole-egg protein can be valuable but probably is not nearly as important to adequate protein nutriture as comparison with reference patterns for the various population groups.

Biological Value

The biological value (BV) of proteins is another method used to assess protein quality. BV is a measure of how much nitrogen is retained in the body for maintenance and growth rather than absorbed. BV is most often determined in experimental animals, but it can be determined in humans. Subjects are fed a nitrogen-free diet for a period of about 7–10 days and then fed a diet containing the test protein in an amount equal to their protein requirement for a similar time period. Nitrogen that is excreted in the feces and in the urine during the period when subjects consumed the nitrogen-free diet is analyzed and compared to amounts excreted when the subjects consumed the test protein. In other words, the change in urinary and fecal nitrogen excretion between the two diets is calculated. The BV of the test protein is determined through the use of the following equation:

$$\text{BV of test protein} = \frac{I - (F - F_0) - (U - U_0)}{I - (F - F_0)} \times 100$$

$$= \frac{\text{Nitrogen retained}}{\text{Nitrogen absorbed}} \times 100$$

where I is intake of nitrogen, F is fecal nitrogen while subjects are consuming a test protein, F_0 is endogenous fecal nitrogen when subjects are maintained on a nitrogen-free diet, U is urinary nitrogen while subjects are consuming a test protein, and U_0 is endogenous urinary nitrogen when subjects are maintained on a nitrogen-free diet.

Foods with a high BV are those that provide the amino acids in amounts consistent with body amino acid needs. The body retains much of the absorbed nitrogen if the protein is of high BV. Eggs, for example, have a BV of 100, meaning that 100% of the nitrogen absorbed from egg protein is retained. Although the BV provides useful information, the equation fails to account for losses of nitrogen through insensible routes such as the hair and nails. This criticism is true of any method involving nitrogen balance studies. A further consideration is that proteins exhibit a higher BV when fed at levels below the amount necessary for nitrogen equilibrium, and retention decreases as protein intake approaches or exceeds adequacy.

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Net Protein Utilization

Another measure of protein quality, similar to nitrogen-balance studies, is net protein utilization (NPU). NPU measures retention of food nitrogen consumed rather than retention of food nitrogen absorbed. NPU is calculated from the following equation:

$$\text{NPU of test protein} = \frac{I - (F - F_0) - (U - U_0)}{I} \times 100$$

$$= \frac{\text{Nitrogen retained}}{\text{Nitrogen consumed}} \times 100$$

where I is intake of nitrogen, F is fecal nitrogen while subjects are consuming a test protein, F_0 is endogenous fecal nitrogen when subjects are maintained on a nitrogen-free diet, U is urinary nitrogen while subjects are consuming a test protein, and U_0 is endogenous urinary nitrogen when subjects are maintained on a nitrogen-free diet.

Although NPU can be measured in humans through nitrogen-balance studies in which two groups of well-matched experimental subjects are used, a more nearly accurate measurement is made on experimental animals through direct analysis of the animal carcasses. In either case, one experimental group is fed the test protein, while the other group receives an isocaloric, protein-free diet. When experimental animals are used as subjects, their carcasses can be analyzed for nitrogen directly (total carcass nitrogen, or TCN) or indirectly at the end of the feeding period. The indirect measurement of nitrogen is made by water analysis. Given the amount of water removed from the carcasses, an approximate nitrogen content can be calculated. NPU involving animal studies is calculated from the following equation:

$$\text{NPU} = \frac{\text{TCN on test protein} - \text{TCN on protein-free diet}}{\text{Nitrogen consumed}}$$

Proteins of higher quality typically cause a greater retention of nitrogen in the carcass than poor-quality proteins and thus have a higher NPU.

Net Dietary Protein Calories Percentage

The net dietary protein calories percentage (NDpCal%) can be helpful in the evaluation of human diets in which the protein-to-calorie ratio varies greatly. The formula is as follows: NDpCal% = Protein kcal/Total kcal intake × 100 × NPU_{op}, where NPU_{op} is NPU when protein is fed above the minimum requirement for nitrogen equilibrium.

Protein Information on Food Labels

Food labels are required to indicate the amount (quantity) of protein in grams and the % Daily Value for protein in a serving of food. As previously mentioned, the protein efficiency ratio (PER) is used to calculate the % Daily Value for infant formulas and baby foods. The U.S. Food and Drug Administration (FDA) specifies the use of the milk protein casein as a standard for comparison of protein quality based on the PER. Specifically, for infant formulas and baby foods, if a test protein has a protein quality equal to or better than that of casein—that is, if the PER is ≥2.5—then 45 g of protein is considered equivalent to 100% Daily Value. If a test protein is lower in quality than casein—that is, if the PER is <2.5—then 65 g of protein is needed to provide 100% Daily Value. For foods other than baby foods, the PDCAAS method is used to establish the protein quality for % Daily Value on food labels. Specifically, 50 g of protein is considered sufficient if the food protein has a PDCAAS equal to or higher than that of milk protein (casein). However, 65 g of protein is needed if the protein is of lower quality than milk protein.

Assessing Protein and Amino Acid Needs

To prevent deficiencies, people need to achieve adequate intakes of energy and protein as well as other essential nutrients. Two techniques—nitrogen balance and indicator amino acid oxidation—are commonly used to assess the adequacy of protein and amino acid intakes.

Nitrogen Balance/Nitrogen Status

Nitrogen-balance studies involve the evaluation of dietary nitrogen intake and the measurement and summation of nitrogen losses from the body. They can be conducted when subjects consume a diet with a protein (nitrogen) intake that is at or near a predicted adequate amount, less than (including protein-free nitrogen) a predicted adequate amount, or greater than a predicted adequate amount. This technique or modified versions of it are often used with hospitalized patients to determine whether protein intake is adequate.

To determine nitrogen balance or status, nitrogen intake and output must be assessed. Assessment of nitrogen intake is based on protein intake. Protein contains approximately 16% nitrogen. Thus, to calculate grams of nitrogen consumed from grams of protein, we can do the following calculation: 0.16 × protein ingested (measured in grams) = nitrogen (measured in grams). Expressed alternately, ingested protein (g)/6.25 = ingested nitrogen (g). So, for example, 70 g of protein intake provides 11.2 g of nitrogen. To reverse the calculations and convert grams nitrogen into grams of protein: protein (g) = nitrogen (g) × 100/16, or protein (g) = nitrogen (g) × 6.25.

Nitrogen losses are measured in the urine (U), feces (F), and skin (S). For example, in the urine, nitrogen is found mainly as urea but also as creatinine, amino acids, ammonia, and uric acid. In the feces, nitrogen may be found as amino acids and ammonia. To

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calculate nitrogen balance/status, nitrogen losses are summed and then subtracted from nitrogen intake (In). Thus, nitrogen balance/status = In − [(U − U_e) + (F − F_e) + S]. The subscript *e* (in U_e and F_e) in the equation stands for *endogenous* (also called obligatory) and refers to losses of nitrogen that occur when the subject is on a nitrogen-free diet.

In clinical settings, nitrogen losses are often estimated. Fecal and insensible (including skin, nail, and hair) losses of nitrogen are thought to account for about 1 g of nitrogen each for a total of 2 g. (Losses should not be estimated in individuals with larger than normal fecal nitrogen losses, as with diarrhea, or large insensible nitrogen losses, as from the skin with burns or high fever.) Urinary losses of nitrogen are measured either as total urinary nitrogen (UN), which gives the most accurate value, or as urinary urea nitrogen (UUN). If urinary urea nitrogen is measured, 2 g of nitrogen is usually added to this value to account for the urinary losses of other nitrogenous compounds such as creatinine, uric acid, ammonia, and so on. Thus, nitrogen balance/status = [protein intake (g)/6.25] − [UN + 2 g], whereby the 2 g accounts for the fecal and insensible nitrogen losses, or nitrogen balance/status = [protein intake (g)/6.25] −[UUN + 2g + 2g], whereby the first 2 g accounts for the losses in the urine of nonurea nitrogen compounds and the other 2 g accounts for the fecal and insensible nitrogen losses.

Nitrogen-balance studies have been criticized for their overall underestimation of protein needs with overestimations of intake and underestimations of losses. In addition, nitrogen balance does not necessarily mean amino acid balance; that is, a person may be in nitrogen balance but in amino acid imbalance.

Indicator Amino Acid Oxidation

Studies assessing protein and amino acid requirements have relied on the indicator amino acid oxidation (IAAO) technique. This method involves feeding test amino acids individually to a person in graded amounts in the presence of an indicator amino acid. The amounts of the test amino acid that are provided include quantities below, at, and above the expected requirement. The method is based on a few principles, including (1) if a test amino acid is not provided, oxidation of the indicator amino acid will be maximal and protein synthesis will be minimal; (2) at an intake above the expected requirement of the test amino acid, oxidation of the indicator amino acid will diminish; and (3) at an intake that is the requirement for the test amino acid, oxidation of the indicator amino acid will be fairly constant. Thus, changes in the oxidation of the indicator amino acid reflect metabolism of the limiting amino acid in the body since if one amino acid is limiting for protein synthesis, all other amino acids (including the indicator amino acid) would be "extra" and oxidized. As the intake of the limiting amino acid increases, the rate of oxidation of the other amino acids decreases, with higher amounts of amino acids being used for protein synthesis. Additionally, any "losses" of the amino acid associated with digestion, absorption, and cellular metabolism are accounted for. Most studies have relied on (1-^{13}C) phenylalanine in the presence of excess tyrosine, lysine, and leucine as the indicator amino acid. The technique has enabled the examination of the requirements for protein as well as the essential amino acids and some conditionally essential amino acids. Mean protein requirements using this technique have been typically higher than those determined using nitrogen-balance methodology.

Recommended Protein and Amino Acid Intakes

Protein and amino acid requirements of humans are influenced by age, body size, and physiological state, as well as by the level of energy intake. Multiple studies using multiple methods, especially indicator amino acid oxidation, nitrogen-balance studies, and the factorial method, have been used over the years to determine protein and amino acid needs. The Estimated Average Requirement for protein for adults (men and women age 19 years and older) is 0.66 g of protein per kg of body weight, or 105 mg of nitrogen per kg of body weight per day [25]. This value represents the lowest continuing dietary protein intake necessary to achieve nitrogen equilibrium or a zero nitrogen balance in a healthy adult [25]. The Recommended Dietary Allowance (RDA) for protein for adults is set at 0.8 g of protein per kg of body weight per day [25]. The protein RDAs for children, adolescents, and adults, including women during pregnancy and lactation, are provided on the inside cover of this book. Instead of RDAs, the recommendations for protein for infants from birth to 6 months of age are given as an Adequate Intake (AI). The AI was derived from data from infants fed human milk as the primary nutrient source for the first 6 months [25].

While the RDA includes a safety variance of over 20%, it does not necessarily provide for maximum protein utilization and it has been suggested that the current RDA for protein is not adequate, especially for older adults [9–14,26]. Habitual intakes of protein in amounts in excess of 1.0 g per kg body weight and up to about 1.5 g protein per kg body weight have been proposed to maintain or accrue muscle mass in older (greater than 65 years) adults [26]. Additionally, a meal threshold effect for protein has been suggested to maximize muscle protein synthesis [7,9,11,27,28]. An intake of 20 g of protein (whey) per meal maximally stimulated muscle protein synthesis in rested muscle and exercised (resistance training) muscle in young men [28]. For older adults, intakes of 25–35 g of high-quality protein per meal have been recommended [9–14]. Furthermore, if consumption is solely from plant-based protein foods, it

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has been suggested that higher protein intakes at each meal may be needed or the meal may need to be supplemented with animal protein and perhaps small amounts (2.5–3 g) of leucine [9].

The current RDA for protein is also not thought to be adequate to support new muscle protein synthesis, to repair muscle damage, and to maintain lean body mass in athletes who are strenuously training and competing [26]. Further information about protein and exercise is provided in Chapter 7.

In addition to the RDA for protein, RDAs for the indispensable amino acids have also been established (see Figure 6.42) based on a variety of methods including amino acid balance and indicators of amino acid oxidation studies. The reader is directed to the Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Protein, and Amino Acids [25] for in-depth information on the methods used in determining the recommendations for the amino acids and protein.

The Institute of Medicine's Acceptable Macronutrient Distribution Range for protein is 10–35% of energy intake. Most Americans consume about 16% of energy from protein, with higher dietary protein intakes (up to about 24% of energy) often consumed by athletes or those who are ill. Use of this distribution range is appropriate as long as energy intake is adequate. If, for example, a person only ingests 800 kcal per day, then 10–35% of energy as protein equals 80–280 kcal; since protein provides 4 kcal/g, this translates into 20–70 g of protein. An intake of 20 g of protein is not sufficient for an adult to maintain nitrogen balance; however, depending on the age, gender, and body weight of the individual, 70 g may be more than adequate.

No Tolerable Upper Intake Level for protein has been established, as no adverse consequences (including cancer, kidney disease, kidney stones, and osteoporosis) were identified from studies of high-protein diets reviewed by the Food and Nutrition Board of the Institute of Medicine [25]. Similarly, indispensable amino acids are thought to have high safety limit intakes.

The most commonly cited concerns from ingesting diets high in protein are an increased risk of dehydration and possible detrimental effects on the kidneys and bones. Dehydration, caused by the need to excrete large amounts of urea and other nitrogenous wastes from protein catabolism, can be prevented with appropriate fluid consumption. Increased urea production and changes in glomerular filtration rate do not promote renal damage in people with no prior history of renal problems. Renal function was not affected in athletes, for example, consuming up to 2.8 g of protein per kg body weight [29]. With respect to bone health, intake of protein must be adequate (along with calcium and other nutrients) to build and maintain bone mass. Most studies report positive associations between bone mass and dietary protein; this relationship is better when the diet is also adequate in other nutrients and derived from dairy, meats, grains, fruits, and vegetables.

To help guide decisions in choosing good sources of protein, the U.S. Department of Agriculture (USDA) published the Food Patterns and MyPlate, which include five major food groups. MyPlate is designed for the consumer and can be accessed at *www.choosemyplate.gov*. From this site, an individual can determine the appropriate amount of foods recommended from each of the food groups. The amounts vary based on a person's gender and age. Generally, however, the recommended quantities for adults from the meat, poultry, fish, eggs, beans, nuts, and seed group range from 5 to 6.5 oz per day, and the recommended amount from the dairy group is 3 cups per

Figure 6.42 Recommended Dietary Allowances for indispensable amino acids for adults.

Source: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Food and Nutrition Board, Institute of Medicine, Washington DC, National Academic Press, 2005, p. 680. Reprinted with permission from the National Academies Press.

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day. Foods ingested from these protein-rich food groups should also be low in saturated fat to promote heart health. Grains also provide some protein. Choices from this group should be high in fiber and low in saturated fat; recommended amounts from the grain group for adults range from 6 to 8 oz or the equivalent per day with the further recommendation that 50% of intake should be whole grains. One slice of bread, 1 cup of ready-to-eat cereal, or ½ cup of cooked cereal, pasta, or rice is equal to 1 oz equivalent.

In addition to MyPlate, dietary protein–related guidelines by the American Heart Association promote the consumption of fish, especially oily fish rich in omega-3 fatty acids, at least twice a week, and lean meats and meat alternatives. Similarly, dietary protein–related guidelines by the American Cancer Society address foods rich in protein, specifically suggesting that intakes of processed and red meats be limited. Consumption of fruits, vegetables, and whole grains is advocated by both organizations. Meals higher in dietary protein have been associated with greater satiety in comparison with meals higher in carbohydrates or fats. Higher satiety may result in reduced food consumption, and thus energy intake with implications in diet planning to promote weight loss. The protein leverage hypothesis suggests that food intake is affected by the protein density of the diet via signaling pathways to the hypothalamus.

Protein Deficiency/Malnutrition

Malnutrition is associated with increased morbidity and mortality. The diagnosis is made and the condition is classified as nonsevere (moderate) or severe within the context of three etiologies including acute injury/illness, chronic illness, or social/environmental circumstances (i.e., reduced access to food or knowledge/beliefs that reduce food intake) [30]. Assessment of individuals for malnutrition includes identification of inflammation (such as by increased plasma C-reactive protein concentrations) for determination of an etiologic-based diagnosis, along with evidence of reduced dietary intake, unintended weight loss, loss of subcutaneous fat, loss of muscle, localized or generalized fluid accumulation, and reduced physical functional status (such as grip strength) [30].

Prolonged or chronic reduced access to food may result in **marasmus**, also referred to as wasting. Individuals with marasmus appear extremely thin (emaciated or underweight) with wasted (depleted) muscle mass and adipose tissue. Bones are prominent in appearance and the skin often droops or hangs from the body. Kwashiorkor or edematous malnutrition represents a form of protein malnutrition in which protein intake is typically insufficient, but energy consumption (usually as carbohydrates) is adequate. It is often seen in children in developing countries. Inadequate quantities of proteins in the blood and in cells (due to insufficient dietary protein intake) cause water to diffuse out of the blood (the intravascular space) and out of the cells (intracellular space) into interstitial (intercellular) spaces, causing edema (swelling). The edema usually appears first in the legs but may also be present in the face or more generalized all over the body (called anasarca).

With chronic disease–related malnutrition, mild to moderate inflammation may also be present. The condition occurs with a variety of illnesses such as rheumatoid arthritis and organ failure, to name a few. Other conditions such as burns, sepsis (infection), and trauma result in marked inflammation and are often associated with acute disease/injury-related malnutrition. Individuals with inflammation (acute or chronic) often do not physically look malnourished, which is why assessment of food energy and nutrient intakes and changes in weight, body composition, and functional status need to be assessed. The effects of inflammation and stress, such as that occurring with burns, sepsis, and trauma, on protein are discussed in the Perspective following this chapter.

SUMMARY

Proteins in foods become available for use by the body after they have been broken down into their component amino acids. Nine of these amino acids are considered essential; therefore, the quality of dietary proteins correlates with their content of these indispensable amino acids. In the body, proteins play many vital roles including functions in structural capacities and as enzymes, hormones, transporters, and immunological protectors, among other roles.

An important concept in protein metabolism is that of amino acid pools, which contain amino acids of dietary origin plus those contributed by the breakdown of body tissue. The amino acids comprising the pools are used in a variety of ways: (1) for synthesis of new proteins for growth and/or replacement of existing body proteins; (2) for production of nonprotein nitrogen-containing compounds; (3) for oxidation as a source of energy; and (4) for synthesis of glucose, ketones, or fatty acids.

The liver is the primary site of amino acid metabolism, but no clear picture of the body's overall handling of nitrogen can emerge without considering amino acid metabolism in a variety of tissues and organs. Of particular significance is the metabolism of the branched-chain amino acids in the skeletal muscle, the role of the intestine in citrulline production, and the role of the kidneys in the production of dispensable

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amino acids, nitrogen-containing compounds, and glucose as well as the elimination of nitrogenous wastes.

Of the nonessential amino acids, glutamine, glutamate, and alanine assume particular importance because of the versatility in the overall metabolism of these amino acids. Glutamate and its α-keto acid make possible many crucial reactions in various metabolic pathways for amino acids. An appreciation for the functions performed by glutamine and glutamate and the numerous amino acids functioning as or used to synthesize neurotransmitters, biogenic amines, and neuropeptides makes one realize that the term "dispensable" as applied to many amino acids may be quite misleading.

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STRESS AND INFLAMMATION: IMPACT ON PROTEIN

In the healthy adult, protein synthesis approximately balances protein degradation. However, in conditions affected by a stress and/or an inflammatory response, such as with sepsis (the presence of a pathogenic microorganism or its toxin in the blood and/or body tissues), burns, and injury/trauma (including surgery), protein synthesis and protein degradation are not in balance. The body systems basically prioritize wound repair and host defense (such as from infection) at the expense of body tissues, in essence gambling that convalescence or a return to health will occur before tissue depletion threatens survival.

A coordinated set of actions occurs in the body in response to "insults" such as that occurring with sepsis/infection, burns, injury/trauma including surgery, and some diseases. The endocrine system responds with increased secretion of catecholamines (especially epinephrine) as well as glucagon, cortisol, growth hormone, aldosterone, and antidiuretic hormone (also called vasopressin); the immune system responds, releasing inflammatory cytokines that augment and exacerbate the stress response. Some of these hormones impact organ functions to help restore homeostasis. For example, aldosterone promotes renal sodium and fluid reabsorption and thus increased blood volume, and antidiuretic hormone (ADH) inhibits diuresis (urination) and thus increases blood volume, diminishes fluid losses, and helps to restore circulation if it has been depressed by shock, fever, burns, and/or hemorrhage. Metabolic rate also increases (referred to as a hypermetabolic state) in an attempt to restore homeostasis with the rise driven by elevations in catecholamines, glucagon, and cortisol. Some of these same hormones also impact nutrient metabolism and body tissues (Figure 1).

The actions of catecholamines, glucagon, and cortisol result in catabolism of body proteins (primarily muscle mass by the ubiquitin system), fat, and carbohydrate (glycogen) (Figure 2), and disrupt signaling pathways that normally promote protein synthesis after food ingestion. The catecholamines, especially epinephrine, stimulate adipose tissue lipolysis, and along with growth hormone, stimulate glycogenolysis. Glucagon and especially cortisol (considered the body's major stress hormone) promote muscle proteolysis and gluconeogenesis. Glutamine and alanine release from tissues rises substantially, with alanine used for gluconeogenesis and glutamine used by immune system and intestinal cells for energy and by the kidneys for acid-base balance. While insulin is produced, the body's tissues become resistant to its action, and hyperglycemia persists. Hyperglycemia further increases inflammation, increases risk for infection, reduces wound healing, and increases length of hospital stay and mortality. Growth hormone contributes to hyperglycemia by exerting anti-insulin effects. Cortisol, like epinephrine, also stimulates adipose tissue lipolysis. However, unlike with starvation, the fatty acids generated from lipolysis do not produce ketones (ketogenesis is inhibited by the presence of insulin). With limited availability of ketones, body proteins, especially those from white fast-twitch muscle, continue to be degraded to supply amino acids for gluconeogenesis and for the synthesis of critical acute-phase proteins. Exacerbating the catabolism of body proteins associated with hypermetabolic states is bedrest or physical inactivity. Bedrest further impairs existing muscle function and diminishes signaling via mTOR to promote muscle protein synthesis. Bedrest even in the absence of illness decreases whole-body protein synthesis that normally follows the ingestion of food by about 15–20% [1,2].

Evidence of protein catabolism is apparent with measurements in the urine of 3-methylhistidine and nitrogen. Urinary nitrogen losses frequently total 30 g or more per day. Each gram of nitrogen lost can be translated into the breakdown of *approximately 30 g of hydrated lean tissue* (whereby 1 g nitrogen = 6.25 g protein; muscle is about 80% water, so 80% of 30 g = 24 g, and 30 g muscle = 24 g water + 6.25 g protein).

The inflammatory response begins with tissue injury or cell death and the release of damage-associated molecular patterns (DAMPs) from the necrotic or damaged cells, more specifically their intracellular components such as mitochondrial DNA, histones, and high-mobility group box 1 (nuclear factors bound to DNA), among others. DAMPs, through binding to pattern recognition receptors such as Toll-like receptors found on immune system cells, activate an immune response.

Figure 1 Response to metabolic stress.

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Figure 2 Substrate utilization during metabolic stress with increased responses shown by heavier black arrows.

With a bacterial infection, pathogen-associated molecular patterns (PAMPs)—small molecular components of bacteria including, for example, bacterial lipopolysaccharide (an endotoxin found on bacterial cell membranes)—initiate a similar inflammatory immunocascade as DAMPs.

The body response to such insults is complex, but a brief overview is provided. The bone marrow responds by increasing production of white blood cells, especially neutrophils (phagocytic cells). DAMPs and PAMPs trigger the production of proinflammatory cytokines by macrophages, T-cells, and monocytes, among others. Two cytokines, interleukin (IL)-1 and tumor necrosis factor (TNF) α, are thought to orchestrate much of the inflammatory response. Fibroblasts and endothelial cells magnify the response by secreting IL-6 in addition to more IL-1 and TNF α. IL-6 levels typically correspond to the severity of the insult. IL-1, IL-6, and TNF α increase body temperature (fever), and with other cytokines accelerate the production of white blood cells, especially neutrophils. Metabolic rate also is elevated above normal secondary to fever and increased oxygen consumption needed for phagocytosis by activated white blood cells. Increases in body temperature also promote the synthesis of heat shock proteins. Cytokines, similar to cortisol's effects, promote adipose tissue lipolysis and muscle protein degradation (via the ubiquitin-proteasomal pathway); resulting increases in plasma free fatty acids contribute to tissue insulin resistance. IL-1 and TNF α facilitate white blood cell infiltration into damaged tissues. Similarly, IL-8 recruits neutrophils into inflamed tissues. Edema, redness, and pain occur at the site of injury as a result from vasodilation and increased capillary permeability around the damaged tissues. The vasodilation and increased capillary permeability facilitate entry of white blood cells into the damaged/infected area.

Inflammatory states range from acute and severe to chronic and low grade depending on the nature of the insult and its persistence. Low-grade inflammatory states may be present in several chronic conditions, such as Crohn's disease, heart disease, some connective tissue disorders, diabetes, and obesity (especially excess body fat in the abdominal/visceral region). With obesity, the adipose tissue and the stroma vascular fraction (a group of cells—including preadipocytes, fibroblasts, macrophages, and histocytes, among others—associated with white adipose tissue) secrete cytokines, growth factors, and adipokines (molecules secreted by white adipose tissue, such as resistin, visfatin, adiponectin), which either directly or indirectly promote inflammation. Moreover, macrophages that become embedded within the adipose tissue also induce inflammation.

The cytokines, like many of the hormones, released with inflammation also affect nutrient utilization. Specifically, the hepatic synthesis of proteins such as albumin, retinol-binding protein, and prealbumin is preferentially reduced and proteins such as albumin may migrate from the blood, resulting in further reductions in the blood. In contrast, the synthesis of other proteins is stimulated. Acute-phase reactant/response protein synthesis increases mostly by the liver, but other cells such as macrophages, lymphocytes, and fibroblasts also generate inflammatory proteins. Some examples of these acute-phase response proteins and their functions include:

- C-reactive protein (CRP): a protein that stimulates phagocytosis and activates complement proteins, which are needed for antibody-induced destruction of microorganisms; hsCRP concentrations, which are normally < 1 mg/L, rise dramatically in response to inflammation. Concentrations > 200 mg/L have been reported with severe sepsis and burns.
- Orosomucoid (α1-acid glycoprotein): a protein important in wound healing and immunomodulatory functions. Orosomucoid concentrations rise about two- to fivefold with inflammation.
- Serum amyloid A: a protein that displaces apoprotein A1 on high-density lipoproteins and facilitates cholesterol delivery to cells and cholesterol removal from damaged tissue. The protein also recruits immune cells (neutrophils and monocytes) to inflammatory sites and induces extracellular matrix degrading enzymes. Concentrations may rise 20- to 1,000-fold with inflammation.
- Fibrinogen: a protein that contributes to blood viscosity and can be converted to fibrin by thrombin to promote blood clotting; increased plasma fibrinogen may increase arterial thromboembolism (blood clot formation and dislodgement) risk.

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- Fibrinonectin: a glycoprotein functioning in cell adhesion and wound healing.
- Haptoglobin: a protein that binds hemoglobin that has been released into the blood due to red blood cell hemolysis and inhibits microbial use of iron.
- Ceruloplasmin: a copper-containing protein with the ability to scavenge free radicals and with oxidase activity to promote iron oxidation and thus inhibit microbial iron use.
- α2 Macroglobulin: a protease inhibitor that, for example, inhibits blood coagulation and fibrinolysis.

In addition to the synthesis of these proteins, more metallothionein (a zinc-containing protein) and ferritin (an iron-containing protein) are made in the liver with inflammation. Consequently, hepatic zinc and iron concentrations increase, while plasma zinc and iron concentrations decrease. Such changes diminish the likelihood of microorganisms utilizing the body's zinc and iron for their own proliferation. Another nutrient affected by inflammation is vitamin A; plasma retinol concentrations typically decrease with both infection and trauma.

Restoration of homeostasis following an inflammatory response involves a group of anti-inflammatory compounds including endogenous lipid mediators like resolvins, protectins, lipoxins, and maresins, as well as anti-inflammatory cytokines like IL-10, transforming growth factor β, and cytokine antagonist IL1Ra. While a discussion of the resolution to inflammation and stress is beyond the scope of this Perspective, it is important to note that inadequate energy and nutrient intakes impair the ability to rebuild lost muscle mass as well as diminish the immune and antioxidant defense responses. Research is focused on determining optimal nutrition support required for the treatment of stress and inflammatory conditions, including the identification of nutrients that may serve as immunomodulators. (However, the benefits of physical activity [early ambulation] should not be ignored.) Supplementation of the diet with essential amino acids, including sufficient amounts of leucine, has been shown to offset the catabolic effects associated with bedrest and acute hypercortisolemia [3]. However, the absolute quantities of protein as well as other nutrients needed to reverse the catabolic state associated with stress and inflammation have not yet been determined.

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7 INTEGRATION AND REGULATION OF METABOLISM AND THE IMPACT OF EXERCISE

ENERGY HOMEOSTASIS IN THE CELL
Regulatory Enzymes
INTEGRATION OF CARBOHYDRATE, LIPID, AND PROTEIN METABOLISM
Interconversion of Fuel Molecules
Energy Distribution among Tissues
THE FED-FAST CYCLE
The Fed State
The Postabsorptive State
The Fasting State
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HORMONAL REGULATION OF METABOLISM
Insulin
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EXERCISE AND NUTRITION
Muscle Function
Energy Sources in Resting Muscle
Muscle ATP Production during Exercise
Fuel Sources during Exercise
SUMMARY
PERSPECTIVE
THE ROLE OF DIETARY SUPPLEMENTS IN SPORTS NUTRITION
BY KARSTEN KOEHLER, PhD

METABOLISM IS OFTEN DEFINED as all chemical reactions and pathways that occur in a living organism to maintain life. Such a broad definition may seem overwhelming, although a closer look at metabolism reveals a highly coordinated set of events that occur around the central theme of energy homeostasis. Humans require frequent input of energy to perform mechanical work, including cardiac and skeletal muscle contractions; active transport of molecules and ions; and synthesis of complex molecules from simple precursors. The demand for energy by cells and the intake of energy from food are rarely synchronized, so the body is constantly adjusting metabolic pathways to maintain energy homeostasis. Despite the complexity, metabolic integration is achieved by the cells' ability to use a common energy currency (i.e., ATP) and surprisingly few intermediates (e.g., pyruvate and acetyl-CoA) that tie together metabolic pathways.

Chapters 3, 5, and 6 featured carbohydrate, lipid, and protein metabolism. Those chapters discussed how the pathways are regulated at the level of regulatory enzymes by substrate availability, allosteric mechanisms, and covalent modifications such as phosphorylation. This chapter focuses on the integration of carbohydrate, lipid, and protein metabolism as it occurs in different organs and tissues—and the interconnections among them. The important topics discussed are: (1) the homeostasis of cellular energy and the control between catabolism and anabolism; (2) how the major organs and tissues interact through integration of their metabolic pathways to redistribute energy, (3) hormonal regulation of these metabolic processes; and (4) examples of the body's ability to maintain homeostasis under the daily events of fasting, refeeding, and physical activity. This chapter also discusses **exercise**—planned, structured physical activity to enhance physical fitness—and sports nutrition.

ENERGY HOMEOSTASIS IN THE CELL

Metabolic pathways generally belong to two broad categories: those that yield energy (degradative; catabolic) and those that require energy (synthetic; anabolic). The main purpose of catabolic reactions is to break down macronutrients so their inherent energy can be released and transformed into ATP. To a lesser extent other high-energy molecules such as GTP and UTP are formed, although ATP is ubiquitous and the primary cellular energy carrier. Anabolic reactions, on the other hand, synthesize complex molecules from simple precursors by utilizing the energy from ATP (and the other nucleoside triphosphates in a few key reactions).

Despite the thousands of reactions that occur in the body, there are only a few common pathways in the metabolic roadmap that control whether a

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Figure 7.1 Metabolic pathways involved in the maintenance of energy homeostasis. Bidirectional pathways with separate arrows indicate separate regulatory enzymes controlling each direction. Not all pathway intermediates are shown.

cell or organ is engaged in catabolism or anabolism. As depicted in Figure 7.1, pyruvate and acetyl-CoA are critical junctions in the roadmap that connect the metabolism of carbohydrate, lipid, and protein. The energy status of the cell largely determines the direction in which molecules flow. If cellular energy (ATP) is needed, the pyruvate from glycolysis is sent to the mitochondria, decarboxylated to acetyl-CoA, and oxidized via the TCA cycle to produce ATP through oxidative phosphorylation (see Chapter 3). Similarly, fatty acids may be catabolized to acetyl-CoA in mitochondria, resulting in the production of ATP via the TCA cycle and oxidative phosphorylation. And when carbohydrates and lipids are in short supply, amino acids are converted to pyruvate and acetyl-CoA, thus providing needed energy for ATP production. Amino acids are also used to replenish many of the TCA cycle intermediates to ensure the cycle's continued operation.

Pyruvate and acetyl-CoA may be used to produce more complex molecules when the cellular energy status favors anabolic reactions (Figure 7.1). Pyruvate is converted to glucose via gluconeogenesis, whereas acetyl-CoA is mostly used for fatty acid synthesis. Note that the conversion

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of pyruvate to acetyl-CoA is an irreversible reaction, preventing appreciable amounts of acetyl-CoA from being used for gluconeogenesis. Other metabolic intermediates, particularly those of the TCA cycle, can be diverted into anabolic pathways as needed. TCA cycle intermediates used for anabolic reactions are largely replenished by the conversion of pyruvate to oxaloacetate, although a variety of molecules are available to ensure the TCA cycle continues to function. Examples of TCA cycle intermediates entering anabolic pathways include the following:

- Citrate can move from the mitochondria into the cytosol, where citrate lyase cleaves it into oxaloacetate and acetyl-CoA, the latter being used for fatty acid synthesis.
- Malate, in the presence of $NADP^+$-linked malic enzyme, may provide a portion of the NADPH required for reduction reactions in fatty acid synthesis.
- Succinyl-CoA can combine with glycine in the mitochondria to form Δ-aminolevulinic in initial step in heme synthesis (see Figure 13.7).
- Oxaloacetate may be used for conversion to amino acids or it may enter the gluconeogenesis pathway.
- CO_2 produced by the TCA cycle is a source of cellular carbon dioxide for carboxylation reactions that initiate fatty acid synthesis and gluconeogenesis. This CO_2 also supplies the carbon of urea and certain portions of the purine and pyrimidine rings (see Figures 6.7, 6.27, and 6.31).

It is important to remember that anabolic reactions generally require NADPH to provide reductive power for synthesis of complex molecules. NADPH is the major electron donor in cells that drives anabolic reactions. Most of the needed NADPH is supplied by the pentose phosphate pathway, which also produces ribose-5-phosphate used in the synthesis of nucleotides (see Chapter 3).

Regulatory Enzymes

Maintaining the balance between catabolism and anabolism is achieved by the regulation of distinct enzymes that are highly sensitive to changes in energy status within the cell. Regulatory enzymes are located at strategic points in metabolic pathways and are mostly unidirectional (irreversible). The majority of regulatory enzymes is controlled allosterically and respond immediately to cellular signals, although some are controlled by covalent modification, usually phosphorylation, in response to hormonal signals. Glycogen synthase and phosphorylase are examples of covalently modified regulatory enzymes (see Chapter 3). Table 7.1 summarizes key enzymes that are controlled allosterically in response to immediate changes in cellular signals.

As the energy status of the cell declines, so does the concentration of acetyl-CoA, citrate, and ATP due to decreased glycolysis, lipolysis (β-oxidation), and TCA cycle reactions. At the same time, increased amounts of ADP and AMP indicate that ATP has been used up in anabolic reactions and more ATP is needed. Furthermore, decreased concentration of malonyl-CoA reflects little or no fatty acid synthesis occurring during low energy status. Each of these cellular signals will trigger the allosteric stimulation of regulatory enzymes to increase glycolysis, β-oxidation, and the TCA cycle to replenish ATP.

When cellular ATP is abundant, the concentration of ATP, acetyl-CoA, and citrate are relatively high and act to allosterically inhibit regulatory enzymes that

Table 7.1 Cellular Energy Status, Metabolic Response, and Allosteric Control Sites

Low Energy Status		
Metabolic Response	**Cellular Signals**	**Regulated Enzyme**
↑ Glycolysis	↑ AMP, ↓ ATP, ↓ citrate, ↓ acetyl-CoA	↑ Phosphofructokinase
	↓ ATP, ↓ acetyl-CoA	↑ Pyruvate kinase
↑ TCA cycle	↑ ADP, ↑ pyruvate	↑ Pyruvate dehydrogenase
	↑ ADP	↑ Isocitrate dehydrogenase
↓ Gluconeogenesis	↑ AMP	↓ Fructose-1,6-bisphosphatase
↑ Fatty acid β-oxidation	↓ Malonyl-CoA	↑ Carnitine acyltransferase I
Abundant Energy Status		
Metabolic Response	**Cellular Signals**	**Regulated Enzyme**
↓ Glycolysis	↑ ATP, ↑ citrate	↓ Phosphofructokinase
↓ TCA cycle	↑ ATP	↓ Isocitrate dehydrogenase
	↑ ATP	↓ α-Ketoglutarate dehydrogenase
↑ Gluconeogenesis	↑ Acetyl-CoA	↑ Pyruvate carboxylase
	↑ Citrate	↑ Fructose-1,6-bisphosphatase
↑ Fatty acid synthesis	↑ Citrate	↑ Acetyl-CoA carboxylase
	↑ Malonyl-CoA	↓ Carnitine acyltransferase I

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govern glycolysis and the TCA cycle while stimulating gluconeogenesis and fatty acid synthesis as a way of capturing and storing the energy for later use. Each of the regulatory enzymes highlighted in Table 7.1 have been discussed in more detail in Chapters 3 and 5.

Role of Malonyl-CoA

Malonyl-CoA deserves special mention because of its role in both fatty acid synthesis and β-oxidation. Its regulatory role is expressed through its allosteric control of carnitine acyltransferase I (CAT I), although the cellular levels of malonyl-CoA are mediated by the phosphorylation of AMP-activated protein kinase (AMPK). Recall from Chapter 5 that CAT I is required to transport activated fatty acids (as fatty acyl-CoA) into the mitochondria for oxidation. Increased cellular concentration of malonyl-CoA blocks CAT I and prevents transport and subsequent oxidation, whereas the absence of malonyl-CoA allows CAT I to function. Figure 7.2 illustrates the cellular signals that control the concentration of malonyl-CoA.

Note that malonyl-CoA and acetyl-CoA are part of a rapid cycle in the cytosol that is mediated by two opposing enzymes, acetyl-CoA carboxylase (ACC) and malonyl-CoA decarboxylase (MCD), as depicted in the shaded area in Figure 7.2. If energy is not needed by the cell, the acetyl-CoA will be carboxylated to form malonyl-CoA, which is the first step of fatty acid synthesis (see Figure 5.27). Malonyl-CoA levels are highest in the fed state and decline with fasting (the fed-fast cycle is discussed in detail later in this chapter). The amount of malonyl-CoA in skeletal muscle is increased by glucose and insulin, resulting in a decrease in β-oxidation of fatty acids due to the inactivation of AMPK. In lipogenic tissues such as the liver, adipose tissue, and lactating mammary glands, malonyl-CoA is a cosubstrate for the cytosolic fatty acid synthase system for the *de novo* synthesis of palmitic acid (see Chapter 5). Malonyl-CoA is also involved in the elongation reactions of fatty acids in the ER.

When the energy status of the cell is low, recruitment of fatty acids from the circulation increases the cytosolic

Figure 7.2 Role of malonyl-CoA in fatty acid synthesis and oxidation. Abbreviations: ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; CAT, carnitine acyltransferase (I and II); and MCD, malonyl-CoA decarboxylase.

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fatty acyl-CoA concentration (Figure 7.2). The need for ATP production is accelerated by fasting and muscle contraction. Under these conditions, increased levels of fatty acyl-CoA and glucagon activate AMPK by phosphorylation, which in turn activates MCD and inactivates ACC by phosphorylation. In cardiac muscle 50–80% of the energy is derived from fatty acids. Fatty acids provide less energy following consumption of a high-carbohydrate meal and more following a high-fat meal. Malonyl-CoA is also thought to function as one of the signals for β-cells of the pancreas to secrete insulin in response to elevated blood glucose levels. The elevated malonyl-CoA levels in β-cells inhibit the transfer of fatty acids into the mitochondria, and the increased fatty acid levels in the cytosol act as a coupling factor for insulin secretion.

Malonyl-CoA is also associated with the restraint of food intake. It acts through the hormone leptin released by adipose tissue to signal that triacylglycerol storage in adipose is adequate. This is discussed in Chapter 8 under the section on the control of food intake.

Role of AMP-Activated Protein Kinase

The previous section described how AMPK participates in fatty acid metabolism. AMPK appears to play a much larger role in metabolism and can be viewed as a master energy sensor, controlling both catabolic and anabolic pathways involving all the macronutrients [1]. Regulatory systems that are responsive to AMPK represent another common mechanism of regulation that links carbohydrate, lipid, and protein metabolism. AMPK is activated by an increasing cellular AMP and declining ATP (high AMP:ATP ratio), indicating low energy status of the cell. This can be caused by declining cellular glucose that occurs during fasting, increased ATP utilization that occurs with muscle contraction, and metabolic stresses that interfere with ATP production such as hypoxia. Several dietary phytochemicals can activate AMPK, including capsaicin in peppers, resveratrol in red wine and grapes, curcumin in turmeric, and epigallocatechin gallate in green tea.

AMPK influences glucose metabolism in several ways. In response to increasing AMP:ATP ratio, activated AMPK in turn activates a transporter protein in adipocytes and muscle cells involved in the translocation of GLUT4 to the plasma membrane, thus increasing the uptake of glucose into the cell. A common drug used to treat type 2 diabetes, metformin, exerts part of its hypoglycemic effect by activating AMPK and increasing the translocation of GLUT4 to the cell surface by mechanisms independent of insulin. AMPK also promotes glucose uptake in cells that express only GLUT1 by activating the transporter that is already located in the plasma membrane (see Table 3.2). AMPK stimulates glycolysis mainly in cardiac muscle by phosphorylating phosphofructokinase-2, which produces 2,6-bisphosphate, a potent allosteric activator of phosphofructokinase (reaction 3 in Figure 3.17). Furthermore, activated AMPK prevents cellular energy from being diverted into anabolic pathways by inhibiting glycogen synthase (see Figure 3.13) and the gluconeogenic enzymes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (see Figure 3.30).

Another role of AMPK in lipid metabolism when the cellular AMP:ATP ratio is high includes the promotion of fatty acid uptake into cardiac muscle by increasing the translocation of the fatty acid transporter CD36 to the plasma membrane. Activated AMPK also inhibits acyltransferases involved in triacylglycerol and phospholipid synthesis (see Figure 5.31), and HMG-CoA reductase, the rate-limiting step in cholesterol synthesis (see Figure 5.32). With regard to protein metabolism, AMPK appears to inhibit protein synthesis by phosphorylating at least two enzymes involved in the translation of mTOR, itself a protein kinase that controls many cellular processes including protein synthesis [1].

AMPK also plays a role in energy metabolism through its action in the hypothalamus, the primary appetite control center of the body. Details of the role of AMPK and hormones in appetite regulation are discussed in Chapter 8.

INTEGRATION OF CARBOHYDRATE, LIPID, AND PROTEIN METABOLISM

The chapter thus far has focused mainly on individual pathways and regulation of those pathways by specific enzymes. We now shift our attention to a broader view of metabolism in which many pathways are coordinated simultaneously through common cellular and extracellular signals. In fact, the entire human body must be continually synchronized for normal metabolism to occur. This expanded view of metabolism reveals a type of "communication" within cells, between cells, and even among tissues and organs. The constantly changing metabolic status of various tissues throughout the body requires communication across great distances, facilitated by the nervous, endocrine, and vascular systems. Described here is an overview of how fuel molecules can be interconverted and redistributed among the body's tissues to maintain energy homeostasis. The impact of the fed-fast cycle and skeletal muscle activity (exercise) on these events are discussed later in this chapter.

Interconversion of Fuel Molecules

The body needs a constant supply of energy from macronutrients to function optimally. The amount of food consumed and the frequency of intake have profound effects on metabolic pathways that maintain the balance

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between catabolism and anabolism both short term (minutes, hours) and long term (days, weeks, months), as discussed later in this chapter. Dietary carbohydrate and lipid provide the majority of energy for ATP production. Amino acids are also used for energy, although the high demand for body proteins generally diverts most food-derived amino acids into protein synthesis. Amino acids may be called upon for energy when carbohydrate and lipid are insufficient.

Figure 7.1 illustrates how macronutrients are catabolized to common intermediates (pyruvate and acetyl-CoA), which can be resynthesized into glucose, triacylglycerol, and amino acids as warranted by the metabolic status of the cell. Pyruvate and acetyl-CoA represent key intersections in the metabolic roadmap where interconversion among the nutrients can occur. For example, as explained in Chapter 6, certain amino acids can be synthesized in the body from carbohydrates or fatty acids; conversely, most amino acids can serve as precursors for glucose or fatty acid/triacylglycerol synthesis. Carbohydrates can be used to synthesis fatty acids and triacylglycerols. Fatty acids, in contrast, cannot be used to make glucose because humans lack the necessary enzymes to convert acetyl-CoA to pyruvate or any intermediate along the gluconeogenic pathway. Not evident from the figure, but important to recall, is that the TCA cycle is an **amphibolic pathway**, meaning that it not only functions in the oxidative catabolism of carbohydrates, fatty acids, and amino acids but also provides precursors for many biosynthetic pathways, particularly gluconeogenesis (see Figure 3.31). Amino acids can be converted into several TCA cycle intermediates. When needed, α-ketoglutarate, succinate, fumarate, and oxaloacetate can be used as gluconeogenic precursors.

When ATP is needed, glucose and amino acids may be catabolized to pyruvate, which is translocated from the cytosol into the mitochondria while simultaneously decarboxylating it to acetyl-CoA. The acetyl-CoA can be oxidized to CO_2 and H_2O to produce ATP by the TCA cycle and oxidative phosphorylation. Another fate of pyruvate is its reduction in the cytosol to lactic acid (Figure 7.1). The lactate can be transported to other tissues, converted back to pyruvate and oxidized in the muscle, or used for gluconeogenesis in the liver. Most of the acetyl-CoA is produced in the mitochondria through the β-oxidation of fatty acids. When acetyl-CoA is involved in anabolic reactions, it has to be translocated back to the cytosol across the mitochondrial membrane, which is not permeable to it. Therefore, the acetyl-CoA in the mitochondria combines with oxaloacetate to form citrate (as in the TCA cycle), to which the mitochondrial membrane is freely permeable. The citrate moves into the cytosol and can break down again to oxaloacetate and acetyl-CoA. The acetyl-CoA may undergo a carboxylation reaction catalyzed by acetyl-CoA carboxylase to form malonyl-CoA (see Figure 5.27), the first step of fatty acid synthesis.

Glucose is the precursor for the glycerol moiety of triacylglycerol in adipose tissue. It can be formed from dihydroxyacetone phosphate (DHAP), a three-carbon intermediate in glycolysis (see Figure 3.17). Reduction of DHAP by glycerol-3-phosphate dehydrogenase and NADH produces glycerol-3-phosphate (see Figure 5.35). The fatty acid components of triacylglycerols in adipose tissue can come from the diet, from adipose tissue (via lipolysis), or from the liver, where they are synthesized and packaged for delivery to adipocytes by VLDL. Triacylglycerols are synthesized by the reaction of the glycerol-3-phosphate with CoA-activated fatty acids (Figure 5.29). Recall that muscle and adipose tissue lack the glycerol kinase that can phosphorylate glycerol directly and must obtain the glycerol-3-phosphate through glycolysis.

While carbohydrate can be converted into both the glycerol and the fatty acid components of triacylglycerols, only the glycerol portion of triacylglycerols can be converted into carbohydrate. The conversion of fatty acids into carbohydrate is not possible because the pyruvate dehydrogenase reaction is not reversible. This fact prevents the direct conversion of acetyl-CoA, the sole catabolic product of even-numbered-carbon fatty acids, into pyruvate for gluconeogenesis. In addition, gluconeogenesis from acetyl-CoA as a TCA cycle intermediate cannot occur because for every two carbons in the form of acetyl-CoA entering the TCA cycle, two carbons are lost by decarboxylation in early reactions of the cycle (see Figure 3.18). Consequently, there can be no net conversion of acetyl-CoA to pyruvate or to the gluconeogenic intermediates of the cycle. Acetyl-CoA produced from any source must be used for ATP production, lipogenesis, cholesterol synthesis, or ketogenesis (Figure 7.1).

Although fatty acids that have an even number of carbons are degraded exclusively to acetyl-CoA and therefore are not glucogenic (resulting in glucose production), fatty acids that possess an odd number of carbon atoms are partially glucogenic. Fatty acids with an odd number of carbons can be partially converted to glucose because propionyl-CoA ($CH_3-CH_2-COSCoA$), ultimately formed by β-oxidation, is carboxylated and rearranged to succinyl-CoA, a glucogenic TCA cycle intermediate (see Figure 5.25). Fatty acids with an odd number of carbon atoms are not abundant in the diet, although ruminant milk fat and some fish are known sources.

Metabolism of the amino acids gives rise to a variety of amphibolic intermediates, some of which produce glucose (glucogenic), while others produce ketone bodies (ketogenic) by their conversion to acetyl-CoA or acetoacetyl-CoA. Only the amino acids leucine and lysine are purely ketogenic. The dispensable (nonessential) glucogenic amino acids can be converted to carbohydrate,

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but like the ketogenic amino acids, they can also be converted indirectly into fatty acids by undergoing oxidation to acetyl-CoA. Fatty acids cannot be converted into the glucogenic amino acids for the same reason that fatty acids cannot be converted into glucose—namely, the irreversibility of the pyruvate dehydrogenase reaction. Although metabolically possible, the conversion of the glucogenic amino acids into fatty acids is rather uncommon. Only when protein is supplying a high percentage of calories would glucogenic amino acids be expected to be used in fatty acid synthesis. All the amino acids producing acetyl-CoA directly—isoleucine, threonine, phenylalanine, tryptophan, tyrosine, lysine, and leucine—are indispensable. Tyrosine is conditionally indispensable because it is formed by hydroxylation of phenylalanine. The catabolism of the individual amino acids is covered in Chapter 6.

Energy Distribution among Tissues

The ability to interconvert fuel molecules is crucial for maintaining energy homeostasis in the body, where metabolism in every tissue is unique and markedly different. The metabolic events that occur in one tissue will significantly affect metabolism in other tissues. In this way, fuel molecules are constantly being interconverted and transported among tissues via the bloodstream under all metabolic conditions to provide energy where needed. The following discussion highlights the unique metabolic features and primary differences of tissues that participate in energy distribution.

Liver

The liver plays a central role in metabolism. Most nutrients absorbed by the small intestine first pass through the liver, and many fuel molecules released by extrahepatic tissues travel to the liver for additional processing. Figures 7.3, 7.4, and 7.5 illustrate the fate of glucose-6-phosphate, amino acids, and fatty acids in the liver. In these figures, anabolic pathways are shown pointing up; catabolic pathways are pointing down; and distribution to other tissues is running horizontally. The pathways indicated are described in detail in Chapters 3, 5, and 6, which deal with carbohydrate, lipid, and protein metabolism, respectively.

Glucose entering hepatocytes from the hepatic portal vein and, to a lesser extent, the systemic circulation is phosphorylated by glucokinase to glucose-6-phosphate. Dietary galactose is also phosphorylated and rearranged to glucose-6-phosphate. Figure 7.3 shows the possible metabolic routes available to glucose-6-phosphate. The liver uses relatively little glucose-6-phosphate for its own energy needs and, instead, stores a significant amount

Figure 7.3 Pathways of glucose-6-phosphate metabolism in the liver.

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Figure 7.4 Pathways of amino acid metabolism in the liver.

as glycogen for times when glucose is in short supply. Glycogen synthesis occurs when blood glucose levels are high and the liver takes in more glucose, especially after a meal. About two-thirds of the glucose-6-phosphate entering glycogenesis is derived from glucose absorbed by the small intestine. The remaining glucose-6-phosphate entering glycogenesis is derived, paradoxically, from newly synthesized glucose because gluconeogenesis continues to function under all metabolic conditions. This is due to the constant flow of lactate into systemic circulation that the liver must convert to glucose-6-phosphate to keep blood lactate levels in check. The lactate comes from extrahepatic tissues, notably skeletal muscle and red blood cells.

Figure 7.4 reviews the particularly active role of the liver in amino acid metabolism. The liver is the site of synthesis of many different proteins, both structural and plasma-borne, from amino acids. The liver can also convert amino acids into nonprotein products such as nucleotides and porphyrins. Catabolism of amino acids can take place in the liver, where most are transaminated and degraded to acetyl-CoA and other TCA cycle intermediates. These substances in turn can be oxidized for energy or converted to glucose or fatty acids. Glucose formed from gluconeogenesis can be transported to muscle, brain, nerve cells, red blood cells, and other tissues for energy utilization. Newly synthesized fatty acids can be transported to adipose tissue for storage or used as fuel primarily by cardiac and skeletal muscle. Hepatocytes are the exclusive site for the formation of urea, the major excretory form of amino acid nitrogen.

The fate of fatty acids in the liver is outlined in Figure 7.5. Hepatic fatty acids are derived from chylomicron remnants and from *de novo* synthesis. In humans, most fatty acid synthesis takes place in the liver rather than in adipose tissue. Fatty acids can be assembled into liver triacylglycerols and released into the circulation as plasma VLDL. Circulating VLDL interact with tissues expressing lipoprotein lipase, namely muscle and adipose tissue, where the triacylglycerols are delivered. Adipocytes store the triacylglycerols, whereas muscle will mostly hydrolyze the triacylglycerols and oxidize the resulting fatty acids for ATP production. Under most circumstances, fatty acids

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Figure 7.5 Pathways of fatty acid metabolism in the liver.

are a major fuel supplying energy to the liver via the TCA cycle and oxidative phosphorylation. The acetyl-CoA that cannot be used for energy may be converted to ketone bodies, which are important fuels for certain peripheral tissues such as the brain and heart muscle, particularly during periods of prolonged fasting.

Muscle

Fatty acids and glucose are the major fuels for both skeletal and cardiac muscle. Muscle can also use ketone bodies for energy when the availability of fatty acids and glucose is insufficient. Cardiac muscle requires a continuous supply of energy, whereas skeletal muscle's demand for fuel molecules is quite low when at rest, but will increase as muscle contractions increase. The relative contribution of fatty acids and glucose use in skeletal muscle can change dramatically depending on the duration and intensity of physical activity (discussed later in this chapter).

Skeletal and cardiac muscle expresses GLUT4 on the cell surface when the blood concentration of glucose and insulin are elevated such as following a carbohydrate-rich meal. Recall that GLUT4 is the only GLUT protein whose function is dependent on insulin (see Table 3.2). Consequently, muscle cells can take up large amounts of glucose and will quickly phosphorylate it to glucose-6-phosphate by the enzyme hexokinase. Cardiac muscle has a very limited capacity to synthesize glycogen and therefore uses the glucose-6-phosphate for immediate energy needs. Skeletal muscle, on the other hand, can store large amounts of glycogen for use at a later time. Skeletal and cardiac muscle express other GLUT proteins and can take up glucose from the circulation during fasting or when energy demands exceed incoming dietary sources of carbohydrate. Under these conditions, the blood glucose originates from the liver via glycogenolysis or gluconeogenesis. The liver releases glucose into the circulation after dephosphorylation by the enzyme glucose-6-phosphatase. Muscle cells lack this enzyme and cannot export glucose; therefore, glucose stored in muscle as glycogen is used for glycolysis.

Fatty acids are a primary fuel source for cardiac muscle and resting skeletal muscle. β-oxidation of fatty acids is entirely aerobic, so it is not surprising that cardiac muscle has a high concentration of mitochondria. Cardiac muscle will increase its use of glucose when glucose is abundant, but still favors fatty acids as the main fuel source. Similarly, resting skeletal muscle can increase its use of glucose in the fed state when there is ample glucose, insulin, and GLUT4. The use of fatty acids by active skeletal muscle can be augmented by the ability of muscle to store moderate

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amounts of triacylglycerol adjacent to mitochondria. However, skeletal muscle relies increasingly on glucose for energy as physical activity increases. Most fatty acids used for energy by muscle are derived from the circulation. Cardiac and skeletal muscle express lipoprotein lipase on the cell surface that will bind to circulating chylomicrons (derived from the intestine following a meal) and VLDL (derived from the liver). The triacylglycerols transported by chylomicrons and VLDL are hydrolyzed by lipoprotein lipase and the fatty acids are transferred into the cell. Muscle cells can also utilize free fatty acids released by adipose tissue and transported by serum albumin.

Adipose Tissue

Adipose tissue has the ability to store huge amounts of triacylglycerols and thus serves as an energy reservoir in the body. Triacylglycerols derived from the diet are transported by chylomicrons to adipose tissue where lipoprotein lipase hydrolyzes the triacylglycerols, facilitating the transfer of fatty acids into the cell. In a similar manner, triacylglycerols derived from the liver are transported by VLDL to adipose tissue. Triacylglycerols secreted by the liver come from the catabolism of chylomicron remnants as well as hepatic synthesis of fatty acids from nonlipid precursors, including "excess" glucose and fructose. Immediately following the uptake of fatty acids into adipocytes, the fatty acids are esterified with glycerol-3-phosphate to form triacylglycerols. The action of lipoprotein lipase does not facilitate the transfer of glycerol into the cell, so adipocytes rely on the presence of glucose as the source of glycerol-3-phosphate. The free glycerol may be transported to the liver and used as a precursor for gluconeogenesis.

Adipocytes express GLUT4 on the cell surface, which promotes glucose uptake when blood glucose levels are elevated. Glucose is converted to glucose-6-phosphate and rapidly enters glycolysis. The glycolytic pathway provides glycerol-3-phosphate for triacylglycerol assembly (as mentioned above) and pyruvate that can be converted to acetyl-CoA. Some acetyl-CoA may be oxidized via the TCA cycle to address the energy needs of the cell, while the remaining is used for fatty acid synthesis. Recall that lipogenesis requires the reducing power of NADPH. Some of the glucose-6-phosphate is directed into the pentose phosphate pathway to supply the necessary NADPH. The rate of lipogenesis is higher in the liver than in adipose tissue when measured on a gram-per-gram basis. However, the mass of adipose tissue can be many times greater than liver, especially in obese individuals, demonstrating that an overabundance of carbohydrate can contribute significantly to adiposity.

When dietary energy is in short supply, the triacylglycerols in adipose tissue are hydrolyzed and released as free fatty acids into the circulation where they bind to albumin for transport to other tissues. Many tissues in the body use fatty acids as a fuel, most notably cardiac and skeletal muscle.

Brain

Glucose is the primary fuel used by the brain and nerve cells. Under normal conditions, glucose is the sole energy source, but the brain can adapt to using ketone bodies during prolonged energy deficit as occurs when consuming energy-restricted diets and in starvation. The brain cannot use fatty acids because these large molecules do not transport across the blood–brain barrier. The brain requires a constant and relatively large supply of glucose. Unlike skeletal muscle whose energy requirements are highly variable, mental activity does not increase energy utilization by the brain. The brain accounts for about 20–25% of total energy used in the body and the majority of glucose removed from the blood when at rest.

Maintaining adequate blood glucose levels is imperative for normal brain function. In the short term when the diet is unable to supply adequate amounts of glucose, the liver releases glucose into the circulation. Liver glucose is derived from the breakdown of glycogen and from gluconeogenesis using noncarbohydrate precursors (lactate, glycerol, and certain amino acids). Prolonged energy deficit leads to accelerated breakdown of triacylglycerols in adipose tissue, resulting in an overabundance of fatty acids that the liver oxidizes to acetyl-CoA. The acetyl-CoA is converted to ketone bodies that the brain and other tissues can convert back to acetyl-CoA and use for ATP production via the TCA cycle and oxidative phosphorylation.

Red Blood Cells

Red blood cells rely exclusively on glucose as their only energy source under all metabolic conditions. During their development, red blood cells lose their organelles, including mitochondria. Without mitochondria, anaerobic glycolysis is the only means of producing ATP. The metabolic advantage is that red blood cells are unable to consume any of the oxygen they transport. The disadvantage is that glycolysis is an inefficient means of producing ATP from glucose. However, the end product of glycolysis (pyruvate) is quickly converted to lactate, released into the blood plasma, and taken up by the liver. The lactate is then used to synthesize glucose via gluconeogenesis and released back into the circulation.

Kidneys

The kidneys require about 10% of the total energy used by the body. Their main function is to produce urine and, in the process, remove metabolic waste products from the blood plasma. The kidneys filter the plasma approximately 60 times per day. Most of the constituents of plasma filtered by the kidney are desirable, such as glucose and water, and need to be retained. Reabsorbing these constituents in the kidney tubules requires substantial amounts of energy.

The role of the kidneys in helping maintain energy homeostasis has not been studied as thoroughly as other major organs, although their contribution appears to have

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been underappreciated. It is useful to think of the kidneys as two separate organs, with glucose utilization occurring mostly in the renal medulla and glucose synthesis and secretion occurring in the renal cortex [2]. These separate activities are the result of different enzymes located within each region of the kidney. The renal medulla is similar to the brain in that it requires glucose for energy, whereas the renal cortex uses fatty acids as a primary fuel source under normal conditions. Cells of the renal medulla are able to convert glucose to glucose-6-phosphate for entry into glycolysis; however, they lack glucose-6-phosphatase and are unable to release glucose into the circulation. In contrast, cells of the renal cortex possess gluconeogenic enzymes—they lack phosphorylating capacity and cannot synthesize glycogen—and therefore can make and release glucose. The renal cortex increases glucose synthesis during prolonged starvation and may contribute up to half of the circulating glucose. Interestingly, any lactate produced as a result of glycolysis in the renal medulla can be used by the renal cortex for gluconeogenesis.

THE FED-FAST CYCLE

The best way to appreciate the integration of metabolic pathways and the involvement of different organs and tissues in metabolism is to understand the fed-fast cycle. Humans are "meal eaters" and typically consume food at routine times, followed by periods of not eating. Most meals provide significantly more energy than is needed at that moment, which triggers regulatory hormones and enzymes designed to capture and store the excess energy largely as glycogen and triacylglycerols in tissues equipped to handle such molecules. Because glucose is a major fuel for tissues, it is important that glucose homeostasis be maintained, whether the person has just consumed food or is in a fasting state. If the period since the last meal is short (less than 18 hours), the mechanisms used to maintain glucose homeostasis are different from those used if the fasting state is prolonged. The extent to which different organs are involved in carbohydrate, fatty acid, and amino acid metabolism varies within the fed-fast cycles that underlie the eating habits of humans. A fed-fast cycle can be divided into four states, or phases:

- the fed state, lasting about 3 hours after a meal is ingested and characterized by insulin secretion
- the postabsorptive state, occurring from about 3 to 18 hours following the meal and accompanied by a rise in glucagon secretion
- the fasting state, lasting from 18 hours to about 2 days without additional intake of food and accompanied by further increases in glucagon
- the starvation state or long-term fast, a fully adapted state of food deprivation lasting longer than about 2 days.

The time frames assigned to each phase are only approximate and are strongly influenced by factors such as activity level, the caloric value and nutrient composition of the meal, and the person's metabolic rate. While somewhat variable, the established time frames represent distinctive metabolic events that characterize each phase. The following discussion highlights these hallmark events that occur as time extends beyond a person's last meal. In a normal eating routine, only the fed and postabsorptive (early fasting) states will apply, although prolonged energy deprivation will occur in extreme dieting, involuntary starvation, and certain metabolic diseases.

The Fed State

Figure 7.6 illustrates the disposition of glucose, fat, and amino acids among the major tissues during the fed state. A primary indicator of the fed state is the release of insulin by the β-cells of the pancreas in response to increased blood glucose levels (discussed in detail later in this chapter). The liver is the first tissue to have the opportunity to use dietary glucose. Only some glucose is retained in the hepatocyte on first pass, while about two-thirds passes into the systemic circulation. Glucose that is retained may enter glycolysis or, to a lesser extent, be converted into glycogen. Liver glycogen is preferentially made from newly synthesized glucose. Even in the fed state, when ample dietary glucose is present, gluconeogenesis continues to function as a result of lactate returning to the liver mainly from glycolysis occurring in red blood cells and, under certain conditions, skeletal muscle (discussed later in this chapter). Red blood cells do not have mitochondria and therefore cannot oxidize fatty acids or glucose aerobically; they can oxidize glucose only anaerobically and produce lactate. The preferential use of gluconeogenic precursors for glycogen synthesis is further encouraged by the low phosphorylating activity (high K_m) of hepatic glucokinase at physiological concentrations of glucose.

Most dietary glucose enters the systemic circulation and is delivered to red blood cells, skeletal muscle, the brain and nervous tissue, adipose tissue, and other tissues of the body. Red blood cells and the brain rely on glucose for energy and have no metabolic mechanisms by which glucose or fatty acids can be converted to energy stores. These tissues cannot make glycogen or store triacylglycerols. Glucose available to these tissues is oxidized immediately to produce ATP. On the other hand, skeletal muscle can store glucose as glycogen and a moderate amount of fatty acids and triacylglycerols in the fed state. With the exception of red blood cells, all of the tissues included in Figure 7.6 actively catabolize glucose for energy by glycolysis and the TCA cycle.

When available glucose or its gluconeogenic precursors exceed the glycogen storage capacity of the liver, the excess glucose can be converted to fatty acids (and triacylglycerols), as shown in Figure 7.3. The conversion

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Figure 7.6 Disposition of dietary glucose, amino acids, and triacylglycerols in the fed state. Abbreviations: RBC, red blood cells; TAG, triacylglycerols.

of glucose to fatty acids appears to occur only in the fed state when energy intake exceeds energy expenditure. Chronic overconsumption of carbohydrate can therefore lead to triacylglycerol accumulation in the liver as well as increased secretion of triacylglycerol-rich VLDL into the circulation. The VLDL deliver their lipid cargo to adipose tissue for storage and may contribute to increased body fat. Adipose tissue itself uses glucose as a precursor for both the glycerol and fatty acid components of triacylglycerols, although most triacylglycerols are delivered to adipocytes from circulating lipoproteins.

The Postabsorptive State

With the onset of the postabsorptive state, tissues can no longer derive energy directly from ingested macronutrients, but instead must begin to depend on fuel sources already in the body (Figure 7.7). During the short period of time marking this phase (3–18 hours after eating), hepatic glycogenolysis is the major provider of glucose to the blood, which transports it to other tissues for use as fuel. When glycogenolysis is occurring, the synthesis of glycogen and triacylglycerols in the liver is diminished, and the *de novo* synthesis of glucose (gluconeogenesis) becomes a more important contributor in maintaining blood glucose levels. Each of these events is controlled largely by glucagon secreted by the pancreas in response to declining blood glucose.

Lactate, formed in and released by red blood cells, is a constant noncarbohydrate carbon source for hepatic gluconeogenesis. Skeletal muscle may also contribute lactate resulting from anaerobic glycolysis. The glucose–alanine cycle, in which alanine returns to the liver from muscle cells, also becomes important (see Figure 6.35). The alanine is then converted to pyruvate by the transfer of the amino group to α-ketoglutarate as the first step in the gluconeogenic conversion of alanine in the liver. Alanine cannot be converted to glucose in skeletal muscle. In the postabsorptive state, glucose provided to the muscle by the liver comes primarily from the recycling of lactate and alanine and, to a lesser extent, from hepatic glycogenolysis. Muscle glycogenolysis provides glucose as fuel only for muscle cells in which the glycogen is stored because muscle lacks the enzyme glucose-6-phosphatase, which converts glucose-6-phosphate to free glucose. Once phosphorylated in the muscle, glucose is trapped there and cannot leave except as lactate or alanine.

The brain is an extravagant consumer of glucose, oxidizing it for energy and releasing no gluconeogenic precursors in return. At rest, the brain uses about 20–25% of the available energy even though it is only about 2% of the body by weight. Mental activity does not increase energy utilization by the brain. The rate of

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Figure 7.7 Distribution of fuel molecules in the postabsorptive state. Abbreviations: RBC, red blood cells; TAG, triacylglycerols.
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glucose use in the postabsorptive state is greater than the rate of glucose production by gluconeogenesis, and thus the stores of liver glycogen begin to diminish rapidly. In the course of an overnight fast, nearly all reserves of liver glycogen are depleted.

Fatty acids released from adipose tissues are another valuable source of energy for tissues that can oxidize fatty acids via the TCA cycle. The brain, nerve cells, and red blood cells are unable to use fatty acids, but both cardiac and skeletal muscle are well adapted to using fatty acids. The liver can also oxidize fatty acids for energy in the absence of insulin, which promotes fatty acid synthesis rather than oxidation. The glycerol that results from triacylglycerol hydrolysis in the adipose tissue is released into the circulation and used as a gluconeogenic precursor by the liver. Free glycerol—that which is not

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phosphorylated or attached to fatty acids—is not used in the adipocyte and is released to the blood. Plasma free glycerol levels have thus been used as an indication of triacylglycerol turnover in adipose tissue.

The Fasting State

The postabsorptive state evolves into the fasting state after 18–48 hours of no food intake. Particularly notable in the liver is the increase in gluconeogenesis that occurs in the wake of hepatic glycogen depletion (Figure 7.8). Amino acids from muscle protein breakdown provide the chief substrates for gluconeogenesis during this time, although the glycerol from lipolysis and the lactate from red blood cells (and skeletal muscle if exercising anaerobically) continue to provide gluconeogenic precursors. The release of fatty acids from adipose tissues continues to occur during the early fasting state at about the same or slightly higher rate as during the postabsorptive state. This supplies many tissues with fatty acids for ATP production, while the glycerol is converted to glucose in the liver.

Figure 7.8 Distribution of fuel molecules in the fasting state.

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The shift to gluconeogenesis using amino acids during the fasting state is mediated by the increased secretion of glucagon and cortisol. Proteins are hydrolyzed in muscle cells at an accelerated rate, providing amino acids for gluconeogenesis. The high rate of breakdown of muscle protein is accompanied by large daily losses of nitrogen through the urine. Of all the amino acids, only leucine and lysine cannot directly contribute to gluconeogenesis because they are ketogenic. However, these two amino acids can nonetheless be used for energy due to their conversion to acetyl-CoA and ketone bodies (acetoacetate and β-hydroxybutyrate), thus providing a source of energy for the brain, heart, and skeletal muscle.

An amino acid of particular significance during the fasting state is alanine, which is involved in the alanine–glucose cycle (see Figure 6.35). During fasting, as muscle protein breaks down to amino acids, the nitrogen from the amino acids is transaminated to α-ketoglutarate (formed in the TCA cycle) to make glutamate. The α-amino group from glutamate is then transaminated to pyruvate (formed from glycolysis) to make alanine. The alanine enters the bloodstream and is transported to the liver, where it again transaminates its amino group to α-ketoglutarate. Alanine is converted to pyruvate, and α-ketoglutarate is converted to glutamate. This cycle serves several functions. It removes the nitrogen from muscle during a period of high proteolysis and transports it to the liver in the form of alanine. This process also transfers the carbon structure of pyruvate to the liver, where it can be made into glucose through gluconeogenesis. The synthesized glucose can be transported back to the muscle and used for energy by that tissue.

Glutamine also plays a central role in transporting and excreting amino acid nitrogen, which is greatly increased during the fasting state. Many tissues, including the brain, form glutamine from glutamate and generate ammonia. In the form of glutamine, the ammonia can then be released from the tissues and carried through the blood to the liver or kidneys for excretion as urea or ammonium ion, respectively. Figure 7.9 gives an overview of organ cooperation and other aspects of amino acid metabolism. See also Chapter 6, "Interorgan 'Flow' of Amino Acids and Organ-Specific Metabolism," for a more detailed discussion of amino acid metabolism, particularly Figure 6.37.

The Starvation State

If the fasting state persists and progresses into a starvation state (often referred to as a long-term fast), a more dramatic metabolic fuel shift occurs, this time in an effort to spare body protein. This new priority is justified by the vital physiological importance of many body proteins such as hemoglobin, which is necessary for the transport of oxygen to tissues. Several important changes in metabolism characteristic of the starvation state occur in order to spare protein: (1) accelerated lipolysis, (2) increased use of fatty acids as fuel in certain tissues, (3) increased use of glycerol for gluconeogenesis, and (4) increased ketone body synthesis and utilization (Figure 7.10).

The protein-sparing shift to lipolysis makes use of the ample triacylglycerol stores in most people. Free fatty acids are released by adipose tissue, becoming the primary fuel for the kidneys, liver, heart, and skeletal muscle. Hydrolysis of triacylglycerols also provides glycerol, now the primary source of carbon atoms for gluconeogenesis in the liver. Lactate is still a gluconeogenic precursor because red blood cells continually produce lactate from glycolysis under all metabolic conditions. And while muscle protein breakdown is significantly diminished, muscle cells still release some amino acids (notably alanine and glutamine), which can be used for gluconeogenesis. During this time the kidneys become a major supplier of glucose through gluconeogenesis, apparently using glutamine and possibly glycerol as substrates, although research on the role of the kidney is somewhat sparse [2]. Total production of glucose in humans during starvation is about 80 g/day.

Eventually, the use of TCA cycle intermediates for gluconeogenesis depletes the supply of oxaloacetate (see Figure 3.31). Low levels of oxaloacetate, coupled with rapid production of acetyl-CoA from fatty acid catabolism, cause acetyl-CoA to accumulate, favoring formation of acetoacetyl-CoA and ketone bodies in the liver. The ketone bodies are then released into the blood for delivery to tissues. Skeletal muscle, heart, and brain preferentially oxidize the ketone bodies instead of glucose via the TCA cycle. In fact, after several weeks of starvation, about two-thirds of the biological fuel for the brain comes from β-hydroxybutyrate and acetoacetate [3]. Table 7.2 shows the dramatic increase in ketone body utilization that occurs during many days of starvation. The kidneys also produce ammonia (NH_3) that helps neutralize the acidity associated with ketone bodies. Furthermore, although cardiac and skeletal muscle can use fatty acids, they also shift to using ketone bodies when available. These shifts away from using glucose help to conserve the blood glucose for tissues that depend solely on glucose as a fuel source. As long as ketone bodies are maintained at a high concentration by increased lipolysis and hepatic fatty acid oxidation, the need for glucose and gluconeogenesis is reduced, thus sparing valuable protein.

Survival time in starvation depends mostly on the quantity of triacylglycerols stored before starvation. Stored triacylglycerols in the adipose tissue of a person of normal weight and adiposity can provide enough fuel to sustain basal metabolism for about 3 months. A very obese adult probably has enough fat calories stored to endure a fast of

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Figure 7.9 Interchanges of selected amino acids and their metabolites among body organs and tissue.
Source: Modified from Munro, H.N., "Metabolic Integration of Organs in Health and Disease," Journal of Parenteral and Enteral Nutrition 1982, 6; 4:271–279. Copyright © 1982 by Sage Publications. Reprinted by permission of SAGE Publications.

more than a year, but physiological damage and even death could result from the accompanying extreme ketoacidosis. When triacylglycerol reserves are gone, the body begins to use essential protein, leading to the loss of liver and muscle function.

To summarize, Figure 7.11 illustrates the changes that occur in plasma concentration of fuel molecules following a single meal. The gradual decrease in glucose during the postabsorptive state stimulates glucagon and inhibits insulin secretion by the pancreas. In normal eating patterns, the postabsorptive phase would be reversed by food consumption and thus prevent the more dramatic changes that occur with prolonged food deprivation. Continued declines in glucose during fasting and starvation states cause greater secretion of glucagon. Increased lipolysis in adipose tissue and subsequent rise in free fatty acids cause the liver to produce significant amounts of ketone bodies.

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Figure 7.10 Distribution of fuel molecules in the starvation state.

HORMONAL REGULATION OF METABOLISM

The organs of the endocrine system secrete hormones that play a major role in regulating metabolism. Tissues and cells that respond to hormones are called target tissues and cells because they express membrane receptors to which the hormones bind. The act of binding triggers a series of intracellular reactions (referred to as signal transduction) leading to a metabolic response. Refer to Figure 3.10 for a brief overview of the insulin signaling pathway and the metabolic events resulting from insulin binding to its receptor. Hormones that control metabolism may be generally categorized as those promoting anabolic reactions and energy storage (insulin) versus those that promote catabolic reactions and energy utilization (glucagon, epinephrine, and cortisol). Some hormones elicit both anabolic and catabolic responses depending on the target tissue (growth hormone). Table 7.3 summarizes

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Table 7.2 Fuel Metabolism in Starvation

Fuel Exchanges and Consumption	Amount Formed or Consumed in 24 Hours (g)	
	Day 3	Day 40
Fuel Use by the Brain		
Glucose	100	40
Ketone bodies	50	100
All other use of glucose	50	40
Fuel Mobilization		
Adipose tissue lipolysis	180	180
Muscle protein degradation	75	20
Fuel Output of the Liver		
Glucose	150	80
Ketone bodies	150	150

Source: Adapted from Berg JM, Tymoczko JL, Stryer L. Biochemistry. 6th ed. New York: Freeman. 2007 p. 773.

Figure 7.11 Changes in plasma concentration of fuel molecules following a single meal.

the role of key regulatory hormones and the target tissues most affected. Regulatory hormones involved in nutrient digestion and absorption were discussed in Chapter 2 and are not elaborated on further here.

Insulin

Insulin is the major anabolic hormone that impacts glucose, fatty acids, and protein synthesis and storage [4]. It is a protein secreted by the β-cells of the pancreas in response to rising blood glucose and has a half-life in the circulation of 4–6 minutes. The impact of insulin is critical in the fed state when large amounts of blood glucose must be removed to prevent hyperglycemia. Insulin promotes the uptake of glucose into muscle and adipose tissue by stimulating the translocation of GLUT4 from storage vesicles to the cell surface (see Figure 3.10). It also increases glycogen synthesis in the liver and skeletal muscle and inhibits gluconeogenesis in the liver. With regard to controlling blood glucose levels, insulin is a master hormone without peer. Failure of insulin to function properly causes chronic hyperglycemia and increases the risk of cardiovascular diseases (discussed in detail in Chapter 8).

Insulin also stimulates fatty acid synthesis—using excess glucose and fructose as precursors—that leads to increased triacylglycerol assembly for energy storage. Newly synthesized triacylglycerols in the liver are packaged in VLDL and shipped out to adipose tissue. As an anabolic hormone, insulin inhibits lipolysis in adipose tissue and proteolysis in muscle, while promoting protein synthesis in muscle, liver, and many other tissues expressing insulin receptors.

Table 7.3 Hormonal Regulation of Energy Metabolism

	Insulin	Glucagon	Epinephrine	Cortisol	Growth hormone
Gluconeogenesis	↓ Liver	↑ Liver		↑ Liver	
Glycogenolysis		↑ Liver	↑ Liver, skeletal muscle	↑ Liver, skeletal muscle	↑ Liver
Glycogenesis	↑ Liver, skeletal muscle	↓ Liver	↓ Liver, skeletal muscle		
Glycolysis	↑ Liver, skeletal muscle	↓ Liver	↑ Skeletal muscle		
Glucose uptake by GLUT4	↑ Liver, heart, skeletal muscle				
Fatty acid oxidation		↑ Liver			
Fatty acid synthesis	↑ Adipose tissue, skeletal muscle, liver	↓ Adipose tissue, liver	↓ Adipose tissue, skeletal muscle, liver		
Fatty acid uptake from plasma lipoproteins	↓ Skeletal muscle; ↑ Adipose tissue		↑ Skeletal muscle		↑ Skeletal muscle, liver
Triacylglycerol synthesis	↑ Adipose tissue	↓ Liver			
Triacylglycerol breakdown (lipolysis)	↓ Adipose tissue	↑ Adipose tissue	↑ Adipose tissue, skeletal muscle	↑ Adipose tissue	↑ Adipose tissue
Protein synthesis (translation)	↑ Skeletal muscle, liver				↑ Skeletal muscle, liver
Protein breakdown (proteolysis)	↓ Skeletal muscle			↑ Skeletal muscle	↓ Skeletal muscle
Ketone body production		↑ Liver			

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Glucagon

All metabolic effects of glucagon reflect the need to liberate stored energy for ATP production while maintaining blood glucose levels in the absence of dietary carbohydrate. The metabolic responses elicited by glucagon oppose those of insulin. Therefore, it is a prominent hormone in nonfed states and its concentration in the blood increases as starvation approaches. Glucagon is a protein secreted by the α-cells of the pancreas when blood glucose levels decline and has a half-life in the circulation of 3–6 minutes. The main tissues expressing glucagon receptors are the liver and adipose tissue. In the liver, glucagon causes an increase in gluconeogenesis and glycogenolysis, while inhibiting glycogen synthesis, so that more glucose can be released into the circulation and thus reverse the effects of insulin (Table 7.3). Additional effects include increased lipolysis in adipose tissue (for release of free fatty acids into the circulation) and increased fatty acid oxidation and ketone body production in the liver as starvation progresses. Glucagon also stimulates thermogenesis in brown adipose tissues, presumably to maintain body heat during periods of low or no food intake [5].

Skeletal muscle does not make glucagon receptors and is unresponsive to the hormone. The kidneys do have receptors, although the effect of glucagon in the kidney is not well studied. It is possible that glucagon contributes to the increased gluconeogenesis known to occur in the kidney during starvation.

Epinephrine

Epinephrine is a catecholamine produced in the adrenal medulla from the amino acids phenylalanine and tyrosine (see Figure 6.10). It functions both as a neurotransmitter in the nervous system and as a stress hormone in the circulation. Epinephrine has a half-life in the circulation of 1–2 minutes. It binds to two classes of adrenergic receptors on cell membranes, α and β receptors. The receptors function as part of a cAMP signal transduction cascade, an example of which is shown in Figure 1.9. As a stress hormone, epinephrine can increase cardiac muscle contractions and increase vasodilation and blood flow to skeletal muscle and the liver. Epinephrine levels are known to increase during exercise.

The binding of epinephrine to α receptors in the pancreas inhibits insulin secretion; in the liver and skeletal muscle it stimulates glycogen breakdown and inhibits glycogen synthesis; and in skeletal muscle it stimulates glycolysis. Epinephrine binding to β receptors in the pancreas stimulates glucagon secretion; and in adipose tissue and skeletal muscle it stimulates lipolysis and inhibits fatty acid synthesis. Each of these responses leads to increased blood glucose and free fatty acids, allowing stored fuels to be used when dietary sources are insufficient.

Cortisol

Cortisol is a corticosteroid hormone produced in the adrenal cortex from cholesterol (see Figure 5.9). It is released from the adrenal cortex in response to low blood glucose levels and has a half-life of about 1 hour in the circulation. Cortisol travels in the circulation bound to albumin and corticosteroid-binding globulin (also called transcortin). After being delivered to target cells, cortisol passes freely through plasma membranes, then binds to intracellular cortisol receptors residing in the cytosol.

In the liver, cortisol stimulates gluconeogenesis and glycogenolysis. It also increases the activity of glucose-6-phosphatase, thus promoting the release of free glucose into the circulation. In skeletal muscle, cortisol stimulates glycogenolysis and inhibits the translocation of GLUT4 to the cell membrane. Cortisol also stimulates lipolysis in adipose tissue, thus providing free fatty acids for energy use in the liver, kidneys, and cardiac and skeletal muscle. Persistently high levels of cortisol, as seen in prolonged fasting (starvation) and vigorous exercise, stimulate protein breakdown in skeletal muscle so amino acids may be used for gluconeogenesis.

Growth Hormone

Growth hormone (GH), also known as somatotropin, is a protein hormone produced by the anterior pituitary gland. It is transported in the circulation bound to GH-binding protein and has a half-life of 12–16 minutes. GH is secreted in response to a variety of stimuli, including fasting and strenuous exercise. GH receptors are present in the liver, adipose tissue, heart, skeletal muscle, kidney, brain, and pancreas.

In adipose tissue, GH stimulates lipolysis and the release of fatty acids into circulation. This lipolytic action occurs predominantly in the visceral adipose tissue and to a lesser extent in the subcutaneous adipose tissue. It also stimulates lipoprotein lipase in skeletal muscle, thus promoting triacylglycerol uptake from circulating VLDL. This may seem paradoxical in view of increased lipolysis in adipocytes, but GH is tissue-specific and has no effect on lipoprotein lipase in adipose tissue.

In the liver, GH increases triacylglycerol uptake from VLDL by inducing the expression of lipoprotein lipase and hepatic lipase. Once again, the action of GH seems paradoxical since the liver produces VLDL. Apparently increased lipase activity in the liver occurs mainly in starvation as a way to recapture energy from the circulation. GH also functions to conserve protein by inhibiting protein breakdown while stimulating protein synthesis [6].

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EXERCISE AND NUTRITION

Movement of the human body requires the contraction of skeletal muscle. Significant amounts of energy may be needed to support muscle function, especially in people who are physically active. Some people may be physically active due to the nature of their jobs, while others engage in exercise—defined as planned, structured physical activity to enhance physical fitness. Whether it be an average person wishing to stay physically fit or an elite athlete, the amount and type (and timing) of nutrient intake can influence health and performance outcomes. Only in recent years has the connection between exercise and nutrition been fully appreciated, which has led to an increase of research on the topic. The following sections address the energy demands of skeletal muscle, the fuel sources available to muscle under different types of exercise, and the special application of sports nutrition.

Muscle Function

Skeletal muscle is composed of striated cells (called myocytes or muscle fibers) that generally extend the length of the muscle. The main proteins in muscle are actin and myosin; upon stimulation, myosin ATPase hydrolyzes ATP that provides the energy for muscle contraction. Muscle fibers also contain myoglobin that can store oxygen to be quickly used when needed. The typical red color of muscle is due to the presence of myoglobin. On the basis of their metabolic characteristics, muscle fibers are classified as type I, type IIa, and type IIx. Type I muscle fibers are also called oxidative, slow-twitch fibers. Type I fibers contain a large number of mitochondria and a relatively high concentration of myoglobin, both features designed to support aerobic metabolism. These fibers are surrounded by more capillaries than other fiber types in order to facilitate oxygen transport. Type I fibers are capable of oxidizing fatty acids and glucose to CO_2 and H_2O via the TCA cycle and oxidative phosphorylation. Because of their reliance on aerobic metabolism, the speed of contraction of type I fibers is considered slow but resistant to fatigue. In contrast are type IIx fibers, also called glycolytic, fast-twitch fibers. Type IIx fibers have significantly fewer mitochondria and less myoglobin, giving these fibers a white appearance. This type of muscle fiber has increased myosin ATPase and an active glycolytic pathway for rapid ATP replenishment in the absence of oxygen. Type IIx fibers have an increased ability to store glycogen and higher phosphofructokinase activity to support glycolysis. The metabolic characteristics of type IIa fibers lie between those of types I and IIx fibers, having both glycolytic (fast) and oxidative (slow) function. Type IIa fibers are red in appearance and contain intermediate levels of both mitochondria and myoglobin. They are resistant to fatigue, but have relatively high myosin ATPase activity and can contract rapidly when necessary.

The combination of fibers is an important property of skeletal muscle that allows the human body to respond to a variety of physical demands. The presence of fast-twitch fibers allows for a rapid and intense muscle contraction, whereas the presence of slow-twitch fatigue-resistant fibers allows for muscular endurance. When a low amount of force is required, muscle contraction involves predominantly type I fibers. Increasing force requirements will recruit progressively more type IIa fibers and lastly type IIx fibers when the greatest force is required. Each muscle in the body exhibits different proportions of fiber types depending on its function. For example, muscles involved in maintaining posture engage in prolonged but relatively low-force contractions and thus have a high proportion of type I fibers. Muscles that engage in rapid or high-force contractions, such as jaw muscles, have a high proportion of type IIa and IIx fibers. The proportion (relative number) of each type of muscle fiber a person has is genetically determined; however, with appropriate exercise training, the metabolic potential of muscle can be influenced by effecting changes in fiber size and its components.

Exercising muscle causes several changes in hormones and other regulatory molecules. Such changes are required to support the increasing energy demands of muscle while simultaneously maintaining blood glucose levels for other tissues that rely on glucose for energy. It is well known that exercise increases the circulating levels of epinephrine, cortisol, and growth hormone. Collectively these regulatory molecules increase gluconeogenesis, glycogenolysis, and lipolysis, thus promoting the release of more glucose and fatty acids into the bloodstream. Most of the fatty acids and some glucose can be taken up by muscle and used for energy, whereas the remaining glucose is intended for the brain and red blood cells. Exercise of higher intensity also activates AMPK, resulting in increased lipolysis and fatty acid oxidation, as well as increased GLUT4 translocation to the muscle cell surface for glucose uptake independent of insulin (discussed earlier in this chapter). Furthermore, physical inactivity is associated with chronic inflammation and related conditions, including atherosclerosis and insulin resistance. Recent studies have suggested that exercise, even a single bout of exercise in untrained adults, stimulates the secretion of cytokines from skeletal muscle that promotes the clearance of glucose and lipoproteins from the circulation and may improve insulin sensitivity [7].

An important tool used to measure exercise capacity is the concept of maximum oxygen consumption (**VO_2 max**). As physical work increases, the volume of oxygen taken up by the body also increases. The VO_2 max is defined as the point at which a further increase in the intensity of the exercise no longer results in an increase in the volume of oxygen uptake. VO_2 max is unique for each person and is generally expressed in milliliters of oxygen consumed per kilogram of body mass per minute ($mL \times kg^{-1} \times min^{-1}$). As a person goes from an untrained state to a trained state,

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the VO_2 max increases. A sedentary (untrained) person may have a VO_2 max of 30 or 40, whereas a trained runner may have a VO_2 max of 80 or 90. Consequently, VO_2 max can be used as a measure of cardiovascular fitness. Another application of VO_2 max is in quantifying the intensity of exercise. If the VO_2 max for an individual is known, then the intensity of exercise can be expressed as a percentage of VO_2 max. When at rest, the relative intensity is < 10% VO_2 max; exercise intensity during light physical activity such as slow walking might correspond to 25% VO_2 max. Techniques used to measure VO_2 max are discussed in Chapter 8.

Energy Sources in Resting Muscle

Energy utilization by skeletal muscle varies greatly depending on the level of physical activity. Even at rest, skeletal muscle needs a minimum of energy to maintain basal function that includes active transport, replenishing glycogen and triacylglycerol stores, and the continuous synthesis and breakdown (turnover) of proteins. Skeletal muscle, despite comprising nearly half of the body's mass in nonobese people, accounts for only 20–25% of the body's energy use when at rest. These energy needs are met primarily by the oxidation of fatty acids and glucose, although their relative contribution depends on the phase of the fed-fast cycle. Glucose is the preferred fuel source in the fed state when ample glucose is available. Blood glucose is the major source rather than stored glycogen. In the postabsorptive and fasting states, resting skeletal muscle will shift to using fatty acids, as glucose becomes more precious to other tissues. Free fatty acids released from adipose tissues provide the major energy source under these conditions, although resting muscle can also obtain some fatty acids from circulating VLDL via lipoprotein lipase on the cell surface. The shift in fuel sources that occurs in resting muscle is regulated by factors induced by the fed-fast cycle, as previously discussed in this chapter.

Muscle ATP Production during Exercise

Contracting muscle fibers require ATP. Skeletal muscle is somewhat unique among the body's tissue because its need for ATP is highly variable and entirely dependent on the intensity and duration of muscle contraction. In order to accommodate such wide-ranging energy demands, skeletal muscle possesses three energy systems that supply ATP:

- the ATP-phosphocreatine system
- the lactic acid system (anaerobic glycolysis)
- the oxidative system (aerobic metabolism).

The ATP-Phosphocreatine System

The ATP-phosphocreatine system is a cooperative system in muscle cells using the high-energy phosphate bond of phosphocreatine to quickly regenerate ATP (see Figure 3.22 and Figure 6.24). When the body is at rest, energy needs of skeletal muscle are fulfilled by glucose and fatty acid oxidation because the low demand for oxygen can easily be met by oxygen exchange in the lungs and by the oxygen carried to the muscle by the cardiovascular system. At the onset of physical activity, the energy requirement of contracting muscle is initially met by existing ATP. However, stores of ATP in muscle fibers are limited, providing enough energy for only a few seconds of maximal exercise. As ATP levels diminish, they are replenished rapidly by the transfer of high-energy phosphate from phosphocreatine to ADP to regenerate ATP. The muscle fiber concentration of phosphocreatine is only four to five times greater than that of ATP, and therefore most energy furnished by this system is diminished after the first 15–25 seconds of strenuous exercise. As the ATP-phosphocreatine system is exhausted, the lactic acid system (anaerobic glycolysis) picks up to produce more ATP. Performance demands of high intensity and short duration such as weightlifting, 100-m sprinting, gymnastics, and various short-duration field events benefit most from the ATP-phosphocreatine and lactic acid systems. Lower-intensity activity may allow skeletal muscle to use the combined ATP-phosphocreatine and lactic acid systems for several minutes.

The Lactic Acid System

This system involves the glycolytic pathway, which anaerobically produces ATP through substrate phosphorylation by the incomplete breakdown of one molecule of glucose into two molecules of lactate in skeletal muscle (see Figure 3.17). The sources of glucose are primarily muscle glycogen and, to a lesser extent, circulating glucose. The system can generate ATP quickly for high-intensity exercise. The rapid rise in cellular AMP resulting from ATP hydrolysis is a strong allosteric stimulator of phosphofructokinase, the most prominent regulatory enzyme in glycolysis (Table 7.1). As pointed out in Chapter 3, the lactic acid system is not efficient from the standpoint of the quantity of ATP produced. However, because the process is so rapid, the relatively small amount of ATP is produced quickly and supplies important energy for a short duration.

The lactate produced by this system rapidly crosses the muscle cell membrane into the bloodstream, from which it can be cleared by other tissues (including the liver) for aerobic production of ATP or gluconeogenesis. If the rate of production of lactate exceeds its rate of clearance, blood lactate accumulates. The quantity of lactate released at the onset of strenuous exercise is low, but when lactate

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accumulates, it lowers the pH of the blood and is one cause of fatigue. Under such circumstances, exercise cannot continue for long periods.

Muscle fibers engage the lactic acid system to provide a rapid source of energy in the absence of oxygen. When an inadequate supply of oxygen prevents the aerobic system from furnishing sufficient ATP to meet the demands of exercise, the lactic acid system will continue to function for a brief time, resulting in what is called "oxygen debt." Although the lactic acid system is operative as soon as strenuous exercise begins, it becomes the primary supplier of energy only after phosphocreatine stores in the muscle are depleted, which occurs after about 15–25 seconds at maximal exercise. As a backup to the ATP-phosphocreatine system, the lactic acid system becomes important in high-intensity anaerobic power events that last from about 20–75 seconds, such as sprints of up to 800 meters and swimming events of 100 or 200 meters. During such events the anaerobic lactic acid system and the aerobic oxidative system each supply about 50% of the energy at maximal exercise [8].

The Oxidative System

The oxidative system involves the TCA cycle and oxidative phosphorylation to completely catabolize glucose, fatty acids, and some amino acids to CO_2 and H_2O. During the onset of maximal exercise, glucose and fatty acids provide nearly all of the aerobic energy for ATP production. The source of glucose and fatty acids may be drawn from that which is stored in skeletal muscle or it may be taken up from the circulation. The oxidative system is highly efficient from the standpoint of the quantity of ATP produced. Because oxygen is necessary for the system to function, a person's level of cardiovascular fitness, as measured by VO_2 max, becomes an important factor in exercise capacity. Contributing factors to VO_2 max include the ability of blood to deliver oxygen, glucose, and fatty acids to exercising muscle; pulmonary ventilation; oxygenation of hemoglobin; and release of oxygen from hemoglobin at the muscle. Inefficiencies in any of these metabolic processes become rate limiting for long-duration exercise at maximal output. The oxidative system is the predominant supplier of energy for forms of exercise lasting longer than 2 or 3 minutes, depending on the intensity of the exercise. Many types of endurance exercise meet these criteria, including distance running, distance swimming, and cross-country skiing.

The contribution of the three energy systems during the first 5 minutes of exercise at maximal output is depicted in Figure 7.12. All systems function at all times, but to varying degrees depending on the intensity and duration of exercise. At the onset, the anaerobic systems (the ATP-phosphocreatine and lactic acid systems) dominate in order to provide "quick energy" for skeletal muscles engaged in immediate bursts of activity. This represents an evolutionary adaptation, called flight or fight, which provides skeletal muscle with readily available ATP to allow an individual to escape danger. The ATP-phosphocreatine system is most important during the first few seconds, followed by the lactic acid system. The oxidative system becomes increasingly more important as exercise duration increases. Not surprisingly, the speeds achieved by runners in 100 m are faster than 800 m simply because the oxidative system requires more time to completely metabolize glucose and fatty acids to CO_2 and H_2O, being fully dependent on the body's ability to deliver oxygen to muscle cells.

Figure 7.12 Relative contribution of energy systems during the onset of exercise.

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Fuel Sources during Exercise

Exercise Intensity and Duration

The intensity and duration of exercise dictates which fuel sources are used. Skeletal muscle utilizes mostly glucose and fatty acids, but will use amino acids as the exercise conditions warrant. Recall that resting muscle uses blood glucose as the preferred fuel source in the fed state, but shifts to using circulating fatty acids released from adipose tissue in the postabsorptive and fasting states. When normal daily physical activity is light (20–40% VO_2 max), skeletal muscle preferentially uses fatty acids as the primary fuel, much like in the nonfed states when at rest as a way to conserve blood glucose and muscle stores of glycogen and triacylglycerols. Continued energy expenditure at this low activity level causes adipose tissue to release free fatty acids into the circulation. The rate of lipolysis may increase three times the basal rate when at rest. Triacylglycerol synthesis is also inhibited, further promoting fatty acid release into plasma to meet the energy demand while conserving glucose. As illustrated in Figure 7.13, fatty acid utilization at 25% VO_2 max provides 80–90% of total energy expenditure [9].

As exercise intensity increases to 65% VO_2 max, skeletal muscle relies heavily on stored glycogen and triacylglycerols to meet the increased energy demand (Figure 7.13). Plasma fatty acids from the circulation still contribute significant energy, although it is slightly reduced. With the added contribution of muscle triacylglycerols, fatty acids from both sources provide about half of the total energy for skeletal muscles at this level of exercise intensity. Fatty acids are the favored substrates for exercise intensities of up to about 50% VO_2 max. Within the exertion range of 60–75% VO_2 max, fatty acids are typically being oxidized at their maximum rate due to the limited transfer rate of fatty acids into mitochondria, and therefore glucose becomes an important fuel as exercise intensity increases. Muscle glycogen, rather than plasma glucose, becomes the principal fuel at exercise intensity of 65% VO_2 max, which is equivalent to playing basketball or swimming at a vigorous pace. Although plasma glucose utilization does increase somewhat, it is still a minor contributor to total energy expenditure at this level of exercise.

Figure 7.13 Utilization of fuel sources after 30 minutes of exercise at different exercise intensity.
Source: Adapted from Coyle EF. Substrate utilization during exercise in active people. Am J Clin Nutr. 1995; 61:968S–79S.

As exercise intensity increases to 85% VO_2 max, the relative contribution of carbohydrate oxidation to total metabolism increases sharply (Figure 7.13). The degradation of muscle glycogen, because of its immediate availability, continues to increase and supplies the majority of fuel at 85% VO_2 max. Exercise intensity at this level is very high, as seen in cross-country skiers and middle-distance runners, and cannot be sustained for prolonged periods due to the depletion of glycogen. The contribution of plasma glucose increases approximately three to four times compared to low-intensity exercise as a result of increased hepatic glycogenolysis and gluconeogenesis. Maintenance of blood glucose levels is a priority, even during high-intensity exercise, and several gluconeogenic substrates are available to the liver. Because the high rate of muscle contraction at 85% VO_2 max requires greater involvement of anaerobic glycolysis, lactate accumulates in muscle when oxygen levels are insufficient for the complete oxidation of pyruvate to CO_2 and H_2O. The lactate is released into the circulation and travels to the liver where it is converted back to pyruvate and used for glucose synthesis. The ability of the liver to convert muscle-derived lactate to glucose, and for muscle to take up that glucose and use it in glycolysis, constitutes the Cori cycle (see Chapter 3). In addition, lipolysis in adipose tissue generates glycerol that the liver converts to glucose. Once released into the blood, the glucose can be taken up by the muscle and used for energy. Finally, skeletal muscle produces alanine during normal metabolism that the liver converts to glucose as part of the alanine–glucose cycle (see Figure 6.35).

The use of fatty acids at exercise intensity of 85% VO_2 max declines. Fewer fatty acids are released by adipose tissue into the plasma, resulting in a decreased concentration of plasma fatty acids. This decrease occurs despite a continued high rate of lipolysis in adipose tissues, which causes free fatty acids to accumulate in adipocytes. The buildup of fatty acids has been attributed to insufficient blood flow and albumin delivery of fatty acids from adipose tissue into the systemic circulation [10]. The cessation of exercise is followed by a rapid rise in plasma fatty acid levels after adequate blood flow and albumin transport is restored. The duration of exercise at different levels of intensity also influences the fuel sources used by skeletal muscles. As mentioned earlier, normal daily activities generally represent light work (< 25% VO_2 max), and skeletal muscle preferentially uses fatty acids as the primary fuel. High-intensity exercise

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at 65% VO_2 max for 30 minutes causes muscle to utilize both glucose (glycogen) and fatty acids (triacylglycerols) stored in muscle, as shown in Figure 7.13. In comparison, Figure 7.14 depicts the changes in fuel sources during prolonged exercise lasting 4 hours at a high intensity of 65–75% VO_2 max. Muscle stores of glycogen and triacylglycerols provide about two-thirds of the energy during the first 30 minutes. After 4 hours of high-intensity exercise, glycogen is completely depleted and half of the muscle triacylglycerol stores are used up. This means that for high-intensity exercise to be sustained for prolonged periods, plasma sources of fuel are critical. Plasma fatty acids released from adipose tissue become the main fuel for high-intensity exercise lasting more than 1 hour, as is typical of marathon runners. Plasma glucose also becomes a critical fuel source, although hepatic gluconeogenesis alone cannot keep pace and ingestion of carbohydrates is necessary to sustain high-intensity exercise [9].

Skeletal muscle is limited in its ability to directly use amino acids for energy. However, muscle liberates amino acids as a result of normal protein turnover in the body. Skeletal muscle is a large depot of protein and accounts for 25–35% of all protein turnover, as discussed in detail in Chapter 6. Some of these amino acids can be converted to TCA cycle intermediates (mostly branched-chain amino acids), whereas some can be transported to the liver for gluconeogenesis (mostly alanine and glutamine). Moreover, exercise can affect muscle protein breakdown and synthesis. Endurance exercise tends to increase muscle protein breakdown (possibly through the action of cortisol), while decreasing protein synthesis. Eating protein immediately after endurance exercise can augment protein synthesis. Eating carbohydrate after exercise causes an increase in insulin, which in turn stimulates protein synthesis and inhibits breakdown (see Table 7.3). Resistance training has little effect on protein turnover during exercise, but both protein synthesis and breakdown are accelerated after strenuous resistance training. Based on these observations, it seems logical to consume both protein and carbohydrate to optimize the benefits of resistance training.

Figure 7.14 Utilization of fuel sources during prolonged exercise at 65–75% VO_2 max. Carbohydrate ingestion is needed after 2 hours to maintain plasma glucose concentration and oxidation.

Source: Adapted from Coyle EF. Substrate utilization during exercise in active people. Am J Clin Nutr. 1995; 61:968S–79S

Fatigue

Muscle fatigue has a variety of causes, some of which are related to substrate availability. For example, fatigue occurs when the supply of glucose is inadequate, such as with muscle glycogen depletion or hypoglycemia. Thus, the consumption of glucose is necessary in prolonged exercise such as marathon running and may temporarily delay fatigue. As muscle fatigue begins to set in, exercise intensity must be reduced to match the muscle's ability to oxidize predominantly fatty acids, possibly as low as 30% VO_2 max. The reason for this limitation, and thus the dependence of muscle upon glucose as an energy source, may be based on two factors: (1) oxidation of fatty acids is limited by the enzyme carnitine acyltransferase I (CAT I), which catalyzes the transport of fatty acids across the mitochondrial membrane; and (2) CAT I is known to be inhibited by malonyl-CoA. When availability of glucose to the muscle is high, fatty acid oxidation may be reduced by the inhibition of CAT I by glucose-derived malonyl-CoA [10].

Benefits of Exercise Training

Endurance training increases the ability to perform more aerobically at the same absolute exercise intensity. Several factors aid in this increase. First, endurance-trained muscle exhibits an increase in the number and size of mitochondria. Cardiovascular and lung capacity also increase, and type I muscle hypertrophies. The activity of oxidative enzymes in endurance-trained subjects has been shown to be 100% greater than in untrained subjects at 65% VO_2 max. Endurance training also results in an increased use of fatty acids as an energy source during submaximal exercise. In skeletal muscle, fatty acid oxidation inhibits glucose uptake and glycolysis. For this reason, the trained athlete benefits from the carbohydrate-sparing effect of enhanced fatty acid oxidation during competition because muscle glycogen and plasma glucose are depleted more slowly. This effect largely accounts for the training-induced increase in endurance for prolonged exercise.

Trained athletes tend to have lower plasma fatty acid concentrations and exhibit less adipose tissue lipolysis than untrained counterparts at similar exercise intensity. This finding suggests that the primary source of fatty acids used by the trained athlete is intramuscular triacylglycerol stores, rather than adipocyte triacylglycerols. After exercise, the intramuscular triacylglycerols are replaced,

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utilizing plasma fatty acids supplied by lipolysis in adipose tissues. This process can result in a reduction in the size of the adipose tissue.

Endurance training appears to result in an increased capacity for muscle glycogen storage. Therefore, the trained athlete benefits not only from a slower use of muscle glycogen (as explained earlier), but also from the capacity to have higher glycogen stores at the onset of competition. High muscle glycogen levels allow exercise to continue longer at a submaximal workload. Even in the absence of carbohydrate loading (see the following section), a strong positive correlation exists between initial glycogen level and time to exhaustion, level of performance, or both during exercise periods that last more than 1 hour. The correlation does not apply at low levels of exertion (25–35% VO_2 max), or at high levels of exertion for short periods, because glycogen depletion is not a limiting factor under these conditions. It has been suggested that the importance of initial muscle glycogen stores is related to the inability of glucose and fatty acids to cross the cell membrane rapidly enough to provide adequate substrate for mitochondrial respiration [9].

Carbohydrate Loading (Supercompensation)

Carbohydrate loading is a dietary and exercise strategy to maximize the storage of glycogen in muscle and liver for the purpose of enhancing endurance performance. Glycogen depletion is strongly associated with muscle fatigue, and carbohydrate loading provides greater energy reserves for individuals engaged in prolonged endurance events such as distance running and cross-country skiing. Figure 7.15 illustrates graphically the amount of muscle glycogen formed as a result of the classical regimen and a modified regimen.

The classical regimen for carbohydrate loading resulted from investigations in the late 1960s by Scandinavian scientists [11]. This regimen involved two sessions of intense exercise to exhaustion to deplete muscle glycogen stores, separated by 2 days of a low-carbohydrate diet (< 10%) to "starve" the muscle of carbohydrate. This interval was followed by 3 days of a high-carbohydrate diet (> 90%) and rest. The event would be performed on day 7 of the regimen. The classical method yielded muscle glycogen levels approaching 220 mmol/kg wet weight, more than double the athlete's resting level. However, because of various undesirable side effects of the classical regimen, such as irritability, dizziness, and a diminished exercise capacity, a less stringent regimen of diet and exercise has evolved that produces comparably high muscle glycogen levels. In the modified regimen, runners perform tapered-down exercise sessions over the course of 5 days, followed by 1 day of rest. During this time, 3 days of a 50% carbohydrate diet are followed by 3 days of a 70% carbohydrate diet, generally achieved by consuming large quantities of pasta, rice, or bread. The modified regimen, which can increase muscle glycogen stores 20–40% above normal, has been shown to be as effective as the classical approach, with fewer adverse side effects [12].

In contrast to carbohydrate loading, emerging research suggests that a low-glycogen approach to endurance training may also enhance performance. The strategy is to deliberately train in conditions of low carbohydrate intake to limit glycogen storage. This promotes adaptations in skeletal muscle that increases mitochondria and improves oxidative capacity, particularly fatty acid oxidation. Then a high-carbohydrate meal is consumed immediately prior to an important competition. Some athletes have claimed success using a "train low, compete high" approach, although strategies that create optimal conditions are unknown and a common protocol has not been established [13].

Figure 7.15 Schematic representation of the "classical" and modified regimens of muscle glycogen supercompensation.

Source: From Sherman, W.M., Carbohydrate, muscle glycogen, and muscle glycogen supercompensation, In *Ergogenic Aids in Sport* by Williams, M.H., Champaign, IL: Human Kinetics Publishers, 1983, p. 14. Reprinted by permission.

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SUMMARY

Metabolic pathways are constantly adjusting in response to the energy status of cells, tissues and organs, and the whole body. Humans require frequent input of energy from dietary sources to perform mechanical work, including active transport at the cell level, synthesis of complex molecules, and muscle contractions. Dietary carbohydrates and fats (triacylglycerols) are the primary fuel molecules, although amino acids from dietary protein can also be used for energy when necessary.

All cells of the body require energy to function. The liver, cardiac and skeletal muscle, kidneys, and adipose tissue can use both glucose and fatty acids for energy. The brain and nerve cells cannot use fatty acids and rely on glucose, but can adapt to ketone bodies made from fatty acids during long-term fasting. Red blood cells lack mitochondria and have no oxidative capacity, so they depend solely on glucose and anaerobic glycolysis for energy.

In the fed state, ample energy is consumed in excess of immediate needs, resulting in energy storage as triacylglycerols in adipose tissue and muscle, and as glycogen in liver and muscle. The fed state is characterized by high insulin levels that stimulate anabolic reactions by allosteric regulation of key enzymes. In the postabsorptive state, insulin diminishes and glucagon increases, which causes the release of stored molecules to provide the energy for cellular function between meals or sleeping through the night. Long-term energy deprivation that occurs in starvation can result in severe loss of body fat and muscle mass as the body sacrifices protein to meet critical energy needs.

Energy demands during exercise are strongly influenced by the intensity and duration of exercise. Contracting muscles that require an immediate burst of energy depend on ATP and phosphocreatine inherently present in the muscle fibers, then shift to anaerobic glycolysis as required in the first several seconds of maximal activity. Aerobic oxidation of fatty acids and glucose becomes the major source of energy as muscle contractions continue beyond 2 or 3 minutes.

Skeletal muscle engaged in normal daily activities at low intensity uses primarily fatty acids derived from adipose tissue for energy. As exercise intensity increases, the muscle uses glycogen stores until it is depleted. Skeletal muscle engaged in long-duration, high-intensity exercise becomes increasingly dependent on plasma glucose for energy and continues to use free fatty acids released from adipose tissue.

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Suggested Readings

Brown WMC, Davison GW, McClean CM, Murphy MH. A systematic review of the acute effects of exercise on immune and inflammatory indices in untrained adults. Sports Med. 2015; 1:35.

The article reviews the transient changes in immune and inflammatory markers evoked by a single bout of exercise and the resulting health benefits, including reduced risk of cardiovascular disease.

McArdle WD, Katch FL, Katch VL. *Exercise Physiology: Nutrition, Energy, and Human Performance*. 8th ed. Baltimore: Wolters Kluwer, 2015.

A textbook that provides in-depth coverage of the metabolic principles connecting nutrition, muscle function, energy metabolism, and exercise performance.

Schnyder S, Handschin C. Skeletal muscle as an endocrine organ: PGC-1a, myokines and exercise. Bone. 2015; 80:115–25.

A review article that describes the regulatory molecules secreted by exercising muscle and their metabolic impact on health.

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THE ROLE OF DIETARY SUPPLEMENTS IN SPORTS NUTRITION BY KARSTEN KOEHLER, PhD

At least since the Ancient Olympics, athletes have been exploring possible ways to gain a competitive edge. While the diet provides ample opportunities to maximize performance and recovery during training and competition, it has become an appealing option for many athletes to supplement their diet with isolated nutrients in highly concentrated form. Supplements, particularly those with claimed ergogenic effects, appear as a legal and healthy alternative to performance-enhancing drugs, which are prohibited by the strict antidoping regulations in competitive sports. Who would not be intrigued by "miracle pills" that promise one to run faster, jump higher, and be stronger, all while being safe and legal?

Considering this appeal, it is not surprising that athletes are much more prone to using supplements than the average population. In fact, depending on the sport and the level of competition, it may be hard to find a single athlete who does not use supplements on a regular basis [1]. Many athletes use supplements for the obvious motive of improving their performance and health, but supplements use is also often done in an attempt to emulate the behavior of opponents and peers [2]. These trends can be observed not only in competitive athletes but also in the world of recreational sports, which is eminent by the ever-increasing market of "sport supplements." This market has been expanding both in revenue as well as in the number of products on the market. Due to varying product definitions and categorizations, it is difficult to estimate the true size of the market, but it is safe to assume that annual revenue from sport supplements is in the multibillion-dollar range.

However, contrary to popular beliefs about supplements among competitive and recreational athletes, scientific evidence for potential ergogenic effects is rather scarce. In fact, only a few selected substances are unanimously considered as performance enhancing, and their ergogenic properties are limited to certain sports and activities. Furthermore, for most substances available on the market, research has failed to demonstrate the claimed ergogenic effects or, more importantly, scientifically valid studies on these effects are completely lacking. Despite the lack of concrete evidence, many supplements are heavily advertised using anecdotal reports from athletes or pseudoscientific publications, which makes it difficult for the layperson to tell fact from fiction. Considering that many athletes obtain their supplement knowledge from coaches, athletic trainers, physical therapists, or team physicians (and not from peer-reviewed scientific journals), it is not surprising that most athletes are inadequately educated about the true effects of supplements [3].

SUPPLEMENTS WITH CONFIRMED ERGOGENIC EFFECTS

Only for a handful of substances available as supplements is there sufficient scientific evidence available to confirm performance-enhancing effects: caffeine, creatine, buffering agents, and nitrate-containing, carbohydrate, and protein and amino acid supplements.

Caffeine

Caffeine is probably the most accepted dietary compound used to enhance performance, even in the nonathletic population. Caffeine is available in various forms, including food and beverages as well as supplements and drugs. As an adenosine receptor antagonist, caffeine has numerous central, neuronal, and metabolic effects. The ergogenic properties of caffeine are most likely modulated through neuromuscular effects that include improved neuromuscular coupling, increased recruitment of motor units, and reduced fatigue. To a lesser extent, caffeine may also influence metabolism by increasing the rate of lipolysis. At doses of approximately 3 mg/kg body weight and higher, caffeine improves endurance exercise performance. Ergogenic effects are likely for other modes of exercise, such as team and racquet sports as well as sports involving prolonged high-intensity exercise, even though scientific data is limited [4]. Despite the well-documented performance-enhancing effects, caffeine is currently not banned by the World Anti-Doping Agency [5]. Possible side effects of caffeine include insomnia, gastrointestinal bleeding, muscle tremor, and coordinative impairments. The diuretic properties of caffeine are minimal during exercise.

Creatine

Creatine, a nonessential nutrient and a component of phosphocreatine involved in intracellular energy storage, is a very popular supplement among strength athletes. In doses of 3 g/d and greater, creatine supplementation is associated with improved contractile performance during high-intensity exercise as well as muscle **hypertrophy**. Furthermore, creatine may also improve intramuscular glycogen storage [6]. For healthy individuals, creatine supplementation is considered safe, even though larger doses of 20–30 g/d, which are frequently endorsed for initial "charging" or "loading" phases, are not recommended [7].

Buffering Agents

During high-intensity exercise, buffering agents may improve performance by increasing fatigue resistance. Extracellular buffers with demonstrated ergogenic properties include sodium bicarbonate, which improves performance during repeated high-intensity interval exercise at doses of 0.3 g/kg and greater [8]. However, sodium bicarbonate supplementation is frequently associated with severe gastrointestinal distress, including nausea and vomiting. Beta-alanine, a precursor of the intracellular buffer carnosine, has been shown to increase performance and attenuate fatigue during short, high-intensity interval exercise at doses of 4–6 g/day. Potential side effects of beta-alanine supplementation include **paresthesia** [9].

Nitrate-Containing Supplements

The ingestion of nitrate, either from supplementation or through nitrate-rich foods such as beetroot juice, is associated with increased nitric oxide generation and an increase in metabolic efficiency during submaximal exercise. However, it remains to be determined whether these effects translate into meaningful improvements in athletic performance, particularly in highly trained individuals [10].

Carbohydrate Supplements

The ingestion of carbohydrates during prolonged aerobic exercise can improve endurance performance, and performance benefits are maximized at intakes of 60–80 g/h when multiple absorbable carbohydrates such as glucose and fructose are utilized. Carbohydrates are obviously not limited to supplements, as many sport-specific products such as beverages, bars, or gels as well as conventional foods can provide similar amounts and types of carbohydrates, and the ergogenic effects appear to be independent of their form of presentation. However, the ingestion of carbohydrates in highly concentrated form has been linked to increased gastrointestinal distress [11].

Protein and Amino Acid Supplements

Protein and amino acids supplements are extremely popular among athletes who wish to increase their muscle mass or strength. It is well established that the ingestion of protein or essential amino acids in association with resistance training can support anabolic adaptations to training. However, there is currently no scientific evidence to suggest that protein or amino acid supplementation is superior to protein from conventional food sources [12].

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POPULAR SUPPLEMENTS WITHOUT SCIENTIFIC EVIDENCE OF ERGOGENIC EFFECTS

For products such as ribose or β-hydroxy-β-methylbutyrate (HMB), which are popular among strength athletes, as well as L-carnitine or medium-chain triglycerides (MCT), which are claimed to improve fatty acid oxidation and promote weight loss, scientific data are mixed. Whereas preliminary studies and early publications acknowledged ergogenic effects, subsequent research failed to confirm these effects [1]. Other popular supplements with mixed or limited findings include quercetin, exotic berries, glutamine, and glucosamine. For many other supplements, sound scientific data is mostly lacking. As new products enter the supplement market almost on a daily basis, a list of supplements without sufficient evidence will always remain incomplete.

CHALLENGES IN THE EVALUATION OF ERGOGENIC EFFECTS

Research addressing the effects of supplements on performance is often complicated by the fact that most studies are exclusively laboratory based and typically employ standardized tasks such as treadmill running, bicycle ergometry, or isometric strength tests to assess physical and physiological measures of performance. While these tests are mostly validated and well accepted, they may not adequately reflect true sports performance in a competitive setting. It has further been questioned whether these laboratory-based tests as well as the statistical approaches employed in laboratory-based research are sufficiently sensitive to detect differences in performance that decide between victory and defeat, which can be as minute as hundreds of a second or millimeters [13]. Another caveat is that most research is conducted in moderately trained or untrained subjects; whereas scientific studies explicitly conducted in elite athletes are scarce. For several supplements, including buffering agents and nitrate-containing supplements, the ergogenic properties appear to be more pronounced in untrained or moderately trained individuals. As such, study results may not be transferable across the whole fitness spectrum.

POTENTIAL NEGATIVE EFFECTS OF SUPPLEMENTATION

It has further been questioned whether the use of certain supplements could potentially impair athletic performance. For example, antioxidant supplementation has been shown to attenuate beneficial effects of exercise training in untrained or moderately trained individuals. However, it remains to be determined whether antioxidant supplementation is similarly detrimental in trained athletes [14].

The consumption of supplements further bears the risk of ingesting substances that are not adequately declared on the label. Despite their form of presentation (i.e., pills, tablets, capsules, or powders), dietary supplements are regulated as food in the United States as well as in many other countries. As such, they are controlled less tightly than pharmaceuticals. There have been numerous cases in which supplements were found to contain substances that were harmful and/or that represented doping agents. For example, numerous rapid weight loss or muscle gain supplements were found to contain large amounts of prohibited stimulants (e.g., ephedrine, sibutramine) or anabolic steroids [15]. In addition, there have also been findings of supplements containing trace amounts of doping agents, most likely due to cross-contamination during the production process. Even though these minute doses were rarely pharmacologically relevant, they were sufficient to trigger a positive doping test [15]. Consequently, athletes enrolled in antidoping programs must be particularly cautious when using supplements. Several countries have fortunately adopted programs in recent years to better protect athletes from adulterated and contaminated supplements.

SUMMARY

Based on current scientific evidence, there is only a handful of dietary supplements, including caffeine, creatine, buffering agents, and nitrate-containing products, that can improve performance during certain sporting events. In addition, dietary supplements may also serve to improve nutrient intake in situations of special needs, such as during travel or weight loss, as well as in athletes with dietary insensitivities or severe dietary restrictions. Furthermore, supplements may also improve athletic performance through placebo effects. However, the use of supplements may also be associated with adverse events that include physical side effects as well as the unintentional uptake of prohibited substances. Therefore, supplements should only be used following a careful benefit–risk analysis, and athletes are encouraged to limit their use of supplements to specific situations [16,17].

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8 ENERGY EXPENDITURE, BODY COMPOSITION, AND HEALTHY WEIGHT

MEASURING ENERGY EXPENDITURE
Direct Calorimetry
Indirect Calorimetry
Doubly Labeled Water
COMPONENTS OF ENERGY EXPENDITURE
Basal and Resting Metabolic Rate
Energy Expenditure of Physical Activity
Thermic Effect of Food
Thermoregulation
BODY WEIGHT: WHAT SHOULD WE WEIGH?
Ideal Body Weight Formulas
Body Mass Index
MEASURING BODY COMPOSITION
Field Methods
Laboratory Methods
REGULATION OF ENERGY BALANCE AND BODY WEIGHT
Hormonal Influences
Intestinal Microbiota
Environmental Chemicals
Lifestyle Influences
HEALTH IMPLICATIONS OF ALTERED BODY WEIGHT
Metabolic Syndrome
Insulin Resistance
Weight Loss Methods
SUMMARY
PERSPECTIVE
EATING DISORDERS

ENERGY IS CONSTANTLY BEING USED by every cell in the body. Consequently, humans must consume food on a regular basis to meet energy demands. When the amount of food energy matches energy expenditure over time, a person is in **energy balance**. A person who habitually consumes energy in excess of energy needs is said to be in positive energy balance and will convert the unused energy into triacylglycerols for storage as body fat. The previous chapter discussed how the body automatically adjusts to the daily inconsistencies in energy intake and energy expenditure by redistributing fuel molecules among tissues during the fed-fast cycle and during exercise. Over longer periods of time, however, maintaining whole-body energy balance is largely under external control, influenced by how much we eat and how much we exercise. These controllable factors inevitably form the basis of recommendations and interventions aimed at reducing the prevalence of obesity in the United States and other developed countries. This chapter addresses the common methods used to measure energy expenditure and body composition. The chapter also discusses energy balance, the concept of healthy weight, and the genetic and hormonal factors that regulate appetite and body composition.

MEASURING ENERGY EXPENDITURE

Techniques for measuring energy expenditure have been important tools for health professionals in developing dietary and exercise strategies for maintaining healthy weight and improving athletic performance. Energy expenditure can be assessed through direct or indirect calorimetry. Another method utilizes doubly labeled water that compares well with calorimetric methods and is considered by many to be a "gold standard" for determining total energy expenditure. These methods have provided data that have been used to develop formulas by which energy expenditure can be quickly calculated based on body weight, height, gender, and age. Each of these methods of assessment is explained in the following sections.

Direct Calorimetry

Recall from Chapter 1 that metabolic processes in the body result in the production of heat. Figure 1.12 illustrates how the metabolic oxidation of a typical fatty acid releases more energy as heat than is captured in ATP molecules. Consequently, energy expenditure can be quantified by measuring heat dissipated by the body. The technique of **direct calorimetry** is highly accurate and includes both sensible heat loss and heat of water vaporization. Although the concept of direct calorimetry is relatively simple, direct measurement of

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body heat loss is expensive, impractical, cumbersome, and usually rather unpleasant for the subject or subjects involved. Direct calorimetry is seldom used and has been replaced by the indirect methods discussed in the following sections.

Indirect Calorimetry

In addition to heat production, metabolic processes also consume oxygen in a quantifiable manner. Therefore, the heat released by metabolic oxidation can be calculated indirectly by measuring the consumption of oxygen. **Indirect calorimetry** is used most often to assess energy expenditure because the required instrumentation can be portable and, under most conditions, does not interfere with physical activities. The expiration of carbon dioxide is also measured so that the ratio of carbon dioxide produced relative to oxygen consumed (termed the *respiratory quotient*) can be determined. While carbohydrate and fat are the major fuels used in the body, it is recommended that urinary nitrogen excretion also be measured to account for the contribution of protein oxidation to energy expenditure. Oxygen consumption and carbon dioxide production are measured using either portable equipment (Figure 8.1) that can be placed on a person, enabling collection and analysis of gases while mobile, or stationary equipment often referred to as a metabolic cart (Figure 8.2). The relative ease of indirect calorimetry makes it a widely used method in research settings when measured data is desired rather than calculated estimates based on body weight.

The Respiratory Quotient

Measuring gas exchange in indirect calorimetry provides additional information about the fuel sources used in the body. Carbohydrates, fats, and proteins each required different amounts of O_2 to completely oxidize to CO_2 and water because of primary differences in their chemical structures. Thus, the ratio of CO_2 produced relative to O_2 consumed, called the **respiratory quotient (RQ)**, is characteristic for each fuel source. The RQ for carbohydrate, fat, and protein is 1.0, 0.70, and 0.82, respectively. An RQ value that falls somewhere between the lowest (0.70) and highest (1.0) value indicates a mixture of fuels were used for energy. Measuring gas exchange over a known period of time provides the necessary data to calculate not only total energy expenditure, but also the relative contribution of fuel sources. It is assumed that no proteins are oxidized for energy during short-duration activity. Over longer periods, the amount of protein being oxidized can be estimated from the amount of urinary nitrogen excreted, and the remainder of the metabolic energy must be made up of a combination of carbohydrate and fat. Should the principal fuel source shift from mainly fat to carbohydrate, the RQ correspondingly increases, and a shift from carbohydrate to fat lowers the RQ. Table 8.1 includes the thermal (caloric) equivalents of oxygen consumed at RQ values between 0.70 and 1.0, assuming no contribution of proteins to energy expenditure. The use of RQ to calculate energy expenditure also assumes that gas exchange in the lungs reflects the ratio of oxygen consumption and carbon dioxide production at the cell level.

Photo courtesy of the NASA John H. Glenn Research Center

Figure 8.1 A portable device to measure oxygen consumption and carbon dioxide production. The headgear contains oxygen and carbon dioxide sensors on the left side and the flow sensor on the right side, all tethered to an electronics box (to the left of the headgear) that fits into a small wearable pack.

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Courtesy of CareFusion Respitory Diagnostics

Figure 8.2 A metabolic cart (shown here with bicycle ergometer) measures oxygen consumption and carbon dioxide exhaled.

RQ and Substrate Oxidation

An RQ equal to 1.0 suggests that carbohydrate is being oxidized because the amount of oxygen required for the combustion of glucose equals the amount of carbon dioxide produced, as shown here:

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$$
$$RQ = 6\ CO_2/6\ O_2 = 1.0$$

The RQ for fat is <1.0 because fatty acids, compared to carbohydrates, require more oxidation relative to the number of carbon atoms when producing CO_2 and water. For example, a triacylglycerol such as tristearin, shown in the following reaction, requires 163 mol of oxygen and produces 114 mol of carbon dioxide per two tristearin molecules:

$$2\ C_{57}H_{110}O_6 + 163\ O_2 \longrightarrow 114\ CO_2 + 110\ H_2O$$
$$RQ = 114\ CO_2/163\ O_2 = 0.70$$

Calculating the RQ for protein oxidation is more complicated because metabolic oxidation of amino acids requires removing the nitrogen and some oxygen and carbon as urea, a compound excreted in the urine. Urea nitrogen represents a net loss of energy to the body, and only the remaining carbon structure of the amino acid can be oxidized in the body. The following reaction illustrates the oxidation of a small protein molecule into carbon dioxide, water, sulfur trioxide, and urea:

$$C_{72}H_{112}N_{18}O_{22}S + 77\ O_2 \longrightarrow 63\ CO_2 + 38\ H_2O + SO_3 + 9\ CO(NH_2)_2$$
$$RQ = 63\ CO_2/77\ O_2 = 0.818$$

RQ values of 1.0, 0.70, and 0.82 are the generally accepted values for carbohydrate, fat, and protein, respectively. The RQ for an individual consuming an ordinary mixed diet consisting of all three macronutrients will range between 0.70 and 1.0. An RQ of 0.82, as indicated in Table 8.1, represents the metabolism of a mixture of 40% carbohydrate and 60% fat. RQ values that approach 1.0 indicate a higher contribution of carbohydrate for fuel, whereas an RQ closer to 0.70 indicates more fat being used for fuel. In clinical practice, an RQ < 0.8 suggests that a patient may be underfed. An RQ < 0.7 suggests starvation; consumption of low-carbohydrate, high-fat, calorie-restricted diets; or alcoholism, due to ethanol having an RQ of 0.67 [1].

Table 8.1 Thermal Equivalent of O_2 and CO_2 for Nonprotein RQ

Nonprotein RQ	Caloric Value of O_2 (kcal/L)	Caloric Value of CO_2 (kcal/L)	Source of Calories	
			Carbohydrate (%)	Fat (%)
0.707	4.686	6.629	0	100
0.71	4.690	6.606	1.10	98.9
0.72	4.702	6.531	4.76	95.2
0.73	4.714	6.458	8.40	91.6
0.74	4.727	6.388	12.0	88.0
0.75	4.739	6.319	15.6	84.4
0.76	4.751	6.253	19.2	80.8
0.77	4.764	6.187	22.8	77.2
0.78	4.776	6.123	26.3	73.7
0.79	4.788	6.062	29.9	70.1
0.80	4.801	6.001	33.4	66.6
0.81	4.813	5.942	36.9	63.1
0.82	4.825	5.884	40.3	59.7
0.83	4.838	5.829	43.8	56.2
0.84	4.850	5.774	47.2	52.8
0.85	4.862	5.721	50.7	49.3
0.86	4.875	5.669	54.1	45.9
0.87	4.887	5.617	57.5	42.5
0.88	4.899	5.568	60.8	39.2
0.89	4.911	5.519	64.2	35.8
0.90	4.924	5.471	67.5	32.5
0.91	4.936	5.424	70.8	29.2
0.92	4.948	5.378	74.1	25.9
0.93	4.961	5.333	77.4	22.6
0.94	4.973	5.290	80.7	19.3
0.95	4.985	5.247	84.0	16.0
0.96	4.998	5.205	87.2	12.8
0.97	5.010	5.165	90.4	9.58
0.98	5.022	5.124	93.6	6.37
0.99	5.035	5.085	96.8	3.18
1.00	5.047	5.047	100	0

Source: Adapted from McArdle, W.D., Katch, F.I., Katch, V.L., *Exercise Physiology*, 2nd ed., p. 127 (Philadelphia: Lea & Febiger, 1986). Adapted by permission.

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Occasionally the RQ value can be greater than 1. For instance, when you hyperventilate you exhale more CO_2 without using more O_2, resulting in an RQ greater than 1. This may also occur when the body is in acidosis, such as following exhaustive exercise when lactic acid builds up. $NaHCO_2$ neutralizes the lactic acid to form sodium lactate and carbonic acid (H_2CO_3). The carbonic acid is converted to CO_2 and H_2O and exhaled, which results in a loss of CO_2 that is not related to oxygen uptake.

RQ and Energy Expenditure

Once the RQ has been computed from oxygen and carbon dioxide exchange, the calculation of energy expenditure is performed using the caloric value of oxygen at different RQ values as shown in Table 8.1.

For example, if under standard conditions for the determination of BMR a person consumed 15.7 L of oxygen per hour and expired 12.0 L of carbon dioxide, the RQ would be 12.0/15.7, or 0.7643. From Table 8.1, the caloric equivalent of 1 L of oxygen at an RQ of 0.76 is 4.751 kcal. Based on the caloric equivalent for oxygen, calories produced per hour are 15.7 × 4.751, or 74.6 kcal. If we use 75 kcal/h as the caloric expenditure under basal conditions, the basal energy expenditure for the day would be 75 kcal/h × 24 h, or about 1,800 kcal/day. At an RQ of 0.76, fat is supplying almost 81% of energy expended (Table 8.1).

Because under ordinary circumstances the contribution of protein to energy metabolism is so small, the oxidation of protein is ignored in the determination of the so-called nonprotein RQ. If a truly accurate RQ is required, a minimal correction can be made by measuring the amount of urinary nitrogen excreted over a specified time period. For every 1 g of nitrogen excreted, about 6 L of oxygen are consumed and 4.8 L of carbon dioxide are produced. The amount of oxygen and carbon dioxide exchanged in the release of energy from protein can then be subtracted from the total amount of measured gaseous exchange.

Measurement of the energy expended in various physical activities has also been made primarily through indirect calorimetry. The method for measuring gas exchange, however, differs slightly from that used for determining BMR. The subject performing the activity for which energy expenditure is being determined inhales ambient air, which has a constant composition of 20.93% oxygen, 0.03% carbon dioxide, and 78.04% nitrogen. Air exhaled by the subject is collected in a spirometer (a device used to measure respiratory gases) and is analyzed to determine how much less oxygen and how much more carbon dioxide it contains compared with ambient air. The difference in the composition of the inhaled air and the exhaled air reflects the energy release from the body. A lightweight portable spirometer (Figure 8.1) can be worn during the performance of almost any sort of activity, and thus freedom of movement outside the laboratory is possible. In many laboratories and hospitals, gas exchange is measured using a so-called metabolic cart (Figure 8.2).

Doubly Labeled Water

The doubly labeled water method also enables assessment of total energy expenditure. $^2H_2$ (deuterium) and $^{18}O_2$ are stable isotopes of hydrogen and oxygen, respectively. In this technique, stable isotopes of water are given as $H_2{}^{18}O$ and as ^2H_2O (or as $^2H_2{}^{18}O_2$). The isotopes equilibrate throughout the water compartments in the body over about 5 hours. The labeled hydrogen can leave the body as water (^2H_2O) in sweat, urine, and pulmonary water vapor, while the labeled oxygen can leave the body as either labeled water ($H_2{}^{18}O$) or $C^{18}O_2$. The disappearance of the $H_2{}^{18}O$ and ^2H_2O is measured in the blood and urine for about 3 weeks. The disappearance of the $H_2{}^{18}O$ is representative of the flux of water (i.e., water turnover) and of the production rate of carbon dioxide. Because the $^2H_2$ can be excreted only as H_2O, the disappearance of the ^2H_2O represents water turnover alone. Thus, the difference between the disappearance rate of $H_2{}^{18}O$ and that of ^2H_2O corresponds to the production rate of carbon dioxide.

The CO_2 production rate is then used to calculate energy expenditure. However, an RQ is needed to determine the caloric value of CO_2 using Table 8.1. Rather than measuring gas exchange, which would defeat the unrestricted character of the doubly labeled water technique, food records are kept throughout the testing period to estimate the metabolic fuel mix from dietary intake. In subjects maintaining body weight, the recorded *food quotient* is equal to the respiratory quotient and can act as a surrogate RQ [2]. Use of the doubly labeled water method to assess total energy expenditure in free-living individuals produces accurate results that correlate well with those of indirect calorimetry. One source of potential error lies with the use of food records, which requires attention to detail and knowledge of portion size to improve accuracy.

COMPONENTS OF ENERGY EXPENDITURE

Daily total energy expenditure is attributable to three primary components: basal metabolic rate, physical activity, and the thermic effect of food. A fourth component, **thermoregulation**, is sometimes included. The average contribution of energy expenditure among the components is illustrated in Figure 8.3 and is discussed in the following sections.

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Figure 8.3 Components of energy expenditure and their approximate percentage contribution.

Basal and Resting Metabolic Rate

Basal metabolic rate (BMR) represents the amount of energy needed to sustain basic life processes such as respiration, heartbeat, renal function, brain and nerve function, blood circulation, active transport, and synthesis of proteins and other complex molecules. Basal metabolism accounts for the majority of energy expenditure in the human body (Figure 8.3). Most of the energy used at rest is attributed to the liver (27%), brain (19%), kidneys (10%), heart (7%), and skeletal muscle (18%), which even at rest require appreciable amounts of energy for protein synthesis and normal cellular function.

Several factors can affect basal metabolism, including body composition and surface area, age and gender, pregnancy and lactation, environmental temperature, and dietary energy restriction. Many of these factors can be attributed to the amount and proportion of lean body mass, which has higher metabolic activity than adipose tissue. People with greater body weight because of increased lean body mass have a higher BMR. In aging, fat mass increases at the expense of fat-free mass, causing BMR to decrease. Women generally have a higher proportion of body fat relative to fat-free mass and, consequently, have a lower BMR than men of the same age, height, and total body weight. Tall, thin people have more surface area relative to volume, which is associated with greater heat loss and higher BMR. Cold environments can increase BMR due to shivering, which generates internal body heat. Paradoxically, hot environments can also increase BMR, possibly due to increased blood circulation and sweat gland activity. BMR increases during pregnancy and when lactating. BMR decreases during starvation due to the loss of lean body mass. BMR also decreases with aging due to reductions in some body organ functions and mass.

BMR is assessed indirectly by measuring oxygen consumption under carefully controlled conditions that eliminates any contribution of energy expenditure due to physical activity, thermic effect of food, or heat production that occurs in cold environments. BMR is measured when awake and in a postabsorptive state between 12 and 18 hours following food intake, preferably in the morning shortly after waking from sleep. A person must be completely relaxed in a supine position for at least 30 minutes in a thermoneutral environment. Any factors that could influence the person's internal work are minimized as much as possible. Oxygen consumption (recorded as mL per minute) is then measured for at least 10 minutes. The next step is to convert the rate of oxygen consumption into energy expenditure, based on the principle that the oxidation of carbohydrate, fat, and protein yields approximately 5 kcal of energy per liter of oxygen consumed. BMR is often expressed as daily energy expenditure (kcal/day) and, accordingly, is called *basal energy expenditure*.

Measuring BMR accurately requires strictly controlled laboratory conditions, making it difficult to obtain in most people. As an alternative, resting metabolic rate (RMR) is more easily measured and can provide information that is nearly the same as BMR, albeit slightly higher than BMR. To measure RMR, an individual needs to fast only 3–4 hours, much less than the more stringent fasting time required for BMR. Resting comfortably just prior to recording oxygen consumption is required, but it is not necessary to conduct the measurements just after waking in the morning. RMR is usually about 10% higher than BMR because of its less stringent conditions of measurement. The term *resting energy expenditure* is used when RMR is converted to daily energy expenditure (kcal/day).

Predictive Equations for RMR

Several equations have been developed that accurately estimate RMR based on body weight, height, age, and gender. These equations do not require specialized equipment or the expertise needed to conduct calorimetric measurements. Many equations have been developed over the past century, although only a few are commonly used today. In general, predictive equations are convenient and yield reasonably accurate estimates of RMR in a variety of populations. However, predictive equations are more variable in older people and tend to overestimate RMR in people with excess body fat. The following describes a few commonly used equations for adult men and women based on body weight in kilograms (W), height in centimeters (H), and age in years (A).

Harris-Benedict Equations Based on indirect calorimetry, the equations were developed by Harris and Benedict in 1919 using mostly normal-weight white men and women [3]. The Harris-Benedict equations have undergone extensive validation and found to yield reasonably accurate results in nonobese individuals, but the equations

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overestimate RMR in obese individuals. Separate equations are used for men and women:

$$\text{Men: RMR, kcal/day} = 66.5 + (13.7 \times W) + (5.0 \times H) - (6.8 \times A)$$

$$\text{Women: RMR, kcal/day} = 665 + (9.56 \times W) + (1.85 \times H) - (4.7 \times A)$$

Mifflin-St. Jeor Equations Published in 1990, the Mifflin-St. Jeor equations were developed using indirect calorimetry in normal-weight, overweight, obese, and severely obese individuals to improve the accuracy of RMR measurements in people with excess body fat [4]. The Mifflin-St. Jeor equations are used frequently in clinical settings and can accurately predict RMR within 10% of that measured by indirect calorimetry in both nonobese and obese adults [5]. As with Harris-Benedict estimates, separate Mifflin-St. Jeor equations are used for men and women and require body weight, height, and age as data inputs:

$$\text{Men: RMR, kcal/day} = (9.99 \times W) + (6.25 \times H) - (4.92 \times A) + 5$$

$$\text{Women: RMR, kcal/day} = (9.99 \times W) + (6.25 \times H) - (4.92 \times A) - 161$$

A female who is 35 years old, weighs 125 lb (56.8 kg), and is 5 feet, 5 inches tall (165.1 cm) would have a RMR of 1,339 kcal/day using the Harris-Benedict equation and an RMR of 1,266 kcal/day using the Mifflin-St. Jeor equation.

Weight-Only Equations Several predictive equations based only on body weight have been developed from indirect calorimetry data [6,7]. These equations are less accurate, but work reasonably well when information about height or age are unavailable. Perhaps the most frequently used weight-only equation is not based on established gas exchange methodology, but rather on the principle that basal metabolic rate (represented by heat production) is correlated with body mass of most vertebrate animal species. The resulting equation for humans is specific for BMR and is written: BMR, kcal/day $= 70 \times W^{0.75}$

Using the same 125-lb (56.8-kg) female as an example, her estimated BMR is calculated to be $70 \times 56.8^{0.75} = 1{,}448$ kcal/day.

Energy Expenditure of Physical Activity

Skeletal muscle requires significant amounts of energy when physically active. Muscle is also involved in maintaining posture when awake, which requires energy in less obvious ways. The energy expenditure of physical activity is highly variable depending on an individual's activity level. Physical activity typically accounts for about 15–30% of total energy expenditure, but it can be considerably less in a truly sedentary person or much more in a very active person. During physical activity that engages large muscles, energy expenditure can greatly exceed RMR at least for a short time. Such high rates of energy usage cannot be sustained, so the daily average of energy expenditure due to physical activity is usually less than RMR in most people. Physical activity includes all exercise and nonexercise activities associated with daily living.

Quantifying the energy expenditure of physical activity requires measuring RMR (or BMR) and total energy expenditure, then calculating the difference. This can be achieved in a clinical setting by measuring gas exchange (oxygen consumed and carbon dioxide expired) or by predictive equations. Alternatively, practitioners can simply estimate the contribution of physical activity to total energy expenditure by multiplying RMR by a factor that approximates the additional energy usage by skeletal muscle [8]. The multiplication factors—called the *physical activity level* (PAL)—are categorized into four different levels, as described in Table 8.2. For comparison, Table 8.2 also shows the number of miles a person would need to walk per day to match each PAL category.

Once again using the 125-lb female as an example, we start with her RMR of 1,266 kcal/day that was calculated using the Mifflin-St. Jeor equation. We know she works at a home improvement store and walks throughout the day, putting her in the "active" PAL category. Therefore, the combined energy expenditure attributed to RMR and physical activity is $1{,}266 \times 1.75 = 2{,}216$ kcal/day.

Another way of estimating energy expended during physical activity is the use of data tables in which

Table 8.2 Physical Activity Level (PAL) Categories and Walking Equivalence

			Walking Equivalence (miles/day at 3–4 mph)		
PAL Category	PAL Range	PAL Average	Light-Weight Individual (44 kg or 97 lb)	Middle-Weight Individual (70 kg or 154 lb)	Heavy-Weight Individual (120 kg or 264 lb)
Sedentary	1.00–1.39	1.25	~ 0	~ 0	~ 0
Low active	1.40–1.59	1.50	2.9	2.2	1.5
Active	1.60–1.89	1.75	9.9	7.3	5.3
Very active	1.90–2.49	2.20	22.5	16.7	12.3

Source: Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: National Academies Press, 2005.

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Table 8.3 Energy Expended on Various Activities

The values listed in this table reflect both the energy expended in physical activity and the amount used for BMR. To calculate kcal spent per minute of activity for your own body weight, multiply kcal/lb/min (or kcal/kg/min) by your exact weight and then multiply by the number of minutes spent in the activity. For example, if you weigh 142 pounds, and you want to know how many kcal you spent doing 30 minutes of vigorous aerobic dance: 0.062 × 142 = 8.8 kcal per minute; 8.8 × 30 minutes = 264 total kcal spent.

Activity	kcal × lb^{-1} × min^{-1}	kcal × kg^{-1} × min^{-1}
Aerobic dance (vigorous)	.062	.136
Basketball (vigorous, full court)	.097	.213
Bicycling		
13 mph	.045	.099
15 mph	.049	.108
17 mph	.057	.125
19 mph	.076	.167
21 mph	.090	.198
23 mph	.109	.240
25 mph	.139	.306
Canoeing, flat water, moderate pace	.045	.099
Cross-country skiing, 8 mph	.104	.229
Gardening	.045	.099
Golf (carrying clubs)	.045	.099
Handball	.078	.172
Horseback riding (trot)	.052	.114
Rowing (vigorous)	.097	.213
Running		
5 mph	.061	.134
6 mph	.074	.163
7.5 mph	.094	.207
9 mph	.103	.227
10 mph	.114	.251
11 mph	.131	.288
Soccer (vigorous)	.097	.213
Studying	.011	.024
Swimming		
20 yd/min	.032	.070
45 yd/min	.058	.128
50 yd/min	.070	.154
Table tennis (skilled)	.045	.099
Tennis (beginner)	.032	.070
Vacuuming and other household tasks	.030	.066
Walking (brisk pace)		
3.5 mph	.035	.077
4.5 mph	.048	.106
Weightlifting		
Light-to-moderate effort	.024	.053
Vigorous effort	.048	.106
Wheelchair basketball	.084	.185
Wheeling self in wheelchair	.030	.066
Wii games		
Bowling	.021	.046
Boxing	.021	.047
Tennis	.022	.048

Source: Rolfes, Pinna, Whitney, *Understanding Normal & Clinical Nutrition*, 9/e. © Cengage Learning.

the amount of energy expended has previously been determined for a variety of activities. Table 8.3, an example of such a table, indicates the amount of energy (kcal) expended per minute per body weight. This table incorporates the basal energy expenditure, whereas some tables provide data for only physical activity. To calculate the energy expended for a given activity, multiply the kcal by your body weight and then by the number of minutes spent performing the activity. Note that every activity performed during a 24-hour period (and possibly for several days) would need to be recorded if total daily energy expenditure is desired.

Thermic Effect of Food

A third component of energy expenditure is the thermic effect of food. This represents the metabolic response to food and is also called diet-induced thermogenesis, specific dynamic action, or the specific effect of food. The thermic effect of food represents the increase in energy expenditure associated with the body's processing of food, including the work associated with the digestion, absorption, transport, metabolism, and storage of energy from ingested food. The percentage increase in energy expenditure above BMR caused by the thermic effect of food is typically about 10% (see Figure 8.1).

Protein in foods has the greatest thermic effect, increasing energy expenditure 20–30%. Carbohydrates have an intermediate effect, raising energy expenditure 5–10%, and fat increases energy expenditure 0–5%. The value most commonly used for the thermic effect of food is 10% of the caloric value of a mixed diet averaged over a 24-hour period [8]. Because of its relative small contribution, the thermic effect of food is usually not included in calculations of total energy expenditure.

Thermoregulation

An additional component of energy expenditure that is of some importance is thermoregulation, also called adaptive, nonshivering, facultative, or regulatory thermogenesis. Thermoregulation refers to the adjustments in metabolism necessary to maintain the body's core temperature of about 98.2–98.6°F. A drop of temperature of 10°F or an increase of just 5°F can be tolerated, but fluctuations beyond this range can result in death. Most people adjust their clothing and environment to maintain comfort and thermoneutrality, although the body can adjust metabolic heat production when needed by hormonal changes controlled by the hypothalamus. Measurements of BMR or RMR are performed in a thermoneutral setting so that the contribution of thermoregulation can be excluded from calculations of energy expenditure.

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As a cautionary note, muscular activity can generate significant heat and cause a rapid increase in body core temperature beyond the ability of the body to thermoregulate, especially in hot environments. **Heat stress** is a concern among high school athletes, particularly football players who have the highest incidence of heat-related deaths [9]. Coaches, parents, and athletes should take the necessary steps to ensure proper hydration and to avoid conditions that increase the risk of heat stress.

BODY WEIGHT: WHAT SHOULD WE WEIGH?

Monitoring changes in body weight has long been a diagnostic tool of the health practitioner. Whether body weight remains stable, increases, or decreases depends entirely on the extent to which total energy expenditure is being met or exceeded by energy intake. The connections between body weight, body fat, and health were recognized centuries ago; as noted by Hippocrates, "those who are constitutionally very fat are more apt to die earlier than those who are thin" [10]. Today, scientists and health professionals recognize that the risk of many diseases—including heart disease, stroke, diabetes mellitus, hypertension, osteoarthritis, infertility, and some cancers (breast, endometrial, colon, and kidney)—increases with excess body fat. Conversely, low body weight may indicate malnutrition or an eating disorder and may pose risks for other diseases, such as osteoporosis. Many methods have been employed to quantify body fat, as discussed in detail later in this chapter. Most of these methods require specialized equipment that is expensive and time-consuming to operate. Using body weight as a proxy for body fat is convenient, applicable to most people, and can be used by researchers to collect data in large numbers of subjects. An important caveat, however, should be noted when using only body weight as an indicator of health. Some individuals may increase body weight by adding muscle mass rather than fat, as seen in body builders and many competitive athletes.

Comparing body weight to height has become a standard measurement among health professionals. In 1846, English surgeon John Hutchinson published a height-weight table based on a sample of 30-year-old Englishmen and urged that future census-taking include such information, which he believed to be valuable in promoting health and detecting disease [11]. The concept of a *desirable body weight* later emerged from the life insurance industry in the early 20th century. In an attempt to find the most healthy body weight, insurance companies began compiling data from their policyholders whose body weights (relative to height) were associated with the lowest mortality. Eventually height-weight tables were developed that showed the most desirable weight from a health standpoint [12]. Health professionals began using these tables in the 1940s for educational purposes for the general public. Data from the tables have also been subjected to regression analysis, resulting in the use of *ideal body weight* formulas for estimating a person's health status. Although the height-weight tables and ideal body weight formulas are able to convey a general notion of disease and mortality risk, they are limited to the demographic populations on which they are based and require a reference population so that the comparative terms *desirable* and *ideal* can apply. Many health experts have abandoned the use of height-weight tables and formulas in favor of body mass index and waist circumference as better indicators of body fat (and thus health status) because the latter measurements do not require a reference population for comparison.

Ideal Body Weight Formulas

Despite falling out of favor, the use of ideal body weight (IBW) formulas may still have a role in certain situations. As previously mentioned, IBW formulas evolved directly from height-weight tables and are intended to provide guidance to health practitioners when determining overall mortality risk in a given population. IBW is more easily understood by the general public than body mass index and may be the method of choice by some health professionals. Table 8.4 describes several common formulas that have been used for calculating IBW [13,14]. Note that the Broca formula provides a range for IBW at a given height, whereas the other formulas provide a single value for IBW for a given height over 60 inches.

Using the Hamwi formula, the IBW for a male who is 6 feet tall (72 inches) is calculated as 48.1 kg + (2.7 kg × 12 inches over 60) = 80.5 kg or 177 lb . The Hamwi formula yields the highest single-value IBW among all of the formulas in Table 8.4. The Miller formula provides the lowest IBW, at 73.1 kg or 161 lb. If one chooses to use the Broca formula, which provides a range, the IBW is 75–91 kg or 165–200 lb.

Body Mass Index

Body mass index, first described in the 1860s and known as Quetelet's Index, is one of the most widely accepted approaches to categorizing weight for a given height. The body mass index is considered an indication of body adiposity but does not directly measure body fat. BMI is calculated from a person's height and weight as shown in this formula:

$$\text{Body mass index} = \frac{\text{Weight}}{\text{Height}^2}$$

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Table 8.4 Ideal Body Weight (IBW) Formulas

	Men	Women
Broca formula (1871)	Weight(kg) = height(cm) − 100 ± 10%	Weight(kg) = height(cm) − 100 ± 15%
Hamwi formula (1964)	48.1 kg + 2.7 kg/inch over 60 inches	45.4 kg + 2.3 kg/inch over 60 inches
Devine formula (1974)	50.0 kg + 2.3 kg/inch over 60 inches	45.5 kg + 2.3 kg/inch over 60 inches
Miller formula (1983)	56.2 kg + 1.41 kg/inch over 60 inches	53.1 kg + 1.36 kg/inch over 60 inches
Robinson formula (1983)	51.7 kg + 1.85 kg/inch over 60 inches	48.7 kg + 1.65 kg/inch over 60 inches
Deitel-Greenstein formula* (2003)	61.3 kg + 1.36 kg/inch over 63 inches	54.0 kg + 1.36 kg/inch over 60 inches
Kammerer formula* (2015)	64.5 kg + 1.36 kg/inch over 63 inches + 0.45 kg/inch over 71 inches	54.0 kg + 1.36 kg/inch over 60 inches

* IBW formulas were developed in bariatric surgical patients.

with weight measured in kilograms (kg) and height measured in meters (m) and raised to a power of 2. BMI is expressed in units of kg/m^2.

An adult male who weighs 165 lb (74.9 kg) and is 5 feet, 11 inches tall (1.803 m) will have a BMI = 74.9/1.803^2 = 23.0 kg/m^2. The following conversion factors are used: 1 lb = 0.454 kg; and 1 inch = 0.0254 m. Alternatively, BMI can be calculated as 1b/inches^2 × 703 to convert to kg/m^2. In this case, BMI = 165/71^2 × 703 = 23.0 kg/m^2.

Body mass index is considered a good index of total body fat in both men and women and has generally replaced the practice of classifying people as underweight or overweight compared to a reference weight. The National Institutes of Health [15] classification of body weight based on BMI for adults is presented in Figure 8.4.

BMI is also used to assess weight in children, but through comparison to population standards for sex and age. BMI changes with age in healthy children, as demonstrated by the growth charts for boys and girls 2–20 years of age shown in Figure 8.5. BMI < 5th percentile is underweight; BMI between the 5th and 85th percentiles is considered healthy weight; BMI between the 85th and 95th percentiles are classified as overweight; and BMI > 95th percentile is considered obese [16]. Body weight

Figure 8.4 BMI values used to categorize weight.
Source: U.S. Department of Agriculture and Human Services, *Nutrition and Your Health: Dietary Guidelines for Americans*. Washington, DC, 2000, p. 7.

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Figure 8.5 Example of growth curves (2–20 years): boys' and girls' body mass index-for-age percentiles.
Source: www.cdc.gov/growthcharts/.

and recumbent length for boys and girls under 2 years of age are assessed using growth charts similar to those in Figure 8.5.

Although the body mass index is a valuable tool for categorizing body weight, it does not directly determine body fatness. People who have large amounts of lean body mass and a low percentage of body fat may fall into the overweight category. Consequently, intermediate BMI values in the normal and overweight categories are not as strongly correlated with actual body fat percentage as compared to BMI of ≥ 30 kg/m² [17]. The relationship between BMI and body fat has also been shown to vary among different age, sex, and racial/ethnic groups. Nevertheless, measurements of BMI sampled from the U.S. population clearly indicate an alarming trend of increasing obesity prevalence in all age groups (Figure 8.6). Note in the figure that BMI ≥ 25 kg/m² includes both the overweight and obese categories and that the obese category (BMI ≥ 30 kg/m²) is shown separately.

Because of the limitations of using BMI alone, many health professionals measure waist circumference in addition to BMI. By utilizing both of these measurements, disease risk relative to normal weight and waist circumference can be determined, as indicated in Table 8.5. Monitoring changes in waist circumference over time is helpful since it can be an indicator of abdominal fat even in the absence of a change in BMI. Including waist circumference measurements is particularly useful in people who are categorized as normal or overweight on the BMI scale because it helps distinguish those who have increased muscle mass versus excess body fat. For individuals with a BMI ≥ 35 kg/m², waist circumference adds no further predictive power of disease risk beyond BMI alone; therefore, it is unnecessary to measure waist circumference in people with a BMI ≥ 35 kg/m².

Figure 8.6 Obesity prevalence in the United States.
*Surveys of children age 2-5 years began in 1988.
**Survey years were 1960-1962 for adults age 20-74 years; 1963-1965 for children age 6-11 years; and 1966-1970 for adolescents age 12-19 years.
Source: Centers for Disease Control and Prevention, National Health and Nutrition Examination Survey (NHANES)

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Table 8.5 Disease Risk* Relative to Normal BMI and Waist Circumference

BMI Categories	Waist Circumference	
	Men ≤ 40 in (102 cm) Women ≤ 35 in (88 cm)	Men > 40 in (102 cm) Women > 35 in (88 cm)
Underweight (< 18.5 kg/m^2)	—	—
Normal (18.5–24.9 kg/m^2)	—	—
Overweight (25.0–29.9 kg/m^2)	Increased	High
Obese, class I (30.0–34.9 kg/m^2)	High	Very high
Obese, class II (35.0–39.9 kg/m^2)	Very high	Very high
Obese, class III (≥ 40 kg/m^2)	Extremely high	Extremely high

Source: National Institutes of Health. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. Publication No. 98-4083. Bethesda, MD: National Institutes of Health; National Health, Lung, and Blood Institute. 1998.

* Risk for type 2 diabetes, dyslipidemia, hypertension, and cardiovascular diseases.

The simplicity and convenience of measuring height, weight, and waist circumference—and the relative ease of calculating BMI and IBW—has contributed to their widespread use as indicators of body fat, disease risk, and overall health. These simple methods work relatively well as an initial screening tool for the general population and in epidemiological research when large sample sizes are studied. However, as previously discussed, methods based on body weight do not directly quantify fat mass or the proportion of body fat relative to lean body mass, which varies greatly in the human population. Therefore, the development of methods that specifically assess body fat and overall body composition has helped researchers and health practitioners better understand the role of body fat in health and disease. The following section discusses some of the methods used to measure body composition in the laboratory and in the field .

MEASURING BODY COMPOSITION

Early observations linking body weight with disease risk and mortality, as discussed in previous sections, led to the obvious conclusion that excessive body weight was due to the accumulation of body fat. Humans have a tremendous capacity to store triacylglycerols in adipose tissue when in positive energy balance over time. However, using body weight as a proxy for body fat is less accurate at intermediate body weights because of individuals who gain muscle mass rather than body fat. The desire to have a more complete and accurate understanding of body composition prompted the development of methods that directly assess body fat and other components that include bone, muscle, visceral organs, minerals, and water. Assessment of body composition can be approached in different ways. Historically, the gross anatomy and chemical composition of the human body were determined using cadavers. Today, modern techniques allow for detailed analyses that can be performed quickly and accurately.

Conceptual models of body composition are currently used that partition the body into "compartments." The two-compartment model includes fat mass and fat-free mass, whereas the four-compartment model includes fat mass, fat-free mass, bone mineral, and total body water. The four-compartment model is considered the gold standard but requires sophisticated equipment to measure all of the components. In some cases the mass of specific organs and the location of adipose tissue can be determined. The most frequently used methods to assess body composition consider only two compartments, fat mass and fat-free mass. Fat mass consists mostly of triacylglycerols, with relatively small amounts of water and minerals. Fat-free mass is much more diverse and comprised of muscle, bones, and the intra- and extracellular fluids. Muscle itself contains about 73% water. The differences in the chemical and physical properties of the two compartments—which include variations in density, the electrolyte content, the ability to conduct an electrical current, and the X-ray density—form the basis for many of the methods of determining body composition.

Choosing a method for measuring body composition depends on the purpose, access to the equipment, the number of individuals to be measured, age, and cost. Some methods can be performed only in a laboratory or clinical setting because of the large equipment involved, whereas some methods use portable equipment and can be used in the field. The following discussion highlights some of the common techniques used to measure body composition.

Field Methods

Skinfold Thickness

The use of skinfold measurements as an indicator of body fat is based on the assumption that the thickness of subcutaneous fat directly correlates with total body fat. Skinfold measurements can estimate the percentage of body fat and is therefore consistent with the two-compartment model. Skinfold measurements are made at various anatomical sites using a caliper (Figure 8.7). The anatomical sites commonly used for measuring skinfold thickness are the triceps (measured on the back of the upper arm), subscapula (measured just below the tip of the scapula), suprailiac (measured above the hip bone), abdomen (measured 1 inch to the right of the umbilicus), and thigh (measured at the midpoint of the thigh, between the kneecap and the hip). All measurements should be repeated at least two or three times, and the average should be used as the skinfold value. The precision of skinfold thickness measurements depends on the skill of the technician; in general, a precision of within 5% can be obtained by a well-trained and experienced technician. After skinfold thickness is measured, the values are entered into mathematical equations to calculate the percentage of body fat. Because of the convenience and low cost, the use of skinfold measurements is a popular choice of community-based health and wellness practitioners.

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David Young-Wolff/PhotoEdit

Figure 8.7 Measuring triceps skinfold with a Lange caliper to estimate body fat.

Bioelectrical Impedance

Bioelectrical impedance analysis is another commonly used field technique that assesses the two-compartment model. The method is based on the principle that the flow of electricity (conductivity) is facilitated in fat-free tissue high in water and electrolyte content, but is impeded by fat tissue low in water and electrolytes. Electrical conductivity is measured by various techniques. For example, an instrument generates a painless current or multiple electrical current frequencies that are passed through the body by means of the electrodes. Other devices are available in which the subject stands barefoot on a scale or grasps a hand-held device that measures electrical conductivity (Figure 8.8). In each case, opposition to the electric current, called impedance, is detected and measured by the instrument. Impedance is the inverse of conductance. The lowest resistance value of a person is used to calculate conductance and predict lean body mass or fat-free mass. For example, muscle, organs, and blood, which have high water and electrolyte contents, are good conductors. Tissues containing little water and electrolytes (such as adipose tissue) are poor conductors and have a high resistance to the passage of electrical current. When multiple frequencies are used, the higher frequencies can estimate both intracellular and extracellular water because the higher-frequency current can penetrate cell membranes. At lower frequencies, the flow of the current is blocked, and the measured resistance indicates extracellular water.

Bioelectrical impedance is a safe, noninvasive, and rapid means to assess body composition. The equipment is relatively inexpensive, portable, and fairly easy to operate. Bioelectrical impedance readings are affected by hydration and electrolyte imbalances. Thus, the technique is more useful for healthy subjects. Several bioelectrical impedance analysis prediction equations have been developed for various populations. The use of multifrequency techniques provides results that are in good agreement with other body composition methods because this technique estimates both total body water and extracellular water.

Laboratory Methods

Densitometry: Underwater Weighing

The density of body fat is about 0.9 g/mL, whereas the density of fat-free mass is about 1.1 g/mL. Percent body fat of an individual can therefore be calculated if whole-body density is known. The Greek mathematician Archimedes discovered that the volume of an object submerged in water is equal to the volume of water displaced by the object. The density of an object can then be calculated by dividing the object's weight (wt) in air by its loss of weight in water. For example, for a person who weighs 47 kg in air and 2 kg underwater, 45 kg represents the loss of body weight and the weight of the water displaced. After an adjustment for the change in density of water at different temperatures is made, the volume of the person can be calculated. Figure 8.9 illustrates an apparatus for weighing under water. Correction for residual air volume in the lungs (RLV) and gas in the gastrointestinal tract (GIGV) must be made.

Body density is calculated using the following formula:

$$\text{Body density} = \frac{\text{Wt of body in air}}{\dfrac{\left(\text{Wt of body in air} - \text{Wt of body underwater}\right)}{\text{Density of water}} - \text{RLV} - \text{GIGV}}$$

Residual lung volume is thought to be about 24% of vital lung capacity. The volume of gas in the gastrointestinal tract is estimated to range from 50 to 300 mL. This volume typically is neglected, or a value of 100 mL may be used in calculations. The density or the weight of water is known for a wide range of temperatures and must be obtained for the calculation. Once density of the human body is known, an estimation of body fat can be determined. At any known body density, estimating the percentage of body fat is possible using established equations.

Underwater weighing is considered a noninvasive and relatively precise method for assessment of percent body fat. The standard error of body fat measurements

Figure 8.8 Hand-held device used to measure bioelectrical impedance.

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David Young-Wolff/PhotoEdit

Figure 8.9 Apparatus for underwater weighing to determine body density.

using densitometry has been estimated at 2.7% for adults and about 4.5% for children and adolescents [18]. Measurements obtained by underwater weighing correlate well in broad populations with those obtained by other techniques. Limitations of underwater weighing include its relatively high equipment cost, the inability to measure gas volume in the gastrointestinal tract, its impracticality for large numbers of subjects, and the high level of cooperation and time required of subjects, who must be submerged and remain motionless for an extended time. Thus, the technique is not suitable for young children, older adults, or subjects in poor health.

Densitometry: Air Displacement

Another way to determine the volume—and thus density—of the body is with air displacement plethysmography. In the commercially available apparatus shown in Figure 8.10 (BOD POD, Cosmed Inc.), the subject is seated in a sealed chamber of known volume, separated from a second chamber by a membrane. The instrument measures the change in pressure caused by the volume occupied by the person. The person is dressed in a tight-fitting bathing suit and wears a bathing cap (to displace pockets of air in the hair). The measurement takes only a few minutes to complete. The apparatus has an advantage in that it can measure the body composition in age groups that are not suitable for underwater weighing, such as older adults or the very young. A similar instrument called the PEA POD is designed for infants and small children. Once the density of the body is obtained, the calculation of percent body fat is the same as with underwater weighing using established equations.

Dual-Energy X-ray Absorptiometry

Dual-energy X-ray absorptiometry (abbreviated DXA or DEXA) involves scanning subjects with X-rays at two different energy levels, illustrated in Figure 8.11. The subject lies on a table while an X-ray source beneath the table and the detector above the table pass across the subject's body. Attenuation of the beam of X-rays as it passes through the body is calculated by computer. Percentage of fat mass, bone-free fat-free mass, and bone mineral (total body or specific sites) can be calculated based on the restriction in the flux of the X-rays across the fat and the fat-free masses [19].

DEXA is considered to be the gold standard technique for diagnosing osteoporosis and osteopenia and is a commonly used method for body composition measurements. It is widely available and entails relatively low X-ray exposure: 1–10% that of a chest X-ray [19]. Limitations to the use of absorptiometry include the expense of the equipment and the exposure of subjects to radiation. In addition, trained personnel are required to run the instrument and analyze the scans. DEXA

Joe Traver//Time Life Pictures/Getty Images

Figure 8.10 Air-displacement plethysmography determines body density by measuring the amount of air displaced.

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Figure 8.11 Dual-energy X-ray absorptiometry (DEXA) uses two X-ray beams of different energy to determine fat-free mass, fat mass, and bone tissue.

measurements are highly reproducible and correlate with other body composition assessment methods. The technique, however, is not accurate for people with metal implants.

Other Imaging Techniques

Computed tomography, more commonly known as CT or CAT scan, and magnetic resonance imaging (MRI) have been used to measure body composition. Both of these imaging techniques are used extensively in medical diagnostics, but the equipment is very expensive and requires highly trained technicians to operate. Consequently, both imaging methods are used primarily for research purposes.

Knowledge about body composition led to the concept of the reference man and woman as a standard benchmark for educational and research purposes [20]. These reference figures are based on average physical dimensions from thousands of volunteer subjects and provide a frame of reference for comparison. They should not be viewed as "ideal" body composition. The characteristics of the reference man and woman were discussed in Chapter 6 and are summarized in Table 6.8. The reference man has 3% essential fat, 12% storage fat (for a total of 15% body fat), 44.8% muscle, 14.9% bone, and 25.3% other components. The reference woman has 12% essential fat, 15% storage fat (for a total of 27% body fat), 36% muscle, 12% bone, and 25% other components. Essential fat includes the fat that is associated with bone marrow, the central nervous system, internal organs, and the cell membranes. The essential fat in females also includes the fat in mammary glands and the pelvic region. These gender differences must be considered when body composition is evaluated. Normal changes in body composition associated with development and aging are discussed in Chapter 6, in the section "Changes in Body Mass with Age."

REGULATION OF ENERGY BALANCE AND BODY WEIGHT

The concept of energy balance has been discussed throughout this chapter, particularly *positive* energy balance that promotes body fat accumulation. Although the concept of energy balance could apply to the daily shifts in metabolic fuels that occur during the fed-fast cycle or bouts of exercise (discussed in Chapter 7), most health professionals refer to energy balance within the context of body composition and the changes that may occur over weeks, months, or years. When an individual is in positive energy balance over time, the excess energy, regardless of the macronutrient form in which it was consumed, will be stored as triacylglycerols in adipose tissue. Most experts generally agree on this concept, as emphasized in the *Dietary Guidelines for Americans* that recommend eating "within an appropriate calorie level" and engaging in "regular physical activity in a variety of ways" [21]. Even slight imbalances can have a significant long-term impact on body weight. Consuming an energy excess of just 150 kcal per day—the amount found in one 12 oz. soft drink—can result in the gain of one pound of adipose tissue in just 23 days.

Although once thought to be a simple matter of "energy in versus energy out," numerous lines of evidence have shown energy balance to be tightly controlled by several hormones working in concert. Additional internal and external factors can influence the function of these hormones and thus food intake, energy expenditure, and body fat deposition. The following discussion highlights some of the key hormones and contributing factors that impact energy balance and body weight.

Hormonal Influences

Hunger and satiety control meal-to-meal eating behavior, and both are under the influence of hormones. The main target of these hormones is the hypothalamus, in particular the **arcuate nucleus** region and its collection of neurons. There are two distinct populations of neurons in the arcuate nucleus with opposing actions. The first group of neurons express hormone receptors and, upon hormone binding, release **orexigenic** (appetite-stimulating) peptides that include neuropeptide Y (NPY) and agouti-related peptide (AgRP). The other group of neurons produce **anorexigenic** (appetite-inhibiting) peptides that belong to the pro-opiomelanocortin (POMC) family. The released peptides act as neurotransmitters and affect other neurons within the hypothalamus and elsewhere in the brain to produce the feeling of either hunger or satiety [22].

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The major hormones involved in appetite control and energy homeostasis are summarized in Table 8.6. The hormonal effects can be thought of as short term, involving daily fluctuations in appetite, and long term, which impacts energy balance and body weight over time. The so-called "gut hormones" include ghrelin, cholecystokinin, peptide YY, and glucagon-like peptide-1. Hormones secreted by the pancreas, namely insulin and pancreatic peptide, are often included in the list of gut hormones because of the intimate connection between the pancreas and the gastrointestinal tract. In addition, adipose tissue secretes leptin and adiponectin.

Leptin

The discovery of leptin by Jeffrey Friedman in 1994 was a milestone event in understanding the relationships of appetite control and obesity [23]. For many years it was suspected that factors in the bloodstream regulated food intake and energy expenditure, and the discovery of leptin provided the first solid evidence. Leptin is a hormone secreted by white adipose tissue that interacts with the hypothalamus to reduce hunger. When leptin binds to its receptors, the orexigenic neurons are inhibited and the production of NPY and AgRP declines, while the anorexigenic neurons are stimulated and release POMC peptides. Leptin levels in the circulation are directly correlated with the amount of body fat. As the amount of body fat increases, so does the level of leptin, thus suppressing hunger. In contrast, less body fat and lower leptin levels mean hunger is suppressed to a lesser extent. The correlation between BMI and leptin concentration is 0.9, illustrating the important role of leptin in long-term control of body weight. Moreover, mutations in the genes that code for leptin or leptin receptors result in severe obesity [23].

The elucidation of the action of leptin is hoped to be an important tool in the treatment of obesity, but this has yet to be fully realized. On one hand, the small number of obese individuals in the population who lack leptin because of genetic mutations benefit from leptin injections. On the other hand, the vast majority of obese people have normal or increased leptin levels, but the feeling of hunger persists and they do not decrease food intake. This has given rise to the concept of **leptin resistance** in which increased leptin levels fail to bring about weight loss even though the leptin receptor is functional. The mechanism of this resistance is not fully understood. One hypothesis is that part of the resistance is due to the inability of leptin to reach the receptor because of diminished transport across the blood–brain barrier. Another hypothesis points to receptor overstimulation, and thus activation of negative feedback pathways that block leptin signaling. The precise mechanisms of leptin resistance require further study.

Leptin also exerts rapid effects on glucose and lipid metabolism by activating leptin-responsive relays that are initiated in the hypothalamus and transmitted to other tissues via the sympathetic nervous system. The major tissues affected include the pancreas, skeletal muscle, liver, and adipose tissue. Furthermore, leptin receptors are present in tissues other than the hypothalamus, so leptin can exert direct effects on these tissues independent of its role in the hypothalamus. In skeletal muscle, leptin

Table 8.6 Agents of Energy Regulation

Agent	Site of Production	Stimulus	Action	Comments
Leptin	White adipose tissue	Levels increase with overfeeding or increased adipose tissue. Levels decrease with starvation or reduced adipose tissue.	Decreases the urge to eat and increases physical activity to produce a negative energy balance.	Leptin resistance reported in the obese.
Insulin	β-cells of pancreas	Levels increase with elevated blood glucose levels.	Suppresses hunger and stimulates the deposition of triacylglycerols in adipose tissue, which stimulates leptin.	
Adiponectin	Adipocytes	Levels increase with decreased fat mass. Levels decrease with increased fat mass.	Protects against insulin resistance, glucose intolerance, and dyslipidemia.	No role in food intake.
Ghrelin	Stomach and duodenum	Levels increase between meals and decrease after a meal and when absorption begins.	Stimulates hunger and food intake. Promotes digestion.	Only known orexigenic hormone.
Cholecystokinin	Intestine	Levels increase during and after a meal.	Suppresses appetite and promotes satiety.	
Pancreatic polypeptide	Pancreas	Levels increase during a meal.	Suppresses hunger in the short term.	Relatively brief half-life in the circulation.
Glucagon-like peptide-1	Intestine	Levels increase during and after a meal.	Suppresses hunger and inhibits glucagon production.	
Peptide YY	Small and large intestine	Levels rapidly increase after a meal. Levels are low in a fasting state.	Suppresses hunger in the long term.	May influence weight loss following surgery.

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activates AMP-activated protein kinase (AMPK) and thus promotes fatty acid oxidation and utilization for energy. Recall from Chapter 7 (Figure 7.2) that when AMPK is active (phosphorylated), fatty acid oxidation is stimulated and fatty acid synthesis is inhibited. Direct binding of leptin to tissues also regulates glucose metabolism through its effects on insulin. Leptin inhibits the synthesis and secretion of insulin by the pancreas, while insulin signaling is suppressed in the liver and white and brown adipose tissue [24].

Insulin

In a manner similar to leptin, insulin suppresses hunger through its action on orexigenic and anorexigenic neurons, although its effects are less robust than leptin. Insulin receptors are distributed throughout the brain, with high concentrations in the arcuate nucleus. Insulin binding stimulates the release of POMC peptides and inhibits the release of NPY and AgRP. Also similar to leptin, blood insulin levels increase in proportion to body fat, which may be the result of insulin resistance, as discussed later in this chapter.

Previous chapters highlighted the role of insulin in anabolic metabolism and energy storage through its direct action in several peripheral tissues such as muscle, adipose tissue, and liver. Insulin binding to hypothalamic receptors also transmits signals to peripheral tissues, which inhibits gluconeogenesis and proteolysis in the liver and stimulates lipogenesis and triacylglycerol accumulation in adipose tissue. The deposition of triacylglycerols, in turn, stimulates the release of leptin by adipocytes [25]. This metabolic coordination of insulin and leptin is not fully understood, although their action clearly impacts long-term energy homeostasis beyond just daily appetite control.

Adiponectin

Adiponectin is an adipocyte-derived hormone that plays a role in energy homeostasis. Plasma adiponectin levels are negatively correlated with body fat. Circulating levels of adiponectin are low in obese individuals, but increase in response to weight loss. Receptors for adiponectin are located in the hypothalamus, skeletal muscle, liver, and smooth muscle of the cardiovascular system. Although the precise role of adiponectin is not fully understood, plasma levels are inversely correlated with a number of metabolic conditions that include hypertension, type 2 diabetes, plasma triacylglycerol concentration, body mass index, several inflammatory markers, bone mineral density, and the risk of some cancers [26]. The promise of adiponectin as a protective molecule against inflammation, atherosclerosis, diabetes, and cancer has led to dietary and lifestyle strategies that boost the levels of plasma adiponectin levels. Caloric restrictive diets and associated weight loss increase adiponectin, as does the consumption of n-3 fatty acids, monounsaturated fatty acids, garlic, and the Mediterranean diet [27]. Exercise contributes to the beneficial effect of adiponectin by increasing the number of adiponectin receptors [28].

Ghrelin

Ghrelin is produced predominantly in the stomach and, unlikely the other regulatory hormones, stimulates the feeling of hunger. Ghrelin secretion rises between meals when the stomach is empty. It binds to receptors in the orexigenic neurons of the hypothalamus, causing the release of NPY and AgRP. As a meal is consumed and nutrient absorption begins, ghrelin secretion rapidly diminishes and hunger reduces. The half-life of ghrelin in the serum is only about 30 minutes. However, in obese individuals, the postprandial decline in ghrelin does not occur and may contribute to overeating [29]. Ghrelin also promotes digestion by stimulating gastric acid secretion and gastric motility prior to food consumption.

Other Anorexigenic Hormones

Cholecystokinin (CCK) An important role of CCK is to stimulate digestive processes when food is consumed (see Chapter 2). CCK is produced in the small intestine in response to food intake, especially lipids and proteins. Another important function is appetite regulation. CCK binds to receptors in the hypothalamus and inhibits NPY production by downregulating mRNA expression. CCK also binds to receptors in the pylorus, causing contractions that send vagal nerve signals from the stomach to the brain, resulting in decreased hunger and increased satiety.

Pancreatic Polypeptide (PP) The anorexigenic PP is synthesized primarily in the pancreas and secreted in response to food intake. Its mechanisms of action are not well understood, although PP receptors are present in the hypothalamus and binding appears to downregulate NPY mRNA expression, thus decreasing hunger. PP has a relatively short half-life in the circulation and therefore a brief period of action.

Glucagon-Like Peptide (GLP)-1 Production of GLP-1 occurs along the entire length of the intestine in proportion to caloric intake. GLP-1 release is delayed in obese individuals, who also have lower circulating GLP-1 levels. GLP-1 stimulates the pancreas to secrete insulin while inhibiting secretion of glucagon. It also reduces gastric emptying and intestinal motility, the latter causing signals

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to be sent via the vagus nerve to the brain, resulting in decreased hunger. GLP-1 receptors are found in the brain, although the role of GLP-1 binding is unclear.

Peptide YY (PYY) The small and large intestine produces PYY and its action lasts longer than the other anorexigenic hormones. As mentioned in Chapter 2, this characteristic is important in countering the effects of CCK by inhibiting intestinal motility and secretion of digestive juices. In addition, PYY binds to receptors in the hypothalamus, although the anorexigenic mechanisms of action are not fully understood. Circulating levels of PYY are lower in obese individuals.

Intestinal Microbiota

The human gastrointestinal tract is host to trillions of microbial cells that participate in nutrient digestion and utilization (see Chapters 2 and 4). Growing evidence indicates that intestinal microbiota are also linked to obesity development and metabolic dysfunction [30]. Normally the microbial community is dominated by bacteria, with 90% belonging to the phyla *Firmicutes* and *Bacteroidetes*. In obese people, the intestinal microbiota is altered and responds to changes in body weight. Excess body fat is associated with more *Firmicutes* and fewer *Bacteroidetes*. The level of *Bacteroidetes* increases when weight is reduced with energy-restricted diets, although it is unclear whether the microbiota are responding to changes in energy intake or changes in adiposity.

One possible outcome of altered microbiota is their altered ability to ferment non-digestible carbohydrates (fiber) and produce short-chain fatty acids. The microbiota of obese individuals have an increased capacity to ferment fiber and thus "harvest" more energy as short-chain fatty acids that contribute to the total energy absorbed into the body. The additional energy can be stored as body fat. The short-chain fatty acids may also act as signaling molecules in intestinal cells by decreasing the production of the anorexigenic hormones, GLP-1 and PYY, thus removing the feeling of satiety.

Long-term dietary habits have significant impact on intestinal microbiota, as evident in populations that consume different macronutrient profiles [30]. For example, the microbiota resulting from European diets rich in protein and animal fat are quite different compared to high-fiber diets in West Africa, which are rich in cellulose and hemicelluloses. The microbiota in West Africans have adapted to fermenting these fibers to gain maximum energy as short-chain fatty acids.

The interrelationships of intestinal microbiota, diet, and obesity are complex and difficult to study because of the high degree of variability in human microbiota and diet. Nevertheless, it has been established that an individual's diet is a strong determinant of the microbiota composition and that altered microbiota profiles are associated with obesity.

Environmental Chemicals

Exposure to certain chemicals may play a role in body fat accumulation. Recent research suggests that a group of endocrine-disrupting chemicals present in the environment, including the diet, may predispose some people to gain body fat despite appropriate levels of food intake and physical activity [31]. These chemicals (aptly named **obesogens**) interfere with hormone function by binding to hormone receptors and either stimulating or inhibiting the signaling pathway. Obesogens are believed to promote triacylglycerol production and deposition in adipose tissue, as well as disrupting appetite control in the hypothalamus. Some evidence suggests that developmental exposure to obesogens early in life may interfere with epigenetic programming of gene regulation, thus influencing the risk of obesity later in life [32]. Much more research is needed to confirm these findings.

Lifestyle Influences

Diet and physical activity are the most obvious factors that influence energy balance and body weight. Achieving a healthy weight and maintaining energy balance should be a life-long goal. The most successful approaches include establishing both dietary and exercise habits that improve fitness and lower the risk of disease. For the average person, the importance of controlling the amount of food consumed cannot be overstated. For example, an individual can easily consume 2,000 kcal or more in one meal. In comparison, that same individual would need to walk 21 miles at a brisk pace requiring more than 5 hours to expend 2,000 kcal of energy (see Table 8.3). The importance of food intake in maintaining energy balance is further illustrated by the fact that total energy intake in adults has increased approximately 200–300 kcal/day since the 1970s, which closely parallels the increase in obesity as depicted in Figure 8.6 [33].

Daily physical activity and exercise contribute to total expenditure and help maintain energy balance. Unfortunately, nearly half of adults in the United States are physically inactive [34]. In the past 50 years, energy expenditure related to occupations and household activities has declined, thus increasing the importance of planning activities that increase the use of skeletal muscle

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throughout the day. This includes both regular exercise and nonexercise activities such as walking, climbing stairs, standing, fidgeting, cleaning, etc. that can significantly contribute to daily energy expenditure [35].

Many social factors influence eating behaviors and exercise habits. Research has shown that increased food and energy intake occurs when eating away from home, and people tend to eat more when presented with larger portion sizes. Interestingly, though, there is little evidence that portion size *per se* is associated with body weight gain [36]. Living in a socioeconomically deprived area, having limited access to supermarkets, and having greater access to fast-food outlets are associated with higher BMI, but whether these factors directly *cause* body weight gain is unclear [37]. At present, there are relatively few studies that address the impact of social and behavioral factors on food intake, physical activity, and obesity.

HEALTH IMPLICATIONS OF ALTERED BODY WEIGHT

The terms *overweight* and *obesity* are often used to indicate different degrees of body "fatness." The overweight category, as previously mentioned in this chapter, can unintentionally include individuals who have gained muscle mass rather than fat. The obesity category, on the other hand, includes only those with excess body fat. Accumulation of body fat is associated with an increased risk of mortality and morbidity due to hypertension, stroke, coronary artery disease, dyslipidemia, type 2 diabetes, sleep apnea, osteoarthritis, and certain cancers. During the past 50 years, prevalence of overweight and obesity has risen steadily to epidemic levels (see Figure 8.6). Currently in the United States there are 79 million obese adults, projected to increase to 144 million by 2030 with an additional economic burden of $48 billion to $66 billion per year [38].

When assessing the health risks related to excess body fat, the differences in fat distribution between men and women should be considered. Recall from Chapter 6 the concept of the reference man and woman (Table 6.8) in which women have significantly more "essential fat," referring to the subcutaneous fat that accumulates mostly around the hips and thighs and in mammary tissue. On the other hand, excess fat in both men and women can accumulate within the body core (visceral fat) and subcutaneously around the torso. These observations form the basis of definitions for two patterns of obesity. **Gynoid obesity**, also called pear-shape, is associated primarily with women and refers to the excess fat that accumulates around the hips and thighs. **Android obesity**, or apple-shape, describes the central adiposity resulting from the accumulation of excess visceral fat. Android obesity is more strongly associated with disease risk. Consequently, measuring waist circumference or waist-to-hip ratio is a more useful risk assessment tool than body weight or BMI alone [39].

At the cell level, the deposition of triacylglycerols causes adipocytes to become enlarged (hypertrophy). In addition, new adipocytes can be produced (**hyperplasia**) to accommodate more triacylglycerol molecules. The creation of new fat cells increases most rapidly in late childhood and early puberty whenever a positive energy balance exists, although hyperplasia can occur later in life as well. Obese people have more, and larger, fat cells than nonobese people. If body fat is lost, the number of fat cells does not decrease; they just get smaller.

Metabolic Syndrome

The definition of *syndrome* is a clustering of factors that occur together more often than expected based on chance alone, and whose cause is often uncertain. The concept of **metabolic syndrome** refers to a group of risk factors that are associated with increased risk of cardiovascular disease (CVD). The risk factors for metabolic syndrome include central obesity; increased fasting plasma glucose; increased fasting plasma triglyceride; decreased plasma HDL cholesterol; and hypertension. A diagnosis of metabolic syndrome requires a patient to exhibit three of the five conditions as defined in Table 8.7. It is believed that the underlying mechanisms uniting these factors are related to insulin resistance (discussed in the next section).

Metabolic syndrome diagnosis holds promise as a predictor of disease: it is associated with a two-fold increase in risk for CVD, myocardial infarction, stroke, and CVD-related mortality [40]. Metabolic syndrome diagnosis is associated with a 1.5-fold increase in risk of all-cause mortality. Still to be determined, however, is whether a diagnosis of metabolic syndrome is a

Table 8.7 Criteria for Clinical Diagnosis of Metabolic Syndrome

Measure	Categorical Cut Points
Elevated waist circumference*	≥ 102 cm (40 in) for men ≥ 88 cm (35 in) for women
Elevated triacylglycerols	≥ 150 mg/dL (1.7 mmol/L)
Reduced HDL-C	< 40 mg/dL (1.0 mmol/L) in men < 50 mg/dL (1.3 mmol/L) in women
Elevated blood pressure	Systolic ≥ 130 and/or diastolic ≥ 85 mm Hg
Elevated fasting glucose	≥ 100 mg/dL

Source: Modified from Alberti KGMN, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120:1640–45.

* Current recommended thresholds for the U.S. adult population. Thresholds vary among countries.

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better predictor of risk than the sum of the individual risk factors. Furthermore, central (android) obesity has consistently been recognized as a key component of the syndrome and is nearly always one of the diagnostic criteria. This has led to considerable debate about the usefulness of metabolic syndrome as a practical clinical tool in identifying patients in need of treatment. In fact, the American Diabetes Association and the European Association for the Study of Diabetes issued a joint statement emphasizing that the risk of CVD associated with metabolic syndrome diagnosis is no greater than the sum of each factor, calling into question the medical value of diagnosing the syndrome [41]. In many cases, reduction in body weight alone will improve each of the other diagnostic factors.

Insulin Resistance

Insulin resistance is generally defined as the inability of target tissues to respond to insulin, causing elevated blood glucose (hyperglycemia). This often leads to the diagnosis of type 2 diabetes. The pancreas may release more insulin in an effort to maintain normal blood glucose levels. Elevated insulin levels confirm the type 2 diabetes diagnosis and distinguish it from type 1 diabetes in which insulin is very low or absent. There is no single cause for type 2 diabetes; however, many studies have identified excess body fat as the most important factor predicting type 2 diabetes, with 90% of type 2 diabetic patients falling into the overweight or obese category [42].

The insensitivity to insulin is primarily seen in muscle and adipose tissue. Within insulin-resistant muscle, insulin loses its ability to stimulate glucose uptake; in adipose tissue, it no longer inhibits free fatty acid release. These observations can explain the elevated blood glucose and free fatty acid levels that accompany insulin resistance. The liver and kidney retain their sensitivity to insulin, and the elevated insulin levels stimulate liver triacylglycerol synthesis using the excess free fatty acids. As a consequence, the assembly and secretion of VLDL increases, resulting in elevated fasting serum triacylglycerol. Triacylglycerol levels in the liver also increase, leading to nonalcoholic fatty liver disease. The kidney responds to the elevated insulin levels by increasing renal sodium retention and decreasing uric acid clearance. This response results in an increased prevalence of essential hypertension and higher plasma uric acid concentrations.

Each of these metabolic conditions related to insulin resistance—excess body fat, elevated blood glucose, elevated triacylglycerols, and high blood pressure—are diagnostic criteria (along with low HDL cholesterol) for metabolic syndrome. It is easy to see why metabolic syndrome has been called insulin resistance syndrome and why the measurement of insulin resistance was once considered as a diagnostic criterion. However, no simple test exists to determine who is insulin resistant and who is not. Fasting insulin levels, fasting plasma glucose levels, and triacylglycerol:HDL-C ratios have all been used as indicators for insulin resistance, with varying degrees of success.

Considerable evidence demonstrates that if a person loses weight, insulin sensitivity improves. Fortunately, the elevated insulin levels do not prevent weight from being lost. On the contrary, free fatty release from adipocytes is accelerated, so it is vital that positive energy balance be avoided for weight reduction to occur. Interesting to note is that variations in the macronutrient content of isocaloric diets have little effect on insulin sensitivity. One common weight loss strategy is to lower the lipid content of the diet and replace it with carbohydrate. The problem with a low-fat, high-carbohydrate diet for a person with insulin resistance, however, is that the additional carbohydrate requires more insulin to be secreted from the pancreas to maintain glucose homeostasis. If the person is insulin resistant, and the pancreas is functioning properly, insulin levels will be elevated further.

Weight-Loss Methods

Each year many people "go on diets" for the purpose of losing body fat. Depending on the nature of the caloric restriction and the level and type of exercise, both body fat and fat-free mass may be lost. Limited information is available on the proportion of fat and muscle lost on different weight-loss regimes.

Losing body fat requires a negative energy balance over an extended period of time. In a systematic review of weight-loss programs lasting at least 6 months, the authors concluded that moderate weight loss (5–10 kg) that consists of both fat mass and fat-free mass can be achieved by calorie restriction alone [43]. Exercise alone is generally considered to be less effective than calorie restriction with exercise. If exercise is added to the weight-loss program, some or all of the fat-free mass can be spared. Most studies have used an aerobic exercise component, and in those that combined calorie restriction with exercise, most of the weight lost was fat mass and very little (0–1.5 kg) was fat-free mass. Resistance training results in only small losses or even a gain in fat-free mass.

Research has also explored the relationship between diet composition and weight loss. Some controversy remains as to the proper combination of low fat, low carbohydrate, and/or high protein. However, it appears the most important factor in successful weight loss is the intensity of behavior modification, irrespective of the macronutrient composition of the energy-restrictive diet [44].

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SUMMARY

To maintain a state of energy balance, the amount of food energy a person consumes must equal energy expenditure in the body. Techniques have been developed to measure energy expenditure based on the principle that all energy-requiring metabolic processes ultimately produce heat while utilizing oxygen. Methods using direct calorimetry measure heat production by the body, whereas methods using indirect calorimetry measure oxygen consumed and carbon dioxide expired. The ratio of CO_2 produced relative to O_2 consumed is called the respiratory quotient and reflects the relative proportion of carbohydrate and fat being used as metabolic fuel.

Daily total energy expenditure is attributable to three primary components: basal metabolic rate, physical activity, and the thermic effect of food. Basal metabolic rate represents the amount of energy needed to sustain basic life processes such as respiration, heartbeat, renal function, brain and nerve function, blood circulation, active transport, and synthesis of proteins and other complex molecules. Physical activity requires energy to support skeletal muscle contraction and is the most highly variable component of total energy expenditure. Physical activity includes all exercise and nonexercise activities associated with daily living. The thermic effect of food represents the increase in energy expenditure needed to process food, including the work associated with the digestion, absorption, transport, metabolism, and storage of energy from ingested food.

Consuming more energy than needed puts a person in a state of positive energy balance, resulting in body fat accumulation. Health practitioners have long used body weight as proxy for body fat. The concept of a desirable or ideal body weight is based height-weight tables developed by the life insurance industry that indicate body weights associated with the lowest mortality. Height-weight tables have largely been abandoned as a diagnostic tool in favor of the body mass index (BMI in conjunction with measurements of waist circumference. BMI and waist circumference correlate reasonably well with disease risk and are used extensively as an initial screening tool for the general population and in epidemiological research.

Body composition and fat mass can be assessed by several methods. Field methods are portable and relatively easy to use, including skinfold thickness, which measures subcutaneous fat, and bioelectrical impedance, which is based on the principle that a low electrical current is facilitated in fat-free tissue high in water and electrolyte content, but is impeded by fat tissue. Laboratory methods include measuring body volume and density by underwater weighing or by air displacement. Computer-based imaging techniques include dual-energy X-ray absorptiometry, computed tomography, and magnetic resonance imaging.

Energy intake is driven by appetite and is influenced by many social and environment factors. Hunger and satiety control meal-to-meal eating behaviors as a result of hormone regulation. Ghrelin is an orexigenic hormone that stimulates hunger. Anorexigenic hormones that inhibit hunger include leptin, insulin, adiponectin, cholecystokinin, pancreatic polypeptide, glucagon-like peptide-1, and peptide YY. Long-term energy balance may be influenced by intestinal microbiota, environmental endocrine-disrupting chemicals (obesogens), exercise habits and food choices, and socioeconomic conditions.

Excess body fat is associated with an increased risk of disease and mortality. Obesity prevalence has increased dramatically during the past 50 years, leading to the development and use of *metabolic syndrome* as a diagnostic tool. Insulin resistance is thought to be the underlying cause of metabolic syndrome. In many cases, weight loss improves insulin sensitivity.

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EATING DISORDERS

Few things can create as large a sensation in the media as a new weight-reduction diet guaranteed to remove unwanted fat. The authors of the sensational new diet are interviewed on television talk shows, the news media give publicity to the new diet (and its authors), and the book promoting the new and revolutionary diet joins its companions on the shelves of all bookstores. The fact that the new diet book has so many companions on the bookshelves attests to the fact that none of these "new and revolutionary" diets is successful in helping people reduce weight and keep it off. Nevertheless, following some sort of weight-reduction diet appears to be a way of life among many Americans, particularly women.

The desire of girls and women to be thin has a foundation: the ideal female body image is dictated to a large extent by movie and television celebrities, fashion models, and beauty pageant contestants. Society considers thin to be healthy. In fact, although more recent winners of the Miss America pageant have body mass indices at the lower end of the normal range, many of the pageant winners during the 1970s and 1980s were considered undernourished, having body mass indices between 16.9 and 18.5 kg/m^2 [1]. Children as young as 9 years of age have been reported curtailing their food intake to avoid becoming fat [2]. A female's body size too often affects her self-worth and self-esteem. Body image distress results when people become dissatisfied with their weight and, if people believe that their weight and shape are central to their self-worth as a person, an eating-disorder mindset often ensues. Eating disorders affect over 30 million in the United States, with 10–15% being male [3].

The American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders*, fifth edition (DSM-5), is commonly used to define eating disorders [4]. The main eating disorder categories listed in DSM-5 are anorexia nervosa, bulimia nervosa, and eating disorder not otherwise specified. This third category includes all other disorders affecting eating.

ANOREXIA NERVOSA

Being too thin is dangerous, even deadly. Anorexia nervosa is a chronic, relapsing illness for many individuals. Of the many psychiatric disorders, anorexia nervosa possesses the highest mortality rate. If left untreated, up to one-fifth of people with the condition die, often before 30 years of age, and many, despite treatment, die from eating disorder–related complications or suicide.

Anorexia nervosa, described over 100 years ago as a loss of appetite caused by a morbid mental state, is actually misnamed because its victims do not typically experience a loss of appetite. People with anorexia nervosa have a distorted body image and an irrational fear of weight gain. This distorted body image is a perception that they are fat even though they are extremely thin. Furthermore, anorectics are extremely critical of their body as a whole and often more critical about selected body areas (such as thighs, stomach, etc.). Thus, they become obsessed with weight loss and relentlessly pursue thinness, often eating diets providing less than 800 kcal per day.

Eating patterns of people with anorexia nervosa mostly fall into one of two categories: the restricting type or the binge eating–purging type. Anorectics with the restricting type eat to a very limited extent without regularly inducing vomiting or misusing laxatives or diuretics. People with the binge eating–purging type alternate between restricting food intake and bouts of binge eating or purging behavior with laxative or diuretic misuse or self-induced vomiting [4]. However, in addition to these controlled eating behaviors, anorectics often exercise excessively to further weight loss efforts, to prevent possible weight gain, and to try to correct perceived imperfections in body size and shape. Exercise is considered excessive if its postponement is accompanied by intense guilt or when it is undertaken solely to influence weight or shape.

Some of the diagnostic criteria for anorexia nervosa (Table 1) based on DSM-5 include refusal to maintain body weight at or above minimally normal weight for age and height (e.g., at least 85% of expected weight for height; or, from the *International Classification of Diseases*, a body mass index of at least 17.5 kg/m^2), intense fear of gaining weight or being fat, and **amenorrhea** (absence of at least three consecutive menstrual cycles). Self-worth based on weight or shape, preoccupation with food, and abnormal food consumption patterns are also typical of those with anorexia nervosa [5].

The causes of anorexia nervosa are unknown, but the disease is thought to be multifactorial. Genetic vulnerability as well as anxiety, obsessive–compulsive personality disorders, and perfectionism traits are typically present in those who develop anorexia nervosa [5]. Anorectics also may exhibit depression and substance abuse. In addition, those who develop anorexia nervosa often have a poor self-image and want to please others because their perceived self-worth is heavily dependent upon the words and actions of others (such as teachers, coaches, or instructors). Other traits associated with the development of this eating disorder include issues concerning food and body weight, issues concerning relationships with oneself and with others, conflict regarding maturation, and problems with separation, sexuality, self-esteem, and compulsivity [5].

The initial weight loss of the anorectic may not always result from a deliberate decision to diet; initial weight loss may occur unintentionally, for example, as the result of the flu or a gastrointestinal disorder [6]. However, following the initial weight loss, whatever its cause, additional diet restriction (and excessive exercise) is deliberate. Weight loss or control of body weight becomes the overriding goal in life, especially during stressful periods when pressures become overwhelming. The anorectic learns the caloric contents of foods and the energy expenditure associated with various activities. Because anorectics have such a disturbed body image and such an intense fear of becoming fat, they may continue starving themselves to emaciation and even death should intervention be delayed too long.

The effects of anorexia nervosa on the body are similar to the effects of hypometabolic states (such as starvation, protein-calorie malnutrition, or marasmus) and affect all

Table 1 Diagnostic Criteria for 307.1 Anorexia Nervosa

A.	Refusal to maintain body weight at or above a minimally normal weight for age and height (e.g., weight loss leading to maintenance of body weight less than 85% of that expected; or failure to make expected weight gain during period of growth, leading to body weight less than 85% of that expected).
B.	Intense fear of gaining weight or becoming fat, even though underweight.
C.	Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of the seriousness of the current low body weight.
D.	In postmenarcheal females, amenorrhea, i.e., the absence of at least three consecutive menstrual cycles. (A woman is considered to have amenorrhea if her periods occur only following hormone, e.g., estrogen, administration.)

Specify type:

Restricting Type: during the current episode of anorexia nervosa, the person has not regularly engaged in binge-eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas)

Binge-Eating/Purging Type: during the current episode of anorexia nervosa, the person has regularly engaged in binge-eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas)

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parts of the body. Table 2 lists some potential consequences of anorexia nervosa. Growth and development slow. Adipose tissue, lean body mass, and bone mass are lost. Organ mass may be lost, and organ function may become impaired. Loss of heart muscle can weaken the heart and cause, among other serious complications, an irregular heartbeat or a prolonged QT interval (the QT interval is the time that it takes for the heart to contract and refill with blood; with a prolonged QT interval, the heart takes longer to recharge between beats in preparation for the next heartbeat). The gastrointestinal tract atrophies such that peristalsis is slowed, gastric emptying is delayed, and intestinal transit time is lengthened. The secretion of digestive enzymes and of digestive juices also is diminished. Constipation often results, along with abdominal distention after eating just small amounts of food. Hormone and nutrient levels in the blood become altered. Skin typically becomes dry, hair loss from the head occurs while **lanugo**-type (soft woolly) hair may appear on the sides of the face and arms, and body temperature drops. A long-term consequence of the bone loss that occurs with anorexia nervosa is osteopenia and ultimately osteoporosis, which occurs much earlier in those who have (or have had) anorexia nervosa than in those who have not had the condition [7].

Treatment of anorexia nervosa is multidisciplinary (involving a physician, dietitian, nurse, psychologist, psychiatrist, and family therapist, among others) and may be accomplished through outpatient or inpatient care, depending on the severity of the condition. Assessment for inpatient treatment generally includes an evaluation of the person's mental status, how much the person is eating, current weight (inpatient treatment is warranted if weight is < 25–30% of ideal), speed of weight loss, motivation and adherence to treatment, family support, purging behavior, and comorbid complications, especially those affecting the heart [8]. Whether the patient is treated as an inpatient or as an outpatient, goals for the patient's health are established, often with a written contract signed by the patient as well as by members of the health care team.

Summaries of treatment outcomes for anorexia nervosa show that about 40–50% recover completely, about 30% improve, 20–25% continue to experience chronic problems with the condition, and another 10–15% die from medical complications, suicide, or malnutrition. Mortality typically is highest among people who have sustained severe weight loss, who have had the condition for a prolonged duration, and who developed the condition at an older age [9–11].

BULIMIA NERVOSA

Bulimia nervosa, another eating disorder, is a condition characterized by recurring binge eating coupled with self-induced vomiting and misuse of laxatives, diuretics, or other medications to prevent weight gain. Binge eating is marked by a sense of lack of control over eating during the binge episode. A binge is defined as eating an amount of food larger than most people would eat during a similar time period and under similar circumstances [4]. Bulimia denotes a ravenous appetite (or "ox hunger") associated with powerlessness to control eating [6]. Criteria for the diagnosis of bulimia nervosa are given in Table 3.

Table 2 Some Potential Medical Complications of Anorexia Nervosa

Gastrointestinal	**Hematologic**
Gastric distention	Anemia
Constipation	
	Skeletal
Cardiovascular	Stress fractures
Heart muscle atrophy	Premature osteoporosis
Bradycardia	
Hypotension	**Muscle**
Arrhythmias	Depleted muscle mass
Mitral valve prolapse	
Peripheral edema	**Brain**
	Abnormal electrical activity
Endocrine/Metabolic	Confusion
Amenorrhea	
Hypothermia	

Bulimia occurs primarily in young women, especially college-age women who are of normal weight or slightly overweight. The typical bulimic, rather than being overly concerned with losing weight and becoming very thin (like the person with anorexia nervosa), seeks to be able to eat without gaining weight [6]. Other factors associated with the development of bulimia include a history of sexual abuse, psychoactive substance abuse or dependence, a family history of depression or alcoholism, obsessive–compulsive disorder, negative self-evaluation, and a high use of escape-avoidance coping [9].

Bulimia often starts with dieting attempts in which hunger feelings get out of control. These dieting attempts, usually based on food abstinence or excessive food restriction, lead to binge eating. Once binge eaters discover that they can undo the consequences of their overeating by vomiting the ingested food, they begin to binge not only when they are hungry but also when they are experiencing any distressing emotion [6,9]. Most binge eating is done privately in the afternoon or evening, with an intake of about 3,500 kcal; purging behaviors reduce retention of energy to about 1,200 kcal [12]. Favorite foods for binging usually are dessert and snack foods very high in carbohydrates.

Diagnosis is usually dependent upon self-reported symptoms or on treatment for related problems or conditions. Conditions that may develop as the result of bulimia are listed in Table 4. The gastrointestinal tract is greatly affected by repeated vomiting and the use of laxatives. Repeated vomiting also causes other problems, including skin lesions or calluses on the dorsal side of the hands (especially over the joints), severe dental erosion, swollen enlarged neck glands (due to salivary or parotid gland enlargement), reddened eyes, headache, and fluid and electrolyte imbalances. Laxative misuse may exacerbate fluid and electrolyte losses and, when coupled with vomiting, may lead to heart arrhythmias and heart failure. The presence of lesions or calluses on the

Table 3 Diagnostic Criteria for 307.51 Bulimia Nervosa

A. Recurrent episodes of binge eating. An episode of binge eating is characterized by both of the following:
- **(1)** eating, in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than most people would eat during a similar period of time and under similar circumstances.
- **(2)** a sense of lack of control over eating during the episode (e.g., a feeling that one cannot stop eating or control what or how much one is eating).

B. Recurrent inappropriate compensatory behavior in order to prevent weight gain, such as self-induced vomiting; misuse of laxatives, diuretics, enemas, or other medications; fasting; or excessive exercise.

C. The binge eating and inappropriate compensatory behaviors both occur, on average, at least twice a week for 3 months.

D. Self-evaluation is unduly influenced by body shape and weight.

E. The disturbance does not occur exclusively during episodes of anorexia nervosa.

Specify type:

Purging Type: during the current episode of bulimia nervosa, the person has regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas.

Nonpurging Type: during the current episode of bulimia nervosa, the person has used other inappropriate compensatory behaviors, such as fasting or excessive exercise, but has not regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas.

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Table 4 Some Potential Medical Complications of Bulimia Nervosa

Gastrointestinal	**Cardiovascular**
Erosion of the teeth	Arrhythmias
Dental caries	
Sore throat	**Respiratory and Skeletal**
Swollen parotid glands	Aspiration pneumonia
Esophageal rupture or tears	Rib fracture
Stomach tear	
Gastroesophageal reflux disease	**Endocrine/Metabolic**
Constipation	Irregular menses or amenorrhea
Cathartic colon	Electrolyte imbalance

hands (due to the scraping of teeth against the skin while self-inducing vomiting), swollen neck glands, and frequent trips to the bathroom after meals often are recognized by health professionals and family or friends and facilitate detection and diagnosis of the problem.

The treatment of bulimia, like that of anorexia nervosa, is multidisciplinary. Goals typically focus on eliminating binge–purge behaviors, normalizing eating habits, maintaining weight, and resuming normal menses, if they are affected. The patient is most likely to be hospitalized with problems such as electrolyte imbalance, drug (e.g., laxatives, diuretics) dependence, severe depression, or suicidal tendencies [9]. The prognosis of those suffering from bulimia nervosa is generally more favorable than for those with anorexia nervosa; over 50% of those with bulimia nervosa fully recover or achieve good outcomes, while fewer than 10% have poor outcomes [13].

BINGE EATING DISORDER

The American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders* includes a provisional eating disorder diagnosis, binge eating disorder, which is not associated with purging as in bulimia nervosa [4]. Binge eating disorder is characterized by binge eating at least twice a week for at least a 6-month period with no compensatory behaviors. The binge eating episode typically involves eating (usually with a general sense of lack of control) large amounts of highly energy-dense foods (such as dessert and snack food–type items) more rapidly than normal, and continues despite the individual feeling uncomfortably full. Subtypes of the disorder are mainly distinguished by whether the binge precedes dieting or whether dieting precedes the binge eating [14]. Factors associated with binge eating include repeated exposure to negative comments about eating, shape, and weight; depression; negative self-evaluation; and vulnerability to obesity. For those with a binge eating disorder, food often provides comfort and a sense of emotional well-being, especially if the person is feeling stressed, anxious, unhappy, or depressed. Thus, the binge usually occurs when the person is not physically hungry but is emotionally unhappy. The binge usually happens in private because of embarrassment, and, following the binge, feelings of disgust, guilt, and depression are common.

DISORDERED EATING

Eating disorders (Table 5) other than anorexia nervosa, bulimia nervosa, and binge eating disorder are categorized by the American Psychiatric Association as eating disorders not otherwise specified [4]. The characteristics of those with disordered eating are similar to those of individuals with anorexia nervosa and bulimia nervosa and include fear of being fat, restrained eating, binge eating, purging behavior, and distorted body image; however, people with disordered eating do not meet the criteria for anorexia nervosa or bulimia nervosa (Tables 1 and 3). Disordered eating is likely more common than anorexia and bulimia nervosa, and is seen in both males and females, especially female athletes, where it often exists as part of the female athlete triad.

The Female Athlete Triad

The female athlete triad, first described in 1992 and defined as the combination of disordered eating, amenorrhea, and osteopenia, is described as a complex interrelationship between menstrual status, bone health, and energy availability [15]. The condition appears most often in women participating in sports in which physique and body image are important and extra body weight is undesirable. While all female athletes are considered at risk, women most at risk include long-distance runners, figure skaters, gymnasts, ballet dancers, swimmers, and divers [16].

Inadequate energy intakes among athletes may be related to disordered eating—with an estimated prevalence of 10–20% in athletes—or simply to a failure to meet the high energy needs of the sport (unassociated with disordered eating) [17]. The causes of amenorrhea in athletes, as in women with eating disorders, are not clearly understood. They are thought to relate to synergistic effects of excessive amounts of physical activity and training, constant stress or anxiety, low amounts of body fat, weight fluctuations, and poor diet, especially an extreme energy (caloric) deficit [15]. The effect of these various factors is to evoke changes in the release of hormones such as follicular stimulating and luteinizing hormones that then lead to diminished release of estrogen and progesterone (among other hormones), which in turn can cause amenorrhea. The amenorrhea—more specifically, the diminished serum estrogen concentrations—in turn negatively impact the skeletal system, similar to what is observed in those with anorexia nervosa [7]. Premature bone loss, inadequate bone formation, or both with resulting low bone mass, stress fractures, and other orthopedic problems are common among female athletes; in fact, over 50% of athletes with amenorrhea have low bone mass or bone densities at least one standard deviation below the mean [9]. High levels of cortisol in the blood, common in athletes, and extensive training regimens also can contribute to bone loss along with poor energy and nutrient (especially vitamin D and calcium) intakes. Some of the American College of Sports Medicine's recommendations for the prevention and treatment of the female athlete triad emphasize optimizing energy and nutrient availability and providing medications, as needed, to improve bone health and for psychological problems, although the full reversal of low bone mineral density is thought to be unlikely [15].

SUMMARY

The early identification and treatment of the female athlete triad as well as eating disorders is crucial if serious complications are to be avoided. Just as obesity is associated with increased risk for a variety of diseases, eating disorders are associated with a multitude of risks, including death. Combating eating disorders is difficult; not only must the victims be treated, but the values of society, including images of beauty, must also be rehabilitated.

Table 5 307.50 Eating Disorder Not Otherwise Specified

The Eating Disorder Not Otherwise Specified category is for disorders of eating that do not meet the criteria for any specific Eating Disorder. Examples include

1. For females, all of the criteria for anorexia nervosa are met except that the individual has regular menses.
2. All of the criteria for anorexia nervosa are met except that, despite significant weight loss, the individual's current weight is in the normal range.
3. All of the criteria for bulimia nervosa are met except that the binge eating and inappropriate compensatory mechanisms occur at a frequency of less than twice a week or for a duration of less than 3 months.
4. The regular use of inappropriate compensatory behavior by an individual of normal body weight after eating small amounts of food (e.g., self-induced vomiting after the consumption of two cookies).
5. Repeatedly chewing and spitting out, but not swallowing, large amounts of food.
6. Binge-eating disorder: recurrent episodes of binge eating in the absence of the regular use of inappropriate compensatory behaviors characteristic of bulimia nervosa.

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Web Sites

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www.nimh.nih.gov/publicat/eatingdisorders.cfm
www.anad.org/get-information/about-eating-disorders/general-information/

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9 WATER-SOLUBLE VITAMINS

VITAMIN C (ASCORBIC ACID)
THIAMIN (VITAMIN B_1)
RIBOFLAVIN (VITAMIN B_2)
NIACIN (VITAMIN B_3)
PANTOTHENIC ACID
BIOTIN
FOLATE
VITAMIN B_{12} (COBALAMIN)
VITAMIN B_6
For each vitamin, the following main subtopics (when applicable) are discussed:
Sources
Digestion, Absorption, Transport, and Storage
Functions and Mechanisms of Action
Interactions with Other Nutrients
Metabolism and Excretion
Recommended Dietary Allowance or Adequate Intake
Deficiency
Toxicity
Assessment of Nutriture
SUMMARY

PERSPECTIVE
GENETICS AND NUTRITION: THE EFFECT ON FOLIC ACID NEEDS AND RISK OF CHRONIC DISEASE BY DR. RITA M. JOHNSON

THE EARLY PART OF THE 20TH CENTURY was the most exciting era in the history of nutrition science. It was during this time that the discovery of vitamins, or "accessory growth factors," began. Researchers found that for life and growth, animals required something more than a chemically defined diet consisting of purified carbohydrate, protein, fat, minerals, and water. The first of these dietary essentials was discovered as a result of a search to cure a "condition." An "antiberiberi substance," which cured the "condition" now called "**beriberi**," was isolated from rice polishings by Casimir Funk, a Polish biochemist. Funk gave the substance the name *vitamine* because the substance was chemically an amine and was necessary for life (*vita* means "life" in Latin). Very shortly thereafter McCollum and Davis extracted a factor from butter fat that they called fat-soluble A to distinguish it from the previously isolated water-soluble substance. These two essential factors became known as vitamine A and vitamine B.

Much of the initial research used to identify vitamins relied on the historical method, which seeks to explain the cause of past events and to interpret current happenings on the basis of these findings. Sources of information for the historical researcher are primarily documentary, existing in the form of written records and accounts of past events as well as literary productions and critical writings. The researcher relies, if possible, only on primary data, that is, data that are "firsthand" and therefore minimally distorted by the channels of communication. Generally, information gathered by historical research does not need to be analyzed by any form of statistical treatment or data analysis.

As each additional vitamin was discovered, it was assigned a letter. The *e* on *vitamine* was dropped to give the general name *vitamin* because only a few of these essential substances were found to be amines. As the chemical structure of a vitamin became known through its isolation and synthesis, it was given a chemical name. Each chemical name assigned was assumed to apply only to one substance, with one specific activity. We now know that a vitamin may have a variety of functions, and that vitamin activity may be found in several closely related compounds known as vitamers. An excellent example of this range of activity is vitamin A, which has several seemingly unrelated functions and encompasses not only retinol but also retinal and retinoic acid. Additionally, we now that some vitamins, like vitamin D and the B-vitamins, need to be chemically modified in body tissues to function.

Vitamins are organic compounds with regulatory functions. They are required (usually in small quantities) in the diet because of the inability to synthesize them. Thus, vitamins are "essential," with inadequate consumption causing specific syndromes or manifestations. Although the clinician should be able to recognize the deficiency syndrome caused by a lack of a particular vitamin, in a country with an abundant and varied food supply such as the

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United States, the nutrition professional should think in terms of what specific functions a vitamin performs in the body and to a lesser extent what disease it prevents. Table 9.1 provides a list of the water-soluble vitamins along with their functions and other aspects associated with each vitamin. Unfortunately, relating the function of the vitamin directly to its deficiency syndrome is often difficult. Moreover, some of the signs and symptoms associated with the deficiency of one vitamin may also occur with a deficiency of another vitamin and/or mineral. Thus, diagnosis of deficiency is best based on an assessment of the larger clinical picture, including biochemical parameters, versus just one or two signs or symptoms. Table 9.2 summarizes some of the signs associated with deficiencies of water-soluble vitamins.

Vitamins, for the most part, are not related chemically and differ in their physiological roles. The broad classifications of water-soluble vitamins and fat-soluble vitamins are made because of certain properties common to each group. The body handles the water-soluble vitamins differently from the way it handles the fat-soluble vitamins (discussed in Chapter 10). Water-soluble vitamins are absorbed into portal blood, in contrast to fat-soluble vitamins, which initially enter the lymphatic system as part of a chylomicron. Furthermore, with some exceptions, water-soluble vitamins are generally excreted in the urine whenever plasma levels exceed renal thresholds; they are also not stored in large quantities in body tissues. Water-soluble vitamins, with the exception of vitamin C, are members of the B-complex. Most of the B-complex group can be further divided according to some of their general functions such as energy production and nutrient metabolism, hematopoiesis, and gene expression (Figure 9.1).

In this chapter, discussions of the vitamins are presented similarly. Each vitamin is considered (when precise

Table 9.1 The Water-Soluble Vitamins: Coenzyme Forms, Functions, Deficiency Syndromes, Food Sources, and Recommended Intake

Vitamin	Main Coenzyme(s)	Biochemical or Physiological Function(s)	Deficiency Syndrome, Symptoms, or Affected Systems	Major Food Sources	RDA* or AI†
Thiamin (B_1)	Thiamin diphosphate (TDP)	Oxidative decarboxylation of α-keto acids, interconversion of phosphorylated sugars, nervous system functions	Beriberi — Nervous system and cardiac dysfunction	Pork, sunflower seeds, legumes	1.1 mg* 1.2 mg
Riboflavin (B_2)	Flavin adenine dinucleotide (FAD); flavin mononucleotide (FMN)	Electron (hydrogen) transfer reactions for nutrient metabolism and energy production	Ariboflavinosis – Cheilosis, glossitis, angular stomatitis, edema oral cavity, dermatitis	Meats, eggs, yogurt, cheese, milk	1.1 mg* 1.3 mg
Niacin (B_3) (nicotinic acid, nicotinamide)	Nicotinamide adenine dinucleotide (NAD); nicotinamide adenine dinucleotide phosphate (NADP)	Electron (hydrogen) transfer reactions for nutrient metabolism and energy production; ADP ribose transfer	Pellagra — Diarrhea,; dermatitis, mental confusion	Fish, meats, peanut butter	14 mg* 16 mg
Pantothenic acid	Coenzyme A (CoA)	Acyl transfer reactions; acetylation/acylation of proteins, sugars, and other substrates; gene expression	Burning foot syndrome — Burning of the feet, neuritis	Widespread in foods	5 mg†
Biotin	Carboxybiotin	CO_2 transfer/carboxylation reactions for nutrient metabolism and energy production; gene expression	Dermatitis, hypotonia, nervous system dysfunction, alopecia	Liver, soybeans, eggs	30 µg†
Vitamin B_6 (pyridoxine, pyridoxal, pyridoxamine)	Pyridoxal phosphate (PLP)	Variety of reactions for nutrient metabolism and energy production; gene expression	Dermatitis, cheilosis, glossitis, angular stomatitis, nervous system dysfunction	Beef, fish, legumes, potato, banana, whole grains	1.3 mg*
Folate	Tetrahydrofolic acid (THF) derivatives: 5, 10-methylene THF, 10-formyl THF, 5-formimino THF, 5, 10-methylenyl THF, 5-methyl THF	One-carbon transfer reactions for nutrient metabolism and energy production; gene expression; purine and pyrimidine synthesis for DNA and RNA; hematopoiesis	Megaloblastic anemia — Enlarged immature red blood cells, diarrhea	Green vegetables — spinach, asparagus, and greens, legumes, fortified grain products	400 µg*
Vitamin B_{12} (cobalamin)	Methylcobalamin, adenosylcobalamin	Nutrient metabolism; energy production; hematopoiesis	Megaloblastic anemia, degeneration of peripheral nerves	Animal products — meat, fish, shellfish, poultry, milk	2.4 µg*
Ascorbic acid (vitamin C)	None	Antioxidant; cosubstrate for hydroxylating enzymes	Scurvy — Hyperkeratosis of hair follicles, psychological manifestations Impaired collagen — Bleeding gums, ruptured capillaries	Citrus fruits and juices, noncitrus fruits, broccoli, Brussels sprouts, green peppers	75 mg* 90 mg

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Table 9.2 Some Common Signs Associated with Water-Soluble Vitamin Deficiencies

System/Sites	Common Selected Signs	Possible Vitamin Deficit
Gastrointestinal tract		
Oral cavity	Cheilosis and/or glossitis	B_2, B_3, B_6, folate
Stomach and Intestines	Nausea, vomiting, and/or diarrhea	B_1, B_3, biotin, pantothenic acid
Skin	Petechia	C
	Pigmentation changes, erythema	B_2, B_3
	Dermatitis	B_2, B_3, B_6, biotin
	Collagen disturbances	C
Skeletal		
Bone and teeth	Pain and impairments	C
Cardiovascular and Pulmonary	Cardiomegaly, tachycardia	B_1
	Shortness of breath	Folate, B_{12}
Blood vessels		C, B_6
Hematopoiesis	Megaloblastic anemia	Folate, B_{12}
Nervous and Muscular* systems	Neuropathy	B_1, B_2, B_6, B_{12}
	Changes in reflexes	B_1, B_6, B_{12}
	Nervous system — general effects	B_1, B_2, B_3, B_6, B_{12}, biotin, pantothenic acid
	Psychological manifestations	C, B_1, B_3, B_6, biotin
	Hypotonia	Biotin
	Ataxia	B_1, B_6, biotin
Ocular	Ophthalmoplegia, nystagmus	B_1
	Photophobia	B_2

* Muscle weakness and/or fatigue have been reported with deficiencies of B_1, B_6, B_{12}, biotin, folate, and pantothenic acid.

information is available) in terms of structure, sources, absorption (also digestion where applicable), transport, storage, functions and mechanisms of action, metabolism and excretion, recommended intake, deficiency, toxicity, and assessment of nutriture. Specific interrelationships with other nutrients are also noted for selected vitamins. Table 9.3 provides an overview of aspects of water-soluble vitamin absorption, transport in systemic circulation, and storage. From this table, it is clear that most water-soluble vitamins (exception vitamin B_{12}) are absorbed primarily in the duodenum and jejunum (proximal small intestine), and many organs contain small amounts of the vitamins.

Several different types of research methodologies are used to arrive at recommendations for the different nutrients. The experimental method (also sometimes called the control group–experimental group design) is frequently used and involves two or more population groups, with the subjects of each group matched as closely as possible in characteristics to the subjects of the other group(s). One group serves as a control and is not exposed to any extraneous change. The experimental group is exposed to the alteration under study (such as an insufficient amount of a vitamin provided in the diet), and whatever change(s) (such as deficiency symptoms) is noted in this group relative to the subjects of the control group is presumed to be caused by the extraneous variable(s). The experimental method can also use just one group, a method sometimes called a pretest–posttest approach. In its simplest form, a group of subjects is evaluated (pretest), subjected to the experimental variable (test), and reevaluated (posttest). In so-called **cross-over studies**, another common approach, the standard experimental method (with separate control and experimental groups) is performed twice. After the initial phase, in which the experimental group is subjected to the variable and differences in the findings are noted, the subjects switch groups: The original control group is exposed to the variable, thus becoming the experimental group, and the original experimental group becomes the unexposed control group. This approach corrects for any inherent differences in the two groups that might confound the experimental data.

In short, the experimental method is based on cause and effect. It involves intervention on the part of the researcher, who introduces a variable and records its effect. Experimental research designs enable the investigator to

Figure 9.1 The vitamins, including some functional roles of the water-soluble vitamins.

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Table 9.3 Gastrointestinal Tract Absorption, Form in Systemic Circulation, and Storage Sites for the Water-Soluble Vitamins

Vitamin	Absorption Site	Method	Form in Blood	Storage Sites(s)
C	Small intestine, jejunum	SDVT1*, SDVT2, diffusion	Free ascorbic acid, dehydroascorbic acid (minor)	Adrenal and pituitary glands, eyes, brain, white blood cells
Thiamin	Duodenum, jejunum, colon	ThT1*, ThT2, diffusion, other**	Free thiamine, TDP, possibly ThBP	Muscle, heart, brain, kidneys, liver
Riboflavin	Duodenum, jejunum, colon	RFVT3, RFVT1, diffusion	Riboflavin, FMN, and FAD usually bound to proteins (albumin, Ig, possibly RiBP)	Liver, kidneys, heart
Niacin	Small intestine, colon	Carrier (unnamed), diffusion	Free nicotinamide, free nicotinic acid, nicotinic acid bound to proteins	Liver
Pantothenic acid	Jejunum, colon	SMVT, diffusion	Free pantothenic acid	Liver, adrenal glands, kidneys, brain, heart
Biotin	Proximal small intestine, colon	SMVT, diffusion	Free biotin, biotin bound to proteins (albumin, biotinidase,...)	Liver, muscle, brain
Folate	Duodenum, jejunum, colon	PCFT*, RFC, diffusion	Free folate, (protein† bound — folate, THF, 5-methyl THF, 10-formyl THF)	Liver
Vitamin B_{12}	Ileum	IF receptor-mediated, diffusion	Methyl-, adenosyl-, cyano-, hydroxocobalamin-bound transcobalamins	Liver, muscle, pituitary gland, bone, kidneys, heart, brain, spleen
Vitamin B_6	Jejunum	Diffusion	Free PL, PLP bound to albumin	Muscle, liver, brain, kidneys, spleen

Abbreviations: SDVT, sodium-dependent vitamin transporter; ThTr, thiamin transporter; TDP, thiamine diphosphate; ThBP, thiamin binding protein; RFVT, riboflavin vitamin transporter; RiBP, riboflavin binding protein; Ig, immunoglobulin; SMVT, shared multivitamin transporter; PCFT, proton couple folate transporter; RFC, reduced folate carrier; IF, intrinsic factor; PL, pyridoxal; PLP, pyridoxal phosphate.

* Thought to be the primary carrier

** Possibly also organic cation transporter protein

† Proteins include mainly albumin, α2 macroglobulin, and a folate binding protein

control or manipulate one or more variables, designated as dependent or independent, in an effort to examine the relationship between the variables. The independent variable is the variable controlled or manipulated (such as the insufficient amount of the vitamin provided in the diet) by the investigator. The dependent variable (i.e., the deficiency symptoms) occurs as the result of the influence of the independent variable. The experimental design is one of the most common methodologies employed and encountered in nutrition literature, including many of the studies used to determine the recommendations, known as Dietary Reference Intakes, for water-soluble and fat-soluble vitamins.

Dietary Reference Intakes (DRIs) represent quantitative approximations of nutrient needs for the purpose of planning and assessing the diets of healthy people. DRIs include **Recommended Dietary Allowances** (RDAs) as well as **Adequate Intakes** (AIs), Tolerable Upper Intake Levels (ULs), and **Estimated Average Requirements (EARs)**. RDAs represent the average daily dietary intake level that is sufficient to meet the nutrient requirements of about 97% of healthy people. They are based on EARs, which are the amounts of nutrients thought to meet the nutrient requirements of 50% of the healthy people in a specified age and gender group. RDAs are set higher than EARs by either two standard deviations or a coefficient of variation for the EAR. The intake of a nutrient is likely inadequate if it is significantly less than the EAR. Furthermore, an intake above the EAR but less than the RDA may still be inadequate. AIs are provided for nutrients instead of RDAs when scientific data are insufficient to calculate the EARs. AIs are based on nutrient intake levels of healthy people (with adequate nutritional status), and are typically thought to exceed the requirement for the nutrient. Thus, nutrient intakes are likely adequate if they equal or exceed the AI, but may or may not be adequate if they are less than the AI. If nutrient intakes are above the RDA but less than the UL, then they are likely to be adequate. ULs provide the highest intake level for a nutrient that is unlikely to cause any risk of adverse health to almost all people in the age- or gender-specified groups. ULs are viewed as maximum amounts for those consuming fortified foods or supplements in large quantities. For some nutrients, the UL is not known, but the lack of a UL does not mean that large doses of the nutrient are harmless.

Foods, as opposed to supplements, are the preferred sources for the intake of vitamins. Diets rich in fruits, vegetables, and whole grains are associated with reductions in risks for heart disease, type 2 diabetes, metabolic syndrome, and some cancers, among other conditions. Whole grains provide dietary fiber and several micronutrients including vitamin E, thiamin, riboflavin, niacin, pantothenic acid, and vitamin B_6 along with minerals and phytochemicals. Fruits and vegetables are major sources of carotenoids, vitamin C, vitamin E, vitamin K, folate, riboflavin, and niacin, as well as a host of minerals, dietary fiber, and thousands of phytochemicals. These nutrients are likely working synergistically or

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antagonistically through different mechanisms to reduce disease risk [1]. However, scientific evidence supports the consumption of foods (such as fruits, vegetables, whole grains, low-fat dairy products and meats, seafoods, nuts, seeds, and legumes) versus the use of supplements to meet nutrient needs and to reduce the risk of diseases including cancer, cardiovascular disease, and cardiovascular disease events (stroke, heart attack, cardiac revascularization, or cardiovascular disease mortality) [1–5].

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VITAMIN C (ASCORBIC ACID)

Vitamin C is also known as ascorbic acid (AH_2). At physiological pH, the vitamin loses a hydrogen and is usually in its ionized form (AH^-), referred to as ascorbate, ascorbate (mono)anion, or monodehydroascorbate. Oxidation of ascorbate generates the ascorbyl radical $A^{\bullet -}$, which has one electron and one proton less than ascorbate. The oxidized form (with two electrons and two protons/hydrogens removed) of the vitamin is called dehydroascorbic acid (Figure 9.2) and has vitamin activity because it can be converted into ascorbate in body cells.

Vitamin C was isolated in 1928, and its structure was determined in 1933. But this vitamin's deficiency disorder—known as **scurvy**—had been prevalent for centuries. Some of the most notable stories are those of the British sailors who frequently died from scurvy on sea voyages. It was a physician who eventually found that sucking the juice from a lime was protective, and in the late 1790s and early 1800s British sailors at sea began receiving limes (resulting in the nickname "limey" for the sailors) in an effort to prevent scurvy. Szent-Györgyi (1928) and King (1932) are considered co-discoverers of vitamin C. Szent-Györgyi, who isolated the vitamin, and Haworth, who determined its structure, were awarded the Nobel Prize in 1937 for their vitamin C work.

Vitamin C exists as both a D- and L-isomer; however, it is the L-isomer of the vitamin that is biologically active in humans. Humans (as well as nonhuman primates, fruit bats, guinea pigs, and some birds) are unable to synthesize

1. During the oxidation of ascorbic acid, a free radical called ascorbyl radical (also called semidehydroascorbic acid radical, ascorbate free radical, ascorbyl, monodehydroascorbate radical) is formed but has a short half-life and reacts poorly with oxygen, so it does not form reactive oxygen species.
2. Oxidation of the radical forms of vitamin C.
3. Dehydroascorbic acid can be reduced to ascorbic acid with hydrogens provided by the reduced form of glutathione (GSH).

Figure 9.2 Structures and interconversion of ascorbic acid and dehydroascorbic acid.

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Figure 9.3 Synthesis of ascorbic acid.

vitamin C, which is derived from glucose, due to the lack of gulonolactone oxidase, the last enzyme in the vitamin C synthetic pathway (Figure 9.3).

Sources

Food sources of vitamin C (Table 9.4) include primarily fruits (especially citrus) and vegetables. Major sources of the nutrient are asparagus, papaya, oranges, orange juice, cantaloupe, cauliflower, broccoli, Brussels sprouts, green pepper, grapefruit, grapefruit juice, kale, lemons, and strawberries. The camu berry (or camu camu), from Peru, is one of the richest sources with 100 g of the fruit containing up to 2 g of vitamin C. Fortified foods, including breakfast cereals, also contribute some vitamin C to the diet. A cup of Froot Loops®, for example, provides 25% of the Daily Value (which is 60 mg), or about 15 mg of vitamin C.

In foods, vitamin C is found mostly as ascorbic acid, but small amounts of dehydroascorbic acid also may be

Table 9.4 Vitamin C Content of Selected Foods*

Food (serving)	Vitamin C (mg)
Tomato juice, canned (1 c)	170
Green pepper, sweet, sautéed (1/2 c)	102
Strawberries (1 c)	89
Papaya (1 c)	88
Orange, sections (1 c)	87
Kiwi, green (1/2 c chopped)	84
Grapefruit, sections (1 c)	72
Cantaloupe (1 c)	68
Pineapple, chunks (1/2 c)	46
Banana (1)	12
Tomato (1 c)	23
Avocado, raw, pureed (1 c)	40
Potato, baked, with skin (large)	38
Broccoli (1/2 cup cooked)	37
Brussels sprouts (1/2 cup cooked)	36

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov./

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present. Supplements usually supply vitamin C as ascorbic acid, calcium ascorbate, and/or sodium ascorbate. Ester-C® contains calcium ascorbate, dehydroascorbate (the ionic form of dehydroascorbic acid), and some vitamin C metabolites. Absorption of these various forms of vitamin C does not appear to differ substantially, thus no one form is thought to be significantly superior to another.

A fat-soluble form, ascorbyl palmitate (ascorbic acid esterified to the fatty acid palmitate), is also available in soft gels. Additionally, skin creams containing the vitamin are available and promoted for skin health because of vitamin C's role in collagen synthesis and as an antioxidant (discussed later). Percutaneous absorption of the vitamin is concentration dependent (requiring a topical solution containing at least 10–20% for maximal absorption) and requires an acidic pH (usually < 3.5). Not all forms of the vitamin in these creams, however, effectively penetrate the skin.

Vitamin C is destroyed by heat, light, oxidation, and alkaline solutions, but is stable in acidic solutions. Vitamin C is also oxidatively damaged (yielding diketogulonic acid and other products without vitamin C activity) in the presence of oxygen and minerals such as iron and copper. Thus, cooking and storage may reduce the vitamin C content of foods.

Digestion, Absorption, Transport, and Storage

Vitamin C does not require digestion prior to being absorbed into intestinal cells. Absorption of ascorbic acid across the intestinal cell brush border membrane occurs throughout the small intestine, especially in the proximal jejunum, and requires sodium-dependent vitamin C transporters (SVCT) 1 and 2. SVCT1 is the main carrier responsible for intestinal vitamin C absorption. SVCT1 enables absorption of the vitamin in excess of cell needs, but is downregulated by ascorbic acid, which limits the absorption when superphysiological doses are ingested.

The dehydroascorbic acid that is found in foods or that may be formed if ascorbic acid is oxidized in the gastrointestinal tract, is absorbed by glucose transporters (GLUT), mainly GLUT 1 and 3. However, once it is absorbed, dehydroascorbic acid is rapidly reduced to ascorbic acid by dehydroascorbic acid reductase in the intestinal cell. Glutathione (GSH), which is required for the reduction of dehydroascorbic acid, is oxidized (to GSSG) in the process as shown in Figure 9.2. NADPH and the dithiol glutaredoxin can also reduce the dehydroascorbic acid.

Over a range of usual vitamin C intake (such as 30–100 mg/day) from foods, vitamin C absorption is about 70–95%. Absorption of the vitamin decreases with increased intake; for example, 16% is absorbed at high intakes (~12 g) versus 98% absorbed at low intakes (~20 mg). At intakes above about 1 g, less than 50% of vitamin C is typically absorbed.

To enter the blood plasma, vitamin C diffuses out of intestinal cells into the extracellular fluid through anion channels that create openings or pores in the basolateral membrane. Additionally, vitamin C may exit intestinal cells via exocytosis or other means. Ingestion of vitamin C in amounts similar to recommended intakes (75–90 mg) produces plasma concentrations of about 0.8 mg/dL. Slightly higher daily intakes (about 100–200 mg vitamin C) produce plasma concentrations of about 1.0 mg/dL; intakes of about 200–400 mg produce steady-state plasma vitamin C concentrations of about 1.0–1.4 mg/dL. Maximization of the body pool occurs at vitamin C intakes of about 100–200 mg. A vitamin C intake of 1.25 g generated a peak plasma vitamin C concentration of 2.37 mg/dL [1].

Ascorbic acid and dehydroascorbic acid are transported in the blood primarily in free form; however, only small amounts (about 5%) of dehydroascorbic acid are present due to its rapid cellular uptake. Plasma ascorbic acid concentrations vary, but a typical reference range is from about 0.6–2.0 mg/dL; higher plasma vitamin C concentrations can be achieved with intravenous administration of the vitamin than with oral intake since intestinal barriers to the vitamin's absorption are circumvented. In the blood, about 70% of the vitamin is found in the plasma and red blood cells, and about 30% is found in white blood cells.

Ascorbic acid uptake into body cells such as those of the liver and kidneys occurs by SVCT1. SVCT2 is also present in most metabolically active tissues with the exceptions of skeletal muscle and lungs. Dehydroascorbic acid uptake into cells utilizes GLUT transporters. Tissue concentrations of vitamin C usually exceed plasma concentrations, with the magnitude dependent upon the specific tissue. The vitamin C content of white blood cells, for example, can be as much as 80 times greater than plasma concentrations. High concentrations of vitamin C are found in the adrenal and pituitary glands (with each possessing about 30–50 mg/100 g of wet tissue) as well as in the eyes, brain, and white blood cells. Intermediate levels of vitamin C (about 10–15 mg/100 g of wet tissue) are found in the liver, spleen, heart, kidneys, lungs, pancreas, and muscle. Skeletal muscle, however, because of its size, may contain up to about 1,500 mg of vitamin C. The maximal vitamin C pool (total body vitamin C content) is estimated at about 2 g.

Functions and Mechanisms of Action

Despite its uncomplicated structure, vitamin C has complex functional roles in the body. Vitamin C functions as an antioxidant and as a cosubstrate for enzyme activity.

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More specifically in reference to this latter role, some enzymes contain a mineral (copper or iron) cofactor and, for some of these metalloenzymes, vitamin C functions as a reducing agent to maintain the iron and copper atoms in the reduced state. These cosubstrate roles of vitamin C are discussed first.

Collagen Synthesis

Vitamin C functions as a cosubstrate in a number of hydroxylation reactions. Three hydroxylation reactions requiring vitamin C are necessary for the synthesis of collagen, a structural protein found in skin, bones, dentine, tendons, and cartilage (connective tissues). Collagen is made up of three polypeptide chains that are synthesized by fibroblasts (connective tissue cells). In the initial steps of collagen production, tropocollagen, which consists of a triple helix of polypeptide chains twisted around each other and linked by hydrogen bonds to form a coil, is generated. After these tropocollagen chains are made, the vitamin C–dependent hydroxylation reactions occur post-translationally and allow aggregation and further cross-linking of the chains. Prolyl 4-hydroxylase and prolyl 3-hydroxylase (also called dioxygenases) catalyze the hydroxylations of specific proline residues on the newly synthesized chains. Proline and hydroxyproline provide more rigidity to the collagen. Lysine hydroxylation also occurs by lysyl hydroxylase (also called dioxygenase). The formation of hydroxylysyl residues provides for attachment to carbohydrate moieties and allows for additional post-translational modifications, such as glycosylation and phosphorylation, to further strengthen the collagen. Additionally, a copper-dependent (but not vitamin C–dependent) oxidation reaction among collagen chains occurs for added strength.

The cosubstrate role of vitamin C in the hydroxylation reactions relates to the iron cofactor that is part of prolyl hydroxylases and lysyl hydroxylase. During the reactions, the hydroxylases incorporate one atom of O_2 into the product, and the second atom of O_2 into the α-ketoglutarate to form the new carboxyl group of succinate (Figure 9.4). During these reactions, the iron cofactor in the hydroxylase enzymes is oxidized; that is, it is converted from a ferrous ($^{2+}$) state to a ferric ($^{3+}$) state. Vitamin C functions as the reductant, thereby reducing the iron back to its ferrous state ($^{2+}$) in the prolyl and lysyl hydroxylases.

These reactions profoundly impact the normal development and maintenance of skin, tendons, cartilage, bone, and dentine, which depend on an adequate supply

[1]Ascorbate acts as a reducing agent to convert the oxidized iron atom (Fe^{3+}) back to its reduced state (Fe^{2+}) in the enzymes lysyl hydroxylase and prolyl hydroxylase, which incorporate one atom of oxygen (*) in the hydroxyl group of the product and the other in succinate.

Figure 9.4 Ascorbate functions in the hydroxylation of peptide-bound proline and lysine in the synthesis of collagen.

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of vitamin C. Vitamin C is also needed for the formation and maintenance of the basement membranes lining the capillaries, the "intracellular cement" holding together the endothelial cells, and the scar tissue responsible for wound healing.

Carnitine Synthesis

Vitamin C is involved in two hydroxylation reactions required for the synthesis of carnitine, a nonprotein, nitrogen-containing compound essential for the transport of long-chain fatty acids from the cell cytosol into the mitochondria for ß-oxidation (and thus energy production). Carnitine is made from the amino acid lysine, which has been methylated using S-adenosyl methionine. The hydroxylation reactions in carnitine synthesis involving vitamin C are similar to those for proline and lysine hydroxylation. Vitamin C functions as a reducing agent, specifically reducing the iron atom from the ferric state (Fe^{3+}) back to the ferrous state (Fe^{2+}) for the reactions catalyzed by trimethyllysine hydroxylase and 4-butyrobetaine hydroxylase (see Figure 6.23).

Tyrosine Catabolism

Tyrosine, ingested from foods or generated in the body from phenylalanine oxidation, can be degraded if needed to produce energy. During its catabolism, para (p)-hydroxyphenylpyruvate is produced and is further hydroxylated by the iron-dependent enzyme para (p)-hydroxyphenylpyruvate hydroxylase (also called dioxygenase). Vitamin C is a preferred reductant for iron in this reaction, as shown here.

Additionally, in the next step of tyrosine catabolism, vitamin C functions as the reductant as homogentisate is converted to 4-maleylacetoacetate by the iron-dependent enzyme homogentisate dioxygenase.

Neurotransmitter Synthesis

As with the synthesis of carnitine and collagen, vitamin C reduces mineral cofactors that become oxidized during selected reactions in the synthesis of some neurotransmitters and hormones.

Norepinephrine Norepinephrine (a catecholamine) is generated from the hydroxylation of the side chain of dopamine (another catecholamine) in a vitamin C–dependent reaction. The reaction is catalyzed by dopamine β monooxygenase (also called dopamine β hydroxylase), which contains eight copper (Cu^{1+}) atoms and is found primarily in the adrenal glands, but also in the nervous system. During the reaction (see Figure 6.10), the cuprous ions (Cu^{1+}) become oxidized to cupric ions (Cu^{2+}) and require vitamin C for reduction.

Other Neurotransmitters and Hormones Vitamin C also serves as a reductant, keeping the copper atom in peptidylglycine α-amidating monooxygenase in its reduced state, as shown in Figure 9.5. Although most of the substrate peptides for this enzyme have a terminal glycine residue, the enzyme is also active with peptides terminating in other amino acids. Many of the amidated peptides resulting from this reaction are active as hormones, hormone-releasing factors, or neurotransmitters. Examples include bombesin or gastrin-releasing peptide (GRP), calcitonin, cholecystokinin (CCK), thyrotropin, corticotropin-releasing factor, gastrin, growth hormone–releasing factor, oxytocin, and vasopressin. The enzyme is found in neuroendocrine cells of the pituitary, adrenal, and thyroid glands and in the brain. As a reductant for the required amidating enzyme, vitamin C assumes important, although indirect, roles in many regulatory processes.

Microsomal Metabolism

A group of enzymes makes up a microsomal metabolizing system that functions mostly in hepatic microsomes and reticuloendothelial tissues to inactivate both endogenous and exogenous substances. Endogenous substrates include various hormones like aldosterone and cortisol, and steroids such as cholesterol. For example, cholesterol 7α-hydroxylase, found in the microsomes of the liver, is required for the initial step in the synthesis of bile acids from cholesterol. Vitamin C plays an undefined role in this hydroxylation.

Exogenous substrates for the microsomal metabolizing system are usually xenobiotics. *Xenos* means "stranger" in Greek, and **xenobiotics** are foreign chemicals such as drugs, carcinogens, pesticides, food additives, pollutants, or other noxious compounds. The reactions needed to metabolize these substances usually involve hydroxylations followed by conjugations or methylations to produce polar metabolites for excretion. The hydroxylation reactions are catalyzed by monooxygenases

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[1] Vitamin C functions as a reducing agent to convert copper that has become oxidized during the reaction back to a reduced (Cu^{1+}) form.

[2] The enzyme cleaves the carboxyl-terminal residue on the peptide substrate. The residue is released as glyoxylate.

[3] Many of the amidated peptides are active hormones, hormone-releasing factors, or neurotransmitters, as discussed in the text.

Figure 9.5 Amidation of peptides with C-terminal glycine requires vitamin C.

or cytochrome P-450 mixed-function oxidases and require oxygen as well as reducing agents such as vitamin C and NAD(P)H. The exact role of vitamin C, however, has not been established.

Antioxidant Activity

In addition to vitamin C's cosubstrate roles, vitamin C acts in a general capacity as a reducing agent or electron donor and thereby has antioxidant activity or functions. Ascorbic acid (which can be thought of as hydrogen ascorbate or AH_2) acts as a reducing agent in aqueous environments such as the blood and within cells. Stated slightly differently, vitamin C is an antioxidant in that it reverses oxidation. Vitamin C's two hydroxyl groups and carbonyl group facilitate its ability to act as a hydrogen or electron donor. Ascorbate (AH^-) is the ionized form—that is, the form of vitamin C that results when a hydrogen has dissociated from ascorbic acid, as in a solution. The reduction potential of vitamin C is such that it also readily donates electrons/hydrogen ions to regenerate other antioxidants, such as vitamin E and glutathione, and to reduce **free radicals** and reactive species. Free radicals and reactive species, if not "inactivated," attack nucleic acids in DNA, polyunsaturated fatty acids in phospholipids, and amino acids in proteins. Vitamin C reduces several oxygen-centered radicals and reactive species, including **hydroxyl radical** ($^{\bullet}OH$), **hydroperoxyl radical** ($HO_2^{\bullet}$), **superoxide radical** ($O_2^{\bullet}$), **alkoxyl radical** ($RO^{\bullet}$), **peroxyl radical** ($RO_2^{\bullet}$), **singlet oxygen** ($^1O_2$), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl). Reactive nitrogen species, such as peroxynitrite radicals and nitric oxide radicals, also may be reduced by vitamin C.

Ascorbic acid has been shown to interact with these radicals in the aqueous phase before they initiate damage. Furthermore, ascorbic acid appears to be superior to other water-soluble antioxidants. The ability of plasma antioxidants to protect, for example, lipids against peroxidation, has been shown to be vitamin C = thiols > bilirubin > uric acid > vitamin E [2]. Following interaction with vitamin C, the radicals or reactive oxygen species are inactivated (such as with conversion to water), while vitamin C becomes oxidized.

Regenerating vitamin C is crucial for cell protection. To accomplish this regeneration, several different reactions may occur. For example, two ascorbyl radicals may react to form ascorbic acid and dehydroascorbic acid; this is especially likely in situations with high levels of oxidant stress. Alternately, reductases, found in most tissues, can reduce the ascorbyl radical and dehydroascorbic acid to ascorbic acid. A niacin coenzyme and thiols such as dihydrolipoic acid, glutathione, and thioredoxin also assist in vitamin C regeneration. Additional reactions involved in the regeneration of vitamin C, as well as more information on the roles of vitamin C and other antioxidants, are discussed in the Perspective at the end of Chapter 10.

Pro-Oxidant Activity

Paradoxically, vitamin C also may act as a pro-oxidant by reducing transition metals—for example, cupric ions (Cu^{2+}) to cuprous (Cu^{1+}) and ferric ions (Fe^{3+}) to ferrous (Fe^{2+}), as shown here:

$$\text{Ascorbate anion } (AH^-) + Fe^{3+} \text{ or } Cu^{2+} \longrightarrow \text{Ascorbyl radical } (A^{\bullet -}) + Fe^{2+} \text{ or } Cu^{1+}$$

The products—Fe^{2+} and Cu^{1+}—generated from these reactions can cause cell damage by generating reactive oxygen species and free radicals like hydroxyl and superoxide radicals, as shown here:

$$Fe^{2+} \text{ or } Cu^{1+} + H_2O_2 \longrightarrow Fe^{3+} \text{ or } Cu^{2+} + OH_2 + {}^{\bullet}OH$$

$$Fe^{2+} \text{ or } Cu^{1+} + O_2 \longrightarrow Fe^{3+} \text{ or } Cu^{2+} + O_2^{\bullet}$$

It is important to note that vitamin C's pro-oxidant activity is thought to be minimal at usual physiological concentrations, and that vitamin C reacts with *free* ferric or cupric ions. In the body, iron and copper are both *bound* to various proteins (i.e., not free), thus lessening the likelihood of such interactions.

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Other Functions

Many other diverse biochemical functions for vitamin C have been proposed. Possible functions for vitamin C include roles in collagen gene expression; synthesis of bone matrix, proteoglycans, fibronectin, and elastin; regulation of cellular nucleotide (cAMP and cGMP) concentrations; and immune function, including complement synthesis. Experimental evidence supporting these functions varies considerably, and the mechanism by which vitamin C may be involved is generally unclear.

Selected Pharmocological Uses/Other Roles

Much attention has also been directed toward vitamin C and its possible effects on diseases ranging from the common cold to cancer and heart disease, among others.

Colds Colds, affecting the average adult about two to six times each year, are caused most often by viruses including adenovirus, rhinovirus, coronavirus, and respiratory syncytial virus, among others. The ability of vitamin C to enhance immune system functions by promoting chemotaxis and proliferation of immune cells like macrophages and lymphocytes, increasing the activity of natural killer cells, and destroying histamine (associated with some of a cold's symptoms) provides a theoretical basis for the use of vitamin C in the prevention and treatment of colds. Numerous studies, involving tens of thousands of individuals, have been conducted over the last several decades to examine the effectiveness of vitamin C in cold prevention and treatment. Studies continue to find no reduction in the incidence of colds with **prophylactic** (preventative) vitamin C use among the general population. However, regular use of vitamin C (250 mg–1 g daily) reduced the incidence of colds by 50% among some subsets of adults, specifically athletes (such as marathon runners and skiers) and soldiers exposed for short time periods to extreme temperatures or participating in extreme physical activities. A Cochrane review reported that regular use of vitamin C supplements (in amounts of 200 mg or more) modestly and consistently shortened the duration of cold symptoms by about 8% in adults and 14% in children; however, vitamin C supplementation once cold symptoms are present has not been shown to be beneficial [3,4].

Cancer **Epidemiological studies** provide evidence that increased intakes of fruits and vegetables are associated with a decreased risk of some cancers. Studies examining vitamin C status (as opposed to dietary vitamin C intake) and cancer risk also generally report favorable associations. The association between vitamin C and a protective effect against cancer is generally stronger with cancers of the oral cavity (including the pharynx), esophagus, stomach, lung, breast, colon, and rectum. However, reports from studies providing oral vitamin C to prevent or treat cancer are inconsistent with most showing no overall benefits. Some antitumor effects have been documented, however, with the use of high doses of intravenously administered vitamin C (which generates plasma vitamin C concentrations over 100 times that attained from oral ingestion) as an adjunct to other cancer treatments [5]. Additionally, vitamin C (dietary or in gastric juice) as well as vitamin E in the stomach may react with nitrites (ingested in foods) to deter their conversion to nitrosamines, which are carcinogenic.

Cardiovascular Disease Many (but not all) studies report that increased fruit and vegetable intakes, vitamin C intake, and/or plasma vitamin C concentrations are associated with a decreased risk of cardiovascular (heart) disease. Yet, studies providing supplements of vitamin C, and other antioxidants, typically have not reported beneficial effects in the prevention of heart disease or the reduction of heart disease–related mortality [6,7]. Several possible mechanisms suggest the likelihood of beneficial effects from vitamin C. For example, vitamin C's antioxidant abilities may inhibit low-density lipoprotein (LDL) oxidation, which in turn can diminish plaque formation associated with the development of heart disease. Vitamin C also may be helpful by decreasing monocyte adhesion to endothelial cells lining blood vessels; adhesion represents one of the first steps in atherogenesis and is followed by monocyte migration into the arterial intima (the innermost layer of blood vessels), transformation into macrophages, and uptake of oxidized LDL. With continued accumulation of oxidized LDL, macrophages develop into foam cells, and over time fatty streaks develop. Vitamin C, alone and with vitamin E, is also thought to help prevent heart disease by scavenging reactive species/free radicals before they reach and/or initiate oxidative damage to LDLs and cells, and by reducing thrombotic potential and vascular reactivity. Other possible beneficial roles in preventing heart disease include enhancing type IV collagen generation in endothelial cells, preventing endothelial cell apoptosis, enhancing endothelial cell function, inhibiting smooth muscle cell proliferation, and/or reducing blood pressure.

Eye Health It has been suggested that vitamin C is beneficial in diminishing the risks and/or preventing age-related macular degeneration and cataracts, both major causes of blindness, especially in older people. The macula, found in the center of the retina, maintains central vision. Behind the retina is the choroid, which contains blood vessels. In age-related macular degeneration (dry form), cellular debris called drusen accumulates between the retina and the choroid, and in the wet (exudative) form of the condition, abnormal blood vessels grow under the macula. In both forms of age-related macular degeneration, there is a loss of vision in the center of the visual field because of damage to the retina. The condition makes reading and recognition of faces difficult, although peripheral vision remains relatively unaffected. Unfortunately, vitamin C taken alone or

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with other antioxidant nutrients does not appear to prevent the development of age-related macular degeneration; however, supplementation with vitamin C (500 mg), vitamin E (400 IU), beta-carotene (15 mg), zinc (80 mg), and copper (2 mg) (as used in the Age-Related Eye Disease Study, abbreviated AREDS, a large randomized, placebo-controlled 5-year clinical trial) may have modest effects in slowing the progression of the condition [8–10]. A second 5-year trial, AREDS2, supplemented those with macular degeneration with a modification of the original formulation [adding omega-3 fatty acids (1,000 mg), lutein (10 mg), and zeaxanthin (2 mg), removing beta-carotene, and reducing zinc (25 mg)], but found no additional benefits [11].

A cataract affects the lens of the eye, causing it to become cloudy. The lens (which functions to focus light onto the retina and adjust eye focus) is made up of over a million lens fiber cells. These cells have high protein contents (especially the protein α-crystallin) and, unlike other tissues, the proteins in the lens do not turn over. α-Crystallin functions to maintain optical clarity by removing damaged proteins from the lens. However, with age, the activity and concentration of α-crystallin diminish. This change results in the accumulation and aggregation of abnormal proteins in the lens. In addition, with aging, the concentrations of antioxidants diminish and lead to oxidative damage in the eye including lipid peroxidation and protein cross-linking. The damaged proteins aggregate and precipitate, causing the lens to become cloudy and causing vision to become impaired. Because oxygen and oxyradicals are thought to contribute to the development of cataracts, and because vitamin C functions as an antioxidant, it is logical that vitamin C may help in the prevention of cataracts. However, a review of trials concluded that antioxidant multivitamin (not just vitamin C) supplementation does not affect the risk of cataracts nor its progression [12].

Interactions with Other Nutrients

One of vitamin C's most notable interactions is with the mineral iron. Vitamin C enhances the intestinal absorption of nonheme iron most likely by reducing iron ferric (Fe^{3+}) state to a ferrous (Fe^{2+}) state. Vitamin C's benefits are thought to be maximized at about 75 mg. It is commonly suggested that individuals, especially if iron deficient, consume vitamin C–rich foods (providing at least 25 mg vitamin C) such as orange juice when ingesting nonheme iron-rich foods to promote the iron's absorption.

Metabolism and Excretion

Vitamin C in excess of tissue needs and storage capacity is excreted intact or catabolized prior to urinary excretion. As vitamin C intake increases, plasma vitamin C concentrations increase but reach an upper limit as renal handling of the vitamin shifts from active saturable reabsorption by SVCT1 carriers in the renal tubules to a renal threshold in which the maximum reabsorption of the vitamin is achieved. The renal reabsorption threshold occurs at a plasma vitamin C concentration of about 1.3–1.8 mg/dL. With ingestion of vitamin C in amounts of about 500 mg (and sometimes less) and with saturated tissue stores, all of the excess ingested vitamin C is usually excreted in the urine within about 6 hours [13].

Vitamin C, that is not excreted intact, is oxidized primarily in the liver but also to some extent in the kidneys. Oxidation first generates dehydroascorbic acid, which is further degraded with the hydrolysis (opening) of the ring structure to yield 2,3-diketogulonic acid. This metabolite, 2,3-diketogulonic acid, can be excreted in the urine or further hydrolyzed (Figure 9.6). When further hydrolyzed, diketogulonic acid is cleaved by separate pathways into a variety of five-carbon metabolites (xylose, xylonic acid, and lyxonic acid) or into the four-carbon compound threonic acid and oxalic acid. Vitamin C intakes up to about 200 mg do not alter the urinary excretion of oxalic acid (which is normally less than about 40 mg/day) [13]. The four- and five-carbon sugars that are generated can be converted into other cellular compounds or be oxidized and excreted as CO_2 and water. Other urinary vitamin C metabolites may include 2-O-methyl ascorbate, ascorbate 2-sulfate, and 2-ketoascorbitol.

Recommended Dietary Allowance

Vitamin C requirements for adult men and women, based on nearly maximizing tissue concentrations and minimizing urinary excretion of the vitamin, are 75 mg and 60 mg, respectively [15]. Vitamin C's Recommended Dietary Allowance (RDA for adult men and women is 90 mg and 75 mg, respectively [15]. During pregnancy and lactation, recommendations for vitamin C increase to 100 mg and 120 mg, respectively [15]. Furthermore, because smoking accelerates the depletion of the body's ascorbic acid pool, it is recommended that smokers consume an added 35 mg of vitamin C daily [15].

Deficiency: Scurvy

An inadequate intake of vitamin C results in the deficiency condition called scurvy. Scurvy is typically manifested when the total body vitamin C pool falls below about 300 mg and plasma vitamin C concentrations drop to less than about 0.2 mg/dL [16]. Scurvy may develop in as little as 1 month with vitamin C intakes less than 10 mg daily, but the condition is more likely to occur with an inadequate vitamin C intake for a duration of at least 4–6 months.

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*Some of the sugars like xylose can be further metabolized before excretion.

Figure 9.6 Vitamin C and the formation of its metabolites excreted in the urine.

Scurvy is characterized by a multitude of signs and symptoms. Initial symptoms include fatigue and malaise that may be associated with impaired carnitine synthesis. Impaired hydroxyproline and hydroxylysine synthesis leads to defects in collagen-rich body structures, and can diminish vascular wall and bone strength. Additional early manifestations are clogged and enlarged, hyperkeratotic hair follicles (keratin accumulations in the hair follicle), especially on the arms, legs, and buttocks; "corkscrew"-shaping of hair; and small, red skin discolorations caused by ruptured small blood vessels (called **petechiae**) along with splinter hemorrhages (damaged capillaries under the fingernails), easy bruising (characterized by ecchymoses—purple discolorations of the skin due to ruptured blood vessels—and purpurae—dark red to purple spots on the skin due to hemorrhage). Oral changes include swollen, bleeding, necrotic gums; sublingual hemorrhages; and loose and decaying teeth. Additionally, impaired wound healing, easily fractured bones, and bone pain (arthralgia) associated with abnormal collagen formation occur. In some cases changes in behavior/personality also result. Scurvy is fatal if untreated. The four Hs—*hemorrhagic* signs, *hyperkeratosis* of hair follicles, ***hypochondriasis*** (psychological manifestation), and *hematologic* abnormalities (associated mainly with impaired collagen synthesis)—are often used as a mnemonic device for remembering scurvy signs. Scurvy is treated with vitamin C doses of about 100–500 mg daily until symptoms are gone, which usually takes about 3 months.

Although scurvy is rare in the United States, low vitamin C status has been observed among smokers and in older adults. People who have poor diets, especially if coupled with alcoholism or drug abuse, are likely to be deficient. People with malabsorption disorders may exhibit diminished intestinal absorption of the vitamin, and those with conditions such as diabetes mellitus and some cancers are at risk due to increased vitamin C turnover.

Toxicity

Daily intakes of up to 2 g of vitamin C are routinely consumed without adverse effects [1,17]. Because vitamin C absorption is saturable and dose dependent, more vitamin C is absorbed, and thus toxicity is theoretically more likely, if several large (1 g) doses of the vitamin are ingested throughout the day than if the same amount is ingested as one single dose. The most common side effect from the ingestion of large amounts (2 g) of the vitamin is gastrointestinal problems characterized by abdominal pain and osmotic diarrhea. The osmotic diarrhea occurs when the unabsorbed vitamin C in the intestinal tract is metabolized by bacteria within the colon. Based on this side effect, a Tolerable Upper Intake Level of 2 g of vitamin C has been recommended [15].

Two other side effects reported from the use of large amounts of vitamin C are thought to affect (if at all) only selected populations. These side effects include increased risk of kidney stones (nephrolithiasis) for those with renal dysfunction and iron toxicity for those with disorders of iron metabolism. Because vitamin C's metabolism generates oxalic acid, a common constituent of kidney stones, it is logical that high intakes of the vitamin

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may increase the risk of kidney stones. Yet, even with ingestion of high doses of vitamin C, urinary oxalic acid excretion typically remains within a normal range for most individuals [14,17]. Nevertheless, some suggest that people predisposed to calcium oxalate kidney stones avoid high doses (≥500 mg) of vitamin C [17]. Furthermore, it is also sometimes advised that people with uric acid kidney stones avoid ingesting large doses of the vitamin. Vitamin C competitively inhibits the renal reabsorption of uric acid, thereby increasing uric acid excretion and acidifying the urine to promote precipitation of uric acid crystals and the formation of uric acid kidney stones.

In addition to increasing the probability of kidney stones, chronic high doses of vitamin C are also purported to be unsafe for people with disorders of iron metabolism, including **hemochromatosis**, **thalassemia**, and **sideroblastic anemia** [17]. However, others contend that pro-oxidant effects of vitamin C on mobilization of iron stores do not occur *in vivo* [18].

Excessive vitamin C excretion can interfere with some clinical laboratory tests. Vitamin C in the urine, for example, may act as a reductive agent and thus interfere with diagnostic tests using redox chemistry such as those used to test for glucose in the urine (for diabetes testing) or for blood in the feces (for gastrointestinal tract bleeding testing).

Assessment of Nutriture

Plasma vitamin C concentrations respond to changes in dietary vitamin C intake and thus are used to assess recent vitamin C intake; however, white blood cell (leukocyte) content of the vitamin better reflects body stores. Plasma concentrations of vitamin C below 0.2 mg/dL are considered to be deficient. Leukocyte vitamin C concentrations of 10 μg/10^8 or less are considered deficient; however, an analysis of vitamin C among the different types of white blood cells is sometimes beneficial due to variations [14,15].

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THIAMIN (VITAMIN B_1)

The need for thiamin (vitamin B_1) was first recognized in the late 1800s by a Dutchman, C. Eijkman, when it was discovered that fowl fed a diet of cooked, polished rice (devoid of the outer germ and bran layers and containing primarily the starch-rich endosperm) developed neurologic problems (now called beriberi). The substance initially called thiamin*e* that corrected the problems was later isolated from rice bran in 1912 by Casmir Funk. The vitamin's structure was determined by R. Williams from the United States in about the mid-1930s. Thiamin's structure (Figure 9.7) consists of a pyrimidine ring and a thiazole moiety (meaning one of two parts) linked by a methylene (CH_2) bridge. The thiazole moiety contains a sulfur atom.

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Figure 9.7 Structure of thiamin.

Sources

Thiamin is widely distributed in foods (Table 9.5). Meats (especially pork) are major sources of this vitamin. Legumes, seeds, and nuts along with cereals and grain products (whole, fortified, or enriched) also provide thiamin in relatively high amounts. Cereals, for example, provide about 0.38 mg of thiamin per 1 cup serving, which is 25% of the Daily Value. Other sources of the vitamin include yeast, wheat germ, and soy milk. Thiamin in foods can be destroyed (primarily at the methylene bridge) in an alkaline environment (pH of 8 or above) and by heat. Thus, cooking thiamin-rich foods in water can lead to loss of the vitamin.

Most thiamin in the American diet comes from products that have been enriched; such products also contribute to the riboflavin, niacin, and iron contents of the diet. In multivitamin supplements, thiamin is provided in amounts of about 1.5 mg (i.e., the Daily Value) mainly as thiamin hydrochloride or thiamin mononitrate salt. Thiamin and one of its phosphorylated forms thiamin diphosphate (TDP), formerly called thiamin pyrophosphate (TPP), are produced by bacteria in the large intestine; however, the amount of bacterially produced thiamin that is absorbed from the colon is unclear [1].

Table 9.5 Thiamin Content of Selected Foods*

Food (serving)	Thiamin (mg)
Pork loin, boneless (1 chop)	0.95
Salmon (3 oz)	0.2
Nuts, macadamia (1/4 c)	0.2
Beans, baked, black, navy (1/2 c)	0.2
Lentils (1/2 c)	0.2
Seeds, sunflower (1/2 c)	0.2
Pasta, macaroni or spaghetti, cooked (1 c)	0.4
Noodles, egg, cooked (1 c)	0.4
Rice, white, enriched, cooked (1/2 c)	0.1
Bagel, enriched (1)	0.5
Bread, enriched (1 sl)	0.1

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

Digestion, Absorption, Transport, and Storage

Thiamin exists in a free (nonphosphorylated) form in plant foods. In animal products, however, about 95% of thiamin is phosphorylated, primarily as TDP, and about 5% exists as thiamin monophosphate (TMP) and thiamin triphosphate (TTP). Digestion of some of these phosphorylated forms by intestinal phosphatases occurs before absorption. It is primarily free thiamin that appears to be absorbed into the intestinal cells.

While absorption of thiamin from foods is thought to be generally high, some foods contain antithiamin factors that can destroy the vitamin. For example, thiaminases found in some raw fish catalyze the cleavage and destruction of thiamin. These thiaminases are thermolabile, so cooking fish renders the enzymes inactive. Other antithiamin factors include polyhydroxyphenols such as tannic, chlorogenic, and caffeic acids. Polyhydroxyphenols, which are thermostable, are found in coffee, tea, betel nuts, and certain fruits and vegetables such as blueberries, black currants, Brussels sprouts, and red cabbage. These polyhydroxyphenols inactivate thiamin by an oxyreductive process that destroys the thiazole ring; the destructive process can be facilitated by the presence of divalent minerals such as calcium and magnesium. Thiamin destruction may be prevented, however, by the presence of reducing compounds such as vitamin C.

Absorption of free thiamin occurs via diffusion throughout the small intestine, and by transporters found mainly in the duodenum and jejunum. The carriers appear to become saturated with thiamin intakes of about 5 mg, but oral thiamin intakes in varying doses of up to 1,500 mg progressively increase blood thiamin concentrations (consistent with absorption also occurring via passive diffusion) [2]. Two main transporters (ThTr1 and ThTr2) are responsible for thiamin absorption. These carriers exchange thiamin for H^+ ions as part of an antiport carrier system and are pH sensitive. Based on this pH dependence, carrier-mediated thiamin absorption is thought to be greatest in the upper jejunum. Both ThTr1 and ThTr2 are found on the brush border membrane of intestinal cells (including the colon); ThTr2 is also found on the basolateral membrane of intestinal cells. ThTr1 is highly expressed and has a high capacity; ThTr2 has a lower capacity but higher specificity for thiamin than ThTr1 [3,4]. Messenger RNA for ThTr2 (but not ThTr1) increases in response to a low thiamin intake [3].

Defects in the gene SLC19A2, which codes for ThTr1, cause a thiamin responsive deficiency disorder, known as Rogers syndrome. This rare autosomal recessive disorder is characterized by megaloblastic anemia, as well as deafness and glucose intolerance among other manifestations [4,5]. Pharmacological doses (about 25–75 mg) of thiamin improve but do not totally correct the manifestations of the syndrome [4,5].

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Organic cation transporter (OCT1 and OCT3) proteins may also actively transport free thiamin into intestinal cells, especially when intestinal concentrations of the vitamin are low (< 2 μmol/L or μM) [3]. Energy-dependent, sodium independent transport of TDP also occurs within the colon [1]. Alcohol inhibits the intestinal expression of ThT1 and ThTr2, and thus impairs thiamin absorption. This alcohol-associated inhibitory effect on absorption also extends to colonic cells, but the mechanism of the inhibition is unclear.

Thiamin is released from the intestinal cells into the blood typically within 1–2 hours for distribution to tissues. About 90% of the thiamin in the blood is present within the blood cells (mostly red and some white) as TDP (formed within the cells). The normal reference range for blood TDP concentrations is about 70–180 nmol/L. Smaller amounts of thiamin (either bound to albumin or free) and TMP are also found in the plasma. Blood thiamin concentrations usually range from about 2.5 to 7.5 μg/dL, and when combined with TMP, the concentrations range from about 5 to 12 μg/dL [6]. A thiamin binding protein has been identified in the plasma in animal studies and, if present in humans, may be involved in thiamin distribution to tissues [6].

It is free thiamin and TMP that are thought to cross cell membranes to enter cells. Transport of thiamin into the red blood cells among other tissues requires carriers, some of which are energy dependent. ThTr1, found especially in skeletal muscles, and ThTr2, found in the liver, kidneys, and heart (among others), mediate thiamin uptake into some tissues [3,4]. Within cells, a TDP carrier has been demonstrated on the mitochondrial membrane and may be responsible for intracellular TDP transport.

The human body contains approximately 25–30 mg of thiamin, with small concentrations stored in the liver, skeletal muscles, heart, kidneys, and brain. Skeletal muscles are thought to contain about half of the body's thiamin. Thiamin's half-life is estimated at about 9–20 days.

Free thiamin is taken up and phosphorylated mainly by the liver and muscle, but also by other organs, including the brain, heart, and kidneys. This phosphorylation reaction requires energy and is catalyzed by thiamin pyrophosphokinase, also called diphosphokinase. About 80% of the total thiamin in the body exists as TDP.

About 10% of thiamin is found in the body tissues as TTP. TTP is synthesized by action of a TDP-ATP phosphoryl transferase that phosphorylates TDP.

Hydrolysis or dephosphorylation of thiamin's phosphorylated forms occurs in tissues throughout the body. The terminal phosphate on the TTP is hydrolyzed by thiamin triphosphatase to yield TDP (shown above), and TDP can be converted to TMP by thiamin diphosphatase, which cleaves the terminal phosphate on TDP. TMP can then be converted to free thiamin by thiamin monophosphatase.

Functions and Mechanisms of Action

Thiamin is involved in essential coenzyme and noncoenzyme roles, including:

- energy production and nutrient metabolism (coenzyme roles in the *pyruvate dehydrogenase*, the *α-ketoglutarate dehydrogenase*, and the *branched-chain α-keto acid dehydrogenase complexes*)
- nutrient metabolism—the interconversion of phosphorylated sugars for the synthesis of *nucleotides* and some *B-vitamin coenzymes* (a coenzyme role as *transketolase*)
- nervous system functions (in a noncoenzyme capacity).

Coenzyme Roles

As the coenzyme TDP, thiamin functions in energy production from the metabolism of nutrients, especially carbohydrates and amino acids. In addition, TDP serves as a coenzyme involved in nutrient metabolism, more specifically the synthesis of some phosphorylated sugars. The specific reactions are shown as an overview in Figure 9.8 and discussed in detail hereafter.

Energy Production and Nutrient Metabolism TDP is necessary for the oxidative decarboxylation of pyruvate to acetyl-CoA, α-ketoglutarate to succinyl-CoA, and of the three branched-chain amino acids (isoleucine, leucine, and valine) to various CoA metabolites. These reactions are instrumental in generating energy (ATP). Reductions or inhibition of the reactions, especially for pyruvate and α-ketoglutarate, diminish synthesis of ATP. Additionally, diminished pyruvate oxidation reduces the production of acetyl-CoA, which is vital for the synthesis of the neurotransmitter acetylcholine and for the synthesis of fatty acids, cholesterol, and other important compounds. Inhibition also results in the accumulation of pyruvate, lactate, and α-ketoglutarate.

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Figure 9.8 Various vitamin cofactors and their action sites in energy metabolism. The role of thiamin as TDP is shown by an asterisk.

The steps that occur in the oxidative decarboxylation of pyruvate to form acetyl-CoA, shown in Figure 9.9, require a multienzyme complex known as the pyruvate dehydrogenase complex, which is bound to the mitochondrial membrane. Three enzymes make up this complex: a TDP-dependent pyruvate dehydrogenase; a lipoic acid–dependent dihydrolipoyl transacetylase; and an FAD-dependent dihydrolipoyl dehydrogenase. The roles of four vitamins—thiamin (TDP), riboflavin (FAD), niacin (NAD^+), and pantothenic acid (CoA)—in this process are described briefly and are shown in Figures 9.9 and 9.10. ATP and Mg^{2+} are also required.

In the first reaction (Figure 9.10), the carbon-2 atom between the nitrogen and sulfur atoms in the thiazole ring of TDP ionizes to form a carbanion, which then combines with the 2-carbonyl group of pyruvate, α-ketoglutarate, and

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1. CO_2 is removed from pyruvate and the rest of the compound (hydroxyethyl) attaches to TDP to form hydroxyethyl TDP.
2. The hydroxyethyl group is transferred to oxidized lipoamide, which consists of lipoic acid attached by an amide link (CO-NH) to a lysine residue of the enzyme dihydrolipoyl transacetylase. With the transfer of the hydroxyethyl group acetyl lipoamide is generated.
3. Acetyl lipoamide reacts with coenzyme A (CoA-SH) to form acetyl-CoA and reduced lipoamide.
4. Reduced lipoamide is oxidized by the flavo (FAD)-dependent enzyme dihydrolipoyl dehydrogenase.
5. The reduced flavo ($FADH_2$) protein is oxidized by NAD^+, which then transfers reducing equivalents to the respiratory chain.

Figure 9.9 The oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase complex.

other α-keto acids, forming a covalent bond. After forming the adduct (attachment) between pyruvate and TDP, pyruvate dehydrogenase (the first enzyme of the complex) catalyzes the removal of pyruvate's COO group to form hydroxyethyl-TDP (Figure 9.9). The hydroxyethyl group is then transferred to oxidized lipoamide (which is bound to the second enzyme, dihydrolipoyl transacetylase), forming acetyl lipoamide. The acetyl lipoamide then reacts with coenzyme A to form acetyl-CoA and reduced lipoamide. Lipoamide is oxidized by the third enzyme, dihydrolipoyl dehydrogenase, which requires FAD. NAD^+ oxidizes $FADH_2$. Thus, the overall reaction is:

$$\text{Pyruvate} + NAD^+ + CoA \longrightarrow \text{Acetyl-CoA} + NADH + H^+ + CO_2$$

The decarboxylation of α-ketoglutarate by the α-ketoglutarate dehydrogenase complex and the decarboxylation of the branched-chain keto acids by the branched-chain α-ketoacid dehydrogenase complex are similar to that of pyruvate. The α-ketoglutarate dehydrogenase complex in the mitochondria decarboxylates α-ketoglutarate and forms succinyl-CoA. Decarboxylation of the branched-chain α-keto acids, which arise from the transamination of valine, isoleucine, and leucine, is an oxidative process that also requires thiamin as TDP; the decarboxylation reactions generate isobutyryl-CoA (from valine), α-methylbutyryl-CoA (from isoleucine), and isovaleryl-CoA (from leucine) (see Figure 6.36). Failure to oxidize the α-keto acids of leucine, isoleucine, and valine (as occurs in the genetic disorder maple syrup urine disease) causes both the branched-chain amino acids and their α-keto acids to accumulate in blood and other body fluids.

Nutrient Metabolism–Interconversion of Phosphorylated Sugars Thiamin as TDP also functions as the loosely bound prosthetic group of transketolase, a key cytosolic enzyme in the pentose phosphate pathway (hexose monophosphate shunt; see Figure 3.29). The oxidative portion of the pentose phosphate pathway generates NADPH, which is needed for fatty acid synthesis, among other roles (see the section of this chapter addressing niacin's functions). The nonoxidative portion of the pathway relies on transketolase to interconvert phosphorylated sugars containing three to seven carbons. *The phosphorylated sugar ribose 5-PO_4 is used for the synthesis of ATP as well as for some B-vitamin coenzymes (coenzyme (Co)A, NAD, $NADP^+$, and FAD), and for nucleotides that make up DNA and RNA.* Some of the phosphorylated sugars generated in the pentose phosphate pathway are also used to produce intermediates in glycolysis. Two enzymes, transketolase and transaldolase, catalyze the interconversions among the phosphorylated sugars. Transketolase, which requires Mg^{2+} in addition to TDP, transfers (in reversible reactions) carbon fragments among phosphorylated ketoses (sugars containing a ketone group) and phosphorylated aldoses

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Figure 9.10 The first steps in the decarboxylation of pyruvate by thiamin diphosphate.

(sugars containing an aldehyde group). In the reaction (shown hereafter), two carbons from the ketose sedoheptulose 7-PO_4 are transferred by transketolase to the aldose glyceraldehyde 3-PO_4 to form the ketose xylulose 5-PO_4 and the aldose ribose 5-PO_4.

Sedoheptulose 7-PO_4 (7 carbons) + Glyceraldehyde 3-PO_4 (3 carbons) $\xleftrightarrow{\text{Transketolase}}$ Xylulose 5-PO_4 (5 carbons) + Ribose 5-PO_4 (5 carbons)

Further interconversion among the phosphorylated sugars is catalyzed by transaldolase, which converts sedoheptulose 7-PO_4 (7-carbon ketose) and glyceraldehyde 3-PO_4 (3-carbon aldose) to erythrose 4-PO_4 (4-carbon aldose) and fructose 6-PO_4 (6-carbon ketose). In a second reaction catalyzed by transketolase, carbon fragments are transferred such that erythrose 4-PO_4 (4-carbon aldose) and xylulose 5-PO_4 (5-carbon ketose) are used to generate fructose 6-PO_4 (6-carbon ketose) and glyceraldehyde 3-PO_4 (3-carbon aldose). The reaction can be written as:

Erythrose 4-PO_4 + Xylulose 5-PO_4 $\xleftrightarrow{\text{Transketolase}}$ Fructose 6-PO_4 + Glyceraldehyde 3-PO_4

Noncoenzyme Roles: Nervous System Functions

In addition to its coenzyme roles, thiamin is involved in nervous system functions. The exact nature of its involvement, however, is unclear. Thiamin appears to play a role in regulating sodium channels or permeability. It also may be needed on the inner membrane surface to maintain a fixed negative charge [6]. Thiamin, as TTP, also provides phosphate for the phosphorylation of synaptic proteins involved in activating chloride transport.

As in other organs/tissues, thiamin serves as a coenzyme needed for energy production in the brain. The conversion of pyruvate to acetyl-CoA is especially noteworthy as acetyl-CoA not only is used for energy

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production but also is needed for the production of the neurotransmitter acetylcholine in some neurons. Myelin, also requiring acetyl-CoA along with various lipids and proteins for its synthesis, is important for the conduction of nerve impulses. The article by Manzetti et al. [3] provides additional information for the interested reader on thiamin functions in the brain.

Selected Pharmacological Uses / Other Roles

Pharmacological doses of thiamin are provided to individuals diagnosed with maple syrup urine disease (MSUD), which results from genetic mutations in the branched-chain α-keto acid dehydrogenase complex. Large oral doses (usually 100 mg or higher) of the vitamin are generally recommended for those with MSUD for several months to see if vitamin supplementation improves residual enzyme activity.

Metabolism and Excretion

Thiamin, TMP, and TDP in excess of tissue needs and storage capacity are excreted intact or catabolized prior to urinary excretion. Degradation of thiamin begins with cleavage of the vitamin into its pyrimidine and thiazole moieties. The two rings are then further catabolized, generating 20 or more metabolites, including, for example, thiochrome (a major metabolite sometimes used to assess thiamin status), 4-methyl thiazole 5-acetic acid, and 2-methyl 4-amino 5-pyrimidine carboxylic acid.

Recommended Dietary Allowance

Recommendations for thiamin intake are based on the results of studies examining urinary excretion, changes in erythrocyte transketolase activity, and thiamin intake data. The RDA for thiamin for adult men is 1.2 mg/day and for adult women is 1.1 mg/day; the requirements for men and women are 1.0 mg/day and 0.9 mg/day, respectively [7]. Differences in thiamin needs between men and women are based on differences in body size and energy needs. Thiamin recommendations with pregnancy and lactation increase to 1.4 mg/day and 1.5 mg/day, respectively [7]. The inside front cover of the book provides additional RDAs for thiamin for other age groups.

Deficiency: Beriberi

Because of thiamin's roles in several pathways involved in nutrient metabolism, a deficiency of the vitamin profoundly impacts energy production as well as other body processes secondary to reductions in the production of various intermediates normally generated during nutrient metabolism. It can take as little as a few weeks to about one month of consuming a diet inadequate in thiamin to deplete the body stores; signs of thiamin deficiency usually appear shortly thereafter [8,9]. Use of parenteral nutrition devoid of or containing inadequate thiamin or containing excessive amounts of glucose can also cause thiamin deficiency within a few weeks. Initial symptoms of a deficiency are vague and may include fatigue and vomiting.

With depletion of body stores of the vitamin, the nervous system and heart are most likely to be affected by a thiamin deficiency, known as beriberi (*beri* means "weakness"). Three forms of the disorder have been described: dry, wet, and acute. Dry beriberi results from a chronic low thiamin intake, especially if coupled with a high carbohydrate intake. Dry beriberi is characterized by peripheral neuropathy and muscle weakness and cramping, especially in the lower extremities. There is a lack of edema that is found in wet beriberi (hence the term "dry" versus "wet"), although there is often overlap between the wet and dry forms. The neuropathy consists of symmetrical sensory and motor nerve conduction problems mostly affecting the distal parts of the limbs/extremities (i.e., the ankles, feet, wrists, and hands). These problems usually begin in the feet, and progress to the calves and then the thighs. A symmetrical foot drop may be present and associated with tenderness of the calf muscles and paresthesia in the feet; in the arms, wrist drop also may occur.

Wet beriberi results in more extensive cardiovascular system involvement than dry beriberi; cardiomegaly (enlarged heart), rapid heartbeat (tachycardia), right-side heart failure (also affecting the lungs), and peripheral edema are common manifestations. The third form, acute beriberi, occurs mostly in infants, especially those who are breastfed by mothers having poor thiamin status. Acute beriberi is characterized by gastrointestinal symptoms (anorexia, nausea, vomiting) as well as tachycardia, cardiomegaly, and lactic acidosis (inadequate thiamin causes pyruvate to be converted to lactic acid versus acetyl-CoA; the lactic acid accumulates, causing acidosis). (Note: Thiamin corrects the lactic acidosis by increasing pyruvate dehydrogenase activity and enabling more pyruvate to be decarboxylated to acetyl-CoA for entry into the TCA cycle versus being converted to lactic acid.)

Treatment of beriberi depends on the severity of its symptoms and the conditions under which the deficiency occurs. A mild deficiency can be treated with oral thiamin in doses of about 30 mg taken three times daily until symptoms disappear [10]. More severe cases may require higher oral intakes of 100–800 mg of thiamin taken in divided doses until symptoms disappear [10].

While most individuals in the United States consume thiamin in recommended amounts, there are a few exceptions. Individuals with alcohol dependency are more prone to developing thiamin deficiency because of decreased food consumption (and thus thiamin intake), decreased thiamin absorption (which remember is impaired by alcohol), and diminished thiamin utilization (i.e., TDP formation). The chronic thiamin deficiency

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seen in those with alcoholism may be characterized by a neurologic disorder called Wernicke's encephalopathy, although not everyone with alcohol dependency develops this condition (the reasons for these differences have not been identified). Neurological dysfunction may occur within about 2 weeks of insufficient thiamin available to the brain [10]. Symptoms of Wernicke's encephalopathy include **ophthalmoplegia** (weakness or paralysis of the ocular muscles), horizontal **nystagmus** (constant, involuntary eyeball movement), ataxia (impaired muscle coordination), and confusion. Without treatment, additional manifestations may occur including amnesia (memory loss primarily for distant events), problems forming new memories, confabulation, and psychosis (referred to as Wernicke-Korsakoff syndrome or Korsakoff psychosis). Prevention and treatment protocols vary. For example, those at risk may need higher than the practice guidelines of 100 mg of thiamin/day. Intravenous administration of thiamin in amounts of 250 mg or more given several times per day has been beneficial and enables plasma thiamin concentrations to reach sufficient levels for transport across the blood–brain barrier [8,10]. Persistent neurological symptoms may necessitate the provision of higher parenterally administered doses of the vitamin over the course of several months [8,10]. In some cases, following the period of intravenous thiamin administration, additional supplementation provided orally, usually in doses of 100 mg taken up to three times per day, is recommended until symptoms disappear [8,10].

In addition to those with alcohol dependency, thiamin deficiency has been documented in people with congestive heart failure. The higher prevalence in this population is attributable to low thiamin intakes and increased urinary thiamin losses secondary to the use of diuretics, especially furosemides in high (80 mg) doses. Treatment typically requires oral thiamin (50–100 mg) taken a few times per day. However, those with more severe heart failure may require higher oral intakes (about 300 mg) of thiamin or intravenously administered thiamin (~200 mg) for several days to weeks [8].

Older adults may fail to ingest enough thiamin-rich foods and be at risk for deficiency. Additionally, those with increased thiamin needs such as individuals with some cancers and with diseases that impair the vitamin's absorption (i.e., some gastrointestinal tract cancers, liver disease, inflammatory bowel diseases) are at risk for deficiency. Individuals with hyperemesis (excessive vomiting as may occur during pregnancy or other situations), diabetes, and on dialysis for renal failure also may be at risk for deficiency and benefit from supplementation. Thiamin deficiency following bariatric surgery, especially Roux-en-Y gastric bypass, has been documented; however, doses of thiamin needed to prevent deficiency are not clear. Recommendations for the treatment of deficiency vary with symptoms [8,11,12].

Toxicity

No Tolerable Upper Intake Level has been established for thiamin, and no side effects have been reported from oral intakes up to about 500 mg daily. Thiamin (in amounts up to 100 mg) given intravenously or intramuscularly has been associated with skin irritation, headache, convulsions, cardiac arrhythmia, and anaphylactic shock, among other signs [7]. The skin irritation at the injection site may be lessened by providing the vitamin in 100 mL normal saline or 5% dextrose and infusing it over a 30-minute period [13].

Assessment of Nutriture

Thiamin status can be assessed by measuring thiamin and/or TDP in the blood or urine and by measuring erythrocyte transketolase activity in hemolyzed whole blood. Blood thiamin concentrations less than about 2.5 μg/dL and blood TDP concentrations less than about 70 nmol/L may be suggestive of deficiency. Urinary thiamin excretion decreases with decreased thiamin status and is also correlated with intake. Urinary thiamin excretion in amounts less than ~40 μg/day or ~27 μg/g creatinine suggests thiamin deficiency. The activity of transketolase, the thiamin-dependent enzyme of the pentose phosphate pathway, is measured after the addition of thiamin to the incubation medium to assess status. An increase in transketolase activity of >25% indicates thiamin deficiency; an increase in activity of 15–25% suggests marginal status; and an increase of ~15% suggests adequate status. Transketolase concentrations of ~120 nmol/L also have been used to indicate deficiency; concentrations of 120–150 nmol/L suggest marginal thiamin status.

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Suggested Reading

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RIBOFLAVIN (VITAMIN B_2)

In 1917 scientists discovered riboflavin (vitamin B_2), which was originally called vitamin G in the United States. Kuhn and coworkers are credited with determining its structure along with Szent-Györgyi and Wagner-Jaunergy in 1933. Riboflavin consists of a flavin molecule (isoalloxazine ring) with a ribitol (sugar alcohol) side chain attached. The name *riboflavin* signifies the presence of a ribose-like side

Figure 9.11 Structures of riboflavin and its coenzyme forms.

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chain (ribo) and its yellow color (*flavus* means "yellow" in Latin). The structures of riboflavin and its two coenzyme derivatives, FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide), are shown in Figure 9.11.

Sources

Riboflavin is found in a wide variety of foods (Table 9.6), but especially those of animal origin. Milk and milk products such as cheeses contribute the most to dietary riboflavin intakes. Meat provides riboflavin in significant quantities; liver is exceptionally rich, with about 2–3 mg/3-oz serving. Fruits have little riboflavin. Refined grains are enriched with riboflavin because during the milling of grains the removal of the bran and germ layers results in the loss of most (about two-thirds) of its riboflavin. Cereals typically provide about 25% of the Daily Value, which is 1.7 mg, or about 0.42 mg of riboflavin per serving. Riboflavin in foods can be destroyed with exposure to sunlight (one reason milk is usually not sold in glass bottles); even photo (light) therapy, used to treat neonatal hyperbilirubinemia, causes riboflavin destruction. The vitamin is fairly resistant to heat, oxidation, and acid.

Another source of riboflavin is provided by bacterial synthesis of the vitamin in the large intestine. Much of this microbially produced riboflavin appears to be present in a free, absorbable form [1].

The form of riboflavin in food varies. Free and protein-bound riboflavin are found in milk, eggs, and enriched breads and cereals. In most other foods, the vitamin occurs as one or the other of its coenzyme derivatives FMN or FAD, although phosphorus-bound riboflavin and amino acid–bound FAD are also found in some foods. In multivitamin and B-complex vitamin supplements, riboflavin is usually present as free riboflavin or bound to phosphate. The amount of the vitamin provided in the multivitamin supplements is typically 1.7 mg (i.e., 100% of the Daily Value).

Table 9.6 Riboflavin Content of Selected Foods*

Food (serving)	Riboflavin (mg)
Milk, 2% (1 c)	0.4
Cottage cheese (1/4 c)	0.3
Cheese, variety (1 oz)	0.1
Yogurt, Greek, plain, nonfat (1 c)	0.5
Egg (1)	0.2
Meats, variety (3 oz)	0.1–0.3
Cod, cooked (3 oz)	0.1
Legumes, variety, cooked (1 c)	0.1
Spinach, cooked (1/2 c)	0.2
Mushrooms, portabella, grilled (1/2c)	0.3
Nuts, almonds (1/4 c)	0.4
Bread, enriched (1 sl)	0.1
Rice, white, enriched, cooked (1/2 c)	0.1

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

Digestion, Absorption, Transport, and Storage

The riboflavin that is found in foods attached noncovalently to proteins must be released from the protein prior to absorption; this process is accomplished by the actions of hydrochloric acid secreted within the stomach and proteases secreted by the stomach, pancreas, and small intestine. The riboflavin in foods as FAD, FMN, and riboflavin phosphate also must be released prior to absorption. FAD pyrophosphatase converts FAD to FMN, and FMN in turn is converted to free riboflavin by FMN phosphatase.

$$\text{FAD} \xrightarrow{\text{FAD pyrophosphatase}} \text{FMN}$$

$$\xrightarrow{\text{FMN phosphatase}} \text{Riboflavin}$$

Other intestinal phosphatases, such as nucleotide diphosphatase and alkaline phosphatase, are thought to hydrolyze riboflavin from riboflavin phosphate.

Not all bound riboflavin is hydrolyzed and available for absorption. A small amount (~7%) of FAD is covalently bound to either of two amino acids, histidine or cysteine. Thus, following consumption of foods with FAD bound to either of these amino acids, the proteins are degraded; however, the riboflavin remains bound to the histidine or cysteine residues and is unable to be used functionally in the body.

Free riboflavin is absorbed across the intestinal brush border membrane by an energy-dependent riboflavin vitamin transporter (abbreviated RFVT) 3, found mainly in the proximal small intestine, but also the colon [1]. Another riboflavin vitamin transporter, RFVT1, carries riboflavin across the basolateral membrane of the intestinal cell. In addition to carrier-mediated transport, riboflavin may be absorbed by diffusion with ingestion of pharmacological doses. The presence of bile facilitates riboflavin absorption, possibly through effects on intestinal motility or permeability. Alcohol impairs both riboflavin digestion and absorption [1]. About 95% of riboflavin intake from foods is absorbed, up to a maximum of about 25 mg at which point absorption plateaus and plasma concentrations peak [2].

Within the intestinal cell, riboflavin may be phosphorylated to form FMN in a reaction catalyzed by flavokinase and requiring ATP, as shown here and in Figure 9.11.

$$\text{Riboflavin} + \text{ATP} \xrightarrow{\text{Flavokinase}} \text{Flavin mononucleotide (FMN)} + \text{ADP}$$

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However, prior to its efflux into portal blood, FMN is dephosphorylated by a nonspecific alkaline phosphatase to generate free riboflavin. Within the liver and other tissues, riboflavin will again be converted to FMN by flavokinase and to its other predominant flavoenzyme FAD by FAD synthetase (shown here and in Figure 9.11).

Flavins are found in the plasma as riboflavin (50%), FMN (10%), and FAD (40%) and are typically bound to a variety of proteins, including albumin, fibrinogen, and globulins (principally immunoglobulins). Albumin appears to be the primary transport protein, although riboflavin binding proteins have been identified and may be also involved in the distribution of the vitamin to tissues. The usual reference range for plasma riboflavin is about 1–19 μg/L.

Regardless of the form in which the vitamin reaches the tissues, free riboflavin is the form that traverses most cell membranes, primarily by a carrier-mediated process that in some tissues, such as the liver, appears to be regulated by calcium or calmodulin [1,2]. Riboflavin is found in small quantities in a variety of tissues. The greatest concentrations are found in the liver, kidneys, and heart. It is estimated that the body stores enough riboflavin to meet its needs for about 2–6 weeks [2].

Following uptake into cells (and as previously discussed in the intestine and liver), riboflavin is converted to its coenzyme forms by flavokinase and FAD synthetase, both of which are widely distributed in tissues, especially the liver, spleen, small intestine, kidneys, and heart. FMN is the major form (~60–95%) present in cells, followed by FAD (~5–20%). Synthesis of FMN and FAD appears to be influenced by end-product inhibition and hormones including ACTH, aldosterone, and thyroid hormones, which accelerate the conversion of riboflavin into its coenzyme forms by increasing flavokinase activity. Once synthesized, the flavin coenzymes become bound to apoenzymes. FMN and FAD function as prosthetic groups for enzymes called flavoproteins that are involved in oxidation-reduction reactions.

Functions and Mechanisms of Action

FMN and FAD function as coenzymes for a variety of oxidative enzymes and remain bound to the enzymes during the oxidation-reduction reactions. Flavins can act as oxidizing agents because of their ability to accept a pair of hydrogen atoms. The isoalloxazine ring is reduced by two successive one-electron transfers with the intermediate formation of a semiquinone free radical, as shown in Figure 9.12. Reduction of the isoalloxazine ring yields the reduced forms of the flavoprotein, which can be found in $FMNH_2$ and $FADH_2$.

Flavoprotein Roles in Nutrient Metabolism and Energy Production

Flavoproteins exhibit a wide range of redox potentials and therefore can play a variety of roles in intermediary metabolism. Some of these roles of flavoproteins are listed here.

- The role of flavoproteins in the *electron transport chain* is illustrated in Figures 3.24 and 3.28.
- In vitamin B_6 metabolism (seen later in Figure 9.36), *pyridoxine phosphate oxidase*—which converts pyridoxamine phosphate (PMP) and pyridoxine phosphate (PNP) to pyridoxal phosphate (PLP), the primary coenzyme form of vitamin B_6—is dependent upon FMN.
- *L-amino oxidase* uses FMN in the dehydrogenation of L-amino acids to imino acids.
- In the *oxidative decarboxylation of pyruvate* (Figure 9.9) and *α-ketoglutarate*, FAD serves as an intermediate electron carrier, with NADH being the final reduced product.
- *Succinate dehydrogenase* is an FAD flavoprotein that removes electrons from succinic acid to form fumarate, and that forms $FADH_2$ from FAD (see Figure 3.26). The electrons are then passed into the electron transport chain by coenzyme Q (see Figure 3.25).
- In fatty acid beta-oxidation, *acyl-CoA dehydrogenases* require FAD (see Figure 5.23).
- *Sphinganine oxidase*, in sphingosine synthesis, requires FAD.
- As a coenzyme for an oxidase such as *xanthine oxidase* involved in purine catabolism, FAD transfers electrons directly to oxygen with the formation of hydrogen peroxide (see the section on molybdenum in Chapter 13).

Figure 9.12 Oxidation and reduction of isoalloxazine ring.

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- Similarly, *aldehyde oxidase* using FAD reacts with aldehydes such as pyridoxal (vitamin B_6)—to form pyridoxic acid—and retinal (a form of vitamin A)—to produce retinoic acid—while also passing electrons to oxygen and generating hydrogen peroxide.
- Synthesis of a *folate as 5-methyl THF* requires $FADH_2$ (shown later in Figure 9.28).
- A step in *the synthesis of niacin from tryptophan* that is catalyzed by kynureninase monooxygenase requires FAD (see Figure 9.15).
- In *choline catabolism*, several dehydrogenases require FAD.
- Some neurotransmitters (such as dopamine) and other amines (tyramine and histamine) require FAD-dependent *monoamine oxidase* for metabolism.
- Reduction of the oxidized form of glutathione (GSSG) to its reduced form (GSH) depends on FAD-dependent *glutathione reductase*. This reaction forms the basis of one assay used to assess riboflavin status (see the "Assessment of Nutriture" section).
- *Ero1 and sulfhydryl oxidases* are FAD dependent and help to form disulfide bonds involved in the structure or folding of selected secretory proteins. Impaired folding and subsequently impaired secretion of proteins have been observed with riboflavin deficiency [3].
- *Thioredoxin reductase*, a flavo (FAD) enzyme (also containing selenocysteine at its active site) transfers reducing equivalents from NADPH through its bound FAD to reduce disulfide bonds within the oxidized form of thioredoxin. The enzyme works as part of a complex set of reactions with ribonucleotide reductase (which contains thiol groups), as shown here:

- *Ribonucleotide reductase* catalyzes the conversion of ribonucleotides to deoxyribonucleotides (such as dADP, dGDP, dCDP, and dUDP; see Figure 6.28), which are needed for DNA synthesis. In the reaction, the sulfhydryl groups in ribonucleotide reductase become oxidized, forming a disulfide bond. Thioredoxin (or glutaredoxin—a small protein like thioredoxin) provides electrons (H), but upon donation becomes oxidized itself (containing a disulfide bond). The flavoenzyme thioredoxin reductase (or glutaredoxin reductase), which also contains sulfhydryl groups, reduces the thioredoxin (or glutaredoxin). NADPH then reduces the thioredoxin reductase (or glutaredoxin reductase) to eliminate the disulfide bond and regenerate the sulfhydryl groups.
- Hydrogen peroxide production from singlet oxygen ($^1O_2$, which is derived from, e.g., activated white blood cells) and water via an antibody-catalyzed water oxidation pathway also appears to require riboflavin [4]. Hydrogen peroxide assists in the destruction of foreign substances, although it is also destructive to body cells.

Selected Pharmacological Uses / Other Roles

The ingestion of riboflavin in doses of up to 400 mg (for at least a 3-month time period) has been effective in decreasing the frequency and/or intensity of migraine headaches in some (but not all) studies [5].

The autosomal recessive condition glutaric aciduria type 1 results from mutations in FAD-dependent enzyme glutaryl-CoA dehydrogenase, which converts glutaryl-CoA to glutaconyl-CoA as part of the degradation of the amino acids tryptophan and lysine. Some individuals with glutaric aciduria type 1 benefit from the ingestion of pharmacologic doses of riboflavin (about 200 mg per day taken in divided doses), which enhances some residual glutaryl-CoA dehydrogenase activity and reduces the production of glutaric acid and some of its associated side effects.

Beta-oxidation of fatty acids in the mitochondria is catalyzed by several acyl-CoA dehydrogenases, which require FAD as coenzymes. Numerous mutations in these enzymes have been identified, with some mutations more responsive to riboflavin supplementation than others. In responsive cases, riboflavin supplementation in amounts of about 25–150 mg (ingested orally in divided doses) has been needed.

Metabolism and Excretion

Riboflavin (that is not attached to proteins in the blood) and its metabolites are excreted primarily in the urine. With an adequate intake, the vitamin (~60–70%) is filtered by the glomerulus and excreted intact in the urine; the amount excreted is typically at least 120 μg/day or 80 μg/g creatinine. The kidneys can reduce losses of the vitamin, if for example intake is inadequate, through carrier-mediated reabsorption in the proximal tubules.

Metabolites arise from tissue degradation of covalently bound flavins as well as from degradation of the vitamin itself. The metabolites present in the greatest concentrations in the urine include 7α- and 8α-hydroxymethyl riboflavin, 8α-sulfonyl riboflavin, 10-hydroxyethyl flavin, and riboflavinyl peptide ester. Riboflavin bound to cysteine and

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histidine also may be found in the urine if absorbed in such form from the gastrointestinal tract or if generated in body cells from the degradation of flavoenzymes such as succinate dehydrogenase and monoamine oxidase [6]. Furthermore, some metabolites formed in the intestinal tract by the bacterial degradation of the vitamin also may be absorbed, but then are excreted in the urine [6]. Only small amounts of riboflavin are found in the feces following excretion via the bile. Fecal riboflavin metabolites also arise from the catabolism of riboflavin by intestinal bacteria.

Unlike other vitamins, the presence of riboflavin in the urine is often visibly noticeable. Within a couple of hours following the ingestion of riboflavin (which is a fluorescent yellow compound) in a quantity such as 1.7 mg (similar to that found in a multivitamin supplement) or higher, the excretion of riboflavin can be noticed by a deepening of the urine's color from a typical light yellow to a brighter, orangish yellow.

Recommended Dietary Allowance

The RDAs for riboflavin have been estimated through various studies examining urinary excretion of the vitamin, the relationship of dietary intake to clinical signs of deficiency, and the activity of erythrocyte glutathione reductase. Recommendations for riboflavin for adult men and women are 1.3 mg/day and 1.1 mg/day, respectively; the requirements for adult men and women are 1.1 mg and 0.9 mg, respectively [6]. With pregnancy and lactation, recommendations for daily riboflavin intake increase to 1.4 mg and 1.6 mg, respectively [6]. The inside front cover of the book provides additional RDAs for riboflavin for other age groups.

Deficiency: Ariboflavinosis

A deficiency of riboflavin, sometimes called ariboflavinosis, rarely occurs in isolation; if encountered, it is usually accompanied by other nutrient deficits. While no clear riboflavin deficiency disease has been characterized, some clinical signs of deficiency after about 3–4 months of inadequate intake include painful lesions or vertical fissures on the outside of the lips (cheilosis) and corners of the mouth (angular stomatitis); inflammation of the tongue (glossitis), which may appear smooth and magenta in color; and a red or bloody (hyperemia) and swollen (edema) mouth/oral cavity. An inflammatory skin condition, referred to as oculo-orogenital syndrome, also occurs. The condition affects areas of the skin containing high concentrations of sebaceous glands, including the external ear, the nasolabial fold, eyelids, and scrotum (males). In these areas, the skin becomes reddened, scaly, greasy, and painful. The eyes may be affected with conjunctival infection and photophobia, and there may also be peripheral nerve dysfunction (neuropathy). Severe riboflavin deficiency also may diminish the riboflavin-dependent synthesis of the coenzyme form of vitamin B_6 and of niacin from tryptophan. Studies in cell cultures have found that riboflavin deficiency can result in protein and DNA damage and arrest cells in the G1 phase of the cell cycle [3]. Treatment of deficiency in adults usually requires about 5–30 mg of riboflavin daily (given in divided doses) until symptoms resolve. To facilitate absorption and minimize digestive tract problems, oral doses of the vitamin are best taken with food and in amounts of no more than about 25 mg at one time.

Because of limited dietary intake and diminished absorption, people consuming excess alcohol are at risk of deficiency. The likelihood of deficiency also remains fairly common in developing countries. Because riboflavin metabolism is altered with thyroid disease (hypothyroidism) and adrenal insufficiency, and because riboflavin excretion is enhanced with diabetes mellitus, trauma, and stress, people with these conditions are also at risk for deficiency. Tricyclic medications used to treat depression inhibit riboflavin function.

Toxicity

Toxicity associated with ingestion of large oral doses of riboflavin has not been reported, and no Tolerable Upper Intake Level for riboflavin has been established [6]. Oral doses of up to 400 mg of riboflavin have been consumed in those with migraines without side effects [5].

Assessment of Nutriture

The most sensitive method for determining riboflavin nutriture is to measure the activity of the FAD-dependent enzyme erythrocyte glutathione reductase, which catalyzes the following reaction:

$$\text{NADPH} + \text{H}^+ + \text{GSSG} \longrightarrow \text{NADP}^+ + 2\text{GSH}\cdot$$

In this reaction, glutathione in its oxidized form is designated GSSG, and in its reduced form, GSH. In cases of a riboflavin deficiency or marginal riboflavin status, the activity of glutathione reductase is limited, and less NADPH is used to reduce the oxidized glutathione. *In vitro* enzyme activity in terms of "activity coefficients" (AC) is determined both with and without the addition of FAD to the medium. Activity coefficients represent a ratio of the enzyme's activity with FAD to the enzyme's activity without FAD. When the addition of FAD stimulates enzyme activity to generate an AC of 1.2–1.4, riboflavin status is considered low; an AC > 1.4 suggests riboflavin deficiency. Conversely, if FAD is added and AC is ~1.2, then riboflavin status is considered acceptable.

In addition to the use of glutathione reductase activity, the activity of a FMN-dependent enzyme, pyridoxamine phosphate oxidase, needed for interconversions of vitamin B_6 coenzyme forms, also appears to be a biomarker of riboflavin status. Urinary riboflavin excretion is also used to assess status, with urinary excretion of less than about 25 μg riboflavin/g creatinine (without recent riboflavin intake) or less than about 40 μg riboflavin per day indicative of deficiency.

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NIACIN (VITAMIN B_3)

Like thiamin, which was discovered through its deficiency disorder beriberi, niacin (also called vitamin B_3) was discovered through the condition called **pellagra** in humans and a similar condition called black tongue in dogs. In fact, the vitamin was once called the anti–black tongue factor because of its effect in dogs. Pellagra was especially prevalent in the southeastern United States where corn (which contains a relatively unavailable form of niacin) was a main dietary staple in the early 1900s. It was not until about 1937 that Elvehjem isolated the vitamin, which was shown then to cure both pellagra and black tongue. The vitamin was named niacin in the early 1940s. Niacin, however, is a generic term for nicotinic acid and nicotinamide (also called niacinamide), which both provide vitamin activity. Structurally, nicotinic acid is pyridine 3-carboxylic acid, whereas nicotinamide is nicotinic acid amide (Figure 9.13).

Sources

Niacin is found in several food groups, with the best sources being fish and meats (Table 9.7). Beef liver is especially rich, with about 15 mg of niacin/3-oz serving. Enriched cereals and bread products, whole grains, seeds, and legumes also contain appreciable amounts of niacin. Cereals are often fortified with the vitamin at 25% of the 20 mg Daily Value, or 5 mg per serving. Niacin is also found in coffee and tea, and in lesser amounts in green vegetables and milk. In coffee, a compound called trigonelline is converted to niacin by heat (such as with coffee bean roasting) and acid.

The production of nicotinic acid by bacteria in the large intestine has also been confirmed [1]. The overall contribution of this source of the vitamin to the body's need for niacin is unclear.

Nicotinic acid Nicotinamide

Figure 9.13 Structures of nicotinic acid and nicotinamide.

Table 9.7 Niacin Content of Selected Foods*

Food (serving)	Niacin (mg)
Tuna, yellow fin (3 oz)	11.3
Swordfish (3 oz)	7.8
Halibut (3 oz)	6.1
Salmon, Atlantic (3 oz)	8.5
Beef, top sirloin (3 oz)	7.6
Chicken, breast (3 oz)	7.7
Veal, loin (3 oz)	8.5
Peanut butter (2 Tb)	4.3
Peanuts (1 oz)	3.8
Lentils (1/2 c)	1.1
Spaghetti, enriched, cooked (1 c)	2.3
Rice, enriched, cooked (1 /2 c)	1.3
Bread, enriched (1 sl)	1.1

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

Niacin is generally provided as nicotinamide in supplements. Nicotinamide is also the form of the vitamin used to fortify foods. Several different forms of

Figure 9.14 Structures of NAD and NADP.

the vitamin, however, are present naturally in foods. In animal foods, niacin occurs mainly as nicotinamide and the nicotinamide nucleotides—nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Figure 9.14 shows the

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Figure 9.15 NAD^+ and $NADP^+$ synthesis from the amino acid tryptophan.

structures of NAD and NADP. In their oxidized forms, NAD and NADP possess a positive charge and therefore may alternatively be written NAD^+ and $NADP^+$. In plant foods, niacin is present mainly as nicotinic acid. Niacin in foods is fairly stable; minimal losses of the vitamin result from cooking or storage.

In addition to being present as nicotinic acid, nicotinamide, NAD, and NADP in foods, niacin may be bound covalently to complex carbohydrates and called niacytin, or it may be bound to small peptides and called niacinogens. The bound forms of niacin are found primarily in corn, but also in wheat and some other cereal products. Treatment of cereals with bases such as lime water can improve the availability of some bound niacin to release nicotinic acid. Some niacin also may be released from niacytin on exposure to gastric acid.

In addition to dietary sources, niacin may be synthesized in the liver and some other tissues from the amino acid tryptophan. This biosynthetic pathway, which provides an important contribution to the niacin needs of the body, is depicted in Figure 9.15. About 3% of the tryptophan that is metabolized follows the pathway, and an estimated 1 mg of niacin is produced from the ingestion of 60 mg of dietary tryptophan (see the RDA section for niacin to understand how this synthesis is accounted for in niacin recommendations). The contribution of tryptophan to niacin production is evident in those with Hartnup disease, an autosomal recessive genetic disorder. In Hartnup disease, the transporter for tryptophan is defective and thus tryptophan absorption into intestinal cells is severely reduced. It is not uncommon for individuals with this disorder to develop a niacin deficiency due to insufficient tryptophan available for niacin production; however, inadequate intakes of riboflavin (as FAD), vitamin B_6 (as PLP), and iron, which are also required in some of the reactions, can also impair niacin synthesis.

Digestion, Absorption, Transport, and Storage

Digestion of NAD and NADP is needed for niacin's absorption. A pyrophosphatase is required for phosphate hydrolysis from NADP. The NAD is then hydrolyzed by glycohydrolase to release free nicotinamide.

$$\text{NADP} \xrightarrow{\textit{Pyrophosphatase}} \text{NAD} \xrightarrow{\textit{Glycohydrolase}} \text{Nicotinamide}$$

With typical dietary intakes of niacin from foods, nicotinamide and nicotinic acid are absorbed primarily in the small intestine by sodium-dependent, carrier-mediated (facilitated) diffusion, although other carriers may also be involved. Within the colon, nicotinic acid absorption occurs by a sodium-independent, high-affinity carrier [1]. When present in high concentrations (as with the ingestion of pharmacological doses), both forms of niacin are absorbed by passive diffusion in the small intestine.

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In the plasma, niacin is found primarily as nicotinamide, but also as nicotinic acid. Plasma concentrations of niacin range from about 0.5 to 8.5 μg/mL. About 15–30% of nicotinic acid in the plasma is bound to proteins. From the blood, nicotinamide and nicotinic acid move across cell membranes by simple diffusion; however, nicotinic acid transport into the kidney tubules and red blood cells appears to require a carrier, and uptake into the brain is energy dependent. No one organ stores niacin, although the liver as well as other tissues likely contain small amounts.

Nicotinamide serves as the primary precursor of NAD, which is synthesized in all tissues. Nicotinic acid may also be used to synthesize NAD, but this reaction occurs primarily in the liver. Phosphorylation of NAD by NAD kinase using ATP generates NADP (Figure 9.15). These reactions may be reversed, converting NADP to NAD, and NAD to nicotinamide, which then is available for transport to other tissues.

As NAD or NADP, the vitamin is trapped within the cell. Intracellular concentrations of NAD typically predominate over those of NADP. In the liver, excess niacin and tryptophan are converted to NAD, which is stored (but not bound to enzymes) in small amounts. NAD is found primarily in its oxidized form (NAD^+), whereas NADP is found in cells mainly in its reduced form (NADPH).

Functions and Mechanisms of Action

Over 200 enzymes, primarily dehydrogenases, require the coenzymes NAD and NADP, which act as hydrogen donors or electron acceptors and enable nutrient metabolism and energy production. Figure 9.16 demonstrates the oxidation-reduction that may occur in the nicotinamide moiety of the coenzymes. In addition to its coenzyme roles, niacin functions in nonredox roles as a donor of adenosine diphosphate ribose.

Figure 9.16 (a) The oxidation and reduction in the nicotinamide moiety. (b) The role of NAD in dehydrogenation reactions. One H of the substrate goes to NAD.

Coenzyme Roles in Nutrient Metabolism and Energy Production

Although NAD and NADP are similar and undergo reversible reduction in the same way, their functions in the cell are different. The major role of NADH (the reduced formed of NAD) is to transfer its electrons from metabolic intermediates through the electron transport chain (see Figure 3.25), thereby producing ATP. NADPH, in contrast, acts as a reducing agent in many biosynthetic pathways such as fatty acid, cholesterol, and steroid hormone synthesis, among others. NAD and NADP coenzymes are tightly bound to their apoenzymes and can easily transport hydrogen atoms from one part of the cell to another. Reactions in which they participate occur both in the mitochondria and in the cytosol.

Oxidative reactions in which NAD participates and is reduced to NADH (and in turn can be transferred to the electron transport chain for ATP generation) include:

- glycolysis (see Figure 3.17)
- oxidative decarboxylation of pyruvate to acetyl-CoA (see Figure 9.9)
- oxidation of acetyl-CoA in the TCA cycle (see Figure 3.18)
- β-Oxidation of fatty acids (see Figure 5.23)
- oxidation of ethanol (see Figure 5.34).

In addition to its aforementioned roles, NAD is also required by aldehyde dehydrogenase for catabolism of vitamin B_6 as pyridoxal to its excretory product, pyridoxic acid.

NADP can be reduced to NADPH. This reaction occurs as part of the pentose phosphate pathway (see Figure 3.29) and the mitochondrial membrane malate aspartate shuttle (see Figure 3.21). The NADPH produced in these reactions is used in nutrient metabolism, including a variety of reductive biosynthetic processes, such as:

- fatty acid synthesis (see Figure 5.29)
- cholesterol and steroid hormone synthesis
- proline synthesis (see Figure 6.33)
- deoxyribonucleotide (precursors of DNA) synthesis (see Figure 6.28)
- glutathione, vitamin C, and thioredoxin regeneration
- folate coenzyme synthesis (dihydrofolate [DHF], tetrahydrofolate [THF], 5-methyl THF, and 5,10-methylene THF; see Figure 9.28 later in this chapter).

Nonredox Roles

NAD is also involved in several nonredox reactions in which adenosine diphosphate (ADP) ribose is transferred to acceptor molecules. These reactions are generally

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associated with cellular processes such as DNA repair, replication, and transcription; G-protein activity; chromatin structure; and intracellular calcium signaling, among others. Four ADP-ribosylation reactions are described hereafter.

Mono-ADP-Ribosyltransferase The transfer of one (mono) ADP-ribose (ARTs) from NAD to various acceptor proteins occurs by the action of mono-ADP-ribosyltransferase and forms ADP-ribosylated proteins with the release of nicotinamide. The acceptor proteins are found in the cytosol or attached to the inner cell membrane (termed endoARTS) and on the outside of cell membranes (termed ectoARTS). EctoARTS are present on the membranes of many tissues including the lungs, muscles (cardiac and skeletal), and lymph tissues, among others. An example of a substrate for ART1 is defensin, an antimicrobial peptide important in the immune response. Other ADP-ribosylated proteins are involved, for example, with the cell cytoskeleton and in cell signaling.

Poly-ADP-Ribose Polymerases Poly ADP-ribose polymerases (PARP) transfer several (*poly* > 200) polymers of branched ADP-riboses from NAD onto various target proteins. PARP-1, the most abundant of five polymerases, binds to DNA strand breaks. This interaction leads to NAD use, poly ADP-ribose formation, and repair in DNA. Other roles of these enzymes include cell replication, differentiation, and signaling along with apoptosis. Possible related roles for niacin in cancer prevention are also under study.

ADP-Ribosylcyclases Another group of enzymes involved in ADP-ribosylation reaction is ADP-ribosylcyclases. These enzymes form cyclic ADP-ribose also using ADP-ribose. Cyclic ADP-ribose functions in certain cells as a second messenger involved in control of ryanodine receptors and in mobilization of calcium from intracellular stores, especially in neurons.

Deacetylases The class of NAD-dependent deacetylases, known as sirtuins, catalyzes the removal of acetyl groups from target proteins containing acetylated lysine residues. The sirtuins are found in the nucleus, cytosol, and mitochondria. Acetylation of proteins, which occurs post-translationally and involves pantothenic acid, affects numerous functions including enzyme activity, cell signaling, gene expression, and interactions "or crosstalk" with other post-translationally modified proteins in nearby locations [2]. The deacetylation process involves enzymatic cleavage of NAD and the transfer of the acetyl group from the substrate protein (such as an acetylated histone) to the ADP-ribose moiety of NAD generating O-acetyl-ADP-ribose and the deacetylated substrate protein (histone). Sirtuins affect chromatin structure and also play roles in signaling pathways involved in energy metabolism in tissues, the cellular stress response, and in DNA repair, cell differentiation, and cell cycle regulation, among others. Their functions also may affect longevity, delaying aging and the development of some age-related conditions through effects on chromatin and genomic stability.

Selected Pharmacological Uses / Other Roles

Large doses of nicotinic acid (up to 6 g/day in divided doses) are used to treat some types of hyperlipidemias (high blood lipids). Pharmacological doses of nicotinic acid significantly lower total serum cholesterol, triacylglycerols, and low-density lipoproteins (LDLs) and increase high-density lipoproteins (HDLs). It also reduces lipoprotein (a) concentrations. Although the mechanisms of action are not fully understood, nicotinic acid may function to improve serum lipids through interactions with G-protein coupled receptors as well as interactions with various enzymes, among other means [3,4]. The vitamin diminishes lipolysis in adipose tissue and hepatic very low-density lipoprotein (VLDL) synthesis (via inhibition of diacylglycerol acyltransferase) and secretion and thus LDL production [3,4].

Despite the therapeutic benefits of nicotinic acid, some undesirable side effects are associated with its use as a drug, especially in certain forms and in doses of typically 1 g or more per day. Some of these side effects include uncomfortable redness/flushing (usually starting in the face and neck) along with burning, itching (pruritus), tingling, and headaches. The flushing, one of the most common side effects, may occur with the use of nicotinic acid in doses as low as about 30–50 mg per day. Gastrointestinal problems may include heartburn, nausea, and vomiting. Liver injury, high blood uric acid concentrations (hyperuricemia), and possibly gout, along with glucose intolerance, also may occur [3,4]. Extended-release forms of nicotinic acid (such as Niaspan®) with fewer side effects are available; however, hepatic toxicity, headaches, and gastrointestinal distress may still result. Another modified-release form of the vitamin contains laropiprant, a prostaglandin D2 receptor antagonist, which, along with aspirin, may help to reduce the flushing [4].

While nicotinamide in large doses does not reduce blood lipids, this form of niacin is sometimes used topically to reduce inflammation associated with acne vulgaris. In addition, the vitamin has been used orally in the treatment of another skin condition, necrobiosis lipoidica, which is characterized by reddish-brown bruiselike markings most often on the lower legs. Over time the lesions become yellowish in color and atropic plaques develop; the condition, although fairly rare, may occur in those with diabetes mellitus. Lastly, nicotinamide (500 mg doses given twice daily) reduced the rates of new nonmelanoma skin cancers (usually associated with damage due to UV radiation) and actinic keratosis in individuals who had at least two nonmelanoma skin cancers in the previous 5 years [5]. Side

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effects with the use of nicotinamide in doses of about 3 g/day include headache, gastrointestinal distress (heartburn, nausea), impaired glucose intolerance, and liver damage (manifested by jaundice and elevated liver enzymes) [6].

Metabolism and Excretion

NAD and NADP undergo degradation in cells by glycohydrolase to form ADP-ribose and nicotinamide. The released nicotinamide is then methylated and oxidized in the liver into a variety of products that are excreted in the urine. The primary metabolites of nicotinamide are N′ methyl nicotinamide (sometimes abbreviated NMN and representing ~20–30% of niacin metabolites) and N′ methyl 2-pyridone 5-carboxamide (also called 2-pyridone and representing ~40–60%). Small amounts of N′ methyl 4-pyridone carboxamide (called 4-pyridone) also may be present. Nicotinic acid is metabolized mainly to N′ methylnicotinic acid. Little free nicotinic acid or nicotinamide is excreted (with usual physiologic niacin intake), as both compounds are actively reabsorbed from the glomerular filtrate.

Recommended Dietary Allowance

Recommendations for niacin intake include calculations of niacin derived from the amino acid tryptophan, with about 60 mg of tryptophan estimated to generate 1 mg of niacin. Total niacin thus is provided to the body as nicotinic acid and nicotinamide and from tryptophan. The term *niacin equivalent* (NE) is used to account for the provision by tryptophan.

Although recommendations are given in niacin equivalents, food composition tables report only preformed niacin. A rough estimate of niacin equivalents from a protein can be made by assuming that 10 mg of tryptophan are provided for every 1 g of high-quality (complete) protein in the diet; that is, 1 g of complete, high-quality protein = 10 mg of tryptophan. This estimate means that an intake of 60 g of complete protein, for example, would provide 600 mg of tryptophan (10 mg tryptophan/1 g protein × 60 g protein = 600 mg tryptophan. Then, because it takes 60 mg of tryptophan to generate 1 mg of NE, 600 mg of tryptophan would generate 10 NEs (600 mg tryptophan × 1 mg NE/60 mg tryptophan = 10 NEs). The average U.S. diet usually contains about 900 mg of tryptophan daily, and tryptophan provides about 50% of niacin intake in the United States [7].

Information used in estimating niacin requirements and recommendations has come from several studies, including human depletion and repletion studies as well as other studies with primarily urinary metabolites of niacin serving as indicators to base requirements and recommendations. The RDAs for niacin for adult men and women are 16 mg of niacin equivalents and 14 mg of niacin equivalents/day, respectively [8]. Estimated requirements are 12 mg and 11 mg of niacin for adult men and women, respectively. With pregnancy and lactation, the RDA for niacin increases to 18 mg and 17 mg of niacin equivalents, respectively [8]. The inside front cover of the book provides additional RDAs for niacin for other age groups.

Deficiency: Pellagra

A deficiency of niacin results in the condition known as pellagra (*pelle* means "skin" and *agra* means "rough" in Italian). The four Ds—*dermatitis*, *dementia*, *diarrhea*, and *death*—are often used as a mnemonic device for remembering its signs. The dermatitis is similar to sunburn at first and later appears hyperpigmented and scaly. The affected areas are those exposed to the sun, such as the face and neck, and on extremities such as the back of the hands, wrists, elbows, knees, and feet. The presence of the dermatological changes on the neck is sometimes called Casal's collar or necklace. Neurological manifestations include headache, apathy, fatigue, loss of memory, peripheral neuritis, paralysis of extremities, confusion, disorientation, and dementia or delirium. Gastrointestinal manifestations include glossitis, cheilosis, angular stomatitis, nausea, vomiting, and diarrhea. Inflammation, especially in the colon, is typically present; such findings have been attributed to niacin's anti-inflammatory roles in the digestive tract [1]. If pellagra is untreated, death occurs. Treatment of niacin deficiency requires oral intakes of about 300 mg of nicotinamide daily (usually divided into three doses of 100 mg) for about one month.

A niacin deficiency or diminished niacin status can result from the use of several medications and from malabsorptive conditions. The antituberculosis drug isoniazid, for example, binds with vitamin B_6 as PLP and thereby reduces PLP-dependent kynureninase activity required for niacin synthesis from tryptophan. Mercaptopurine, a drug used in cancer treatment, inhibits NAD phosphorylase. Malabsorptive disorders (chronic diarrhea, inflammatory bowel diseases, some intestinal cancers, and Hartnup disease) may impair niacin and/or tryptophan absorption to increase the likelihood of niacin deficiency. People who consume excessive amounts of alcohol typically have poor food, and thus vitamin, intakes and are at risk for niacin deficiency. Individuals with HIV and with cancer undergoing chemotherapy may have higher needs for niacin, and dietary intake of the vitamin may be insufficient among adults over 50 years of age. Supplemental niacin may be beneficial for these individuals.

Toxicity

Because of the vasodilatory effects associated with the use of supplemental niacin (see the section "Selected Pharmacological Uses/Other Roles"), a Tolerable Upper

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Intake Level for adults for niacin (both nicotinic acid and nicotinamide) from supplements and from fortified foods has been set at 35 mg/day [8]. For those being treated for hyperlipidemia or other conditions necessitating ingestion of large doses of the vitamin, the benefits of the vitamin must be weighed against its potential toxic effects.

Assessment of Nutriture

Several methods are employed to assess niacin status. Most methods involve measurement of one or more urinary metabolites of the vitamin. Urinary excretion of ~0.8 mg/day of N′ methyl nicotinamide and of ~0.5 mg of N′ methyl nicotinamide/1 g of creatinine are suggestive of poor (deficient) niacin status [9]. Marginal niacin status is suggested by urinary amounts in the range of 0.5–1.59 mg of N′ methyl nicotinamide/1 g of creatinine, while levels in excess of 1.6 mg reflect adequate status [9]. This ratio, however, has been criticized as being difficult to interpret because of multiple influences on urinary creatinine excretion. It usually is employed during a period of 4 to 5 hours after a 50 mg test dose of nicotinamide. Another ratio employed to assess niacin status is that of urinary N′ methyl 2-pyridone 5-carboxamide (2-pyridone) to N′ methyl nicotinamide (NMN). Although a ratio of ~1 is found with niacin deficiency, this ratio is not thought to be sensitive enough to detect marginal niacin intakes and may better reflect dietary protein adequacy as opposed to niacin status [9].

In addition to measurement of urinary metabolites, serum or red blood cell indicators are used to assess niacin status. NAD concentrations and the ratio of NAD to NADP (~1.0) in erythrocytes have been used. In plasma, concentrations of 2-pyridone drop below the detection level with low niacin intakes and thus may be used as an index of niacin status [9].

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PANTOTHENIC ACID

Pantothenic acid's essentiality was not discovered until 1954, although the vitamin had been isolated in about 1931 by R. J. Williams and its structure determined in 1939. Structurally, pantothenic acid (once called vitamin B_5) consists of β-alanine and pantoic acid joined by an amide linkage. The vitamin is shown at the top of Figure 9.17.

Sources

The Greek word *pantos* means "everywhere," and the vitamin pantothenic acid, as its name implies, is found widely distributed in foods. Because this vitamin is present in virtually all plant and animal foods, a deficiency is unlikely. Selected sources of the vitamin are shown in Table 9.8. Royal jelly from bees also provides relatively large amounts (about 0.5 mg/g) of pantothenic acid.

Pantothenic acid can be destroyed with heating and freezing. It is stable when dry and in solution at a neutral pH, but destroyed in acidic and alkaline solutions. The refining of grains decreases their pantothenic acid content by as much as 75%. Most adults in the United States consume about 4–7 mg of pantothenic acid per day.

Bacteria in the colon also generate pantothenic acid. However, the extent to which the microbial-produced

Table 9.8 Pantothenic Acid Content of Selected Foods*

Food (serving)	Pantothenic acid (mg)
Liver, beef or chicken (3 oz)	6.5
Beef, chuck (3 oz)	0.5
Pork, shoulder (3 oz)	0.5
Chicken, breast, boneless, skinless (3 oz)	1.2
Salmon, Atlantic (3 oz)	1.2
Cheese, feta (1/4 c)	1.1
Yogurt, vanilla, nonfat (1 c)	1.3
Egg, whole, scrambled (1)	0.7
Mushrooms, portabella, grilled (1/2 c)	0.7
Broccoli, cooked (1/2 c)	0.5
Avocado, pureed (1/2 c)	1.6
Lentils, cooked (1/2 c)	0.6
Legumes, variety, cooked (1/2 c)	0.1–0.2
Sunflower seeds (1/4 c)	2.3
Potato, flesh no skin (1/2 c)	0.3
Bread, whole grain (1 sl)	0.2

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

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*Structure shown in Fig. 9.18

1. The synthesis begins with the rate limiting phosphorylation of pantothenic acid by pantothenic acid kinase to form 4'-phosphopantothenic acid. The reaction occurs in the cytosol.

2. Cysteine reacts with 4'-phosphopantothenic acid to form 4'-phosphopantothenyl cysteine. A peptide bond is formed between the carbonyl group of 4'-phosphopantothenic acid and the amino group of cysteine by the enzyme phosphopantothenylcysteine synthase. This reaction and subsequent two reactions occur in the cytosol on a protein complex with multiple catalytic sites.

3. A carboxyl group from the cysteine moiety is removed by phosphopantothenylcysteine decarboxylase to form 4'-phosphopantetheine. This compound is needed by acyl carrier proteins for function.

4. An adenylation occurs in the mitochondrial inner membrane whereby ATP reacts with 4'-phosphopantetheine; adenosine monophosphate (AMP) is attached forming dephosphocoenzyme A with the release of pyrophosphate.

5. Phosphorylation of the 3'-hydroxy group of dephosphocoenzyme A with ATP produces CoA. This reaction also occurs in the mitochondrial inner membrane.

Figure 9.17 Synthesis of coenzyme A from pantothenic acid.

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vitamin is absorbed meets the pantothenic acid needs of the body is unclear.

In supplements, pantothenic acid is usually found as calcium pantothenate or as panthenol, an alcohol form of the vitamin. The amount present in multivitamin supplements is typically similar to the Daily Value, which is 10 mg (and twice its RDA). In skin and hair products, panthenol is sometimes added as a humectant to promote moisture retention.

Digestion, Absorption, Transport, and Storage

Pantothenic acid occurs in foods in free and bound forms. About 85% of the pantothenic acid in food is bound as a component of coenzyme A, abbreviated CoA (shown in Figure 9.18). During digestion in the small intestine, CoA is hydrolyzed by pyrophosphatase to 4′-phosphopantetheine, which is then dephosphorylated by a phosphatase to pantetheine. This later compound is subsequently converted to pantothenic acid by pantotheinase.

Pantothenic acid absorption occurs principally in the jejunum by passive diffusion when present in high concentrations and by a shared multivitamin transporter (SMVT) when present in low concentrations [1]. Pantothenic acid shares the intestinal multivitamin transporter with biotin and lipoic acid. Carrier-mediated absorption occurs mainly in the proximal small intestine, although bacterially produced pantothenic acid is also absorbed using the SMVT in the proximal and midtransverse sections of the colon. Panthenol, from vitamin supplements, is absorbed by diffusion, and is subsequently converted to pantothenic acid within the intestinal cell. Approximately 50% (range 40–61%) of ingested pantothenic acid is absorbed [2]. However, absorption decreases to about 10% with supplement use in amounts of about 10 times recommendations.

Pantothenic acid from the intestinal cells enters portal blood for transport to the liver and other tissues. Pantothenic acid is found free in the blood, primarily within the red blood cells. Blood concentrations of the vitamin usually range from about 30 to 60 μg/dL [2]. The uptake of the vitamin into red blood cells appears to occur by diffusion, while uptake into some tissues/organs requires SMVT. Pantothenic acid, 4′-phosphopantothenic acid, and pantetheine are all present within cells. Most pantothenic acid is used intracellularly to synthesize CoA, which is found in all tissues, but in fairly high concentrations in the liver, adrenal gland, kidneys, brain, and heart.

Functions and Mechanisms of Action

Pantothenic acid is needed by cells for the synthesis of CoA. Additionally, the 4′-phosphopantetheine moiety, upon donation from CoA, is required for the activity of the acyl carrier protein (ACP), a component of the fatty acid synthase complex.

The synthesis of CoA from pantothenic acid is described and shown in Figure 9.17. Regulation of this pathway occurs at the first rate-limiting step catalyzed by panthothenate kinase (PANK), which phosphorylates pantothenic acid to produce phosphopantothenic acid. Feedback inhibition is provided by the pathway's end-product free CoA, as well as by CoA thioesters (i.e., acetyl-CoA, malonyl-CoA, propionyl-CoA, and other longer-chain acyl-CoAs). Three genes code for different panthothenate kinase isoforms, which are found in different subcellular locations [3]. Mutations in genes for panthothenate kinase are associated with a specific autosomal recessive condition known as PANK-associated neurodegeneration, and a link between this condition and CoA synthesis suggests normal brain function is dependent upon adequate CoA concentrations [4,5].

Coenzyme A

Figure 9.18 shows the structure of CoA. Note from this figure that CoA contains several components including 4′-phosphopantetheine. It is through the sulfhydryl group (active site) in 4′-phosphopantetheine that thio ester $(-S-\overset{\overset{\displaystyle O}{\|}}{C}-R)$ bonds form with various carboxylic acids. Some of these acids, which are typically 2–13 carbons in length, include:

- acetic acid (two carbons)
- malonic acid (three carbons)
- propionic acid (three carbons)
- methylmalonic acid (four carbons)
- succinic acid (four carbons).

Most of these carboxylic acids arise in cells during metabolism. Some, like propionic acid, are also obtained by additional means. For example, propionic acid is produced from bacterial fermentation of carbohydrates in the colon and can be absorbed and contribute to the cellular supply.

CoA serves (functions) as a carrier of acetyl/acyl groups, forming thioester derivatives including acetyl-CoA, propionyl-CoA, malonyl-CoA, and succinyl-CoA, among others. It was F. Lipmann who won the Nobel Prize for his work in 1957 showing that CoA facilitated biological acetylation reactions. The levels and ratios among the thioester derivatives are tightly regulated, and impact a wide range of cellular metabolic activities.

Nutrient Metabolism and Energy Production CoA and its thioester derivatives are found in multiple cellular compartments and function in hundreds of metabolic reactions. It is through these reactions that pantothenic acid as CoA and its derivatives participates extensively in nutrient metabolism, including degradation reactions resulting in energy production and synthetic reactions for the production of vital compounds.

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Figure 9.18 Structure of coenzyme A.

The metabolism of carbohydrate, lipids, and protein (energy-producing nutrients) relies to varying degrees on CoA. For example, a crucial reaction in nutrient metabolism is the conversion (oxidative decarboxylation) of pyruvate (formed from the degradation of glucose as well as some amino acids) to acetyl-CoA. This acetyl-CoA, which is also formed from the catabolism of fatty acids and some amino acids, can then condense with oxaloacetate to introduce acetate for oxidation in the TCA cycle (see Figure 9.8). Acetyl-CoA is thus a common intermediate formed from the catabolism of the three energy-producing nutrients, and holds a central position, intersecting both catabolic and anabolic pathways.

Pantothenic acid joins thiamin, riboflavin, and niacin in the oxidative decarboxylation of pyruvate (see Figure 9.9). These same vitamins also participate in the oxidative decarboxylation of α-ketoglutarate to succinyl-CoA, a TCA cycle intermediate and compound used with the amino acid glycine to synthesize the porphyrin ring in heme.

In lipid metabolism, CoA is important in the synthesis of cholesterol, ketone bodies, fatty acids, phospholipids, and sphingolipids. For example, in cholesterol and ketone body synthesis, acetyl-CoA and acetoacetyl-CoA react to form the key intermediate HMG-CoA (see Figure 5.32). Condensation of acetyl-CoA with activated CO_2 to form malonyl-CoA represents the first step in fatty acid synthesis (see Figure 5.28). Additionally, many of the compounds produced using CoA are involved in reactions for the synthesis of other compounds. Cholesterol, for example, once produced is used further for the synthesis of bile and steroid hormones, and sphingolipids that are generated are used further for the production of myelin, which is involved in nerve transmission.

Acetyl-CoA is also important for the production of neurotransmitters. Choline acetyltransferase catalyzes the synthesis of acetylcholine in selected (cholinergic) neurons from acetyl-CoA and choline. Insufficient availability of acetyl-CoA has been linked with neurodegenerative conditions [6].

The aforementioned roles of CoA and its derivatives in nutrient metabolism and energy production have been long recognized. More recently, additional roles of CoA in acetylation (acetate donated by CoA to an acceptor compound) and acylation (the donation of fatty acids by CoA thioester derivatives to an acceptor compound) are being elucidated.

Acylation, Acetylation, and Cellular Processes Pantothenic acid as part of CoA is also involved in the acetylation and acylation of proteins and sugars as well as some drugs. Both

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acylation and acetylation of the proteins by CoA occur post-translationally. Acylation affects protein functions, activity, and location. The fatty acids most often attached to cellular proteins are myristic acid and palmitic acid. Myristolation of proteins appears to be irreversible, while the palmitoylation of proteins can be reversed. Palmitoylation of proteins affects some regulatory functions such as signal transduction with affected proteins including guanosine triphosphate–binding proteins and insulin receptors, among others. Acylation of cytoskeletal proteins and neuronal proteins by palmitic acid also has been demonstrated and influences cell structure and neural development.

The acetylation of cytosolic and mitochondrial (non-nuclear) proteins is extensive, with acetylation detected on almost every enzyme in the liver involved in intermediary metabolism including glycolysis, gluconeogenesis, TCA cycle, amino acid and fatty acid metabolism, and the urea cycle [3,7]. A better understanding of the impact of acetylation on these enzymes is needed; however, effects on enzyme activity and structure (stability) have been documented. Acetylation of enzymes appears to have primarily an inhibitory effect on activity [7]. The means by which these proteins are acetylated are thought to be both enzymatic (such as via p300 acetyltransferase) as well as nonenzymatic. The availability of acetyl-CoA appears to "drive" or control the acetylations and are based largely on the cell's metabolic state [3,7].

In addition to effects on enzymes involved with intermediary metabolism, acetyl-CoA concentrations affect the acetylation of other non-nuclear cell proteins that in turn impact a multitude of cellular events. Higher cellular acetyl-CoA concentrations, for example, have been shown to suppress autophagy through interactions with acyltransferases. In contrast, in the presence of lower acetyl-CoA concentrations, acetylation of cytosolic proteins is reduced and autophagy is induced [5]. Microtubules, made from polymerization of α- and β-tubulin dimers and that make up a part of the cell's cytoskeleton, are also acetylated. Microtubules appear to be stabilized by acetylation and destabilized when deacetylated. The acetylation of some proteins is also thought to affect cell signaling pathways and interactions between or among post-translationally modified proteins [8,9].

In the nucleus, the acetylation of histones induces structural changes in chromatin to enhance gene expression and promote transcription. Acetylation, for example, enables interactions between transcription factors and the promoter regions of genes [8].

In addition to proteins, aminosugars, such as glucosamine and galactosamine, are also acetylated by acetyl-CoA to form N-acetyl glucosamine and N-acetyl galactosamine, respectively. These acetylated aminosugars in turn may function structurally in the cell, for example, to provide recognition sites on cell surfaces or to direct proteins for membrane functions, among other roles.

Acyl Carrier Protein (ACP)

Figure 9.18 shows the components of CoA, including 4′-phosphopantetheine. It is this 4′-phosphopantetheine moiety (donated by CoA, which is synthesized from pantothenic acid) that attaches to apoacyl carrier protein and serves as its prosthetic group. Acyl carrier protein is a small component of the fatty acid synthase complex. Phosphopantetheinyl transferase (also referred to as 4′-phosphopantetheine-apoACP transferase) carries the 4′-phosphopantetheine moiety from CoA to a serine residue in the apoacyl carrier protein. Once bound to the acyl carrier protein, it is the sulfhydryl group in the 4′-phosphopantetheine that binds and transfers acyl groups to another sulfhydryl group located in the enzyme complex. These two groups are located close to each other so that the acyl chain being synthesized can be transferred between them. Thus, the 4′-phosphopantetheine component of ACP is like a "docking station" to which substrates and intermediates in fatty acid synthesis "link to" and undergo a progression of enzymatic modifications to extend the fatty acid chain. See Chapter 5 for a complete discussion of fatty acid synthesis.

Metabolism and Excretion

CoA degradation occurs in a series of reactions first catalyzed by a phosphatase to generate dephospho-CoA, and then by a pyrophosphatase to produce 4′-phosphopantetheine. The latter compound is further catabolized by additional phosphatases to pantothenic acid. Release of 4′-phosphopantetheine from acyl carrier protein is accomplished by an acyl carrier protein hydrolase; the 4′-phosphopantetheine can be reused or further degraded to pantothenic acid.

Pantothenic acid does not appear to undergo degradation prior to its excretion, and is thus excreted intact primarily in the urine. Usual urinary excretion of pantothenic acid ranges from about 1 to 8 mg/day.

Adequate Intake

The Adequate Intake (AI) recommendation for adults for pantothenic acid is 5 mg [2]. AIs for pantothenic acid of 6 mg/day and 7 mg/day are suggested for women during pregnancy and lactation, respectively [2]. The inside front cover of this book provides AIs for pantothenic acid for other age groups.

Deficiency: Burning Foot Syndrome

The pantothenic acid deficiency disorder, referred to as burning foot syndrome, is rare. The disorder has been studied with the provision of a metabolic inhibitor of the vitamin, omega methylpantothenate, and a restricted diet.

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The syndrome is characterized by a sensation of burning in the feet and neuritis (nerve inflammation). The condition is exacerbated by warmth and diminished with cold. Other signs and symptoms of deficiency include vomiting, fatigue, muscle weakness, arm and leg cramping, restlessness, and irritability. Although the vitamin is needed for heme synthesis, problems with hematopoiesis are not usually observed in humans with a pantothenic acid deficiency, but have been shown in some animal studies. Pantothenic acid deficiency is corrected with calcium pantothenate administration.

When pantothenic acid deficiency occurs naturally, it usually is in conjunction with multiple nutrient deficiencies. Some conditions that may increase the need for the vitamin include alcoholism, diabetes mellitus, and inflammatory bowel diseases. Increased excretion of the vitamin has been observed in people with diabetes mellitus. Absorption is likely to be impaired with inflammatory bowel diseases. Intake of the vitamin is typically low in people with excessive alcohol intake.

Toxicity

Pantothenic acid toxicity has not been reported to date in humans. Intakes of about 10 g of pantothenic acid as calcium pantothenate daily for up to 6 weeks have not caused problems; however, higher intakes of 15–20 g have been associated with mild intestinal distress, including diarrhea [2].

Assessment of Nutriture

Blood pantothenic acid concentrations are thought to reflect low dietary pantothenic acid intakes; however, blood concentrations do not correlate well with changes in dietary pantothenic acid intake and status. Urinary pantothenic acid excretion is considered to be a better indicator of status, with excretion of < 1 mg/day considered indicative of poor status.

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BIOTIN

Biotin's discovery was based on research investigating the cause of what was called egg white injury. Eating raw eggs was known to result in hair loss, dermatitis, and various neuromuscular problems. Szent-Györgyi in 1931 found a substance (now called biotin) in liver that could cure and prevent the condition. It was not until about 10 years later (the early 1940s) that Kogl (from Europe) and du Vigneaud and colleagues (from the United States) determined biotin's structure. Biotin consists structurally of two rings—an ureido ring joined to a thiophene ring—with an additional valeric acid side chain (Figure 9.19). Biotin was once called vitamin H (the H refers to *haut* in German and means "skin") as well as vitamin B_7.

Sources

In addition to being made by bacteria inhabiting the colon, biotin is found widely distributed in foods. Major food sources include liver (beef, about 31 μg), milk, soybeans, and egg yolk (about 4 μg), as well as cereals (Cheerios® and Frosted Flakes®, for example, provide 0.03 and 0.04 μg/serving), legumes, and nuts (peanuts 4.9 μg, almonds 1.3 μg/serving) [1]. Canned salmon provides about 3.7 μg/serving [1].

Within foods, biotin is found free (unattached) or bound covalently to protein, usually through a lysine residue. In raw egg whites, a glycoprotein called avidin irreversibly binds biotin in what has been suggested as the tightest noncovalent bond found in nature. This binding to avidin in turn prevents biotin absorption; however, because avidin is heat labile (unstable with heat), eating cooked egg whites does not compromise biotin absorption.

Figure 9.19 Structure of biotin.

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In multivitamin and individual supplements, biotin is usually present in its free form and in amounts typically equal or several times greater than the Daily Value. The Daily Value for biotin is 30 μg.

Digestion, Absorption, Transport, and Storage

Protein-bound biotin requires digestion by enzymes prior to absorption. Proteolysis by pepsin and intestinal proteases yields free biotin and/or biocytin. Biocytin consists of biotin bound to the amino acid lysine (Figure 9.20). While some biocytin may be absorbed intact by peptide carriers, most biocytin is hydrolyzed by biotinidase, which is widely available on the intestinal brush border membrane, in pancreatic and intestinal juices secreted into the small intestine, in the plasma, and in multiple cellular locations including the nucleus. Biotinidase hydrolyzes the biocytin to release free biotin and lysine.

Biotinidase is active over a wide pH range. At a more acidic pH, biotinidase cleaves biocytin to produce biotin and lysine and cleaves covalently bound biotin from any biotinyl peptides that have been released as biotinylated proteins are degraded. The enzyme also cleaves biotin from histones. At a more alkaline pH, the enzyme itself becomes biotinylated; in other words, the biotinidase enzyme becomes attached to the biotin.

Biotinidase deficiency (first discovered in 1983) is caused by an autosomal recessive inborn error of metabolism. Depending on the specific gene mutation, residual biotinidase activity varies. Insufficient intestinal biotinidase activity generally impairs lysine-bound biotin digestion and thus limits some biotin availability for absorption. Insufficient extraintestinal biotinidase activity hampers biotin release and thus recycling in tissues. Some manifestations associated with the genetic disorder include lethargy, hypotonia (reduced muscle tone), seizures, and ataxia. In addition, dermatitis (dry and erythematous, and usually present on the face) and alopecia (loss of hair from the body) may occur. Treatment requires the use of pharmacologic doses of biotin taken orally; however, not all manifestations improve with supplementation [2].

Biotin absorption occurs by both passive diffusion and carrier-mediated transport. Biotin absorption by passive diffusion predominates with consumption of pharmacologic doses of the vitamin, as is needed with some genetic disorders. With physiological intake, biotin absorption is carrier mediated. This carrier, which also transports pantothenic acid and lipoic acid, is called the shared multivitamin transporter (SMVT). SMVT transcription is regulated by biotin concentrations and is negatively affected by alcohol [3–5]. With high cellular biotin, an enzyme holocarboxylase synthetase (discussed further under enzyme roles) translocates to the nucleus, where it biotinylates specific histones to suppress transcription of the vitamin transporter gene. Absorption via SMVT occurs mainly in the proximal small intestine, but also in the proximal and midtransverse colon. In the colon, an accessory protein that interacts with SMVT is also thought to be involved in the absorption of the microbial-generated biotin [3]. Bacterially made biotin, however, cannot totally meet the body's biotin needs [4].

Transport of biotin across the basolateral membrane of the enterocyte for entrance into the blood is carrier mediated [4]. Biotin is found in the plasma mostly (~80%) in a free state, with lesser amounts bound to plasma proteins, including albumin, α- and β-globulins, and biotinidase, which has two binding sites for the vitamin and arises from hepatic secretion [6]. Whole-blood biotin concentrations range from about 200 to 750 pg/mL. Biotin uptake into the liver, and probably other tissues, is thought to involve SMVT as well as monocarboxylate transporter (MCT) 1. Biotin is stored in small quantities in the muscle, liver, and brain.

Functions and Mechanisms of Action

Biotin functions in cells as a coenzyme carrier for the transfer of "activated bicarbonate" to substrates. These reactions are vital for both nutrient metabolism and energy production. In addition, biotin functions in a non-coenzyme capacity in regulating gene expression.

Coenzyme Roles in Nutrient Metabolism and Energy Production

For coenzyme functions within cells, biotin is covalently bound to each of four apocarboxylases. The attachment of biotin (called biotinylation) to these apocarboxylases

Figure 9.20 Structure of biocytin, also called biotinyllysine.

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Figure 9.21 Biotin bound to the lysine residue of carboxylase and functioning as a carrier of activated CO_2.

is catalyzed by holocarboxylase synthetase (HCS), which is found in both the cell cytosol and mitochondria. The attachment of biotin by the enzyme HCS occurs in two steps as shown hereafter.

- Biotin + ATP + HCS ⟶ Biotinyl-5'-AMP-HCS + pyrophosphate
- Biotinyl-5'-AMP-HCS + apocarboxylase ⟶ Holocarboxylase + AMP + HCS

Holocarboxylase refers to biotin attached to any of four carboxylases.

A mutation in holocarboxylase synthetase, as was first discovered in 1981, or in any of the biotin-dependent carboxylases negatively impacts nutrient metabolism and is manifested by vomiting, lethargy, hypotonia (reduced muscle tone), acidosis, and seizures. Pharmacologic doses (ranging from 10 mg to about 200 mg orally per day) of biotin may be needed to enhance residual enzyme activity and help reduce some of the manifestations of the disorder [2].

Each biotinylated carboxylase is a multisubunit enzyme to which biotin is attached by an amide linkage. Specifically, the carboxyl terminus of biotin's valeric acid side chain is linked to the epsilon amino group of a specified lysine residue in the apocarboxylase, as shown in Figure 9.21. The chain connecting biotin and the apoenzyme is long and flexible, allowing the biotin to move from one active site of the carboxylase to another. One active site generates the carboxybiotin enzyme, and the other transfers the activated carbon dioxide (as HCO_3^-) to a reactive carbon on the substrate. Figure 9.22 illustrates the formation of the CO_2-biotin-enzyme complex.

The four biotinylated carboxylases (holocarboxylases), which are synthesized by holocarboxylase synthetase, are pyruvate carboxylase, acetyl-CoA carboxylase (two isoforms), propionyl-CoA carboxylase, and β-methylcrotonyl-CoA carboxylase. Table 9.9 lists these enzymes and their **carboxylation** roles in metabolism.

Pyruvate Carboxylase Pyruvate carboxylase is a particularly important enzyme because of its regulatory function. Specifically, pyruvate carboxylase (a mitochondrial enzyme) catalyzes the carboxylation of pyruvate to form oxaloacetate (Figure 9.23). For its activation, pyruvate carboxylase requires the presence of acetyl-CoA as well as ATP and Mg^{2+}. Acetyl-CoA serves as an allosteric activator, and its presence indicates the need for increased amounts of oxaloacetate. If the cell has a surplus of ATP, the oxaloacetate is then used for gluconeogenesis. However, if the cell is deficient in ATP, the oxaloacetate enters the TCA cycle on condensation with acetyl-CoA.

Acetyl-CoA Carboxylase The importance of biotin in nutrient metabolism is further exemplified by its role in the initiation of fatty acid synthesis. Malonyl-CoA formation from acetyl-CoA by the regulatory and rate-limiting enzyme acetyl-CoA carboxylase 1, found in the

Figure 9.22 The formation of the CO_2-biotin-enzyme complex.

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Table 9.9 Biotin-Dependent Enzymes

Enzyme	Role	Significance
Pyruvate carboxylase	Converts pyruvate to oxaloacetate	Replenishes oxaloacetate for TCA cycle Necessary for gluconeogenesis
Acetyl-CoA carboxylase	Forms malonyl-CoA from acetate	Commits acetate units to fatty acid synthesis
Propionyl-CoA carboxylase	Converts propionyl-CoA to methylmalonyl-CoA	Provides mechanism for metabolism of some amino acids and odd-number-chain fatty acids
β-methylcrotonyl-CoA carboxylase	Converts β-methylcrotonyl-CoA to β-methylglutaconyl-CoA	Allows catabolism of leucine and certain isoprenoid compounds

Figure 9.23 The role of biotin in the synthesis of oxaloacetate from pyruvate.

cytosol, promotes fatty acid synthesis (see Figure 5.28). The isoform acetyl-CoA carboxylase 2, found on the outer mitochondrial membrane, also catalyzes malonyl-CoA formation from acetyl-CoA; however, within the mitochondria the malonyl-CoA serves to inhibit fatty acid uptake for use in beta oxidation.

Propionyl-CoA Carboxylase Propionyl-CoA carboxylase (a mitochondrial enzyme) is important for the catabolism of the amino acids isoleucine, threonine, and methionine, each of which generates propionyl-CoA. Propionyl-CoA also arises from the catabolism of odd-number-chain fatty acids. Propionyl-CoA carboxylase catalyzes the carboxylation of propionyl-CoA to D-methylmalonyl-CoA (Figure 9.24). Deficient or defective propionyl-CoA carboxylase activity, as occurs in the genetic disorder propionic acidemia, results in the accumulation of propionyl-CoA, which is then shifted into an alternate metabolic pathway. This alternate pathway results in increased production and urinary excretion of 3-hydroxypropionic acid (3HPA) and methylcitrate (MCA).

β-methylcrotonyl-CoA Carboxylase β- (or 3-)methylcrotonyl-CoA carboxylase (a mitochondrial enzyme) is important in the degradation of the amino acid leucine. During leucine catabolism (see Figure 6.36 and Figure 9.25), β-methylcrotonyl-CoA is formed and is subsequently carboxylated by the biotin-dependent β-methylcrotonyl-CoA carboxylase to form β-methylglutaconyl-CoA. The latter compound is further catabolized to generate acetoacetate and acetyl-CoA.

Deficient β-methylcrotonyl-CoA carboxylase activity, due to an inborn error of metabolism, causes the accumulation of β-methylcrotonyl-CoA, which is then shunted into an alternate metabolic pathway. This alternate pathway results in increased production and urinary excretion of 3-hydroxyisovaleric acid (3HIA), 3-methylcrotonylglycine (3MCG), and isovalerylglycine (IVG). Increased 3HIA and decreased biotin concentrations in the urine are also indicative of biotin deficiency.

Noncoenzyme Roles: Gene Expression

In addition to biotin's coenzyme roles, biotin functions in the biotinylation of histone proteins involved with gene expression. Histones, of which there are five classes—H1, H2A, H2B, H3, and H4—are small proteins that group together and are found bound to or associated with DNA. DNA base pairs are wrapped in the histones, which when tightly packed together minimize access to gene promoter sequences. Modification of the histones, however, can "open up the packing." Histones consist of a flexible amino (also called N) terminus (often called the histone tail) and a globular domain. It is the tail section of the histones that can be covalently modified (e.g., by biotinylation, acetylation, and methylation) to affect chromatin structure, chromosomal stability, and gene regulation. (Chromatin can be thought of as uncoiled chromosomes consisting of DNA and histones.) Biotinylation of the histones is mediated by holocarboxylase synthetase. This modification causes the

Figure 9.24 The role of biotin in the oxidation of propionyl-CoA.

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Figure 9.25 The role of biotin in leucine catabolism.

histones to "uncoil" and thus creates pores through which transcription factors can enter to reach DNA and activate gene promoter sequences. This biotinylation of histones also affects cell proliferation. Arrest of the cell cycle in the G0/G1 and S phases and reductions in cell proliferation occur when biotin is deficient [7–9].

Effects of biotin on gene expression also occur via multiple cell signaling pathways including by cGMP, nuclear factors (NF)-κB, transcription factors Sp1 and Sp3, and receptor tyrosine kinases and by biotin as biotinyl-5′-AMP [9]. Over 2,000 human genes depend on biotin for expression. Biotin appears to be involved in both the transcription of some genes and the translation of some mRNAs [10]. Holocarboxylase synthetase, for example, can directly interact with chromatin, creating a multiprotein gene repression complex [9]. Other effects of biotin affect glucose metabolism by stimulating the expression (transcription) of glucokinase and inhibiting the expression of phosphoenolpyruvate carboxykinase; biotin also increases mRNA levels of 6-phospho-fructokinase when given to biotin-deficient rats [10]. The expression of genes responsible for the production of some cytokines and for the production of β-methylcrotonyl-CoA carboxylase has also been shown to be regulated by biotinylation of histones.

Selected Pharmacological Uses/Other Roles

The use of pharmacological doses of biotin has mainly been employed in the management of a handful of inborn errors of metabolism. In the genetic disorder propionic acidemia, almost 100 mutations have been identified in the two genes coding for the subunits of propionyl-CoA carboxylase. Individuals with proprionic acidemia are generally supplemented with oral biotin (5–10 mg per day) to determine its impact, if any, on residual enzyme activity. Because it is the alpha subunit of the enzyme that binds biotin, supplementation with the vitamin improves enzyme activity in those with only certain mutations affecting the alpha subunit.

Inborn errors in both biotinidase and holocarboxylase synthetase affect the availability and use of biotin for metabolic functions. Oral biotin supplements in amounts of 5–20 mg daily are used to treat biotinidase deficiency, and oral doses of 10–200 mg of biotin daily may be needed for those with holocarboxylase synthetase deficiency [2].

Metabolism and Excretion

Catabolism of the biotin holocarboxylases by proteases ultimately yields biocytin. The biocytin is then degraded by biotinidase to lysine and free biotin, which may be reused or excreted.

Biotin is excreted from the body mainly in the urine. Small amounts of intact biotin and biocytin are sometimes found in the urine, but the vitamin is typically further degraded before excretion. A few of the urinary metabolites are formed from the oxidation of the sulfur in biotin's ring; these metabolites include biotin sulfoxide and biotin sulfone (Figure 9.26). Most of the metabolites arise from the degradation of biotin's valeric acid side chain by β-oxidation. These metabolites are bisnorbiotin and tetranorbiotin (Figure 9.26) and, to a lesser degree, derived metabolites such as bisnorbiotin methyl ketone and tetranorbiotin methyl ketone. Smoking appears to accelerate biotin catabolism in women [11].

Biotin that has been synthesized by intestinal bacteria but not absorbed is found excreted in the feces. Very little dietary biotin that has been absorbed is excreted in the feces [4].

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Figure 9.26 Selected metabolites from biotin degradation.

Adequate Intake

Because bacterially synthesized biotin is not sufficient to maintain normal biotin status, humans need to obtain biotin from the diet. The Adequate Intake (AI) recommendation for biotin for adults is 30 μg per day [12]. Adequate Intakes for biotin of 30 μg and 35 μg per day are suggested for women during pregnancy and lactation, respectively [12]. The inside front cover of this book provides additional AIs for biotin for other age groups.

Deficiency

Biotin deficiency due to inadequate intake of the vitamin is rare. The deficiency, however, has resulted from excessive consumption of raw egg whites. Some of the neurologic symptoms associated with a biotin deficiency include lethargy, paresthesias in extremities, hypotonia (reduced muscle tone), depression, and hallucinations. The most notable cutaneous symptom is a red, dry, scaly dermatitis found around the eyes, nose, and mouth. In addition, anorexia, nausea, alopecia (body hair loss), brittle nails, and muscle pain may occur. Death may result if biotin deficiency goes untreated. Therapeutic doses of up to 10 mg of biotin daily are typically used to treat deficiency.

Biotin deficiency or poor biotin status may be present in selected populations. People who ingest raw eggs in excess amounts are likely to develop a deficiency because avidin's binding to the biotin prevents the vitamin's absorption. Impaired biotin absorption also may occur with gastrointestinal disorders such as inflammatory bowel disease and in chronic consumers of excessive alcohol. Biotin status has been shown to decline in some women during pregnancy and in those on anticonvulsant drug therapies such as phenobarbital, phenytoin, or carbamazepine [10].

Toxicity

Toxicity from oral biotin ingestion has not been reported, and no Tolerable Upper Intake Level has been established [12]. Fairly large oral doses (up to 200 mg) of biotin have been given daily, without side effects, to people with inherited disorders of biotin metabolism. Biotin supplements taken orally and use of biotin as a hair and skin conditioning agent in cosmetic-type products have been shown to be safe, but scientific studies documenting its effectiveness in treating hair and nail problems are lacking [13]. Several conditions/situations can promote hair loss, such as with rapid weight loss; thyroid conditions; extreme stress; inadequate intakes of protein, zinc, and iron; excessive selenium intake; and the use of certain medications, among others.

Assessment of Nutriture

The evaluation of biotin in the blood and urine is used most often to assess biotin status. While blood biotin concentrations normally exceed 200 pg/mL, low blood biotin concentrations have not been shown to accurately reflect intake or status; they will, however, decrease after about 2–4 weeks of consuming a biotin-deficient diet [12,14]. Reductions in urinary biotin excretion have also been observed with ingestion of a biotin-free diet for about 2–4 weeks [14].

Decreased urinary biotin excretion (~6 μg/day) and increased urinary excretion of 3-hydroxyisovaleric acid and 3-hydroxyisovaleryl carnitine, generated from altered metabolism of β-methylcrotonyl-CoA, are sensitive and early indicators of biotin deficiency [12]. Normal 3-hydroxyisovaleric acid excretion is ~0.2 μmol/mg of creatinine.

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Suggested Reading

Symposium: advances in the understanding of the biological role of biotin at the clinical, biochemical, and molecular level. J Nutr. 2009; 139:152–70.

FOLATE

Folate's and vitamin B_{12}'s discoveries resulted from the search to cure the disorder megaloblastic anemia in the late 1870s and early 1880s. As with some of the other vitamins, eating liver was shown to cure the condition. Mitchell and colleagues are credited with folate's discovery in 1941. Its chemical synthesis in a lab was reported in 1945. The word *folate* from Italian means "foliage." The Latin word *folium* means "leaf." The vitamin is also referred to (although rarely) as vitamin B_9 or folacin.

Folate, shown in Figure 9.27, is composed of three parts that are all required for vitamin activity. The three parts are (1) 2-amino-4-hydroxypteridine, more commonly called pterin or pteridine, that is conjugated by a methylene group ($-CH_2-$) to (2) para-aminobenzoic acid (PABA) to form pteroic acid; the carboxy group of PABA is peptide bound to the amino group of (3) glutamic acid (an amino acid, and also called glutamate as found at physiological pH) forming folate (also called pteroylglutamate or pteroylmonoglutamate). Although humans can synthesize each of these components, they do not have the conjugase enzyme necessary for the coupling of the pterin molecule to PABA to form pteroic acid.

Often attached to the glutamic acid in folate is additional (usually up to another eight) glutamic acids (also referred to as glutamic acid residues). The additional glutamic acids are linked via gamma-peptide bonds to the glutamate in the folate. When these additional glutamates are attached, this form of folate is sometimes referred as pteroylpolyglutamate.

The terms *folate* and *folic acid* are not interchangeable. *Folate* refers to the *reduced form* of the vitamin; it is found naturally in foods and in biological tissues. *Folic acid* refers to the *oxidized form* of the vitamin (Figure 9.27). Folic acid is not found naturally in foods, but is the form used in supplements and to fortify foods.

Sources

Selected food sources of folate are depicted in Table 9.10. Folate is found in significant quantities in vegetables. Other food sources of the vitamin are legumes and lentils, along with some fruits and their juices. Liver (such as from beef) also provides folate, over 200 μg/3-oz serving.

Raw foods are typically higher in folate than cooked foods because of folate losses incurred with cooking. Folate is destroyed by heat, oxidation, and exposure to ultraviolet light. It is also reduced by 50–80% with certain types of food processing and preparation. Thus, consuming folate-rich foods raw or after cooking them quickly in a little water can help to minimize loss of the vitamin.

Fortification of flours, grains, and cereals with folic acid (140 μg of folic acid per 100 g of product) was initiated in 1998. These fortified cereals, breads, and grain products now represent major dietary sources of the vitamin. Enriched white bread provides 2.5–3 μg/slice, and fortified oatmeal provides 100 μg/cup. Bran cereal with raisins has 25% of the Daily Value of 400 μg, or 100 μg folate. Some juices are also fortified with folic acid. Because of this fortification program, more Americans are meeting recommendations for folate intakes.

Bacterial production of folate in the colon has also been demonstrated, and the vitamin is thought to be produced in amounts similar to that consumed from foods [1]. The overall contribution of microbial-derived folate in meeting the body's need for folate is not clear [1].

In foods, the primary form (over 75%) of folate has multiple glutamic acid residues attached. Other principal pteroylpolyglutamates in foods are 5-methyl tetrahydrofolate (THF) and 10-formyl THF, although over 150 different forms of folate have been reported.

In supplements and in fortified foods, the oxidized form of the vitamin, folic acid (Figure 9.27), is present; this

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Figure 9.27 Structures of folic acid, folate, and tetrahydrofolate (THF) and its derivatives.

Table 9.10 Folate Content of Selected Foods*

Food (serving)	Folate (μg DFE)
Spinach, cooked (1/2 c)	130
Asparagus, cooked (1/2 c)	127
Broccoli, cooked (1/2 c)	92
Brussels sprouts, cooked (1/2 c)	78
Greens, cooked (1/2 c)	75
Kidney beans, cooked (1/2 c)	45
Peanuts, roasted (1 oz)	41
Lentils, cooked (1/2 c)	160
Banana (1)	24
Orange (1)	29
Cantaloupe (1/4 c)	20
Egg (1)	22
Milk, 1% (1 c)	12

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

form also has only one glutamic acid attached to the PABA (i.e., folic acid monoglutamate). Folic acid is a very stable form of the vitamin, and as a supplement, folic acid is almost completely absorbed (especially if consumed on an empty stomach). When fortified foods or supplements are consumed with natural food sources of folate, the vitamin is about 85% bioavailable.

The bioavailability of folate from foods varies based on multiple factors including intestinal pH, genetic variability in enzymatic activity needed for folate digestion, the presence of dietary constituents such as inhibitors, and the food matrix. Because of the difference in the efficiency of folate absorption from foods versus folic acid from supplements and fortified products, folate equivalents are used in recommendations for dietary folate intakes (see the "Recommended Dietary Allowances" subsection in this section).

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Digestion, Absorption, Transport, and Storage

Folic acid in fortified foods and supplements does not need to undergo digestion because it is already present in the monoglutamate form. Folate in foods, however, is found in polyglutamate forms and must be digested to the monoglutamate form in order to be absorbed. This hydrolysis or deconjugation is performed by at least two gamma-glutamyl carboxypeptidases, previously referred to as folate hydrolases, pteroylpolyglutamate hydrolases, or folly gamma-glutamyl conjugases. The two carboxypeptidases exhibit separate activities in the jejunum, one soluble (from pancreatic juice and bile), and the other bound to the enterocyte's brush border membrane. The brush border carboxypeptidase is a zinc-dependent exopeptidase that stepwise cleaves the polyglutamate to produce a monoglutamate. It is this brush border carboxypeptidase that is thought to more significantly contribute to folate digestion as long as the lumen pH is about 6.5–7. Zinc deficiency and a more acidic pH diminish carboxypeptidase activity and thus folate digestion [2]. Alcohol ingestion and inhibitors in certain foods such as legumes, lentils, cabbage, and oranges also diminish enzyme activity to impair digestion of the vitamin's polyglutamate forms and thus inhibit folate absorption. Pancreatic exocrine insufficiency, which reduces the bicarbonate content of pancreatic juice, also impairs this folate digestive process.

The main carrier responsible for transporting folate (in its monoglutamate form) as well as 5-methyl THF (as a monoglutamate) into intestinal cells is the proton-coupled folate transporter (PCFT). This high-affinity facilitative folate carrier is found mostly in the proximal jejunum and duodenum. PCFT appears to be the primary transporter of folate and folic acid into cells of the small intestine, although folic acid may also be absorbed when present in high concentrations and in a more acidic environment by passive diffusion. Additionally, while a reduced folate carrier (RFC), which is bidirectional, is also present on the brush border membrane, this carrier is thought to be responsible for folate absorption in the colon [3]. The overal absorption of dietary folate is estimated at about 50%, but may vary widely from 10 to 90%.

Gene expression for PCFT is influenced by vitamin D and retinoic acid, among other transcription factors [3]. Hereditary folate malabsorption, a rare genetic disorder, results from mutations in the gene for PCFT. The condition is characterized by diarrhea, megaloblastic anemia, failure-to-thrive, and in some individuals, neurological problems [4]. High (pharmacological) oral doses or lower intravenously administered doses of the vitamin are needed to overcome the absorptive defect [4].

Within the intestinal cell, folic acid and folate are reduced to dihydrofolate (DHF), which is then reduced to generate THF (shown later in Figure 9.28). The reduction to THF occurs stepwise in the cytosol through the action of NADPH-dependent dihydrofolate reductase. Four hydrogens are added at positions 5, 6, 7, and 8 (Figure 9.27) during the reactions. Folate-binding proteins are thought to transport folate within cells. Transport of folate across the enterocyte's basolateral membrane to enter the blood is active and carrier dependent, likely involving multidrug resistance protein (MRP) 3 and to a lesser extent MRP 5 [3]. Folate is found in portal circulation as folate and 5-methyl THF, although dihydrofolate and formylated forms are also present; all forms are as monoglutamates, and not polyglutamates.

The uptake of folate into the liver and other tissues is carrier mediated. Reduced folate carriers (RFC), which are ubiquitously expressed, deliver folate from systemic circulation into many cells [1,3]. Proton-coupled folate transporter (which is expressed not only in the intestines, but on many tissue membranes including the liver, pancreas, kidneys, and spleen, among others) as well as organic anion transporting polypeptides (OATP1 B1 and B3) also may be responsible for folate uptake into some tissues [3]. Another transporter, folate receptor α, carries folate via endocytosis into the brain; defects in the FOLR1 gene impair the activity of this carrier and are associated with neurological disorders [4,5]. The roles of the folate transporters in various tissues are areas of active investigation [3].

Within liver (and other) cells, THF is found as THF (~33%), converted to 5-methyl THF (~33%) and converted to either 5- or 10-formyl THF (~33%). Bound to these forms of folate are four to eight glutamate residues. Folylpolyglutamate synthetase catalyzes the ATP-dependent additions of the glutamates, which are usually added one at a time to the monoglutamate. The addition of these glutamates traps the folate within the cell. Before being released into systemic blood, however, the glutamate residues are removed by gamma-glutamyl hydrolases.

In systemic blood, folate is found as a monoglutamate either free (~1/3) or bound to proteins (~2/3), including albumin, α2 macroglobulin, and a high-affinity folate-binding protein, which is thought to represent a soluble form of the cell membrane–derived folate receptor. The major forms of folate found in systemic blood are THF, 5-methyl THF, and 10-formyl THF. Typical plasma folate concentrations range from about 3 to 20 μg/L. More folate is found in red blood cells than the plasma. The folate in the red blood cells, however, is attained during erythropoiesis; folate is not taken up by mature red blood cells. Thus, red blood cell folate concentrations represent an index of longer-term (2–3 months) folate status than does plasma.

Body stores of folate range from about 7 to 30 mg, with about one-half stored in the liver. Storage occurs in association with intracellular folate-binding proteins. The main storage forms of folate are the polyglutamate forms of THF and 5-methyl THF. These polyglutamate forms can be

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converted to monoglutamate forms by gamma-glutamyl hydrolase, if needed.

Folate, as a cosubstrate, functions to accept and donate one-carbon units; these reactions are vital to nutrient metabolism, energy production, hematopoiesis, and gene expression, among other roles. The availability of folate to tissues where rapid cell division is occurring appears to be regulated when folate availability is limited. The mechanisms of this regulation are unclear but may involve changes in the rate of synthesis of polyglutamates and the release of folate monoglutamates from less metabolically active tissues to the liver, which then redistributes the folate to the actively proliferating cells.

Functions and Mechanisms of Action

THF derivatives are found in the mitochondria, cytosol, and nucleus, and serve as donors of single- or one-carbon groups in a variety of reactions. These reactions involve the metabolism of several nutrients including choline and some amino acids as well as the production of purines and pyrimidines. Folate is thus critical for DNA synthesis and repair with impacts on cell division and other activities. Moreover, because of its role as a carrier of methyl groups, folate also impacts gene expression. The THF derivatives, their one-carbon units that attach to THF at specific positions (N5 and/or N10 on the pteridine ring, commonly written without the N), and the oxidation states are illustrated as follows:

5- and 10-formyl THF	O═CH	Formate
5-formimino THF	—HC═NH—	Formate
5,10-methenyl THF	═CH—	Formate
5,10-methylene THF	$—CH_2—$	Formaldehyde
5-methyl THF	$—CH_3$	Methanol

The formyl derivatives represent the most oxidized forms, and, excluding THF, 5-methyl THF is the most reduced form. These derivatives, are interconvertible (as depicted in Figure 9.28), except that 5-methyl THF cannot be converted directly back to 5,10-methylene THF.

Genetic polymorphisms have been identified in some enzymes involved in folate metabolism. In fact, over 50 mutations have been characterized in methylene THF reductase (abbreviated MTHFR), an FAD-dependent enzyme which converts 5,10-methylene THF to 5-methyl THF. The ramifications of this defect are discussed further in the Perspective at the end of this chapter. Table 9.11 provides an overview of some of the metabolic roles of folate.

Amino Acid and Choline Metabolism

Folate is involved in the metabolism of several amino acids including serine, glycine, histidine, and methionine and in the metabolism of choline. These reactions are of further significance in that they link with other folate-dependent reactions for purine and pyrimidine synthesis.

Serine and Glycine Degradation Serine represents a major source of one-carbon units for use in folate reactions. The enzyme serine hydroxymethyltransferase, which requires vitamin B_6 as pyridoxal phosphate (PLP) for activity and is found in the cytosol and mitochondria in all tissues (especially the liver and kidneys), transfers a one-carbon unit from serine to THF to generate 5,10-methylene THF as well as glycine.

Some additional reactions involving glycine metabolism occurring in both the cytosol and mitochondria also require folate and generate 5,10-methylene THF. Glycine degradation, for example, requires THF and generates 5,10-methylene THF as well as ammonium and carbon dioxide, as shown here.

Choline Degradation The degradation of choline (which is made in the body and obtained from foods; see Chapter 6) also generates glycine and requires folate. In the liver, choline is initially catabolized in a NAD^+-dependent reaction to form betaine (also called trimethylglycine) and $NADH + H^+$. Further degradation of betaine, which occurs primarily in the liver and kidneys in both the mitochondria and cytosol, releases a methyl group and generates dimethylglycine. Dimethylglycine undergoes further metabolism to produce sarcosine (also called

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Red arrows indicate predominant direction of the pathway in the mitochondria for formate synthesis

Enzymes involved in interconversions of coenzyme forms of THF:

1. Serine hydroxymethyltransferase (coenzyme-PLP)—folate accepts carbon units as 5,10-methylene from serine resulting in 5, 10-methylene THF and glycine generation.
2. Methylene THF dehydrogenase—the generation of 5,10-methylene THF from 5,10-methenyl THF is important given the roles of 5,10-methylene THF in serine synthesis and pyrimidine synthesis.
3. Cyclohydrolase. The production of 10-formyl THF is especially important for purine synthesis.
4. Formate-activating enzyme. 10-formyl THF is especially important for purine synthesis.
5. Methylene-THF reductase—the generation of 5-methyl THF is essential for methionine synthesis from homocysteine.
6. Methionine synthetase (coenzyme-B_{12})—see Figure 9.30 for details on this reaction.
7. Formiminotransferase—folate accepts a formimino group from FIGLU and facilitates the final step in histidine catabolism.
8. Cyclodeaminase.
9. Thymidylate synthetase—5,10-methylene THF provides the formaldehyde group for this reaction needed for pyrimidine synthesis.
10. 10-formyl THF dehydrogenase.

Figure 9.28 Interconversions of coenzyme forms of tetrahydrofolate (THF).

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Table 9.11 Forms of Folate and Their Metabolic Roles in the Body

Folate Form	Roles
10-formyl THF	**Folate transfers formate as 10-formyl THF for purine synthesis:** 5-phosphoribosylglycinamide ribonucleotide (GAR) conversion to 5-phosphoribosyl formylglycinamide (FGAR) by glycinamide ribonucleotide formyltransferase and 5-phosphoribosyl 5-amino 4-imidazole carboxyamide ribonucleotide (AICAR) conversion to 5-phosphoribosyl 5-formamido 4-imidazole carboxamide ribonucleotide (FAICAR) by aminoimidazolecarboxamide ribonucleotide formyltransferase
5, 10-methylene THF	**Folate transfers formaldehyde as 5,10-methylene for pyrimidine synthesis:** Deoxyuridine monophosphate (dUMP) conversion to deoxythymidine monophosphate (dTMP) by thymidylate synthetase **Folate receives formaldehyde from serine and glycine degradation:** Serine conversion to glycine by serine hydroxymethyltransferase Glycine degradation by the glycine cleavage system **Folate receives formaldehyde from choline degradation:** As part of choline degradation, dimethylglycine and its catabolic product sarcosine are degraded to glycine by dimethylglycine dehydrogenase and sarcosine dehydrogenase, respectively
5-formimino THF	**Folate receives a formimino group in histidine degradation:** Formiminoglutamate (FIGLU) conversion to glutamate by formiminotransferase
5-methyl THF	**Folate provides a methyl group for methionine synthesis:** Homocysteine conversion to methionine by methionine synthase

monomethylglycine) in a reaction catalyzed by dimethylglycine dehydrogenase. Sarcosine dehydrogenase converts sarcosine to glycine, with THF functioning as the carbon acceptor and forming 5,10-methylene THF. Riboflavin as FAD is also required by this dehydrogenase.

In addition to its synthesis from choline, betaine is found in the diet in both animal and plant foods. Estimated betaine intake ranges from about 1 to 2.5 g per day. Folic acid supplementation appears to increase endogenous betaine concentrations [6], and supplementation with betaine (which provides methyl groups upon degradation) reduces plasma homocysteine concentrations in those with elevated blood levels [7].

Thus, the catabolism of glycine and serine as well as choline provide one-carbon units for the formation of 5,10-methylene THF. This 5,10-methylene in the cytosol (as well as in the nucleus) is required in pyrimidine synthesis for the conversion of deoxyuridine monophosphate (dUMP) to thymidylate (dTMP); thymidylate is essential for DNA synthesis. In the mitochondria, the 5,10-methylene is used extensively to produce 10-formyl THF; hydrolysis of 10-formyl THF generates THF and formate. Formate can move from the mitochondria into the cytosol where it is used to resynthesize 10-formyl THF for use in the production of purines needed for DNA synthesis. Alternately, the 10-formyl THF can be reduced to form 5,10-methenyl THF and subsequently 5,10-methylene THF for use in methionine resynthesis.

Histidine Degradation The final reaction in histidine catabolism also requires THF. Histidine catabolism begins with its deamination to generate urocanic acid, which undergoes further metabolism to yield formiminoglutamate (FIGLU). The formimino group is removed from FIGLU by formiminotransferase to generate glutamate; THF receives the formimino group to yield 5-formimino THF, as shown here and in Figure 9.29.

Figure 9.29 The role of folate in histidine catabolism.

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This reaction has been used diagnostically as a basis for determining folate deficiency, although other approaches are now more often used. In the diagnostic procedure, subjects ingest an oral histidine load dose, and FIGLU excretion is measured in the urine. With a folate deficiency, FIGLU accumulates in the blood and is excreted in higher than normal concentrations in the urine. With adequate folate (THF) status, the THF is converted into 5-formimino THF and FIGLU is converted to glutamate, with little to no FIGLU appearing in the urine.

The 5-formimino THF generated in the reaction can be converted to 5,10-methenyl THF and subsequently to 5,10-methylene THF if needed by cyclodeaminase and methylene THF dehydrogenase, respectively. Thus histidine, like serine, glycine, and choline, provides a source of one-carbon units.

Methionine and SAM Synthesis Methionine regeneration from homocysteine, which occurs in the cytosol, requires folate as 5-methyl THF as a methyl donor, and the enzyme methionine synthase (also called homocysteine methyltransferase), which must have cobalamin (vitamin B_{12}) as a tightly bound prosthetic group. While bound to methionine synthase, cobalamin picks up the methyl group from 5-methyl THF to generate methylcobalamin and THF. Methylcobalamin then serves as the methyl donor providing homocysteine with its methyl group to generate methionine (Figure 9.30; see also the vitamin B_{12} section "Functions and Mechanisms of Action").

Methionine resynthesis from homocysteine or the further degradation of homocysteine are influenced by S-adenosyl methionine (SAM) concentrations and by the availability of folate. SAM is an important methyl donor, involved in over 100 reactions, including, for example, DNA, RNA, and histone methylation, myelin maintenance, neural function, and polyamine, carnitine, and catecholamine synthesis, among others. SAM concentrations increase with increased cellular methionine concentrations. These higher SAM concentrations stimulate the transsulfuration pathway in which homocysteine is further degraded in

1. Cobalamin, which is bound to the enzyme methionine synthase, picks up the methyl group on 5-methyl THF, forming THF and methylcobalamin.
2. Methylcobalamin, which is still bound to the enzyme methionine synthase, gives the methyl group to homocysteine, which then forms methionine and reforms cobalamin.
3. THF must be reconverted to 5-methyl THF for the reaction to proceed again. This process requires two reactions catalyzed first by serine hydroxymethyl transferase to generate 5,10-methylene THF. Second, methylene THF reductase converts 5,10-methylene THF to 5-methyl THF, which can once again donate its methyl group to cobalamin.

Figure 9.30 The resynthesis of methionine from homocysteine, showing the roles of folate and vitamin B_{12}.

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an irreversible reaction, to cystathionine by cystathionine synthase (Figure 9.30). Higher SAM concentrations (as well as 5-methyl THF) also inhibit methylene THF reductase, which converts 5,10-methylene THF to 5-methyl THF. This inhibition decreases 5-methyl THF availability and decreases remethylation of homocysteine. Thus, higher SAM reduces methionine resynthesis, while lower SAM concentrations encourage the remethylation of homocysteine to methionine. Adequate availability of 5-methyl THF is needed for the synthesis of methionine and SAM.

Gene Expression

Methylation of DNA influences gene expression. Adequate provision of folate as 5-methyl THF enhances the availability of SAM for its methylation functions, including methylation of DNA. Folate deficiency or poor folate status is associated with increased breaks in chromosomes and decreased DNA methylation. Aberrant methylation is thought to activate oncogenes, promoting cancer. Decreased methylation of tumor suppression genes associated with folate deficiency also may promote cancer.

Associations with Disease Folate deficiency or poor folate status is suspected in the development (initiation) of some cancers, especially colon cancer but also lung, esophageal adenocarcinoma, and other gastrointestinal cancers. In addition to affecting DNA methylation, which alters gene expression, folate deficiency may promote cancer by increasing DNA strand breaks (associated with misincorporation of uridylate for thymidylate in DNA, as discussed in the next section) [8,9].

Purine and Pyrimidine Synthesis/Nucleotide Metabolism

The involvement of THF derivatives in purine and pyrimidine synthesis makes folate essential for DNA synthesis and cell division. The synthesis of cells with short life spans, such as enterocytes and red blood cells (erythrocytes), is particularly affected if folate is inadequate.

In pyrimidine synthesis, thymidylate synthetase uses 5,10-methylene THF to convert deoxyuridine monophosphate (dUMP) to thymidylate (dTMP) and dihydrofolate (DHF) (see Figure 6.28 and Figure 9.28). This reaction occurs both in the cytosol and nucleus. Thymidylate is required for DNA synthesis; the reaction is rate limiting to DNA replication. Inadequate folate restricts this conversion and normal cell cycle progression, causing intracellular uracil accumulation, misincorporation of uracil into DNA, and ultimately increased DNA strand breakage. Both thymidylate synthetase and dihydrofolate reductase (which converts DHF to THF) are especially active in cells, including tumor cells, undergoing division. The drug methotrexate is used in the treatment of some cancers and psoriasis, among other conditions, because it binds to dihydrofolate reductase's active site to prevent resynthesis of THF needed for actively dividing cells. Such effects may help to diminish tumor growth.

In purine synthesis (see Figures 6.29 and 6.30), folate as 10-formyl THF provides formate in two reactions needed for purine (adenine and guanine) ring formation. The formate (which is formed mainly in the mitochondria but which moves into the cytosol) condenses with THF in an ATP-dependent reaction to generate 10-formyl THF; the reaction is catalyzed by 10-formyl THF synthetase, which is part of a multienzyme complex. Purine-ring carbon atom 2 is acquired by formylation of 5-phosphoribosyl 5-amino 4-imidazole carboxamide ribonucleotide (AICAR) by aminoimidazolecarboxamide ribonucleotide formyltransferase. Purine ring carbon 8 is also acquired by 10-formyl THF, which donates the formyl group to 5-phosphoribosylglycinamide, also called glycinamide ribotide (GAR), to form 5-phosphoribosyl formylglycinamidine ribotide (FGAR) in a reaction catalyzed by glycinamide ribonucleotide formyltransferase.

Interactions with Other Nutrients

A synergistic relationship exists between folate and vitamin B_{12}. This relationship is sometimes called the methyl-folate trap. The following sequence of events leads to the methyl-folate trap (tracing the reactions shown in Figures 9.28 and 9.30 is helpful). In the synthesis of methionine from homocysteine, the methyl group from 5-methyl THF is normally transferred to vitamin B_{12} (cobalamin) that is attached to the enzyme methionine synthase. Without vitamin B_{12} to accept the methyl group from 5-methyl THF, the 5-methyl THF accumulates and "is trapped" and other forms of folate, like THF, cannot be produced. Thus, the cells have folate, but not in a form that can be used for DNA synthesis. With adequate vitamin B_{12} status, the THF resulting from the methionine resynthesis can be used to make the forms of folate needed for DNA synthesis, including 10-formyl THF (which is needed for purine synthesis) and 5,10-methylene THF (which is needed for thymidylate synthesis in pyrimidine metabolism).

Association with Disease

Folate and vitamin B_{12} participate in the regeneration of methionine from homocysteine and vitamin B_6 is needed for the catabolism of homocysteine (Figure 9.30); low intakes of these three vitamins, especially folate, are inversely associated with plasma homocysteine concentrations. Elevated plasma homocysteine concentrations (>11 μmol/L, which is toward the upper end of the normal range of about 5–15 μmol/L) are associated with premature heart disease, occlusive vascular disease, and cerebral (i.e., stroke) and peripheral vascular diseases. The mechanisms by which hyperhomocysteinemia increases disease risk are not clear, but may result from impairing endothelial

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function, promoting the growth of smooth muscle cells leading to vascular lesions, promoting an autoimmune response, and/or promoting platelet adhesiveness and clotting, among other hypotheses. A 5 μmol/L increase in serum homocysteine concentrations increases the risk for heart disease by 20–30% [10]. Folic acid supplementation in people with hyperhomocysteinemia improves endothelial function. Supplementation of folic acid, vitamin B_{12}, and vitamin B_6 in people (both healthy and with heart disease) with hyperhomocysteinemia normalizes or reduces blood homocysteine concentrations, but does not consistently diminish the risk of cardiovascular events or stroke [11–14].

Elevations in plasma homocysteine concentrations have also been positively correlated with cognitive dysfunction and dementia, but lowering these concentrations has not always improved cognitive function [11,15,16]. Alzheimer's dementia has been linked to poor folate status, and both memory and abstract thinking as well as several neurologic conditions appear to be influenced by folate [5,8,11,16]. Further trials are needed to examine the roles and benefits of folate on cognitive functions.

Metabolism and Excretion

Folate is excreted from the body in both the urine and the feces. Within the kidneys, folate-binding proteins present in the renal brush border coupled with tubular reabsorption of the vitamin help the body retain folate, if needed. Excess folate is excreted in the urine with some folate excreted intact and some catabolized in the liver prior to urinary excretion. Oxidative cleavage of folate is thought to occur between C9 and N10 of polyglutamate forms of the vitamin. This cleavage generates para-aminobenzoyl polyglutamate and pteridine. All but one of the glutamates is then hydrolyzed, and usually the compound is acetylated to form the major urinary metabolite N-acetyl para-aminobenzoyl glutamate. Smaller amounts of para-aminobenzoyl glutamate also are found in the urine.

In addition to urinary losses, folate (up to about 100 μg) is secreted by the liver into the bile. MRP2 and another transporter, known as breast carrier resistance protein (BCRP), move folate across the apical bile canicular membrane from the hepatocyte. Most of this folate, however, is reabsorbed with enterohepatic recirculation, so fecal vitamin losses are minimal. Folate of microbial origin that has not been absorbed, however, may appear in the feces in relatively high amounts.

Recommended Dietary Allowance

The establishment of recommendations for folate intake consider its bioavailability as well as several indices of nutriture. Folate requirements are estimated at 320 μg per day [17]. Recommendations for folate are provided as dietary folate equivalents (DFE), which account for the higher bioavailability of folic acid taken as supplements versus the lower bioavailability of folate in foods. The RDA for adults for folate is 400 μg DFE per day. One DFE is equal to 1 μg of food folate, 0.6 μg of folic acid from a supplement or fortified food consumed with a meal, or 0.5 μg of folic acid from a supplement taken without food (on an empty stomach) [17]. Stated alternately, DFE = μg food folate + (1.7 × μg folic acid); the definition is based on the assumption that the bioavailability of folic acid supplemented in foods is greater than folate found naturally in foods by a factor of 1.7, while that of synthetic folic acid, when ingested on an empty stomach, is two times as much [17]. As an example, if a person consumed a vitamin containing 100 μg of folic acid along with some bread containing 50 μg of folic acid as part of a meal, the DFE = natural food folate + (1.7 × 150 μg).

Because of evidence that folic acid supplementation during the periconceptional period of pregnancy may reduce the incidence of neural tube defects, the Centers for Disease Control and Prevention (CDC) suggests 400 μg of synthetic folic acid/day for women capable of becoming pregnant. Foods that are good sources of folate (i.e., that provide ≥ 10% of the 400 μg Daily Value or at least 40 μg/serving) are permitted by the U.S. Food and Drug Administration (FDA) to make the health claim "Healthful diets with adequate folate may reduce a woman's risk of having a child with a neural tube (brain or spinal cord) defect" [18]. RDAs for folate of 600 μg and 500 μg of DFE per day are suggested for pregnancy and lactation, respectively [17]. Additional RDAs for folate for other age groups are provided on the inside front cover of the book.

Deficiency: Megaloblastic Macrocytic Anemia

Folate deficiency results in megaloblastic macrocytic anemia—the release into circulation of red blood cells that are fewer than normal in number as well as large and immature. Some other signs and symptoms of the condition include fatigue, weakness, headaches, irritability, difficulty concentrating, shortness of breath, and heart palpitations.

The deficiency is characterized initially (within about a month or two if the diet is devoid of folate) by reductions in plasma folate and then by increases in plasma homocysteine concentrations. Red blood cell folate concentrations diminish after about 3–4 months (remember red blood cells live about 90–120 days) of low folate intake. After approximately 4–5 months, rapidly dividing cells such as those in the gastrointestinal tract and blood become megaloblastic. Mean cell volume (MCV) increases, and hypersegmentation (increased lobes) of white blood cells (neutrophils) occurs, along with decreased blood cell counts. The reduced and

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abnormal red blood cells in turn diminish the oxygen-carrying capacity of the blood and may result in shortness of breath, fatigue, and weakness. Treatment of the deficiency usually requires oral ingestion of 1–5 mg folate daily.

Megaloblastic macrocytic anemia, also called megaloblastic anemia, is relatively common in the United States. It may result from a deficiency of either folate or vitamin B_{12}, both of which disrupt DNA synthesis (replication) and thus cell division. As discussed under the functions of folate, the 10-formyl THF coenzyme form of folate is needed for purine synthesis, and the 5,10-methylene THF coenzyme form is needed for thymidylate (pyrimidine) synthesis. Without folate, these compounds are not made, and DNA synthesis becomes impaired. In the bone marrow, precursor red blood cells exhibit deranged DNA synthesis and defective cell maturation and division, resulting in abnormally large (macrocytic) and immature red blood cells (megaloblasts) with shortened life spans. Over time, the megaloblasts steadily increase in number in the blood while the numbers of healthy red blood cells decrease in the blood, with negative effects on the blood's oxygen-carrying capacity. Figure 9.31 reviews the formation and maturation of erythrocytes.

Impaired DNA synthesis is also evident in other body cells, especially those of the gastrointestinal tract, which also normally exhibit rapid turnover. Folate deficiency is associated with a bright red tongue, glossitis, and the shortening of the villi height and thinning of the layers of the gastrointestinal tract. The latter two changes may impair nutrient absorption and promote diarrhea.

Some conditions associated with an increased need for folate include excessive alcohol ingestion (which inhibits folate digestion and thus absorption) and malabsorption disorders such as inflammatory bowel diseases. Malabsorption secondary to gastric bypass procedures used in the treatment of obesity can also cause a folate deficiency. Several medications affect folate. The diuretic furosemide—used to treat hypertension, among other conditions—decreases intestinal folate absorption. Folate deficiency has been observed in people taking diphenylhydantoin or phenytoin, anticonvulsants used to treat epilepsy. Folate and phenytoin each inhibit the gastrointestinal cellular uptake of the other. Methotrexate, used to treat rheumatoid arthritis and some cancers, among other conditions, binds to dihydrofolate reductase and thus prevents THF synthesis. Other drugs, including cholestyramine (used to treat high blood cholesterol concentrations) and sulfasalazine (used to treat

Figure 9.31 Genesis and maturation of the red blood cells (left); red blood cells characteristic of microcytic and megaloblastic anemias (right).

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inflammatory bowel diseases), interact with folate and can increase the likelihood of folate deficiency.

Because inadequate folate status in women increases the risk of neural tube defects in infants, specific recommendations are provided for women capable of becoming pregnant, as discussed in the section "Recommended Dietary Allowances." Folic acid supplements taken before or about the time of conception reduce the risk of neural tube defects; however, the mechanism(s) by which folate plays a role in the etiology of neural tube defects is unclear [19].

Toxicity

A Tolerable Upper Intake Level for adults of 1,000 μg (1 mg) for synthetic folic acid in supplements or from fortified foods (not natural foods) is based on the ability of folate to mask the neurological manifestations of vitamin B_{12} deficiency [17]. Folic acid supplements can alleviate the megaloblastic anemia caused by a vitamin B_{12} deficiency, but not the neurological damage, which progresses undetected and is irreversible (see the "Deficiency" section for vitamin B_{12}).

Side effects have been documented with folate intakes of 15 mg (i.e., 15 times the Tolerable Upper Intake Level); these side effects include insomnia, malaise, irritability, and gastrointestinal distress [17,19]. Additionally, intakes of supplemental folic acid in amounts exceeding about 2½ times recommendations have been shown to increase cancer risk and cancer mortality, especially in individuals with precancerous tumors [11]. Such findings may lead to a reevaluation of the level of folic acid fortification in foods in the United States.

Assessment of Nutriture

Folate status is most often assessed by measuring folate concentrations in the plasma, serum, or red blood cells. Serum or plasma folate levels reflect recent dietary intake; thus, true deficiency must be interpreted through repeated measures of serum or plasma folate. Serum folate concentrations less than about 3 μg/L typically suggest deficiency. Red blood cell folate concentrations are more reflective of folate tissue status than is serum folate and represent vitamin status at the time the red blood cells were synthesized. Red blood cell folate concentrations less than about 140 μg/L suggest folate deficiency; however, concentrations are also lowered with a vitamin B_{12} deficiency [17].

Formiminoglutamate (FIGLU) excretion may also be used to measure folate nutriture because folate as THF must be available for the formimino group to be removed from FIGLU and glutamate to be formed (see Figure 9.29). FIGLU excretion is measured in a 6-hour urine collection after ingestion of 2–5 g oral L-histidine. Normal FIGLU excretion is ~35 μM/day in folate-adequate adults, whereas with folate deficiency it rises to > 200 μM/day [19]. A deficiency of vitamin B_{12}, however, also elevates FIGLU excretion.

The deoxyuridine suppression test, another method for assessing folate status, measures the availability of folate for thymidine synthesis. In this test, the activity of thymidylate synthetase is measured in cultured lymphocytes or bone marrow cells. The reaction catalyzed by thymidylate synthetase is dependent on folate and, indirectly, on vitamin B_{12}; therefore, the change in activity elicited by adding one or the other vitamin allows the deficiency to be identified. In other words, if a person were folate deficient, adding folate—but not vitamin B_{12}—would normalize enzyme activity. Likewise, if a person were vitamin B_{12} deficient, adding vitamin B_{12}—and not folate—would normalize thymidylate synthetase activity. In the case of a deficiency of both vitamins, enzyme activity could be normalized only by adding both vitamins [19].

A functional marker of folate and vitamin B_{12} deficiencies is elevated plasma homocysteine concentrations. Remember, both vitamins are required for the remethylation of homocysteine to methionine and with a deficiency of either vitamin (as well as vitamin B_6) plasma homocysteine concentrations become elevated.

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Suggested Reading

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VITAMIN B_{12} (COBALAMIN)

Vitamin B_{12} (also called cobalamin) was the last vitamin to be discovered. It was isolated in 1948 by Smith (from England) and by Rickes and others (from the United States). Its structure was discovered by Hodgkin; however, Minot and Murphy in 1926 showed that eating large amounts of liver could help correct pernicious anemia associated with deficiency of the vitamin. It took about two decades to identify the vitamin in liver.

Vitamin B_{12} (sometimes referred to as a corrinoid because of the corrin nucleus) consists of a macrocyclic ring made of four reduced pyrrole rings linked together. In the ring's center is an atom of cobalt (Co) to which is attached, at almost right angles, the nucleotide 5,6-dimethylbenzimidazole. Also attached to the cobalt atom in vitamin B_{12} is one of the following:

Group Attached	Resulting Compound
—CN	Cyanocobalamin
—OH	Hydroxocobalamin
$-H_2O$	Aquo- or hydrocobalamin
—5′-deoxyadenosyl	5′-deoxyadenosylcobalamin
$-CH_3$	Methylcobalamin

The structure of cyanocobalamin is shown in Figure 9.32. Only 5′-deoxyadenosyl-cobalamin (subsequently called adenosylcobalamin) and methylcobalamin are active as coenzymes in humans.

Figure 9.32 Structure of vitamin B_{12} (as cyanocobalamin).

Sources

Dietary sources of vitamin B_{12} come primarily from animal products (Table 9.12). Foods of plant origin do not naturally contain the vitamin. Major sources of vitamin B_{12} are meat and meat products. Milk and milk products such as cheese, cottage cheese, and yogurt contain less of the vitamin; however, absorption may be better due to the presence of binders in dairy products. Plant-derived foods, such as ready-to-eat cereals and soymilk, are sometimes fortified with the vitamin. For example, bran cereal with raisins (1 cup) contains 1.5 μg of vitamin B_{12}, which is 25% of the vitamin's Daily Value of 6 μg. Vitamin B_{12} is fairly stable and, resistant to light, heat, and oxidation.

Cyanocobalamin and hydroxocobalamin forms of the vitamin are commonly used in multivitamin supplements and to fortify foods, although some supplements contain the methyl form. Orally dissolving sublingual products and nasal sprays containing vitamin B_{12} are also available. The cyano- and hydroxocobalamin forms are readily converted to the forms of the vitamin (methylcobalamin and adenosylcobalamin) used within body cells.

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Table 9.12 Vitamin B_{12} Content of Selected Foods*

Food (serving)	Vitamin B12 (μg)
Beef, sirloin (3 oz)	1.3
Pork, loin (3 oz)	0.6
Chicken, breast (3 oz)	0.3
Cod (3 oz)	0.9
Salmon (3 oz)	2.6
Clams, mixed species (3 oz)	84
Oysters, Eastern (3 oz)	11
Egg (1)	0.6
Milk, low fat (1 c)	1.0
Cheese, Swiss (1 oz)	0.9
Cottage cheese (1/2 c)	0.7
Yogurt, low fat (1 c)	0.9

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

Digestion, Absorption, Transport, and Storage

Several steps are required for the digestion and absorption of vitamin B_{12} (Figure 9.33). Ingested cobalamins (from foods) must be first released from the food proteins to which they are bound. This digestion occurs through the actions of pepsin and hydrochloric acid in the stomach. In contrast to vitamin B_{12} ingested through foods, vitamin B_{12} ingested from supplements and fortified foods is not bound to proteins and thus does not require this initial hydrolysis.

Within the stomach, the now "free" vitamin B_{12} (all forms) next binds to an R protein (also called haptocorrin or transcobalamin I). This R protein originates primarily from the saliva and is thought to protect vitamin B_{12} from bacterial use. The R protein–vitamin B_{12} complex next moves from the stomach into the duodenum. Within the alkaline environment of the duodenum, the R protein is hydrolyzed by pancreatic proteases, and free vitamin B_{12} is released. Next (and still within the duodenum), the free vitamin B_{12} (all forms) binds to intrinsic factor (IF), a glycoprotein that is synthesized by parietal cells in the stomach but which interacts with vitamin B_{12} in the duodenum.

From the duodenum, the vitamin B_{12}–IF complex travels to the distal ileum, where it interacts with a cubam complex receptor on the brush border membrane of ileal cells. The cubam receptor is formed from two proteins, cubilin and amnionless, and possibly may involve another protein, megalin [1]. Binding of the vitamin B_{12}–IF complex to the receptor triggers active endocytotic

1. Vitamin B_{12} is released from food in the acid environment of the stomach and with the help of pepsin.
2. Vitamin B_{12} binds to R proteins found in saliva and gastric juice. The B_{12}•R protein complex travels from the stomach to the duodenum.
3. Within the alkaline environment of the duodenum, R protein is digested to release vitamin B_{12}.
4. In the duodenum, vitamin B_{12} binds and forms a complex with intrinsic factor (IF) which was made by gastric parietal cells.
5. Within the ileum, vitamin B_{12}•IF complex binds to specific receptor and is internalized by endocytosis.

Figure 9.33 Vitamin B_{12} absorption.

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internalization. Defects in the receptor result in vitamin B_{12} malabsorption. Within the enterocyte, the vitamin is released from its complex with IF. It is carried across the ileum's basolateral membrane, possibly by an ATP-binding cassette drug transporter, and binds to the protein transcobalamin II for transport in portal blood. Several conditions can interfere with this absorptive process, as discussed under the section "Deficiency."

Carrier-mediated intestinal absorption of vitamin B_{12} (i.e., bound to intrinsic factor) is saturated with vitamin intakes of about 1.5–2.0 μg. However, about 1–3% of intake may be absorbed by passive diffusion when pharmacological doses of vitamin B_{12} are ingested. Thus, if 1,000 μg were consumed, about 2 μg would be absorbed from IF-mediated absorption plus another 30 μg from passive diffusion (3% of 1,000 μg). Overall absorption of vitamin B_{12} with usual intake is estimated at 50% (range: about 11–65%), with decreased absorption efficacy as intake increases [2,3].

Enterohepatic circulation is important in vitamin B_{12} nutriture, accounting in part for the vitamin's long biological half-life. The vitamin is excreted in the bile; however, it can bind to IF in the duodenum and be reabsorbed in the ileum. Thus, malabsorption syndromes not only decrease absorption of ingested cobalamin but also interfere with its enterohepatic circulation, thereby increasing the amount of vitamin B_{12} required to meet body needs.

Following its absorption, the vitamin appears in the blood in about 3–4 hours, with peak levels occurring after about 8–12 hours. Methylcobalamin comprises about 60–80% and adenosylcobalamin perhaps up to 20% of total blood cobalamin. Other forms in the blood include small amounts of cyanocobalamin and hydroxocobalamin. Vitamin B_{12} circulates in the blood bound to transporter proteins, transcobalamin (TC) and haptocorrin (HC)-like proteins. TCII, which is made in many body cells including enterocytes, carries primarily newly absorbed cobalamin in a one-to-one ratio (referred to as holoTCII) in the blood. TCII accounts for about 20% of cobalamin in the blood and holoTCII has a half-life of less than 2 hours. Other proteins—haptocorrin, TCI, and TCIII—transport most of vitamin B_{12}; however, the exact functions of these proteins are unknown. Much of vitamin B_{12} is transferred from TCII to TCI. TCI, which transports up to about 80% of vitamin B_{12} and has a half-life of about 10 days, is thought to function as a circulating storage form of the vitamin; it is also thought to prevent bacterial use of the vitamin. TCIII may function in the delivery of cobalamin from peripheral tissues back to the liver.

A fairly common genetic mutation in TCII results in the substitution of cytosine (C) for guanine (G) at base pair 776 (written as TC 766C G). This substitution results in the insertion of arginine instead of proline, and in turn diminishes the protein's (TCII's) ability to bind and transport B_{12} to tissues. An estimated 20% of the population is homozygous for the GG variant, which is associated with low serum vitamin B_{12} and high serum homocysteine concentrations (a risk factor for heart disease) and neuropsychiatric conditions [4,5].

Uptake of vitamin B_{12} into tissues is receptor dependent. All tissues appear to have receptors for TCII, and nonspecific receptors have been shown to take up the TCI-B_{12} complex. The TCII-cobalamin complex, upon binding to TCII cell receptors, appears to enter cells by endocytosis with subsequent fusion to lysosomes that provide for proteolytic degradation of TCII and release of the vitamin within the cell cytosol. **Chaperones**, intracellular transport proteins, are thought to carry or escort the vitamin within the cell's various compartments and organelles.

Metabolism of the various forms of the vitamin occurs within cells. Hydroxocobalamin, for example, may undergo cytosolic methylation to generate methylcobalamin, or may undergo reduction and subsequent reaction with ATP in the mitochondria to yield adenosylcobalamin. Cyanocobalamin is typically converted to aquo- or hydroxocobalamin. However, individuals with an inherited cobalamin C defect exhibit impaired conversion of cyanocobalamin into either of the two coenzymes due to a defect in cyanocobalamin decyanase, which catalyzes the removal of the cyano group from cyanocobalamin.

Vitamin B_{12}, unlike other water-soluble vitamins, can be stored (retained) in the body for relatively long periods of time. About 2–3 mg of the vitamin is stored in the body, mainly (~50%) in the liver. The muscles store about 30% of the vitamin, with lesser amounts found in the pituitary gland, bone, kidneys, heart, brain, and spleen. Adenosylcobalamin is the main form of the vitamin in most of these storage sites (such as the liver, kidneys, and brain), but small amounts of hydroxocobalamin and methylcobalamin may be present. The amount of the vitamin available in stores in an adult is estimated to be sufficient to prevent a deficiency (if no further intake of vitamin occurs) for about 3–5 years.

Functions and Mechanisms of Action

Two enzymatic reactions requiring vitamin B_{12} have been recognized in humans. One of these reactions requires methylcobalamin as a coenzyme for methionine synthase, and the other relies on adenosylcobalamin as a coenzyme for L-methylmalonyl-CoA mutase. These reactions facilitate nutrient metabolism and energy production, as well as indirectly (via interactions with folate) the synthesis of purines and pyridimidines for use in nucleic acids.

Methionine Synthase

Two reactions are responsible for converting homocysteine to methionine. One uses betaine to supply the needed methyl group; however, the enzyme betaine-homocysteine methyltransferase does not require vitamin B_{12}. The other reaction requires methylcobalamin as a coenzyme for methionine synthase (also called homocysteine methyltransferase) (see Figure 9.30). This reaction, which occurs in the cytosol, is shown as a two-step process to facilitate an understanding of the sequential nature of the reaction.

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- First, cobalamin bound to the methionine synthase picks up the methyl group from 5-methyl tetrahydrofolate (THF), forming methylcobalamin bound to methionine synthase and THF.

- Next, methionine synthase releases the methyl group from its bound methylcobalamin for transfer to homocysteine, producing methionine and cobalamin.

In this reaction, the transfer of the methyl group from methylcob(III)alamin results in the formation of cob(I)alamin. Cob(I)alamin, however, is easily oxidized and, with its oxidation, methionine synthase becomes inactive. Reactivation of methionine synthase is accomplished by the NADPH-dependent flavoenzyme methionine synthase reductase. Interestingly, polymorphisms in this reductase (causing reduced methionine synthase activity) have been linked with an increased risk of neural tube defects in individuals with suboptimal vitamin B_{12} status as well as in individuals with 5,10-methylene tetrahydrofolate reductase mutations.

L-methylmalonyl-CoA Mutase

The second of the vitamin B_{12}–dependent reactions requires adenosylcobalamin for the activity of methylmalonyl-CoA mutase, which converts (rearranges the skeleton of) L-methylmalonyl-CoA to succinyl-CoA (Figure 9.34) in the mitochondria. L-methylmalonyl-CoA is made from D-methylmalonyl-CoA, which in turn is generated from propionyl-CoA. Propionyl-CoA arises from the oxidation of methionine, isoleucine, and threonine and of odd-number-chain fatty acids. The conversion of propionyl-CoA to D-methylmalonyl-CoA is an ATP-, Mg^{2+}-, and biotin-dependent reaction (previously discussed in the section on biotin; see Figure 9.24). Methylmalonyl-CoA mutase (a dimer) requires two adenosylcobalamin molecules (one per subunit) to convert L-methylmalonyl-CoA to succinyl-CoA (Figure 9.34).

With a deficiency of vitamin B_{12} or a genetic defect in the enzyme, mutase activity is impaired and methylmalonyl-CoA and methylmalonic acid, formed from hydrolysis of methylmalonyl-CoA, accumulate in body fluids. High methylmalnonic acid concentrations are thought to destabilize myelin or promote the formation of abnormal myelin, which is normally wrapped around nerve axons and serves like an electrical insulator to assist with nerve conduction. Such changes may be responsible in part for some of the neurological manifestations associated with a vitamin B_{12} deficiency. The response of serum methylmalonic acid to vitamin B_{12} depletion and repletion is useful in the diagnosis of vitamin B_{12} deficiency and in monitoring the response to treatment.

Selected Pharmacological Uses / Other Roles

Supplementation with oral vitamin B_{12}, usually in pharmaceutical amounts of 1–2 mg daily, can sometimes enhance residual methylmalonyl-CoA mutase activity in those with the genetic disorder methylmalonic acidemia. Pharmacological doses of the vitamin are also often needed to overcome problems with carrier-mediated absorption of the vitamin.

Large doses of the vitamin, however, are also purported to cure a variety of other problems, including fatigue and weight gain. Unfortunately, scientific studies supporting

Figure 9.34 Role of vitamin B_{12} in oxidation of L-methylmalonyl-CoA.

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the use of the vitamin to boost energy and enhance weight loss (among other claims) are generally lacking.

Metabolism and Excretion

Vitamin B_{12} undergoes little to no degradation (metabolism) prior to excretion. About 0.1% (2 μg) of vitamin B_{12} is excreted through the bile. However, most (about 75%) is reabsorbed in the ileum after binding to intrinsic factor in the proximal small intestine. About 0.25 μg of the vitamin is excreted daily in the urine. Trace dermal losses of vitamin B_{12} may also occur.

Recommended Dietary Allowance

Recommendations for vitamin B_{12} intake are based on estimates of the vitamin's intake and turnover, and on amounts of the vitamin needed for the maintenance of normal serum vitamin indices and hematological status. The RDA for adults for vitamin B_{12} is 2.4 μg per day, although intakes of 4–20 μg per day have been recommended [2,3,6,7]. Increases of 0.2 μg and 0.4 μg per day above the RDA are suggested for women during pregnancy and lactation, respectively [2]. The requirement for the vitamin for adults is 2.0 μg/day [2]. People age 51 years and older are counseled to consume foods fortified with the vitamin or consume vitamin B_{12} supplements (usually between 25 and 100 μg per day) because 10–30% of older people have changes to the gastrointestinal tract that limit their ability to absorb food-bound forms of the vitamin [2]. The inside front cover of the book provides additional recommendations for vitamin B_{12} intake for other age groups.

Deficiency: Megaloblastic Macrocytic Anemia

Deficiency of vitamin B_{12}, like that of folate, results in megaloblastic macrocytic anemia, as described in the folate deficiency section. The condition occurs with vitamin B_{12} because of the development of the methyl-folate trap, also previously discussed in the "Folate" section in the "Interactions with Other Nutrients" subsection. Consequently, with a vitamin B_{12} deficiency, DNA synthesis becomes deranged, along with cell differentiation and maturation; and these events negatively impact cells, especially those with rapid turnover such as blood cells.

Manifestations of vitamin B_{12} deficiency occur in stages. Initially, serum vitamin B_{12} concentrations diminish; serum B_{12} concentrations, however, may remain normal until vitamin stores become depleted. Second, cell concentrations of the vitamin diminish, affecting the activities of both vitamin B_{12}–dependent enzymes. DNA synthesis decreases and plasma homocysteine and methylmalonic acid concentrations increase. Finally, morphological and functional changes occur in blood cells, resulting in megaloblastic macrocytic anemia.

Most of vitamin B_{12}'s deficiency signs and symptoms affect the body's hematologic and neurologic systems, although there is emerging evidence that deficiency of vitamin B_{12} may be involved in the etiology of neural tube defects in developing embryos [8]. Initially, mild deficiency may be associated with fatigue, skin pallor, and some hematological effects (anemia and low blood leukocyte and thrombocyte counts), and cardiorespiratory effects such as shortness of breath and palpitations. Neurologic problems, which may be irreparable, are manifested by clumsiness, poor coordination, numbness and/or pain in extremities, abnormal gait, increased loss of coordination, loss of a sense of relative position (proprioception), loss of vibration sense or touch in the ankles and toes, swelling of myelinated fibers, and demyelination, along with irritability, memory loss, disorientation, hallucinations, psychosis, and dementia. The mechanisms of the neuropathy and demyelination have not been clearly elucidated. The neurological problems, which occur in about 75–90% of those with vitamin B_{12} deficiency, are not responsive to folate therapy. About 20% of individuals display neurological signs but not anemia [9].

Vitamin B_{12} deficiency is especially prevalent with aging, and may affect as many as 15% of older adults. The vitamin deficiency, however, can result from several situations (not just aging), as listed hereafter.

- *Inadequate intake* is most likely to occur in a strict vegetarian (vegan), especially in an infant or young child with minimal stores of the vitamin.
- *An altered (too high/alkaline) gastric pH* may occur due to diminished *(hydrochlorhydria)* or absent (**achlorhydria**) hydrochloric acid production by parietal cells. Parietal cell functions are diminished with conditions such as *atrophic gastritis* (characterized by a loss and inflammation of gastric cells) and *pernicious anemia* (an autoimmune condition in which the body produces antibodies that attack the gastric parietal and mucosal cells). *Medications*—H_2 blockers and proton pump inhibitors—for the treatment of ulcers and gastroesophageal reflux disease also diminish hydrochloric acid production to increase gastric pH. Such changes in pH impair vitamin B_{12} release from food protein.
- *Parietal cell destruction causing insufficient intrinsic factor* may result from *atrophic gastritis*, *pernicious anemia*, and other conditions. Without sufficient intrinsic factor produced by the parietal cells and present in the intestine, carrier-mediated vitamin B_{12} absorption is impaired.

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- *Altered (too low/acidic) duodenal pH* may occur with *impaired pancreatic exocrine function* (which causes insufficient release of bicarbonate) as well as with *Zollinger-Ellison syndrome*. Zollinger-Ellison syndrome occurs with the presence of a gastrin-producing tumor and results in excessive hydrochloric acid production secondary to the high gastrin (remember gastrin stimulates the parietal cells to release hydrochloric acid). The overproduction of acid with Zollinger-Ellison syndrome and the underproduction of bicarbonate with pancreatic insufficiency negatively impact the duodenum, lowering its luminal pH. The lower than normal pH in the duodenum impairs the release of vitamin B_{12} from R protein. If the vitamin is not released from R protein in the duodenum, it cannot bind to intrinsic factor.
- *Impaired intestinal integrity or function*, especially if affecting the ileum, occurs with celiac disease and Crohn's disease (among other conditions) and may decrease the absorptive surface and prevent the vitamin from binding to receptors in the ileum. Intact ileal cells are needed for receptor-mediated absorption of the vitamin-intrinsic factor complex.
- *Resection of portions of the stomach and/or small intestine* reduces the secretions and the sites needed for the digestion and absorption of the vitamin.
- *Competition*. People with *parasitic infections* such as tapeworms may develop a vitamin B_{12} deficiency because the parasite uses the vitamin and consequently limits the vitamin's availability to the infected person. Similarly, the prolonged use of H_2 blockers and proton pump inhibitors (used to treat ulcers and gastroesophageal reflux disease) is associated with diminished absorption of vitamin B_{12} because of *bacterial overgrowth*. Bacterial overgrowth in the more alkaline intestinal environment occurs when the medication diminishes acid production. Furthermore, the bacteria use vitamin B_{12} for their own growth, which limits the vitamin's availability to the individual. Treatment with antibiotics is usually needed to retard bacterial overgrowth and subsequently improve vitamin absorption and status.
- *Use of nitrous oxide* (an anesthetic agent), primarily in people who have poor vitamin B_{12} status, may result in deterioration of nervous system function, especially demyelination problems. Mechanisms by which nitrous oxide alters vitamin B_{12} metabolism and induces deficiency are under investigation but may involve inactivation of methionine synthase [10].

Vitamin B_{12} deficiency due to inadequate intake of the vitamin and without neurologic symptoms is typically treated with up to 1 mg vitamin B_{12} for the first week or so, followed by a slightly lower dose of the vitamin for about 1 or 2 months. Treating pernicious anemia or deficiency secondary to malabsorption often requires monthly intramuscular injections of vitamin B_{12} in amounts of 500–1,000 μg, or oral ingestion of pharmacologic amounts (1,000–2,000 μg) of the vitamin [2]. Vitamin B_{12} nasal sprays are also available. Nascobal®, for example, provides the vitamin as cyanocobalamin (500 μg/spray) in a nasal spray that is beneficial to people with malabsorptive disorders. A hematological response to supplementation may take up to 2 months; however, improvements in some parameters (such as the reticulocyte count) may be evident within about 10 days of beginning treatment. Serum methylmalonic acid concentrations may also start to diminish within the first week of supplementation [10].

Toxicity

Although no clear toxicity from massive doses of vitamin B_{12} has been reported, neither has any benefit been noted from an excessive intake of the vitamin by people with adequate vitamin status [2]. No Tolerable Upper Intake Level for vitamin B_{12} has been established [2].

Assessment of Nutriture

Vitamin B_{12} status may be assessed using several indices. Serum vitamin B_{12} concentrations, which include cobalamin bound to TCI, TCII, and TCIII, are commonly measured and reflect both intake and status. Increases in plasma holotranscobalamin TCII concentrations provide an indication of vitamin B_{12} absorption. Below normal serum holotranscobalamin concentrations (less than about 20 pmol/L) thus suggest inadequate absorption [11]. What is considered as "normal" serum vitamin B_{12} concentrations vary, but concentrations less than about 200 pg/mL (based on a radioassay method) are generally considered deficient, while those between 200 and 300 pg/mL suggest borderline deficiency [9,12]. However, because serum vitamin B_{12} concentrations can be maintained at the expense of tissues, a person may exhibit normal serum concentrations but have low tissue concentrations. Thus, assessment that includes use of indices in addition to serum concentrations is beneficial.

Measurement of methylmalonic acid is also used to assess vitamin B_{12} status, and may be the most representative marker of metabolic insufficiency [9]. Normally, no or only trace amounts of methylmalonic acid are excreted in the urine; however, with vitamin B_{12} deficiency, methylmalonic acid concentrations increase in the serum (greater than ~350 or 400 nmol/L) and excretion exceeds about 300 mg per day [9,11]. A breath test to assess vitamin B_{12} status has also been developed; carbon dioxide concentrations in the breath are measured following ingestion of labeled

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propionate. Deficiency of the vitamin is indicated by subnormal production of labeled carbon dioxide.

Other tests used to assess vitamin B_{12} nutriture include the deoxyuridine suppression test, discussed previously in the "Assessment of Nutriture" subsection of the "Folate" section, and the Schilling test. The Schilling test involves orally administering radioactive vitamin B_{12} and measuring urinary excretion of the vitamin. Below-normal urinary excretion of the vitamin suggests impaired absorption. In lieu of the Schilling test, the presence of antibodies to intrinsic factor and/or parietal cells may be directly measured in the blood as an indicator of an autoimmune response and pernicious anemia.

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Suggested Readings

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VITAMIN B_6

Vitamin B_6 was isolated in 1934 and its structure confirmed in 1939. Some of the initial research was aimed at correcting dermatitis in rats. Kuhn and Szent-Györgyi are credited with isolating the vitamin (which was called pyridoxine due to its structural homology to pyridine) in 1938 that cured the dermatitis. The pyridoxal and pyridoxamine forms of the vitamin were identified in the mid-1940s.

Vitamin B_6 exists as six vitamers, the structural formulas of which are given in Figure 9.35. These vitamers are interchangeable and comparably active. Pyridoxine represents the alcohol form, pyridoxal the aldehyde form, and pyridoxamine the amine form. Each has a 5′-phosphate derivative (i.e., phosphorylated form); it is some of these phosphorylated vitamers that function as coenzymes in the body.

Figure 9.35 Vitamin B_6 structures.

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Sources

All B_6 vitamers are found in food. Pyridoxine, the stablest of the compounds, and its phosphorylated form are found almost exclusively in plant foods. In some plants, the pyridoxine may be conjugated and present as a glycoside. Pyridoxal and pyridoxamine and their phosphorylated derivatives are found primarily in animal products.

The main sources of vitamin B_6 (Table 9.13) are animal products, especially beef, fish, pork, and chicken. Of the plant foods, whole-grain products, some vegetables (such as potatoes), some fruits (e.g., bananas), and nuts as well as fortified cereals represent major contributors of vitamin B_6 in the diet. Cheerios®, for example, provides 25% of the Daily Value of 2 mg, or 0.5 mg/cup. Most multivitamins provide the vitamin in amounts similar to the Daily Value, and the form of vitamin B_6 in supplements and in fortification of foods is generally pyridoxine hydrochloride.

The bioavailability of vitamin B_6 from foods is influenced by the food matrix and by the extent and type of processing to which the foods are subjected. The vitamin is fairly stable with cooking; however, much of the vitamin originally present in foods can be lost if exposed to prolonged high heat (especially with sterilizing and canning). Vitamin losses from plant foods are generally less than from animal products. Loss of the vitamin also may occur with food storage as well as with milling and refining of grains.

Table 9.13 Vitamin B_6 Content of Selected Foods*

Food (serving)	Vitamin B_6 (mg)
Liver, beef (3 oz)	0.9
Tuna, yellowfin (3 oz)	0.9
Salmon (3 oz)	0.5
Chicken, breast (3 oz)	0.6
Chicken, dark meat (3 oz)	0.3
Beef, sirloin (3 oz)	0.3
Pork, loin (3 oz)	0.4
Chickpeas, canned (1/2 c)	0.6
Banana (1)	0.4
Watermelon (1 c)	0.1
Spinach, cooked (1/2 c)	0.1
Broccoli, cooked (1/2 c)	0.1
Zucchini, cooked (1/2 c)	0.1
Carrots, raw (1 c)	0.1
Potato (1 medium)	0.6
Pecans (1 oz)	0.1
Nuts, mixed (1 oz)	0.1
Seeds, sunflower (1/4 c)	0.2
Bread, whole grain (1 sl)	0.1

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

Digestion, Absorption, Transport, and Storage

For vitamin B_6 to be absorbed, the phosphorylated vitamers must be dephosphorylated. Alkaline phosphatase, a zinc-dependent enzyme found at the intestinal brush border, or other intestinal phosphatases, hydrolyze the phosphate from the phosphorylated vitamers to yield free pyridoxine (PN), pyridoxal (PL), or pyridoxamine (PM).

At physiological intakes free pyridoxine, pyridoxal, and pyridoxamine are absorbed primarily in the jejunum by passive diffusion. Absorption of some pyridoxine glucosides may also occur by passive diffusion, although glucosidase within the intestinal cell typically hydrolyzes the glucosides to free the vitamin (pyridoxine). Little additional metabolism of the vitamin occurs within the intestinal cell, although some pyridoxine may be converted to pyridoxine phosphate (PNP) and pyridoxal phosphate (PLP). Most pyridoxine, pyridoxal, and pyridoxamine are released directly into portal blood. Overall absorption of vitamin B_6 is about 75%, with a range of about 61–92% [1].

The liver is the main organ that takes up (by passive diffusion) and metabolizes newly absorbed vitamin B_6. Figure 9.36 shows these reactions and other interconversions of the B_6 vitamers. Unphosphorylated forms of the vitamin typically are phosphorylated by a kinase using ATP within the cytosol of hepatocytes and other organs. PNP and PMP are then generally converted by the action of an FMN-dependent oxidase to the main vitamer and coenzyme form PLP; the oxidase that catalyzes this reaction is dependent upon adequate riboflavin (a coenzyme) and is found mainly in the liver and intestine and to lesser extents in the muscle, kidneys, brain, and red blood cells.

Intracellular PLP concentrations are dependent, in part, upon the availability of binding proteins. With saturation of binding proteins, unbound PLP is hydrolyzed to PL, which is released into the blood for use by other tissues. From the liver, mostly PLP and PL (with possibly smaller amounts of the other vitamers) are released into the blood for transport to extrahepatic tissues.

PLP and PL are the main (75–90% of the total) forms of the vitamin found in systemic blood. Most PLP in the plasma is bound to albumin but the absolute concentrations found in the plasma are very small, about 5–50 μg/L. Because it is the unphosphorylated vitamers that are usually taken up by body tissues, PLP is typically hydrolyzed by a phosphatase to PL prior to cellular uptake. Red blood cells, for example, take up PL and a kinase subsequently converts it to PLP; the PLP then binds to hemoglobin to prevent its degradation.

The body contains about 40–185 mg of vitamin B_6. The liver stores only about 5–10% of the vitamin. Muscles represent the major (75–80%) storage site where the vitamin is found, primarily as PLP bound to glycogen

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*Oxidase—riboflavin (FMN)-dependent.

Figure 9.36 Vitamin B_6 metabolism in the liver.

phosphorylase. Phosphorylation of the vitamin prevents its diffusion out of the cell, and the binding of the vitamin to protein (as occurred in the red blood cell) prevents hydrolysis by phosphatases. Other tissues with substantial amounts of the vitamin are the brain, kidneys, and spleen; in these tissues the vitamin is also found in its phosphorylated form, typically bound to enzymes in the cytosol and mitochondria.

Functions and Mechanisms of Action

The PLP form of vitamin B_6 functions as a coenzyme for over 100 enzymes, the majority of which are involved in nutrient (amino acid) metabolism but also affect the production of neurotransmitters, nucleic acids, heme, sphingolipids, carnitine, and glucose in the body. Vitamin B_6's noncoenzyme role affects gene expression.

Coenzymes

As a coenzyme in reactions involving amino acids, PLP, through the formation of a Schiff base (the product formed by an amino group and an aldehyde), labilizes all the bonds around the α-carbon of the amino acid. The specific bond that is broken is determined by the catalytic groups of the particular enzyme to which PLP is attached. The covalent bonds of an α-amino acid that can be made labile by its binding to specific PLP-containing enzymes are shown in Figure 9.37.

Some of the reactions involving amino acids that are catalyzed by PLP include transamination (which can also be catalyzed by PMP), dehydration (elimination)/deamination, decarboxylation, transulfhydration, transelenation, cleavage, racemization, and synthesis. In addition to its participation in reactions, vitamin B_6 functions by a different mechanism in the initial step of glycogen catabolism, important in the liver for glucose production. Each of these types of reactions is discussed briefly along with some examples related to nutrient metabolism.

Transamination Transamination reactions involve the transfer of an amino group ($^{-}NH_2$) from one amino acid to an α-ketoacid, and, like the deamination reactions discussed next, are important for the *synthesis of nonessential amino acids* and for the use of amino acid carbon skeletons for *energy and glucose production* (e.g., gluconeogenesis).

The most common aminotransferases for which PLP or PMP is a coenzyme are aspartic amino transferase (AST; also called glutamate oxaloacetate transaminase or GOT) and alanine aminotransferase (ALT; also called glutamate pyruvate transaminase or GPT) (see Figure 6.5 for the reactions catalyzed by these transaminases). Figures 9.38a and b show the two phases of transamination and demonstrate how the coenzyme forms a Schiff base. In the first phase, the corresponding α-keto acid of the amino acid is produced along with PMP. In the second phase, the transamination cycle is completed as a new α-keto acid substrate receives the amino group from the PMP. The corresponding amino acid is generated, along with regeneration of the PLP.

Dehydration, Elimination, or Deamination PLP also participates in reactions in which an amino group is removed from a compound such as an amino acid and released as

Figure 9.37 The covalent bonds of an acid that can be made labile by its binding to PLP-containing enzymes.

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(a)

(b)

Figure 9.38 (a) The role of vitamin B_6 in transamination, phase 1. (b) The role of vitamin B_6 in transamination, phase 2.

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ammonia or ammonium ion. Such reactions may be called dehydration, elimination, or deamination reactions. Threonine dehydratase is an example of a PLP-dependent enzyme that is involved in such a reaction, specifically removing water and the amino group from the amino acid threonine; the reaction is shown in Figure 6.6. These types of reactions produce substrates that, for example, may be further catabolized for energy production.

Decarboxylation Decarboxylation reactions involve the removal of the carboxy (COO^-) group from an amino acid or other compound. Many of these reactions are involved in the *production of neurotransmitters* for nervous system function and other body processes. Some examples of some common decarboxylation reactions include the formation of *γ-aminobutyric acid (GABA)* from the amino acid glutamate (see Figure 6.38), the production of *serotonin* from 5-hydroxytryptophan (see Figure 6.39), and the synthesis of *histamine* from the amino acid histidine (see Figure 6.14). *Dopamine* is formed following decarboxylation of dihydroxyphenylalanine, which is generated from the amino acid tyrosine (see Figure 6.10). *Taurine*, another neuromodulatory compound, is generated during cysteine metabolism in a vitamin B_6–dependent reaction (see Figure 6.12).

Transulfhydration PLP is required for two enzymes catalyzing reactions in the transulfhydration pathway in which *cysteine, a nonessential amino acid, is synthesized* from methionine. The two PLP-dependent enzymes in this pathway are cystathionine β-synthase and cystathionine lyase; both are shown in Figure 6.12, which depicts methionine transulfhydration and degradation.

Transelenation Similar to transulfhydration, selenomethionine may be converted through the transelenation pathway to selenocysteine (see Figure 13.17). γ-lyase, which is PLP dependent, directly cleaves the selenium from selenomethionine to generate selenide. Selenocysteine β-lyase, also PLP dependent, generates selenide from selenocysteine. Selenide is converted into selenophosphate, an important intermediate in the synthesis of the body's *selenium-dependent enzymes*, which serve in various *antioxidant roles*.

Cleavage An example of a cleavage reaction requiring PLP is the removal of the hydroxymethyl group from serine. In this reaction, PLP is the coenzyme for a transferase that transfers the hydroxymethyl group of serine to tetrahydrofolate (THF) to form glycine and another THF coenzyme (see Figure 9.28). This reaction is also significant for the *production of nucleic acids*, as discussed further under the section "Other Synthetic Reactions."

Racemization PLP is required by racemases that catalyze the interconversion of D- and L-amino acids. Although such reactions are more prevalent in bacterial metabolism, some occur in humans.

Other Synthetic Reactions Vitamin B_6 is also necessary as a coenzyme in the first step in the *synthesis of heme* (see Figure 13.7). Heme is needed not only for hemoglobin formation but also is a component of many enzymes. PLP is required for aminolevulinic acid synthetase, which catalyzes the condensation, followed by decarboxylation, of glycine with succinyl-CoA to form aminolevulinic acid (ALA). ALA is used next to synthesize porphobilinogen (PBG), the parent pyrrole compound in porphyrin synthesis. Through a series of reactions, PBG is converted into protoporphyrin IX, which, with the addition of Fe^{2+} by ferrochelatase, forms heme. Defects in heme synthesis from a vitamin B_6 deficiency can result in microcytic anemia (a problem usually only seen in infants, but accounts for vitamin B_6 sometimes being listed along with vitamins B_{12} and folate as being needed for hematopoiesis).

PLP functions as a cofactor for another condensation reaction necessary for *sphingolipid synthesis*. Specifically, the amino acid serine condenses with palmitoyl-CoA in a reaction catalyzed by a PLP-dependent transferase to form 3-dehydrosphinganine, a compound that serves as a precursor for sphingolipids. Sphingolipids have many roles in the body (see Chapter 5), including the production of myelin for nerve transmission.

In fatty acid metabolism, the PLP-dependent enzyme δ-6-desaturase catalyzes the synthesis of selected polyunsaturated fatty acids through desaturation of linoleic and γ-linolenic acids. Also related to lipid use in the body is vitamin B_6's coenzyme role in the synthesis of carnitine, a nitrogen-containing nonprotein required for *fatty acid oxidation* and thus *energy production*.

Niacin synthesis from tryptophan also requires an important PLP-dependent reaction. Specifically, kynureninase required for the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid requires vitamin B_6 (PLP) as a coenzyme (see Figure 9.15).

Nucleic acid production involves the vitamin B_6 (PLP)–dependent enzyme serine hydroxymethyltransferase. This enzyme transfers a one-carbon unit from serine to THF to generate 5,10-methylene THF and glycine. The 5,10-methylene THF in turn is used to synthesize thymidylate (dTMP) (pyrimidine), which is needed for DNA synthesis and to synthesize other forms of THF needed for the remethylation of homocysteine to methionine. Furthermore, in the mitochondria, the 5,10-methylene is used to produce 10-formyl THF, which upon hydrolysis generates formate for purine synthesis.

Glycogen Degradation Glycogen is catabolized by glycogen phosphorylase to form glucose-1-PO_4 (see Figure 3.15); vitamin B_6 is required for glycogen phosphorylase activity. The mechanism of action of the coenzyme appears to be

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different from that exerted with other enzymes. The phosphate of the coenzyme is believed to stabilize the compound and permit covalent bonding of the phosphate to form glucose-1-PO_4. Most vitamin B_6 found in muscle is present as PLP, which in turn is bound to glycogen phosphorylase. It is this role of the vitamin that is thought to account for the use of more than 50% of the body's vitamin B_6.

Noncoenzyme Role: Gene Expression

Although the coenzyme roles of vitamin B_6 have been more thoroughly investigated, PLP also affects gene expression. The vitamin modulates steroid hormone binding as well as transcription factor binding to regulatory regions on DNA. The vitamin affects the synthesis of albumin by interacting with DNA ligand–binding sites; such interactions suppress gene transcription and reduce albumin mRNA production [2]. Similar interactions from PLP binding to regulatory regions on DNA negatively impact mRNA production for a number of other proteins.

Selected Pharmacological Uses / Other Roles

Those possibly benefiting from pharmacological doses of vitamin B_6 include individuals with primary hyperoxaluria (type 1), which results from a mutation in the vitamin B_6–dependent enzyme alanine glyoxylate aminotransferase and increases the risk for oxalate kidney stone formation. Vitamin B_6 (in amounts up to about 20 mg/kg body weight per day) may improve residual enzyme activity (in some individuals) and reduce the production of oxalic acid that occurs secondary to the disorder. Some infants born with an inherited, intractable seizure disorder also have been shown to benefit from pharmacologic doses of vitamin B_6.

Homocysteinuria results from mutations in cystathionine β-synthase, a PLP-dependent enzyme. The enzyme is one of many needed to oxidize the amino acid methionine. Cystathionine β-synthase activity in many individuals with homocysteinuria (close to 50%) improves to some extent with vitamin B_6 supplementation. Recommended doses vary considerably, ranging from about 100–500 mg given orally; however, individuals responding to supplementation should be monitored for signs of toxicity (the Tolerable Upper Intake Level for adults is 100 mg/day).

Metabolism and Excretion

Vitamin B_6 is excreted primarily in the urine, with very little excreted in the feces. 4-Pyridoxic acid is the major urinary metabolite and results from the oxidation of pyridoxal by either NAD-dependent aldehyde dehydrogenase, found in all tissues, or FAD-dependent aldehyde oxidases, found in the liver and kidneys. Ingesting larger doses (about 100 mg) of the vitamin as pyridoxine may result in urinary excretion of intact pyridoxine and 5-pyridoxic acid, and lower urinary 4-pyridoxic acid excretion.

Recommended Dietary Allowance

The RDA for vitamin B_6 for adult men age 19–50 years is 1.3 mg per day (requirement 1.1 mg) and for men age 51 years and older, 1.7 mg per day (requirement 1.4 mg) [1]. For adult women age 19–50 years, the RDA for vitamin B_6 is also 1.3 mg per day (requirement 1.1 mg), and for women age 51 years and older it is 1.5 mg daily (requirement 1.3 mg) [1]. With pregnancy and lactation, recommendations for vitamin intake increase to 1.9 mg and 2.0 mg, respectively [1]. Recommendations are based largely on the maintenance of adequate plasma vitamin concentrations [1]. Some have suggested the recommendations need to be raised [3]. The inside front cover of the book provides additional recommendations for vitamin B_6 for other age groups.

Deficiency

Vitamin B_6 deficiency is relatively rare in the United States. In the 1950s, deficiency occurred in infants because of severe heat treatment of infant milk. The heat processing resulted in a reaction between the PLP and the epsilon amino group of lysine in the milk proteins to form pyridoxyl-lysine, with little vitamin activity. Signs and symptoms of vitamin B_6 deficiency (which can occur in as little as 2–3 weeks but may take up to ~2½ months) include a **seborrheic dermatitis**/rash on the face (cheeks and nasolabial fold), neck, shoulders, and buttocks areas; weakness; fatigue; cheilosis; glossitis; angular stomatitis; and neurological problems such as confusion, peripheral neuropathy, and (especially in infants) seizures and convulsions. A hypochromic, microcytic anemia (seen usually in infants) may also occur due to impaired heme synthesis. Deficiency also impairs niacin synthesis from tryptophan, and inhibits the metabolism of homocysteine, which may result in hyperhomocysteinemia, a risk factor for heart disease. While supplementation with vitamin B_6, folate, and vitamin B_{12} has been shown to lower plasma homocysteine concentrations, evidence showing lowered risk of heart disease is conflicting [4]. A vitamin B_6 deficiency is usually treated with daily oral supplements of the vitamin in amounts of 2.5–25 mg (or up to about 100 mg if needed) for a few weeks.

Groups particularly at risk for vitamin B_6 deficiency are older adults, who may have a poor intake of the vitamin and may also have accelerated hydrolysis of PLP and oxidation of PL; and people who consume excessive amounts of alcohol (alcohol can impair the conversion of pyridoxine and pyridoxamine to PLP, and the presence of acetaldehyde formed from alcohol metabolism enhances coenzyme degradation). Systemic inflammation appears to both alter tissue distribution and increase catabolism of vitamin B_6, suggesting higher needs for the vitamin in this situation. People on a variety of drug therapies may also

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be at risk. For example, isoniazid used to treat tuberculosis interferes with vitamin activity. Penicillamine used to treat some autoimmune conditions and Wilson's disease inactivates the vitamin. Corticosteroids, used to suppress the immune system in some inflammatory conditions such as Crohn's disease, promote the loss of the vitamin from the body, and anticonvulsants, used to diminish seizures, inhibit vitamin activity. Oral contraceptive use also may result in suboptimal vitamin B_6 status, perhaps by affecting the metabolism and/or altering tissue concentrations of the vitamin.

Toxicity

Pharmacological doses of vitamin B_6 have been advocated to prevent or treat a variety of states, including hyperhomocysteinemia, carpal tunnel syndrome, morning sickness, premenstrual syndrome, depression, and muscular fatigue. Although some beneficial results from megadoses of the vitamin have been noted with selected conditions, indiscriminate use of the vitamin is not without risk. Excessive pyridoxine use (>~200 mg/day but sometimes lower in some individuals) causes sensory and peripheral neuropathy. Some signs and symptoms include unsteady gait, paresthesias in the extremities, and impaired tendon reflexes. Intakes in excess of 2 g/day are usually associated with not only paresthesia in the feet and hands, but also impaired motor control or ataxia. High intakes also may cause degeneration of neurons (dorsal root ganglia) in the spinal cord, loss of myelination, and degeneration of sensory fibers in peripheral nerves [3]. The Tolerable Upper Intake Level for vitamin B_6 is 100 mg/day for adults to minimize the development of neuropathy [1]. Vitamin B_6 supplementation, at even low doses, is not recommended for individuals with Parkinson's disease who are being treated with L-dopa; the vitamin interferes with the effectiveness of drug therapy.

Assessment of Nutriture

Plasma PLP concentrations are thought to be the best indicator of vitamin B_6 tissue stores, with concentrations of less than about 20 nmol PLP/L suggestive of vitamin deficiency, concentrations of 20–30 nmol PLP/L suggestive of marginal status, and adequacy indicated by plasma concentrations > 30 nmol PLP/L [1]. Plasma vitamin B_6 concentrations also may decrease below normal, < 50 μg/L, with deficiency, but are not typically used to assess status.

Urinary vitamin B_6 (measured over several days for a period of 1–3 weeks) and urinary 4-pyridoxic acid are commonly used to assess the status of vitamin B_6. Urinary vitamin B_6 excretion of ~0.5 μM/day or ~20 μg/g creatinine and urinary 4-pyridoxic acid concentrations of ≤ 3.0 μM/day are thought to indicate deficiency [5]. Urinary 4-pyridoxic acid excretion, however, is considered to be a short-term indicator of vitamin B_6 status, and cutoff values are controversial [1].

A functional test for the vitamin measures xanthurenic acid excretion following tryptophan loading (2 g or 100 mg of tryptophan/kg body weight). Abnormally high xanthurenic acid excretion is found in vitamin B_6 deficiency because 3-hydroxykynurenine, an intermediate in tryptophan metabolism, cannot lose its alanine moiety and be converted to 3-hydroxyanthranilic acid, as should occur (see Figure 9.15). Instead, 3-hydroxykynurenine is converted to xanthurenic acid, which is excreted in the urine in greater than normal amounts—that is, > 25 mg/6 hours.

Measuring transaminase activity before and after adding vitamin B_6 is an additional technique for determining vitamin B_6 nutriture, especially longer-term vitamin status. However, because of a variety of limitations with the assays, these tests are better used as an adjunct to other tests. The erythrocyte transaminase index examines the activity of glutamic oxaloacetic transaminase (abbreviated GOT or AST) and glutamic pyruvic transaminase (abbreviated GPT or ALT) after the addition of vitamin B_6. Deficient vitamin B_6 status is suggested by GOT/AST activity of >1.85 following the addition of the vitamin and by GPT/ALT activity of >1.25 following the addition of the vitamin [5].

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GENETICS AND NUTRITION: THE EFFECT ON FOLIC ACID NEEDS AND RISK OF CHRONIC DISEASE BY DR. RITA M. JOHNSON

"Genomic medicine holds the ultimate promise of revolutionizing the diagnosis and treatment of many illnesses." [1]

Since the completion of the Human Genome Project over 10 years ago, the research in genetics and its impact on disease risk has exploded. Academic libraries stock books and research-based journals about topics that were only a dream a decade ago. Books and articles about genetics and disease risk are widely available from libraries, bookstores, magazines, and the World Wide Web. While interest in these findings continues to grow, in the past decade few advances directly related to humans and disease treatment have originated from the discovery of the human genome [2].

Similarly, the scientific literature and mass media also overflow with information about the relationship among food choices, an individual's genome, and disease risk. Nutrigenomics and nutrigenetics are increasingly discussed in the study of nutrition, and specialty career paths in these areas exist. The report *The Future of Nutrigenomics* states that an understanding of the interaction among our genes, our diets, and our individual differences will lead to both personalized nutrition and personalized medicine. In addition, the businesses that provide food and dietary supplements will change to meet these newly identified individualized needs [3]. Nutrigenetics, a substudy of nutrigenomics, studies the influence of a nutrient on a risk factor when the individual has a variation in the genetic code [4]. Nutrition professionals in the near future may need to both understand and practice nutrigenomics to provide evidence-based and individualized medical nutrition therapy for their clients' unique genetic traits [5].

Discoveries about folate metabolism provide an often-used example of nutrigenetics [6]. The identification of genetic variations in folate metabolism preceded the sequencing of the human genome, and these variations have been linked to neural tube defects, fetal malformations, coronary heart disease, colorectal cancer, dementia, and other health problems. The purposes of this perspective are to (1) describe the most common types of genetic variants in the enzyme methylene tetrahydrofolate reductase, (2) review the prevalence of the genetic variation in different ethnic groups, (3) briefly summarize the research that links these variants to disease risk, and (4) discuss the relationship between folic acid and choline requirements.

N^5, N^{10} METHYLENE TETRAHYDROFOLATE REDUCTASE AND ITS GENETIC VARIANTS

As shown in Figures 9.29 and 9.31, methylene tetrahydrofolate reductase (abbreviated as MTHFR) catalyzes the unidirectional conversion of 5,10-methylene THF to 5-methyl THF. The activity of MTHFR, along with adequate amounts of NADPH and $FADH_2$, is essential to maintain the concentration of 5-methyl THF in the cell. As discussed in Chapter 9, 5-methyl THF provides the methyl group for the synthesis of methionine.

If MTHFR activity is low, intracellular 5-methyl THF concentrations will decrease and an impaired conversion of homocysteine to methionine will result. A lack of methionine results in a lack of the methyl groups necessary for reactions that include the methylation of RNA and DNA and the synthesis of carnitine, creatine, epinephrine, purines, and nicotinamide. At the same time, an accumulation of homocysteine is believed to increase risk for cardiovascular disease and dementias. Clearly, impairing the formation of 5-methyl THF has an effect on the body's ability to synthesize methlyated products and remove homocysteine.

Many genetic variations of MTHFR have been reported. These variants are caused by substitutions in the DNA sequence that codes for the enzyme. When a variation is shared by more than 1% of the population, it is called a *genetic polymorphism* [6]. The genetic polymorphisms of MTHFR cause a decrease in its activity and the subsequent formation of 5-methyl THF. This Perspective discusses the most common MTHFR polymorphism, which is caused by a substitution of the nucleotide thymine (abnormal) for the nucleotide cytosine (normal) at position 677. Thus, the DNA contains a thymine when it should contain a cytosine. This polymorphism is abbreviated as MTHFR 677C ⟶ T or MTHFR 677C>T. The result of this alteration in the DNA sequence is that a molecule of valine (abnormal) is inserted into the amino acid sequence during the synthesis of MTHFR, instead of a molecule of alanine (normal) [7]. This polymorphism is an example of a single-nucleotide polymorphism (SNP), pronounced "SNIP." More than 3.7 million SNIPs have been identified [8].

The MTHFR 677C ⟶ T variant can be heterozygous or homozygous. An individual with the heterozygous genotype, abbreviated 677CT, has one normal and one abnormal allele, while someone with the homozygous genotype, abbreviated 677TT or 677CC, has either two abnormal or two normal alleles, respectively [5,7]. The homozygous individual with two abnormal alleles (677TT) synthesizes MTHFR that is only 30–35% as active as that of the homozygous individual with the unaltered 677CC genotype, who is considered to have the normal or "wildtype" MTHFR activity [9,10]. Someone with the heterozygous (677CT) genotype exhibits 70% of the normal MTHFR activity [11].

The individual with the 677TT genotype may have an increased plasma homocysteine level and a decrease in the methylation reactions described above. Research has determined that these individuals have increased hypomethylation of DNA, lower serum and RBC folate levels, and an increased risk of neural tube defects [12]. This increased disease risk is less associated with the 677CT heterozygote, and most research has studied the 677TT homozygote. Since these polymorphisms in MTHFR were identified in the early 1990s, many researchers have investigated the folic acid needs of individuals who do not have normal MTHFR activity and the effect of intake on their individualized risk of chronic diseases. Thus, the identification of the 677C ⟶ T MTHFR polymorphism has implications related to the individualization of nutrition care.

ETHNIC DIFFERENCES IN GENETIC VARIATIONS IN MTHFR AND FOLATE NEEDS

Data from three studies show that the 677TT homozygous genotype is distributed by ethnic groups. Africans, African Americans, and Asians (but not Japanese) have the lowest prevalence of less than 5%. The occurrence of the 677TT genotype in Northern Europeans, Japanese, and Caucasian Americans is between 9% and 14%. The greatest prevalence is reported in Italians (18%), Hispanics living in California (21%), Mexican women (18%), and Mexicans (36%) [9,10,13].

While the literature infers a differing risk for disease in those with the 677TT genotype, it is not clear that these individuals have greater folic acid requirements. Using a depletion/repletion design with Mexican women that had either the CC (normal), CT (heterozygote), or TT (homozygote) genotype, Guinotte et al. [11] measured serum folate, RBC folate, plasma homocysteine, and urinary folate excretion. The subjects all showed moderate folate deficiency after the 7-week depletion phase and returned to folate sufficiency after 7 weeks of repletion with 400 µg DFE/day, with no differences in plasma homocysteine. These data support the supposition that the RDA of 400 µg DFE met the needs of these subjects.

Not all research shares these findings, however. In a study of 126 healthy subjects with different MTHFR genotypes, the 677TT subjects had similar plasma folate, but higher plasma homocysteine levels than the CT or CC subjects when

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consuming a folate-rich diet that contained an average of 660 μg DFE/day [14]. These differences in plasma homocysteine levels disappeared when a folic acid supplement was added, boosting folate intake to an average of 814 μg DFE/day [14]. Based on these data and the assumption that serum homocysteine reflects folate status, Ashfield-Watt et al. concluded that the 677TT subjects needed between 575 and 830 μg DFE/day.

Hung et al. [15] reported similar results in their study of 32 women with either the 677TT or 677CC genotype. With all folate coming from food, one group consumed 400 μg/d and the other 800 μg/d for 12 weeks. While the 677CC group had significantly higher serum folate and red blood cell folate and lower serum homocysteine than the 677TT group, the authors summarized that a diet containing 800 μg/d improves the folate status of both groups [15].

Solis et al. [16] also challenged the adequacy of the RDA of 400 μg DFE/d for individuals with the 677TT genotype. In a carefully controlled feeding study of male Mexican Americans, 29 with the 677TT and 31 with the 677CC genotypes, a diet that provided 438 μg/d was consumed by the subjects for 12 weeks. Both groups started the study with mean folate status measures within normal values and not significantly different from one another. At the end of the study all subjects had decreased serum and red cell folate levels, but the 677TT subjects had significantly lower levels than the 677CC subjects. Similarly, plasma homocysteine levels were significantly different, with the 677CC subjects remaining relatively stable, while the 677TT subjects experienced a dramatic increase, with 23 of the 29 subjects having high plasma homocysteine levels. These data indicate that the RDA of 400 μg DFE/day does not meet the needs of Mexican American men of either genotype [16].

Results from a large clinical trial of 932 northern Chinese women of childbearing age showed that even after ingesting a folic acid supplement of 4000 μg/day for 6 months, 21% of the 327 subjects with the 677TT genotype had a high plasma homocysteine level (defined as > 10.4 μmol/L) [17]. Overall, the indicators of folate status of the 677TT subjects who received the supplement significantly increased (serum and red cell folate) or decreased (plasma homocysteine), but none reached the levels of the 677CC subjects. These results support the idea that these Chinese women with the 677TT genotype have much higher needs than others [17].

An analysis of data collected from 6,793 NHANES III subjects, however, leads to different conclusions for the U.S. population [18]. NHANES III, conducted between 1991 and 1994, used a complicated subject selection process to choose a representative sample of Americans. Importantly, NHANES III occurred prior to the 1998 folic acid fortification of flour and grain products in the United States and Canada. The NHANES III data showed that there were no differences in serum folate between the 677TT and 677CC genotypes when folic acid intake exceeded 400 μg/d. Furthermore, when the authors divided folate intake into quintiles, the three quintiles of 677TT subjects that consumed ≥308 μg/ day had mean serum homocysteine values that were within normal levels. These authors conclude that even before fortification, the combination of diet and supplemental folic acid was already protecting the 677TT individual and that fortification "may have attenuated the effect of the polymorphism" [18].

In conclusion, the MTHFR 677TT polymorphism has its greatest impact on folate status when folate and folic acid intakes are low and supplements are not taken. The fortification policies of the United States and Canada ensure that folic acid intake has increased since 1998.

MTHFR VARIATIONS AND RISK FOR CHRONIC DISEASE

Since the identification of the MTHFR polymorphisms, a great deal of research has investigated and reported risks for neural tube defects, cardiovascular disease, colorectal cancer, and dementias in people with poor folate status [12,19]. This research has an extra dimension when the MTHFR polymorphisms are also considered.

Neural Tube Defects (NTDs)

The impact of folic acid fortification in the United States, Canada, and other nations has been widely reported as beneficial. Different studies of the U.S. population ". . . reported an 11–20% decline in anencephaly and a 21–34% reduction in the occurrence of spina bifida . . ." [20, p 10]. Mills and Carter [21] reported that the Canadian health care system provided more complete data about neural tube defects and found a 50% reduction in NTDs after a folic acid fortification program similar to that in the United States was implemented.

Botto and Yang [13] pooled data from 15 studies from Europe and North America. They calculated the odds ratio of parents having the 677TT genotype to their children having an NTD and found a significant relationship. The increased risk is more related to the mother's genotype than the father's. But, as discussed above, this increased risk does not mean that a woman with the 677TT genotype will automatically have poor folate status. A diet rich in folate and folic acid can normalize folate status in most people with the 677TT genotype. Thus, it is very important to include questions about specific folate-rich foods, folic acid–fortified foods, and folic acid supplements when assessing an individual's intake.

Coronary Heart Disease

While the increased risk for coronary heart disease (CHD) in individuals with hyperhomocysteinemia is well documented in case-control studies, large prospective trials have not found the same relationship. It's not clear if hyperhomocysteinemia is a cause or a predictor of heart disease [22]. This uncertainty is reinforced by the general finding that individuals with the 677TT genotype have higher plasma homocysteine levels, but do not have a greater risk of cardiovascular disease. A meta-analysis of 80 studies including 26,000 subjects with the 677TT genotype and 31,813 677CC control subjects reported a small increase in the chances of 677TT subjects developing CHD. However, "This meta-analysis provides no evidence of a causal relation between homocysteine and heart disease risk in European, North American, and Australian populations" [23].

Dementias

If homocysteine accumulates inside cells, the cells will evict this metabolic oxidant into the bloodstream. Homocysteine crosses the blood–brain barrier and is theorized to be neurotoxic and cause increased oxidative stress [24]. Many people with dementias also have high serum homocysteine levels and these are positively correlated with lesions in the brain. Since research indicates that high plasma homocysteine is a risk factor for dementias and other cognitive problems (depression, psychosis), it logically follows that individuals with MTHFR genetic polymorphisms are at a greater risk. However, this relationship has not been verified [19,24].

Using a meta-analysis design, Ho et al. [25] identified 1,432 articles that met the search criteria, but used only 17 that provided enough data deemed necessary by the authors. They report that data from thousands of subjects do not support a causal relationship between high serum homocysteine and a later diagnosis of dementia. The genotype of the subjects is not mentioned. However, another meta-analysis that compared subjects with (n = 672) and without n = 1,038 vascular dementia (the most common type of dementia after Alzheimer's disease) noted their MTHFR 677C ⟶ T genotype. Six studies included Caucasian subjects, while five were of Asian subjects. The overall odds ratio for vascular dementia among those with the 677TT genotype was 1.41, showing an increased risk, and the condition was significantly associated with the 677TT genotype for the Asian subjects [26].

Colorectal Cancer

Poor folate status is positively correlated with risk of colorectal cancer. This can be theoretically explained because a decrease in folate leads to DNA hypomethylation (due to a lack of 5-methyl THF) and increased uracil (instead of thymine) incorporation into DNA. In addition, DNA hypomethylation negatively affects gene expression. The presence of uracil increases the activity of DNA repair mechanisms, but the repair may not be totally effective. In summary, poor folate status increases the risk of cancer due to the combination of DNA hyopmethylation and increased uracil incorporation [12].

But this seemingly straightforward relationship of poor folate status to an increased risk of colorectal cancer does not seem to hold when the MTHFR polymorphism is considered. The 677TT genotype, thought to increase risks for other diseases, may actually protect individuals from colorectal cancer. In a recent meta-analysis, Taioli et al. [27] sought to include all published and unpublished studies conducted since the original report about MTHFR 677TT individuals' decreased risk of colon cancer. Twenty-nine of the identified 199 articles that met the inclusion criteria for the study included 11,936 cases of colorectal cancer and 18,714 control subjects. Results from this statistical analysis showed that the 677TT genotype was related to a reduced risk of colorectal cancer, even when smoking, body mass index, and moderate alcohol intake (not alcohol abuse) were considered. When an odds ratio was calculated based on ethnic groups, whites and Asians with the 677TT genotype still had a reduced colorectal cancer risk, but Latinos and blacks did not. This may be due to few studies being done in these two groups.

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These authors acknowledge the absence of information about the folic acid intake of any of the subjects.

Readers may wonder why this imbalance in thymine synthesis and DNA methylation might protect against colorectal cancer in the 677TT homozygote. Researchers state that the reported differences may be due to chance, but several meta-analyses share similar findings about this relationship. Perhaps the body's access to methyl groups, which are available from dietary methionine or choline, may explain the relationship.

THE RELATIONSHIP BETWEEN THE MTHFR 677C ⟶ T VARIANT AND CHOLINE STATUS

In 1998 the Institute of Medicine recognized the Adequate Intake (AI) of choline as between 425 and 550 mg/d for adults. Humans obtain choline by ingesting foods rich in phosphatidylcholine (abundant in milk, liver, eggs, and peanuts) or through *de novo* synthesis. In the body, choline is degraded to betaine, which functions as a methyl donor. In fact, the methyl group needed to convert homocysteine to methionine can come from either 5-methyl THF or betaine. How does good or poor choline status affect methylation reactions in people with the MTHFR 677C ⟶ T polymorphism?

Shin and coauthors [28] fed Mexican-American men with the 677TT (n=29) or 677CC (n=31) genotype a diet containing the RDA of folate combined with 300, 550, 1,100, or 2,200 mg/d of choline for 12 weeks. A variety of "functional biomarkers of 1-carbon metabolism" including plasma S-adenosylmethionine (SAM) and DNA methylation were measured. The results showed no difference in SAM levels, but the 677TT subjects needed 1,100 or 2,200 mg/d of choline to maintain DNA methylation and to prevent an increase in DNA damage. The authors acknowledge that their results do not automatically apply to health or a decrease in disease risk; they urge continued investigation into this relationship.

SUMMARY

This brief discussion about the effect of MTHFR polymorphisms currently has limited application to the general public for two reasons. First, there is currently little individualized genetic mapping of the population. A list of companies that do assay for the MTHFR variant is available from the Gene Tests page from the National Center for Biotechnology Information [29]. Second, the impact of an MTHFR polymorphism on an individual's health or on that of his or her children is not clear. This is especially true in the United States and Canada, where grain products are fortified with folic acid. However, research findings do strongly suggest that individuals with the 677TT MTHFR genotype should eat foods rich in folate, folic acid, and choline.

These two current limitations will decline with enhanced technology and continued research. Future nutrition professionals will probably consider MTHFR polymorphisms, among many other genetic traits, in their assessments and recommendations. Understanding how slight alterations in the human genome might affect nutrient needs and disease risk sheds light into the future provision of personalized nutrition care.

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10 FAT-SOLUBLE VITAMINS

VITAMIN A AND CAROTENOIDS
VITAMIN D
VITAMIN E
VITAMIN K
For each vitamin, the following subtopics (when applicable) are discussed:
Sources
Digestion and Absorption
Transport, Metabolism, and Storage
Functions and Mechanisms of Action
Interactions with Other Nutrients
Metabolism and Excretion
Recommended Dietary Allowance or Adequate Intake
Deficiency
Toxicity
Assessment of Nutriture
PERSPECTIVE
ANTIOXIDANT NUTRIENTS, REACTIVE SPECIES, AND DISEASE

THIS CHAPTER ADDRESSES EACH of the four fat-soluble vitamins—A, D, E, and K—and the carotenoids. Like the water-soluble vitamins (discussed in Chapter 9), the fat-soluble vitamins have several similar characteristics. Their absorption and transport, in contrast to those of the water-soluble vitamins, are closely associated with the absorption and transport of ingested lipids. Thus, digestion and absorption are greatest when some dietary fat is present with the fat-soluble vitamins in the digestive tract and when there is sufficient production and delivery of bile as well as pancreatic enzymes and secretions. Absorption of the fat-soluble vitamins occurs most rapidly from the duodenum and jejunum (proximal small intestine), but quantitatively, absorption appears to be greatest from the jejunum, as destruction or resection of the jejunum is often associated with fat-soluble vitamin deficiencies. Additionally, destruction or resection of the ileum disrupts the enterohepatic recirculation of bile; in such cases, the synthesis of new bile often cannot compensate for the loss of bile in the feces, causing malabsorption of fat-soluble vitamins and fat. The enterocyte also must be functional to synthesize the chylomicrons, which initially transport all fat-soluble vitamins along with fat out of the intestine and into the lymphatic system. Also unlike water-soluble vitamins, the fat-soluble vitamins are stored in greater quantities in body tissues—mainly in the liver, adipose, and/or cell membranes, although the amount stored varies widely among the fat-soluble vitamins. Table 10.1 provides an overview of the functions, deficiency syndrome, food sources, and Recommended Dietary Allowance (RDA) or Adequate Intake (AI) of each of the fat-soluble vitamins. The RDAs and AIs for all nutrients and for all age groups are provided on the inside front cover of the book.

As with the discussion of the water-soluble vitamins, each of the fat-soluble vitamins is considered (when information is available) in terms of structure, sources, absorption (also digestion where applicable), transport, storage, functions and mechanisms of action, metabolism and excretion, recommended intake, deficiency, toxicity, and assessment of nutriture. Specific interrelationships with other nutrients are also noted for selected vitamins. Table 10.2 provides an overview of some of the manifestations associated with fat-soluble vitamin deficiencies. Yet, while treatment of deficiency (especially if severe) is typically best accomplished with supplements, it is consumption of diets rich in fruits, vegetables, and whole grains (and not ingestion of supplements) that is most often associated with reduced risk of diseases [1–6].

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Table 10.1 The Fat-Soluble Vitamins: Function, Food Sources, and Recommended Dietary Allowance (RDA) or Adequate Intake (AI)

Vitamin	Biochemical or Physiological Function	Food Sources	RDA or AI
Vitamin A Retinol, retinal, and retinoic acid Provitamin Carotenoids	Synthesis of rhodopsin; cell growth, cell differentiation; bone development; and immune function Antioxidant	Liver, dairy products, and fortified foods Sweet potato, carrots, spinach, butternut squash, greens, broccoli, and cantaloupe	900 µg RAE[a] 700 µg RAE[b]
Vitamin D D_2 –ergocalciferol and D_3 –cholecalciferol Provitamin – 7-dehydrocholesterol	Regulator of bone mineral metabolism, blood calcium homeostasis, cell differentiation, proliferation, and growth	Fatty fish and their oils, and fortified foods	15–20 µg[c, d]
Vitamin E Tocopherols and tocotrienols	Antioxidant	Vegetable oils, nuts, and seeds	15 mg α-tocopherol[c]
Vitamin K Phylloquinones and menaquinones	Activates blood-clotting factors and proteins in bone by γ-carboxylating glutamic acid residues	Vegetables, especially leafy vegetables, and legumes	120 µg[a, e] 90 µg[b, e]

[a] Adult males
[b] Adult females
[c] Both males and females
[d] Varies with age for adults; see text
[e] Adequate intake

Table 10.2 Some Common Signs Associated with Fat-Soluble Vitamin Deficiencies

System/Sites	Common Selected Signs	Possible Vitamin Deficit
Skin	Rough, red, "bumpy" skin, keratinization of epithelium	A
Muscle	Weakness, pain	D
Skeletal Bone	Excessive bone growth Inadequate bone mineralization, pain	A D, K
Blood vessels/ cells	Red blood cell fragility Hemolytic anemia Prolonged blood clotting	E E K
Ocular Retina	Pigmented retinopathy Nyctalopia, night blindness	E A
Conjunctiva	Xerosis, Bitot's spots	A
Cornea	Xerosis, ulcerations/keratomalacia	A

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VITAMIN A AND CAROTENOIDS

Vitamin A was initially found to be an essential growth factor in animal foods and was called fat-soluble A. McCollum and Davis, followed by Osborne and Mendel, are credited with its discovery in about 1915. Today, the term *vitamin A* (also called preformed vitamin A or retinoids) is generally used to refer to a group of compounds that possess the biological activity of all-*trans* retinol. The retinoids are structurally similar and include retinol, retinal, retinoic acid, and retinyl ester, as well as synthetic analogues. Structurally, retinoids contain a β-ionone ring and a polyunsaturated side chain with either an alcohol group (retinol, shown in Figure 10.1a), an aldehyde group (retinal, also called retinaldehyde, Figure 10.1b), a carboxylic acid group (retinoic acid, Figure 10.1c), or an ester group (retinyl ester [Figure 10.1d], such as retinyl stearate or palmitate [Figure 10.1e]). The side chain is made up of four **isoprenoid** units with a series of conjugated double bonds. The double bonds may exist in a *trans* (as in all-*trans* retinol) or a *cis* configuration.

Carotenoids are red, orange, and yellow lipid-soluble pigments found mainly in plants. Of the over 600 carotenoids found in nature, less than about 10% are in commonly consumed foods, and less than 20 are found in human blood and tissues. Structurally, carotenoids consist of an expanded carbon chain containing conjugated double bonds, with usually but not always an unsubstituted β-ionone ring at one or both ends of the chain. Three carotenoids, which can be converted into retinal in the body, are known as provitamin A carotenoids and include β-carotene (Figure 10.1f), α-carotene (Figure 10.1g), and β-cryptoxanthin (Figure 10.1h); these carotenoids are found most often in the all-*trans* form but can occur as *cis* isomers. Of the three,

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β-carotene exhibits the greatest amount of provitamin A activity. Some other important carotenoids, while not vitamin A precursors, include lycopene (an open-chain analog of β-carotene; Figure 10.1i) and many oxycarotenoids (also called oxygenated carotenoids or xanthophylls), such as canthaxanthin (Figure 10.1j), lutein (Figure 10.1k), and zeaxanthin (discussed later under "Functions").

Sources

Both preformed vitamin A (retinoids) and carotenoids are found naturally in foods. Vitamin A (preformed) is found primarily in selected foods of animal origin, especially liver, dairy products (including milk, cheese, and butter), eggs, and fatty fish and their oils (such as tuna, sardines, and herring) (Table 10.3). Some products, such as margarine and breakfast

Table 10.3 Vitamin A and β-carotene Contents of Selected Foods*

Food (serving)	Vitamin A (μg)	Food (serving)	β-carotene (mg)
Liver, beef (3 oz)	6,600	Spinach, cooked (1/2 c)	6.9
Herring (3 oz)	220	Carrots, cooked (1/2 c)	6.5
Milk (1 c)	150	Collards, cooked (1/2 c)	5.8
Margarine (1 tbsp)	150	Carrot, raw (1 medium)	5.1
Sardines (3 oz)	92	Cantaloupe (1 c)	3.2
Egg (1)	84		
Cheeses (1 oz)	60–85		
Tuna (3 oz)	55		
Herring oil (1 tbsp)	13.6		
Cod liver oil (1 tbsp)	13.5		

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov. Also see http://lpi.oregonstate.edu/mic/dietary-factors/phytochemicals/carotenoids.

Figure 10.1 Vitamin A and carotenoid structures. *(Continued on next page)*

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(h) β-cryptoxanthin

(i) Lycopene

(j) Canthaxanthin

(k) Lutein

Figure 10.1 *(Continued)* Vitamin A and carotenoid structures.

cereals, are fortified with vitamin A. Wheat bran cereal with raisins (1 c), for example, provides 15% of the Daily Value of vitamin A (which is 5,000 IU) or 750 IU (225 μg). In the United States, foods containing preformed vitamin A (versus carotenoids) contribute most to vitamin intake. Retinoids can undergo oxidation if exposed to varying degrees of, for example, oxygen, light, heat, and some metals.

The main form of preformed vitamin A in foods is as retinyl esters in which the vitamin is attached to a long-chain fatty acid such as palmitate (Figure 10.1e). Pharmaceutical vitamin preparations typically contain all-*trans* retinyl acetate and all-*trans* retinyl palmitate. Aquasol A, a water-miscible form of the vitamin, is available for people with fat malabsorptive disorders.

Carotenoids are synthesized by a wide variety of plants. The most common dietary carotenoids are β-carotene, α-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin. Generally, the carotenoids are found in significant amounts in yellow, orange, and red (brightly colored) fruits and vegetables such as carrots, watermelon, papayas, tomatoes, tomato products (ketchup, chili sauce, tomato paste, tomato juice, spaghetti sauce), squash, pink grapefruits, and pumpkins. Green vegetables also contain some carotenoids, but the pigment is masked by (green) chlorophyll. Carrots typically represent a major source of both α- and β-carotene in the American diet. Other major dietary contributors of β-carotene are listed in Table 10.3. Alpha-carotene is also found in most of the same foods as β-carotene, including pumpkin, squash, plantains, collard greens, and tomatoes, among others. Fruits as well as pumpkin, peppers, carrots, and yellow corn contribute substantially to dietary β-cryptoxanthin intake. Tomatoes, along with tomato juice and sauces, watermelon, dried apricots, guava, papaya, and pink grapefruit are major sources of dietary lycopene, a carotenoid that is red in color. Good sources of zeaxanthin and lutein include kale, spinach, broccoli, peppers (yellow), zucchini, corn, Brussels sprouts, green beans, kiwi, and eggs. Canthaxanthin, a red-orange carotenoid, is found in plants as well as in fish and seafood such as sea trout and crustaceans. Meat and fish are not major sources of carotenoids, but because animals and fish feed on plants, they can accumulate some carotenoids.

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Carotenoids also may be added to foods. β-Carotene and canthaxanthin, for example, are approved by the Food and Drug Administration (FDA) for use as food color additives.

Digestion and Absorption

Because it is bound to other food components, vitamin A requires some digestion before it can be absorbed from the small intestine. Retinol, for example, is typically bound to a fatty acid as a retinyl ester (see retinyl palmitate previously shown in Figure 10.1e) and may also be bound, like carotenes in foods, to protein from which they must be released. Although heating plant foods weakens some complexes, such as protein–carotenoid complexes, enzymatic digestion is still required. Carotenoids and retinyl esters are initially hydrolyzed from protein by pepsin in the stomach. Because of their fat solubility, the freed (i.e., no longer bound to protein) retinyl esters and carotenoids typically coalesce, along with other lipids, to form fat globules in the stomach. These fat globules containing the vitamin are emptied into the duodenum, where bile emulsifies them (emulsification results in large fat globules being broken up into smaller droplets).

Proteolytic enzymes in the duodenum can hydrolyze any remaining protein-bound retinyl esters or carotenoids not freed in the stomach. Hydrolysis of retinyl and carotenoid esters by various hydrolases and esterases occurs at the same time that triacylglycerols, phospholipids, and cholesteryl esters are being hydrolyzed by pancreatic enzymes. Pancreatic lipase and pancreatic cholesterol ester hydrolase, secreted into the lumen of the small intestine, free the bound vitamin. Additionally, enzymes such as retinyl ester hydrolase on the intestinal brush border membrane digest the vitamin. Pancreatic hydrolases cleave shorter-chain retinyl esters, whereas intestinal brush border hydrolases act on longer-chain retinyl esters.

Micelles form within the lumen of the small intestine from the bile, digested lipids, retinol, and carotenoids. The micellar solution diffuses through the lumen and unstirred water layer adjacent to the enterocytes, enabling close contact with the brush border membrane and the passive diffusion of the vitamins into intestinal cells. Absorption occurs most rapidly in the duodenum and jejunum, but continues to a large extent throughout the jejunum. In addition to this diffusion, carotenoids may also be absorbed by the transporter scavenger receptor class B type 1 (SR-B1) which is found in the small intestine, among other tissues. This uptake of carotenoids may be regulated through an intestine-specific homeobox (ISX) transcription factor that responds to intestinal carotenoid concentrations [1].

The efficiency of absorption differs between vitamin A and carotenoids. Approximately 70–90% of dietary vitamin A is absorbed as long as the meal contains some (~10 g or more) fat [2]. Carotenoid absorption varies considerably depending on food processing, and may range from <5% for carotenoids in uncooked vegetables or non-heat-processed vegetable juices to about 60% if present as a pure oil or as part of an aqueous dispersion supplement [2,3]. β-carotene absorption generally ranges from about 20 to 50%. Fiber (especially pectin) intake as well as excessive vitamin E consumption can diminish carotenoid absorption [2]. Pectin appears to diminish absorption by interfering with micelle formation.

Within the enterocyte (as well as the liver, adipose, lungs, and kidneys, among other organs), some carotenoids, including α- and β-carotene and β-cryptoxanthin, undergo metabolism, although 15–60% of β-carotene may leave enterocytes intact (and get taken up via scavenger receptor class B type 1 into target peripheral tissues such as the eyes for local production of retinoids). The extent to which these carotenoids are converted to retinoids in enterocytes is influenced by several factors, such as an individual's vitamin A status and the amounts and forms of the carotenoids consumed. For example, with higher vitamin A intake, receptor-mediated carotenoid absorption and conversion to vitamin A are decreased likely through an ISX transcription factor [1].

The synthesis of vitamin A (specifically all-*trans* retinal) from β-carotene (and other provitamin A carotenoids) is accomplished by either noncentral cleavage (by 9´10´-dioxygenase) or central cleavage (by 15,15´-mono-oxygenase, an iron-dependent cytosolic enzyme that is also found in the liver, lungs, kidneys, and retina). Noncentral cleavage generates several metabolites (alcohols, aldehydes, etc.) and is more common with non-provitamin A carotenoids, whereas central cleavage can convert, for example, one molecule of β-carotene into two molecules of retinal (Figure 10.2). The overall efficiency of β-carotene conversion to retinal is estimated at about 50%. An estimated 12 μg of β-carotene or 24 μg of α-carotene or β-cryptoxanthin produces about 1 μg of retinol (which is generated from retinal, as discussed next) [1,2].

Within enterocytes, retinoids also undergo metabolism (Figure 10.3). Retinal, produced from β-carotene or other carotenoids, is reduced by retinal/retinaldehyde reductase, an NADH-dependent enzyme, to retinol. The retinol is then re-esterified for incorporation into chylomicrons. Retinol, whether formed by carotenoid oxidation or originating from dietary retinyl esters, follows one of two metabolic pathways for re-esterification within the enterocyte. The primary pathway involves cellular retinol-binding protein (CRBP) II, one of a group of low-molecular-weight lipid-binding proteins that help regulate retinol use in cells. CRBPII, present in the cytosol of the enterocyte, binds both retinol and retinal and directs the reduction of retinal to retinol and subsequent esterification to a fatty acid. CRBPII-bound retinol is esterified by lecithin retinol acyl transferase (LRAT) to form mainly retinyl palmitate, but also retinyl stearate, retinyl oleate, and retinyl linoleate, among others. LRAT specifically transfers sn-1 fatty acids from membrane-associated phosphatidylcholine to retinol that is bound to CRBPs. LRAT is thought to be the main

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Figure 10.2 Central cleavage of β-carotene to retinal.

enzyme responsible for esterification in the small intestine, liver, pigment epithelium of the retina, and likely other tissues. The second, minor pathway for re-esterification is by acyl-CoA retinol acyl transferase (ARAT). The fatty acids used to esterify retinol are not reflective of the fatty acid content of the meal consumed.

Additional metabolism of vitamin A may also occur within the enterocyte, as shown in Figure 10.4, and within other cells,

1. β-carotene is converted into two retinal molecules. See Figure 10.3 for details of this reaction.
2. Cellular retinol-binding protein (CRBP) II binds to both retinol and retinal in the intestinal cell.
3. Retinal, while attached to CRBPII, is reduced to retinol by retinal/retinaldehyde reductase to form CRBPII-retinol.
4. Lecithin retinol acyl transferase (LRAT) esterifies a fatty acid (palmitic acid) onto the CRBPII-bound retinol to form CRBPII-retinylpalmitate.
5. Retinyl esters are incorporated along with phospholipids, triacylglycerol, cholesterol esters, carotenoids, and apoproteins to form a chylomicron.
6. Chylomicrons leave the intestinal cell and enter the lymph system and ultimately the blood.
7. Retinoic acid can directly enter the blood, where it attaches to albumin for transport to the liver.

Figure 10.3 Digestion and absorption of carotenoids and vitamin A, and re-esterification of retinol in the intestinal cell.

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Figure 10.4 Retinoid metabolism.

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as shown later in Figures 10.5 and 10.10. Retinol, for example, may be conjugated (especially in the liver but also in the intestine) to glucuronic acid to form retinoid β-glucuronide, which is excreted in the bile. In addition, some of the retinal may be irreversibly oxidized to retinoic acid within the intestinal cell. Some of this retinoic acid may also then be conjugated to glucuronic acid to form retinoyl β-glucuronide. Retinoyl β-glucuronide and retinoic acid, in contrast to retinol, can enter the circulation through the portal vein. Retinoic acid is transported in the plasma bound tightly to albumin (Figure 10.3). Retinoyl β-glucuronide concentrations in the plasma are typically low, but the metabolite appears to function in part like retinoic acid in tissues, where it promotes growth and cell differentiation, but does not bind to nuclear retinoic acid receptors. Retinol is also used within some (primarily extraintestinal) tissues to produce 11-*cis* retinal that is needed for vision and 9-*cis* retinoic acid that functions in gene expression, as discussed in more detail under the section on vitamin A's functions. All three retinoids—retinol, retinal, and retinoic acid—also may undergo metabolism, especially in the liver, by the cytochrome P-450 system to generate a variety of oxidized metabolites.

Transport, Metabolism, and Storage

To leave the enterocyte for transport to tissues, the newly formed retinyl esters, along with small amounts of unesterified retinol and any unmetabolized carotenoids, are incorporated into chylomicrons, also containing cholesterol esters, phospholipid, triacylglycerols, and apoproteins. Small quantities of unesterified retinol, however, may also directly enter portal blood. The chylomicrons, once formed, enter first into the lymphatic system and then into general circulation (i.e., the blood) via the thoracic duct (thereby initially bypassing the liver).

Chylomicrons deliver retinyl esters, some unesterified retinol, and carotenoids to extrahepatic tissues, including especially bone marrow, but also blood cells, spleen, muscle, lungs, kidneys, and adipose tissue. In fact, the adipose tissue, which takes up retinyl esters from chylomicrons, stores about 15–20% of the body's vitamin A. Retinyl esters and carotenoids not taken up by peripheral tissues are transported to the liver as part of the chylomicron remnant, which is taken up by receptor-mediated endocytosis and degraded by lysosomes. About 65–75% of chylomicron retinoids are cleared from circulation by the liver [4].

The handling of retinyl esters that reach the liver is shown in Figure 10.5. However, most cells of the body metabolize retinol generated from the retinyl esters through a number of metabolic pathways. The retinyl esters are hydrolyzed by a retinyl ester hydrolase following their uptake by the hepatic **parenchymal cells** (functional cells of an organ). Within the hepatic cell, retinol binds to a CRBP. CRBPs are found throughout the body. For example, CRBPI is present in all tissues but is found in especially high concentrations in the liver and kidneys. CRBPII is abundant in the intestine, especially the jejunum. CRBPIII is present in relatively high concentrations in the liver, skeletal muscle, kidneys, and heart; CRBPIV is found mostly in the heart, kidneys, and colon. As in the enterocyte, CRBPs in hepatic cells function both to help control cytosolic concentrations of free retinol and thus prevent its oxidation and to direct the vitamin, through a series of protein–protein interactions, to specific enzymes. CRBPs may also assist in the transfer of retinal across organelles for metabolism. As in the enterocyte, retinol metabolism includes possible esterification by enzymes such as LRAT if retinol is bound to CRBP, or ARAT if retinol is not bound to CRBP. Unbound CRBP concentrations are thought to inhibit LRAT and thereby prevent esterification for storage. CRBP-bound retinol may also be oxidized to retinal by NAD(P)H-dependent retinol dehydrogenase or phosphorylated to retinyl phosphate by ATP for glycoprotein functions.

Retinol that has been esterified may be stored in the liver. Small amounts of retinol are stored in the parenchymal cells, but most (80–95%) is stored in the liver's perisinusoidal cells called **stellate cells** (also known as Ito cells), which constitute less than 15% of total liver cells. (Note that retinoic acid does not accumulate in appreciable amounts in the liver or other tissues.) In the stellate cells, vitamin A (retinol) is stored as retinyl esters (primarily retinyl palmitate, but also retinyl stearate, oleate, and linoleate) with lipid droplets. Hydrolases can release the retinol from its stores as needed for use. With adequate liver stores of vitamin A (minimum 20 μg of vitamin A per gram of liver), plasma vitamin A concentrations remain fairly constant over a wide range of dietary intakes. When hepatic vitamin A concentrations are less than 20 μg of vitamin A per gram of liver, plasma retinol concentrations usually decline; in contrast, hepatic concentrations in excess of 300 μg of vitamin A per gram of liver cause plasma retinol concentrations to rise. Once hepatic stellate cells can accept no more retinol for storage, hypervitaminosis A (vitamin toxicity) occurs.

Retinol transport in the blood requires two specific proteins, retinol-binding protein (RBP) and transthyretin, also known as prealbumin and as thyroxine-binding globulin. Both proteins are synthesized by hepatic parenchymal cells. Initially, for hepatic release into the blood, one molecule of retinol released by a hydrolase from its ester form combines with one RBP to form holo-RBP (Figure 10.5). The synthesis of RBP depends not only on vitamin A but also on zinc and protein; RBP synthesis diminishes with inadequate protein, retinol, or zinc status. Holo-retinol-RBP (which contains the vitamin A bound in an interior, hydrophobic region of the complex since it is fairly insoluble in the aqueous environment of the blood) combines next with transthyretin, a tetrameric thyroid hormone (T_4) transport protein.

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1. Retinyl esters are taken up into the liver cells and the retinol and fatty acids are released by retinyl ester hydrolase.
2. Free retinol can be esterified via ARAT or it can bind to CRBP and be esterified by LRAT to form retinyl esters.
3. Retinyl esters are stored in stellate cells until needed.
4. CRBP-retinol can be (a) converted into CRBP-retinal, which is then converted into retinoic acid, or (b) it can be attached to retinol-binding protein for release into the blood, or (c) it can be conjugated with glucuronic acid to form retinyl β-glucuronide for excretion in the bile.
5. Retinoic acid binds to cellular retinoic acid–binding proteins (CRABPs), which can then function in the nucleus in gene expression, or be conjugated to glucuronic acid and excreted in the bile, or be converted into 4-OH retinoic acid and then to 4-oxoretinoic acid.
6. 4-oxoretinoic acid may function in cells like retinoic acid, or it may be conjugated to glucuronic acid for excretion in the bile.
7. Retinol attaches to retinol-binding protein in the liver. The complex called holo-retinol-RBP is then released into the blood where it binds to transthyretin and thyroxine to form a trimolecular complex.

Figure 10.5 Vitamin A metabolism in the liver.

This holo-retinol-RBP-transthyretin ternary complex represents the circulating source of the vitamin for extrahepatic tissues; it has a half-life of about 11–15 hours in the plasma and, as a complex, is too large to be cleared by the kidneys for excretion. Blood/serum concentrations of the complex remain fairly constant even when total hepatic retinol concentrations vary greater than 15-fold [5]. Normal plasma retinol concentrations range from about 1.05 to 3 μmol/L (30–86 μg/dL) but decrease below normal with chronic consumption of inadequate vitamin A.

Uptake of retinol from the holo-retinol-RBP-transthyretin complex by target tissues is mediated by a protein transporter called "stimulated by retinoic acid 6" (STRA6), which binds to the RBP portion of the complex to enable cellular retinol absorption. Apo-RBP (with no retinol attached) returns to the blood where it is either reused or taken up and degraded by the kidneys. Some of the many tissues that use vitamin A from the complex include adipose tissue, skeletal muscle, heart, lungs, kidneys, eyes, white blood cells, and bone marrow.

Carotenoids reaching the liver also typically undergo metabolism, including either storage, cleavage, and/or incorporation into and release as part of very-low-density lipoproteins (VLDLs) or other lipoproteins for transport to other tissues. The most common carotenoids in the blood are β-carotene, α-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin. Some carotenoids, such as β-carotene, concentrate in the hydrophobic core of the lipoproteins, whereas other carotenoids with polar groups are found partly covering the lipoprotein surface. In a fasting state, β-carotene and α-carotene are transported mainly in low-density lipoproteins (LDLs) and VLDLs, while lutein, zeaxanthin, and β-cryptoxanthin are transported mainly in LDLs and high-density lipoproteins (HDLs) with smaller amounts in VLDLs. Overall, the transport of carotenoids in the blood is accomplished

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mainly by LDLs (55%), followed by HDLs (31%) and VLDLs (14%) [1]. The half-life of the carotenoids in the blood varies with β-carotene at less than 2 weeks, lycopene at about 12 days to 1 month, and lutein and zeaxanthin at about 1–2 months [1]. Serum carotenoid concentrations reflect intake and not body stores.

The uptake of carotenoids into target tissues differs from that of retinol. Carotenoids are taken up as part of the lipoprotein, with uptake mediated by specific apoprotein receptors found in a variety of tissues. Scavenger receptor class B type 1 also may be involved in transporting the carotenoids from the lipoproteins to tissues and vice versa. Carotenoids, like vitamin A, are stored mainly in the liver, followed by adipose tissue, with lesser amounts in the kidneys, skin, and lungs; however, some tissues concentrate specific carotenoids. For example, the retina of the eye is relatively rich in lutein and zeaxanthin.

In contrast to retinol, which is generally released by the liver for transport to and uptake by target tissues, retinoic acid is thought to be produced in small amounts from retinal or β-carotene by individual cells and used locally within those cells. Within the cell cytosol, the retinoic acid binds to cellular retinoic acid–binding proteins (CRABPs). CRABPs are thought to function in a capacity similar to that described for CRBPs. Although both CRBPs and CRABPs are often found in the same tissues, their relative distribution in the tissues differs. CRABPs solubilize retinoic acid, regulate cellular retinoic acid metabolism, and direct retinoic acid usage intracellularly. Metabolism of retinoic acid occurs in part through the cytochrome P-450 enzyme system CYP26, which is found in the liver and brain, among other tissues, and whose expression appears to be positively regulated by vitamin A [5]. This system also catalyzes the oxidation and glucuronidation of retinoic acid to generate polar metabolites such as 4-oxoretinoic acid (shown in Figures 10.4 and 10.5), which appears to be involved along with retinoic acid in the synthesis of **gap junction** proteins needed for cell growth (see the "Growth" subsection in the "Functions and Mechanisms of Action" section for vitamin A).

Functions and Mechanisms of Action

Vitamin A

Vitamin A is recognized as being essential for vision as well as for cellular differentiation, growth, reproduction, bone development, and immune system functions. In the case of vision, it is the 11-*cis* retinal form that binds to the protein opsin of visual pigments to mediate phototransduction (the process by which light is converted into electrical signals for the brain). For the other biological processes, it is all-*trans* retinoic acid and 9-*cis* retinoic acid that are needed. This section reviews each of these functions; the functions of the carotenoids are addressed in a later section.

Vision Several parts of the eye work together to ensure vision. For example, light enters the eye through the cornea, the outermost tissue that covers the front of the eye. The muscles of the iris adjust the size of the pupil in response to the dimness or brightness of the light. The light then passes through the lens and the vitreous humor (which shapes the eye) and hits the retina, the inner lining at the back of the eye. The retina contains specialized cells, called rods and cones, which act as photo- or light receptors. Cones are found near the center of the retina and function especially during the day in bright light. As darkness falls or light dims, the rods serve as the photo- or light receptors. Vitamin A is needed to form **rhodopsin** (a vitamin A–containing pigment protein) found in the rods. Rhodopsin is made up of vitamin A as 11-*cis* retinal and the protein opsin.

In basic terms (shown in Figure 10.6), when a flash of light hits the retina (as may occur when leaving a dark movie theater), a series of events occurs: the *cis*-retinal portion of rhodopsin is converted to *trans*-retinal, the rhodopsin molecule is cleaved, and signals are sent to the part of the brain involved with eyesight. To regain vision in the dark (e.g., reentering the dark movie theater), the rhodopsin molecule must be remade using vitamin A as *cis*-retinal. The *trans*-retinal that is released is transported from the photoreceptor rod cells into the pigment epithelium of the retina, where it is converted back to *cis*-retinal. The *cis*-retinal is then transported back to the rod cell, where it reattaches to opsin to form rhodopsin. A failure of vision in the dark (dim light) is called night blindness and may be caused by inadequate vitamin A.

Figure 10.7 shows the visual cycle (the formation and reformation of rhodopsin after its degradation) in more detail. This section focuses on how vitamin A is metabolized as part of the visual cycle. Retinol is transported to the retina via the blood as part of the holo-retinol-RBP-transthyretin complex. Following the binding of the complex to receptors on the pigment epithelium (a pigmented cell layer) adjacent to the photoreceptor rod cells (Figures 10.7 and 10.8), retinol enters the pigment epithelium cells and binds to CRBP. Much of the retinol is then converted by LRAT to all-*trans* retinyl esters, and some is isomerized into 11-*cis* retinyl esters. The all-*trans* retinyl esters in turn may be stored in the pigment epithelium and metabolized by an all-*trans* retinyl ester isomerohydrolase, as needed, to generate 11-*cis* retinol plus a fatty acid. Similarly, the 11-*cis* retinyl esters may be hydrolyzed, as needed, to 11-*cis* retinol and a fatty acid by retinyl ester hydrolase.

For the visual cycle to operate effectively, the oxidative conversion of 11-*cis* retinol to 11-*cis* retinal is necessary. The 11-*cis* retinal is next transported from the pigment epithelium into the photoreceptor rod cells (and thus across the interphotoreceptor matrix/space) by interphotoreceptor (also called interstitial) retinol–binding protein (IRBP); this protein resides within the retina's

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Figure 10.6 An overview of the role of vitamin A as a part of rhodopsin in vision.
Source: Beerman/McGuire, Nutritional Sciences, 1/e. © Cengage Learning.

interphotoreceptor space that lies between the pigment epithelium and the photoreceptor rod cells.

Within the photoreceptor rod cells, 11-*cis* retinal binds as a protonated Schiff base to a lysine amino acid residue in the protein opsin (Figure 10.6) to produce the rhodopsin. Rhodopsin is embedded in disks located in the rod's outer segment, which is enclosed within a restricted compartment of the retina created by tight junctions between cells (Figure 10.8). The cells on the blood side are one layer thick and form the pigment epithelium. The "outer limiting membrane" is formed on the vitreal side of the photoreceptor cells by specific junctions between the photoreceptor cells and the Müller cells (Figure 10.8).

Within the photoreceptor cells, the rhodopsin responds to small amounts of light. When a quantum of light (hv) hits the rhodopsin, changes occur in the vitamin A portion of the molecule such that 11-*cis* retinal is photoisomerized to generate all-*trans* retinal (Figures 10.6 and 10.7). The term *bleaching* is often used to describe this event because a loss of color occurs. A chain of events triggered by the absorption of light by 11-*cis* retinal results in an electrical signal or impulse to and along the optic nerve to the brain. To transmit through the cell to the plasma membrane the message that light has hit the rhodopsin, a cascade of reactions thought to involve **transducin** (a G protein), phosphodiesterase, and cGMP occurs. This reaction cascade causes sodium channels in the plasma membrane to be blocked and the rod cell to hyperpolarize, resulting in signals by the optic nerve leading to specific areas of the brain.

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* IRBP: Interphotoreceptor retinol–binding protein.
** Stored until needed.

1. All-*trans* retinol moves out of the blood [where it is found as part of a complex with transthyretin (TTR) and retinol-binding protein (RBP)] and into the pigment epithelium adjacent to the rod cell. In the pigment epithelium it attaches to CRBP (cellular retinol–binding protein).
2. All-*trans* retinol is converted into all-*trans* retinyl esters by LRAT.
3. All-*trans* retinyl esters are converted to 11-*cis* retinol, which is then attached to cellular retinal–binding protein (CRALBP).
4. 11-*cis* retinol is converted to 11-*cis* retinal while attached to CRALBP.
5. 11-*cis* retinal detaches from CRALBP and attaches to interphotoreceptor retinol–binding protein (IRBP) for transport across the interphotoreceptor space and into the photoreceptor rod cell. IRBP releases the 11-*cis* retinal upon delivery into the photoreceptor rod cell.
6. 11-*cis* retinal attaches to opsin to form rhodopsin.
7. Light hits the rod cell causing the cleavage of rhodopsin.
8. All-*trans* retinal is first converted to all-*trans* retinol before being ultimately converted back to 11-*cis* retinal.
9. All-*trans* retinol attaches to IRBP for transport across the interphotoreceptor space and into the pigment epithelium.
10. All-*trans* retinol is released from IRBP and attaches to CRBP in the pigment epithelium to enter the cycle again at 2.

Figure 10.7 The visual cycle.

The all-*trans* retinal formed as a result of light must be converted back to 11-*cis* retinal, and the rhodopsin must be regenerated. The steps for this conversion are thought to involve hydrolysis of all-*trans* retinal from the rhodopsin, reduction of all-*trans* retinal to all-*trans* retinol, and transport of all-*trans* retinol across the interphotoreceptor matrix and into the retinal pigment epithelium by IRBP (Figure 10.7). Within the pigment epithelium, the all-*trans* retinol may be metabolized as initially described—that is, in the same manner as upon uptake into the pigment epithelium—to repeat the visual cycle.

Gene Expression Two forms of vitamin A, all-*trans* retinoic acid and the isomer 9-*cis* retinoic acid (generated from retinol), exert genomic effects to regulate gene expression following their transport into the nucleus and their binding to specific nuclear receptors. Transport of the vitamin into the nucleus for interactions with nuclear receptors is carried out by two cytosolic intracellular lipid–binding proteins (iLBPs): cellular retinoic acid–binding protein (CRABP) II and fatty acid–binding protein (FABP) 5.

Once within the nucleus, the vitamin interacts with nuclear receptors. Nuclear receptors include retinoic acid receptors (RAR) and retinoid X receptors (RXR) (with each group made of three isotypes designated α, β, and γ) as well as peroxisome proliferator–activated receptors (PPAR) and retinoid-related orphan receptors (ROR). All-*trans* retinoic acid binds all three RAR isotypes as well as ROR and PPAR isotype β/δ. The isomer 9-*cis* retinoic acid binds RAR as well as the three RXR isotypes. The binding of all-*trans* and 9-*cis* retinoic acid to their respective

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Figure 10.8 Photoreceptor (rod) cells, their structure, and their surroundings.

nuclear receptors results in conformational changes that alter the receptor functions (Figure 10.9).

The vitamin–receptor complex functions (i.e. activity), however, are also influenced by other factors. For example, some vitamin–receptor complexes, such as all-*trans* retinoic acid–ROR, function as a monomer, interacting with ROR response elements (RORRE) located in the promoter regions of specific genes on DNA. In contrast, other vitamin–receptor complexes must interact with another receptor complex, forming either **homodimers** or **heterodimers**, before interacting with the DNA. A homodimer is formed when two of the same receptors interact, such as RXR-RXR. A heterodimer is formed between two or more different receptors, such as RXR-RAR, VDR (vitamin D receptor)–RXR, RXR-PPAR, and RXR-TR (thyroid hormone receptor). The ability of the heterodimer to enable transcription upon interacting with specific domains within the response elements occurs in part through the actions of histone acetyl transferase. This enzyme acetylates histone proteins, allowing the chromatin to "open" and transcription activity to proceed [6].

Another factor influencing vitamin–receptor complex activity is the need for coactivators and/or the removal of corepressors that are attached to the DNA receptor. Typically, as vitamin A binds to its receptor, the resulting conformational change causes the release of the corepressors—and in some cases, the recruitment of coactivators. Some additional examples of nuclear corepressors include silencing mediator for retinoid and thyroid hormone receptors, among others. When no vitamin A is present, the corepressors are typically found attached to the nuclear receptors and bound to the response elements on the DNA. Additionally, histone deacetylase functions to deacetylate the histones, which promotes chromatin condensation and represses gene transcription [6].

The genes affected by retinoic acid are mostly unknown but are thought to code for multiple proteins including enzymes, growth factors, and transcription factors, among others, and thus impact a wide variety of cellular processes. *Cell death*, or *apoptosis*, is an example of a cellular process regulated in part by retinoic acid. The vitamin is delivered by CRABPII to the cell nucleus, where it binds to RAR and interacts with a RARE to upregulate the expression of caspase 9 and Bcl2, two proteins involved in the apoptotic pathway (see Chapter 1 for further information on apoptosis). Another process affected in part by the genomic actions of vitamin A is *osteogenesis*, which appears to be repressed by the RORβ. *Lipolysis* is activated by vitamin A interactions with the nuclear PPARβ/δ receptors, while *adipocyte differentiation* is inhibited by retinol, retinal, and β-carotene, among others.

Retinoic acid, typically as all-*trans* retinoic acid, may also indirectly affect gene expression by initiating events that lead to changes in gene expression. For example, all-*trans* retinoic acid as well as retinol may stimulate (via kinase activity) the phosphorylation of selected binding proteins, which (once phosphorylated and translocated

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1. All-*trans* or 9-*cis* retinoic acid moves into the nucleus of the cell bound to binding proteins.
2. All-*trans* retinoic acid binds to retinoic acid receptors (RAR) and 9-*cis* retinoic acid binds to retinoid X receptors (RXR).
3. Binding of the vitamin to the nuclear receptors and interaction with response elements on the DNA enhances the transcription of selected genes. The absence of bound vitamin A usually results in the repression of gene transcription.

Figure 10.9 Hypothesized mode of action for retinoic acid on gene expression.

to the nucleus) bind to response elements to affect gene expression. Another nongenomic action is known as retinoylation, in which retinoic acid is attached post-translationally to selected proteins. One impact of retinoylation appears to be enhanced *cell differentiation*.

Signaling Vitamin A, as retinol, in complex with RBP and transthyretin, serves as an extracellular signal compound upon attachment to the cell membrane receptor protein STRA6. This attachment, which promotes cellular uptake of retinol, also induces phosphorylation of a specific tyrosine residue on STRA6. This phosphorylation then activates a specific signaling cascade (called signal transducers and activators of transcription [STAT]/janus kinase [JAK]–mediated signaling cascade) to affect gene expression and various cellular processes. Certain activated STATs (several are present in cells, but only certain ones are activated by this cascade), after a series of additional reactions, form dimers that translocate to the nucleus and serve as transcription factors for the expression of genes involved in the regulation of the *insulin response* and *lipid homeostasis* [6].

Cellular Differentiation, Proliferation, and Growth Vitamin A, as retinoic acid, is also involved in cell differentiation, the process by which an immature cell is transformed into a specific type of mature cell. Epithelial cells, found as part of our skin and in all internal body tracts, such as the respiratory, gastrointestinal, and urogenital tracts, are one example of cells that require vitamin A. Retinoic acid helps maintain both the normal structure and the functions of the epithelial cells. For example, retinoic acid directs the differentiation of **keratinocytes** (immature skin cells) into mature epidermal cells through interactions with nuclear chromatin and thus changes in gene expression. With vitamin A deficiency, keratin-producing cells replace mucus-secreting cells in many body tissues, especially the eyes, skin, gastrointestinal tract, and trachea and disrupt normal tissue properties and functions.

In addition to epithelial cells, retinoic acid is thought to regulate the proliferation and differentiation of some myeloid precursor cells in the bone marrow. Progenitor cells in the bone marrow, for example, differentiate into immature dendritic cells with adequate retinoic acid. The dendritic cells, which mature after exposure to an antigen, function to present antigens to other immune system cells, such as T-cells, to augment the body's immune response.

Retinoids also have been associated with a variety of other cell processes. They can induce the arrest of the cell cycle and apoptosis. In hematopoietic cells, retinoic acid mediates insulin receptor substrate (IRS) 1 levels by stimulating the binding of the ubiquitin-proteasomal complex to IRS 1; this attachment induces the degradation of IRS 1. IRS 1

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regulates cell proliferation and survival in selected cells. The vitamin's ability to impact growth appears to be cell-specific, whereby it enhances proliferation and survival in some cells whereas, in others, it induces differentiation, cell cycle arrest, and/or apoptosis. Interactions between retinoic acid and RAR receptors, for example, can inhibit cell growth of some tumors. Alternately, retinoic acid interactions with PPARβ/δ stimulate cell growth and inhibit apoptosis. Another possible means by which growth is influenced may involve effects on gap junctions. Retinoic acid and 4-oxoretinoic acid, generated from retinoic acid, have been shown to increase the synthesis of specific gap junction connexin proteins by stabilizing their mRNA. Gap junctions, cell-to-cell channels formed from connexin proteins, are important for the exchange of small signaling compounds and thus for cell-to-cell communication. A lack of gap junction communication results in uncontrolled cell growth, as can occur with cancer cells. Thus, vitamin A, in preserving this communication, plays a role in the control of cell growth.

Retinoic acid also may be able to modify cell surfaces, possibly by increasing glycoprotein synthesis at the gene level or by improving the attachment of glycoproteins to cell surfaces to induce cell adhesion. Retinol may also play a more direct role in glycoprotein synthesis. Retinyl phosphate (generated from retinol and ATP) can be converted to retinyl phosphomannose (also called mannosyl retinyl phosphate) in the presence of GDP mannose. Retinyl phosphomannose can donate its mannose to glycoprotein acceptors, which then become mannosylated glycoproteins. Such changes in the glycan portion of the glycoprotein can affect differentiation of cells through their effects on cell recognition, adhesion, and cell aggregation. These reactions (which have been suggested as a coenzyme role) involving vitamin A and glycosylation are shown here.

Retinoids and Cancer Because of its ability to induce differentiation and apoptosis and to inhibit proliferation, retinoids have been used as chemotherapeutic agents for treating some cancers. Some retinoids (and chemically related derivatives of retinoids) effectively promote cell cycle arrest and terminal differentiation of some cancer cells [6]. All *trans*-retinoic acid (tretinoin), for example, is used in the treatment of lymphomas, leukemia, cervical cancer, lung cancer, neuroblastoma, and glioblastoma, while 13-*cis* retinoic (isotretinoin) and other synthetic retinoids are undergoing clinical trials for the treatment of other cancers [6]. Transformations and modifications of retinoids are being tested for their antiproliferative and/or pro-differentiative activities and cytotoxic actions on various cancer cell lines.

Other Functions Vitamin A, as retinol but not as retinoic acid, is essential for *reproductive processes* in both males and females, although the mechanism(s) of its action(s) is unclear. *Bone development and maintenance* also require vitamin A; either too much or too little of the vitamin negatively impacts bone mineral density. Vitamin A is thought to be involved in the regulation of **osteoblasts** (bone-forming cells) and/or **osteoclasts** (cells participating in bone resorption). Although the mechanism of action is unclear, vitamin A deficiency results in excessive deposition of bone by osteoblasts and reduced bone degradation by osteoclasts. Excess vitamin A, in contrast, stimulates osteoclasts and inhibits osteoblasts, decreasing bone mineral density and increasing fracture risk. Vitamin A appears to be involved in *hematopoiesis*, a function likely mediated by inhibition of erythropoiesis and impaired iron distribution among tissues; iron-deficiency anemia is often observed in those with chronic vitamin A deficiency. It is characterized by both low red blood cell counts and low hemoglobin.

Several aspects of *immune system function*, both humoral and cell-mediated, are also influenced by vitamin A. Retinoic acid, produced in some immune cells such as antigen-presenting cells, stimulates phagocytic activity, cytokine production, and natural killer cell activity. Depletion studies suggest that vitamin A appears to be needed for T-lymphocyte function and for antibody response to viral, parasitic, and bacterial infections. Thus, people with vitamin A deficiency can have an impaired ability to resist and fight infections and exhibit increased morbidity and mortality. Supplementation with vitamin A has been shown to improve death rates associated with measles and diarrhea in developing countries.

Another role of vitamin A, likely mediated by effects on gene expression, cell differentiation, and growth, is in *morphogenesis/embryogenesis*. Specifically, retinoic acid is thought to act as a morphogen in embryonic developments; nuclear retinoic acid receptors have been found in different cells during different times of development in the embryo.

Carotenoids

Carotenoids are found in lipoproteins and in cell membranes (with some like β-carotene and lycopene in interior sections due to their hydrophobic structure, and some with polar functional groups like zeaxanthin present closer to the membrane surface). The mechanism by which carotenoids function is linked in part to their structure, more specifically their extended system (often nine or more) of conjugated double bonds that make them soluble in lipids and capable of quenching singlet oxygen ($^1O_2$) and free

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radicals (atoms or molecules with one or more unpaired electrons). The antioxidant roles of carotenoids and their relationship to diseases are discussed first, followed by some of the other postulated roles of carotenoids.

Antioxidant The carotenoid's ability to react with and quench singlet oxygen and free radicals is an antioxidant function. Because of their location in membranes, carotenoids are especially valuable in the protection of cell membranes, whereas vitamin C, which is water soluble, is more active in aqueous environments such as the cytosol of cells and the blood.

Quenching is a process by which electronically excited molecules or atoms, such as singlet oxygen, are inactivated. Singlet oxygen possesses higher energy and is more reactive than ground state molecular oxygen, which typically exists in triplet ($^3O_2$) rather than singlet ($^1O_2$) form. Singlet oxygen is generated from lipid peroxidation of membranes; transfer of energy from light (photochemical reactions); the respiratory, also called oxidative, burst occurring in some phagocytic cells (enzymatic reactions); and the dismutation of superoxides (spontaneous reaction). Singlet oxygen, like free radicals, readily reacts with organic molecules such as protein, lipids, and DNA and thus can damage cellular components unless removed. Carotenoids can react with (quench) singlet oxygen, and it is the conjugated double-bond systems within the carotenoids that permit the quenching. The singlet oxygen ($^1O_2$) transfers its excitation energy and returns to the ground state ($^3O_2$), and the carotenoid receiving the energy enters an excited state. Resonance states in the excited carotenoid allow some stabilization. Carotenoids then release the energy in the form of heat and thus do not need to be regenerated like some other antioxidants (e.g., vitamins C and E).

$$^1O_2 + \beta\text{-carotene} \longrightarrow {}^3O_2 + \text{excited } \beta\text{-carotene}$$
$$\longrightarrow \beta\text{-carotene} + \text{heat}$$

In addition to quenching singlet oxygen, β-carotene and other carotenoids have the ability to react directly with free radicals (radical scavenge), especially peroxyl radicals ($ROO^{\bullet}$), which cause lipid peroxidation. Such actions by carotenoids decrease circulating peroxide concentrations and reduce lipid peroxidation (thus helping to minimize damage to cells). Radical scavenging by carotenoids involves the donation of a hydrogen atom or an electron and results in the formation of a carotenoid radical. Electron delocalization over the carotenoid's conjugated double bond system stabilizes the carotenoid.

Although most studies have focused on β-carotene, other carotenoids have higher capacities to donate an electron or hydrogen. Of those studied, lycopene appears to have the highest capacity, followed in descending order by α-tocopherol (vitamin E), α-carotene, β-cryptoxanthin, zeaxanthin, β-carotene, and lutein [7]. However, carotenoids—for example, lycopene and lutein—and vitamin E can act synergistically and, in combination, are more effective than any one carotenoid itself. Additional interactions between antioxidant nutrients are discussed in the Perspective at the end of this chapter.

Because of the ability of carotenoids to react with free radicals and quench singlet oxygen, carotenoids are thought to be protective against several diseases. Some epidemiological studies have shown that people with high intakes of fruits and vegetables, which are also rich in carotenoids, and people with higher serum carotenoid concentrations have a lower incidence of diseases.

Carotenoids and Eye Health Carotenoids, especially zeaxanthin and lutein, are important in visual protection and acuity and in shape discrimination. These carotenoids are found in the macula and function to absorb portions of sunlight; they may also protect against ultraviolet light [8].

Cataracts, which affect the lens of the eye, is a common cause of blindness, especially among older adults. As previously described in the "Vitamin C" section in Chapter 9, accumulation and aggregation of abnormal proteins in cells in the eye's lens (which functions to focus light onto the retina and adjust eye focus) cause the lens to become cloudy and vision to become impaired with cataracts. Free radicals are thought to contribute to the cataract's development, and because lutein and zeaxanthin are found in the eye and function as antioxidants, these carotenoids as well as β-carotene are purported to prevent and/or lower the risk of developing cataracts. Age-related macular degeneration is also a common cause of blindness in older people. The macula, found in the center of the retina, maintains central vision; this region can become damaged, as described in the "Eye Health" section under vitamin C in Chapter 9. Lutein and zeaxanthin, which are found in the macula, as well as β-carotene can inhibit the oxidation of cell membranes and thus may be protective against eye damage. Mixed results have been documented in studies examining dietary intakes and/or plasma concentrations of carotenoids (lutein, zeaxanthin, and/or β-carotene) and risk of cataracts and macular degeneration; while inverse correlations have sometimes been reported, several meta-analyses have concluded that carotenoids as part of antioxidant supplementation do not lower the risk or slow the progression of cataracts or of age-related macular degeneration [9–12]. However, the Age-Related Eye Disease Study (AREDS), a large randomized, placebo-controlled 5-year clinical trial, which provided a combination of antioxidants [including vitamin C (500 mg), vitamin E (400 IU), beta-carotene (15 mg), zinc (80 mg), and copper (2 mg)], reported modest effects in slowing the progression of age-related macular degeneration [13]. A second 5-year trial, AREDS2, which supplemented those with macular degeneration with a modification of the original formulation [adding omega-3 fatty acids (1,000 mg), lutein (10 mg), and zeaxanthin

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(2 mg), removing β-carotene, and reducing zinc (25 mg)], found no additional benefits [14]. Consumption of a Mediterranean-type diet has also been shown to reduce risk of progression to advanced macular degeneration [15].

Carotenoids and Heart Disease Many, but not all, studies have demonstrated inverse associations between dietary carotenoids and/or serum carotenoid concentrations and risk of coronary heart disease. In the events associated with the development of atherosclerosis, the oxidation of cholesterol in LDLs increases the likelihood of a monocyte binding to and being taken into the blood vessel walls/endothelium (in comparison with native/unoxidized cholesterol). Monocytes, once present within the blood vessel endothelium, are called macrophages. These macrophages continue to take up oxidized LDL through a specific cell surface scavenger receptor and, when filled with cholesterol, become foam cells. Fatty streaks (an accumulation of foam cells) lead to atherosclerotic plaque in blood vessels and thus contribute to heart disease. Carotenoids, including β-carotene, lycopene, lutein, and α-carotene, can prevent the oxidation of LDL and cell membrane lipids and thus theoretically prevent or slow the development of atherosclerosis. However, supplementation with carotenoids has not been shown to prevent or improve heart disease in most studies and at present, supplementation is not recommended [see reviews 16 and 17].

Other Roles Carotenoids, like retinoids, also inhibit *cell proliferation* and stimulate *cell differentiation* while also affecting *growth and apoptosis*. Lycopene, for example, inhibits the growth and proliferation of various cancer cells and induces cell differentiation, and canthaxanthin and β-carotene have been shown *in vitro* to inhibit carcinogen-induced neoplastic transformation. Carotenoids, like retinoids, also enhance or induce gap junction communication. Some carotenoids, for example, upregulate the *gene expression* of proteins that enable connections (bridges) to form between cells; such actions in turn affect cell proliferation and growth.

Another means by which some carotenoids (such as lycopene and various carotenoid metabolites) affect gene expression is through nuclear factor E_2-regulated factor 2 (Nrf2). Nrf2 is a transcription factor that is found in the cell cytosol typically in association with a specific protein. Cytosolic increases in selected carotenoids or carotenoid metabolites are thought to facilitate the uncoupling of the protein from Nrf2, which enables the Nrf2 to translocate to the nucleus and act as a transcription factor to enhance the expression of genes coding for a group of proteins known as phase II/antioxidant detoxifying enzymes. Nrf2 plays significant roles during carcinogenesis and is considered a molecular target for cancer prevention. The phase II/antioxidant detoxifying enzymes provide defense in response to oxidation and other "stresses" on cells [1]. Although much additional research is needed, the expression of other genes such as those coding for enzymes involved in apoptosis may also be affected by the carotenoid metabolites.

Carotenoids and Cancer Carotenoids are thought to deter or protect against cancer via several means, including regulation of cell growth and cell cycle progression, inhibition of cell proliferation, enhancement of cell differentiation, stimulation of intercellular gap junction communication, immune modulation, and apoptosis, among others [18]. Intervention trials using β-carotene, however, have failed to show protective effects on health. Moreover, two intervention trials conducted back in the 1990s providing 20 mg of β-carotene along with other antioxidants (including vitamin A) found an increased risk of lung cancer and of mortality from cancer and heart disease in a high-risk population (smokers and those with asbestos exposure) [19,20]. Additional studies in less high-risk populations have not found beneficial effects from β-carotene supplementation in reducing the risk of cancer or mortality from cancer. The Institute of Medicine states that "beta-carotene supplements are not advisable for the general population" [3].

Carotenoids and Health Claims Health claims that are approved by the FDA are targeted at carotenoid-rich foods (fruits and vegetables) as well as grain products. These claims require the food to be low in fat and a good source of dietary fiber without fortification. An example model claim is "Low-fat diets rich in fiber-containing grain products, fruits and vegetables may reduce the risk of some types of cancer, a disease associated with many factors" [21]. Another sample claim for fruits, vegetables, or grain products that are low in (saturated and total) fat and cholesterol and contain at least 0.6 g of soluble fiber per reference amount without fortification is "Diets low in saturated fat and cholesterol and rich in fruits and vegetables and grain products that contain some types of dietary fiber, particularly soluble fiber, may reduce the risk of heart disease, a disease associated with many factors" [21]. Another model claim—"Low-fat diets rich in fruits and vegetables (foods that are low in fat and may contain dietary fiber, vitamin A, or vitamin C) may reduce the risk of some types of cancer, a disease associated with many factors"—has also been approved for fruits and vegetables that are a good source of vitamin A or C or dietary fiber [21].

Interactions with Other Nutrients

Vitamin A and carotenoids interact with vitamins E and K. Excess dietary vitamin A intake interferes with vitamin K absorption. High β-carotene intake, in turn, may decrease plasma vitamin E concentrations.

Protein and zinc influence vitamin A status and transport. First, the activity of carotenoid dioxygenase, which cleaves β-carotene, is depressed by an inadequate protein intake. Second, overall vitamin A metabolism is closely related to protein and zinc status because the

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Figure 10.10 Metabolism of vitamin A, showing some excretory products that are secreted into the bile for removal from the body.

transport and use of the vitamin depend on two vitamin A–binding proteins and because zinc is required for protein synthesis as well as for alcohol dehydrogenase activity, which converts retinol to retinal. Impairments in the synthesis of retinol-binding protein and transthyretin have been shown to diminish retinol mobilization from the liver.

Iron metabolism is also interrelated with that of both carotenoids and vitamin A. Iron is a cofactor for 15,15' mono-oxygenase, the enzyme responsible for the conversion of β-carotene into retinal. Vitamin A (likely as retinoic acid) also may directly affect iron absorption, metabolism, or storage or red blood cell differentiation. Vitamin A deficiency is associated with decreased incorporation of iron into red blood cells and diminished mobilization of iron from stores. Thus, vitamin A deficiency can be associated with iron-deficiency anemia, and in this situation supplementation of vitamin A (as opposed to supplementation with iron) improves indices of iron status.

Metabolism and Excretion

Vitamin A is excreted in both the urine and feces with the relative amounts varying based on vitamin intake. Urinary excretion of vitamin A metabolites usually accounts for up to about 60% of vitamin A excretion, and fecal excretion accounts for the remaining 40%; however, with higher intakes, fecal excretion generally exceeds urinary excretion [2]. For urinary excretion, retinol and retinoic acid are typically oxidized at the β-ionone ring and then conjugated to generate polar, water-soluble metabolites. Many of these metabolites, especially those that are short chain and acidic, are excreted by the kidneys. Small amounts of vitamin A, however, may be expired by the lungs as CO_2. Oxidized products of vitamin A that contain intact chains and have been conjugated to glucuronic acid, such as retinoic acid glucuronide and 4-oxoretinoic acid glucuronide (Figure 10.10), or to taurine are generally secreted into the bile for ultimate fecal excretion. Some polar vitamin A metabolites secreted into the bile, such as 4-oxoretinoic acid glucuronide, however, can be absorbed and returned to the liver through the enterohepatic circulation. This recycling helps to partially conserve the body's supply of vitamin A.

Carotenoids are metabolized, depending on the individual carotenoid, into a variety of compounds for excretion. Carotenoid metabolites are excreted primarily into the bile for fecal elimination.

Recommended Dietary Allowance

The Recommended Dietary Allowance (RDA) for vitamin A is based on the requirement plus twice the coefficient of variation (20%), with the value rounded to the nearest 100 μg and are expressed as retinol activity equivalents (RAE) to account for differences in the biological activities of the carotenoids [2]. The RDAs for vitamin A for adult men and women are 900 μg RAE (3,000 IU) and 700 μg RAE (2,310 IU), respectively, if the food or supplement source is a retinoid (i.e., preformed vitamin A) [2]. The RDA of 900 μg RAE is also equivalent to 6,000 IU of supplemental β-carotene, 18,000 IU of food-derived β-carotene, and 36,000 IU of food-derived α-carotene or β-cryptoxanthin [2,17]. See the inside cover of the book for additional recommendations for women during pregnancy and lactation. The requirements for vitamin A for adult men and women are 625 μg RAE and 500 μg RAE, respectively [2].

Older units of measure for vitamin A such as international units (IU) and retinol equivalents (RE) are still sometimes used. **International units**, which, for example, may be found on food and supplement labels, represent the quantity of a nutrient that produces a particular biologic effect, but do not account for differences in biological activities among related compounds like carotenoids. Conversion factors are 1 IU of retinol = 0.3 μg RAE;

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I IU of β-carotene from food = 0.05 μg RAE and if from a supplement = 0.15 μg RAE. Finally, 1 IU of the other provitamin A carotenoids (α-carotene or β-cryptoxanthin) = 0.025 μg RAE [17].

Deficiency

Vitamin A deficiency is less common in the United States than in developing countries, where inadequate intake occurs frequently in children under 5 years of age. Selected signs and symptoms of deficiency include anorexia, retarded growth, increased susceptibility to infections, obstruction and enlargement of hair follicles, and keratinization of epithelial cells with accompanying failure of normal differentiation. With vitamin A deficiency, the skin becomes dry and scaly and may appear to have "goose bumps." In the eyes, the effects of vitamin A deficiency impact the retina, conjunctiva, and cornea. Initially, an inability to adapt to dim light occurs (and may be one of the first symptoms of deficiency). If not corrected, the condition progresses to an inability to see in dim light, called night blindness or nyctalopia. The problems result from impaired production of rhodopsin in the rod cells in the retina of the eye. Xerosis or **xerophthalmia** (dryness found in the conjunctiva and cornea because of inadequate mucus production) also occurs and is associated with the disappearance of goblet cells in the conjunctiva and the enlargement and keratinization of epithelial cells. This keratinization of the epithelial cells in the conjunctiva also promotes Bitot's spots. **Bitot's spots** are small, white, foamy-looking accumulations of sloughed cells and secretions. As the xerosis worsens, corneal scarring and ulcerations as well as keratomalacia (softening of the cornea) may occur; these may ultimately lead to corneal perforation and blindness. Vitamin A deficiency can also result in bone overgrowth with excessive new bone deposition and reduced bone degradation.

Treatment of a deficiency may require daily supplementation of 2,000–200,000 IU (606–60,600 μg RAE) of vitamin A depending on the person's age and the severity of the deficiency. Some of vitamin A's deficiency manifestations such as night blindness, xerosis, and Bitot's spots usually begin to improve within a few days following supplementation, but several weeks to months may be required for more complete healing.

Individuals with an increased need for vitamin A include people with malabsorptive disorders in which steatorrhea (excessive fat in the feces or fat malabsorption) occurs; fat malabsorption is most commonly found in those with conditions affecting the small intestine (ileum), pancreas, liver, or gallbladder. Additionally, people with chronic nephritis (affecting the kidneys), acute protein deficiency, intestinal parasites, or acute infections may also become vitamin A deficient. Measles infections, for example, in developing countries are associated with high mortality. Measles is thought to depress vitamin A status (which may already be low in children in developing countries due to inadequate vitamin A intake and absorption) by diminishing the vitamin's function as well as by increasing urinary vitamin A excretion. Vitamin A supplements are recommended by the World Health Organization and the United Nations Children's Fund for the routine treatment of measles in populations in which vitamin A deficiency is likely.

Toxicity

The Tolerable Upper Intake Level (UL) for preformed vitamin A is 3,000 μg RAE (10,000 IU) per day [2]. Ingesting larger amounts (such as 50,000 IU) of vitamin A (even a single dose) may result in acute hypervitaminosis A. Symptoms of acute hypervitaminosis A include nausea, vomiting, double or blurred vision, increased intracranial pressure, headache, dizziness (vertigo), diarrhea, skin desquamation (peeling), and muscle incoordination [22].

Chronic intake (daily for months or years) of vitamin A in excess of recommended amounts can also lead to hypervitaminosis A. For example, a chronic oral intake of retinol in amounts of three to four times the RDA (about 9,000–50,000 IU) can cause toxicity. Chronic vitamin A toxicity is manifested by a variety of maladies, including anorexia; dry, itchy skin with increased desquamation; alopecia (hair loss) and coarsening of the hair; ataxia; headache; bone and muscle pain; conjunctivitis and ocular pain; nausea, vomiting, and abdominal pain; and liver damage [22]. Additionally, bone mineral density decreases and bone fracture risk increases. In bone, excess vitamin A promotes premature epiphyseal closures and increases cortical bone resorption and periosteal bone formation (periosteum covers bone), causing a thinning of long bones and effects on metatarsals (bones in the feet) and metacarpals (bones in the hands).

The effects of hypervitaminosis A on the liver, the primary storage site for vitamin A, are multiple. They include fat-storing cell **hyperplasia** (excessive cell proliferation) and hypertrophy, fibrogenesis, sclerosis of veins, portal hypertension, and congestion in perisinusoid cells, which leads to hepatocellular damage and cirrhosis or a cirrhosislike hepatic disorder. Some toxic effects of excess vitamin A intake are thought to be mediated by changes in the regulation of vitamin A (retinoid) receptors in the nucleus and by effects on cell differentiation and growth, among other means.

Another mechanism by which excess vitamin A is thought to exert toxic effects is related to its transport in the blood. When vitamin A intake is in large excess, serum retinol levels rise (often above 200 μg/dL, whereas normal is 30–86 μg/dL), and retinol is no longer transported exclusively by RBP but instead is carried as retinyl esters by plasma lipoproteins to the tissues. It has been suggested that when retinol is presented to the cell membranes in a

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form other than in an RBP complex, the released retinol produces toxic effects [2,22].

Excessive intake of vitamin A in its natural form or in a synthetic form (as found in the oral acne treatment medications Accutane® and Roaccutane® [isotretinoin]) is also **teratogenic** (causes birth defects). For example, an oral intake of more than 4,500 μg RAE daily by pregnant women has been associated with an increased risk of malformations in infants born to the women [23]. Furthermore, because the use of synthetic retinoids for acne by women in the early months of their pregnancy causes a number of birth defects among the infants born to these women, dermatologists prescribe contraceptives for patients in their childbearing years taking the drug.

Carotenoids, in contrast to vitamin A, appear to have few side effects. In fact, β-carotene is listed on the Generally Recognized as Safe (GRAS) list with the FDA for use as a dietary and nutrient supplement as well as for use as a colorant in foods, drugs, and cosmetics [24]. The most commonly cited problem with supplemental carotenoid use is hypercarotenosis, also called carotenodermia; this problem is most often seen in people ingesting about 30 mg or more of β-carotene daily for at least 1–2 months and with plasma β-carotene concentrations in excess of about 250 μg/dL. Hypercarotenosis results in a yellow discoloration of the skin, especially in the fat pads or fatty areas of the palms of the hands and soles of the feet. The condition usually disappears once carotenoid intake is decreased. While no Tolerable Upper Intake Level has been established for β-carotene or other carotenoids [3], supplementation has been shown to be detrimental to some populations such as smokers [19,20]. Consequently, carotenoid supplements are not advised for the general public [3]; instead, the public is encouraged to consume at least 2½ cups each of fruits and vegetables per day.

Assessment of Nutriture

Vitamin A status may be assessed in a variety of ways. To assess for night blindness, electrophysiological measurements, made by electroretinograms, directly measure the level of rhodopsin and its rate of regeneration in the eye. Other eye problems may be detected using conjunctival impression cytology, a histological method of assessment that involves examining morphological changes in the epithelial cells of the conjunctiva. A reduction in goblet cells and the derangement (enlargement and flattening) of epithelial cells in the conjunctiva suggest vitamin A deficiency.

Plasma retinol concentrations are frequently measured as a biochemical indicator of vitamin A status. Plasma retinol levels reflect status best if the person has exhausted his or her stores (primarily in the liver) of the vitamin, as with deficiency, or if the stores are filled to capacity, as with toxicity. Use of plasma retinol concentrations, however, also depends on the adequacy of dietary energy, protein, and zinc because of their roles in the synthesis of retinol-binding protein. Moreover, use of plasma retinol is unreliable as an indicator of vitamin A status in people with infection or inflammation, both of which depress plasma vitamin concentrations. Plasma retinol concentrations less than ~20 μg/dL (0.7 μmol/L) are usually considered deficient, and concentrations of 20–30 μg/dL (0.7–1.05 μmol/L) are suggestive of marginal status. Plasma retinol concentrations of 30–86 μg/dL (1.05–3 μmol/L) are considered adequate, while concentrations above 86 μg/dL (3 μmol/L) are thought to be excessive (toxic). Serum retinyl ester concentrations are also used to assess toxicity, with concentrations greater than 0.2 μmol/L suggestive of excessive hepatic vitamin A stores.

Adequacy of vitamin A stores in the liver can be assessed by the relative dose response (RDR) test or the modified relative dose response (MRDR) test. The RDR test involves measuring changes in plasma retinol concentration before and 5 hours after oral administration of retinyl esters (usually as acetate or palmitate). Blood is taken initially, vitamin A is ingested, and 5 hours later blood is taken again. Retinol concentrations of the blood are determined, and the difference in concentration is calculated and divided by the 5-hour concentration. RDR is then expressed as a percentage.

$$\text{RDR (\%)} = \frac{\text{5-hour plasma retinol} - \text{initial plasma retinol}}{\text{5-hour plasma retinol concentration}} \times 100$$

A % RDR equal to or greater than 20% suggests inadequate liver vitamin A stores [2]. The MRDR test involves measuring the ratio of 3,4 didehydroretinol to retinol in the blood after administering a single dose of 3,4 didehydroretinyl acetate. This test, unlike the RDR, requires that only one blood sample be taken, about 4–6 hours after the vitamin is ingested. An MRDR ratio at 5 hours of less than 0.04 in healthy adults indicates adequate vitamin status [25,26].

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Suggested Readings

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VITAMIN D

Through the years vitamin D (also known as calciferol) has been associated with skeletal growth and strong bones. This association arose because early in the 20th century it was shown that **rickets**, a childhood disease characterized by improper bone development, could be prevented by a fat-soluble factor D in the diet or by body exposure to ultraviolet light. Emphasis was placed on the dietary factor; therefore, any compound with curative action on rickets was designated as vitamin D. E. McCollum is credited in 1922 with the vitamin's discovery.

Structurally, vitamin D is derived from a steroid and is considered to be a seco-steroid because one of its four rings is broken. Vitamin D contains three intact rings (A, C, and D) with a break in the B ring between carbons 9 and 10 (see the structure of previtamin D_3 in Figure 10.11). The two main forms of the vitamin (Figure 10.11), D_2 (also called ergocalciferol) and D_3 (also called cholecalciferol) differ in the structure of their side chains, but not in their general metabolism or functions in the body.

Sources

Vitamin D is provided as D_3 by a relatively small number of foods of animal origin. In foods, the vitamin is fairly stable and thus not prone to cooking, storage, or processing losses. The largest quantities of the vitamin are found in fatty fish (and their oils) such as swordfish, salmon, tuna, and sardines (Table 10.4). A few foods of plant origin, such as shitake mushrooms (0.5 μg/½ cup cooked), provide some vitamin D as D_2. Yet, because so few foods in the United States contain much vitamin D, selected foods—including milk, yogurt, cheese, butter, and margarine, as well as some orange juice, breads, and breakfast cereals—may be fortified with the vitamin, usually as D_3 but sometimes as D_2. In the United States, for example, milk and orange juice may be fortified with 2.5 μg (100 IU) of vitamin D_3/cup. The vitamin D content of foods is expressed as international units (IU) or μg, with 1 μg vitamin D = 40 IU or 1 IU = 0.025 μg vitamin D.

Most vitamin D supplements provide the vitamin as D_3, although some still use D_2. Supplementation with the D_2 form, however, is less potent at higher doses versus with the D_3 form

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Table 10.4 Vitamin D Content of Selected Foods*

Food (serving)	Vitamin D (μg)
Cod liver oil (1 tbsp)	34
Swordfish (3 oz)	14
Salmon, sockeye (3 oz)	11
Sardines, canned (3 oz)	4.1
Tuna fish, canned (3 oz)	3.8
Herring (3 oz)	2.4
Liver, beef (3 oz)	1
Milk, fortified (1 c)	3
Yogurt, fortified (1 c)	2.6
Margarine, fortified (1 tbsp)	1.5
Egg yolk (1)	1
Cheeses (1 oz)	0.1–0.2
Butter (1 tbsp)	0.2

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

[1]. The amount provided in the multivitamin supplements is typically equal to or twice the Daily Value, which for vitamin D is 10 μg (400 IU). Over-the-counter supplements of just vitamin D may contain up to 50 μg (2,000 IU).

Another major source of vitamin D_3 is that made in the body from the steroid 5,7-cholestradienol, commonly called 7-dehydrocholesterol, which is derived from cholesterol (Figure 10.11). Specifically, 7-dehydrocholesterol is made in the skin's sebaceous glands and secreted onto the skin's surface, where it becomes incorporated into the skin's various layers, including the dermis and especially the epidermis. The conjugated set of double bonds (five to seven) in ring B of 7-dehydrocholesterol allows the absorption of specific wavelengths of light found in the ultraviolet range. Thus, during direct exposure to sunlight (the photons do not go through glass), ultraviolet B (UVB) photons (wavelength ~285–320 nm) penetrate into the epidermis and dermis, allowing 7-dehydrocholesterol in the

Figure 10.11 Production of ergocalciferol (vitamin D_2) and vitamin D_3 (cholecalciferol).

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plasma membranes of skin cells to absorb the photons; this event causes ring B to open, forming previtamin D_3 (also called precholecalciferol). The unstable double bonds in previtamin D_3 are rearranged (a process also called thermal isomerization) over a period of several hours to a few days, resulting in the synthesis of vitamin D_3/cholecalciferol (Figure 10.11). Excess production of vitamin D_3 in the skin is prevented through the generation of inactive metabolites, especially lumisterol, which is also produced from 7-dehydrocholesterol in the presence of ultraviolet light, and tachysterol, which is generated by further irradiation of previtamin D_3. Because neither lumisterol, tachysterol, nor previtamin D_3 has much affinity for vitamin D's main blood transport protein (vitamin D–binding protein), these compounds are lost as the skin cells slough off.

This cutaneous production of vitamin D_3, which diffuses from the skin into the blood, represents a key source of the vitamin for many persons. A 15- to 30-minute exposure of the skin (in a fair-skinned individual wearing a bathing suit) between about 10:00 AM and 3:00 PM during the spring, summer, and fall produces about 10,000–20,000 IU (250–500 μg) of vitamin D_3, an amount 100 times more than is present in fortified milk [2]. Even a 5- to 10-minute exposure of the arms and legs at midday by a fair-skinned Caucasian individual can generate about 3,000 IU (75 μg) (about four times the RDA for adults) [3]. However, cutaneous production of the vitamin is affected by time of day, season, latitude, altitude, cloud cover, air pollution, skin pigmentation, sunscreen use, and age. The seasonal, time of day, and latitudinal effects are related to the zenith angle of the sun. During the winter, the morning and late-afternoon hours, and above and below latitudes of about 35°, there is an increase in the zenith angle of the sun, which results in a longer UVB photon path and fewer UVB photons reaching the surface of the earth. Such effects diminish vitamin D production in the skin. Additionally older adults may produce up to 75% less vitamin D_3 in the skin than younger adults due to diminished 7-dehydrocholesterol content in the skin. In those with darker skin color (higher melanin content), longer sun exposure time is needed to generate the vitamin than in those with lighter skin color, since melanin blocks some of the UVB rays.

Absorption

Dietary vitamin D (both D_3 and D_2) requires no digestion and is absorbed from a micelle, in association with fat and with the aid of bile, by passive diffusion into the intestinal cell. About 50% of dietary vitamin D is absorbed. Although the rate of absorption is most rapid in the duodenum and proximal jejunum, the largest amount of vitamin D is absorbed in the jejunum. The absence of a jejunum (secondary to surgical resection) has been associated with a five-fold increased likelihood of vitamin D deficiency [4]. Within the intestinal cell, vitamin D is incorporated primarily into chylomicrons, which then enter the lymphatic system with subsequent entry into the blood. As with vitamin A, conditions associated with fat malabsorption negatively impact the intestinal absorption of vitamin D.

Transport, Metabolism, and Storage

Chylomicrons transport vitamin D that came from the diet to nonhepatic tissues throughout the body. Chylomicron remnants deliver the vitamin to the liver, which takes up the remnants by endocytosis. In contrast to dietary vitamin D, vitamin D_3 that is made in the skin slowly diffuses from the skin and is picked up for transport in the blood by a vitamin D–binding protein (DBP).

Vitamin D reaching the liver either by way of chylomicron remnants or by DBP must be hydroxylated by cytochrome P-450 hydroxylases to begin the generation of vitamin D's active form. These hydroxylases are collectively referred to as mixed-function oxidases (the enzymes reduce one atom of molecular oxygen to water and one to the hydroxyl group) and abbreviated CYP followed by numbers and letters; reviews of these enzymes are available [5]. In the liver, 25-hydroxylase (primarily CYP2A1), which is NADPH-dependent, functions in the mitochondria to hydroxylate vitamin D_3 at carbon 25 to form 25-OH (vitamin) D, also called calcidiol or 25-OH cholecalciferol (Figure 10.12). Another 25-hydroxylase (CYP2R1) found in microsomes also hydroxylates D_3 as well as D_2. While the liver expresses most of the 25-hydroxylases, the enzyme is found in other organs, including the lungs, intestine, and kidneys. While the enzyme is largely unregulated, 25-hydroxylase is more efficient during periods of vitamin D deprivation than when normal amounts of the vitamin are available. With usual physiological intake, most vitamin D is converted to 25-OH vitamin D (hereafter designated 25-OH D) and released into the blood; however, with supraphysiological intake, more vitamin D is stored in the body's adipose tissue and then released, although very slowly, as needed [6].

After its hepatic synthesis, most 25-OH D is secreted from the liver and transported in the blood by DBP. The blood is the largest single pool and represents the major "storage site" of the vitamin as 25-OH D, which has a half-life of about 2–3 weeks or more. Vitamin D is also stored in adipose tissue, which contains primarily nonhydroxylated vitamin D, and muscle, which contains both vitamin D and 25-OH D. Individuals with greater than normal amounts of body fat (as may be found in those who are overweight or obese) appear to store more of the vitamin in adipose tissue than those with less body fat.

Circulating 25-OH D concentrations reflect vitamin D status, which varies depending on both dietary vitamin D intake and sunlight exposure. Serum 25-OH D concentrations between about 30 and 40 ng/mL are generally thought to be sufficient to maintain health [7,8]; note in the text

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serum 25-OH D are expressed as ng/mL; to convert ng/mL to nmol/L, multiply by 2.496. The optimal serum 25-OH D concentration is unclear; recommendations range from about 30 to 80 ng/mL. Several genes code for proteins needed for vitamin D activation and transport in the blood. Polymorphisms in these genes are thought to contribute in part to observed variations in serum 25-OH D and DBP concentrations. Levels of both, for example, tend to be lower among African Americans than among Caucasians.

From the blood, 25-OH D is taken up by tissues (mostly by the kidneys) in response to increased concentrations of parathyroid hormone (PTH). Specifically, the 25-OH D–DBP complex binds to a cubulin-megalin membrane receptor system on the kidneys' proximal tubule cell's plasma membrane to form a megalin-DBP-25-OH D complex. The complex is internalized by endocytosis into the cells, where the 25-OH D is released and is then hydroxylated at position 1 to form the vitamin's active form, 1,25-$(OH)_2$ (vitamin) D (also called calcitriol or 1,25 dihydroxycholecalciferol; Figure 10.12). The 1-hydroxylase (CYP27B1), an NADPH-dependent mitochondrial enzyme, is expressed in the highest concentrations in the kidneys, but is also present in macrophages, skin, the intestine, and bone, among other tissues. Much of the calcitriol that is made in the kidneys is ultimately released into the blood for other tissues to use. However, many other tissues (besides the kidneys) are thought to use 25-OH D from the plasma to make their own calcitriol, provided that enough 25-OH D is available in the blood serum.

The renal synthesis of calcitriol is tightly regulated by two hormones, PTH and fibroblast-like growth factor (FGF) 23, as well as other factors. PTH (secreted primarily

Figure 10.12 Hydroxylations of vitamin D.

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when serum calcium concentrations are low but also to a lesser extent when serum magnesium concentrations are low) stimulates the synthesis of the 1-hydroxylase, and thus the synthesis of calcitriol. In contrast, FGF23, which is secreted by osteocytes, reduces 1-hydroxylase expression (to reduce calcitriol production) and stimulates 24-hydroxylase to promote the synthesis of 24,25-$(OH)_2$ D. High serum concentrations of calcium (hypercalcemia) and phosphorus (hyperphosphatemia) also inhibit calcitriol synthesis. Additionally, the concentration of the enzyme's end product, 1,25-$(OH)_2$ D (i.e., calcitriol), influences the enzyme's production by binding to a vitamin D response element (VDRE) on the promoter region of the 1-hydroxylase gene. Low concentrations of the end product stimulate 1-hydroxylase synthesis, while high concentrations inhibit 1-hydroxylase synthesis (thereby decreasing the vitamin's activation) and stimulate the production of another mixed-function oxidase, 24-hydroxylase. The 24-hydroxylase generates 24,25-$(OH)_2$ D from the hydroxylation of 25-OH D, and 1,24,25-$(OH)_3$ D from hydroxylation of 1,25-$(OH)_2$ D (Figure 10.12); these 24-hydroxylation reactions represent steps in the vitamin's inactivation. These metabolites may be further oxidized to generate a variety of excretory products.

Vitamin D_2, although less frequently consumed, is metabolized in the body to calcitriol by the same 25-hydroxylase and 1-hydroxylase as vitamin D_3. The metabolism of D_2, however, produces additional metabolites not generated by D_3 and has been suggested to be less efficient in calcitriol production than D_3 [1].

The calcitriol that is made in the kidneys is released into the blood, where it binds to DBP for transport. Thus, DBP serves to transport several forms of the vitamin to various target tissues. DBP's binding to calcitriol, however, is loose to facilitate the vitamin's release to tissues; this is in contrast to its tight binding of 25-OH D. Calcitriol in the blood has a half-life of about 2–6 hours; normal plasma calcitriol concentrations range from about 20 to 40 pg/mL, considerably lower than plasma 25-OH D concentrations. On reaching its target tissues, calcitriol is easily released from the DBP for cellular uptake.

Calcitriol's target tissues were once believed to be limited initially to the intestine, bone, and kidneys, but it is now known that receptors for the vitamin are found in many tissues, including the heart, muscle, pancreas (β-cells), brain, skin, colon, prostate, breast, hematopoietic system, central nervous system, and immune system.

Functions and Mechanisms of Action

Calcitriol has several functions; its most widely recognized role is in serum calcium homeostasis with effects on the kidneys, small intestine, and bone. Additional roles include serum phosphorus homeostasis; cell differentiation, proliferation, and growth; and muscle structure and function. The mechanisms by which calcitriol performs these functions may be divided into two categories—nongenomic and genomic—although the details of these mechanisms have not been clearly elucidated. This section first describes some of the possible nongenomic, and then the genomic, mechanisms of action of the vitamin and then discusses the functions of vitamin D.

Many nongenomic actions of calcitriol are mediated by the activation of signal transduction pathways (also called intracellular signaling) linked to cell membranes and are thought to account for vitamin D's rapid (seconds to minutes) cellular response effects. Calcitriol binding to cell membrane receptors in selected tissues (especially intestine, parathyroid, liver, and pancreatic β-cells) triggers a series of events through signal transduction pathways to evoke relatively rapid changes in some body processes. One such vitamin membrane receptor that has been identified and linked with these nongenomic actions of vitamin D is known as membrane-associated rapid-response steroid-binding (MARRS) protein. The many actions initiated from these intracellular signaling pathways include increased calcium uptake, increased intracellular calcium concentration, and/or transcellular calcium flux in cells such as enterocytes, osteoblasts, adipocytes, and skeletal muscle. These cellular events are thought to be mediated primarily by the generation of second messengers and other compounds such as mitogen-activated protein (MAP) kinase, protein kinase C, cyclic AMP, tyrosine kinase, phospholipase C, diacylglycerol, inositol phosphate, and arachidonic acid. Additional details on possible vitamin D–mediated signal transduction pathways can be found in the article by Fleet [9]. Some of the details of vitamin D–mediated nongenomic intestinal cell calcium uptake are discussed in the "Calcitriol and the Intestine" section.

Calcitriol also exerts its functions through genomic mechanisms of action (and thus regulates gene expression in target cells). Calcitriol's genomic mechanism of action is similar generally to that described for retinoic acid. Vitamin D, as calcitriol, diffuses from the cytosol into the nucleus, where it (behaving like a hormone) binds to and induces conformational changes to nuclear vitamin D receptors (VDRs; Figure 10.13). Nuclear VDRs have been found in over 30 organs, including the bone, intestine, kidneys, lungs, muscle, and skin. These nuclear VDRs are part of a so-called superfamily of receptors that also includes receptors for retinoic acid and thyroid and steroid hormones. The conformational changes in the VDRs as well as perhaps phosphorylation enables dimerization of the VDR with another receptor (commonly retinoid X receptor, or RXR) forming a heterodimer.

Interactions between the complex (calcitriol-VDR-RXR) and specific DNA nucleic acid sequences called

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vitamin D response elements (abbreviated VDRE) that are found in the promoter regions of target genes in turn affect gene expression. However, as with vitamin A, additional comodulatory (either coactivator or corepressor) proteins may further interact to influence (enhance or inhibit) gene transcription. The mechanisms by which these comodulatory proteins function are mostly unknown, but they may help link the receptor to enzymes, such as RNA polymerase II, or to other components such as factors necessary for gene transcription. Some of the comodulatory proteins are thought to include steroid receptor coactivators (SRC-1, SRC-2, and SRC-3), nuclear coactivator proteins, histone acetyl transferase, vitamin D–receptor interacting protein, and silencing mediators for retinoid and thyroid hormone receptors, among others [10]. Over 200 genes are thought to have VDRE and to be directly or indirectly influenced by calcitriol. Through these genomic interactions, vitamin D exhibits multiple biological actions with many of the effects influencing body calcium and its functions.

One of the most investigated roles of calcitriol relates to serum calcium homeostasis. In fact, in addition to its genomic interactions with VDRE on genes, calcitriol also appears to exert its effects through interactions with messenger (m)RNA to enhance or inhibit translation of selected proteins. The proteins are typically, but not exclusively, involved in calcium homeostasis and/or functions and include, for example, osteocalcin, osteopontin, 24-hydroxylase (CYP24), the epithelial transient receptor potential cation channel vanilloid-type subfamily member 6 (TRPV6), calbindin, and Ca^{2+}-ATPase. Vitamin D's role in calcium homeostasis is discussed first, followed by calcitriol's effects on phosphorus homeostasis; on cell differentiation, proliferation, and growth; and on muscle. Finally, some of calcitriol's other roles in the body are discussed.

Serum Calcium Homeostasis—Actions to Increase Serum Calcium

Calcitriol synthesis is stimulated in response to changes in serum calcium concentrations and the release of PTH. Normal serum calcium concentrations range from about 8.5 to 10.5 mg/dL (2.12–2.62 mmol/L). Hypocalcemia (low serum calcium concentrations, i.e., < 8.5 mg/dL) initially stimulates the secretion of PTH from the parathyroid gland through activation of calcium-sensing receptors. PTH concentrations then rise in the blood. Low serum concentrations of 25-OH D (especially less than about 30 ng/mL) and magnesium also promote increases in serum PTH concentrations [11,12]. (Note: 25-OH D, when present in high concentrations, diminishes serum PTH concentrations by interacting with VDRE in the promoter region of the PTH gene to inhibit its transcription.) The PTH travels to the kidneys, where it stimulates 1-hydroxylase to convert 25-OH D to calcitriol. Once synthesized, some calcitriol remains and functions within the kidneys, and some calcitriol is released into the blood bound to DBP. The vitamin then acts alone or with PTH on its other target tissues (the intestine and bone), causing serum calcium concentrations to rise to within the normal range. The effects of calcitriol on its target tissues—kidneys, intestine, and bone—are discussed next and are shown in Figure 10.14.

Calcitriol and the Kidneys Calcitriol induces calcium reabsorption in the kidneys. More specifically, calcitriol is transported into the nucleus of renal cells, where it interacts with nuclear VDRs to enhance the transcription of the genes coding for calbindin D28k. Calbindin D28k binds calcium and enhances its reabsorption in the kidneys. Calcitriol also enhances the expression of a calcium channel protein, called TRPV5, and an ATP-dependent calcium transporter

Figure 10.13 Proposed role of calcitriol bound to VDR on DNA in gene expression.

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at the kidneys' basolateral membrane to enhance calcium reabsorption from the glomerular filtrate. These actions serve to increase the reabsorption of calcium back into blood, and thus to raise serum calcium concentrations to within a normal range.

Calcitriol and the Intestine The primary function of calcitriol in the intestine is to increase the absorption of calcium (as well as phosphorus, as discussed later). Serum 25-OH D concentrations of 30–40 ng/mL appear to maximize calcium absorption in men [13,14], which is normally about 30% (of ingested calcium). However, with a vitamin D deficiency (serum 25-OH D concentrations <10 ng/mL), only about 10–15% of ingested calcium is absorbed. The vitamin is believed to act through genomic and nongenomic mechanisms to exert its effects.

With respect to genomic effects on intestinal cell calcium absorption, calcitriol is transported into the enterocyte and carried into the nucleus, where it interacts with nuclear VDRs to directly regulate specific genes encoding for proteins involved in calcium uptake and transport. As the result of this interaction, selective DNA transcriptions occur that result in the biosynthesis of new mRNA molecules. These mRNA molecules are then translated on the endoplasmic reticulum into selected proteins. The proteins act at the brush border, in the cytosol, and at the basolateral membrane of the intestinal cells, especially in the duodenum and jejunum, to promote calcium absorption. At the brush border membrane, calcitriol enhances uptake of calcium by increasing calcium channel transporters (TRPV6). Second, it enhances cellular calcium transport in enterocytes by its genomic effects, specifically interacting with VDREs associated with the gene for a calcium-binding protein called calbindin D9k, a smaller form of the protein calbindin D28k that is found in the kidneys. Calbindin D9k, which binds two calcium atoms, transports over 90% of calcium through the cytosol of enterocytes. Finally, vitamin D enhances the extrusion of calcium across the basolateral membrane of the enterocyte and into the plasma by enhancing the synthesis of Ca^{2+}-ATPase pumps. Interestingly, however, in laboratory studies, mice unable to make calbindin D9k or the calcium channel transporters exhibited adequate calcium absorption if provided with adequate dietary calcium intake [15]. Such findings suggest that other absorptive mechanisms (such as paracellular absorption) for calcium are of significance.

Calcium may be absorbed in the intestine by paracellular (meaning occurring between the enterocytes) transport that is also regulated in part by vitamin D. Vitamin D enhances the expression of genes that code for a specific

Figure 10.14 Calcitriol, 1,25-$(OH)_2$ D, synthesis and actions with parathyroid hormone (PTH).

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group of transmembrane proteins, called claudins, found in the tight junctions between cells, including those of the intestine. The synthesis of claudins 2 and 12, which have been shown to be essential for paracellular calcium absorption in the intestine, is activated by vitamin D [16]. A possible (but not well-defined) nongenomic process by which vitamin D may rapidly initiate intestinal calcium absorption is thought to involve calcitriol binding to a membrane-associated rapid-response steroid-binding protein (MARRS) on the enterocyte's basolateral membrane.

Calcitriol and the Bone Elevated serum PTH concentrations, along with calcitriol, direct the mobilization of calcium out of bone to raise serum calcium concentrations to within a normal range (Figure 10.14). More specifically, calcitriol interacts within the nucleus of mature osteoblasts to induce the expression of a cytokine called receptor activator of NFκβ ligand (RANKL). The RANKL released from the osteoblasts then interacts with the receptor protein RANK, found on the cell surface of immature osteoclast precursors (also called preosteoclasts), to stimulate (via signal transduction) the differentiation, production, and maturation of the osteoclasts. The mature osteoclasts in turn release hydrochloric acid, alkaline phosphatase, collagenase, and other hydrolytic enzymes and substances, which dissolve and catabolize (eat away at) the bone matrix. The net effect of these (bone demineralization) actions is an increase in serum calcium (and phosphorus) concentrations at the expense of the bone.

The findings, however, from meta-analyses of trials examining vitamin D supplementation failed to support its use to reduce fracture risks, but did suggest the combined use of vitamin D and calcium marginally reduced fracture risks among older adults [17]. However, more recent randomized controlled trials providing vitamin D in doses of 800 IU decreased fracture risk in older adults (>65 years), suggesting benefits may require the use of higher doses [18].

Serum Calcium Homeostasis—Actions to Decrease Serum Calcium

Although calcitriol is not thought to be directly involved in decreasing serum calcium concentrations should they rise above normal (hypercalcemia), calcitriol is indirectly involved through effects on PTH. Elevations in the serum concentrations of both calcitriol and ionized serum calcium downregulate fibroblast growth factor 23 (FGF 23) and decrease PTH production through long and short feedback loops. The long feedback loop is indirect, resulting from elevated serum ionized calcium's inhibitory effect on the parathyroid gland's secretion of PTH. The short feedback loop is direct: calcitriol decreases the transcription of the gene for preparathyroid hormone by interacting with the VDR bound to VDRE for the gene for PTH. In addition, with above-normal elevations in serum calcium concentrations, calcitonin (a hormone produced by parafollicular endocrine clear [C] cells of the thyroid gland) is released. Calcitonin blocks calcium mobilization from bones by inhibiting osteoclast activity. In addition, it inhibits the tubular resorption of calcium in the kidneys leading to increased urinary calcium excretion. Furthermore, but in contrast to their detrimental effects when present in high concentrations, calcitriol and PTH when present in low concentrations and with sufficiently available calcium and phosphorus promote bone anabolism and mineralization.

Phosphorus Homeostasis

Calcitriol affects phosphorus homeostasis. In the intestine, calcitriol is thought to increase the activity of brush border alkaline phosphatase, which hydrolyzes phosphate ester bonds to free the phosphorus and thus enable its absorption in the small intestine. Calcitriol also upregulates the number of carriers responsible for the sodium-dependent absorption of phosphorus at the brush border membrane of intestinal cells (especially of the jejunum and ileum). In bone, calcitriol promotes the resorption of phosphorus out of bone and into the blood. (See Chapter 11's section "Phosphorus Homeostasis" for a more complete discussion.)

Cell Differentiation, Proliferation, and Growth

The local presence of the calcitriol within many non-calcium-regulating tissues is thought to help maintain normal cell growth, promote differentiation, and inhibit proliferation. Some examples of cells affected by vitamin D include premyeloid white blood cells and stem cells, which differentiate into macrophages and monocytes in the presence of adequate calcitriol. Calcitriol also induces cell differentiation of stem cell monocytes in the bone marrow, which become mature osteoclasts. Vitamin D's ability to stimulate skin epidermal cell differentiation while inhibiting proliferation has been applied in the treatment of psoriasis (a disorder marked by enhanced proliferation and failed differentiation of keratinocytes). Specifically, calcitriol binds to VDRs in keratinocytes to alter the expression of regulatory proteins that serve to inhibit proliferation of the epidermis and induce normal differentiation in those with psoriasis.

Other mechanism(s) by which vitamin D affects aspects of cell differentiation, proliferation, and growth are not clear but are likely related to effects on genes coding for various regulatory factors, including ones that control cell growth and death. Calcitriol, for example, slows cell cycle progression by inhibiting key regulators of the transition from the gap (G)1 phase to the synthesis (S) phase of the cell cycle. The vitamin also affects cell growth by altering concentrations of regulatory proteins involved in apoptosis such as caspases and bcl-2.

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Calcitriol and Muscle

Muscle dysfunction (myopathy) is well documented in individuals with vitamin D deficiency. The most commonly reported problems include muscle weakness and pain, difficulty rising from a squatting or sitting position, difficulty walking (especially up stairs), and increased falls. Biopsies of muscle in those with vitamin D deficiency show atrophy of primarily type II (fast-twitch) muscle fibers (note that it is these fibers that are utilized to prevent falls). Vitamin D supplementation, in those with deficiency, in turn increases the type II muscle fiber area and diameter, as well as the synthesis of muscle cytoskeletal proteins such as calmodulin needed for muscle contraction.

In muscle, calcitriol is thought to function through genomic mechanisms to enhance calcium uptake and thus intracellular calcium concentrations. Specifically, the vitamin is thought to increase the transcription of genes for calcium ATPase pumps (that actively transport calcium into the sarcoplasmic reticulum and sarcolemma), and for voltage-sensitive calcium channels. Such changes in intracellular calcium concentrations are needed for contraction and relaxation of muscle. Calcitriol is also thought to modulate muscle contractility through nongenomic mechanisms. For example, through binding to cell surface receptors, calcitriol activates second messenger pathways to rapidly increase intracellular calcium concentrations by promoting calcium release from intracellular stores. Additionally, through second messenger pathways, calcitriol enhances myogenesis, cell proliferation, differentiation, and apoptosis. Optimal muscle function is thought to require serum 25-OH D concentrations of at least 30 ng/mL [19]. Antifall and antifracture benefits from vitamin D are thought to require daily vitamin D intakes of at least 800 IU [20].

Other Roles

Many additional biological actions have been attributed to vitamin D and linked to disease prevention. Low vitamin D status has been associated with increased risks of hypertension, cardiovascular disease, type 2 diabetes, certain cancers, and some autoimmune disorders, among other conditions. Similarly, higher serum 25-OH D concentrations have been found in observational studies to be associated with decreased risk of many of these same conditions, decreased all-cause mortality, and diminished risk of inflammation.

Vitamin D exhibits several cardioprotective functions. Deficiency of the vitamin is associated with upregulation of the renin-angiotensin-aldosterone system, hypertrophy of vascular smooth muscle cells, and left ventricular hypertrophy [12,21,22]. Vitamin D also impacts blood pressure, promoting endothelial cell–dependent vasodilation; it also affects arterial pressure and myocardial contractility via elevations in PTH [12,21,22]. Intervention studies, however, have not consistently demonstrated benefits from vitamin D supplementation in the absence of vitamin deficiency.

A strong inverse association has been documented between serum 25-OH D concentrations and type 2 diabetes in meta-analyses of prospective studies [23]. Among other roles, vitamin D is thought to protect pancreatic β-cells (via decreasing apoptosis) and modulate insulin synthesis (via interacting with VDRE in the promoter region of the gene for insulin). Insulin secretion also appears to be affected secondary to vitamin D–induced changes in calcium concentrations.

Reductions in the growth of cancer cells by vitamin D have been associated with the vitamin's role in promoting cell differentiation and inhibiting cell proliferation. Additionally, the production of calcitriol from 25-OH D within specific tissues may also inhibit cancer by promoting apoptosis, inhibiting invasiveness/metastases and angiogenesis (blood vessel growth in cancerous tissues), and affecting the synthesis of other proteins/enzymes that in turn protect tissues. Most often cited are reductions in the risk for colon and breast cancers with higher serum 25-OH D concentrations; however, the vitamin's beneficial effects may extend to reductions in all-cause cancer risk and cancers of the lungs, kidneys, and prostate [2,19,24]. Results from a systematic review, however, did not find clear benefits of vitamin D supplementation in decreasing cancer occurrence [25].

Autoimmune conditions, including rheumatoid arthritis, Crohn's disease, multiple sclerosis, and type 1 diabetes mellitus, as well as inflammation have been linked with inadequate vitamin D status. Many of these disorders are more common among individuals living in the United States in northern latitudes where less cutaneous vitamin D is synthesized. Protective effects against developing some of these diseases have also been found with the use of vitamin D supplements in amounts up to about 50 μg (2,000 IU) daily [26,27]. In other cases, reducing disease activity and a slowing of disease progression (except in multiple sclerosis and rheumatoid arthritis) have resulted from vitamin D supplementation. These findings are thought to be linked (at least partially) with vitamin D's role in immune-modulating and anti-inflammatory actions. Several immune cells—including activated T- and B-cells, antigen-presenting dendritic cells, monocytes, macrophages, and cytotoxic T-cells—express VDRs. Many of these cells also produce calcitriol. Vitamin D generally appears to upregulate the production of many anti-inflammatory cytokines and downregulate the synthesis of several pro-inflammatory cytokines. Such roles impact both autoimmune conditions as well as heart disease. Vitamin D also enhances the production of antimicrobial peptides like cathelicidin and defensins. These peptides increase tight junctions between intestinal mucosal cells to help protect against bacterial invasion and reduce inflammation in the digestive tract. Autoimmune

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disease development is thought to be also affected by vitamin D's effects on T-cell differentiation, proliferation of activated B-cells, and inhibition of antigen presentation. Excellent reviews of study findings addressing vitamin D and a variety of diseases are available; unfortunately, the results of intervention supplementation trials with vitamin D to treat many of these conditions (such as cardiovascular disease) and to improve quality of life have not shown benefits [8,28,29].

Interactions with Other Nutrients

Vitamin D functions and relationships with calcium and phosphorus have been discussed previously. The vitamin also interacts with vitamin K–dependent proteins, a topic discussed in the "Vitamin K and Bone" section of this chapter.

Metabolism and Excretion

Calcitriol hydroxylation at carbon 24 by a 24-hydroxylase generates the trihydroxy metabolite 1,24,25-$(OH)_3$ D (see Figure 10.12), which may be further oxidized to 1,25-$(OH)_2$ 24-oxo D. Subsequent reactions, including side-chain cleavage, yield the major end-product calcitroic acid (see Figure 10.12). Other vitamin D metabolites are also formed after additional hydroxylation, oxidation, and conjugation. Calcitroic acid and vitamin D metabolites are excreted primarily through the bile in the feces. Little (<30%) of the vitamin is excreted via the urine.

Recommended Dietary Allowance

An RDA for vitamin D, which was published for the first time in 2010 [7], assumes minimal sun exposure and suggests an intake of 600 IU (15 μg) of vitamin D for children (age 1 year and older), adolescents, and adults, including women who are pregnant or lactating. Recommended intakes increase to 800 IU (20 μg) of vitamin D for adults older than 70 years of age. Requirements are estimated at 400 IU (10 μg) for these age groups. Sufficient amounts of vitamin D are thought to be obtainable by exposure to sunlight for about 5–15 minutes at midday a few times per week. Such exposure is thought to be able to raise serum 25-OH D concentrations to 45 ng/mL and to reduce more deaths than would occur from skin cancers [19,24].

Deficiency: Rickets and Osteomalacia

Vitamin D deficiency is widespread across the United States and the world. Depending on the cutoff values used for serum 25-OH D, the prevalence of vitamin deficiency may be as high as 80% [11]. Vitamin D deficiency most notably affects bone. In infants and young children, vitamin D deficiency causes rickets, a condition characterized primarily by failure of bone to mineralize. Signs of rickets appear at about 6 months of age in affected infants. With deficiency, the epiphyseal cartilage continues to grow and enlarge without sufficient replacement by bone matrix and minerals. The widening of the epiphyseal plates is especially visible at the ends of long bones (i.e., wrists, ankles, and knees) and at costochondral junctions (referred to as rachitic rosary and resembling beading at the junction of the ribs and cartilage). In addition, because in infants and young children little mineral is present in the skeleton, the long bones of the legs bow and knees knock as weight-bearing activities such as standing and walking begin. The spine also becomes curved, and pelvic and thoracic deformities occur. Frontal bossing (a prominent protruding forehead), widening of the fontanelles (the soft membranous space between the cranial bones where ossification is not yet complete), and softening of the fontanelle borders appear sometime between about 6 and 14 months of age. Other manifestations may include bone pain in the legs or back, delayed age of standing or walking, frequent falling, delayed growth, neuromuscular irritability, and hypocalcemic seizures. Biochemical indicators of deficiency may include elevated total or bone alkaline phosphatase and PTH concentrations, low 24-hour urinary calcium excretion, and low serum calcium and/or phosphorus and 25-OH D concentrations. Radiography of long bones at the knees and wrists shows widening of the growth plates and fraying of the margin of the metaphysis, indicating impaired mineralization [2].

In adults and older children, insufficient vitamin D leads to **osteomalacia** (*malacia* means "softening," and *osteo* refers to bone). Osteomalacia results from the failure to mineralize already formed bone (i.e., unlike in infants and young children, the epiphyseal plates are closed and enough mineral in the bone is present to prevent deformities with weight-bearing activities in adults and older children). Insufficient vitamin D over time induces secondary hyperparathyroidism, which increases bone turnover and mineral loss. Bone resorption is characterized by urinary excretion of bone collagen by-products such as hydroxyproline, N-telopeptide, pyridinoline, and deoxypyridinoline. Additionally, as new bone is produced by osteoblasts (remember bone is constantly being remodeled), it does not get mineralized sufficiently. Thus, over time, the amount of nonmineralized bone exceeds mineralized bone. The progressive demineralization results in bone pain (characterized as throbbing or aching), especially at the joints (such as the shoulders), and increased risk of fractures. Muscle weakness and pain and gait instability may also be present. Biochemical indicators of deficiency are usually the same as those seen in rickets. Radiographic findings may include low bone density and skeletal pseudofractures and/or nontraumatic fractures (fragility).

While exposure of skin (without sunscreen) to sunlight can maintain adequate vitamin D nutrition for most

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of the world's population, people with insufficient sun exposure and those with certain diseases or conditions may be at risk for vitamin D deficiency. Older adults represent a population group at risk due to insufficient vitamin D intake, low sunlight exposure, age-associated 7-dehydrocholesterol content reductions in the skin, and reduced renal 1-hydroxylase activity in response to PTH. Impaired vitamin D absorption may occur in disorders characterized by fat malabsorption, such as Crohn's disease, celiac disease, cystic fibrosis, pancreatitis, and liver disease as well as with some bariatric surgical procedures used to treat obesity. Disorders affecting the parathyroid, liver, or kidneys impair synthesis of the active form of the vitamin. People on anticonvulsant drug therapy may develop an impaired response to vitamin D secondary to increased hepatic metabolism of the vitamin to inactive forms and subsequent reductions in calcium absorption. Infants may be at risk for deficiency because human milk is low in vitamin D and exposure to sunlight typically is minimal; vitamin D (400 IU or 10 μg and equal to the RDA) supplements are recommended daily for exclusively or partially breast-fed infants. The Endocrine Practice Guideline Committee recommends intakes of 1,500–2,000 IU of vitamin D for adults at risk for vitamin D deficiency [8].

For those with liver and/or renal dysfunction who cannot generate the 25-OH D or 1,25-$(OH)_2$ D forms of the vitamin, a number of supplements are available. For example, Rocaltrol (Hoffman-LaRoche) for oral use and Calcijex (Abbott Laboratories) for intravenous use are commonly prescribed brands of calcitriol for those with kidney disease. Other preparations, such as Calderol (Organon-USA), which provides 25-OH D, and vitamin D_2 and D_3 (i.e., unhydroxylated forms) are also available.

A deficiency of vitamin D in adults with serum 25-OH D concentration <10 ng/mL and normal organ function is often treated with high loading doses of the vitamin—such as 50,000 IU (1,250 μg) given once per week for 8–12 weeks to achieve a serum 25-OH D concentration of above 30 ng/mL [8,19,30]. Other regimens for adults who are vitamin D insufficient (20–29 ng/mL) provide lower doses of the vitamin such as 1,000–2,000 IU (25–50 μg) daily for 2–3 months. Because the vitamin is sequestered in adipose and thus less available, obese individuals may require about two to three times higher doses of the vitamin to prevent and treat deficiency [8,19,20]. Intakes of up to about 2,000 IU (50 μg) are also recommended for those with malabsorption conditions and those on medications affecting vitamin D metabolism [8]. Serum 25-OH D concentrations and perhaps other biochemical indicators associated with rickets and osteomalacia should be rechecked after about 3 months of supplementation. Some radiological healing may be evident after 1 month, but complete healing usually requires a longer time frame [31].

It is estimated that for every 1,000 IU of vitamin D ingested, serum 25-OH D concentrations increase by about 6–10 ng/mL after a few weeks and in those with serum 25-OH concentrations less than about 15 ng/mL, concentrations may rise by 20–30 ng/mL [19]. Once repletion has occurred, maintenance intakes of about 800–2,000 IU (20–50 μg) daily are thought to be needed for adults to maintain serum 25-OH D concentrations in excess of 30 ng/mL [8,30].

Toxicity

The Tolerable Upper Intake Level established by the Institute of Medicine for vitamin D has been set at 4,000 IU (100 μg) for children age 9 years and older, adolescents, and adults [7]. However, many believe this upper level is too low. The Endocrine Practice Guidelines Committee, for example, suggests an upper level of 10,000 IU (2,500 μg) for adults over 19 years of age [8].

Toxicity of vitamin D results from ingestion of oral supplements in large quantities. In fact, vitamin D, when ingested in large amounts, is one of the most likely of all vitamins to cause overt toxicity. Excessive exposure to sun, however, does not result in overproduction of endogenous vitamin D_3. Cutaneous vitamin D production reaches a maximum of about 20,000 IU (500 μg), and extensive whole-body ultraviolet light irradiation generally raises serum 25-OH D concentrations to about 40–80 ng/mL [26]. A safe upper limit for serum 25-OH D concentrations is unclear. A U-shaped relationship has been suggested with detrimental consequences associated with both low and high concentrations of 25-OH D. Serum concentrations of 25-OH D over 60 ng/mL have been associated with increased mortality and some cancers (especially pancreatic cancer) [11,12,32]. The Endocrine Society suggests a preferred serum 25-OH D range from 40 to 60 ng/mL [8].

Toxicity is seen especially in those with vitamin D intakes of 10,000 IU (or more) per day for several months. Serum 25-OH D concentrations greater than 80 ng/mL are considered suggestive of possible toxicity; however, concentrations greater than 150 or 200 ng/mL are more commonly used diagnostically [7,17,26]. Manifestations of toxicity in adults include high ionized serum calcium (greater than about 5.7 mg/dL) as well as hypercalciuria and hyperphosphatemia. The high serum calcium concentrations lead to calcinosis, that is, calcification of soft tissues including organs such as the kidneys, heart, and lungs, along with blood vessels. The calcification damages the blood vessels and tissues, resulting in manifestations such as hypertension, headache, renal dysfunction (characterized by polyuria, polydipsia, azotemia, and possibly nephrolithiasis and renal failure), heart damage, and, in some cases, death. In the 1950s an epidemic of "idiopathic hypercalcemia" among English infants was traced to a daily intake of vitamin D between 2,000 and 3,000 IU. Symptoms of toxicity in the infants included anorexia, nausea, vomiting, hypertension, renal insufficiency, and failure to thrive. Because so many

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foods are now fortified with vitamin D and many over-the-counter products also contain the vitamin, the consumer needs to consider all sources of the vitamin to ensure intake is not excessive.

Assessment of Nutriture

Serum 25-OH D concentrations are most often used to assess vitamin D status, with concentrations less than 20 ng/mL indicating deficiency and concentrations in the range of 21–29 ng/mL constituting vitamin D insufficiency. Target serum 25-OH D concentrations of 40 ng/mL are suggested, although PTH concentrations plateau at about serum 25-OH D concentrations between 30 and 40 ng/mL [8,26]. Toxicity is apparent when serum 25-OH D concentrations exceed about 200 ng/mL, although some evidence suggests that concentrations greater than 60 ng/mL may be adverse to health [7,17,26,32].

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VITAMIN E

Vitamin E encompasses eight compounds (vitamers). Each of these eight compounds contains a phenolic functional group on a chromanol/chromane ring (sometimes called the head of the molecule) and an attached phytyl side chain (sometimes called the **phytyl tail** of the molecule). The eight compounds (Figure 10.15) are usually divided into two classes:

- the tocopherols, which have saturated side chains with 16 carbons
- the tocotrienols (also called trienols), which have unsaturated side chains with 16 carbons.

Each class is composed of four vitamers that differ in the number and location of methyl groups on the chromanol ring. Vitamers in both classes are designated as α, β, γ, or δ. Only α-tocopherol has biologic activity and can meet the body's need (requirement) for the vitamin, as the body cannot interconvert the vitamers.

Tocopherols and tocotrienols contain three chiral centers with a configuration of "R" at three positions. R and S are used to designate stereoisomers of asymmetrical molecules such as vitamin E. The naturally occurring and the most biologically active form of α-tocopherol exists as one stereoisomer, RRR α-tocopherol, which is also referred to as D- or d-α-tocopherol.

Synthetic forms of the vitamin may contain a mixture of the eight possible stereoisomers. Of the eight stereoisomers, four are in the 2R-stereoisomeric form (RRR, RSR, RRS, and RSS) and four are in the 2S-stereoisomeric form (SRR, SSR, SRS, and SSS) [1]. The form of the vitamin should be listed on the food or supplement label. Synthetic forms of α-tocopherol are shown on supplement labels as DL- or dl-α-tocopherol. The Food and Nutrition Board recommends that vitamin E activity be limited to naturally occurring RRR α-tocopherol and to three synthetic stereoisomeric forms: RSR-, RRS-, and RSS-α-tocopherol; however, in establishing a Tolerable Upper Intake Level, all supplemental forms of the vitamin were considered [1].

The term *tocopherol* is derived from the Greek word *tokos*, which means "childbirth," and *phero*, which means "to bear or bring forth." This terminology is based on the vitamin's discovery by H. Evans and K. Bishop in 1922, when they found that rats could not reproduce when given a diet of rancid lard. Wheat germ oil provided the needed vitamin; the oil was later purified, and the vitamin was extracted and named vitamin E (following D, which had been previously discovered).

Tocopherols

	R_1	R_2	R_3
α-tocopherol	CH_3	CH_3	CH_3
β-tocopherol	CH_3	H	CH_3
γ-tocopherol	H	CH_3	CH_3
δ-tocopherol	H	H	CH_3

Tocotrienols

	R_1	R_2	R_3
α-tocotrienol	CH_3	CH_3	CH_3
β-tocotrienol	CH_3	H	CH_3
γ-tocotrienol	H	CH_3	CH_3
δ-tocotrienol	H	H	CH_3

Figure 10.15 The structures of the various forms of the tocopherols and tocotrienols.

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Sources

Vitamin E, in its various forms, is found primarily in plant foods, especially nuts, foods made from nuts (e.g., nut butters), seeds, and the oils from plants. As seen in Table 10.5, which provides the α-tocopherol content of some of these plant foods, wheat germ oil provides significant quantities of vitamin E; lesser (but still relatively high) amounts are also present in other oils. Some oils, including soybean, canola, and corn, contain less α-tocopherol and more δ-tocopherol. Foods (especially full-fat varieties) made from vegetable oils, such as salad dressings, mayonnaise, and margarine, also represent major sources of α-tocopherol. Other plant sources of vitamin E include some fruits and vegetables. The green (chloroplast) portions such as the leaves of plants contain more α-tocopherol, while the other portions of the plant provide some γ-, δ-, and β-tocopherols. Cereals that are whole grain provide some vitamin E. Raisin Bran® and Frosted Mini Wheats®, for example, provide 0.45 mg and 0.34 mg of α-tocopherol/cup, respectively.

Tocotrienols are found in small quantities in seeds, legumes especially soybeans, palm and coconut oils, cocoa butter, and cereal grains, especially the bran and germ fractions of barley, rice, and oats. Palm oil, one of the more saturated oils from a plant source, is also one of the richest natural sources of tocotrienols, with 70% of its vitamin E as tocotrienols and 30% as tocopherols. Rice bran oil is also a good source of δ-tocotrienol.

In foods of animal origin, vitamin E, primarily α-tocopherol, is found concentrated in fatty tissues. Higher-fat meats, such as ground beef that is 20% fat/80% lean, provide about 0.4 mg of α-tocopherol/3-oz portion. Animal products, however, when compared to plants, represent an inferior source of vitamin E.

While the FDA does not require vitamin E to be listed on food labels unless the product has been fortified with the vitamin, the vitamin E content, when present on the label, is given as a percentage of the Daily Value, which for vitamin E is 30 IU. While the use of IU has been officially discontinued, it is still found on some labels. Conversion factors to calculate intake provided by IU versus mg are given in the section on Recommended Dietary Allowances.

Supplements vary in amount and form of vitamin E. Most supplements provide vitamin E in amounts ranging from 100 to 800 IU; note 100 IU is about five times higher than the RDA for vitamin E for adults. The form of vitamin E in supplements also varies, with some containing α-tocopherol and others providing mixtures of the eight possible stereoisomers. Synthetic vitamin E is usually provided as all-racemic (or all-rac, meaning that it has a synthetic mixture of all eight possible stereoisomers) α-tocopheryl acetate or all-rac α-tocopheryl succinate. These forms may also be used to fortify foods. Supplements containing the stereoisomer mixtures are not as active (about 50% less) as the naturally occurring RRR d-α-tocopherol. The form of the vitamin should be listed on the food or supplement label.

Vitamin E, usually as vitamin E acetate, is also found in many creams designed for topical use. While concentrations of the vitamin in these products vary considerably, credible scientific studies have not supported the use of these creams in the prevention of scarring (a frequently encountered claim).

Vitamin E, like other fat-soluble vitamins, is susceptible to destruction during food preparation and storage. The processing of some foods also significantly reduces their vitamin E content; for example, the germ is removed in the milling of wheat to make white flour, and thus substantial amounts of the vitamin are lost. Tocopherols are also oxidized with lengthy exposure to air. In addition, exposure of the vitamin to light and heat can also lead to increased destruction. Thus, for example, the roasting of nuts reduces their vitamin E content.

Dietary vitamin E intakes by Americans are generally estimated to be low, with over two-thirds of adults not meeting recommendations and in some cases requirements for the vitamin [2,3]. It is especially likely that people following a low-fat diet will limit foods that are major sources of vitamin E unless wise food choices are made [1].

Table 10.5 Vitamin E Content of Selected Foods*

Food (serving)	Vitamin E (mg)
Wheat germ oil (1 tbsp)	20.3
Sunflower oil (1 tbsp)	5.6
Canola oil (1 tbsp)	2.4
Safflower oil (1 tbsp)	4.6
Sunflower oil (1 tbsp)	5.6
Soybean oil (1 tbsp)	1.1
Corn oil (1 tbsp)	1.9
Peanuts (1 oz)	2.2
Peanut butter (2 tbsp)	2.9
Cashews (1 oz)	0.3
Hazelnuts (1 oz)	4.3
Almonds (1 oz)	7.0
Sunflower seeds (1 oz)	7.4
Margarine (1 tbsp)	1.6
Mayonnaise (1 tbsp)	0.7
Broccoli, cooked (1/2 c)	1.1
Collards, cooked (1/2 c)	0.8
Carrots, raw (1/2 c)	0.4
Avocado, raw (1/2 c)	1.5
Kiwifruit (1 medium)	1.1
Tomato (1 medium)	0.7

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

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Digestion and Absorption

Whereas the tocopherols are found free in foods, the tocotrienols are found esterified and must be hydrolyzed before absorption. Similarly, synthetic ester forms of the tocopherols such as tocopheryl acetate must be digested before absorption. Pancreatic esterase and especially duodenal mucosal esterase (also called carboxyl ester hydroxylase) function in the lumen or at the brush border membrane of enterocytes to hydrolyze tocotrienols and synthetic ester α-tocopherols so they can be absorbed.

Vitamin E is absorbed primarily in the jejunum by passive diffusion; bile salts are required for emulsification, solubilization, and micelle formation, thus allowing the vitamin to diffuse across the membrane of the enterocyte. Simultaneous digestion and absorption of dietary lipids with vitamin E improves the vitamin's absorption, which ranges from about 20 to 70%. Higher intake of the vitamin appears to reduce its absorption. Little is known about tocotrienol absorption; however, the involvement of a Niemann-Pick C1-like 1 (NPC1L1) transporter in δ-tocotrienol absorption has been suggested.

Transport, Metabolism, and Storage

In the enterocyte, absorbed tocopherols and tocotrienols are incorporated into chylomicrons for transport through the lymph and then into circulation. During transport in the chylomicrons, the vitamin (tocopherols and tocotrienols) is transferred among the other lipoproteins, especially HDLs and LDLs. LDLs, which possess the highest concentrations of the vitamin, are thought to contain about five to nine α-tocopherol molecules per LDL [4]. The half-life of RRR α-tocopherol in the plasma is about 48 hours, whereas the stereoisomer SRR α-tocopherol has a half-life of about 13–15 hours [5].

Hepatic uptake of vitamin E occurs following the delivery of the tocopherols and tocotrienols via chylomicron remnants. The liver metabolizes the various forms of vitamin E, but "trafficks" only RRR α-tocopherol for incorporation into nascent VLDLs. An α-tocopherol transfer protein (αTTP), made in the liver, carries out this transfer, and the α-tocopherol-rich VLDL is then released into the blood. A deficiency or absence of αTTP caused by gene defects leads to a vitamin E deficiency. Additionally, conditions affecting the liver, such as nonalcoholic fatty liver disease and metabolic syndrome, among others, may disrupt the "trafficking" of the vitamin and affect its use [6]. It is because of the specificity of αTTP that other forms of the vitamin are not resecreted into the circulation. Thus, α-tocopherol is the primary form of vitamin E found in the blood; the vitamin, however, is exchanged, as previously mentioned, among the various lipoproteins, which maintain plasma concentrations and distribute the vitamin to tissues. The exchange of the vitamin among the lipoproteins is mediated by a phospholipid transfer protein. LDLs and HDLs carry most of the vitamin in the blood. Plasma α-tocopherol (bound within lipoproteins) concentrations usually range from about 5 to 20 mg/L, with higher serum total lipids resulting in a longer plasma vitamin E half-life [7].

Tocopherol uptake into cells occurs in association with the lipoproteins: as receptor-mediated uptake of LDLs occurs, through lipoprotein lipase-mediated hydrolysis of chylomicrons and VLDLs, through HDL-mediated nutrient delivery, and possibly by other mechanisms. A phospholipid transfer protein may facilitate vitamin E transfer from the lipoproteins to the cell membranes.

Within the cytosol as well as other locations, including the nucleus, vitamin E binds to specific tocopherol-binding proteins for intracellular transport. ATP-binding cassette (ABC) A1 is thought to be involved in cellular trafficking and efflux of the vitamin from cells. The protein is also known to transport cholesterol and phospholipids. Within cells, vitamin E is primarily incorporated in the phospholipid bilayer of membranes including the plasma, mitochondrial, and microsomal membranes. Vitamin E's chromanol group is likely directed toward the membrane surface (near the phosphate region of the phospholipid), and its phytyl tail is directed toward the hydrocarbon region.

There is no single storage organ for vitamin E. The largest amount (over 90%) of the vitamin is concentrated in an unesterified form in fat droplets in adipose tissue. The concentration of vitamin E in adipose tissue increases linearly with absorbed dietary vitamin E; however, the release of the vitamin from adipose tissue is slow, even during periods of low vitamin E intake. Other tissues that take up smaller amounts of vitamin E include the liver, lungs, heart, muscle, adrenal glands, spleen, and brain. The vitamin E concentration in these tissues remains relatively constant or increases only at a slow rate with increased ingestion of the vitamin. Yet, during times in which vitamin E intake is low, the liver and plasma provide a more available source of the vitamin, in addition to skeletal muscle, which, because of its large mass, contains appreciable amounts.

Functions and Mechanisms of Action

The principal function of vitamin E (both tocopherols and tocotrienols) is as an antioxidant. It is in this capacity that the vitamin maintains the integrity of membranes and lipoproteins, protecting them (and other components) from oxidation. The mechanism by which vitamin E protects the membranes from destruction is through its ability to prevent the oxidation (peroxidation) of unsaturated fatty acids contained in the phospholipids of the membranes. Though the phospholipids of the mitochondrial membrane and endoplasmic reticulum contain more

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unsaturated fatty acids than the cell's plasma membrane and thus are at greater risk of oxidation, cell membranes are still vulnerable. Tissues with cell membranes especially susceptible to oxidation include the lungs, brain, and erythrocytes. Erythrocyte membranes, for example, are vulnerable because they are high in polyunsaturated fatty acids and are exposed to high concentrations of oxygen. A discussion of vitamin E's role as an antioxidant follows, with a brief description of the generation of carbon-centered and peroxyl radicals. More information on how free radicals are generated and how they can damage cell membranes is found in this chapter's Perspective.

Antioxidant Role

As an antioxidant, vitamin E can destroy singlet oxygen and can stop reactions involving free radicals (sometimes called free radical termination or chain-breaking). This section addresses each of these aspects of vitamin E function.

Singlet Molecular Oxygen Destruction Singlet molecular oxygen, also called singlet oxygen, $^1O_2$, is a very reactive and destructive compound that may be formed in the body from lipid peroxidation of membranes, transfer of energy from light (photochemical reactions), or the respiratory burst occurring in neutrophils (enzymatic reactions). Singlet oxygen readily reacts with organic molecules such as protein, lipids, and DNA and thus can damage cellular components unless removed. As discussed earlier in this chapter in the section on the antioxidant functions of carotenoids, quenching is a process by which electronically excited molecules, such as singlet oxygen, are inactivated. Specifically, physical quenching occurs when the singlet excited oxygen is deactivated without light emission and generally involves electron energy transfer. Like carotenoids, vitamin E has oxygen-quenching abilities. The ability of vitamin E to physically quench singlet oxygen is related to the free hydroxyl group in position 6 of vitamin E's chromane ring (Figure 10.15). Yet, all tocopherols are not equal in their quenching abilities: α-tocopherol was found to be as or more effective in the quenching of singlet oxygen than β-tocopherol and γ-tocopherol, followed in descending order by δ-tocopherol.

Free Radical Termination The structure of vitamin E, specifically the phenolic hydroxyl group, provides hydrogen ions to terminate the actions of free radicals and thus limit their destruction of cell membranes and other cell components. Reactions involving free radicals often occur in three phases: initiation, propagation (ongoing generation), and termination, with the last involving vitamin E. A description of the three phases, including the reactions occurring in each, is presented next.

Initiation typically begins with a free radical, for example a hydroperoxyl ($HO_2^{\bullet}$) radical, which is highly reactive and rapidly takes electrons from its surrounding organic molecules. If the organic molecule is a polyunsaturated fatty acid (PUFA) present in the phospholipid portion of the cell membrane, the membrane is damaged. In fact, membrane lipid peroxidation is thought to represent a primary event in oxidative cellular damage. Specifically, hydrogen atoms from the methylene groups ($—CH_2—$) found between double bonds in polyunsaturated fatty acids ($—CH{=}CHCH_2CH{=}CH—$) are primary targets for proton abstraction by radicals. Examples of initiation reactions follow:

- The reaction between lipid compounds (LH) such as PUFA and free hydroxyl radicals ($^{\bullet}OH$) leads to the formation of a lipid carbon-centered or alkyl radical ($L^{\bullet}$) and water, as shown here and in Figure 10.16:

$$LH + {}^{\bullet}OH \longrightarrow L^{\bullet} + H_2O$$

- Alternately, lipid compounds (LH) can react with molecular oxygen (O_2) to generate lipid carbon-centered or alkyl radicals and the hydroperoxyl radical $HO_2^{\bullet}$, as follows:

$$LH + O_2 \longrightarrow L^{\bullet} + HO_2^{\bullet}$$

Once lipid carbon-centered or alkyl radicals are formed, they may react to form additional radicals in propagation reactions. Propagation is the second step in the process of lipid peroxidation.

- Lipid carbon-centered or alkyl radicals can react with molecular oxygen in a propagation reaction to form a lipid peroxyl radical $LOO^{\bullet}$ and promote peroxidation, as shown here and in Figure 10.16:

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet} \text{ (Also written } LO_2^{\bullet}\text{)}$$

Lipid peroxyl radicals ($LOO^{\bullet}$), once formed, can abstract a hydrogen atom from other organic compounds including more polyunsaturated fatty acids (L´H) in membranes or

Figure 10.16 Initiating and chain reactions caused by hydroxy free radical attack on unsaturated fatty acid.

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in lipoproteins to generate lipid hydroperoxides (LOOH) and a chain reaction with the $L'^{\bullet}$, as shown here and in Figure 10.16:

$$LOO^{\bullet} + L'H \longrightarrow L'^{\bullet} + LOOH$$

Termination of chain reactions (also referred to as free radical scavenging) is the final step. Without termination, one initiating event could result in the generation of thousands of lipid peroxides and massive cellular damage. Vitamin E located in or near membrane surfaces can react with peroxyl radicals ($LOO^{\bullet}$) before they interact with fatty acids in cell membranes or other cell components. Thus, vitamin E terminates chain-propagation reactions. While much research has focused on α-tocopherol, γ-tocopherol also exhibits superior antioxidant properties but the vitamin is more effective against some types of free radical versus others. Specifically, vitamin E (EH, reduced state), because of the reactivity of the phenolic hydrogen on its carbon 6 hydroxyl group and the ability of the chromanol ring system to stabilize an unpaired electron, provides a hydrogen for the reduction of lipid peroxyl radicals, as shown here:

$$LOO^{\bullet} + EH \longrightarrow LOOH + E^{\bullet}$$

Vitamin E (EH) also provides a hydrogen for the reduction of lipid carbon-centered radicals:

$$L^{\bullet} + EH \longrightarrow LH + E^{\bullet}$$

In these reactions, vitamin E, after hydrogen donation, becomes oxidized. $E^{\bullet}$ represents oxidized vitamin E (also called an α-tocopherol radical or a tocopheroxyl radical). This tocopheroxyl radical can react with another peroxyl radical to form an inactive product such as tocopherylquinone. Alternately, the tocopheroxyl radical that is generated may be reduced; such a reaction is important since it enables the vitamin's reuse. Regeneration of the reduced form of vitamin E (Figure 10.17) requires reducing agents, such as vitamin C, reduced glutathione (GSH), NADPH, ubiquinol, or dihydrolipoic acid; see also the Perspective at the end of this chapter. Vitamin E is only one line of defense against oxidative tissue damage and, consequently alone or with other antioxidants may be protective against disease.

Vitamin E and Heart Disease Heart disease is thought to arise, in part, with the oxidation of LDLs, which contributes to plaque formation along with an accumulation of lipid-laden foam cells in the blood vessel walls (arterial intima), described previously in the section "Carotenoids and Heart Disease." The antioxidant properties of vitamin E enable it to inhibit the oxidation of LDLs. The vitamin also helps diminish platelet aggregation and thus blood clot formation, and helps suppress the release of inflammatory cytokines [1,4]. Thus, it is logical that diets rich in vitamin E or supplementation with vitamin E may be associated with a reduced risk of heart disease. Unfortunately, randomized clinical trials among other types of studies have not shown beneficial effects of vitamin E supplementation (generally providing 400 IU, 600 IU, or 600 μg of vitamin E) on reducing risk for heart disease or cardiovascular events in healthy middle-aged adults or in those with one or more risk factors for heart disease. (see [8] for a review of the findings of some of these studies).

Vitamin E and Cancer Vitamin E's antioxidant abilities also form the basis for its suggested benefits in reducing the risk of cancer. Free radical–induced damage is thought to trigger cancer through the activation of certain signaling pathways, alterations in gene expression, disruption of normal repair systems, alterations in enzyme activity, alterations in cell growth or differentiation, and/or direct production of toxic compounds, among other theories [9]. Unfortunately, the results of the majority of vitamin E supplementation trials (providing about 400 IU) (see [8] for a review of the findings of some of these studies) show no benefits in reductions in risk of cancer or in deaths from cancer. Moreover, a few studies found an increased risk of prostate cancer. Thus, the use of vitamin E supplements is not recommended at present for cancer prevention.

Vitamin E and Eye Health Reduced antioxidant status and/or dietary intake, especially of vitamins E and C, has been shown in many, but not all, studies to be associated with an increased risk of development of cataracts and age-related macular degeneration (see the "Eye Health" section of vitamin C in Chapter 9 for a brief discussion of these two conditions). Because radical-induced damage is thought to contribute to the development of these ocular conditions,

Figure 10.17 The regeneration of vitamin E (α-tocopherol).

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and because vitamin E functions as an antioxidant, it is logical that vitamin E, like vitamin C and β-carotene, may help in their prevention or treatment. The Age-Related Eye Disease Study (a large randomized, placebo-controlled 5-year clinical trial) provided supplementation with vitamin E (400 IU), vitamin C (500 mg), β-carotene (15 mg), zinc (80 mg), and copper (2 mg) found modest effects in slowing the progression of age-related macular degeneration [10], but most other studies and meta-analyses of studies providing vitamin E alone or with other antioxidants have not reported benefits from supplementation [11–13].

Vitamin E and Other Diseases/Conditions Vitamin E supplementation has also been suggested to diminish oxidation in those individuals with conditions characterized by increased metabolic stress and thus lipid peroxidation such as iron toxicity, diabetes, metabolic syndrome, and nonalcoholic fatty liver disease, among others. By maintaining plasma cell membranes, vitamin E may also enhance cellular glucose uptake (which is especially important for those with diabetes). Additionally, vitamin E's antioxidant function has been linked to improved bone health and reduced fracture risk.

In neurodegenerative diseases such as Alzheimer's disease, protein aggregates typically accumulate and are coupled with the loss of specific neuronal cell populations. Because oxidative stress (free radicals) is thought to be linked (at least in part) with the development of some neurodegenerative conditions, provision of antioxidants such as vitamin E is theorized to be beneficial. Yet, as with other conditions, consistent benefits of vitamin E supplementation in older adults with cognitive decline and in individuals with Alzheimer's disease as well as those with other neurodegenerative diseases such as Parkinson's disease and other forms of dementia have not been documented [8,14–17]. Benefits from topical vitamin E use to prevent scar formation have also not been documented in scientific studies; however, vitamin E creams, usually containing α-tocopherol acetate, may improve skin dryness.

Cell Signaling, Gene Expression, and Other Roles

Vitamin E functions as a cell signaling molecule. In this capacity it interacts with cell receptors and transcription factors, thereby affecting signaling cascades as well as gene expression, enzyme activity, and protein concentrations. One of the most investigated roles is vitamin E's inhibition of protein kinase C, a serine/tyrosine kinase that functions in the transduction of signals from G protein–coupled tyrosine kinase receptors and nonreceptor tyrosine kinases to the nucleus by hydrolysis of phospholipids [18]. Genes transcriptionally regulated by vitamin E are involved in steroidogenesis (including cholesterol synthesis), lipid uptake, antioxidant defense, the cell cycle, inflammation, cell adhesion, and blood coagulation, among other activities, as reviewed by Rimbach and others [19]. Vitamin E also impacts specific micro (mi)RNAs, a group of noncoding RNAs that bind to untranslated regions on mRNA to inhibit translation of the mRNA into protein. One miRNA is suggested to be able to interact with 100 different target mRNAs and thus inhibit multiple genes and silence entire pathways [19,20].

Tocotrienols: Additional Comments

Research focusing on tocotrienols is expanding. Tocotrienols, especially δ-tocotrienol, functions in cell signaling (involving estrogen and insulin receptors) like other forms of vitamin E. Tocotrienols, like tocopherols, also function as antioxidants in the body, but some may be more evenly distributed within the plasma membrane and may be more efficient in scavenging free radicals than tocopherols. Additionally, the tocotrienols exhibit cholesterol-lowering abilities by suppressing the activity of the rate-limiting enzyme 3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase in cholesterol synthesis. This form of the vitamin also inhibits platelet aggregation and monocyte adhesion as well as exhibits anti-inflammatory properties. These cardioprotective benefits of tocotrienols are being actively investigated. Other studies suggest that tocotrienols also exhibit antiproliferative, antiapoptotic, and antiosteoporotic properties with implications in the prevention of cancer, diabetes, osteoporosis, and neurodegenerative conditions [21–24].

Interactions with Other Nutrients

Because the antioxidant functions of vitamin E in the body are closely tied to those of selenium-dependent glutathione peroxidase (an enzyme that converts lipid peroxides into lipid alcohols), an interrelationship exists between vitamin E and selenium. The actions of both nutrients are complementary, and higher concentrations of one nutrient can reduce the effects of lower concentrations of the other nutrient. Similarly, some of vitamin C's functions also complement vitamin E, and vitamin C can regenerate vitamin E following its oxidation.

A relationship between vitamin E and dietary polyunsaturated fatty acids has been suggested because the requirement for the vitamin increases or decreases as the degree of unsaturation of fatty acids in body tissues rises or falls; body tissue lipids, in turn, are influenced by dietary lipid intake [1]. However, foods high in polyunsaturated fatty acids also tend to be relatively good sources of vitamin E.

High intake of vitamin E can interfere with other fat-soluble vitamins. For example, vitamin E inhibits β-carotene absorption and its metabolism in the intestine [25,26]. Vitamin E also impairs vitamin K absorption and its metabolism, including the conversion of phylloquinone to menaquinone [27]. The effects of vitamin E may increase the risk for bleeding secondary to interference in vitamin K's role in blood clotting.

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Metabolism and Excretion

Hepatic metabolism of vitamin E begins with an ω-hydroxylation reaction, requiring cytochrome P-450, to form hydroxychromanol. Next, a series of reactions analogous to β-oxidation of fatty acids follows to effectively truncate vitamin E's phytyl side chain. The end products include a group of carboxyethyl hydroxychromans (abbreviated CEHC). Prior to urinary or fecal excretion, these carboxyethyl hydroxychromans are usually conjugated to glucuronic acid or sulfate. Urinary excretory products of the vitamin also include α-tocopheronic acid and α-tocopheronolactone conjugated to either glucuronic acid or sulfate.

Recommended Dietary Allowance

The RDA for vitamin E for adult men and women (including during pregnancy for women) is 15 mg (22.4 IU) of naturally occurring RRR α-tocopherol (or if consuming the synthetic (labeled as dl) form about 33 IU is needed) [1]. During lactation, recommendations are slightly higher, with an RDA of 19 mg (28.4 IU) of α-tocopherol for women [1]. The RDA for vitamin E for adults is based on the vitamin E requirement plus twice the coefficient of variation, rounded to the nearest mg [1]. People with oxidative stress and/or inflammatory conditions (such as smokers or those with metabolic syndrome) may have higher requirements for vitamin E, but specific recommendations for these population groups have not been made [6].

Recommendations for vitamin E are based on intake of the natural (labeled D- or d-) RRR α-tocopherol form and the synthetic all-rac 2R-stereoisomeric forms (RSR, RRS, and RSS) of α-tocopherol used in fortified foods and vitamin supplements [1]. The equivalent of 1,500 IU of RRR α-tocopherol is 1,000 mg of α-tocopherol. Conversions from IU to mg are 1 IU=0.67 mg α-tocopherol if the vitamin E is from natural sources, whereas 1 IU=0.45 mg α-tocopherol if a synthetic form of vitamin E is used [8,28]. Conversely, to convert from mg to IU: 1 mg α-tocopherol = 1.49 IU from natural sources and 1 mg α-tocopherol = 2.22 IU from synthetic sources [8,28].

Deficiency

A deficiency of vitamin E resulting in clinical manifestations in humans is rare. A few population groups, however, are at risk for deficiency, including premature infants (who typically exhibit impaired fat utilization secondary to prematurity) and individuals with fat malabsorption disorders such as cystic fibrosis (characterized by pancreatic lipase deficiency) and hepatobiliary system disorders, particularly chronic cholestasis (characterized by decreased bile production). Individuals with genetic defects in either lipoproteins or the α-tocopherol transfer protein are also at risk. For example, individuals with abetalipoproteinemia may develop a vitamin E deficiency because of a lack of microsomal transfer protein needed to assemble or secrete lipoproteins containing apolipoprotein B, while those with an αTTP defect cannot appropriately release α-tocopherol into the blood for distribution to tissues. Treatment of both disorders necessitates the ingestion of large (gram) doses of the vitamin.

Because vitamin E functions to maintain the integrity of cell membranes, a deficiency of the vitamin negatively impacts cells. The effects are apparent especially in red blood cells, which become fragile and lyse, causing hemolytic anemia. Degeneration of nerve cells and effects on muscle also may occur, causing peripheral neuropathy (pain or numbness in extremities), ataxia (poor muscle coordination), loss of vibratory sense, loss of coordination of limbs, skeletal muscle pain (myopathy), and weakness. In the skin, ceroid pigments accumulate and appear as visible brown spots. Plasma α-tocopherol concentrations less than 5 mg/L are suggestive of deficiency.

Toxicity

Vitamin E appears to be one of the least toxic of the fat-soluble vitamins, although mild gastrointestinal problems may occur with vitamin intakes between 200 and 800 mg. However, it is because of an increased tendency for bleeding/impaired blood coagulation (due to antiplatelet effects and/or abnormal blood clotting) that a Tolerable Upper Intake Level of 1,000 mg of α-tocopherol (1,500 IU of natural RRR α-tocopherol or 1,100 IU of synthetic all-rac tocopherol/day) for adults has been established by the Food and Nutrition Board [1]. This recommendation for an upper level of intake includes any form of supplemental α-tocopherol [1]. In addition to increased bleeding, higher intakes of the vitamin (1,000 mg or greater) have also been associated with gastrointestinal distress, including nausea, diarrhea, and flatulence; possible increased severity of respiratory infections; and occasional reports of muscle weakness, fatigue, and double vision.

Assessment of Nutriture

Evaluation of vitamin E status relies primarily on blood analyses. Plasma concentrations are responsive to dietary intake under deficiency and toxicity situations, with concentrations <5 mg/L suggesting deficiency and concentrations exceeding about 20 mg/L reflecting possible toxicity. A crude estimate of vitamin E status can also be obtained from an erythrocyte hemolysis test that compares the amount of hemoglobin released by red blood cells during incubation with dilute hydrogen peroxide, with the amount released during distilled water incubation. The result is expressed as a percentage, with >20% indicating deficiency and generally associated with plasma α-tocopherol concentrations below 5 mg/L; however, variables other than vitamin E status influence *in vitro* hemolysis.

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Suggested Reading

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VITAMIN K

Vitamin K was named after the Danish word *koagulation*, which means "coagulation." In the 1920s, H. Dam discovered that chicks fed a low-fat and cholesterol-free diet became hemorrhagic (i.e., they bled excessively) and that their blood took a long time to clot. The missing vitamin called K that corrected the problem was identified in about 1935. Dam (along with Doisy) was recognized with a Nobel Prize in medicine in 1941 for the discovery.

Compounds with vitamin K activity have a 2-methyl 1,4-naphthoquinone ring with a substitution at position 3. The naturally occurring forms of vitamin K are phylloquinone (vitamin K_1), which has a phytyl group at position 3 of the ring (2-methyl 3-phytyl 1,4-naphthoquinone), and menaquinone (vitamin K_2), which has an unsaturated multiprenyl group at position 3. There are several menaquinones based on the number of isoprenoid groups. Menaquinones (abbreviated MK) are designated as MK-*n*, with *n* indicating the number of isoprenoid units in the side chain. Some menaquinones, such as MK-4 (also called menatetrenone), can be synthesized in the body from phylloquinone; most others, including MK-7, cannot. However, the compound menadione (2-methyl 1,4-naphthoquinone), a synthetic water-soluble form of vitamin K sometimes used in animal feeds, can be alkylated by tissue enzymes to generate MK-4. Figure 10.18 depicts menadione, phylloquinone, and menaquinone-7 (MK-7).

Figure 10.18 Structures of vitamin K.

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Sources

Dietary vitamin K is provided mostly as phylloquinone from ingestion of plant foods. The richest sources and the main dietary sources of vitamin K include leafy green vegetables, especially collard greens, spinach, turnip greens, some salad greens, and broccoli. Oils and margarine from plants represent the second major source of the vitamin and a more bioavailable source than plant foods [1]. Rapeseed and soybean oils are particularly rich (142–200 μg phylloquinone/100 g), while olive oil contains 55 μg of phylloquinone/100 μg of oil. Sunflower, safflower, walnut, and sesame oils provide only 6–15 μg of phylloquinone/100 g, and peanut and corn oils contain <3 μg/100 g [2]. Smaller amounts of phylloquinone are found in cereals, fruits, dairy products, and meats. Table 10.6 provides information about selected vitamin K–rich foods. The average adult is thought to consume up to several hundred micrograms of phylloquinone per day [1,3]. Vitamin K's Daily Value, used to express the nutrient's content on food and supplement labels, is 80 μg; however, the vitamin K content of foods is seldom listed on food labels. Exposure of the vitamin to light and heat can result in significant vitamin K destruction.

Menaquinones are synthesized by a variety of facultative and obligate anaerobic bacteria that reside in the body's intestines, although small amounts of MK-4 also are found in a few foods such as liver, fish, fermented cheeses, eggs, milk, butter, and soybean products. MK-7 is found in fermented soybeans (natto) and cheeses. Examples of menaquinone-producing obligate anaerobes include *Bacteroides, Bacillus fragilis, Eubacterium, Propionibacterium*, and *Arachnia*; the facultative anaerobe *Escherichia coli* also generate menaquinone [4]. Bacterial synthesis of vitamin K is not sufficient to meet the needs of the body for the vitamin [1].

Table 10.6 Vitamin K Content of Selected Foods

Phylloquinone (μg/100 g)			
<10	10–50	>100	>200
Milk	Asparagus	Cabbage	Broccoli
Butter	Celery	Lettuce	Kale
Eggs	Green beans	Brussels sprouts	Swiss chard
Cheese	Avocado	Mustard greens	Turnips
Meats	Kiwi		Watercress greens
Fish	Pumpkin (canned)		Collards
Corn	Peas		Spinach
Cauliflower	Peanut butter		Salad greens
Grains	Lentils		
Fruits (most)	Kidney beans		
Tea (brewed)	Pinto beans		
	Soybeans		
	Coffee (brewed)		

Source: Adapted from Booth et al., Vitamin K1 content of foods. Journal of Food Composition and Analysis 1993; 6: 109–20. Reprinted by permission.

Supplements of vitamin K are available as phylloquinone (such as Mephyton and Konakion) and as MK-4 and MK-7. Water-soluble forms of the vitamin (such as AquaMephyton, Synkayvite, and Kappadione) are also manufactured for people with fat malabsorptive disorders.

Absorption

Phylloquinone, which requires no digestion, is absorbed from the small intestine, particularly from the jejunum. Like the other fat-soluble vitamins, the vitamin is absorbed by diffusion and its absorption is enhanced by the presence of dietary fats, bile salts, and pancreatic enzymes and juices, and is negatively impacted by fat malabsorption disorders that are associated with impaired secretion of bile and/or pancreatic enzymes and juices. Some phylloquinone, however, may be absorbed by active transport from the proximal small intestine (i.e., the duodenum and jejunum).

Menaquinones that are synthesized by bacteria in the lower digestive tract are absorbed by passive diffusion from the ileum and colon. However, the ability to absorb and use the bacterially produced vitamin varies considerably among individuals and has been difficult to determine accurately [1].

Transport, Metabolism, and Storage

Within the enterocytes, phylloquinone is incorporated into chylomicrons that enter the lymphatic and then the circulatory system for transport to tissues. Chylomicron remnants deliver any vitamin K not taken up by tissues to the liver. Phylloquinone can be metabolized by the liver among other tissues to MK-4. The conversion is thought to involve the removal of the phytyl tail and the production of the intermediate menadione; this intermediate is thought to undergo prenylation requiring the enzyme prenyltransferase and to ultimately form MK-4 [5]. While the pathway has not been clearly delineated, it has been estimated that 5–25% of ingested phylloquinone is converted into MK-4 [5]. From the liver, the vitamin is incorporated into very-low-density lipoproteins (VLDLs) for secretion into the blood and transport to extrahepatic tissues.

Phylloquinone is the main circulating form of the vitamin in the blood, although small amounts of some menaquinones may also be present. The appearance and peak concentrations of the vitamin in the blood after eating differ based on the form of the vitamin ingested; for example, plasma MK-4 concentrations peak a few hours earlier than plasma phylloquinone and MK-9 concentrations, and peak concentrations of MK-7 appear to be greater than those of phylloquinone [5]. These differences are thought to relate to differences in lipoprotein transport and exchange of the various vitamin forms and differences in tissue uptake [5]. Normal plasma

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phylloquinone concentrations range from about 0.2 to 3.2 ng/mL (0.5–6.4 nmol/L). Most vitamin K is taken up for use by tissues within 24 hours of the vitamin's appearance in circulation [6].

Vitamin K is stored primarily in cell membranes in several tissues, including the lungs, kidneys, bone marrow, and adrenal glands. The liver rapidly metabolizes, but retains little of the vitamin. Hepatic concentrations of phylloquinone range from about 2 to 20 ng/g of liver, and are about 10 times lower than those of the menaquinones [7]. MK-4 is also found in a variety of tissues, including the pancreas, salivary glands, brain, and bone. Nonhepatic tissues also convert phylloquinone to MK-4; however, the quantity of MK-4 synthesized by these tissues has not been established. The body's pool of vitamin K, estimated at 50–100 mcg, is low among the fat-soluble vitamins and smaller than that of vitamin B_{12}[8]. Turnover of the vitamin K pool is fairly rapid at about 1.5 days [8].

Functions and Mechanisms of Action

Vitamin K is necessary for the post-translational carboxylation of specific glutamic acid (glutamyl) residues in proteins to form γ-carboxyglutamic acid (GLA) residues. These interactions are necessary for blood clotting (hemostasis) and bone mineralization, among other processes

1. Initial step: Factor XII adsorbs onto a substance such as collagen, which becomes exposed with injury to a tissue. Upon contact, factor XII becomes activated (denoted by an "a").
2. Factor XIa, which becomes activated by XIIa, activates factor IX, a vitamin K–dependent protein.
3. Factor IXa activates factor X, another vitamin K–dependent protein.
4. Factor Xa converts factor II, prothrombin (vitamin K–dependent) into thrombin.
5. Thrombin alters fibrinogen to produce fibrin for clot formation.
6. Factor XIIIa, fibrin stabilizing factor, creates insoluble fibrin for final clot formation.
7. In the extrinsic pathway, factor VII, a vitamin K–dependent protein, becomes activated in the presence of thromboplastin released by injured tissue.
8. Factor VIIa works with factor IXa to activate factor X.

Figure 10.19 An overview of blood clotting. Vitamin K–dependent proteins are boxed.

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including apoptosis, growth, and signal transduction. The role of vitamin K in blood clotting and vitamin K's roles in bone and nonosseous tissues are reviewed next.

Vitamin K and Blood Clotting

The vitamin K–dependent post-translational carboxylation of glutamic acid residues forms γ-carboxyglutamic acid on several major proteins required for the coagulation of blood. The four most well-studied vitamin K–dependent blood-clotting proteins, called factors, are factors II (prothrombin), VII, IX, and X. In addition, proteins C, S, Z, and M, also involved in blood clotting, require vitamin K for carboxylation.

Overview of Blood Clotting For blood to clot, fibrinogen, a soluble protein, must be converted to fibrin, an insoluble fiber network. Two pathways, intrinsic and extrinsic, lead to clot formation, as shown in Figure 10.19 and briefly described here.

In the intrinsic pathway, the coagulation process is initiated by the adsorption of factor XII, which circulates in the blood, onto a substance such as collagen, which becomes exposed with tissue injury. Upon contact, factor XII becomes *activated*, as indicated by the letter *a* next to the factor. Through a series of reactions in the intrinsic pathway and the extrinsic pathway (which is activated by the release of thromboplastin by injured tissue), vitamin K–dependent factor X becomes activated by factors IXa and VIIa, respectively. It is factor Xa that in turn activates another vitamin K–dependent factor, factor II (prothrombin), to produce IIa (thrombin). Thrombin catalyzes the proteolysis of fibrinogen to yield fibrin. Fibrin molecules aggregate to form a mesh-like polymer, which then undergoes cross-linking by fibrin stabilizing factor to form an insoluble fibrin clot and stop bleeding (hemorrhage).

Other blood-clotting proteins, designated C, S, Z, and M, also have been identified as vitamin K–dependent carboxylated proteins. The function of protein M is unknown, but the other three proteins inhibit the blood-clotting process and thus exhibit anticoagulant functions. Protein Z inhibits factor Xa. Protein C inactivates factors VIIIa and Va and, along with protein S, enhances fibrinolysis to disrupt the clotting process. Protein S has also been found in bone, suggesting other functions in the body.

The Role of Vitamin K in Carboxylation of Glutamic Acid Residues This section uses prothrombin as a model to describe the carboxylation process; however, remember that in addition to prothrombin (factor II), blood-clotting factors VII, IX, and X; proteins C, S, M, and Z; and proteins presented in the section discussing bone and nonosseous roles of vitamin K depend on vitamin K for carboxylation.

Proteins like prothrombin require a vitamin K–dependent enzyme for the carboxylation of 10–13 glutamic acid residues residing in the N-terminal. Once carboxylated, this glutamic acid portion is referred to as γ-carboxyglutamic acid (Gla), as shown in Figure 10.20. The carboxylation is required for the protein to become functional. The enzyme responsible for the γ-carboxylation, called vitamin K–dependent γ-glutamyl carboxylase, is associated with the rough endoplasmic reticulum (where vitamin K–dependent proteins are carboxylated), primarily in the liver. The liver is also where the hemostatic factors are synthesized. The enzyme, however, is found in all human tissues. This widespread occurrence of γ-glutamyl carboxylase suggests that the need for carboxylated proteins that can bind calcium is broad.

Gla residues are synthesized post-translationally. All glutamic acid residues must be carboxylated for the protein to function. Gla residues on the blood-clotting proteins function to bind calcium. The calcium then mediates the binding of Gla proteins to negatively charged phospholipids on membrane surfaces of blood platelets and endothelial cells at the site of injury. This adsorption is essential in hemostasis.

The participation of vitamin K in the carboxylation of proteins is a cyclic process, often called the vitamin K cycle. The γ-glutamyl carboxylase enzyme requires vitamin K in its reduced form, referred to as dihydrovitamin KH_2, dihydroxy, or hydroquinone vitamin K. However, vitamin K is generally present in the body in its oxidized quinone

Figure 10.20 Production of γ-carboxylglutamic acid (Gla) via vitamin K–dependent carboxylation.

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form because of the presence of oxygen in the blood. The steps of the vitamin K cycle, in which vitamin K is converted to its reduced form and functions in the carboxylation process, are shown in Figure 10.21 and briefly reviewed here.

- Reduction of vitamin K quinone to dihydroquinone KH_2 is accomplished by quinone reductases that require either dithiol (RSH-HSR) or NAD(P)H. The dithiol-dependent quinone reductase appears to be the main physiological pathway for generating dihydroquinone KH_2 from the quinone. (See steps 1 and 2 of Figure 10.21.)
- Once dihydroquinone KH_2 is present, along with oxygen and carbon dioxide as the carboxyl precursor, γ-glutamyl carboxylase can carboxylate (add a CO_2 onto) the glutamic acid residues of specific proteins. The carboxylation enables the protein to interact with calcium. (See steps 3 and 4 of Figure 10.21.)
- The carboxylation of glutamic acid is believed to be coupled with the formation of vitamin K 2,3-epoxide (Figure 10.21, step 5). No energy is required for the carboxylation reaction; the reaction is probably accomplished by the free energy produced through the oxidation of dihydroquinone KH_2 to vitamin K 2,3-epoxide, whereby vitamin K provides reducing equivalents [9].
- To complete the cycle (step 6 of Figure 10.21), vitamin K 2,3-epoxide is converted back to vitamin K quinone by an epoxide reductase.

Anticoagulants Warfarin (Coumadin, Jantoven) is an anticoagulant that may be prescribed to people at risk for a thrombotic event (e.g., a heart attack). Anticoagulants antagonize the synthesis of vitamin K by interfering with the activity of the reductases that convert vitamin K 2,3-epoxide to vitamin K quinone and that convert vitamin K quinone to vitamin K dihydroquinone (Figure 10.21). Ingestion of diets high in vitamin K, as obtained from about a pound of broccoli daily, override the effects of warfarin [10]. Thus, people who are taking anticoagulant medications are instructed to maintain a consistent, yet adequate intake of vitamin K, but also to avoid consumption of large quantities of foods rich (about 700–1,500 μg or more of phylloquinone) in vitamin K at a single meal. Vitamin K can also serve as an antidote for warfarin, if the drug is present in higher than acceptable concentrations in the blood.

Vitamin K and Bone

Bones take up vitamin K from circulating lipoproteins in a receptor-dependent manner. Within the bone, the vitamin is used for carboxylation reactions similar to those described in the section "Vitamin K and Blood Clotting." The most

Figure 10.21 The vitamin K cycle.

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prevalent of the vitamin K–dependent proteins in bone, cartilage, and dentine include osteocalcin (also sometimes called bone Gla protein) and matrix Gla protein (MGP). These proteins, once carboxylated, function much like those involved in blood clotting, that is, they bind calcium only in bone it is to **hydroxyapatite**. Osteocalcin, one of the most abundant of the noncollagen proteins in bone, is secreted by osteoblasts during bone extracellular matrix (protein) formation, around the onset of hydroxyapatite and mineral deposition. Osteocalcin comprises about 10–20% of noncollageneous protein in bone. With vitamin K–dependent carboxylation, the three Gla residues on osteocalcin facilitate the binding of calcium ions to the hydroxyapatite lattice in the extracellular matrix of bone. Osteocalcin appears to be involved in bone remodeling and prevents overcalcification; plasma osteocalcin is often used as an index of bone formation. Undercarboxylation of osteocalcin occurs with insufficient vitamin K. Osteocalcin is also released into circulation and functions as a hormone when interacting with target tissues. It is thought to affect insulin secretion and tissue sensitivity to insulin, among other roles [11].

Matrix Gla protein is synthesized in bone, dentine, cartilage, and blood vessels. Matrix Gla protein appears to prevent deposition of calcium in blood vessels and other soft tissues. In fact, a lack of matrix Gla protein is associated with extensive arterial calcification. In bone, matrix Gla protein is associated with the extracellular (protein) matrix, and may promote calcification of bone. Lack of matrix Gla protein in bones is associated with osteopenia and fractures. Given that mRNA for matrix Gla protein has been found in a variety of tissues, including the brain, heart, kidneys, liver, lungs, and spleen, broader roles for the protein are likely. The synthesis of both osteocalcin and matrix Gla protein appears to be stimulated by calcitriol and by retinoic acid as well as by other hormones.

Vitamin K and Bone Health Associations between serum vitamin K and bone mineral density or fracture risk as well as knee osteoarthritis are numerous in the scientific literature; however, evidence showing a protective influence of supplemental vitamin K (ranging from about 45 mg of MK-4 to 500–5,000 μg of phylloquinone/day) on bone health remains equivocal [6,7,11–15]. Supplemental vitamin K in the aforementioned quantities can, however, increase gamma carboxylation of osteocalcin, but data at present are not sufficient to endorse vitamin K supplementation to prevent bone loss or fractures [15]. Research investigating the effectiveness of MK-7 for increasing bone density is ongoing; it is also being investigated for possible benefits in preventing heart disease.

Vitamin K and Nonosseous Tissue Proteins

In nonosseous tissues, other vitamin K–dependent proteins have been identified, including growth arrest-specific protein (Gas) 6 (in smooth muscle, endothelial cells, and natural killer cells), atherocalcin (in atherosclerotic blood vessels), periostin (in the heart and tissues undergoing remodeling), transforming growth factor β-inducible protein (in multiple tissues), transmembrane Gla proteins (in multiple extrahepatic tissues), and renal Gla protein (in the cortex of the kidneys) [16,17]. The functions of these proteins, which are γ-carboxylated primarily in extrahepatic tissues, have not been clearly defined, but their presence indicates that vitamin K performs several different roles in the body. Additional possible roles for the vitamin, which require further investigations, include sphingolipid and energy metabolism and neuroprotection against oxidative stress [5].

Interactions with Other Nutrients

Vitamins A and E are known to antagonize vitamin K; excesses of both interfere with vitamin K absorption. Vitamin E's antagonistic effects also include possible interference with vitamin K metabolism. Specifically, vitamin E is thought to inhibit metabolism of phylloquinone and to increase hepatic oxidation and excretion of all forms of vitamin K [18].

Metabolism and Excretion

Phylloquinone is almost completely metabolized to a variety of metabolites (many uncharacterized) before being excreted. The metabolism usually involves stepwise oxidation of the phytyl side chain at position 3 with subsequent conjugation. Most of phylloquinone's metabolites are conjugated with glucuronic acid for excretion primarily in the feces by way of the bile; however, some metabolites are also excreted in the urine.

Relatively little is known about the metabolism and excretion of menaquinone. Metabolism of menadione (the synthetic form of the vitamin) generates menadiol, which then reacts with phosphate, sulfate, or glucuronide. Menadiol phosphate and menadiol sulfate are excreted in both the feces and urine, while menadiol glucuronides are excreted mostly in the feces.

Adequate Intake

Lack of data has hampered the efforts of the Food and Nutrition Board to estimate requirements for vitamin K [1]. Adequate Intake (AI) recommendations are set at 120 and 90 μg/day for adult males and females (including those who are pregnant or lactating), respectively. Bacterial-generated menaquinones cannot maintain adequate vitamin K status [1]. Metabolic studies suggest that the current vitamin K recommendations, which are based on the vitamin's need for coagulation function, may be inadequate to maximize carboxylation of proteins needed for bone health [13].

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Deficiency

While a deficiency of vitamin K is relatively rare in healthy adults, a subclinical vitamin K deficiency is more likely, and has been induced with the daily provision of about 10 μg of phylloquinone. A deficiency is characterized by serum vitamin K concentrations less than about 0.2 ng/mL (which drop below normal after about 2 weeks of inadequate intake) and serum undercarboxylated osteocalcin concentrations greater than or equal to 4.0 ng/mL. Undercarboxylated prothrombin concentrations also may significantly rise. Subclinical vitamin K deficiency may be associated with diminished bone mineral density and increased fracture rates, although the results of studies are not consistent (as previously discussed in the "Vitamin K and Bone Health" section). Additionally, vitamin K deficiency has been linked to cardiovascular disease, arterial calcification, and inflammation, among other conditions [16].

Severe vitamin K deficiency is associated with bleeding episodes (hemorrhage). The undercarboxylated blood-clotting factors cannot effectively bind calcium and interact with cell membrane phospholipids exposed on tissue injury, an interaction necessary for thrombin generation and clot formation. The bleeding disorder is most likely to occur in early infancy and is referred to as hemorrhagic disease of the newborn, although it is now more commonly called vitamin K deficiency bleeding (VKDB). The condition is characterized by intracranial bleeding, which may lead to neurologic damage or death. Newborns are particularly at risk because breastmilk is low in vitamin K; their vitamin K stores are low because inadequate amounts cross the placenta; and their intestinal tract is not yet populated by vitamin K–synthesizing bacteria. Consequently, intramuscular injection of 0.5–1 mg of phylloquinone shortly after birth is recommended for all infants [1].

Other population groups that are at risk for a vitamin K deficiency are older adults and those being treated chronically with antibiotics or with severe gastrointestinal malabsorptive disorders. Older adults tend to consume inadequate amounts of the vitamin; however, the problem of inadequate intake may be more widespread in the United States, where intakes of the vitamin are often less than 80 μg daily [1]. People consuming vitamin K–poor diets and on prolonged sulfonamides and broad-spectrum antibiotic drug therapy are at risk for vitamin K deficiency owing to the coupled effects of low dietary intake and antibiotic-induced destruction of gastrointestinal tract bacteria that manufacture and contribute a source of vitamin K. Reductions of over 70% in bacterial-produced vitamin K can occur with the use of broad-spectrum antibiotics. Individuals with fat malabsorptive disorders such as cystic fibrosis, obstructive jaundice, Crohn's disease, chronic pancreatitis, and liver disease are also at risk of deficiency. Because vitamin K is fat-soluble, it is absorbed best with dietary fat. Consequently, fat malabsorption results in fat-soluble vitamin malabsorption.

Toxicity

Ingestion of large amounts of phylloquinone and menaquinone has not been shown to cause toxicity, and no Tolerable Upper Intake Level for vitamin K has been established [1]. The synthetic form of the vitamin, menadione, is no longer generally sold as a supplement for humans because of its toxic effects including hemolytic anemia and liver damage (indicated by hyperbilirubinemia and severe jaundice). Menadione is thought to combine with sulfhydryl groups such as those in glutathione, resulting in glutathione oxidation and ultimately membrane damage induced by phospholipid oxidation.

Assessment of Nutriture

Multiple biomarkers are generally used to assess vitamin K status since no single index or biomarker clearly indicates deficiency and adequacy. Plasma or serum concentrations of phylloquinone reflect recent (within about 24 hours) intake of the vitamin; concentrations less than about 0.2 ng/mL are associated with deficiency. Whole-blood clotting times and prothrombin (or other blood-clotting proteins) time are used to identify potential deficiency of vitamin K. Prothrombin time, which measures the time required for a fibrin clot to form following the addition of calcium and other substances to citrated plasma, is normally between about 11 and 14 seconds; times greater than 25 seconds are associated with major bleeding and may indicate possible vitamin K deficiency. This test, however, is relatively insensitive because plasma prothrombin concentrations must usually decrease considerably (sometimes 50% or more) before any effects on prothrombin time are observed.

Another fairly sensitive means of assessing vitamin K status is to measure the percentage of undercarboxylated vitamin K–dependent proteins, such as prothrombin or osteocalcin, or the ratio of under- to fully carboxylated proteins. Vitamin K deficiency results in the secretion of under- or partially carboxylated proteins into the blood from either the liver (as with prothrombin) or the bone (as with osteocalcin). However, one protein (such as prothrombin) may be 100% carboxylated whereas another protein (such as osteocalcin) is only 10–40% carboxylated; the physiological significance of these differences is not clear at present [16].

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Suggested Reading

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ANTIOXIDANT NUTRIENTS, REACTIVE SPECIES, AND DISEASE

Although different sections in several chapters of this book have discussed nutrients with antioxidant functions, nowhere within those chapters is this information brought together to provide a more comprehensive review of how these individual nutrients function together to protect the body from destructive radicals and nonradical species. That is the purpose of this Perspective, which first reviews free radical chemistry and formation; followed by a discussion of the damage caused by these species; and finally explains how the antioxidants function together to eliminate radical and nonradical species.

Table 1 Some Reactive Oxygen and Nitrogen Species

Reactive Oxygen Species		Reactive Nitrogen Species	
Oxygen-Containing Radicals	**Oxygen-Containing Nonradicals**	**Nitrogen-Containing Radicals**	**Nitrogen-Containing Nonradicals**
Superoxide $O_2^{\bullet}$	Ozone O_3	Nitric oxide $NO^{\bullet}$	Nitrous acid HNO_2
Hydroxyl $^{\bullet}OH$	Singlet oxygen $^1O_2$	Nitrogen dioxide $^{\bullet}NO_2$	Peroxynitrite $ONOO^-$
Hydroperoxyl $HO_2^{\bullet}$	Hypochlorous acid HOCl		$LOONO^{\bullet}$
Alkoxyl $LO^{\bullet}$ or $RO^{\bullet}$	Hydrogen peroxide H_2O_2		
Peroxyl RO_2^{2-} or $LO_2^{\bullet}$ or $RO_2^{\bullet}$			

FREE RADICAL CHEMISTRY AND THE PRODUCTION OF REACTIVE SPECIES

Back in probably one of your first chemistry courses, you learned about atoms. It is here that a brief review of free radical chemistry begins. Atoms contain protons and neutrons, which are found in the nucleus. You may remember that the atomic weight of an element is a function of its number of protons and neutrons, whereas the atomic number represents solely the number of protons. Atoms also have electrons, which revolve in orbitals (also called shells) around the nucleus. An atomic orbital holds a maximum of two electrons, which are generally found in pairs in the orbitals. The term *free radical* represents an atom, molecule, or molecular fragment that has one or more unpaired electrons. The unpaired electron is found alone in the outer orbital and is usually denoted by a superscript dot. The imbalance in electrons in the orbitals results in most cases in the high reactivity of the free radicals and their pro-oxidant activities. Free radicals that are derived from oxygen are called reactive oxygen species (ROS), and free radicals that are derived from nitrogen are called reactive nitrogen species (RNS). The term *reactive* is most appropriately used when comparing different radicals because reactivity with other compounds is relative. The terms *reactive oxygen species* and *reactive nitrogen species*, however, include not only free radicals but also nonradicals, as shown in Table 1.

Table 1 does not include all free radical or reactive species. Oxygen itself is a biradical because it has two unpaired electrons, residing in separate orbitals that cannot form a pair. An example of a reactive sulfur species radical is thiyl ($RS^{\bullet}$), generated from amino acids and thiols. Trichloromethyl ($^{\bullet}CCl_3$), formed during metabolism of carbon tetrachloride (CCl_4) by cytochrome P-450 enzymes in the liver, is a chloride-based carbon-centered radical, meaning that the unpaired electron resides on the carbon atom.

Free radicals and reactive species are generated daily at multiple sites in the body (Figure 1). In general, they are formed on exposure to substances such as smog, ozone, chemicals, drugs, and radiation, among other environmental factors, as well as on exposure to high levels of oxygen. Normal physiological processes, including reactions occurring as part of oxidative phosphorylation in the mitochondria and as part of immune defense in neutrophils, macrophages, monocytes, and eosinophils, also produce reactive species. Additionally, the peroxisomes and cytochrome P-450 enzymes generate both nonradicals and radicals. Radicals also breed more radicals, as seen in several of the reactions shown in this Perspective. The overproduction of oxygen-derived radicals causes what is commonly referred to as "oxidative stress." Similarly, the overproduction of nitrogen-derived radicals results in "nitrosative stress."

The Superoxide Radical

The superoxide radical (usually designated as $O_2^{\bullet}$ or as $O_2^{-\bullet}$) is an oxygen-centered radical (e.g., the unpaired electron resides on the oxygen). The addition of an electron to molecular oxygen (which has two unpaired electrons in different orbitals) results in one unpaired electron.

Superoxide radicals are formed when molecular oxygen (O_2) reacts with different compounds, such as with the catecholamines epinephrine and dopamine or with folate's coenzyme form tetrahydrofolate. The electron transport chain also produces superoxide radicals as a result of autoxidation reactions and the leaking of electrons from the electron transport chain onto oxygen—that is, a one-electron reduction of oxygen to generate the superoxide radical. This leaking of electrons onto oxygen occurs during the passage of electrons from $CoQH^{\bullet}$ (coenzyme Q), as shown here:

Cytochrome P-450 enzymes also generate superoxide radicals. These heme enzymes, found in the endoplasmic reticulum membrane, consist of a cytochrome P-450 reductase that transfers electrons from NADPH, and a second cytochrome P-450 that binds molecular oxygen and the substrate being hydroxylated. A variety of substrates, including fatty acids, steroids, and therapeutic drugs, are hydroxylated by this system, and the reactions catalyzed by some of the cytochrome P-450 enzymes convert nonpolar compounds to polar compounds, which enables elimination (fecal or urinary) of the compound from the body.

Superoxide radicals are also produced in substantial quantities (up to 10^{-11} M) in activated white blood cells, such as macrophages, monocytes, and neutrophils conducting phagocytosis. The superoxide radicals heighten the inflammatory response by acting as chemoattractants for other neutrophils and generate other toxic reactive species, such as hydrogen peroxide (H_2O_2), to further help destroy foreign bacteria and other organisms. Superoxide radical production in activated white blood cells is thought to begin with the action of NADPH oxidase while a foreign substance is being engulfed by a white blood cell. Specifically, the NADPH oxidase reduces oxygen to generate the superoxide radical, as shown here:

The extensive oxygen-requiring process by which white blood cells destroy organisms is sometimes called the *respiratory* or *oxidative burst*.

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Abbreviations

- LH = unsaturated fatty acid
- $L^{\bullet}$ = carbon-centered lipid radical
- RH = organic compound (e.g., amino acid and nucleic acid)
- $O_2^{\bullet}$ = superoxide radical
- ${}^{\bullet}OH$ = hydroxy radical
- $R^{\bullet}$ = carbon-centered nonlipid radical
- H_2O_2 = hydrogen peroxide
- $ROO^{\bullet}$ = nonlipid peroxy radical
- $LOO^{\bullet}$ = peroxy radical
- $HO_2^{\bullet}$ = hydroperoxyl radical

Figure 1 Generation of reactive species.

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Superoxide radicals can also produce secondary reactive species, such as hydrogen peroxide, hydroperoxyl radicals, and the **peroxynitrite** ($ONOO^-$) anion. Fortunately, superoxides are not lipid soluble and thus do not diffuse too far away from their site of production, and the radicals can react with each other to form oxygen and hydrogen peroxide.

Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a reactive oxygen species and not a radical, since it does not have any unpaired electrons. Hydrogen peroxide is generated through the action of several enzymes. A major hydrogen peroxide producer (found extracellularly and intracellularly, in both the cell cytosol and mitochondria) is superoxide dismutase (SOD). Extracellular and cytosolic superoxide dismutases require both zinc and copper, whereas mitochondrial superoxide dismutase is manganese dependent. The reaction catalyzed by this enzyme, which removes superoxide radicals, is shown here:

$$O_2^{\bullet} + O_2^{\bullet} + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2$$

Vitamin C (AH_2), normally present in the body in its reduced state AH^-, can also generate hydrogen peroxide and an ascorbyl radical ($A^{\bullet}$) while eliminating superoxide radicals as shown:

$$AH^- + O_2^{\bullet} + H^+ \longrightarrow A^{\bullet} + H_2O_2$$

Hydrogen peroxide is also produced in large quantities during the oxidation of compounds in peroxisomes. Peroxisomes are cytoplasmic organelles responsible for the degradation of molecules such as very long-chain (20+ carbons) fatty acids, among others.

Additionally, reactive oxygen species including hydrogen peroxide are generated with injury or damage, especially if it involves ischemia (inadequate blood flow and, thus, oxygen supply). Possible reasons for ischemia-induced reactive species production include (1) neutrophil activation by compounds released by the damaged tissues, (2) disruption of the electron transport chain, and (3) secondary production associated with the generation of xanthine oxidase, especially if the ischemia affects the intestine or endothelial cells of blood vessels (see Chapter 13 under "Molybdenum Functions" for discussion of xanthine oxidase). Other cellular oxidases such as amine oxidase (which is copper dependent) also generate hydrogen peroxide. The reaction catalyzed by amine oxidase, found in the blood and body tissues, is as follows:

$$RCH_2NH_2 \xrightarrow{O_2 \;\rightarrow\; H_2O_2} RC(=O)H + {}^{+}NH_4$$

While now thought to perhaps function as a transient signaling molecule, hydrogen peroxide concentrations need to be controlled in the body cells. Hydrogen peroxide easily diffuses in water and in lipid environments within cells and to tissues, and damages cellular components either directly or by interacting with superoxide radicals to produce the hydroxyl radical.

The Hydroxyl Radical

The hydroxyl radical ($^{\bullet}OH$) is a highly reactive oxygen-centered radical. It can be produced when the body is exposed to γ rays, low-wavelength electromagnetic radiation. These rays "split" water in the body to form the hydroxyl radical:

$$H_2O \longrightarrow H^+ + {}^{\bullet}OH$$

Hydroxyl radicals also are produced when hydrogen peroxide interacts with a superoxide radical (known as the Haber-Weiss reaction), as shown here:

$$H_2O_2 + O_2^{\bullet} \longrightarrow O_2 + OH^- + {}^{\bullet}OH$$

or from other electrons and protons, as shown here:

$$H_2O_2 \xrightarrow{e^-,\; H^+} H_2O + {}^{\bullet}OH$$

Hydrogen peroxide in contact with free ferrous iron also generates hydroxyl radicals. Iron, however, is normally bound to proteins, and not free, in cells. If free reduced iron is generated (protein-bound $Fe^{3+} + O_2^{\bullet} \longrightarrow$ free $Fe^{3+} + O_2^{\bullet} \longrightarrow O_2 + Fe^{2+}$); as may occur, for example, during periods of extensive oxidative stress, the Fenton reaction, shown here, may be triggered:

$$H_2O_2 \xrightarrow{Fe^{2+} \;\rightarrow\; Fe^{3+}} OH^- + {}^{\bullet}OH$$

In this reaction, the hydrogen peroxide is functioning as an iron-oxidizing agent, and a hydroxyl radical is produced. The iron in the reaction can be substituted with copper, as both can react with hydrogen peroxide; however copper, like iron, is typically bound to proteins *in vivo.*

The hydroxyl radical, thought to be one of the most potent and reactive radicals in spite of its short 1 nanosecond life span, rapidly attacks (by taking electrons) virtually all molecules in close proximity. The hydroxyl radical is a major initiator of lipid peroxidation. It also reacts with nucleic acids in DNA, forming 8-hydroxyguanosine (a compound used to estimate DNA damage). Hydroxyl radicals fragment proteins, primarily at proline and histidine residues, triggering damage and premature degradation of the protein.

Peroxyl Radicals, Carbon-Centered Radicals, and Lipid Peroxides

Peroxyl radicals ($ROO^{\bullet}$ or O_2^{2-}) represent another group of reactive oxygen-derived free radicals that are especially destructive to polyunsaturated fatty acids. The simplest of the peroxyl radicals, hydroperoxyl ($HO_2^{\bullet}$) or perhydroxyl radicals, is formed from superoxide radicals reacting with an additional electron and hydrogen, as shown here:

$$\underset{\text{Superoxide radical}}{O_2^{\bullet}} \xrightarrow{e^-} \underset{\text{Peroxyl radical}}{O_2^{2-}} \xrightarrow{H^+} \underset{\text{Hydroperoxyl radical}}{HO_2^{\bullet}}$$

The initial "attack" of polyunsaturated fatty acids (LH) in the phospholipids of membranes, for example by hydroxyl ($^{\bullet}OH$) or hydroperoxyl ($HO_2^{\bullet}$) radicals, generates lipid carbon-centered radicals ($L^{\bullet}$). The initiation peroxidation reaction may be written as follows:

$$LH + {}^{\bullet}OH \longrightarrow L^{\bullet} + H_2O \text{ (initiation)}$$

Alternately, the reaction may be viewed showing part of the polyunsaturated fatty acid, as shown here:

Propagation follows the initiation step, with products formed in one reaction being used as reactants in another reaction. Oxygen, for example, can react with the lipid carbon-centered radical to generate a lipid peroxyl ($LOO^{\bullet}$) radical as follows:

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}$$

or alternately, this reaction may be expressed as follows:

Oxygen also can react with polyunsaturated fatty acids to form carbon-centered radicals and hydroperoxyl radicals:

$$LH + O_2 \longrightarrow L^{\bullet} + HO_2^{\bullet}$$

In addition to propagation reactions, lipid peroxyl radicals ($LOO^{\bullet}$) may attack (abstract a hydrogen atom or proton from) other polyunsaturated fatty acids (L'H) in cell membranes to generate lipid peroxides (LOOH) and another carbon-centered radical.

$$LOO^{\bullet} + L'H \longrightarrow LOOH + L'^{\bullet}$$

This reaction may also be depicted as follows:

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Should lipid peroxides (LOOH), also known as peroxidized fatty acids, come in contact with free iron, amplification results with alkoxyl ($LO^{\bullet}$) and peroxyl ($LOO^{\bullet}$) radicals also generated, as shown in these two reactions:

$$LOOH + Fe^{2+} \longrightarrow LO^{\bullet} + OH^{-} + Fe^{3+}$$
$$LOOH + Fe^{3+} \longrightarrow LOO^{\bullet} + H^{+} + Fe^{2+}$$

Like peroxyl radicals, the alkoxyl radical can in turn initiate chain reactions with other polyunsaturated fatty acids in membranes, as follows:

$$LO^{\bullet} + L'H \longrightarrow LOH + L'^{\bullet}$$

Singlet Molecular Oxygen

Singlet molecular oxygen, also called singlet oxygen ($^1O_2$), possesses higher energy and is more reactive than ground-state oxygen. Specifically, in singlet oxygen, the peripheral electron in the oxygen structure is excited to an orbital above the one it normally occupies. This excited form of oxygen can be generated from lipid peroxidation of membranes by enzymatic reactions, such as that occurring between hydrogen peroxide and hypochlorous acid in the respiratory burst in white blood cells (i.e., $H_2O_2 + HOCl \longrightarrow {}^1O_2 + H_2O + HCl$), or through photochemical reactions, as shown here:

$$O_2 \xrightarrow{h\nu} {}^1O_2$$

Singlet oxygen, being a reactive oxygen species (although not a free radical), can damage cells and tissues unless it is removed from the body.

Nitric Oxide

Nitric oxide ($NO^{\bullet}$) is one of many free nitrogen-derived radicals. Nitric oxide is generated in the body from nitric oxide synthase and easily diffuses through the cytosol and plasma membrane due to it solubility in both aqueous and lipid environments. In spite of its life span of just a few seconds, nitric oxide affects numerous, diverse physiological processes in the body. Some of these physiological processes include blood pressure regulation, neurotransmission, immune regulation, and smooth muscle relaxation. Nitric oxide's role as a vasorelaxant is applied in medicine. Nitroglycerin, for example taken for ischemic chest pain (angina), generates nitric oxide in the body, which relaxes coronary blood vessels and increases blood (and thus oxygen) flow to the heart.

Nitric oxide, however, can cause damage. The compound can react with oxygen to form **nitrogen dioxide** ($NO_2^{\bullet}$), another reactive nitrogen species, and can react with superoxide radicals to generate the peroxynitrite ($ONOO^-$) anion. Thiols (RSH) also can react with nitric oxide, as shown in the general reaction: $NO^{\bullet} + RSH \longrightarrow RSNO + O_2 + H^+$. The **nitrosothiol** (RSNO) that is produced is harmful in that it may attack other compounds unless it is terminated by combining with another thiol (R'SH) to produce RSH + R'SNO or RSSR' + HNO. In contrast to these radical-producing reactions, nitric oxide, by removing superoxide radicals and other radicals, can be viewed as an eliminator or terminator of free radicals. It also appears to work directly with uric acid to generate nitrosated uric acid; the nitroso group is then transferred to glutathione for transport to other molecules.

Peroxynitrite

Peroxynitrite ($ONOO^-$), an anion (not free radical) formed by the reaction between nitric oxide and a superoxide radical, is a strong oxidizing agent. Once produced (often at the site of tissue injury), it quickly and directly attacks amino acids such as cysteine, methionine, and tyrosine in proteins, causing protein **nitrosylation** and extensive functional damage to the protein. It also attacks polyunsaturated fatty acids and DNA. Moreover, when peroxynitrite decomposes, it generates **nitrogen dioxide** ($^{\bullet}NO_2$) and the highly destructive hydroxyl ($^{\bullet}OH$) radical. Alternately, and more likely in human tissues and fluids, peroxynitrite reacts with carbon dioxide (CO_2) to produce carbonate ($CO_3^{-\bullet}$) and nitrogen dioxide ($^{\bullet}NO_2$). These compounds, however, are also destructive, reacting with lipids and with amino acids within proteins (primarily tyrosine, tryptophan, and cysteine) to form nitrated molecules, which disrupt normal cellular processes.

Nitrogen Dioxide and Peroxynitrate

Nitrogen dioxide ($^{\bullet}NO_2$) is a free radical and a fairly potent oxidant. It is formed when nitric oxide reacts with oxygen ($^{\bullet}NO + O_2 \longrightarrow {}^{\bullet}NO_2$). Nitrogen dioxide, for example, in addition to coacting with carbonate ($CO_3^{-\bullet}$) radicals to produce nitrated compounds, reacts with unsaturated fatty acids by abstracting a hydrogen atom and induces isomerization of *cis*-double bonds in unsaturated fatty acids by a reversible addition reaction. These actions damage the lipids and, if the lipids are part of a cell membrane, damage the membrane. Peroxynitrate ($O_2NOO^{\bullet}$) is made from the reaction between nitrogen dioxide and a superoxide radical. It typically decomposes to form singlet oxygen and $NO_2^{-\bullet}$.

DAMAGE DUE TO REACTIVE SPECIES

Once formed, free radicals attack by taking electrons from cell constituents (including nucleic acid in DNA in the nucleus of cells). They also take electrons from proteins (especially amino acids such as tyrosine, tryptophan, proline, histidine, or arginine and those with sulfhydryl groups, such as cysteine) and polyunsaturated fatty acids in cell membranes or in the membranes of intracellular organelles, such as the nucleus, mitochondria, or endoplasmic reticulum. Hydroxyl radical–induced changes in purine and pyrimidine bases in DNA may lead to mutations or breakages, which if not repaired may result, for example, in cancer. Attacks on amino acids in proteins by reactive oxygen species may break the peptide bonds in the protein backbone or disrupt the protein structure. Oxidative damage to proteins may cause cross-linking between amino acids or aggregation, resulting in changes in the secondary or tertiary structures. Such events may even lead to premature degradation of the protein. Free radical attack on polyunsaturated fatty acids present in the phospholipid portion of the cell membranes can lead to degradation of the lipid. Extensive damage in a red blood cell, for example, may cause hemolysis of the membrane and thus the cell. Aqueous peroxyl and peroxynitrite radicals may induce oxidation of LDLs. Furthermore, radicals give rise to more radicals and thus, more damage.

ANTIOXIDANT FUNCTIONS

Overproduction of reactive oxygen and nitrogen species and their attack on DNA, proteins, and polyunsaturated fatty acids have been implicated as a cause of or contributor to a variety of conditions and diseases. Vitamins, along with several other antioxidant compounds, help control or eliminate free radicals. However, the term *antioxidant* is a bit of a misnomer because once it works, the antioxidant itself becomes a radical. Some have suggested that the term *redox agent* be used instead of the term *antioxidant*. Whether or to what extent an overproduction of vitamin radicals may be associated with diseases is unclear; however, the results of many clinical trials providing antioxidant vitamins to treat or prevent various diseases have failed to show beneficial results, and some have even found that supplementation was detrimental to health. The destruction of reactive species by some of the antioxidant nutrients and compounds is reviewed in this section and shown in Figure 2.

Elimination of Superoxide Radicals

Several antioxidant nutrients help dispose of superoxide radicals, including vitamin C and three minerals (zinc, copper, and manganese) that function as cofactors for enzymes involved in oxidant defense. Vitamin C (present under physiological conditions as ascorbate AH^-), being water soluble and hydrophilic, is found in aqueous regions, such as the blood and the cytosol of cells. The vitamin provides electrons to reduce the superoxide radical and forms hydrogen peroxide, as shown here:

$$AH^- + O_2^{\bullet} + H^+ \longrightarrow A^{\bullet} + H_2O_2$$

Superoxide radicals may also be eliminated by the action of the enzyme superoxide dismutase (SOD), which works considerably faster than vitamin C to inactivate superoxide radicals. The extracellular form of superoxide dismutase is found in exceptionally high concentrations in arterial blood vessels. As previously mentioned, both the extracellular and the cytosolic forms of the enzyme require zinc and copper as cofactors; the

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Abbreviations

EH = vitamin E	$L^{\bullet}$ = carbon-centered lipid radical	$A^{\bullet}$ = ascorbyl radical
AH_2 = vitamin C	RH = organic nonlipid compound	AH^- = ascorbate
SOD = superoxide dismutase	$R^{\bullet}$ = carbon-centered nonlipid radical	DHAA = dihydroascorbic acid
GSH = reduced glutathione	H_2O_2 = hydrogen peroxide	UA = uric acid
GSSG = oxidized glutathione	$ROO^{\bullet}$ = nonlipid peroxy radical	$UA^{\bullet}$ = uric acid radical
$O_2^{\bullet}$ = superoxide radical	ROOH = nonlipid peroxides	
$LOO^{\bullet}$ = peroxy radical	LOOH = lipid peroxides	
LH = unsaturated fatty acid	DHLA = dihydrolipoic acid	
	LA = lipoic acid	

Figure 2 The interactions among selected antioxidant nutrients to prevent cell damage.

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mitochondrial form depends on manganese for activity. Thus, zinc, copper, and manganese are important minerals in the body's oxidant defense system. Superoxide dismutase eliminates superoxide radicals but generates hydrogen peroxide, as shown here:

$$O_2^{\bullet} + O_2^{\bullet} + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2$$

Elimination of Hydrogen Peroxide

Hydrogen peroxide disposal occurs through several means in cells and tissues. In a reaction catalyzed by ascorbate peroxidase, vitamin C (as AH^-), found in the blood and cell cytosol, provides the needed electrons to convert hydrogen peroxide into water and dehydroascorbic acid.

Glutathione peroxidase, found in the plasma as well as the cell's cytosol and mitochondria, reduces hydrogen peroxide as well as other peroxides. The enzyme is selenium dependent, and enzyme activity is diminished with poor selenium status. The reaction also requires glutathione (composed of glycine, cysteine, and glutamic acid) in its reduced form (GSH). Glutathione is one of many thiols found in both aqueous and lipophilic environments in the body. Thiols are characterized by the presence of sulfhydryl groups (R-SH) and include glutathione, thioredoxin, and lipoic acid, among others. Glutathione (GSH) serves as a reducing agent, donating hydrogen from its sulfhydryl group (SH). A radical center is formed on the sulfur atom ($GS^{\bullet}$) until two glutathiyl radicals join to form a disulfide bond between the two now oxidized glutathione molecules (designated as GSSG or GS-SG).

$$H_2O_2 + 2\,GSH \xrightarrow{\text{Glutathione peroxidase}} 2\,H_2O + GSSG$$

Catalase is another key enzyme in hydrogen peroxide removal. This heme iron–dependent enzyme is found mostly in cell peroxisomes (cytosolic organelles where lots of hydrogen peroxide is produced during the oxidation of very long-chain fatty acids, among other molecules). Smaller amounts of the enzyme are also found in the cytosol, mitochondria, and microsomes of cells. Neutrophils and other white blood cells contain fairly high quantities of catalase to dispose of hydrogen peroxide no longer needed in the respiratory burst required for the phagocytosis of foreign bacteria, viruses, and fungi. Higher concentrations of hydrogen peroxide are required for catalase activity than for glutathione peroxidase activity. The reaction catalyzed by catalase is shown here:

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

The accumulation of H_2O_2 in the body is thought to be prevented primarily by catalase and glutathione peroxidase. Glutathione peroxidase, because of its dual (mitochondrial and cytosolic) cellular locations and its greater activity at lower hydrogen peroxide concentrations, is thought to be more active than catalase in cellular hydrogen peroxide removal. Figure 2 shows the complex interaction among components of the oxidant defense system, including the roles of iron-dependent catalase, selenium-dependent glutathione peroxidase, and copper-, zinc-, and manganese-dependent superoxide dismutase.

A third enzyme, myeloperoxidase, which is also heme iron dependent, uses hydrogen peroxide for the respiratory burst involved in the destruction of bacteria and viruses, among other harmful foreign substances. Myeloperoxidase, found mostly within activated white blood cells, is released from granules into vacuoles that contain the engulfed foreign substance. In the vacuoles, the hydrogen peroxide, produced from the superoxide radical, is used to generate a potent toxic acid, hypochlorous acid (HOCl).

Hypochlorous acid, along with other potent compounds, helps to destroy the foreign bacteria's cell membrane to promote death (lysis) of the foreign substance.

Elimination of Hydroxyl Radicals

Vitamin C and other water-soluble compounds, such as uric acid, thiols including glutathione and dihydrolipoic acid, and possibly other substances such as metallothionein, defend against hydroxyl radicals. In aqueous solutions such as blood, vitamin C rapidly and effectively reacts with hydroxyl radicals before they can initiate oxidative damage.

$$^{\bullet}OH + AH^- \longrightarrow H_2O + A^{\bullet}$$

Glutathione may also react directly with hydroxyl radicals in aqueous or lipid environments, as shown here:

$$^{\bullet}OH + GSH \longrightarrow H_2O + GSSG$$

Dihydrolipoic acid (DHLA), the reduced form of lipoic acid (also called thioctic acid), functions as a reducing agent. Dietary or endogenously generated lipoic acid is reduced in cells to dihydrolipoic acid by dihydrolipamide dehydrogenase, glutathione reductase, or thioredoxin reductase. Dihydrolipoic acid in turn functions in aqueous and lipophilic environments to eliminate radicals such as hydroxyl radical (among others), as shown here:

Ubiquinol (also called coenzyme Q, CoQ_{10}, or Q_{10} and part of the quinone system; see Figure 3.27) and uric acid (Figure 3) also may act as reducing agents in aqueous solutions. Both ubiquinol and uric acid scavenge various free oxygen radicals, including $^{\bullet}OH$. Metallothionein, a protein rich in cysteine residues and thus sulfhydryl groups, is also thought to scavenge hydroxyl radicals.

Elimination of Peroxyl, Hydroperoxyl, and Carbon-Centered Radicals, Lipid Peroxides, and Some Reactive Nitrogen Species

Several nutrients and compounds, including vitamin E, carotenoids, manganese, ubiquinol, vitamin C, uric acid, and glutathione, along with the selenium-dependent enzyme glutathione peroxidase, actively eliminate carbon-centered, peroxyl, and hydroperoxyl radicals as well as lipid peroxides and some reactive nitrogen species. Vitamin E, being lipid soluble and located near or in membranes, effectively reacts with many radicals, especially carbon-centered radicals and those that initiate peroxidation, such as peroxyl radicals. Specifically, vitamin E donates its phenolic hydrogen on the carbon 6 hydroxyl group. Vitamin E's chromanol ring then stabilizes the unpaired electron.

- Vitamin E (EH) terminates carbon-centered radicals (as shown below) before they abstract further hydrogens from other polyunsaturated fatty acids:

$$L^{\bullet} + EH \longrightarrow LH + E^{\bullet}$$

- Vitamin E (EH) prevents peroxidation of polyunsaturated fatty acids by reacting with peroxyl radicals ($LOO^{\bullet}$), as illustrated in this reaction:

$$LOO^{\bullet} + EH \longrightarrow LOOH + E^{\bullet}$$

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Figure 3 Uric acid.

Thus, vitamin E (as well as carotenoids) terminates chain-propagation reactions.

Some transition metals, such as manganese, may be able to scavenge peroxyl radicals, as shown here:

In addition to the aforementioned nutrients, ubiquinol, the reduced form of coenzyme Q_{10} ($CoQH_2$), also acts as a potent antioxidant. Ubiquinol is a small fat-soluble molecule that transports electrons and ultimately generates ATP in the electron transport chain in the mitochondria. Ubiquinol has been found in small quantities in lipoproteins, where it is thought to be used before vitamin E in the termination of peroxyl radicals (as shown hereafter) and thus prevent LDL oxidation.

$$CoQH_2 + LOO^{\bullet} \longrightarrow CoQH^{\bullet} + LOOH$$

Vitamin C effectively scavenges alkoxyl ($RO^{\bullet}$) and nonlipid peroxyl ($ROO^{\bullet}$) radicals to produce a hydroxyl acid (ROH) and nonlipid peroxide (ROOH), as shown here:

$$AH^{-} + RO^{\bullet} \longrightarrow A^{\bullet} + ROH$$
$$AH^{-} + ROO^{\bullet} \longrightarrow A^{\bullet} + ROOH$$

Nitric oxide ($NO^{\bullet}$) can also act as an antioxidant to terminate lipid alkoxyl ($LO^{\bullet}$) and peroxyl ($LOO^{\bullet}$) radicals, as shown here:

$$^{\bullet}NO + LO^{\bullet} \longrightarrow LONO$$
$$^{\bullet}NO + LOO^{\bullet} \longrightarrow LOONO$$

Most of the resulting LOONO homolyzes to produce nitrogen dioxide, $^{\bullet}NO_2$, and an alkoxyl radical, $LO^{\bullet}$, and then recombines to produce alkylnitrates ($LONO_2$); however, a small percentage remains as free radicals. Additionally, uric acid removes peroxynitrite ($ONOO^{-}$) before it causes protein ***nitrosation***.

Although eliminating peroxyl radicals ($LOO^{\bullet}$) is helpful, the often simultaneous generation of lipid peroxides/peroxidized fatty acids (LOOH) can cause problems if the peroxidized fatty acids are within hydrophobic regions of cell membranes. The problems occur because peroxidized fatty acids are polar compounds, and the polarized peroxidized fatty acids, once liberated from the phospholipid in the membrane by the actions of phospholipase A_2, destroy the normal architecture of the cell as they migrate from the nonpolar region where they are generated.

Thiols and the selenium-dependent enzyme glutathione peroxidase help to eliminate lipid peroxides. Thiols like glutathione and thioredoxin [Trx $(SH)_2$] act in both aqueous and lipid environments as antioxidants by providing reducing equivalents (hydrogen ions). Glutathione peroxidase uses glutathione in its reduced form (GSH) and catalyzes the conversion of the peroxides (LOOH) to hydroxy acids (LOH) as follows:

Elimination of Singlet Oxygen

Carotenoids as well as vitamin C, uric acid, and thiols (especially lipoic acid) may quench singlet oxygen. Carotenoids such as β-carotene and lycopene have the ability to directly quench hundreds of singlet oxygen molecules either in solution or in membrane systems. *Quenching* is a process by which electronically excited molecules, such as singlet oxygen, are inactivated. The ability of carotenoids to quench singlet oxygen is attributed to the conjugated double-bond systems within the carotenoid structure. The carotenoids can absorb energy from the singlet oxygen without chemical change to return the "excited" $^1O_2$ to its ground state. Carotenoids then release the energy in the form of heat (as shown hereafter) and thus do not need to be regenerated.

$$^1O_2 + \beta\text{-carotene} \longrightarrow {}^3O_2 + \text{excited } \beta\text{-carotene} \longrightarrow \beta\text{-carotene} + \text{heat}$$

REGENERATION OF ANTIOXIDANTS

When antioxidants provide reducing equivalents, the antioxidants are oxidized. Regenerating the antioxidants is important for further defense against free radicals. Without the recycling of antioxidants, the body would quickly succumb to oxidative stress. Uric acid, which is thought to account for almost half of the antioxidant capacity of human plasma, depends on vitamin C for its regeneration [1]. It has been estimated that fewer than nine vitamin E molecules exist for every 1,000–2,000 unsaturated fatty acids in cell membrane phospholipids or lipoprotein molecules. Thus, regenerating or recycling oxidized vitamins is critical to prevent massive damage.

Redox potential (or reduction potential) is used to predict some reactions between radicals and antioxidants. Substances that have been oxidized can abstract an electron (hydrogen) from a reduced substance with a lower redox potential; or, stated in reverse, a reduced substance (with its specific redox potential) can donate an electron (hydrogen) to an oxidized substance with a more positive reduction potential. The carotenoids, for example, have higher redox potentials than vitamins C and E. Consequently, the carotenoids readily take an electron (hydrogen) from either of these two vitamins. The following section discusses the recycling of some antioxidants.

Vitamin E Regeneration

The regeneration of vitamin E is thought to initially require the migration of the vitamin to the membrane surface. At the cell surface, several compounds regenerate vitamin E. Vitamin (AH^{-}) can regenerate α-tocopherol from its radical form ($E^{\bullet}$), but it too will then need to be regenerated.

Ubiquinol ($CoQH_2$) also recycles vitamin E, as shown here:

Reduced glutathione (GSH) may donate its hydrogen atom to reform vitamin E, as follows:

Vitamin C Regeneration

Niacin, dihydrolipoic acid, and thiols such as glutathione and thioredoxin help to regenerate some, but not all, vitamin C. In a reaction catalyzed by semidehydroascorbic acid reductase, niacin in its coenzyme form, NADH, provides for the regeneration of vitamin C, as follows:

$$2\,A^{\bullet} + NADH + H^{+} \longrightarrow 2\,AH^{-} + NAD^{+}$$

Dihydrolipoic acid recycles the ascorbyl radical to ascorbate:

The next few reactions depict some of the recycling efforts contributed by glutathione (GSH) and thioredoxin.

$$2\,GSH + 2\,A^{\bullet} \longrightarrow GSSG + 2\,AH^{-}$$

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An interaction between two vitamin C radicals also permits the regeneration of vitamin C, as follows:

$$2\,A^{\bullet} \longrightarrow AH_2 + DHAA$$

Ubiquinol, Thioredoxin, and Glutathione Regeneration

Regeneration of vitamin E by ubiquinol ($CoQH_2$) results in the formation of ubisemiquinone ($CoQH^{\bullet}$). Ubisemiquinone can be converted back to ubiquinol through the electron transport chain in the mitochondria, by dihydrolipoic acid (DHLA), as shown here:

or by the thioredoxin-thioredoxin reductase system. This system, ubiquitous in the body, includes thioredoxin (Trx), a small protein with a dithiol (two sulfhydryl groups —$[SH]_2$), and the selenium-dependent flavoenzyme (FAD) thioredoxin reductase with a selenocysteine residue at its active site. The recycling of ubiquinol is shown here:

Thioredoxin reductase (TrxR) then functions to reduce thioredoxin, using reducing equivalents from NADPH, as shown here:

Glutathione reductase, a flavoprotein that requires FAD as a cofactor, regenerates oxidized glutathione (GSSG) with niacin as NADPH providing the reducing equivalents, as shown here:

Dihydrolipoic acid (DHLA) is also thought to be able to regenerate glutathione, as shown here:

ANTIOXIDANTS AND DISEASE

Oxidative or nitrosative stress resulting from the overproduction of reactive species leads to an imbalance within the body's defense system, and is thought to contribute to aging and the development of several diseases and conditions, including some cancers, heart disease, cataracts, diabetes mellitus complications, rheumatoid arthritis, and ischemia-**reperfusion** injury, among others. Nutrients *in vitro* often demonstrate specific functions or abilities, such as inhibiting lipoprotein cholesterol oxidation, inhibiting cell proliferation or transformation, or modulating the immune system, that are thought to provide protection against the development of disease. Despite the promising results of many *in vitro* studies, the results of supplementation trials *in vivo* to prevent or treat disease are not consistent, and sometimes have shown that vitamin supplementation may be detrimental to health. Similarly, although many studies have shown that people who either consume diets rich in foods (especially fruits and vegetables) that contain antioxidant nutrients or who have high plasma antioxidant nutrient concentrations have a reduced risk of many diseases or conditions, others studies do not support such associations. New supplementation trials are being conducted, as are *in vitro* and *in vivo* studies, to better elucidate the roles and effects of antioxidants. As these studies continue to clarify the roles of the antioxidant nutrients, scientists and other health professionals will continue to reevaluate current recommendations and perhaps develop new guidelines defining optimal levels of nutrients to prevent diseases. However, enjoying a diet rich in a variety of fruits and vegetables and whole grains is always encouraged to help prevent disease and maintain health.

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Web Sites

National Cancer Institute
www.cancer.gov
American Heart Association
www.heart.org
American Cancer Society
www.cancer.org
Prevent Cancer Foundation
www.preventcancer.org
Produce for Better Health Foundation
www.fruitsandveggiesmorematters.org

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11 MAJOR MINERALS

CALCIUM
PHOSPHORUS
MAGNESIUM
For each major mineral, the following subtopics (when applicable) are discussed:
Sources
Digestion, Absorption, and Transport
Regulation and Homeostasis
Functions and Mechanisms of Action
Interactions with Other Nutrients
Excretion
Recommended Dietary Allowance
Deficiency
Toxicity
Assessment of Nutriture
PERSPECTIVE
OSTEOPOROSIS AND DIET

THE IMPORTANCE OF MINERALS IN NUTRITION and metabolism cannot be overstated, despite the fact that they constitute only about 4% of total body weight. Their functions are many and varied. They provide for normal cellular activity, determine the osmotic properties of body fluids, impart hardness to bones and teeth, regulate vital processes, and function as obligatory cofactors in metalloenzymes, among several other roles.

Minerals can be defined as inorganic elements that come from the earth (water, soil, and plants). Minerals are classified as major (also called macro) or trace (also called micro), although the trace minerals are sometimes further divided into an ultratrace category. The major minerals, in contrast to the trace and ultratrace minerals, are found in greater abundance in the body and are required by adults in amounts greater than 100 mg/day. Trace minerals are required by adults in amounts of about 1–100 mg/day, and ultratrace elements are required by adults in amounts less than 1 mg/day. Minerals are referred to as **ions** when they carry a charge, as occurs with a loss or gain of electrons. Minerals that lose electrons have a positive charge and are called cations, while those that gain electrons have a negative charge and are called anions.

The major minerals include calcium, phosphorus, magnesium, sodium, potassium, and chloride, shown on the periodic table in Figure 11.1. Because of their role in maintaining electrolyte balance in body fluids, sodium, chloride, and potassium are discussed in association with water in Chapter 12. Sulfur is not discussed in the chapters addressing minerals because it does not function independently in the body as a nutrient. Sulfur is found in the body (about 175 g) as part of the structures of vitamins (thiamin and biotin) and of sulfur-containing amino acids (methionine, cysteine, and taurine) and lipoic acid, among other compounds. Because of its presence in amino acids, sulfur is commonly found in protein-containing foods and within body proteins, especially those in skin, hair, and nails.

Table 11.1 provides an overview of the major minerals, including information on general functions, approximate body content, deficiency, food sources, and recommended intakes. Note from Table 11.1, the difference in body content between the major and trace minerals, with the major mineral content of the body ranging from ~28 to 1,200 g, and the trace mineral content ranging from <1 mg to ~4 g. In considering the body's mineral content, keep in mind that 1 pound is equal to 454 g and an ounce is about 28.4 g.

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Figure 11.1 The periodic table highlighting the body's major (macro) minerals.
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CALCIUM

Calcium is the most abundant divalent cation, Ca^{2+}, in the body, representing about 40% of the body's mineral mass and 1.5–2% of total body weight, or between ~900 and 1,200 g in the human body. Bones and teeth contain about 99% of the body's calcium. The other 1% is found in extracellular fluid (i.e., the blood) and soft tissues.

Sources

The best food sources of calcium include dairy products, especially milk, cheese, and yogurt, and some seafood, such as salmon and sardines (with bones), clams, and oysters. Selected vegetables—such as turnip and mustard greens, broccoli, cauliflower, and kale—as well as nuts and legumes and legume products—especially tofu (soybean curd)—also provide calcium, as shown in Table 11.2.

Table 11.1 Major Minerals*: Functions, Approximate Body Content, Deficiency Symptoms, Food Sources, and Recommended Dietary Allowances (RDA)

Mineral	Selected Physiological Functions	Body Content	Deficiency Symptoms	Selected Food Sources	RDA
Calcium	Component of bones and teeth; role in cellular processes, muscle contraction, blood clotting, enzyme activation, other	1,200 g	Rickets, osteoporosis, tetany	Dairy products, canned sardines, clams, oysters, turnip and mustard greens, broccoli, legumes	1,000 mg, 19–50 years
Phosphorus	Component of bone, phospholipids, signaling compounds, nucleic acids, nucleotides, ATP-ADP phosphate transfer; pH regulation	850 g	Neuromuscular, skeletal, hematologic, and cardiac manifestations; rickets, osteomalacia	Meat, poultry, fish, eggs, dairy products, nuts, legumes	700 mg, 19+ years
Magnesium	Component of bones, nerve impulse transmission, enzymatic reactions, ATP use, calcium regulation, ion transfer, other	28 g	Neuromuscular hyperexcitability, cardiovascular effects, central nervous system effects	Nuts, legumes, whole-grain cereals, leafy green vegetables	400 mg males; 310 mg females; 19–30 years

Note: Abbreviations: ATP, adenosine triphosphate; and ADP, adenosine diphosphate.
* Sodium, chloride, and potassium are discussed in Chapter 12.

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Table 11.2 Calcium Content of Selected Foods*

Food (serving)	Calcium (mg)
Milk and yogurt, variety (1 c)	200–400
Cheeses, variety (1 oz)	100–225
Salmon, canned, with bones (3 oz)	190
Sardines, with bones (3 oz)	200
Orange juice, fortified (1 c)	250–350
Turnip greens, cooked (1/2 c)	99
Collard greens, cooked (1/2 c)	175
Kale, cooked (1/2 c)	47
Broccoli, cooked (1/2 c)	21
Cauliflower, cooked (1/2 c)	40
Tofu (soybean curd) (1/2 c)	125–227
Navy beans, cooked (1 c)	126
Pinto beans, cooked (1 c)	79
Kidney beans, cooked (1 c)	64
Chick peas/garbanzo beans (1/2 c)	77
Edamame, shelled, cooked (1/2 c)	90
Almonds (1 oz)	75
Walnuts (1 oz)	28
Pecans (1 oz)	20
Peanuts (1 oz)	15

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

Other significant food sources of calcium include those fortified with the mineral, such as breads and fruit juices. The Daily Value for calcium, which is used on food and supplement labels, is 1,000 mg. Thus, one-half cup of low-fat cottage cheese providing 10% of the Daily Value contains 100 mg of calcium.

In contrast to the aforementioned foods, meats and grains (not fortified) are relatively poor sources of calcium. Some vegetables, such as spinach, rhubarb, and Swiss chard, are also poor sources because they contain large amounts of oxalic acid, which binds calcium and inhibits its absorption, as discussed in the "Factors Influencing Absorption" section.

Small amounts, usually less than 200 mg, of calcium are found in multivitamin/mineral supplements. Several single nutrient supplements of calcium are available, with the forms of calcium in these supplements found as calcium carbonate, calcium citrate, calcium acetate, calcium lactate, calcium gluconate, calcium monophosphate, tricalcium phosphate, calcium amino acid chelates, and calcium citrate malate. Two widely available forms are calcium citrate and calcium carbonate. Calcium citrate is beneficial for those with limited gastric acid production (as may occur in older individuals) and does not need to be ingested with food. Calcium carbonate, which is also commonly found in antacid medications like Tums®, is a relatively inexpensive form, but its use can be associated with gastrointestinal side effects such as gastric pain, constipation, gas, and/or bloating. Because the percentage of calcium by weight in different supplements varies, different amounts of supplements must be consumed to obtain a specific amount of calcium. For example, to obtain 500 mg of calcium from calcium carbonate, 1.26 g would need to be ingested, whereas a person would need to ingest 5.49 g of calcium gluconate, 3.53 g of calcium lactate, 2.37 g of calcium citrate, or 2.16 g of calcium acetate. Sources of calcium used for supplements, however, should also be considered. For example, calcium carbonate from fossilized oyster shell or dolomite may be contaminated with aluminum and lead, and thus should not be used. Bone meal preparations may also contain lead and should be avoided. The amount of calcium found in the single nutrient supplements ranges from about 200 to 750 mg per tablet.

Digestion, Absorption, and Transport

Digestion

Calcium is present in foods and dietary supplements as relatively insoluble salts. It takes about 1 hour at an acidic pH (as occurs in the stomach) for calcium to be solubilized (to exist as free Ca^{2+}) from most calcium salts. Solubilization does not necessarily ensure better absorption, however, because free calcium can bind to other dietary constituents, limiting its bioavailability, as discussed in the "Factors Influencing Absorption" section.

Absorption

Calcium absorption occurs in the small intestine via two mechanisms: (1) saturable, carrier-mediated, active, transcellular transport and (2) diffusion. The main transcellular transport system for calcium operates primarily in the duodenum and proximal jejunum, is saturable (at low to moderate calcium intakes), requires energy, and is regulated by calcitriol (the active form of vitamin D). At a calcium intake of up to about 400–500 mg/meal and daily intakes of about 1,000 mg, this active transport system accounts for about 50–60% of total calcium absorption in the small intestine. Absorption of calcium using this system involves a channel protein called transient receptor potential (TRP) vanilloid (V)6 (abbreviated TRPV6 and also called calcium transporter 1 or CaT1) to get the calcium across the brush border membrane of the enterocyte. Synthesis of this TRPV6 transporter is enhanced by calcitriol and estrogen; its expression declines with age (which accounts in part for the higher calcium recommendations for older adults). Next, a cytosolic binding protein called calbindin D9k, which binds two calcium ions, transports the mineral within the enterocyte. Calbindin shuttles calcium across the cytosol and releases it near the enterocyte's basolateral membrane. The release of calcium

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is thought to require fusion of vesicles containing the calbindin-bound calcium with lysosomes. Synthesis of calbindin is enhanced by calcitriol and, like TRPV6, calbindin expression declines with age. Following its release by calbindin, calcium extrusion across the enterocyte's basolateral membrane and into the blood involves a high-affinity (limited-capacity) Ca^{2+}-ATPase pump (also referred to as PMCA1b) or a low-affinity Na^{+}/Ca^{2+} exchanger (referred to as NCX1). NCX1 exchanges three Na^{+} ions for one Ca^{2+}; PMCA1b pumps Ca^{2+} out at the expense of ATP. Calcitriol enhances the synthesis of the Ca^{2+}-ATPase pump. Calcitriol may also enhance calcium transport through binding to selected basolateral membrane receptors, such as membrane-associated rapid-response steroid-binding (MARRS) protein [1]. This process is referred to as transcaltachia, and is characterized by the rapid absorption of calcium through calcium channels in the enterocyte's brush border membrane, then across the cytosol of the enterocyte, and finally across the enterocyte basolateral membrane.

The second major route for calcium absorption occurs paracellularly by diffusion, a passive, nonsaturable, nonregulated process (i.e., no carriers or energy needed) that is concentration dependent and occurs throughout the small intestine but mostly in the jejunum and ileum. Paracellular absorption occurs between cells, rather than through/across them. Tight junctions between enterocytes affect paracellular absorption. Tight junctions are controlled by integral and membrane-spanning proteins, including occludins and claudins, and are thought to exhibit properties similar to ion channels. Paracellular absorption occurs typically when high calcium concentrations are present in the lumen, and thus a gradient of calcium concentrations exists between the lumen and the basolateral side of the enterocyte. Increases in the concentration of intracellular calcium ions are thought to mediate the process through a series of reactions that ultimately increase permeability through the junctions between cells to facilitate calcium absorption. Calcitriol may be involved in this process by enhancing the expression of genes that code for some transmembrane proteins. Claudin-2, for example, forms charged selective pores that facilitate paracellular intestinal absorption of calcium, magnesium, and other solutes.

While the overwhelming majority of calcium is absorbed in the small intestine as described above, small amounts, about 4–10% (or ~8 mg), of dietary calcium may be absorbed by the colon. Calcium becomes available for absorption in the colon as bacteria degrade highly fermentable fibers such as pectins to which calcium is bound.

Overall, calcium absorption in adults averages about 25–30%. Calcium absorption from calcium supplements (providing 250 mg of calcium) varies from about 27 to 39%, depending on the specific calcium salt used in the supplement, the amount of calcium ingested, and whether it is consumed under fasting conditions or with food; ingestion with food generally enhances absorption. Absorption also tends to be higher when calcium is consumed in amounts less than about 400–500 mg. Relative to calcium absorption from milk as a reference and under fasting conditions, calcium absorption from calcium lactate exceeded that from milk, while absorption from calcium carbonate, citrate, and succinate was similar to milk [2]. Relative to calcium absorption from milk as a reference and testing with coingestion of a meal, calcium absorption from calcium carbonate, lactate, citrate malate, and gluconate was similar to milk; in contrast, calcium absorption from tricalcium phosphate was well below that from milk [2]. In a food/supplement matrix, calcium absorption from calcium citrate malate and calcium sulfate appears to be best [2]. The anion (bicarbonate, citrate, etc.) portion of the supplement is absorbed by various solute-linked carrier anion exchangers found in the digestive tract.

Factors Influencing Absorption Several factors influence calcium absorption, including stage of life. Infants, children, adolescents, and women during pregnancy and lactation exhibit greater calcium absorption than other age groups. (Note that calcium requirements are also increased in these groups.) Infants and young children, for example, absorb up to about 60% of dietary calcium, in contrast to adults, who absorb up to about 30%. With aging (and with lower levels of estrogen in females), calcium absorption diminishes to about 15–20%; this decline may be associated with age-related decreases in both gastric acid production and calcitriol production along with its secondary effects reducing TRPV6 channels and calbindin synthesis.

Dietary factors also affect calcium absorption (Table 11.3). Protein intake enhances calcium absorption [3,4]. Ingesting food along with the calcium source improves calcium absorption, possibly by improving its solubility. The presence of lactose and sugar alcohols is also thought to improve calcium solubility and thus absorption, although the effect of lactose is thought to be more pronounced in infants than in adults.

Table 11.3 Interactions between Calcium and Selected Nutrients/Substances

Nutrients/Substances Enhancing Calcium Absorption	Nutrients/Substances Inhibiting Calcium Absorption
Vitamin D	Fiber
Sugars and sugar alcohols	Phytic acid
Protein	Oxalic acid
	Excessive divalent cations (Zn and Mg)
	Unabsorbed fatty acids
Nutrients Enhancing Urinary Calcium Excretion	**Nutrients Whose Absorption May Be Inhibited by Excessive Calcium**
Sodium	Phosphorus
Protein	Iron
Caffeine	Fatty acids

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Oxalic acid

Figure 11.2 The binding of calcium by oxalic acid.

Several dietary components diminish calcium absorption or promote its secretion from the blood back into the gastrointestinal tract. Ingestion of caffeine, for example, increases the secretion of calcium into the gut, thereby leading to increased endogenous fecal calcium losses. Absorption of calcium is strongly inhibited by the presence of oxalic acid (oxalate [$C_2O_4^{2-}$] is the salt-forming ion of oxalic acid) (Figure 11.2) in the digestive tract. Oxalate chelates ionized calcium, has a very low solubility (<0.1 mmol/L; optimal solubility is thought to range from about 0.1 to 10.0 mmol/L), and increases fecal calcium excretion. Oxalic acid is found in a variety of vegetables (e.g., spinach, rhubarb, Swiss chard, beets, celery, eggplant, greens, okra, squash), fruits (e.g., currants, strawberries, blackberries, blueberries, gooseberries), nuts (pecans, peanuts), and beverages (tea, Ovaltine, cocoa), among other foods. Absorption of calcium from meals rich in oxalic acid is less than 5%.

Phytic acid (phytate or inositol hexaphosphate; shown later in Figure 11.7), found in whole-grain breads, seeds, and legumes, also inhibits intestinal calcium absorption, but not to the same extent as oxalic acid. Specifically, phytic acid binds calcium and decreases its availability, especially when present in a phytic acid:calcium molar ratio >0.2. Some fibers, such as wheat bran, may also bind to calcium and decrease its absorption. However, most individuals in the United States do not consume enough phytic acid and fiber to profoundly affect calcium absorption.

Divalent cations, especially magnesium and zinc, can inhibit calcium absorption. Magnesium and calcium compete with each other for intestinal absorption whenever an excess of either is present in the gastrointestinal tract. Similarly, zinc diminishes calcium absorption, especially when the diet is low in calcium and contains an excess of zinc (usually when taken in the form of a supplement).

Unabsorbed dietary fatty acids found in significant quantities in the gastrointestinal tract can interfere with calcium absorption by forming insoluble calcium "soaps" (calcium–fatty acid complexes) in the lumen of the small intestine. These calcium soaps cannot be absorbed and are excreted in the feces. The presence of relatively large amounts of unabsorbed fatty acids in the feces is referred to as steatorrhea (usually characterized by the presence of greater than about 7 g of fecal fat per day). Steatorrhea is common in several gastrointestinal tract disorders, such as inflammatory bowel diseases, as well as with liver disease (cirrhosis) and pancreatic disorders, such as pancreatitis and cystic fibrosis.

Finally, diminished gastric acid production (as may occur with aging and the use of some medications like proton pump inhibitors for the treatment of gastroesophageal reflux disease or ulcers) may reduce calcium absorption. Insufficient gastric acid may limit the solubilization of some calcium salts in the stomach and thereby reduce the transport of calcium into the intestinal cells. Some low-molecular-weight forms of calcium, however, may not need to be solubilized before being absorbed, especially through paracellular routes.

Transport

Calcium is transported in the blood in three forms. Some calcium (~40–45%) is bound to proteins, mainly albumin, but also prealbumin and globulins. Some calcium (up to ~10%) is complexed with anions such as sulfate, phosphate, citrate, and/or bicarbonate, and about 45–50% of calcium is found free (also referred to as ionized) in the blood. Protein-bound calcium is in equilibrium with ionized calcium. Thus, as ionized calcium is taken out of the blood and enters tissues/cells, some calcium in the blood that is bound to blood proteins is released. Figure 11.3 provides an overview of calcium digestion, absorption, and transport.

Regulation and Homeostasis

Calcium concentrations are tightly controlled both intracellularly and extracellularly. Extracellular regulation is discussed first, followed by intracellular regulation.

Extracellular Calcium Concentration Regulation

Calcium concentrations are tightly maintained in the plasma/serum (i.e., extracellular fluid). Total serum calcium concentrations normally range from about 8.5 to 10.5 mg/dL, and ionized serum calcium concentrations range from about 4.6 to 5.2 mg/dL. Three main hormones are involved in this calcium homeostasis: PTH, calcitriol, and calcitonin. PTH is released by the chief cells of the parathyroid gland in response to primarily low serum concentrations of calcium. Calcitriol is the active form of vitamin D in the body; the kidneys synthesize and release this hormonal form of the vitamin into circulation. Calcitonin is synthesized by the parafollicular (also called C) cells of the thyroid gland. An overview of calcium regulation follows and is shown in Figure 11.4.

Ionized calcium is removed from the blood with secretion into the digestive tract, excretion in the urine, and uptake by tissues. PTH and calcitriol respond to this decrease, functioning to increase serum calcium concentrations.

- Calcium-sensing receptors (CaSR) found on the parathyroid gland (as well as on renal cells, among others) monitor calcium concentrations in the blood.

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Figure 11.3 Calcium digestion, absorption, and transport.

CaSR detect a lowering (fluctuation) of the serum concentration of calcium (and to a lesser extent to calcitriol and other minerals like magnesium and phosphorus) and within minutes the parathyroid gland releases PTH into the blood.

- PTH increases serum calcium concentrations through interactions with the kidneys and the bone. In the kidneys, PTH stimulates the transcription of 1-hydroxylase to promote the synthesis of calcitriol from 25-OH vitamin D. Calcitriol production results in increased renal reabsorption of filtered calcium by interacting with nuclear vitamin D receptors to induce the transcription of the gene that codes for calbindin D28k, a calcium transporter found in the kidneys. In bone, PTH interacts with receptors on osteoblasts (bone-building cells). Osteoblasts perform several functions, including some that are regulated by calcitriol; one such function is stimulating the production of osteoclasts (bone-degrading cells). Osteoclasts promote the release of calcium from the bone into the blood, enabling an increase in serum calcium concentrations (see also an overview of bone under "Bone Mineralization").
- Calcitriol acts in the small intestine to enhance calcium absorption. First, calcitriol interacts with nuclear vitamin D receptors to induce the transcription of the gene that codes for calbindin D9k. Calbindin D9k, like calbindin D28k, functions as a calcium-binding protein to promote calcium transport through the cytosol. Second, calcitriol enhances calcium absorption at the brush border membrane by increasing TRPV6 channels and at the basolateral membrane by increasing calcium-ATPase pumps. The vitamin may also facilitate paracellular absorption by promoting the synthesis of specific claudin proteins.

The net effect of these actions by PTH and calcitriol is to increase serum calcium concentrations into the normal range. Once the serum concentrations of calcium are appropriate, the secretion and actions of PTH and calcitriol are suppressed as follows.

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1 Low blood calcium signals the parathyroid gland to release parathyroid hormone (PTH) into the blood.

2 PTH binds to bone cell receptors and triggers the resorption or breakdown of bone mineral for the release of calcium into the blood.

3 PTH acts on the kidneys to synthesize the active form of vitamin D, calcitriol.

4 PTH and calcitriol promote the reabsorption of calcium from the kidneys and into the blood.

5 Calcitriol leaves the kidneys and travels to the intestine, where it promotes calcium absorption across the brush border membrane, its transport in the cell cytosol, and egress into the blood.

6 Calcium enters the blood a after release from bone, b after release from kidneys, and c after absorption from intestinal cells.

Figure 11.4 An overview of blood calcium regulation by parathyroid hormone (PTH) and calcitriol (also called 1,25(OH)$_2$ vitamin D) in response to low blood calcium concentrations.

- As serum calcium concentrations rise, calcium binds to CaSR on the parathyroid gland, and via second messengers, signals for a reduction in PTH release. Calcitriol also suppresses PTH synthesis through interactions with nuclear receptors in the parathyroid gland. CaSR on kidney cells also monitor serum calcium. Elevated serum calcium concentrations result in reduced renal reabsorption of calcium and thus increased urinary calcium loss.
- Calcitonin is released from the thyroid gland in response to elevations in serum calcium. Calcitonin suppresses PTH production and release and inhibits osteoclast activity. Thus, calcitonin functions in opposition to PTH to lower serum calcium concentrations. Table 11.4 summarizes the actions of parathyroid hormone, vitamin D, and calcitonin.

Table 11.4 A Summary of the Effects of Parathyroid Hormone (PTH), Calcitriol, and Calcitonin on Calcium Balance

	PTH	Calcitriol	Calcitonin
Serum calcium	↑	↑	↓
Bone calcium	↓	*	↑
Renal calcium reabsorption	↑	↑	↓
Intestinal calcium absorption	↑	↑	No effect

* Works with PTH

Intracellular Calcium Concentration Regulation

Low free Ca^{2+} concentrations (100 nmol, or approximately 0.0001 times the concentration in the extracellular fluid) are tightly maintained within the cytosol of cells in its normal resting state. However, for calcium to carry out many of its functions, it must gain entry into and be present in higher concentrations in the cell cytosol. Then, as the cell can be restored to its resting state, the excess calcium must be removed from the cytosol.

Calcium gains entry into the cytosol of cells from extracellular sites through cell membrane channels (such as voltage-dependent slow channels, ligand-gated channels, or stretch-activated channels) as well as from intracellular sites such as the endoplasmic (or sarcoplasmic) reticulum and the mitochondria, among other organelles. The efflux of organelle-sequestered calcium into the cytosol typically requires a Ca^{2+}-ATPase pump or release channel (such as the **ryanodine receptor** of the sarcoplasmic reticulum in skeletal muscle).

Once calcium has gained entry into the cell cytosol to perform its functions, the cell's normally low resting calcium concentration must be reestablished (i.e., calcium must be exported from the cell cytosol). The main system responsible for moving calcium out of cells is the $Ca^{2+}/3Na^{+}$ exchanger, which exhibits a low affinity but

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high capacity for calcium. A $Ca^{2+}/2H^{+}$ (proton)-ATPase pump, which exhibits high affinity but a low capacity for calcium, also exports calcium, extruding it in exchange for two hydrogen ions (protons); this pump, however, is thought to account for only minor adjustments in cellular calcium concentrations. (Some of the ATPases responsible for maintaining calcium concentrations are shown later in Figure 11.6.) In addition to being transported out of the cell to restore resting cytosolic concentrations, calcium can be sequestered (stored/restored) in cellular organelles such as the mitochondria, endoplasmic (or sarcoplasmic) reticulum, nucleus, and vesicles. This function is performed by Ca^{2+}-ATPase, which pumps Ca^{2+} from the cytosol into the organelles for storage until needed in the cell cytosol again, either for function or to maintain cytosolic concentrations in the event that concentrations drop below resting levels. Within organelles, calcium binds to proteins (such as calsequestrin in the sarcoplasmic reticulum) or complexes with phosphate (as in the mitochondrion). Calcium-binding proteins function as an important buffer within cells to prevent excessive free calcium.

Functions and Mechanisms of Action

Calcium plays several vital roles in the body, including bone (skeleton) mineralization, muscle contraction, blood clot formation, signal transduction (mediating signaling from activated cell membrane receptors) to enable neurotransmission, and enzyme activation, among other roles.

Bone Mineralization

Calcium's most widely known function is in the mineralization of bone, of which there are two main types: cortical and trabecular. Some characteristics of cortical and trabecular bone include the following :

Cortical Bone	Trabecular Bone
• Is compact or dense	• Has a spongy appearance
• Represents about 75–80% of total bone in the body	• Represents about 20–25% of total bone in the body
• Consists of layers of mineralized proteins	• Consists of an interconnected system of mineralized proteins
• Is found mainly on the surfaces of all bones and the shaft of long bones of the limbs.	• Is found in relatively high concentrations in the axial skeleton (vertebrae and pelvic region) and the ends of long bones.

Most bones possess a dense outer layer of cortical bone that surrounds trabecular bone. Some bones also contain a cavity for bone marrow. Trabecular bone is more active metabolically, with a high turnover rate, and thus can be more rapidly depleted of calcium with poor calcium intake than is cortical bone. Consequently, trabecular bone is more susceptible to osteoporosis, as discussed in the Perspective at the end of this chapter.

Approximately 99% of total body calcium is found in teeth and bones. About 50–66% of the weight of bones is minerals, with the remaining 34–50% being water, ground substance, and protein. Minerals, or the mostly inorganic portion of bone, consist largely of calcium and phosphorus but also include magnesium, potassium, sodium, fluoride, and strontium. Calcium comprises about 37–40% and phosphorus about 50–58% of the weight of the minerals in bones. Some of these minerals along with hydroxyl groups make up hydroxyapatite, a crystal latticelike substance found bound to proteins and ground substance in bone. Carbonate is also found in bone, comprising about 2–8% of the weight of bones. This carbonate is usually associated with calcium, potassium, and sodium, but the amount present in bone varies especially with changes in systemic acid-base balance. Of the minerals present in bone, phosphorus is thought to be laid down first; calcium is thought to then bind to the phosphorus. Calcium is initially present in bone as Ca^{2+} and then as amorphous (noncrystal or poorly crystalline) calcium forms, such as $Ca_3(PO_4)_2$, that are deposited between collagen and noncollagen proteins and ground substance. Other amorphous forms of minerals in bone include $Ca_3(PO_4)_2$ (tricalcium phosphate), $Mg_3(PO_4)_2$ (trimagnesium phosphate), and $CaHPO_4 \bullet 2H_2O$ (brushite). These salts ultimately are converted to more crystalline compounds such as $Ca_8H_2(PO_4)_6 \bullet 5H_2O$ (octacalcium phosphate) and hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$.

An Overview of Bone The minerals in bone are embedded in protein and ground substance, which together form the bone matrix or scaffolding (also called the extracellular matrix). It is the protein matrix that provides bone's shape and three-dimensional structure. Proteins in bone include primarily type I collagen (about 85–90% of proteins), with smaller amounts of several other proteins. Many of these noncollagenous bone proteins contain the amino acid glutamic acid, which has been carboxylated (a COO^- group has been added) in the gamma position by vitamin K–dependent enzymes. These glutamic acid carboxylated proteins are referred to as Gla proteins. Examples of some Gla proteins found in bone are osteocalcin (also called bone Gla protein), fibronectin, matrix Gla protein, osteonectin, osteopontin, and bone sialoprotein-1 (BSP1). The Gla proteins are important in mineralization, facilitating interactions between the bone proteins and minerals. For example, osteonectin, a phosphoprotein, binds both calcium and collagen. Osteopontin binds to hydroxyapatite. Osteocalcin and matrix Gla protein bind calcium and hydroxyapatite. Bone sialoprotein-1, produced by osteoblasts in relatively large quantities versus other noncollagen proteins, also exhibits a high affinity for hydroxyapatite. In fact, its multiple Gla residues are thought to bind numerous (close to 100) calcium ions to in essence dock the bone sialoprotein-1 to the hydroxyapatite and contribute to $Ca_3(PO_4)_2$ formation.

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In addition to protein, the bone matrix is made up of ground substance, which consists mostly of glycoproteins and proteoglycans. Glycoproteins are composed of proteins covalently bound to typically short chains of carbohydrate. Proteoglycans are similar to glycoproteins but typically are larger, consisting of a core protein covalently conjugated to one or more glycosaminoglycans (made up of long chains of repeating disaccharides). It is the glycosaminoglycan portion of the proteoglycan that interacts with matrix proteins to enhance bone strength and resilience. Hyaluronic acid and chondroitin sulfate are two glycosaminoglycans associated with bone and cartilage.

Proteins and ground substance are synthesized by bone cells. Among the main types of bone cells (osteoblasts, osteocytes, and osteoclasts), osteoblasts, which originate from the bone marrow, are bone-building cells with about a 3-month life span. Under the influence of PTH, calcitriol, and estrogen, among other hormones, osteoblasts secrete collagen and other proteins as well as ground substance. Osteoblasts also release matrix vesicles, which attach to the underlying bone matrix and release a variety of substances onto the bone surface that enhance the precipitation or deposition of calcium and other minerals. These substances also may break down substances released by osteoclasts that prevent bone mineralization. The process of mineralization, which has not been completely delineated, also requires the appropriate concentrations of minerals, especially calcium and phosphorus, along with the presence of other substances in the extracellular fluid.

As the osteoblasts secrete the proteins and ground substance and mineralization occurs, the osteoblasts become embedded in the matrix. With further embedding in the matrix, some osteoblasts undergo apoptosis and others undergo differentiation and morphological changes to become either osteocytes or lining cells. Osteocytes—that is, osteoblasts that have been incorporated into bone matrix—represent over 90% of all the cells in bone where they monitor and maintain the integrity of the surrounding bone (including new bone matrix formation and mineralization). These cells produce, for example, sclerostin, which inhibits osteoblasts from generating new bone, as well as other substances like transforming growth factor β, which inhibits osteoclast activities. Osteoclasts are also inhibited by osteoprotegerin, whose gene expression is downregulated by a calcitriol-VDR-RXR heterodimer. (Some of these compounds are the targets of drug therapies used to treat osteoporosis.) Lining cells, which have a relatively flat shape, form a membrane (called the periosteum) that covers the bone surface. Lining cells regulate the flux of minerals into and out of bone. They are also involved to a lesser extent in bone remodeling, functioning to degrade the nonmineralized surface layer at specific sites to form bone-remodeling compartments where the osteoclasts will function.

Osteoclasts, another type of bone cell, are large, multinucleated (with about 2–10 nuclei) cells that resorb (break down or degrade) previously made bone. Osteoclasts arise from the actions of osteoblasts, which produce two proteins: macrophage colony stimulating factor (MCSF) and receptor activator of nuclear factor κ B ligand (RANKL). RANKL initiates osteoclastogenesis. RANKL, upon binding to RANK receptors found on osteoclast precursor cells (and in the presence of MCSF), stimulates precursor cell proliferation and differentiation into osteoclasts as they relocate to areas of bone targeted for resorption. At the designated sites, a sealing zone is formed that restricts bone resorption activities to an exposed area beneath the osteoclasts. Several phosphorylation cascades lead to the expression of genes that enable the osteoclasts to synthesize and release proteases, acids, and other substances that resorb bone. Bone resorption is facilitated by the release of hydrochloric acid (via vacuolar ATPase proton pumps) and carbonic anhydrase into selected regions within the bone. Other acids, including citric and lactic acids, are also released to dissolve the amorphous mineral complexes. Lysosomes in osteoclasts release enzymes such as cathepsin K, matrix metalloproteinases, and hydrolases that break down the bone protein matrix. Remnants from the resorption are released into extracellular fluid.

Osteoclasts respond to PTH, calcitriol, and calcitonin, among other hormones and signaling compounds. Osteoclasts play an important role in increasing serum calcium concentrations to a normal level in times of inadequate calcium intake but also contribute to bone fragility and osteoporosis if not balanced by adequate bone formation by osteoblasts.

Bones are constantly undergoing remodeling. In children and adolescents, skeletal turnover occurs such that formation of bone exceeds resorption of bone. Skeletal turnover continues into adulthood, with peak bone mass occurring in early adulthood. During the fifth decade, bone mass begins to decline. Although the need for calcium in bone modeling is continuous, its greatest benefits in promoting the formation of a sturdy skeletal mass occur during linear bone growth and the years immediately following.

Other Roles

The small amount (1%) of body calcium that is nonosseous (not associated with bone) is responsible for a variety of functions in the body. The calcium needed for these functions is taken out of the blood (extracellular sites) or taken from sites within cell organelles where it has been stored (intracellular sites). Magnesium (discussed later in the chapter) often opposes calcium, including its release from intracellular sites, its flux within the cytosol, and its cellular actions. It is the ionized calcium that is needed, which means that the numerous regulatory functions of calcium are performed by <0.5% of the total body calcium.

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Most of calcium's nonosseous roles are involved with regulation of cellular events and metabolic processes, and often require interactions with one or more proteins. A few of these roles are briefly reviewed. One such role is blood clotting. Specifically, calcium is needed for clot formation, which involves the binding of cell membrane phospholipids with Gla residues of specific blood-clotting proteins (see Chapter 10).

Skeletal muscle contraction necessitates increased intracellular calcium concentrations, which are typically achieved by calcium secretion via calcium release channels in the membranes of intracellular storage sites such as the sarcoplasmic reticulum. The released calcium then binds to troponin C, which has four calcium binding sites. The binding of calcium to troponin C results in a conformational change in the protein and alters interactions with other proteins to enable an interaction between actin and myosin, resulting in muscle contraction. Once the plasma membrane repolarizes, calcium is pumped back into cisternae of the sarcoplasmic reticulum via a Ca^{2+}-ATPase and bound to calsequestrin, and myosin and actin can no longer interact to sustain muscle contraction. Visceral smooth muscle contraction also requires increased intracellular calcium concentrations; however, the calcium influx into the cytosol of visceral smooth muscle cells comes from the extracellular fluid via gated channels rather than from intracellular organelles.

Calcium can also promote vasorelaxing and membrane-stabilizing effects on the smooth muscle cells, possibly through interactions with the central and peripheral sympathetic nervous systems. These, among other, actions of calcium may account for its inverse association in some studies with blood pressure.

Membrane permeability is affected by calcium, which binds to both membrane proteins and phospholipids. Changes in membrane fluidity occur with calcium–protein interactions that affect protein cross-linking. Enhanced membrane rigidity and electrical resistance may result from calcium's interaction with membrane phospholipids.

Ion channels (porelike protein structures in membranes) are involved in the generation of action potentials in nerve cells. Various neurotransmitters interact with receptors on nerve cells to open ion channels, especially calcium and sodium channels. Calcium, for example, can enter the nerve ending and trigger the release of acetylcholine. The acetylcholine in turn diffuses to and binds to receptors to trigger another series of events, ultimately including depolarization to generate an action potential.

In many body cells, calcium carries out its functions in association with various binding proteins. Calmodulin, a cytosolic calcium-binding protein operative in most body cells, consists of two similar globular lobes (each with two Ca^{2+}-binding sites) joined by a long helix. The binding of calcium activates calmodulin by changing its conformation (Figure 11.5); this conformational change allows the protein to stimulate or interact with a variety of enzymes, such as:

Figure 11.5 Schematic representation of the structural change that occurs in calmodulin following the binding to calcium (Ca^{2+}) ions.

- calcineurin, a phosphatase that dephosphorylates and inactivates calcium channels
- myosin light-chain kinase, which phosphorylates the light chain of myosin and, following a sequence of events, causes smooth muscle contraction
- phosphorylase kinase, which activates phosphorylase (the enzyme responsible for glycogenolysis, i.e., degrading glycogen to glucose-1-PO_4)
- calcium calmodulin kinases, of which there are several, with several functions.

Calcium, either by itself or as part of calmodulin or other calcium-binding proteins, acts as a messenger within many cells. For example, increased intracellular concentrations of calcium can activate phospholipase A_2, which hydrolyzes fatty acids like arachidonic acid from phospholipids in cell membranes. The released arachidonic acid can in turn be metabolized to form thromboxanes, prostaglandins, or leukotrienes. Phosphodiesterase, which hydrolyzes cyclic AMP (cAMP) to 5′AMP, is also dependent on calcium. Cyclic AMP, formed from ATP by adenylate cyclase, activates protein kinases, thereby influencing intermediary metabolism. Calcium also activates protein kinase C, which is involved in a number of cellular processes such as phosphorylating enzymes to activate or inactivate metabolic pathways. Some other enzymes that may be affected either directly by increased free cytosolic Ca^{2+} or through increases in protein-bound Ca^{2+} are listed in Table 11.5. Figure 11.6 shows some of calcium's intracellular actions and mechanisms by which cytosolic calcium concentrations are maintained. Given these roles of intracellular calcium, it is not surprising that it is involved in a host of physiologic processes.

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Table 11.5 Selected Enzymes Regulated by Calcium and/or Calmodulin

Adenylate cyclase	Myosin kinase
Ca-dependent protein kinase	NAD kinase
Ca/Mg-ATPase	Nitric oxide synthase
Ca/phospholipid-dependent protein kinase	Phospholipase A_2
Cyclic nucleotide phosphodiesterase	Phosphorylase kinase
Glycerol-3-phosphate dehydrogenase	Pyruvate carboxylase
Glycogen synthase	Pyruvate dehydrogenase
Guanylate cyclase	Pyruvate kinase

Interactions with Other Nutrients

Nutrients or substances that inhibit or enhance calcium absorption were discussed in the section on calcium absorption. Additional interactions (also listed in Table 11.3) discussing the effects of calcium on phosphorus, iron, and fatty acids are addressed here.

Ingestion of large amounts of calcium along with phosphorus-containing foods inhibits phosphorus absorption. The use of calcium supplements (about 2–3 g/day, or in a calcium-to-phosphorus ratio >3:1) to inhibit intestinal phosphorus absorption has been employed in the medical management of hyperphosphatemia (high blood phosphorus concentrations) secondary to kidney failure. This strategy, however, is used less often than in the past due to its propensity to increase calcium phosphate deposition in soft tissues.

Calcium from dietary sources as well as from supplements (especially in doses of 800 mg or more) can decrease iron absorption. The inhibitory effect, however, appears to be of short duration, such that iron status is not negatively impacted. Calcium is thought to transiently inhibit iron absorption by promoting the temporary relocation of the iron transporter ferroportin from the enterocyte's basolateral membrane to the cytosol. Additionally, calcium may affect the availability of DMT1 (which transports nonheme iron across the brush border membrane) and/or alter membrane fluidity to reduce iron absorption [5].

Calcium also diminishes the absorption of fatty acids. This in turn may impact bile's fatty acid profile. Calcium, when consumed in gram amounts with a meal, inhibits

Figure 11.6 Some of calcium's intracellular actions and mechanisms by which cytosolic calcium concentrations are maintained.

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some bile acid reabsorption in the ileum and promotes its excretion. Calcium may also directly bind to fatty acids in the small intestine and form insoluble soaps that are excreted in the feces. Calcium supplementation of 2 or 3 g daily may decrease chenodeoxycholate concentrations in bile and the lithocholate:deoxycholate ratio in the feces. Such changes are favorable to the colonic environment and may reduce the risk of colon cancer.

Excretion

Calcium losses from the body occur via the feces and urine, with lesser amounts (up to about 25 mg/day) lost through insensible routes (dermal, hair, nails). Extreme sweating, however, can increase calcium losses about six-fold. Fecal losses of calcium from endogenous sources (the sloughing of mucosal cells and calcium that is not reabsorbed from digestive juices—saliva, gastric juice, pancreatic juice, and bile) range from about 45 to 200 mg/day.

The kidneys filter ionized calcium and calcium that is bound to anions. Of this filtered calcium, about 98% is reabsorbed by the kidneys. Most (about 70% of the 98%) of the filtered calcium is reabsorbed passively in the proximal tubule, with another 20% of the 98% reabsorbed passively in the ascending limb of the loop of Henle. The basolateral membrane of cells in the ascending limb of the loop of Henle contains CaSR that respond to high serum calcium concentrations and can suppress ion movement through various channels to reestablish serum calcium homeostasis. The distal convoluted tubule can actively reabsorb another 8% of calcium. The active reabsorption occurring in this region is a major contributor to calcium balance in the body. Calcium enters the renal cell through a membrane transporter called transient receptor potential calcium channel vanilloid (TRPV) 5. Calcium is then transported across the cytosol by calbindin D28k (remember both TRPV5 and calbindin D28k are influenced by calcitriol) to the basolateral membrane where it is extruded by Na^+-Ca^{2+} exchanger (NCX1) or Ca^{2+}-ATPase (PMCA1b). Urinary calcium excretion normally ranges from about 100 to 240 mg/day.

Urinary calcium losses are increased by dietary protein, caffeine, and sodium. (The effect of phosphorus on urinary calcium excretion appears to be negligible, although it once was thought to be of significance.) Protein's stimulation of urinary calcium excretion is well documented, but offset by its ability to enhance intestinal calcium absorption and decrease endogenous calcium secretion into the gastrointestinal tract. Urinary calcium losses associated with caffeine consumption are small, about 2–3 mg calcium loss in the urine per cup of ingested coffee (providing <100 mg of caffeine). Neither protein nor caffeine intake is thought to compromise calcium balance (especially if calcium intake is adequate). High sodium intake is also associated with increased urinary calcium excretion (while high potassium intake is associated with decreased urinary calcium), but the impact of high sodium intake on bone mineral density and fracture risk is unclear.

Recommended Dietary Allowance

In 2010, the Recommended Dietary Allowance (RDA) for calcium was set at 1,000 mg daily for adult men age 19–70 years and women age 19–50 years, including pregnant and lactating women [6]. For females age 51 years and older and males age 71 years and older, the recommendations for calcium intake are slightly higher at 1,200 mg/day. The inside covers of this book provide additional recommendations for calcium for other age groups.

The U.S. Food and Drug Administration (FDA) has approved several health claims related to calcium. One example claim is "Regular exercise and a healthy diet with enough calcium help teen and young adult white and Asian women maintain good bone health and may reduce the risk of osteoporosis later in life" [7]. Another claim added to the aforementioned statement on foods providing 40% or more of the Daily Value (1,000 mg) states, "Adequate calcium intake is important, but daily intakes above about 2,000 mg are not likely to provide any additional benefit" [7]. Foods citing these claims should not provide more phosphorus than calcium on a weight-for-weight basis [7].

Deficiency

Inadequate calcium intake, poor calcium absorption, excessive calcium losses, or a combination of these factors contribute to calcium deficiency. Individuals with a disorder associated with fat malabsorption (such as exocrine pancreatic conditions, cirrhosis, and intestinal conditions) exhibit diminished vitamin D absorption, which negatively impacts vitamin D status and thus calcium absorption. Immobilization—for example, due to injury—also promotes calcium loss from bone. Conditions that decrease gastrointestinal transit time, such as diarrhea, diminish intestinal calcium absorption. Use of thiazide diuretics increases urinary calcium excretion.

Infants and children develop rickets with insufficient calcium, and usually also a codeficiency of vitamin D. Rickets is characterized by defective mineralization of bone matrix and growth plate cartilage (before epiphyseal closure). The resulting weak and misshaped or deformed bone is especially noticeable in the legs, which curve or bow outward (seen when the infant begins to stand and bear weight) and in the ribs at the costochondral junction (see Chapter 10 under "Vitamin D Deficiency").

Inadequate calcium intake in adults (who, in contrast to infants and children, already have formed bones) decreases serum calcium concentrations, leading to chronic elevations in serum PTH (termed *secondary hyperparathyroidism*); the higher PTH promotes bone resorption to correct the

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low serum calcium concentrations. Thus, over time, bones lose more and more calcium. In addition, the bone matrix is also typically progressively lost, and osteopenia (reduced bone mineral density but not as severe as osteoporosis) and ultimately osteoporosis develop. Osteoporosis increases bone fragility and fracture risk. Unfortunately, much of the U.S. population, particularly females over 12 years of age, fails to consume the recommended amount of calcium and many are also deficient in vitamin D. Osteoporosis and diet are discussed further in the Perspective at the end of the chapter.

While serum calcium concentrations are tightly regulated, as previously discussed, **hypocalcemia** can occur, for example, with parathyroid gland conditions that reduce PTH secretion and/or with impaired vitamin D status. Should serum calcium concentrations drop below about 7 mg/dL, increased neuromuscular excitability results with an action potential triggered with a smaller stimulus. The condition is characterized by neuronal excitability and intermittent contractions of muscle that fail to relax; muscles of the hands and feet (extremities) are usually most affected. Paresthesia (numbness or tingling, especially in the hands and feet) may also be present. **Tetany** is characterized by continuous severe muscle spasms, and can cause respiratory dysfunction and death. Some clinical signs include a positive Chvostek sign (spasm of facial muscles elicited by tapping the facial nerve near the parotid gland) and Trousseau phenomenon/sign (spasmodic contractions of muscles of the hand initiated by pressure on the brachial artery). Treatment of tetany and hypocalcemia generally requires intravenous calcium administration, usually as calcium chloride or gluconate. Mild-to-moderate reductions in serum calcium concentrations may respond to oral calcium supplements in those with adequate gastrointestinal tract function.

Inadequate dietary calcium intakes have been associated with increased risks of several conditions including colon cancer, type 2 diabetes mellitus, metabolic syndrome, and obesity. The mechanisms by which calcium is thought to exert beneficial effects are not clear. The reduced risk for colorectal cancers and/or for the formation of its precursor colorectal adenomas is thought to be related to calcium-induced reductions in proliferation and reductions in the normal differentiation of colonic cells. Additionally, calcium's precipitation of bile acids is thought to prevent negative impacts on colonic cells. At present, while data demonstrating these positive associations are deemed promising, the data are considered insufficient to recommend the routine use of a calcium supplement to reduce or prevent colon cancer [6,8].

Inverse associations between the risk for type 2 diabetes and calcium intake and/or dairy product consumption have also been demonstrated. While the mechanisms are not clear, the relationship may be mediated by increased circulating concentrations of insulin-like growth factor-1, which promotes glucose uptake and/or reductions in inflammation, among other possible factors.

Low intakes of calcium and/or dairy products have been associated with gains in body weight and/or an increased risk of obesity. The association is thought to result from (in part) increased serum concentrations of vitamin D and PTH, which occur secondary to low calcium intake and low serum calcium concentrations. In this situation, vitamin D–induced openings of calcium channels in cell membranes lead to increased entry of calcium into adipocytes; the resulting elevation in cytosolic calcium affects gene expression, increasing de novo lipogenesis and inhibiting lipolysis. Conversely, increases in dietary calcium reduce serum PTH and vitamin D concentrations, reduce calcium uptake into adipocytes, and reduce intracellular calcium concentrations. This "lower-calcium environment" in the cell promotes lipolysis and inhibits expression of genes that enhance lipogenesis. While the modulation of body weight by calcium and/or dairy has been shown to be beneficial in some trials, meta-evaluations of randomized clinical trials along with findings from additional studies report no consistent effect from the ingestion of calcium and/or dairy products on weight and/or alterations in fat metabolism.

Toxicity

A Tolerable Upper Intake Level of 2,500 mg of calcium has been recommended for adults 19–50 years of age [6]. The recommendation decreases to 2,000 mg of calcium for those age 51 years and older [6]. This upper level was set based on the results of some studies showing positive associations between use of supplemental calcium and risk of kidney stones. High calcium intakes from dietary sources have not been typically associated with higher risk of stone formation, although this is not the case in those with idiopathic **hypercalciuria** and fat malabsorption disorders.

Idiopathic hypercalciuria is a medical condition characterized by high urinary calcium levels (>4 mg/kg body weight per day or about 250–300 mg/day) without a known cause (i.e., there this an absence of disease causing the hypercalciuria). Individuals with this condition who consume excessive amounts of calcium are at increased risk of developing calcium-containing kidney stones. Another group at increased risk of developing kidney stones is individuals who have fat malabsorption syndromes (such as those with chronic pancreatitis, cystic fibrosis, Crohn's disease, liver failure); such conditions enhance intestinal oxalic acid absorption and calcium oxalate stone formation in the kidneys. Several other factors, including inadequate fluid consumption, however, also contribute to stone formation in susceptible individuals.

Calcium-alkali syndrome (once called milk-alkali syndrome) has been documented in those consuming excessive quantities of calcium (formerly in the form of milk but more commonly now as antacids or supplements

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containing calcium carbonate and sodium bicarbonate). The condition is characterized initially by hypercalcemia and metabolic alkalosis. The excessive calcium intake (especially in excess of 4 g/day) promotes hypercalcemia. The hypercalcemia decreases PTH secretion. Retention of bicarbonate also occurs and causes a systemic alkalosis. Hypercalcemia and systemic alkalosis may be associated with lethargy, anorexia, nausea, vomiting, and heart arrhythmias. Hypercalcemia may also be observed with some cancers (especially those affecting the bone), immobility, and adrenal insufficiency, as well as from conditions affecting the parathyroid gland. Symptoms of hypercalcemia vary depending on the etiology and severity, but may include bradycardia, ventricular arrhythmias, coma, and death.

Calcium ingestion, usually when taken as a supplement, has been linked with increased risks of cardiovascular disease, ischemic heart disease, myocardial infarction (i.e., heart attack), and cardiovascular mortality, but also a decreased risk of all-cause mortality. Study findings supporting these associations, however, have not been consistent and the strength of some of the findings has been questioned. It has been suggested that acute increases in serum calcium concentrations that result for a few hours following the ingestion of large intakes of calcium may "overwhelm" the usual mechanisms that regulate serum calcium concentrations. The resulting periods of hypercalcemia in turn trigger changes (such as calcification of blood vessels, among other events) that increase cardiovascular disease risk. (See references [9–12] for a few of the many articles available on this topic.)

Assessment of Nutriture

No routine biochemical method is available to directly assess calcium status. Serum calcium is so exquisitely regulated that it indicates little about either calcium status or intake. Abnormal serum concentrations of calcium, however, can occur with various diseases, especially renal failure and cancers, and secondary to changes in blood protein (albumin) concentrations and pH. Thus, when evaluating serum calcium, it is important to check the individual's serum albumin concentration. In the presence of a normal serum albumin concentration, the ratio between bound calcium and ionized calcium remains constant. However, when serum albumin concentrations are depressed, corrections are needed to adjust for the corresponding decrease that occurs in the protein-bound fraction of calcium. For each 1 g/dL decrease in serum albumin, total serum calcium decreases 0.8 mg/dL. The following equations can be used for estimating protein-bound calcium: Protein-bound calcium (mg/dL) = 0.44 + [0.76 × albumin (g/dL)] and total serum calcium (mg/dL) = [0.8 × (normal albumin – actual albumin)] + measured calcium (mg/dL). Additionally, as blood pH increases (becomes more alkaline), protein-bound calcium increases and ionized calcium decreases in the blood. Conversely, with acidosis or a lower blood pH, serum ionized calcium increases and protein-bound calcium decreases.

Because the majority of calcium is found in bone, it is common to assess bone mineral density, especially in those at risk for osteoporosis. Assessment of bone is accomplished by several methods. Measurement by dual-energy X-ray absorptiometry (abbreviated DEXA or DXA), a widely used method, involves scanning specific sites at two different energy levels using an X-ray tube. Radiation exposure is low, and the procedure is relatively quick. Bone density can also be assessed through computerized tomography (CT) scans, which measure variances in tissue density. X-rays are taken as the person is held in a scanner. Radiation pulses are emitted, collected, and processed to reconstruct the image and calculate bone density. This method, however, is less precise and accurate than DEXA. Single-photon absorptiometry exposes a portion of a limb, usually the radius (forearm) or os calcis (heel), to radiation. The quantity of bone mineral is inversely proportional to the amount of photon energy transmitted from the bone, as measured by a scintillation counter. More information on osteoporosis is provided in the Perspective at the end of this chapter.

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PHOSPHORUS

Among the minerals, phosphorus is second only to calcium in abundance in the body. The human body contains about 550–850 g of phosphorus, representing about 0.8–1.4% of body weight. Of total body phosphorus, about 85% is in the skeleton, 1% is in extracellular fluids, and the remaining 14% is associated with soft tissues.

Sources

Phosphorus is widely distributed in foods (Table 11.6). The best food sources include protein-rich foods like meat, poultry, fish, eggs, milk, and milk products. Cola-type soft drinks, which contain 25–40 mg of phosphorus per 12 oz, can contribute substantially to dietary intake depending on consumption habits. Coffee and tea contain fairly little phosphorus, less than 10 mg/cup. The Daily Value for phosphorus is 1,000 mg.

Another source contributing what may be significant amounts of phosphorus to the diet is additives in foods. Phosphorus-containing additives, usually as inorganic phosphate salts, are found in a variety of foods including frozen foods, dry food mixes, bread and baked goods, canned vegetables, condiments, and sauces, among many others. While phosphorus-containing additives are indicated on the food label's ingredient list, manufacturers are not required to include the amount of phosphorus provided from the additives on the nutrition facts panel. Thus, dietary phosphorus intakes may be considerably higher than that given in reported studies depending on a person's use of processed foods.

Table 11.6 Phosphorus Content of Selected Foods*

Food (serving)	Phosphorus (mg)
Milk and yogurt, various types (1 c)	185–350
Cheeses, various types (1 oz)	85–155
Egg, poached (1)	98
Beef, loin, top sirloin, grilled (3 oz)	197
Beef, ground, 75% lean, 25% fat, broiled (3 oz)	161
Chicken, thigh, meat only, roasted (1)	267
Chicken, breast, meat only, roasted (3 oz)	200
Tuna, bluefin, cooked, dry heat (3 oz)	277
Halibut, cooked, dry heat (3 oz)	244
Salmon, Atlantic, farmed, cooked (3 oz)	214
Mixed nuts, oil roasted (1 oz)	123
Walnuts, dry roasted (1 oz)	98
Pecans, dry roasted (1 oz)	79
Peanut butter (2 tbsp)	114
Legumes, cooked (1/2 c)	230–250
Bread, whole wheat (1 sl)	57
Cola-type soft drinks (12 oz)	25–40

*The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

Although usually only needed for individuals with specific medical conditions, phosphate-containing supplements, including K-Phos Neutral® and Phospha 250 Neutral®, are available commercially. In multivitamin/minerals supplements, phosphorus is usually provided in amounts around 100 mg.

Phosphorus in foods occurs as organic and inorganic forms. In its organic form, phosphorus is bound to proteins, sugars, and lipids. The relative amounts of inorganic and organic phosphorus vary within foods and the diet. For example, meats contain phosphorus that is largely bound to organic compounds; milk has about one-third of the phosphorus as inorganic phosphates, and the remaining two-thirds bound to organic nutrients. Over 80% of the phosphorus in grains as well as some in legumes is found as part of phytic acid. The bioavailability of phosphorus from phytic acid is limited (see "Factors Influencing Absorption").

Digestion, Absorption, and Transport

Digestion

Regardless of its dietary form, most phosphorus is absorbed from the gastrointestinal tract as free inorganic phosphate ions. Thus, bound phosphorus must be digested enzymatically to release inorganic phosphate (P_i) to enable absorption. Several enzymes help to release the phosphorus. Phospholipase C, a zinc-dependent enzyme, for example, hydrolyzes the glycerophosphate bond in phospholipids. Alkaline phosphatase, another zinc-dependent enzyme whose activity is stimulated by calcitriol, functions at the brush border membrane of the enterocyte to free phosphate from some, but not all (such as phytic acid), bound forms.

Absorption

Phosphorus absorption occurs throughout the small intestine, but primarily in the jejunum. About 50–80% of dietary phosphorus is absorbed, with absorption from animal products at the upper end of the range and that from phytic acid–containing foods at the lower end. About 80–100% of inorganic phosphates in foods as additives are absorbed.

Phosphorus absorption occurs by two processes: (1) saturable, carrier-medicated, active transport and

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(2) diffusion. The absorption of most phosphate from the diet occurs by passive diffusion that is likely paracellular. Active transport contributes to absorption primarily when phosphate intake is low, and involves a sodium-phosphate, Na^+-Pi, cotransporter (NPT type 2b also written as NaPi2b), which carries three sodium ions for each phosphate (as either $H_2PO_4^-$ or HPO_4^{2-}). Calcitriol and low-phosphorus diets increase the numbers of the cotransporter in the intestinal brush border membrane; however, the involvement of calcitriol may vary within different regions of the intestine. The role or need for proteins for intracellular phosphate transport is not known. Transport of phosphorus across the enterocyte's basolateral membrane is thought to occur by facilitated diffusion.

Factors Influencing Absorption A number of factors influence phosphorus absorption. Calcitriol stimulates carrier-mediated intestinal phosphate absorption, but the extent to which absorption is enhanced is relatively small when compared with the effects of calcitriol on the absorption of calcium.

Phosphorus bioavailability is low from certain foods, especially grains (like wheat, corn, and rice) and legumes, which contain phosphate groups that are part of phytic acid (Figure 11.7). The bioavailability of phosphorus from phytic acid is relatively poor (less than about 50%) because humans do not produce phytase, a phosphate esterase that liberates phosphate from phytic acid. (Note: Yeasts in breads possess phytase that hydrolyzes phytates to yield some phosphorus available for absorption.) Moreover, when phytic acid–containing foods are consumed with foods rich in Ca^{2+} or Zn^{2+}, the phytic acid forms a cation–phytic acid complex that prevents these nutrients from being absorbed. Soaking legumes in water that is slightly acidic may partially reduce the phytic acid content.

Several minerals, including magnesium, aluminum, and calcium, form insoluble complexes with phosphorus and impair its absorption. Phosphorus and magnesium are thought to form a $Mg_3(PO_4)_2$ complex within the gastrointestinal tract to render each other unavailable for absorption. Aluminum hydroxide (3 g) given with a meal can substantially reduce phosphorus absorption from 70 to 35%. Aluminum and magnesium (as hydroxides) and calcium (as carbonate or acetate) are common components of antacids, and for years were given in pharmacological doses to bind dietary phosphate in people with **hyperphosphatemia** (high serum phosphorus concentration, which is greater than the upper limit of normal) caused by kidney disease. Today, nonionic binders and other types of agents are typically used for the treatment of hyperphosphatemia in individuals with renal failure.

Figure 11.7 Phytic acid (phytate).

The use of extended-release niacin in amounts of at least 2 g daily (as may be done to treat hypercholesterolemia) also reduces phosphorus absorption. High doses of niacin in this form appear to downregulate the active intestinal transport of phosphorus by the sodium-phosphorus cotransporter.

Transport

Phosphorus is quickly absorbed from the intestine, appearing in the blood within about an hour after ingestion in animal studies. Phosphorus is found in the blood in several inorganic forms. Most (about 55%) is present as HPO_4^{2-} due to its greater solubility in blood than $H_2PO_4^-$ and the trivalent anion PO_4^{3-} (which is present in trace amounts). Up to about 35% of inorganic phosphate, mainly HPO_4^{2-}, is found complexed with calcium, magnesium, or sodium as salts in the blood. About 10–15% is bound to proteins in the blood. Figure 11.8 provides an overview of phosphorus digestion, absorption, and transport.

Plasma inorganic phosphate concentrations usually range from about 2.5 to 4.5 mg/dL. Circulating plasma phosphate is in equilibrium with skeletal and cellular inorganic phosphate as well as with organic phosphates formed in intermediary metabolism. Uptake of phosphorus into cells is thought to occur passively (driven by the chemical gradient), but the mechanism is not clear.

Regulation and Homeostasis

While not maintained within a narrow a range as serum calcium, plasma/serum phosphate concentrations are regulated to an extent in adults. Parathyroid hormone (PTH) and fibroblast growth factor 23 influence phosphorus balance, although calcitriol and calcitonin also contribute. Fibroblast growth factor (FGF)23 is a phosphatonin secreted by osteoblasts. PTH (as you may remember from the section on calcium) is a hormone released by the parathyroid gland in response to primarily low serum concentrations of calcium, but to a lesser extent in response to low serum phosphorus, magnesium, and calcitriol. Calcitriol synthesis (specifically 1-hydroxylase activity) is enhanced by low serum calcium and phosphate. The hormone calcitonin is released by the thyroid gland in response to elevations in serum calcium, among other factors. These hormones and growth factors regulate phosphorus through actions on the bone, intestine, and kidneys. To increase serum phosphate concentrations:

- PTH and calcitriol stimulate resorption of phosphate from bone, possibly through enhanced alkaline phosphatase activity.

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Figure 11.8 Digestion, absorption, and transport of phosphorus.

- Calcitriol stimulates phosphorus absorption (to a limited extent) in the small intestine.
- Calcitriol enhances phosphorus reabsorption in the kidneys by upregulation of the NaPi2a and NaPi2c transporters.

FGF23, in response to elevated serum phosphate concentrations, primarily reduces serum phosphate concentrations through its actions on the kidneys and to a lesser extent through actions in the intestine. Calcitonin also contributes to these efforts through its actions on bone.

- FGF23 stimulates the urinary excretion of phosphate by downregulating the NaPi2a and NAPi2c transporters in the kidneys. PTH also stimulates the urinary excretion of phosphate.
- FGF23 diminishes intestinal phosphate absorption by diminishing intestinal phosphate transporters. Additionally, both FGF23 and a high plasma phosphate concentration reduce 1α-hydroxylase activity and thus calcitriol synthesis to reduce some phosphate absorption.
- Calcitonin inhibits osteoclast activity and thus decreases phosphate resorption/release from bone. Calcitonin also suppresses PTH release.

Functions and Mechanisms of Action

Phosphorus, which is found in all cells of the body, has many functions and is a component of several biologically important compounds. Some examples include roles in bone mineralization, energy transfer and storage, nucleic acid formation, cell membrane structure, and acid-base balance. This section briefly discusses each of these roles.

Bone Mineralization

Phosphate, like calcium and magnesium, is importance in bone (skeletal) tissue, which in itself accounts for 85% of body phosphorus. In bone, phosphorus is found in amorphous calcium phosphate forms, including, for example, $Ca_3(PO_4)_2$, $CaHPO_4 \bullet 2H_2O$, and $Ca_3(PO_4)_2 \bullet 3H_2O$, and in more crystalline forms such as hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, which is laid down on collagen in the ossification process of bone formation. In amorphous bone, the ratio of calcium to phosphorus is about 1.3:1, similar to extracellular fluid; however, in crystalline bone, the ratio is about 1.5 to 2.0:1. Phosphorus concentrations in the fluid surrounding bone are important both for osteoblast function as well as for osteoclast response to PTH.

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Figure 11.9 Some examples of important phosphorus-containing compounds in the body.

Phosphorus that is not part of bone or in the plasma is found within cells, where it serves as the cell's major anion. It is also a structural component of many important compounds, some of which are shown in Figure 11.9 and described hereafter.

Nucleotide/Nucleoside Phosphates

Structural Roles Phosphate is an important component of the nucleic acids DNA and RNA (Figures 11.9a and b), alternating with pentose sugars to form the linear backbone of these molecules.

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Energy Storage and Transfer Phosphorus is of vital importance in the intermediary metabolism of the energy nutrients in the form of high-energy phosphate bonds, such as those in adenosine triphosphate (ATP) (Figure 11.9c). The energy released as the phosphate bond of ATP is broken provides for numerous cellular functions (e.g., active transport pumps for nutrient absorption and for maintenance of ion concentrations, muscle cell contraction). In addition to its presence in ATP, phosphorus is found in creatine phosphate (also called phosphocreatine; Figure 11.9d). Creatine phosphate, synthesized in muscle from ATP and creatine, provides and replenishes energy to muscles as needed (e.g., during exercise) by transferring its PO_4 to ADP using creatine kinase.

Another nucleoside triphosphate, uridine triphosphate (UTP), activates substances in intermediary metabolism. For example, UTP hydrolysis enables the coupling of uridine monophosphate (UMP) and glucose-1-phosphate to form uridine diphosphate (UDP)–glucose. UDP-glucose is critical for the synthesis of glycogen.

Intracellular Second Messenger/Signaling Compounds Phosphorus as part of cyclic adenosine monophosphate (cAMP) (Figure 11.9e), which is derived from ATP, functions as a second messenger to affect cellular metabolism. cAMP, which acts within cells by activating selected protein kinases, is generated in response to the binding of certain hormones to cell receptors. Another phosphorus-containing second messenger is cyclic guanosine monophosphate (cGMP), which activates protein kinases. Inositol triphosphate (IP_3; Figure 11.9f) also functions as a second messenger to trigger intracellular calcium release from cellular organelles. Its actions are mediated by protein kinases. The role of protein kinases as they function in enzyme activation is discussed next.

Phosphoproteins and Phosphorylated Forms of Vitamins

Phosphorus is also of vital importance in intermediary metabolism of the energy nutrients through the phosphorylation of different substrates in the body. Protein kinases activated by cAMP, a phosphate-containing second messenger, phosphorylate specific target proteins within the cell, thereby changing cellular activities. Many enzymatic activities, for example, are controlled by alternating phosphorylation or dephosphorylation. An example of the role of phosphorylation and dephosphorylation of enzymes can be found in the discussion of glycogen degradation (see Chapter 3). In addition to phosphorylating proteins, phosphorus is needed for the actions of some vitamins, including thiamin and vitamin B_6. The active coenzyme forms of both of these vitamins—thiamin as thiamin diphosphate and vitamin B_6 as pyridoxal phosphate, pyridoxamine phosphate, and pyridoxine phosphate—require phosphorus.

Phospholipids

Cell membranes are made up, in part, of lipids, including phospholipids, which (as their name implies) contain phosphorus. Phospholipids, with their polar and nonpolar regions, are important to the bilayer structure of cell membranes. Each phospholipid contains a glycerol backbone with two fatty acyl chains attached at carbons 1 and 2; attached at glycerol's carbon 3 is a phosphorus-containing base (Figure 11.9g). The bases include choline (forming phosphatidylcholine), inositol (forming phosphatidylinositol), serine (forming phosphatidylserine), and ethanolamine (forming phosphatidylethanolamine). See Chapter 5 for more information on phospholipids.

Acid-Base Balance

Phosphate also functions in acid-base balance. Within cells, phosphate serves as an intracellular buffer. Within the kidneys, for example, filtered phosphate reacts with secreted hydrogen ions, releasing sodium ions in the process, as shown here:

$$Na_2HPO_4 + H^+ \longrightarrow NaH_2PO_4 + Na^+$$

This action removes free hydrogen ions and therefore increases pH. The following reaction also increases pH: $HPO^{2-} + H^+ \longrightarrow H_2PO_4^-$. These reactions may be reversed to lower pH.

Oxygen Availability

Phosphate is involved indirectly in oxygen delivery. In red blood cells, the synthesis of 2,3-diphosphoglycerate (2,3-DPG), which regulates oxygen release from hemoglobin to tissues, requires phosphorus. Decreased 2,3-diphosphoglycerate associated with phosphorus deficiency can diminish release of oxygen from hemoglobin to tissues.

Excretion

Phosphorus losses from the body occur via the feces and urine. Endogenous fecal losses of phosphorus, in amounts usually up to about 300 mg, result from the sloughing of mucosal cells and phosphorus that is not reabsorbed from digestive juices—saliva, gastric juice, pancreatic juice, and bile.

Urinary excretion is the primary means of excreting phosphate and maintaining homeostasis in the body. Phosphate that is not bound to proteins in the blood is filtered by the glomerulus. The proximal tubule actively reabsorbs about 75% of this filtered phosphate via two (type 2a and 2c) sodium phosphate cotransporters [Na^+–$H_2PO_4^-$ or HPO_4^{2-}]. The distal convoluted tubule reabsorbs up to about 10% of phosphate, and about 15% is typically excreted. If phosphate intake and plasma phosphate concentrations are low, then most filtered phosphate is reabsorbed. However, the carriers in the

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proximal tubule have a limited capacity to reabsorb phosphorus, referred to as the tubular maximum for phosphorus (TmP). The tubular maximum for phosphorus (TmP) is the amount (mmol) of phosphorus reabsorbed per unit time. If the amount of phosphate filtered is greater than the tubular maximum for phosphorus, more phosphate will be excreted. Thus, high serum phosphate promotes urinary phosphate excretion (i.e., tubular reabsorption of phosphorus is inhibited). PTH and FGF23 mediate this process; both promote urinary phosphate excretion by diminishing phosphate's active reabsorption in the proximal tubule via sodium-phosphate cotransporters. In contrast, peptides/hormones, especially insulin-like growth factor 1 (IGF-1), promote renal phosphate reabsorption. Urinary phosphorus excretion in adults ranges from about 170 to 1,600 mg/day.

Recommended Dietary Allowance

The RDA for phosphorus is 700 mg/day for males and females (including those who are pregnant or lactating) age 19 years and older [1]. An estimated requirement (580 mg/day) for phosphorus was determined based on the relationship between dietary phosphorus intake and plasma phosphorus concentrations as well as a known efficiency of intestinal absorption [1]. A coefficient of variation of 10% was added to the requirement and rounded to establish this recommended intake. The inside covers of the book provide the RDAs for phosphorus for other age groups.

Deficiency

Phosphorus deficiency is rare. Premature infants can be at risk for deficiency because of their higher needs for the mineral and the insufficient amount found in human milk. Additionally, people who are malnourished and are being refed enterally through a tube or parenterally (intravenously) without being given additional phosphorus can exhibit hypophosphatemia as part of a condition called refeeding syndrome. Alkalosis as well as critical illness and chronic alcoholic consumption may also be associated with hypophosphatemia. Genetic disorders associated with phosphorus deficiency include X-linked hypophosphatemia and hypophosphatemic rickets (also called Dent's syndrome). These genetic disorders result from defects in phosphorus reabsorption in the kidneys, and thus cause excessive urinary phosphorus loss (see reference [2]).

A phosphorus deficiency, especially if chronic, affects bone. Rickets occurs in infants and children and results from an inadequate mineralization of the bone matrix and growth plate cartilage (see "Vitamin D Deficiency" section in Chapter 10). In adults, a chronic phosphorus deficiency promotes osteomalacia characterized by bones that are soft due to the failure to mineralize the bone matrix. Typically with osteomalacia, the proportion of the mineral content of the bone is reduced in comparison to the protein matrix of the bone (see also "Vitamin D Deficiency"). The effects on bone are more likely to occur when serum phosphorus concentrations decrease below about 1.5 mg/dL.

Individuals with moderate phosphorus deficiency, indicated by serum phosphorus concentrations between about 2 and 2.5 mg/dL, can be asymptomatic. Increasing intake of phosphorus-rich foods is usually sufficient to correct the depletion, but supplements of phosphate (usually as potassium phosphate) may be needed in some situations. (Oral phosphate supplementation, however, may be associated with diarrhea.) Severe phosphorus deficiency, manifested biochemically by serum phosphorus concentrations less than about 1.0 mg/dL, is associated with reduced oxygen transport and delivery, reduced cardiac output, arrhythmias, decreased diaphragmatic contractility, respiratory failure, skeletal muscle and cardiac myopathy, and neurological problems (ataxia and paresthesia), as well as possible death. Repletion of severe deficiency requires intravenous administration of phosphorus, usually as sodium or potassium phosphate.

Toxicity

While toxicity from phosphorus is rare, a Tolerable Upper Intake Level of 4 g of phosphorus has been recommended for those age 9–70 years to minimize the likelihood of hyperphosphatemia [1]. After age 70 years, the tolerable level drops to 3 g of phosphorus daily; this decrease is associated with an increased likelihood of impaired renal function that often occurs with aging and reduces urinary phosphorus excretion [1]. For pregnant and lactating women, the Tolerable Upper Intake Levels are 3.5 g and 4 g, respectively [1].

Toxicity from phosphorus occurs most commonly in individuals with impaired renal function, especially when the glomerular filtration rate decreases below about 25 mL/minute. The resulting high serum phosphate concentrations (hyperphosphatemia) promote the formation and deposition of $Ca\text{-}PO_4$ crystals in the body's soft tissues including blood vessels and organs. The risk of $Ca\text{-}PO_4$ precipitation and crystal formation increases when the calculated product of the serum calcium concentration multiplied by the serum phosphorus concentration is greater than about 55 or 60 mg/dL. Other conditions increasing the risk for hyperphosphatemia include immobility, acidosis, vitamin D toxicity, and hypoparathyroidism. Chronic hyperphosphatemia (greater than about 5.5 mg/dL) associated with renal failure is treated with medications to bind dietary phosphorus and a phosphorus-restricted diet. In other cases, the underlying cause(s) of the elevated serum phosphorus concentrations must be addressed.

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Hyperphosphatemia and plasma phosphorus concentrations at the upper end of the normal physiologic range have been linked with atherosclerosis and left ventricular hypertrophy (a risk factor for heart disease). The mechanism(s) responsible for the increased risk is not clear.

Assessment of Nutriture

Serum concentrations and urinary excretion of phosphorus are most often assessed to examine mineral status; however, their specificity and sensitivity are low. Serum phosphate concentrations, for example, can be maintained at the expense of tissues.

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MAGNESIUM

Of the major minerals, magnesium ranks sixth ($Ca^{2+} > P > K^{+} > Na^{+}$ and $C1^{-} > Mg^{2+}$) in overall abundance in the body, but intracellularly the cation is second only to potassium. The human body contains about 21–28 g of magnesium (close to 1% of body weight), of which approximately 50–60% is located in bone, another 39–49% in soft tissues (of which about 25% is found in skeletal muscle), and about 1% in extracellular fluids.

Sources

Magnesium is found in a wide variety of foods. Foods particularly high in magnesium include nuts, seeds, and whole-grain cereals (especially oats and barley) (Table 11.7). Food processing such as refining whole wheat, which removes the germ and outer bran layers, substantially reduces its magnesium content (by over 75%). Green leafy vegetables also provide significant amounts of magnesium; it is the chlorophyll that is found in the green leafy vegetables that contains magnesium. Legumes, lentils, spices, seafood, and dairy products also contribute to dietary magnesium. Tap water (which is "hard") may also add small amounts of the mineral to the diet (versus soft water that is higher in sodium). The Daily Value for magnesium, used on food and supplement labels, is 400 mg.

Table 11.7 Magnesium Content of Selected Foods*

Food (serving)	Magnesium (mg)
Halibut, cooked (3 oz)	90
Cod, cooked (3 oz)	36
Oysters, eastern, cooked (3 oz)	30
Clams, mixed species, cooked (3 oz)	15
Legumes, cooked (1/2 c)	35–60
Lentils, cooked (1/2 c)	35
Sunflower seeds, dry roasted (1/4 c)	43
Almonds, dry roasted (1 oz)	80
Cashews, dry roasted (1 oz)	74
Mixed nuts, oil roasted (1 oz)	65
Walnuts, dry roasted (1 oz)	42
Peanut butter (2 tbsp)	50
Edamame, shelled, cooked (1/2 c)	50
Spinach, cooked (1/2 c)	78
Peas, green, cooked (1/2 c)	23
Broccoli, cooked (1/2 c)	12
Avocado, raw (1/2 c)	22
Banana (1)	30
Potato, baked, with skin (1)	50
Rice, white, cooked (1/2 c)	10
Rice, brown, cooked (1/2 c)	42
Oatmeal, cooked (1/2 c)	25
Bread, whole wheat (1 sl)	25
Milk and yogurt (1 c)	25–40
Chocolate, varying types (2 oz)	36–51
Blackstrap molasses (1 tbsp)	43
Coffee, espresso (2 oz)	48
Cocoa, hot chocolate, instant (6 oz)	25

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

Multivitamin/minerals supplements contain magnesium in amounts of about 100 mg. Single-nutrient forms of magnesium are also available and provide magnesium usually as a magnesium salt—such as magnesium sulfate ($MgSO_4$, or Epsom salts), magnesium oxide (MgO), magnesium chloride ($MgC1_2$), magnesium lactate, magnesium acetate, magnesium gluconate, magnesium citrate, and magnesium hydroxide. The single-nutrient supplements vary in magnesium content, ranging from about 25 to 300 mg of magnesium/tablet. Liquid and sustained-release forms of magnesium are also available. Absorption of magnesium from magnesium oxide and magnesium sulfate tends to be less than that from other magnesium salts. Absorption also may be better from effervescent tablets than from capsules. To maximize absorption, magnesium supplements should not be taken

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at the same time as other mineral supplements, such as iron. The use of magnesium supplements, however, can result in toxicity; it also acts as a cathartic, promoting defecation (see "Toxicity" section).

Digestion, Absorption, and Transport

Digestion and Absorption

Dietary magnesium does not require digestion prior to absorption. Two processes facilitate magnesium absorption throughout the small intestine; however, the colon also absorbs magnesium, especially if disease has interfered with magnesium absorption in the small intestine. Magnesium is absorbed in the small intestine through (1) saturable, carrier-mediated active transport and (2) diffusion. The active transporter of magnesium is a transient receptor potential (TRP) melastatin divalent cation-permeable channel protein (abbreviated TRPM6). This channel protein is found on the brush border membrane of enterocytes, and is inhibited by high cytosolic magnesium concentrations. Thus, absorption decreases with increased intracellular magnesium concentrations. This carrier system operates mostly in the distal small intestine (lower jejunum and ileum) with low magnesium intakes.

Paracellular diffusion of magnesium (the second means of absorption occurring throughout the small intestine) increases as intraluminal magnesium concentrations increase. The majority of dietary magnesium is thought to be absorbed by this passive, concentration-dependent route when magnesium intakes are high. Calcitriol may enhance paracellular absorption by increasing the expression of genes that code for transmembrane proteins called claudins. Claudin-2, for example, forms charged selective pores that facilitate permeability and thus paracellular intestinal absorption of magnesium, as well as calcium, and other solutes.

About 30–60% of dietary magnesium is typically absorbed from the intestine. Absorption declines to less than 30% as magnesium intake increases above about 550 mg. Conversely, magnesium absorption may increase above 60% (up to ~75%) when magnesium intake is low (less than about 40 mg). Efflux of magnesium from cells is thought to occur by a Na^+/Mg^{2+} antiport system that depends on a Na^+/K^+-ATPase to sustain the sodium gradient. Figure 11.10 illustrates magnesium absorption and transport.

1. Mg^{2+} crosses the brush border membrane of the enterocyte through a magnesium channel, TRPM6.
2. Mg^{2+} also may be absorbed between cells; this transport is influenced by the electron chemical gradient and solvent drag.
3. Mg^{2+} is pumped out of the cell across the basolateral membrane by a sodium-dependent ATPase.

Figure 11.10 Magnesium absorption and transport.

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Factors Influencing Absorption Magnesium absorption is influenced by several dietary factors, listed in Table 11.8. For example, unabsorbed fatty acids present in high quantities in the gastrointestinal tract, as with steatorrhea, may bind to magnesium to form soaps. These magnesium–fatty acid soaps are excreted in the feces. Minerals such as phosphorus also inhibit magnesium absorption. Magnesium and phosphorus form a complex, $Mg_3(PO_4)_2$, within the gastrointestinal tract to render each other unavailable for absorption. The inhibition is most apparent when magnesium consumption is low and intake of phosphorus is high. Additionally, phytic acid and nonfermentable fiber (such as cellulose) may reduce magnesium absorption, but only to a small extent with usual intakes in the United States.

Vitamin D, in pharmacological doses, and protein, in some but not all studies, may increase magnesium absorption and/or its retention. Carbohydrates, such as fructose and oligosaccharides, may also increase magnesium absorption to a small extent.

Transport

In the plasma/serum, most magnesium (50–55%) is found free in its ionic form as Mg^{2+}, about 20–30% is bound to protein (mostly albumin with lesser amounts bound to globulins), and about 5–15% is complexed with citrate, phosphate, sulfate, or other negatively charged anions. Like calcium, it is ionized magnesium that is physiologically active and bound magnesium is released as ionized magnesium is taken up by cells/tissues for use. Plasma magnesium concentrations are maintained tightly; however, the lower level of the "accepted" reference range for total plasma magnesium is under scrutiny [1,2]. While it has been well established that plasma magnesium concentrations are not indicative of total body magnesium stores, the lower end of the reference range for plasma magnesium is frequently used in clinical practice to guide nutrition support. Thus, this reevaluation is important since what is considered "normal" may actually be "low"; in other words, plasma magnesium concentrations of 1.7–2.1 mg/dL, and possibly even a concentration as high as 2.4 mg/dL, may reflect mild hypomagnesemia [1–3].

Regulation and Homeostasis

Maintenance of body magnesium depends on gastrointestinal absorption and renal excretion; however, it is primarily the kidneys that control (increase or decrease) urinary losses of magnesium in response to plasma concentrations of the mineral. Bones also provide a needed reservoir of the mineral. Several hormones influence magnesium balance. PTH, for example, increases intestinal magnesium absorption, diminishes renal magnesium excretion, and enhances bone magnesium resorption, thereby raising plasma magnesium concentrations. Renal excretion of magnesium is affected by dietary magnesium intakes and plasma concentrations as well as by PTH, as discussed further in the section on excretion.

Table 11.8 Substances/Nutrients Affecting Intestinal Magnesium Absorption

Substances Enhancing Absorption	Substances Inhibiting Absorption
Vitamin D	Phytic acid
Protein	Fiber
Carbohydrates–fructose and oligosaccharides	Unabsorbed fatty acids

Plasma magnesium concentrations, like calcium, can be maintained at the expense of bone. Of the 50–60% of magnesium in the body that is found associated with bone, about 30% is located on the surface in an amorphous form, which provides an exchangeable magnesium pool that is able to maintain plasma concentrations. This "bone pool" of magnesium, however, is released slower in comparison to the "intracellular pool."

Free intracellular concentrations of magnesium are rigidly maintained—at about 0.2–1.0 mmol/L—by altering membrane uptake, intracellular storage in organelles, and cellular flux. Most intracellular magnesium is bound to nucleic acids, ATP, proteins, and phospholipids. Several plasma membrane magnesium transporters assist in maintaining intracellular concentrations of the mineral; some of these transporters include TRPM7 (in the heart, adipose tissue, and bone), MagT1 (in epithelial cells), NIPA Mg^{2+} transporter, and SLC41 Na^+/Mg^{2+} exchanger. The Na^+/Mg^{2+} exchanger, which is ubiquitously expressed, is responsible for cellular magnesium efflux. TRPM7 is an active ion channel that transports both magnesium and calcium; it is regulated by intracellular magnesium concentrations. MgT1 and 2 appear to control magnesium metabolism within the Golgi complex and post-Golgi vesicles [4]. TRPM6 regulates magnesium transport in the small intestine and kidneys with effects on total body magnesium concentrations.

Functions and Mechanisms of Action

Magnesium plays numerous roles in the body including functions in bone mineralization, enzymatic reactions, nucleic acids, platelet activity, hormone receptor binding and signal transmission, cell membrane ion transfer, and calcium regulation, among others. Within cells, magnesium is thus associated with phospholipids as part of cell membranes (plasma, endoplasmic reticulum, and mitochondrial), as well as with proteins and nucleic acids. A few of these roles are discussed hereafter.

Bone Mineralization

Most (about 50–60%) magnesium in the body is found in bone. Several forms of magnesium are found associated with bone, including, for example, $Mg(OH)_2$ and $Mg_3(PO_4)_2$. While about 30% of bone magnesium is found

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on the surface in an amorphous form and serves as an available pool to maintain plasma magnesium concentrations, the rest of magnesium in bone is found with calcium and phosphorus as part of the crystal lattice. This magnesium is likely deposited at the time of bone formation and is important for mineralization.

Enzymatic Reactions

Magnesium within cells is associated with numerous proteins including enzymes. In fact, magnesium is required for over 300 different enzymatic reactions either as a structural cofactor to stabilize the enzyme or as an allosteric activator of enzyme activity. Additionally, intracellular magnesium is found linked to the oxygen atoms of the phosphate groups of ATP and ADP, and assists in the transfer of a phosphate group. Figure 11.11 depicts magnesium as a ligand for the phosphate groups. Protein kinases transfer the γ-phosphate of magnesium ATP to a substrate. Listed hereafter are some of magnesium's enzymatic functions, including roles in glucose, fat, protein, vitamin, and nucleic acid metabolism:

- glycolysis: hexokinase, glucokinase, and phosphofructokinase
- TCA cycle: oxidative decarboxylation
- pentose phosphate pathway (hexose monophosphate shunt): transketolase reaction
- creatine phosphate formation: creatine kinase
- β-oxidation: initiation by thiokinase (acyl-CoA synthetase)
- activities of alkaline phosphatase and pyrophosphatase
- amino acid activation and protein synthesis (e.g., with ribosomal aggregation and binding messenger RNA to ribosome subunits)
- hydroxylation of vitamin D in the 25-position; this hydroxylation is the first of two reactions needed to produce the active form of vitamin D

Figure 11.11 Modes by which Mg^{2+} provides stability to ATP.

- pyrimidine and purine synthesis (for DNA and RNA synthesis)
- DNA replication/synthesis and degradation, as well as the physical integrity of the DNA helix and conformation of nucleic acids
- RNA transcription.

Other Roles

Magnesium also affects several other physiological processes, including:

- blood clotting, reducing platelet aggregation and thus thrombosis formation
- hormone receptor binding and activation of second-messenger signaling. Magnesium bound to nucleotides such as GTP, following activation of membrane receptors, is involved in the activation of adenylate cyclase, which leads to cAMP production and its second-messenger intracellular signaling effects. Magnesium thus mediates, in part, the effects of numerous hormones, including parathyroid hormone.
- ion channel regulation, especially potassium and calcium channels, where intracellular magnesium plays a role in the active transport of these ions across cell membranes to affect cardiac and smooth muscle contractibility, normal heart rhythm, nerve impulse conduction and neuromuscular conduction, vasomotor tone, and normal blood pressure
- antagonism of intracellular calcium, whereby following cell stimulation, magnesium inhibits the release of calcium from its intracellular stores in organelles and stimulates the reuptake of calcium by Ca^{2+}-ATPase pump into the sarcoplasmic reticulum to decrease cytosolic Ca^{2+} concentrations. Magnesium may also decrease the flux of calcium across cell membranes. Furthermore, magnesium may mimic or displace calcium from calcium-binding sites, or compete with calcium for nonspecific binding sites on troponin C and myosin to alter muscle contraction. In smooth muscle, magnesium, if bound to sites that are normally occupied by calcium, can inhibit contraction.
- insulin production, release, and action/signaling, with magnesium promoting cellular glucose uptake and utilization.

Interactions with Other Nutrients

Magnesium interacts with phosphorus to inhibit phosphorus absorption. As magnesium intake increases, phosphorus absorption decreases. The two minerals are thought to precipitate as $Mg_3(PO_4)_2$. Ingestion of large doses of magnesium (600 mg), for example, reduce phosphorus absorption by almost 50%.

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An interrelationship also exists between magnesium and potassium. Magnesium influences the balance between extracellular and intracellular potassium, but its mechanism of action is unclear. Magnesium depletion is associated with increased potassium efflux from cells (i.e., cellular potassium depletion) and subsequent renal potassium excretion. When magnesium and potassium deficiencies coexist, as may occur with some diuretic drug therapies, magnesium infusions—but not potassium infusions—normalize cell potassium.

Excretion

Magnesium is excreted from the body mainly via the kidneys, which help to maintain magnesium homeostasis. Lesser amounts of magnesium are excreted in the urine when plasma magnesium is low. Conversely, increased plasma magnesium concentrations, which are monitored by renal divalent cation sensors, inhibit magnesium reabsorption from the distal convoluted tubule and thus promote urinary magnesium excretion. Activation of these sensors also reduces the kidney's response to hormones such as PTH, which normally reduces magnesium excretion (promoting reabsorption of magnesium in the loop of Henle and distal convoluted tubule).

Of the filtered magnesium (which includes magnesium in the blood that is not bound to proteins), about 95–97% is reabsorbed and about 3–5% is excreted in the urine. Of this reabsorbed magnesium, about 50–60% is reabsorbed actively in the ascending limb of the loop of Henle, another 20–30% is reabsorbed by passive diffusion in the proximal convoluted tubule, and the remaining about 5–7% is reabsorbed in the distal convoluted tubule. Claudin-16 (also called paracellin-1), present in the ascending limb of the loop of Henle, and TRPM6, found in the distal convoluted tubule, are involved in urinary magnesium excretion. Mutations in claudin-16 have been shown to contribute to the development of magnesium deficiency. Diuretic loop medications, high serum aldosterone concentrations, as well as protein, alcohol, and caffeine consumption, increase urinary magnesium excretion. Urinary magnesium excretion ranges from about 60 to 185 mg/day, with excretion reduced to less than about 24 mg/day with low plasma magnesium concentrations.

Fecal magnesium concentrations represent unabsorbed magnesium and a small amount of endogenous magnesium. About 25–50 mg of magnesium from endogenous sources is usually excreted daily in the feces [5]. Magnesium may also be lost from the skin into sweat, in amounts estimated at up to approximately 15 mg/day [5,6].

Recommended Dietary Allowance

The RDA for magnesium varies in adults; among those 19–30 years of age, males need 400 mg and females need 310 mg magnesium per day, and among those 31 years and older, males need 420 mg and females need 320 mg magnesium daily [5]. Requirements used to establish these recommendations, however, are thought to be high by some researchers [7]. Pregnant women age 19–30 years should ingest 350 mg daily, and those age 31–50 years should consume 360 mg of magnesium [5]. During lactation, women age 19–30 years should ingest 310 mg daily, and those age 31–50 years should consume 320 mg of magnesium [5]. The inside covers of the book provide the RDAs for magnesium for other age groups.

Deficiency

Magnesium deficiency, which is traditionally diagnosed based on total plasma/serum magnesium concentrations, is relatively common among hospitalized individuals; it may also be underestimated among the general population due to the use of an elevated serum reference range for magnesium in the diagnosis of deficiency and due to the inability to detect small reductions in tissue magnesium [3]. In other words, plasma magnesium concentrations may appear within the normal range at the expense of tissue magnesium. Furthermore, individuals are often asymptomatic as plasma magnesium concentrations begin to decline below normal, or they have only vague symptoms such as fatigue, lethargy, and weakness along with anorexia, nausea, and vomiting. Thus, the diagnosis of deficiency is often delayed.

Metabolic effects occurring with hypomagnesemia can include decreased serum concentrations of PTH, calcium, potassium, and calcitriol. The hypocalcemia is likely to result from reduced PTH levels and inhibition of calcium release from cells caused by the hypomagnesemia. The **hypokalemia** (low blood potassium) likely results from altered cellular transport systems (such as impaired Na^+-K^+-ATPase) that maintain the potassium gradient and from increased urinary potassium losses. Calcitriol synthesis may be altered by decreases in PTH secretion or renal resistance to PTH.

Symptoms of a magnesium deficiency are usually more pronounced as plasma concentrations decrease below about 1.2 mg/dL; these symptoms are also more likely if the decrease occurs more rapidly versus declining gradually. Hypomagnesemia (and accompanying drops in serum potassium and calcium concentrations) can promote a state of neuromuscular hyperexcitability. Neuromuscular symptoms include muscle weakness, tremors, muscle fasciculations (brief spontaneous contractions of a small number of muscle fibers), latent tetany (such as may be indicated by a positive Chvostek's sign and Trousseau's phenomenon; see "Calcium Deficiency"), paresthesia, hyperreflexia, and myoclonic jerks. Some cardiovascular symptoms associated with magnesium deficiency (and accompanying drops in serum potassium and calcium concentrations) include EKG changes, vasospasms, higher

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blood pressure, and arrhythmias—rapid heart rate (tachycardia; ventricular tachycardia), skipped heartbeats, or irregular heartbeat (fibrillation; ventricular fibrillation, premature ventricular beats). Some central nervous system symptoms are headache, nystagmus (rapid involuntary eye movement), irritability, restlessness, disorientation, mental confusion, personality changes, psychosis, hallucinations, and seizures.

Treatment of deficiency depends on the severity of depletion and the underlying cause(s). Correction of mild deficits characterized by plasma magnesium concentrations between about 1.1 and 1.4 mg/dL is usually accomplished with increased intake of magnesium-rich foods, but supplements in amounts ranging from about 300 to 600 mg/day may also be needed. To minimize the risk of diarrhea that can occur with oral supplementation, the total daily magnesium dose being provided as a supplement should be divided into at least three smaller quantities to be taken throughout the day (e.g., one 300 mg magnesium per day dose can be divided into three 100 mg doses). Intravenous provision of magnesium (as magnesium sulfate or chloride) is usually needed if plasma concentrations decrease below about 1.0 mg/dL, but may also be needed due to the gastrointestinal side effects associated with oral supplementation. Corrections of hypokalemia and hypocalcemia may also be needed following repletion with magnesium.

Several factors can contribute to the development of a magnesium deficiency. First is inadequate intake, which is common among most adults in the United States. Excess alcohol use is also associated with inadequate magnesium intake and increased urinary magnesium excretion. Deficiency also occurs secondary to other conditions such as malabsorptive disorders (bariatric surgery, ileostomy, Crohn's disease, pancreatic insufficiency, and liver failure, among others), which reduce magnesium absorption and/or promote increased magnesium losses through vomiting or diarrhea/steatorrhea. Many medications promote hypomagnesemia such as cyclosporine and amphotericin B, as well as proton pump inhibitors. Chronic loop and thiazide diuretic use increases urinary magnesium losses; increased renal magnesium excretion also results with the use of some chemotherapy drugs like cisplatin. Parathyroid and thyroid conditions also alter magnesium, and burns result in excessive dermal loss of magnesium. Intracellular shifts in the mineral, such as occurs with refeeding syndrome, myocardial infarction, diabetic ketoacidosis, among other conditions, also affect plasma concentrations of magnesium. Additionally, uncontrolled diabetes and metabolic syndrome increase urinary magnesium losses; urinary magnesium increases secondary to the osmotic effects induced by high urinary glucose concentrations.

Chronic latent (without symptoms) deficiency of magnesium and/or low magnesium intakes have been associated with a number of conditions, including hypertension, cardiac arrhythmias, cardiovascular disease, diabetes, and migraines. How magnesium directly impacts blood pressure is not clear (see reference [8] for a review). Possible mechanisms include its actions as a natural calcium channel blocker, as well its ability to increase nitric oxide, improve endothelial dysfunction and promote relaxation of vascular smooth muscle, which are known to reduce blood pressure [8]. Supplementation trials with magnesium (about 500–1,000 mg), however, have not consistently shown significant reductions in blood pressure.

Heart disease appears to develop more quickly in the presence of magnesium deficiency; this accelerated development may result from increased oxidative stress, increased blood cholesterol concentrations, increased thrombosis, and/or inflammation, among other possible mechanisms. Typically individuals in the highest quartiles for dietary magnesium intake and/or plasma magnesium concentration (within the normal physiologic range) exhibit lower heart disease risk than those in the lowest quartile.

Significant inverse associations have been demonstrated between dietary magnesium intake and/or serum magnesium concentrations versus risk of diabetes. Links between diabetes and magnesium are plausible given the many roles that magnesium plays in glucose metabolism, including, for example, insulin secretion, interactions between insulin and the insulin receptor, involvement in tyrosine kinase activity at the insulin receptor, intracellular calcium concentrations, and ATP-dependent reactions, among others. While some supplementation trials providing magnesium (in amounts from ~300 to 600 mg/day) to individuals with type 2 diabetes have shown beneficial effects (e.g., on glucose metabolism, reducing fasting plasma glucose), others have not. According to the American Diabetes Association, at present, evidence for the use of magnesium in the treatment of type 2 diabetes is insufficient [9]. Thus, magnesium supplementation in the absence of deficiency is not currently recommended.

In addition to the previously discussed conditions, magnesium deficiency or low plasma magnesium has been linked to headaches, including migraine headaches. Enhanced vasoconstriction and/or altered neurotransmitter release, among other processes associated with inadequate magnesium, may be contributing factors to the migraine headaches. Supplementation trials providing magnesium (in amounts of usually 600 mg/day) to individuals with migraines have shown beneficial effects (reduced headache frequency), but the number of conducted studies is extremely small. A joint report by the American Academy of Neurology and the American Headache Society states that magnesium supplementation is "probably effective" (level B) for migraine prevention [10].

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Toxicity

An excessive intake of magnesium from food sources has not been shown to cause toxicity in healthy individuals since the kidneys can normally excrete magnesium fairly rapidly (thus preventing significant increases in plasma concentrations). However, consumption of magnesium from nonfood sources, including supplements and medications, can produce side effects. A Tolerable Upper Intake Level of 350 mg magnesium from nonfood sources has been recommended for people age 9 years and older (including during pregnancy and lactation) [5].

Excessive intake of magnesium from magnesium salts (3–5 g), such as from $Mg(OH)_2$ and $MgSO_4$, results in a cathartic effect, characterized predominantly by diarrhea (along with nausea, abdominal cramping, and possible dehydration). Magnesium is thus found in a number of laxatives such as Phillips' Milk of Magnesia®. It is also found in antacids such as Gaviscon®, Gelusil®, Maalox®, and Mylanta®. These gastrointestinal side effects along with hypermagnesemia are even more likely in individuals with impaired renal function.

Acute magnesium toxicity (such as from excessive intravenous magnesium administration or excessive oral magnesium intake) may cause nausea, vomiting, depression, muscle weakness, and paralysis. Grams doses (>1 g) of magnesium (given intravenously), however, are often helpful in the treatment of preeclampsia and eclampsia. Diminished and eventual loss of deep tendon reflexes may be associated with plasma magnesium concentrations of about 4.9–7.3 mg/dL. In addition to these neuromuscular effects, toxicity affects the cardiopulmonary systems, causing hypotension, apnea, and EKG changes. Magnesium toxicity also produces flushing, double vision, muscle weakness, and slurred speech; these manifestations may not appear until plasma magnesium concentrations rise to about 9–12 mg/dL. Muscular paralysis and cardiac and/or respiratory failure (death) are likely if plasma magnesium concentrations exceed about 15 mg/dL. Intravenous administration of calcium chloride or gluconate and dialysis in those with renal failure or diuretics in those with adequate renal function may be employed to help reduce the hypermagnesemia.

Assessment of Nutriture

Assessment of magnesium status is difficult because extracellular magnesium represents only about 1% of total body magnesium and appears to be regulated. Despite low sensitivity and specificity (e.g., normal plasma/serum levels may persist despite severe intracellular deficits), plasma/serum magnesium concentrations are routinely measured to assess magnesium status and, when the plasma/serum magnesium concentration is below normal, an inadequate amount of intracellular magnesium is a certainty. The exact "cutoff" value constituting a "low" plasma/serum magnesium concentration is under review, with suggested lower end of the reference range values of either 2.1 or 2.4 mg/dL [1–3]. While erythrocyte magnesium concentrations are higher, and decrease more slowly than plasma/serum concentrations with magnesium deficiency, they are not deemed as a better alternative to measurement of plasma/serum magnesium concentrations.

Measurement of renal magnesium excretion alone or as part of a magnesium retention test (loading test) is also sometimes used to assess magnesium. Changes in excretion are measured following oral or parenteral magnesium administration. Normally, greater than about 80% of an intravenous magnesium load is excreted within 24 hours; however, with deficiency, more is retained and less is excreted. Renal magnesium excretion should be measured before and after the administration of the magnesium load.

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Suggested Readings

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OSTEOPOROSIS AND DIET

One in every two women and one in every four men over the age of 50 years will suffer a fracture because of osteoporosis sometime during their lives [1,2]. Treating the fracture, however, does not necessarily restore health. About 20% of people with hip fractures attributable to osteoporosis die within 1 year of sustaining the fracture [3]. About 33% of people who have had an osteoporosis-induced hip fracture are no longer able to care for themselves and move into nursing homes within the year following the fracture, and another 17% (although they do not require nursing home care) are not able to return to their previous, prefracture lifestyle [4]. An estimated 54 million Americans (over 80% of whom are women) age 50 years and older have osteoporosis or low bone mass, and this number is expected to reach 64.4 million by 2030 [5]. Over 9 million osteoporosis-induced fractures occur each year worldwide at an estimated cost of about $100 billion [6].

Osteoporosis is a systemic skeletal disease characterized by decreased bone strength associated with the deterioration of the microarchitecture of bone tissue (bone quality) and low bone mineral density, as shown in Figure 1. In osteoporosis (as in osteopenia, which occurs prior to the development of osteoporosis), the ratio of the mineral content to the protein matrix content in the bone is within the normal range but reduced; this is in contrast to osteomalacia (associated with mainly vitamin D deficiency) in which the proportion of the mineral in bone is reduced in relation to the protein matrix. Osteoporosis results in brittle, porous fragile bones at increased risk for fracture. Fragility fractures are fractures that occur in the absence of major trauma, or from injury that would not normally be sufficient to cause fractures in normal bones. They usually affect the spine, ribs, hip, pelvis, and/or wrist.

Bone turnover occurs throughout life. Yet, after about age 30–35 years, bone resorption (breakdown) exceeds bone formation. This bone resorption, including mineral loss, occurs at a rate of up to ~10% per decade in both men and women. However, during the first 5–10 years after menopause, the rate of loss accelerates considerably in women. Women, for example, may lose nearly 200 mg of calcium per day in the first several years after the start of menopause [7]. The menopause-related decline in estrogen production and the generally smaller body and bone mass of women contributes to the higher prevalence of osteoporosis in women than in men.

Osteoporosis affects both cortical and trabecular bone, although trabecular bone has a higher turnover rate (~25%/year) and is affected to a greater extent than is cortical bone (with a turnover rate of about 4%/year). Cortical or compact bone is found mostly in the shaft of long bones of the limbs but also on the outer walls of all bones. Trabecular (or cancellous) bone is the honeycomb or lattice-type bone found in the vertebrae of the spine, the pelvis (hip area), and the ends of long bones (such as the wrist). Thus, sites containing trabecular bone—the vertebral bodies (~95% trabecular bone), the femoral neck in the pelvis (~45% trabecular bone), and the distal radius (~5% trabecular bone)—are the principal sites affected with osteoporosis (Figure 2). In addition, teeth may become loose or fall out because of loss of some trabecular bone in the jaw. Osteoporosis that affects the vertebrae is associated with loss in height, vertebral pain, and rounding of the shoulders (**kyphosis**, a hunchback-type curvature of the spine, also called **dowager's hump**). The kyphosis in turn reduces the space in the chest and abdominal cavity, resulting in decreased lung capacity and thus shortness of breath, abdominal pain, reduced appetite, and premature satiety. Osteoporosis-induced vertebral fractures also typically limit mobility. The limited mobility causes excessive bedrest, which further weakens muscles and bones, and predisposes the person to falling and suffering further fractures. The excessive bedrest also increases the person's risk for pressure sores, also called decubitus ulcers.

Figure 1 Normal bone/osteoporotic bone.

Diagnosis of osteoporosis is based on measurement of bone mineral density, primarily by dual-energy X-ray absorptiometry (DXA or DEXA). DEXA scans use X-rays at two energy levels to assess bone mineral content and bone area; bone mineral density is then calculated using software by dividing bone mineral content by bone area in various regions of interest. Peripheral DEXA scans typically measure the extremities, including the heel, wrist, or finger. Axial (central) DEXA scans focus on the spine/vertebrae and hip. Bone mineral density thus represents the average concentration of minerals per unit area, or how tightly the bone mineral tissue is compressed in a given area (usually the lumbar spine, femoral neck, or proximal femur). It does not, however, account for bone thickness, which is often greater in individuals of higher body weight. (Quantitative computed tomography, which assesses bone density in three dimensions, may provide a better approach.)

Bone density is reported for comparison purposes as a T score or Z score. T scores represent the number of standard deviations away from the mean bone density of gender- and race-matched young (usually 25- to 45-year-old) adults. A T score equal to 0 means that the person's bone mineral density is at the mean for young adults (considered to represent peak bone mass) of the same gender and race. Osteoporosis is diagnosed if bone mineral density is greater than 2.5 standard deviations below the bone mineral density of young adults. Alternately, osteoporosis may be diagnosed with the presence of a fragility fracture or a vertebral compression fracture. A T score of –1 to –2.49 is classified as osteopenia, while a T score of 0 to –0.99 is normal. For every 1 standard deviation below the mean, the risk of fracture doubles. Thus, those with osteoporosis are at considerable risk for fracture, and those with osteopenia are at risk for fracture as well as for the development of osteoporosis. Z scores are similar to T scores but represent the number of standard deviations from the mean bone density of age-matched as well as gender- and race-matched people. Thus, for older people, T scores will likely be lower than Z scores. The Fracture Risk Assessment Tool (FRAX), developed by the World Health Organization, is a risk assessment tool that is also used to examine an individual's risk for fracture. Factors considered as part of the tool include age, gender, height, weight, family history of

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Figure 2 Major sites affected by osteoporosis.

fractures, use of glucocorticoids, and lifestyle factors including smoking and alcohol consumption with or without femoral neck bone mineral density. FRAX estimates 10-year probability of hip and major osteoporotic fractures.

Individuals most at risk for osteoporosis are female, have a family history of the disorder, are Caucasian or Asian, have a small frame size or low body mass index (especially < 19 kg/m^2), and are of advanced age. People using medications such as glucocorticoids, thyroid hormones (taken in excess), and antiepileptic drugs are also at increased risk for bone loss and thus osteoporosis. Estrogen deficiency and inadequate weight-bearing physical activity also increase risk. Although the effects of aging and genetic factors (and sometimes drug therapies) cannot be eliminated, other factors contributing to the development of osteoporosis may be modified. Some of the main nutritional factors include inadequate intakes of calcium and vitamin D, with other factors such as intakes of sodium, protein, acid load, potassium, and magnesium, and a few other nutrients thought to play lesser roles.

CALCIUM AND VITAMIN D

Both calcium and vitamin D are critical for the attainment of the full genetic expression of peak skeletal mass that occurs sometime in early adulthood, and it is the attainment of dense bones during these early years that offers the best protection against weakened, osteoporotic bones in later years. However, adequate intakes of calcium and vitamin D are needed throughout life for function in bone as well as in other body tissues. Many (but not all) studies have found that supplementation with calcium alone (1,000–1,200 mg or more) or in combination with vitamin D (400–1,000 IU or more) reduces the loss of bone mineral density, especially in the spine and sometimes in the hip, in postmenopausal women; however, significant reductions in fracture risk have not consistently been demonstrated. Consequently, calcium (together with vitamin D) is generally deemed beneficial to prevent bone loss, mainly from the hip and spine, but is considered to have small or even questionable long-term effectiveness in reducing fracture risk [8,9]. Additionally, this preservation of bone mineral density by calcium may only delay the loss of bone; studies of longer duration (3 years) indicate the calcium-associated preservation of bone may be only temporary [10,11]. Intervention studies of longer duration are important to advance dietary recommendations for the prevention of both bone loss and fractures. The National Osteoporosis Foundation recommends 800–1,000 IU of vitamin D and 1,200 mg of calcium per day, and suggests serum 25-OH vitamin D concentrations of 30 ng/mL or higher [5]. Maximum serum 25-OH vitamin D concentrations of 50 ng/mL, however, have also been suggested [12].

SODIUM

Increased dietary sodium intake is associated with increased urinary calcium excretion, with an excess sodium intake of about 1,000 mg associated with urinary calcium losses of about 20 mg. Reductions in urinary calcium and sodium excretion have also been demonstrated following decreases in sodium intake among study subjects consuming high dietary sodium [13]. Generally, however, the impact of sodium intake on bone is less clear, with inconsistent or no associations between bone mineral density at various sites and sodium intake [14,15].

PROTEIN, DIETARY ACID LOAD, POTASSIUM, AND MAGNESIUM

An adequate protein intake is necessary for bone health, yet concerns have been raised that high protein intakes may be detrimental to bone and thus be a risk factor for osteoporosis. While it has been demonstrated that doubling protein intake without changing intake of other nutrients increases urinary calcium, this rise in urinary calcium is associated with increased calcium absorption and is not associated with increased bone resorption. Dietary protein is generally thought to promote bone growth by providing amino acids needed for bone proteins and by stimulating insulin-like growth hormone 1, among other mechanisms. Adequate ingestion of base-rich foods such as fruits and vegetables, however, should not be neglected.

The pH of body cells and fluids is tightly regulated, with normal blood pH ranging from about 7.35 to 7.45. Most acid, an excess of hydrogen ions, is produced in the body during normal cellular metabolism throughout the day. Ingestion of protein-rich foods such as meat, fish, eggs, and cheese (and to a lesser extent, grain products) generates more acid than ingestion of other food groups. Most of this generated acid is thought to arise from the oxidation of the sulfur-containing amino acids (such as methionine and cysteine) in the proteins.

The blood is thought to become mildly acidic if acid production exceeds the body's ability to buffer and/or remove the acids (via the lungs and kidneys; see Chapter 12's section "Acid-Base Balance"). However, whether the acid load generated from protein (sulfur-containing amino acid) ingestion is large enough to cause a metabolic acidosis that induces bone demineralization (contributing to osteoporosis) is unclear [10,16]. Furthermore, while bone culture experiments and a few controlled studies suggest unfavorable effects, direct *in vivo* evidence of low-grade acidosis negatively impacting bone mass and size is scarce [17]. Additionally, the findings from a meta-analysis and systematic review of over 50 trials as well as from prospective **observational studies** failed to find a causal association in adults between the acid load of the diet and risk of osteoporosis [18]. The analysis also failed to find a protective effect on bone from an alkaline diet [18].

Buffers are present in the blood and body cells, and are also provided by the diet, especially those containing sufficient potassium and magnesium and organic anions like citrate. Such nutrients and anions, found in fruits and vegetables as well as in supplements like potassium citrate or bicarbonate, favorably affect acid-base balance, bone mineral density, and bone metabolism. Diets rich in potassium also enhance renal retention of calcium and improve calcium balance. Potassium intakes of at least 3,400–3,600 mg are recommended for bone health [19]. Unfortunately, most Americans fail to meet the recommended Adequate Intake for potassium, or even consume the 3,400 mg of potassium suggested for bone health. They also fail to ingest the recommended servings of fruits and vegetables. Such imbalances, especially if renal function is

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impaired, may increase bone resorption and/or decrease bone formation [20]. The urinary loss of calcium associated with the excretion of acidic urine has been estimated at 66 mg calcium/day [21]. Over time, this daily loss of 66 mg of calcium has been calculated as a 24 g loss per year, or a 480 g loss of calcium over 20 years; this 480 g loss is almost half of the skeleton's calcium content and is consistent with severe osteoporosis [21].

VITAMINS C AND K

Vitamins C and K are important for the synthesis and function of various proteins found in bone. Remember that vitamin C is needed for the synthesis of hydroxylysine and hydroxyproline, which contribute to triple helix formation of collagen, one of the main proteins in bone. Vitamin K is needed for the formation of Gla proteins in bone; the Gla proteins are important for bone strength and mineralization. Positive correlations between intakes of both vitamins C and K and bone mineral density have been demonstrated in some studies. Similarly, some (but not all) studies have shown higher intakes of these nutrients are associated with reduced fractures. Supplementation trials, however, have not consistently improved bone mineral density or prevented fractures [22–26].

OTHER LIFESTYLE FACTORS

Smoking negatively affects bone health. Smoking is associated with lower bone density and, in women, with earlier menopause, increased postmenopausal bone loss, and increased risk of fracture, especially of the hip and spine.

Caffeine minimally affects calcium balance and therefore is thought to be weakly associated with the development of osteoporosis. Caffeine reduces the renal reabsorption of calcium, which leads to a temporary (~1- to 3-hour) increase in urinary calcium losses, but has no net effect on daily (24-hour) urinary calcium excretion. Caffeine in amounts of 100–400 mg (about one to four cups of coffee) increases urinary calcium by up to about 10 mg/day. However, caffeine may also promote increased secretion of calcium into the gut to enhance calcium loss from the body; whether the secreted calcium is reabsorbed, and the extent of the secretion, have not been determined. Caffeine intake has been positively associated with fracture risk and/or bone density in women in some, but not all, studies.

SUMMARY

Maintenance of desirable skeletal status is clearly multifactorial. A person's genetic makeup cannot be changed, nor can the physiological changes accompanying aging be reversed. People usually can choose, however, a lifestyle that includes good nutrition (i.e., eating a variety of foods and getting recommended intakes of all nutrients) and regular weight-bearing exercise. Clearly, consuming recommended intakes of calcium and vitamin D as well as vitamins C and K is important. Reductions in dietary sodium consumption if excessive are also worthwhile, and should be coupled with efforts to increase the ingestion of potassium- and magnesium-rich foods, especially fruits and vegetables, which are also rich in buffers. Prevention of osteoporosis is a much better goal than managing its treatment, and such prevention should begin in childhood.

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12 WATER AND ELECTROLYTES

WATER FUNCTIONS
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ACID-BASE BALANCE: CONTROL OF HYDROGEN ION CONCENTRATION
Chemical Buffer Systems
Respiratory Regulation
Renal Regulation
SUMMARY
PERSPECTIVE
MACROMINERALS AND HYPERTENSION

WATER ACCOUNTS FOR ABOUT 60% of an adult's total body weight, making it the most abundant constituent of the human body. Water is vital for life and must be maintained in appropriate amounts and locations in the body. Three minerals—sodium, potassium, and chloride—substantially influence water distribution and movement. Information on these minerals and their roles in fluid balance and acid-base balance follows discussions of water functions, body water content and distribution, and water losses, sources, absorption, needs, and regulation.

WATER FUNCTIONS

Water plays many critical roles in the body. Some of these functions include:

- Chemical reactions. Water is needed for chemical reactions such as those involved in nutrient catabolism.
- Body temperature regulation. Water has a high specific heat and thus does not readily change temperature as heat is released during normal cellular metabolism; additionally, sweat (consisting largely of water) that is generated facilitates heat removal and body temperature regulation as it evaporates from the skin.
- Lubrication and protection. Water is a necessary component of several secretions such as mucus, synovial fluid, and spinal fluid, among others; these secretions facilitate body processes as well as cushion and protect tissues.
- Solvent and transport medium. Water in body fluids contains numerous dissolved substances (solutes); examples of body fluids containing the solutes include the blood and urine, along with gastrointestinal tract secretions such as saliva, pancreatic juice, and bile, among others.
- Maintenance of blood volume. Water, as a large component of the blood, helps to maintain blood pressure and sustain cardiovascular system function.
- Acid-base (pH) balance. Water is needed for reactions involving buffers responsible for maintaining pH balance such as $H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$.

BODY WATER CONTENT AND DISTRIBUTION

While body water contributes typically over one-half (range ~40–80%) of a person's body weight, the amount varies based on age and body size and composition. Body water, for example, decreases both with age and body size. It is also affected by body composition. The body's organ mass varies relatively little

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in water content, and thus does not have substantial effects on body water. For example, the bone contains about 25% water, the liver and kidneys are about 85% water, and the brain and muscle mass are about 75% water. The most significant contribution to variations in body water is the amount of fat mass. Fat mass has only about 10% water. Thus, a person with a large fat mass of, for example, 50% fat may have 40% body water, whereas a person with lower body fat at 6% may have 70% water. It is also because males generally have a higher percentage muscle mass and lower percentage fat mass than females that they also have a higher percentage of body water.

Total body water can be theoretically compartmentalized into two major reservoirs: the intracellular compartment, which includes water enclosed within cell membranes, and the extracellular compartment, which includes water external to (outside of) cell membranes. The content of primarily sodium in the extracellular compartment affects its volume, with increases in total body sodium in this compartment increasing its fluid volume. The approximate distribution of the 42 L (or about 11.2 gallons) of total body water in a 70-kg (154-lb) man is:

The extracellular compartment primarily includes interstitial fluids and the plasma (also called intravascular fluid), but also fluids found in two smaller compartments, the lymph and transcellular spaces.

- The interstitial fluid (ranging from about 10.5 to 11.2 L) directly bathes the cells and provides the medium for the passage of nutrients and metabolic products back and forth between the plasma and cells.
- The plasma/intravascular fluid (ranging from about 2.8 to 3.5 L) represents the fluid portion of the blood. Cell membranes separate intracellular fluid from interstitial fluid, and blood vessel walls separate the interstitial fluid from the plasma.
- The lymph is fluid that is being redirected (recirculated back) to the blood plasma from interstitial spaces.
- Transcellular fluid (a water-containing and sometimes viscous fluid) is found sequestered in small quantities in various cavities or spaces such as the pericardial, pleural, peritoneal, gastrointestinal tract, and synovial spaces or cavities, and the aqueous humor of the eyes, among other areas.

Table 12.1 Body Fluid Compartments

	Approximate Percentage of Body Weight	Approximate Percentage of Total Body Water	Approximate Volume (L) in 70-kg Man
Total body water	60	—	42
Extracellular water	20	33	14
Plasma	5	8	3
Interstitial fluid	15	25	11
Intracellular water	40	67	28

Fluid compartment volumes for a 70-kg man are summarized in Table 12.1.

Distributed throughout the fluid compartments of the body are *electrolytes*, including both anions and cations (Table 12.2). Electrolytes are distributed in such a way that within a given compartment—the blood plasma, for example—electrical neutrality is always maintained, with the anion concentration balanced by the cation concentration. The cationic electrolytes include sodium, potassium, calcium, and magnesium (Ca^{2+} and Mg^{2+} are discussed in Chapter 11). These cations are electrically balanced by the anions, which include chloride, bicarbonate, and negatively charged proteins, along with relatively lower concentrations of organic acids, phosphate, and sulfate.

WATER LOSSES, SOURCES, AND ABSORPTION

Water is lost from the body each day primarily through the urine, with lesser amounts excreted in the feces. Additionally, losses occur via insensible (not normally noticeable) routes—evaporation from the respiratory tract and

Table 12.2 Electrolyte Composition of Body Fluids

	Plasma (mEq/L)	Interstitial Fluid (mEq/L H_2O)	Intracellular Fluid (mEq/L H_2O)
Cations	153	153	195
Na^+	142	145	10
K^+	4	4	156
Ca^{2+}	5	2–3	3.2
Mg^{2+}	2	1–2	26
Anions	153	153	195
Cl^-	103	116	2
HCO_3^-	28	31	8
Protein	17	—	55
Others	5	6	130
Osmolality (mOsm/kg H_2O)	280–295	~300	~300

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nonsweat diffusion from the skin. Urinary losses of water, the largest component, usually range from about 1 to 2 L/day. Fecal losses are usually small, less than about 200 mL/day (in the absence of diarrhea). Another 350–400 mL of water is lost daily from the respiratory tract as part of insensible losses. Insensible nonsweat losses of water from the skin are typically small, but sensible dermal losses of water as sweat (which serves as a means of eliminating heat that has been generated in the body) vary considerably depending on the environment and level of physical activity. Thus, total water losses from skin may range from as little as about 100 mL (up to about 300 mL insensible only) to well over 1 L (with extensive sweating).

Beverages and foods are the major sources of water to the body, and are needed to replace its losses. Water from the ingestion of beverages usually contributes about 75–80% of intake and water from foods the remaining 20–25%. The water content of foods, however, varies tremendously with, for example, nuts and seeds containing very little water to some fruits and vegetables at over 95% water. A third, but minor, source of water to the body is referred to as metabolic water. Metabolic water is the water formed as part of some cellular biochemical reactions that occur in the body. Daily metabolic water generation totals about 200–300 mL. Estimates of metabolic water based on individual nutrient oxidation of 100 g of fat, carbohydrate, and protein are about 100 mL, 55 mL, and 41 mL of generated water, respectively.

In addition to water present in the digestive tract after the ingestion of foods and beverages, a large amount of water (about 7 L) is released into the gastrointestinal tract each day as part of secretions (i.e., saliva, gastric juice, pancreatic juice, bile, intestinal juice, etc.). Just about all (over 98%) of this water, as well as that which is consumed, is absorbed from the intestines.

The majority (about 85%) of water is absorbed in the small intestine, primarily in the jejunum and the ileum, and the remainder is absorbed from the colon, especially the ascending and transverse regions. Of the ~1–1.5 L of water entering the colon each day, about 90–95% is absorbed, with only about 50–200 mL excreted daily as part of the feces. Absorption of water from the digestive tract is passive and occurs transcellularly and paracellularly; absorption is strongly influenced by osmolarity. Thus, sodium absorption enhances water absorption, and the two main systems responsible for most of sodium's absorption also promote the absorption of water.

RECOMMENDED WATER INTAKE

Recommendations for water are published as an Adequate Intake (AI) with large variations in needs based not only on age and gender, but also on the environment, level of physical activity, and rate of metabolism, among other factors. The AI recommendation for adult females is 2.7 L (about 91 oz) and for adult males is 3.7 L (about 125 oz), and includes water that can be obtained both from eating foods and from drinking beverages. Thus, assuming 25% of the water is derived from foods and 75% from beverages, beverage consumption by an adult female should total at least 8 cups and by an adult male at least 12 cups each day. Large variations in individual needs, however, mean that some individuals need more and some individuals need less than these recommendations. Other approaches used to estimate fluid needs for adults are based on energy intake, with 1 mL of water recommended for each 1 kcal ingested. Recommendations based on body weight suggest an intake of about 25–40 mL of water per kg body weight, depending on age and gender. Physically active adults may need to consume fluid intakes in excess of 3 L/day. Needed water may be consumed (derived) from the ingestion of foods and a number of beverages such as water, milk, fruit juices, broth, soft drinks, coffee, and tea, among others. Healthier beverage choices would be those that are lower in simple sugars and fat.

WATER (FLUID) AND SODIUM BALANCE

Water moves among the various body compartments but in a regulated manner that is strongly affected by the presence of sodium in the extracellular fluid (ECF) compartment. Thus, water and sodium balance are interrelated. For example, water moves between the extracellular compartment's interstitial space (crossing the cell plasma membrane) and the intracellular space. The water movement between these compartments is regulated by *osmotic pressure*. Water also moves across the capillary blood vessel walls that separate the plasma and interstitial space. Differences in *hydrostatic pressure (fluid or capillary blood pressure)* and *colloidal osmotic pressure* govern this movement. The balancing of these different pressures and the regulation of ECF volume and ECF osmolarity enable water (fluid) balance maintenance.

Osmotic Pressure

Osmotic pressure affects water passage across the cell plasma membrane. The osmolarity of the ECF is regulated to reduce deleterious osmotic movement of water between the intracellular and extracellular compartments. In the body, water moves from areas where solute concentrations are low to areas where solute concentrations are high. **Osmosis** refers to the movement of water across a semipermeable membrane in response to differences in solute concentrations across the membrane. In the body, cell plasma membranes are selectively permeable and allow the

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passage of water in and out of cells; differences in osmotic pressure associated with solute concentrations influence this water movement, which occurs via osmosis.

The nature and distribution of solutes, especially sodium, potassium, and chloride, differ considerably between the intracellular and ECF compartments and play important roles in maintaining osmolarity and water distribution. Osmolarity can be thought of as the solute (particle) concentration of a fluid. As shown in Table 12.2 and Figure 12.1, within cells (intracellular), potassium represents the major cation (only small amounts of sodium are found) and phosphate is present as the major anion along with smaller amounts of protein anions. Potassium maintains primarily the osmotic balance of the intracellular fluid compartment. The ECF, in contrast to the intracellular fluid, contains sodium as the major cation (not potassium) and contains chloride (not phosphate) as its major anion. The presence of the sodium in the ECF contributes most to its osmolarity and ECF volume. The intracellular fluid and ECF are electrically balanced, and it is the sodium-potassium ATPase pumps in the cell membranes that maintain the appropriate intracellular potassium and extracellular sodium concentrations. Other transporters and channels also facilitate the movement of solutes across the membrane to maintain electrical neutrality.

A comparison between the electrolyte composition of plasma and the interstitial fluid (which both make up the ECF) shows few differences (Figure 12.1). In both, it is sodium that is the major cation with only small amounts of potassium present and the anions include mostly chloride and bicarbonate. The main difference between the plasma and interstitial fluid is the presence of protein anions in the plasma but not in the interstitial fluid.

Disruptions in osmotic pressure affect fluid balance between the extracellular and intracellular compartments. Usually it is changes in ECF (and not intracellular fluid) osmolarity that result in osmotic pressure changes and water movement between compartments. Gains of *water without solutes* in the ECF compartment (such as the plasma) decrease ECF osmolarity (making it hypotonic), and are associated with overhydration and cell expansion/swelling (including cerebral edema). An excessive overconsumption of water, for example, dilutes the plasma, resulting in **hyponatremia** (low serum sodium less than 135 mEq/L), and reduces the plasma's approximate 0.9% salt. Conversely, losses of *water without solutes* from the ECF compartment increases serum sodium (hypernatremia) and ECF osmolarity (making it hypertonic) and results in dehydration. Cells in turn may shrink in this hypertonic environment.

Gains and losses of *fluid along with solutes (salts)* from the ECF compartment cause isotonic imbalances (in spite of the gains/losses, the fluids are still similar in composition to normal ECF). Hypervolemia (volume overload) results with gains in salt and water into the plasma, and hypovolemia (volume depletion) occurs with losses of both. Hypovolemia, for example, may occur with excessive vomiting and diarrhea, among other conditions. While a discussion of fluid and electrolyte disturbances is beyond the scope of this chapter, an understanding of the distribution of water and the role of solutes enables an understanding of how infusions (administered to correct deficits, for example) distribute in the body. *Water containing no salts*, for example, if administered would distribute across both extracellular fluid and intracellular fluid compartments. Thus, an intravenous administration

Figure 12.1 Electrolytes in the plasma, interstitial, and intracellular compartments of the body.

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of 1,000 mL of such fluid would result in 667 mL of water entering cells (i.e., intracellular 2/3) and 333 mL of water in the extracellular (i.e., 1/3) compartments. The 333 mL of water would further distribute with about 73 mL (22% of the 333 mL) remaining in the plasma and 260 mL of water (78% of the 333 mL) entering the interstitial space. In comparison, the intravenous infusion of *1,000 mL of normal saline (containing solutes, 0.9% sodium chloride)* distributes only into the extracellular compartments—780 mL interstitial and 220 mL intravascular.

Hydrostatic (Fluid/Capillary) Pressure

Hydrostatic (fluid/capillary) pressure affects water movement within the two main ECF compartments, between the interstitial fluid and the plasma. Just as water moves from areas of lower solute concentration to higher solute concentration (discussed in the previous section), water also moves from areas of higher pressure to areas of lower pressure. This is referred to as hydrostatic (fluid/capillary) pressure. Water distribution across the capillary endothelial surface is controlled by the balance of forces that tend to move water from the plasma into the interstitial fluid (filtration forces) and forces that move water from the interstitial fluid into the plasma (reabsorption forces). The major filtration force in the capillaries is hydrostatic pressure (P_{pl}) which is about 25 mm Hg and caused by the pumping of the heart. Weaker filtration forces include both interstitial fluid colloid osmotic pressure (II_{isf}) at 5 mm Hg (note that this force is weak because of negligible protein present in the interstitial fluid) and interstitial fluid hydrostatic pressure (P_{isf}) which is negative at –6 mm Hg. The major reabsorption force countering the filtration force is the plasma osmotic pressure (II_{pl}), which is approximately 28 mm Hg. The net result of these four forces at the arteriolar end of the capillaries can be described by Starling's equation:

$$\text{Filtration pressure} = (P_{pl} + II_{isf}) - (II_{pl} + P_{isf})$$

or after substituting the average values, we have

$$\begin{aligned}\text{Filtration pressure} &= (25 + 5) - (28 + [-6]) \\ &= (25 + 5) - (28 - 6) \\ &= (30) - (22) \\ &= 8 \text{ mm Hg}\end{aligned}$$

This positive filtration pressure indicates that a net filtration of water from the plasma into the interstitial fluid occurs at the arteriolar end of the capillaries. When filtration pressure is negative, a net reabsorption of water from the interstitial fluid into the plasma occurs. This situation exists at the venule end of the capillaries, where P_{pl} is substantially reduced while the concentration of plasma protein, and therefore II_{pl}, correspondingly increases. The net effect of these forces on the water distribution between plasma and interstitial fluid along the course of the capillary is shown in Figure 12.2.

Figure 12.2 Starling's hypothesis of water distribution between plasma and interstitial fluid compartments. The relative magnitudes of the pressures, Ppl (plasma hydrostatic pressure) and IIpl (plasma osmotic pressure), are represented by the thickness of their respective arrows. There is a positive net filtration pressure at the arteriolar end of the capillary and a negative net filtration pressure at the venule end.
Source: *Clinical Chemistry: Theory, Analysis, and Correlation*, 2nd ed., Kleinman, L.I., Lorenz, J.M., "Physiology and Pathophysiology of Body Water and Electrolytes," p. 373. Copyright © Elsevier 1989.

Colloidal Osmotic Pressure

Colloidal osmotic pressure, like hydrostatic pressure, affects water movement across the capillary walls (endothelium) that separates the plasma and the interstitial fluid. Because proteins are too large to pass through the capillary endothelium, the concentration of proteins is much higher in the plasma than in the interstitial fluid; this confers on the plasma a relatively high osmotic pressure and a water-attracting property. Proteins and other macromolecules that are too large to traverse the capillary endothelium are sometimes called **colloids**, and the osmotic pressure attributed to them is appropriately termed the *colloid osmotic pressure*.

Extracellular Fluid Volume and Osmolarity and Hormonal Controls

ECF volume, like osmolarity, is also critical in the control of fluid balance. Sustaining ECF volume is additionally vital to blood pressure and cardiovascular system functions. To maintain plasma volume and keep blood pressure from dropping should ECF osmolarity increase or hypovolemia occur, water must move into (increase in) the plasma. Conversely, in the event of decreased ECF osmolarity, hypervolemia, and elevated blood pressure, water needs to move out of the plasma. Shifts in ECF volume and osmolarity serve as one of many triggers for the release of hormones responsible in part for correcting imbalances.

Several hormones including vasopressin, the renin-angiotensin-aldosterone system, and natriuretic peptides influence renal function, and in some cases the cardiovascular system, among others, to correct imbalances in the body's fluid and sodium levels. Sodium balance is mainly regulated by the hormone aldosterone (which is part of the renin-angiotensin-aldosterone system), and water balance is mostly controlled by the hormone vasopressin.

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Vasopressin

The osmolarity of the blood ranges from about 280 to 295 mOsm/L (its **osmolality** is similar at about 280–295 mOsm/kg of water). **Osmoles** (osm) represent the number of moles of each particle in solution. The hypothalamus responds to slight increases in ECF osmolarity and signals the release of vasopressin, also called arginine vasopressin or antidiuretic hormone (ADH). It is osmoreceptor neurons present in the anterior hypothalamus that detect increasing ECF osmolarity and trigger vasopressin release. Vasopressin release is also stimulated to a lesser extent by reductions in plasma volume, which are monitored by receptors in the left atrium of the heart. Additionally, vasopressin release is stimulated by angiotensin II (the role of angiotensin II is addressed under the section "Renin-Angiotensin-Aldosterone System"). Vasopressin's effects on the body (see Figure 12.4) include:

- stimulation of the reabsorption of water in the kidneys, which reduces urine output and increases ECF volume
- stimulation of thirst, which serves to increase fluid consumption and increase ECF volume
- stimulation of vasoconstriction of the arterioles, which increases blood pressure.

In the kidneys, vasopressin's effects are confined to the distal tubule and collecting duct, which absorb about 20% of water independent of sodium and chloride. (The hormone does not affect passive water reabsorption occurring in the proximal tubule and loop of Henle; about 80% of water is reabsorbed from these regions and is associated with sodium and chloride reabsorption.) Vasopressin acts by binding to hormone-binding sites called V_2 receptors; the binding stimulates the production of cAMP and the movement of specific aquaporins (AQP-2) from intracellular vesicles to the cell surface. These aquaporins are water channels, and facilitate water movement from the filtrate across the tubule cell and into the peritubular capillaries. The absorbed water "dilutes" the ECF and reduces the osmotic pressure. The urine that is excreted under conditions of elevated vasopressin is low in volume and concentrated.

Under conditions of hypervolemia (increased ECF volume), decreased ECF osmolarity, and higher blood pressure, vasopressin is not released and the aquaporin water channels are endocytosed back into the cell cytosol. Consequently, the distal tubule and collecting duct reabsorb little water. Thus, in this situation, most of the 20% of the water does not get reabsorbed and is excreted as urine. The urine is now higher in volume and less concentrated than when vasopressin concentrations were elevated.

Before discussing the effects of other hormones on fluid and sodium balance, a brief discussion of kidney physiology and function is provided. For a more complete understanding of renal anatomy and physiology, it is suggested that the reader consult a physiology text.

The Kidneys: A Brief Review

The kidneys are responsible for eliminating metabolic wastes, regulating pH, and regulating the body's electrolyte and water content to maintain homeostasis; the latter is accomplished through adjustments in urine volume and osmolarity. The functional unit of the kidney is the nephron. Each kidney contains about 1–1.5 million nephrons. Each nephron in turn consists of tubular and vascular components. The tubular components of the nephron are the Bowman's capsule (a capillary network), proximal (convoluted) tubule, loop of Henle, distal (convoluted) tubule, and collecting duct. The main vascular components include the glomerulus, the afferent and efferent arterioles, and the peritubular capillaries. The glomerulus is a tuft of about 50 capillaries that filters water and solutes from the blood as it passes through this region of the nephron. About 20% of the plasma entering the glomerulus is filtered. Glomerular filtration occurs at a rate of about 125–130 mL/minute. It is the afferent arterioles that transport blood into the glomerulus and it is the efferent arterioles that carry blood out of the glomerulus. The 80% of the plasma not entering the glomerulus flows through the efferent arterioles. The efferent arterioles branch into another network of capillaries called the peritubular capillaries. These capillaries surround the tubules and function in the exchange of substances; they can selectively reabsorb into the blood substances that were filtered (and present in the fluid in the lumen of the tubules) and thus serve as a salvage mechanism. These capillaries may also secrete certain substances from the blood through the tubules and into the filtrate. The peritubular capillaries coalesce to drain into the renal vein.

The Bowman's capsule, which surrounds the glomerulus, collects the filtrate, that is, the fluid and substances that were filtered from the blood. The filtrate next moves into the proximal convoluted tubule, and progressively through the loop of Henle (both its thin descending limb and its thick ascending limb), the distal tubule, and the collecting duct (Figure 12.3). Collecting ducts gather and direct the filtrate from the distal tubules of many nephrons into the ureter, a "tube" that leads the urine to the bladder. The cells making up the walls of the nephron and that surround the tubular lumen are only a single layer thick; the basolateral side of these cells is surrounded by interstitial fluid and the peritubular capillaries (Figure 12.3).

Each segment of the nephron is functionally distinct in its permeability to water and the solutes present in the filtrate. The ultimate composition of the urine depends on three processes, filtration, reabsorption, and secretion, which occur as the filtrate moves through the nephron.

- Filtration occurs as blood passes through the glomerular capillaries; these capillaries differ from other capillaries in the body in that the hydrostatic pressure within them is approximately three times greater than in other

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Figure 12.3 A nephron.
Source: Adapted from L. Sherwood. *Human Physiology from Cells to Systems*, 9th ed. Cengage Learning. 2013. pp. 494, 506.

capillaries. They also have larger pores. These characteristics enable the glomerular capillaries to act as a filter, removing most everything from the blood except blood cells and proteins that exceed a molecular weight of about 50,000 daltons. The entire plasma volume (of an adult) is filtered over 60 times each day. The formed filtrate, which amounts to over 185 L (or nearly 49 gallons) per day, contains water and other substances, including electrolytes, glucose, amino acids, and metabolic waste products.

- Reabsorption (passive and active) of selected substances from the filtrate and into the blood occurs via the peritubular capillaries; this reabsorption takes place as the filtrate passes through the tubular components of the nephron. Of the 185 L of filtrate formed daily, about 183.5 L is reabsorbed and 1.5 L is excreted as urine. This difference in the volumes means that only about 1% of the filtrate is excreted as urine, and the remaining 99% is reabsorbed into the blood. The proximal tubule reabsorbs most nutrients including about 90% (or more) of sodium, chloride, potassium, glucose, amino acids, bicarbonate, phosphate, and water. Additionally, reabsorption of water, sodium, chloride, potassium, calcium, magnesium, and bicarbonate, among other substances, occurs in the loop of Henle, distal tubule, and collecting duct. Active transport, either primary or secondary, is responsible for most of the nutrient reabsorption. As the nutrient carriers become saturated, transport maximums are reached for some nutrients. This situation occurs most often when nutrient concentrations in the blood are high (as with hyperglycemia in those with diabetes) and can result in increased urinary excretion of the nutrient (in this case glucose).
- Secretion allows for the selective movement of substances from the blood in the peritubular capillaries and ultimately into the filtrate. This process enables the excretion of selected substances that were not a part of the 20% of the plasma that was originally filtered through the glomerulus. The ability of the tubules to secrete nutrients/substances such as potassium, hydrogen ions, creatinine, and various organic cations and anions is important in maintaining homeostasis. Secretion occurs throughout the nephron, but mostly in the proximal tubule, distal tubule, and collecting duct.

Fluid and solutes (both filtered and not reabsorbed, and secreted) remaining as filtrate in the collecting duct enter the renal pelvis and then the ureter for excretion as urine.

Renin-Angiotensin-Aldosterone System and Sodium Balance

The major regulatory factor controlling sodium (and chloride) balance in the body is the renin-angiotensin-aldosterone system (RAAS). This system enhances

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Figure 12.4 The effects of vasopressin and the renin-angiotensin-aldosterone system on water and sodium balance.
Abbreviations: ACE-angiotensin converting enzyme; GFR- glomerular filtration rate.

sodium and chloride reabsorption, with water (fluid) balance also affected since water reabsorption follows the reabsorption of the electrolytes. The RAAS is active when ECF volume and blood pressure are low, and is initiated by the actions of renin.

Renin Renin, a hormone with enzymatic functions, is secreted by granular cells in the juxtaglomerular apparatus (near or adjoining the glomerulus) of the kidneys. The granular cells are innervated by the sympathetic nervous system and are stimulated to release renin by increased circulating catecholamines and when blood pressure and plasma fluid volume are reduced/low. The resulting decreased renal perfusion pressure is sensed by distention receptors and baroreceptors within the juxtaglomerular apparatus. Renin secretion into the blood starts a series of reactions that initially involves hydrolysis of angiotensinogen and ultimately results in the release of aldosterone from the adrenal cortex. This sequence of events and its effects are illustrated in Figure 12.4.

Angiotensinogen and Angiotensin Renin functions to hydrolyze angiotensinogen, an inactive protein synthesized and released by the liver into the blood. Hydrolysis of angiotensinogen generates angiotensin I, another inactive decapeptide. Angiotensin I is subsequently hydrolyzed by angiotensin-converting enzyme (ACE), which is present in the capillaries of the lungs. Cleavage of angiotensin I produces angiotensin II, an active octapeptide.

Angiotensin II performs several functions affecting sodium and water balance and blood pressure. Angiotensin II:

- serves as a potent vasoconstrictor of systemic arteries, increasing peripheral resistance and blood pressure. In fact, one group of blood pressure medications, known as ACE inhibitors, is used to treat hypertension and functions by inhibiting ACE and thus reducing the conversion of angiotensin I to angiotensin II.
- increases blood pressure by stimulating the hypothalamic thirst center to increase thirst (which increases water intake and thus plasma volume)
- stimulates vasopressin release (which increases water reabsorption by the kidneys and thus increases plasma volume)
- reduces the glomerular filtration rate and therefore filtered sodium
- interacts with receptors on the adrenal cortex, leading to the release of the hormone aldosterone.

Aldosterone release is also increased by angiotensin III, which is produced from angiotensin II after the hydrolytic removal of an aspartic acid residue by a plasma aminopeptidase. Angiotensin III, however, is present in considerably lower concentrations than that of angiotensin II, and is thus thought to have lesser effects on maintaining fluid and sodium balance.

Aldosterone In addition to being stimulated by angiotensin, aldosterone release is stimulated by decreased concentrations of natriuretic peptides (discussed in the next section), by increased plasma potassium, and by decreased plasma sodium concentrations. Aldosterone promotes the reabsorption of sodium and the excretion of potassium

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Table 12.3 Electrolytes: Functions, Body Content, Deficiency Symptoms, Food Sources, and Adequate Intakes (AIs)

Mineral	Selected Physiological Functions	Approximate Body Content	Deficiency Symptoms	Selected Food Sources	AI
Sodium	Water, pH, and electrolyte regulation; nerve transmission, muscle contraction	105 g	Anorexia, nausea, muscle atrophy, poor growth, weight loss	Table salt, processed and snack foods, cured meats, seafoods, condiments, milk, cheese, and bread	1,500 mg, 19–50 years
Potassium	Water, electrolyte, and pH balances; cell membrane polarization	130 g	Muscular weakness, cardiac arrhythmias, paralysis	Fruits, vegetables, legumes, nuts, and dairy products	4,700 mg, 19+ years
Chloride	Fluid and pH balance, component of gastric hydrochloric acid	105 g	Weakness, lethargy, hypokalemia, metabolic acidosis	Table salt, seafood, meat, and eggs	2,300 mg, 19–50 years

in the distal tubule; chloride is passively reabsorbed with the sodium. Water reabsorption follows the sodium and chloride. More specifically, aldosterone exerts its functions by promoting the insertion of more Na^+ channels into the luminal membrane and more Na^+-K^+ pumps into the basolateral membrane. These actions increase the reabsorption and thus retention of salt and water. It also promotes an increase in blood volume, an increase in blood pressure, and an increase in urinary potassium excretion, and hence reverses the conditions that initially triggered renin release.

The RAAS is less active once blood volume and blood pressure have been returned to normal or if elevated. Under these conditions, renin secretion is inhibited and the sequence of activations diminished to thus reduce aldosterone secretion and reduce the reabsorption of Na^+ in the distal and collecting tubules. The sodium (and chloride) is consequently excreted in the urine. Although aldosterone regulates only about 8% of filtered sodium, the reduction in sodium reabsorption with low aldosterone levels can account for a large daily loss of sodium because of the large amount of fluid filtered by the glomerulus and the typically large consumption of dietary salt. The kidney's ability to respond to changes in the RAAS and adjust salt excretion and retention throughout the day help the body maintain sodium and fluid balance.

Natriuretic Peptides

Opposing the RAAS and promoting the loss of sodium and the lowering of blood volume and pressure are atrial natriuretic peptide (ANP), a peptide hormone synthesized in atrial cells of the heart, and B-type natriuretic peptide (BNP), a peptide synthesized by myocytes in the ventricles of the heart. Both peptides are released into circulation when the heart muscle is stretched by an expansion of ECF (plasma) volume that occurs with sodium and water retention and increased arterial blood pressure. The peptides function to increase glomerular pressure and filtration rate, which increase the amount of sodium and water excreted (**natriuresis** and diuresis, respectively), and in turn decrease the plasma volume and lower blood pressure. The natriuretic peptides also reduce renin and aldosterone release and promote the dilation of blood vessels to reduce peripheral vascular resistance and thus blood pressure.

SODIUM

The body's total sodium content is estimated at about 105 g. The majority (60–70%) of this sodium is found in ECF. About 30–40% of body sodium is located on the surface of bone, and a much lower level (less than ~10%) is found intracellularly in mostly nerve and muscle tissue. Sodium constitutes about 93% of the cations in the body fluids, making it by far the most abundant member of this family (shown in Table 12.2). An overview of sodium, along with potassium and chloride, functions, body content, food sources, deficiency symptoms, and recommendations for intake are provided in Table 12.3.

Sources

The major source (over 75%) of the sodium in the diet of Americans is salt (in the form of sodium chloride) that has been added to processed foods. Sodium comprises 40% by weight of sodium chloride. One teaspoon of salt provides 2,300 mg (2.3 g) or 100 mmol of sodium. Cold cuts/cured meats, soups, pizza, mixed dishes (with pasta and/or meat), condiments, pickled foods, and savory snacks (chips, pretzels, crackers, etc.) are particularly high in added salt and contribute substantially to sodium intake. For example, a half-cup of soup or stew or a tablespoon of condiments such as ketchup and mustard contain 400–500 mg of sodium. Smoked, processed, or cured meats (such as luncheon meats, ham, corned beef, hot dogs), processed cheeses, and canned fish provide about 400–800 mg of sodium in a 2- to 3-oz serving. Moreover, some condiments, like soy sauce, contain >1,000 mg of sodium/tablespoon.

Naturally occurring sources of sodium such as milk, meat, eggs, and most vegetables furnish about 10% of consumed sodium. Milk, for example, provides about 120 mg of sodium per cup. Meats, poultry, and fish (not processed) provide about 25 mg of sodium per ounce. Breads provide about 160 mg of sodium/slice, although quick breads (muffins, biscuits) contain >300 mg sodium in a biscuit or a 2-oz muffin. Fresh vegetables provide typically less than 40 mg of sodium per half-cup, although celery is an exception, containing about 100 mg of sodium per cup. In contrast, canned vegetables

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contain >200 mg of sodium per half-cup. Instant pasta and rice dishes are exceptionally high in sodium, often providing over 700 mg of sodium per half-cup, as prepared. Water can supply up to about 10% of sodium intake. Salt added from the salt shaker during cooking and at the table provides roughly 15% of total sodium. Note that sodium obtained from the use of table salt (which is usually derived and refined from salt mines) does not differ from sodium found, for example, in sea salt; sea salt, however, may contain other minerals such as iodine, potassium, magnesium, and zinc, among others.

Estimates of sodium intake by American adults range from approximately 3,000 to 5,000 mg/day, with a mean of about 3,371 mg and with over two-thirds of adults consuming more sodium than is recommended [1]. While this text uses primarily milligrams (or grams) in discussions of intake and recommendations, electrolytes are sometimes expressed in terms of milliequivalents (mEq). Milliequivalents are determined by dividing the number of millimoles (mmol) of the ion by its valence (electrical charge). For Na^+ (and K^+ and Cl^-) the valence is one, so the mmol and mEq are the same, whereas for ions like Mg^{2+} or Ca^{2+} the valence is two, so the number of mmol must be divided by 2. Electrolyte concentrations provided in terms of mEq account for cations and anions of different valences since they must balance out. One (1) mEq or mmol of sodium is equal to 23 mg; 1 mEq or mmol of potassium is equal to 39 mg, and 1 mEq or mmol of chloride is equal to about 35.4 mg.

Terms such as *free, very low, low, reduced*, or *light* in conjunction with sodium on food labels are associated with specific amounts of sodium per serving. For example, "free" means +5 mg of sodium per serving, "very low" means <35 mg per serving, and "low" means <140 mg of sodium per serving. The term *reduced* or *less* indicates at least 25% less sodium per serving than the appropriate reference food. The term *light* may be used if the food is low in calories and fat, and the sodium content has been reduced by at least 50%. The Daily Value for sodium used on food labels is 2,400 mg.

In addition to sodium obtained from food ingestion, the secretions of the digestive tract also contain appreciable amounts of sodium among other electrolytes. The sodium content of bile (about 145 mEq/L) and intestinal and pancreatic secretions (both containing about 140 mEq/L) are greatest with lesser amounts in gastric and colonic secretions (both about 60 mEq/L). Much of this sodium is absorbed.

Absorption and Transport

About 95–100% of sodium is absorbed from two sites, the small intestine and proximal portion of the colon. Three main mechanisms enabling intestinal sodium absorption (shown and described in Figure 12.5) are: (a) Na^+/glucose (nutrient) cotransporter which functions throughout the small intestine but especially the jejunum; (b) electroneutral Na^+ and Cl^- cotransport exchange transporter which is active in both the small intestine and the colon; and (c) an electrogenic system, which is a lesser used system and operates principally in the colon. Additionally, solvent drag, associated with paracellular water absorption especially in the jejunum and ileum, increases the absorption of sodium and other electrolytes.

Once absorbed into the body, sodium is transported freely in the blood. The serum's sodium concentration is maintained within a fairly narrow range (135–145 mEq/L). Serum sodium concentrations are indicative of fluid balance, with low concentrations (hyponatremia) reflecting excessive fluid in the plasma and high concentrations (hypernatremia) indicative of fluid loss. Electrolyte as well as fluid balance is regulated by several hormones, including vasopressin, angiotensin II, aldosterone, and natriuretic hormones, as discussed previously under the section on Water (Fluid) and Sodium Balance.

Functions and Interactions with Other Nutrients

Within the body, sodium plays important roles in the maintenance of osmotic pressure for fluid balance. It also works with potassium and calcium in nerve transmission/impulse conduction and muscle contraction. In nerve transmission and muscle contraction, sodium functions as part of the Na^+/K^+-ATPase pump found in the plasma membrane of cells. With the exchange of sodium for potassium and the hydrolysis of ATP, an electrochemical potential gradient generates nerve impulse conduction.

In addition to sodium's interrelationships with potassium and chloride, it has been recognized for several decades that increased dietary sodium intake is associated with increased urinary calcium excretion. Chapter 11's Perspective on Osteoporosis addresses sodium and bone health.

Excretion

Because nearly all ingested sodium is absorbed and dietary sodium intake is generally high, much larger amounts enter the body than are required. The kidneys provide the primary means of excreting excess sodium, with its excretion influenced by several hormones. The proximal tubule reabsorbs up to about 67% of filtered sodium (and chloride) by paracellular and transcellular mechanisms. The loop of Henle reabsorbs an additional 25–30% via a Na^+-K^+-$2Cl^-$ cotransporter. It is the distal tubule and collecting ducts that are under hormonal control, responding to the RAAS by reabsorbing sodium. Aldosterone specifically increases sodium reabsorption by inserting additional Na^+ channels

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Figure 12.5 Intestinal sodium absorption.

into the luminal membrane, which improves passive reabsorption from the lumen into the renal cells, and by inserting Na^+/K^+-ATPase pumps into the basolateral membrane, which improves the active transport of the sodium from the renal cells into the plasma. In the absence of RAAS stimulation as occurs with higher sodium, ECF volume, and blood pressure, sodium is not reabsorbed and is excreted in the urine. Additionally, natriuretic peptides promote urinary sodium excretion.

In addition to urine losses, sodium is excreted in small amounts (usually less than about 125 mg) in the feces (except in the case of diarrhea or other intestinal illnesses in which losses can be much higher). Sodium losses via the skin are variable. Under conditions of moderate temperature and low levels of exercise, sodium losses in sweat are small. However, because the sodium content of sweat is ~50 mEq/L, conditions of high temperature or sustained vigorous exercise, which can generate large amounts of sweat, can account for significant sodium losses.

Adequate Intake, Deficiency, Toxicity, and Assessment of Nutriture

The National Research Council (in 2004) recommends an Adequate Intake of 1,500 mg (65 mmol) of sodium (or 3.8 g of salt) and a Tolerable Upper Intake Level of 2,300 mg (100 mmol) of sodium (or 5.8 g of salt) for adults per day [2]. The American Heart Association recommends 1,500 mg

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of sodium/day. The World Health Organization recommends a daily sodium intake of less than 2,000 mg. The Dietary Guidelines also endorse consuming less than 2,300 mg of sodium/day, yet an expert panel of the Institute of Medicine (IOM) in 2013, after evaluating research addressing sodium and its direct relationship with health outcomes, reported that data were insufficient (both in quality and quantity) to conclude whether sodium intakes of less than 2.3 g/day was associated with risk (increased or decreased) of cardiovascular disease in the general population [3,4]. The minimum amount of sodium needed to replace losses (with no sweat and maximal adaptation) is estimated at about 180 mg (8 mmol); however, this amount is not thought to represent the sodium requirement [2]. Additional recommendations for sodium for other age groups are provided on the inside front cover of the book. The Perspective at the end of this chapter provides a further discussion of sodium and hypertension.

A dietary deficiency of sodium does not normally occur because of the abundance of the mineral across a broad spectrum of foods. However, excessive sweating, involving a loss of more than about 3% of total body weight, may result in sodium deficiency. Symptoms include muscle cramps, nausea, vomiting, dizziness, shock, and coma.

The U.S. Food and Drug Administration has approved health claims stating "Diets low in sodium may reduce the risk of high blood pressure, a disease associated with many factors" as well as "Development of hypertension or high blood pressure depends on many factors. [This product] can be part of a low-sodium and low-salt diet that might reduce the risk of hypertension or high blood pressure" [5].

Sodium is measured routinely in clinical laboratories. A 24-hour urinary sodium excretion level is most often used as a reflection of sodium intake. Urinary sodium in amounts less than about 5–10 mmol/L suggests sodium depletion. Collection of multiple 24-hour urine samples is best for assessment of sodium intake because intake typically varies greatly from day to day. Plasma sodium concentrations provide an indication of hydration status.

POTASSIUM

Potassium is the major intracellular cation, with about 98% of the body's potassium found within cells and only about 2% present extracellularly. Potassium constitutes up to about 0.19% of total body weight, or up to about 130 g of potassium in a 70-kg human.

Sources

Potassium is fairly widespread in the diet, and is especially abundant in fruits and vegetables, which provide the mineral along with anions like phosphate and citrate. Foods exceptionally rich in potassium (usually greater than 300 mg per cup) include fruits such as prune juice, avocados, bananas, cantaloupe, honeydew melon, mango, and papaya, and some vegetables (winter squash, leafy green vegetables, and yams). Other major sources of potassium, containing between about 200 and 300 mg of potassium per cup, are legumes, nuts and seeds, nut butters like peanut butter, and other selected vegetables (such as potatoes, asparagus, mushrooms, and okra) and fruits (like oranges, grapefruits, peaches, pears, kiwi, and nectarines). Milk and yogurt also provide potassium—about 300 mg per cup.

Potassium may also be added to some processed foods (but not in the large quantities as found with sodium) and found as part of salt substitutes such as potassium chloride. Multivitamin/minerals supplements may or may not contain potassium. If included, potassium is present in amounts up to a maximum of about 99 or 100 mg. Individual nutrient potassium supplements are available in normal and extended-release tablets and capsules as well as dissolvable tablets, elixirs, and powders for suspension. The form is provided as a salt, usually potassium gluconate, citrate, phosphate, or bicarbonate. Use of higher-dose supplements is recommended only to correct deficiency under medical supervision. Because potassium supplements can be irritating to the gastrointestinal tract, smaller divided doses, for example taken two to three times daily, are often better tolerated. Additionally, consumption of enteric-coated forms of the mineral with meals may help reduce intestinal side effects.

Potassium, unlike sodium, is consumed in amounts considerably less than current recommendations. Mean potassium intake among adults in the United States is estimated at 2,631 mg [1].

Absorption, Secretion, and Transport

Over 85% of ingested potassium is absorbed. Absorption occurs throughout the small intestine and to a lesser extent in the colon. Depending on concentration in the lumen, potassium is absorbed by passive diffusion or actively by a K^+/H^+-ATPase. This pump exchanges intracellular H^+ for luminal K^+. Alternatively, potassium may be absorbed across the brush border membrane by potassium channels that also serve as secretory pathways in the colon. To enter the blood, the potassium accumulated in the enterocyte diffuses across the basolateral membrane through a potassium channel.

Plasma potassium concentrations are maintained within a narrow range of about 3.5–5.0 mEq/L. This ECF concentration is regulated primarily through hormonal and renal functions. The large, rapid rises in plasma potassium concentrations that could routinely occur after eating potassium-rich foods are managed hormonally to a large extent by the action of insulin, which promotes active potassium uptake by hepatic and muscle cells. High intracellular potassium concentrations are maintained

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by Na^+/K^+-ATPase pumps. The presence of dietary potassium in the digestive tract is also thought to signal, by an unclear mechanism, the kidneys, which in turn respond by increasing urinary potassium excretion. During the periods between meals, muscles release potassium back into the plasma, renal potassium reabsorption increases, and renal potassium secretion decreases to ensure plasma potassium concentrations remain within the normal range. Conditions disrupting renal (as with renal failure) and hormonal functions can have profound effects on plasma potassium concentrations and body functions.

Functions and Interactions with Other Nutrients

Potassium's intracellular to extracellular ratio is needed for its function in the maintenance of the cell's resting membrane potential. The mineral influences depolarization as well as contractility of excitable tissues, especially of smooth, skeletal, and cardiac muscle and nerve tissue. Potassium's role as the main cation within cells is also central to its function in water balance (discussed earlier in the chapter) and its role in maintaining acid-base balance (discussed at the end of this chapter). Potassium also plays a role in cellular metabolism, where it is needed, along with magnesium, for the activity of pyruvate kinase, which converts phosphoenolpyruvate to pyruvate during glycolysis and is coupled with the substrate-level phosphorylation (ATP production). Potassium is thought to be needed to enhance the binding of substrates at the enzyme's active site.

Potassium, like sodium, interacts with calcium, and affects the urinary excretion of calcium. However, its effect is opposite to that of sodium; whereas sodium increases calcium excretion, potassium decreases it.

Excretion

Potassium is excreted from the body mainly via the kidneys, with typically only small amounts excreted in the feces (about 195–390 mg) and in the sweat (except under extreme physical activity). However, considerably larger amounts of potassium are lost in the feces with excessive diarrhea or intestinal drainage (as with inflammatory bowel disease or an ileostomy, among other conditions); the increased losses of potassium associated with diarrhea are often secondary, in part, to high aldosterone secretion (which is released to reduce urinary water and sodium losses, but which promotes potassium loss).

Most potassium in the glomerular filtrate is actively reabsorbed in the proximal tubule; this reabsorption is unregulated. In the distal and collecting tubules, potassium can be both actively secreted into the filtrate as well as reabsorbed as needed. Reabsorption of potassium in these regions is mediated by increased H^+/K^+-ATPase activity. Urinary potassium excretion is reduced when potassium needs to be conserved, as with lower intakes (or with high losses from diarrhea, vomiting, etc.) and lower plasma concentrations. Most potassium that is excreted in the urine entered the filtrate via secretion associated with increased Na^+/K^+-ATPase activity, increased tubular flow, and increased aldosterone. Urinary potassium rises with higher potassium intakes and thus higher plasma concentrations in the peritubular capillaries. Aldosterone release (which as discussed earlier is stimulated by increased plasma potassium concentrations along with decreased plasma sodium, increased angiotensin II, and decreased natriuretic peptides) enhances sodium reabsorption and stimulates potassium secretion. Changes in pH also influence potassium excretion, with acidosis reducing excretion and alkalosis increasing potassium excretion.

Adequate Intake, Deficiency, Toxicity, and Assessment of Nutriture

The Adequate Intake recommendation for potassium is 4,700 mg (120 mmol) per day for adults [2]. Recommendations for potassium for other population groups are provided on the inside front cover of the book. Potassium intakes of at least 3,600–3,800 mg/day have been suggested for heart and bone health; this recommendation, although lower than the Adequate Intake recommendation, is considerably higher than is currently consumed by most Americans [1,6].

Deficiency of potassium most often results from situations causing profound loss of fluids and electrolytes, such as excessive vomiting and diarrhea. Additionally, a magnesium deficiency, which promotes potassium excretion, and the use of some medications such as thiazide and loop diuretics (used to treat high blood pressure) and glucocorticoids (used to suppress inflammation, for example) increase urinary potassium excretion and may cause deficiency. The condition can also result from various nephropathies and from increased distribution of potassium into cells. This latter situation sometimes occurs as part of refeeding syndrome, a condition that can develop when insufficient potassium is provided in the nutritional treatment of those with malnutrition.

Deficiency of potassium is manifested biochemically by decreases in serum potassium concentrations (hypokalemia). Some manifestations of hypokalemia (especially when concentrations drop below about 3.0 mEq/L) include muscle weakness, lethargy, ascending paralysis, cardiac arrhythmias, and possibly death. The reduction in potassium leads to changes in resting membrane potential and excitability, with lower plasma potassium concentrations associated with membrane hyperpolarization and reduced tissue excitability. Treatment usually requires potassium supplementation as potassium phosphate, bicarbonate, or citrate.

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Insufficient dietary potassium intakes (in the absence of hypokalemia), especially when coupled with high dietary sodium intakes, have been linked with hypertension and cardiovascular disease (see this chapter's Perspective). Because of these well-documented associations, the Food and Drug Administration has approved the health claim "Diets containing foods that are good sources of potassium and low in sodium may reduce the risk of high blood pressure and stroke" [5]. To use this claim, a food must contain at least 350 mg (10% Daily Value) of potassium; must contain ≤140 mg of sodium, ≤3g of total fat, ≤1g of saturated fat, and ≤20 mg of cholesterol; and must provide ≤15% of energy (kcal) from saturated fat [5]. For food labeling purposes, to be considered a "rich," "excellent," or "high" source of potassium (or any other nutrient), a food must contain 20% or more of the Daily Value; to be a "good" source of potassium, the food must contain 10–19% of the Daily Value [5].

Too much potassium in the blood/plasma is referred to as **hyperkalemia**. While the condition is commonly associated with renal failure since it is the kidneys that are responsible for eliminating excess potassium from the body, there are several other causes of the hyperkalemia, including the use of potassium supplements in large doses. Hyperkalemia, like hypokalemia, impacts resting membrane potential and tissue excitability, specifically reducing both. Heart function is negatively affected (as with hypokalemia), resulting in many types of arrhythmias and possible death. Some additional manifestations of hyperkalemia (especially when concentrations exceed about 5.5 mEq/L) include muscle weakness, twitching and cramping, and ascending paralysis. While no Tolerable Upper Intake Level has been established for potassium from foods, the use of potassium supplements should only be under the supervision of medical personnel.

Potassium status is typically assessed based on plasma/serum potassium concentrations. Both depletion and excess potassium in the blood can be lethal.

CHLORIDE

Chloride is the most abundant anion (at 88%) in the ECF; about 12% is found intracellularly. Chloride's negative charge neutralizes the positive charge of the Na^+ with which it is usually associated. In this respect, it is of great importance in maintaining electrolyte balance. Total body chloride content is similar to that of sodium, representing about 0.15% of body weight, or about 105 g in a 70-kg human.

Sources

Nearly all the chloride consumed in the diet is associated with sodium in the form of sodium chloride, or salt. Salt, which is about 60% chloride, is abundant in a large number of foods, particularly in snack items and processed foods. Chloride is also found in eggs, fresh meats, and seafood. The average adult consumes an estimated 2,000–8,000 mg (2–8 g) of chloride each day.

In addition to chloride obtained from food ingestion, the secretions of the digestive tract also contain appreciable amounts of chloride. The chloride content of gastric juice is greatest (about 130 mEq/L); the chloride content of bile and intestinal secretions is each about 100 mEq/L, with lesser amounts in pancreatic secretions and colonic secretions (about 75 mEq/L and 40 mEq/L, respectively). Much of this chloride is absorbed.

Absorption, Secretion, and Transport

Chloride is almost completely absorbed in the intestines. Its absorption closely follows that of sodium for the establishment and maintenance of electrical neutrality and osmotic balance. In the Na^+/glucose (nutrient) cotransport system (described in the section on sodium), chloride follows the actively absorbed Na^+. The absorbed Na^+ creates an electrical gradient that provides the drive for the accompanying inward diffusion of Cl^- between cells. In the electroneutral Na^+/Cl^- cotransport absorption system, chloride is absorbed in exchange for bicarbonate as sodium is absorbed in exchange for H^+. In the electrogenic Na^+ absorption mechanism, chloride follows the absorbed sodium passively. Thus, regardless of the absorptive mechanism, basically wherever sodium goes, chloride is not far behind to maintain neutrality and osmotic balance.

Secretory mechanisms for the electrolytes, especially chloride, potassium, and bicarbonate, are also found throughout the gastrointestinal tract. One mechanism for intestinal chloride secretion is active and electrogenic (Figure 12.6). Intestinal cells take up chloride from the blood across the basolateral membrane by way of a $Na^+/K^+/Cl^-$ cotransport pathway. Chloride accumulating in the enterocyte exits via a Cl^- channel across the brush border membrane into the lumen; potassium channels on the basolateral membrane allow the potassium present in the enterocyte to reenter the blood.

Intestinal cells (especially those in the crypt) also secrete chloride into the lumen via a cyclic AMP–dependent chloride channel called the cystic fibrosis transmembrane conductance regulator (CFTR). In this pathway, activation of adenyl cyclase and production of cyclic AMP stimulate the CFTR, which secretes the chloride through a channel into the lumen. Mutations in the CFTR gene result in the disorder cystic fibrosis. However, dysfunction of this channel may also occur secondary to the presence of bacterial toxins, such as the cholera toxin. Toxins, which activate adenyl cyclase and elevate cAMP, cause these channels to continuously release chloride accompanied by sodium and water (fluid) into the lumen, resulting in severe diarrhea and ultimately dehydration. Other channels also enable chloride secretion. Secretion of chloride in some

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Figure 12.6 One mechanism for intestinal chloride secretion.

tissues may be calcium-induced and occur via calcium-activated Cl^- conductance and by ligand gated anion channels, among other means. Cl^- channels in colonic cells also provide for chloride transport. The secretion of chloride may be accompanied by sodium secretion (both paracellularly and transcellularly) and water movement via osmosis.

Functions

Chloride has multiple functions in addition to its role along with sodium in maintaining fluid balance. The formation of gastric hydrochloric acid requires chloride, which is secreted along with protons from the parietal cells of the stomach. Chloride is released by white blood cells during phagocytosis to assist in the destruction of foreign substances. Also, chloride acts as the exchange anion for HCO_3^- in red blood cells. This process, sometimes called the *chloride shift* and shown later in Figure 12.7, allows the transport of tissue-derived CO_2 back to the lungs in the form of plasma HCO_3^-.

Excretion

Chloride excretion occurs through three routes—the gastrointestinal tract, the skin, and the kidneys—with losses through each route closely reflecting those of sodium. Excretion of chloride via the feces normally is minimal, approximately 35–70 mg/day. Losses through the skin are similar quantitatively to sodium losses, that is, normally quite small except in cases of high temperature and vigorous exercise. The major route of chloride excretion is through the kidneys, where it is primarily regulated indirectly with sodium. Sodium reabsorption promotes paracellular chloride reabsorption, although some chloride is also reabsorbed by active transcellular mechanisms. About two-thirds of chloride reabsorption occurs in the proximal tubule and another 20–25% occurs by both passive transport and active sodium-coupled transport in the loop of Henle. The remaining chloride is reabsorbed in the distal tubule and collecting ducts via sodium-coupled transport. Chloride secretion can also occur (with sodium) as needed to maintain ECF.

Adequate Intake, Deficiency, Toxicity, and Assessment of Nutriture

The Adequate Intake recommendation for chloride for adults is 2,300 mg (65 mmol) per day [2]. Additional recommendations for chloride for other age groups are provided on the inside front cover of the book. A Tolerable Upper Intake Level for chloride is 3.6 g (100 mmol) [2].

Dietary deficiency of chloride does not occur under normal conditions. As is the case for the other electrolytes, imbalances and deficiency arise chiefly through gastrointestinal tract disturbances such as severe diarrhea and vomiting. The condition results in weakness, lethargy, and metabolic acidosis. Convulsions also may occur with chloride deficiency.

Chloride status is assessed through evaluation of its concentration in the serum, which usually ranges from about 101 to 111 mEq/L. However, like that of other serum solutes, chloride concentration depends on the plasma volume. It is possible, for example, for total body chloride to be diminished but plasma chloride concentrations to appear normal or even be elevated (if body water was lost with the chloride loss).

ACID-BASE BALANCE: CONTROL OF HYDROGEN ION CONCENTRATION

The maintenance of the hydrogen ion concentration in body fluids within a narrow range is vital to normal physiological function. In fact, acid-base (pH) regulation is one of the most important aspects of homeostasis since

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even slight deviations from normal can cause marked alterations in enzyme-catalyzed reaction rates, cellular uptake and regulation of metabolites and minerals, and oxygen uptake and release from hemoglobin. Because the body regulates acid-base balance in conjunction with fluid-electrolyte balance, it is addressed in this chapter.

The degree of acidity or alkalinity of fluids is determined by its concentration of protons (H^+). The **hydrogen ion** concentration in body fluids is generally quite low, regulated at approximately 4×10^{-8} mol/L, with a range of a low of about 1.0×10^{-8} mol/L to a high of about 1.0×10^{-7} mol/L; values outside this range are not compatible with life.

Because expressing H^+ in terms of its actual concentration is awkward, the concept of pH, which is the negative logarithm of the H^+ concentration, was devised to simplify the expression. It enables concentrations to be expressed as whole numbers rather than as negative exponential values: $pH = -\log[H^+]$ with bracketed values symbolizing concentrations. Throughout this discussion, this designation is used to signify concentrations of substances other than protons. The pH of ECF, in which the H^+ concentration may be assumed to be approximately 4×10^{-8} mol/L, can therefore be calculated as follows:

$$pH = -\log\ (4 \times 10^{-8})$$

or

$$pH = \log\ (1/(4 \times 10^{-8}))$$

(dividing)

$$pH = \log\ (0.25 \times 10^{8})$$

$$pH = \log\ 0.25 + \log\ 10^{8}$$

(taking logs)

$$pH = -0.602 + 8$$

$$pH = 7.4$$

As the molar concentration of H^+ becomes smaller and the value of the negative exponent of 10 becomes larger, the pH correspondingly increases. High acidity is associated with high H^+ concentration and low pH; the term *acidosis* refers to a rise in extracellular (principally plasma) H^+ concentration beyond the normal range. Low acidity is associated with low H^+ concentration and high pH, and when referring to the plasma is often called *alkalosis*.

An acid, as it relates to fluid acid-base regulation, may be defined as a substance capable of releasing protons (H^+). The metabolism of the macronutrients continuously generates organic acids such as lactic and pyruvic acids (see Chapter 3) and acetoacetic acid and β-hydroxybutyric acid (see Chapter 5), which must be neutralized. Acidic salts of sulfuric and phosphoric acids are also generated metabolically from sulfur- and phosphorus-containing substances, respectively. Additionally, carbon dioxide, the product of the complete oxidation of energy nutrients, is itself indirectly acidic because, on combination with H_2O, it forms carbonic acid (H_2CO_3). To guard against such fluctuations in pH,

- chemical buffer systems operate in the blood and in cells
- the lungs respond through changes in respiration
- the kidneys respond through changes in substrate excretion and reabsorption.

In addition to these systems, the bones serve as a "bank," accepting and releasing cations and anions to facilitate balance.

The chemical buffer systems in the blood and cells are the quickest to respond to changes in pH, working typically in less than a minute to remove H^+ from the environment or to release H^+ into the environment as needed to reestablish the appropriate pH. The actions of the lungs, which serve to eliminate or retain carbon dioxide, CO_2 (and therefore carbonic acid, H_2CO_3), usually take minutes to hours to respond. The actions of the kidneys, which function in the secretion of H^+, the reabsorption and regeneration of bicarbonate (HCO_3^-), and the production of ammonia enabling ammonium ion formation, are the slowest, requiring hours to days, to respond.

Chemical Buffer Systems

A buffer is a substance that reversibly binds protons. In the body, buffers function to resist changes in pH despite the addition of acids or bases to the environment. An explanation of buffers is presented hereafter followed by a discussion of the body's chemical buffer systems.

Principles of Buffers

Buffers exist as pairs, consisting usually of a weak acid (represented as HA) and its conjugate base (A^-). The conjugate base is the residual portion of the acid following the release of the proton (H^+). The conjugate base of a weak acid is basic because it tends to attract a proton and to regenerate the acid. The dissociation of a weak acid and the reunion of its conjugate base and proton comprise an equilibrium system:

$$HA \longleftrightarrow H^+ + A^-$$

The equilibrium expression for this reaction, called the acid dissociation constant (K_a), is represented as

$$K_a = \frac{[H^+]\,[A^-]}{[HA]}$$

The equation can be rearranged to

$$[H^+] = \frac{K_a\,[HA]}{[A^-]}$$

Taking the negative logarithm of both sides of the equation gives

$$-\log\,[H^+] = -\log K_a - \log\frac{[HA]}{[A^-]}$$

These values become

$$pH = pK_a + \log\frac{[A^-]}{[HA]}$$

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This equation, referred to as the Henderson-Hasselbalch equation, shows how a buffer system composed of a weak acid and its conjugate base resists changes in pH if a strong acid or base is added to the system. For example, if the molar concentrations of the conjugate base and the acid are equal, then the ratio of $[A^-]$ to [HA] is 1.0, and the logarithm of this ratio is 0, making the pH of the system equal to the pK_a of the acid.

The pK_a, which is the negative logarithm of the acid dissociation constant (K_a), of any weak acid is a constant for that particular acid and simply reflects its strength (i.e., its tendency to release a proton). If a strong acid or a strong base is added to this system, the ratio of $[A^-]$ to [HA] changes and therefore the pH changes, but only slightly. Suppose, for example, that both the conjugate base and the free acid are present at 0.1 mol/L concentrations, and suppose also that the pK_a of the acid is 7.0. As shown previously, if the ratio is 0.1:0.1, the pH is 7.0. The addition of enough hydrochloric acid (a strong acid) to the buffer in the example to make its final concentration 0.05 mol/L shifts the equilibrium to the left (to make HA). The 0.05 mol/L of H^+ (from the fully dissociated HCl) will combine with an equal molar amount of A^- to form HA. The new $[A^-]$ concentration therefore becomes 0.05 mol/L (0.1 – 0.05), and [HA] is 0.15 mol/L (0.1 + 0.05). The logarithm of this new ratio (0.05:0.15, or 0.33) is –0.48. Inserting this value into the Henderson-Hasselbalch equation, we can see that the pH decreases by only 0.48. In other words, the pH decreased from 7.0 to 6.52 by making the system 0.05 mol/L hydrochloric acid. In contrast, this same concentration of HCl in an unbuffered, aqueous solution would produce an acid pH between 1.0 and 2.0.

The Body's Chemical Buffers

Several physiologically important chemical buffers maintain the narrow pH range of ECF at approximately 7.35–7.45. One such buffer is the bicarbonate (HCO_3^-)–carbonic acid (H_2CO_3) system. Additional buffers that help to maintain acid-base (pH) balance include hemoglobin, other proteins present in cells as well as in ECF, and the phosphate system, found mainly in cells. Additionally, potassium (K^+) plays a role through ion exchange with H^+ between compartments.

Bicarbonate–Carbonic Acid The bicarbonate–carbonic acid buffer system is composed of the weak acid carbonic acid (H_2CO_3) and its salt or conjugate base, the bicarbonate ion (HCO_3^-). The carbonic acid dissociates reversibly into H^+ and HCO_3^-, as shown here:

$$H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$$

The buffering capacity of this reaction arises from the fact that either added protons or added hydroxide ions will be neutralized by corresponding shifts in the equilibrium. The H_2CO_3 can be formed both from the acidification of HCO_3^- (i.e., $H^+ + HCO_3^-$) and from the reaction of dissolved CO_2 with water (i.e., $CO_2 + H_2O$). The lungs regulate the CO_2 and the kidneys control HCO_3^-. The overall reaction involving carbon dioxide, carbonic acid, and bicarbonate ion (shown later in Figures 12.7 and 12.8) is:

$$\underset{\text{(gas)}}{CO_2} \longleftrightarrow \underset{\text{(dissolved)}}{CO_2} \longleftrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$$

Normally, the ratio of the concentration of HCO_3^- to H_2CO_3 in plasma is 20:1, and the apparent pK_a value for H_2CO_3 is 6.1. Using the Henderson-Hasselbalch equation, we can also show how a normal plasma pH of 7.4 results from these values:

$$\begin{aligned} pH &= pK_a + \log \frac{[HCO_3^-]}{[H_2CO_3]} \\ &= 6.1 + \log \frac{20}{1} \\ &= 6.1 + 1.3 \\ pH &= 7.4 \end{aligned}$$

Alterations in the 20:1 ratio of $[HCO_3^-]$ to $[H_2CO_3]$ change the pH, as discussed later in the sections on the respiratory and renal regulatory systems.

Proteins and Hemoglobin Proteins are found both within the blood and within cells. As **amphoteric** substances (substances that possess both acidic and basic groups), proteins are capable of neutralizing acids or bases due to the presence of carboxylic acid (R—COOH) and amino ($R—NH_3^+$) groups, which dissociate as:

1. $R—COOH \longleftrightarrow R—COO^- + H^+$
2. $R—NH_3^+ \longleftrightarrow R—NH_2 + H^+$

At physiological pH, the carboxylic acid is largely dissociated into its conjugate base ($R—COO^-$) and a proton (H^+), so the equilibrium is shifted strongly to the right. At that same pH, however, the amino group, being much weaker as an acid (a stronger base), is only weakly dissociated, and its equilibrium greatly favors to the left. If protons, in the form of a strong acid, are added to a protein solution, they are neutralized by reaction 1 because their presence will cause a shift in the equilibrium toward the undissociated acid (right to left). Strong bases, as contributors of hydroxide (OH^-) ions, will likewise be neutralized because, as they react with the protons to form water, the equilibrium of reaction 2 shifts to the right to restore the protons that were neutralized.

The protein hemoglobin (Hb) serves as an important physiological buffer because of its high concentration in red blood cells and its ability to bind H^+ forming HHb, a weak acid. Hemoglobin's binding of H^+ is shown and described further in Figure 12.7.

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In red blood cells (rbc), as Hb releases oxygen (O_2) to tissues, it can react 1 with carbon dioxide (CO_2) and 2 with hydrogen ions (H^+) and serve as a buffer. The CO_2 in the rbc enters via diffusion from tissues and may 3 remain dissolved, or 4 bind to Hb, or 5 react with water forming carbonic acid (H_2CO_3). The latter then dissociates into H^+ and HCO_3^-. The HCO_3^- is then 6 released into the plasma in exchange for chloride (Cl^-) in a process referred as the chloride shift. The bicarbonate–carbonic acid buffer system, like Hb, is important for acid-base balance in cells and the blood.

Figure 12.7 Red blood cell hemoglobin (Hb) as a buffer for hydrogen ions and the release of the buffer bicarbonate (HCO_3^-).

Phosphate The phosphate buffer system operates mainly within cells (only small amounts are present in ECF). The system consists of dibasic phosphate, HPO_4^{2-}, and monobasic phosphate, $H_2PO_4^-$, complexed with sodium as Na_2HPO_4 and NaH_2PO_4, respectively. In the kidneys, filtered HPO_4^{2-} reacts with H^+ in the filtrate as:

$$HPO_4^{2-} + H^+ \longleftrightarrow H_2PO_4^-$$

The $H_2PO_4^-$ (NaH_2PO_4) is then excreted in the urine, and thus enables the removal of "bound H^+" from the body. (See also Figure 12.8.)

Potassium Potassium helps maintain acid-base balance as part of a cellular ion exchange system. The electrolyte shifts in and out of cells in exchange for H^+ as needed based on blood pH. With acidosis (blood pH <7.35), K^+ moves out of cells and into the plasma and H^+ moves out of the plasma and into cells. The movement reduces the blood H^+ concentration, and thus increases its pH. Conversely, with alkalosis (blood pH >7.45), K^+ moves into cells and H^+ moves out of cells into the plasma. This movement increases the blood H^+ concentration, and thus decreases its pH. The resulting changes in serum potassium concentrations in these circumstances, however, can be life-threatening if too substantial.

Respiratory Regulation

When the chemical buffer systems are not sufficiently managing acid-base balance, the lungs can respond within minutes, acting as a regulator of the HCO_3^-/H_2CO_3 system, by retaining or exhaling CO_2. Increases in respiration are triggered by both rises in blood H^+ and by rises in CO_2 concentrations. Increases in blood H^+ trigger an increase in respiration, and thus the exhalation of more CO_2 (i.e., more CO_2-generated H^+ if one thinks of CO_2 as acid/H_2CO_3-forming). Breathing rate is restored (slowed) as more CO_2 is exhaled and less is present in the blood. Similarly, if plasma levels of CO_2 rise, perhaps because of accelerated metabolism, more H_2CO_3 is formed. This reaction, in turn, causes a fall in pH as the acid dissociates to release protons. The elevated CO_2 itself, as well as the resulting increase in H^+ concentration, is detected by the respiratory center of the brain, resulting in an increase in the respiratory rate. This hyperventilation increases CO_2 loss through the lungs substantially and therefore decreases the amount of H_2CO_3. This mechanism increases the ratio of HCO_3^- to H_2CO_3 by reducing H_2CO_3, thus elevating the pH to a normal value. Conversely, if plasma pH rises for any reason (because of either an increase in HCO_3^- or a decrease in H_2CO_3), the respiratory center is signaled accordingly and causes a slowing of the respiration rate. As CO_2 then accumulates, the H_2CO_3 concentration rises and the pH decreases.

Renal Regulation

In contrast to the more rapid actions of the chemical buffer systems and the lungs, the kidneys take hours or days to effect change in pH. They act mainly by controlling the secretion of H^+, by conserving or producing HCO_3^-, and by synthesizing and excreting ammonia, which reacts with H^+ to form ammonium ions for excretion. Filtered phosphate also facilitates H^+ excretion.

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With consumption of a normal diet, about 50–100 mEq of H^+ ions are generated daily and must be removed from the body to prevent a progressive metabolic acidosis. Filtered H^+ ions are not reabsorbed. The proximal, distal, and collecting tubules also actively secrete H^+, removing it from the blood in the peritubular capillaries and into the filtrate. The H^+s in the filtrate are used initially to form carbonic acid needed for bicarbonate conservation (as described later in this section). Any additional H^+ get excreted in the urine typically in association with ammonia or phosphates. The pH of the urine is usually around 6, with a range from about 4.5 to 6.5.

The presence of substances in the filtrate to combine with the H^+ enables the excretion of more H^+ and minimizes direct effects on urine pH. Two substances functioning in this capacity ("neutralizing or buffering" the H^+) include dibasic phosphate (HPO_4^{2-}, discussed in the section on phosphate buffers) and ammonia (NH_3) (Figure 12.8). In the renal tubule cells, ammonia, which is generated from glutamine degradation by glutaminase, diffuses into the

Filtered bicarbonate, HCO_3^-, is conserved by its ability to react with secreted H^+ in the tubule lumen (1) through a reaction catalyzed by carbonic anhydrase (found on the luminal membrane), to form carbonic acid, H_2CO_3.

(2) The carbonic acid disassociates in the filtrate into H_2O and CO_2.

(3) Both H_2O and CO_2 diffuse into the tubule cell.

(4) The H_2O and CO_2 in the tubule cell form carbonic acid, H_2CO_3, through the action of intracellular carbonic anhydrase.

(5) The carbonic acid, H_2CO_3, then disassociates in the tubule to form "new" bicarbonate, HCO_3^-.

(6) The bicarbonate next enters the peritubular capillary blood, which may occur in exchange with chloride.

(7) HPO_4^{2-} which is present in the filtrate when plasma phosphate concentrations are elevated can also react with secreted H^+ to form monobasic phosphate, $H_2PO_4^-$; this compound is excreted in the urine.

(8) Ammonia, which is generated in the tubule cells by the glutamine catabolism, enters the filtrate where it can also react with secreted H^+ to form an ammonium ion, $^+NH_4$. The ammonium ion is subsequently excreted in the urine.

Figure 12.8 Acid-base balance by the kidneys.

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tubular lumen of the collecting ducts where it combines with H^+ to form ammonium ions ($^+NH_4$). The ammonium ions remain in the filtrate and are excreted primarily as chloride salts in the urine.

Bicarbonate is critical for acid-base balance. Renal conservation (production) of bicarbonate occurs by an indirect process (shown in Figure 12.8) because of bicarbonate's relatively large size and charge. Briefly, in the filtrate, H^+ and HCO_3^- form H_2CO_3 by the action of carbonic anhydrase. The H_2CO_3 then disassociates into CO_2 and H_2O. The CO_2 and H_2O diffuse into the tubular cell, where carbonic anhydrase enables H_2CO_3 formation. The relatively high tubular cell pH allows the dissociation of the H_2CO_3 into HCO_3^- and H^+, after which the bicarbonate can reenter peritubular capillary blood and the H^+ can be actively secreted by the Na^+/H^+ carrier back into the filtrate. The net result enables H^+ secretion and bicarbonate "reabsorption," even though it is not the same bicarbonate ion.

Like respiratory regulation, renal regulation of pH is directed at maintaining a normal ratio of $[HCO_3^-]$ to $[H_2CO_3]$. In alkalosis, for example, in which the plasma ratio of $[HCO_3^-]$ to $[H_2CO_3]$ increases as the pH rises above 7.4, a net increase occurs in the excretion of bicarbonate. This increase occurs because the high extracellular HCO_3^- concentration increases its filtration, while the relatively low concentration of H_2CO_3 decreases the secretion of H^+. Therefore, the fine balance between HCO_3^- and H^+ that normally exists in the tubules no longer is in effect. Also, because no HCO_3^- ions can be reabsorbed without first reacting with H^+ (Figure 12.8), all the excess HCO_3^- passes into the urine, neutralized by sodium ions or other cations. In effect, therefore, HCO_3^- is removed from the ECF, restoring the normal ratio of HCO_3^- to H_2CO_3 and pH. In acidosis, the ratio of plasma $[HCO_3^-]$ to $[H_2CO_3]$ decreases, meaning that the rate of H^+ secretion rises to a level far greater than the rate of HCO_3^- filtration into the tubules. As a result, most of the filtered HCO_3^- is converted to H_2CO_3 and reabsorbed as CO_2 (Figure 12.8), while the excess H^+ is excreted in the urine. As a consequence, the ECF ratio of $[HCO_3^-]$ to $[H_2CO_3]$ increases, as does the pH.

SUMMARY

Maintaining body fluids and electrolytes is vitally important for health and nutrition. Intracellular fluid provides the environment for myriad metabolic reactions that take place in cells. The interstitial fluid compartment of the ECF mass allows nutrients to migrate into cells from the bloodstream and metabolic waste products from the cells to return to the bloodstream. These fluids contain the electrolytes, dissolved minerals that have important physiological functions. Their concentrations and their intracellular and extracellular distribution must be precisely regulated, and the mechanism for achieving this regulation is exerted largely through the kidney. The homeostatic maintenance of fluid volume is also the responsibility of this organ.

Fluid volume control by the kidneys is mostly hormone mediated. Vasopressin, produced in the hypothalamus, stimulates the tubular reabsorption of water from the glomerular filtrate. Aldosterone, a product of the adrenal cortex, increases the reabsorption of sodium, which indirectly stimulates vasopressin release through the resulting rise in ECF osmotic pressure. Thirst centers in the brain, which respond to fluctuations in blood volume or ECF osmolality, are also important regulators of fluid balance by their influence on the amount of fluid intake.

The macrominerals sodium, potassium, and chloride along with other ions of nutritional importance such as calcium and magnesium are freely filtered by the glomerulus but are selectively conserved by tubular reabsorption through active transport systems. Potassium is an example of a mineral that is regulated in part by tubular secretion into the filtrate. Secretion of the ion from the distal tubular cells increases as its concentration in those cells rises because of increased dietary intake. Potassium, like sodium, is regulated by aldosterone. Elevated plasma potassium stimulates the release of aldosterone, which exerts opposing renal effects on the two minerals—enhanced reabsorption of sodium and an increase in potassium excretion. Normal physiological functions depend on the body's fluid and acid-base balance.

The plasma is well buffered against changes in pH, primarily by proteins and by the bicarbonate–carbonic acid system. However, conditions of acidosis or alkalosis can result in situations such as an overproduction of organic acids, as would occur in diabetes or starvation, or respiratory aberrations that may cause abnormal carbon dioxide ventilation. Therefore, restoration of normal pH may be necessary and is accomplished through compensatory mechanisms of the kidneys and lungs. These organs function to maintain a normal ratio of bicarbonate to carbonic acid. The bicarbonate concentration is under the control of the kidneys, which can either conserve the ion by reabsorbing it to a greater extent or increase its excretion. The lungs control carbon dioxide and thus carbonic acid concentrations with hyperventilation "blowing off" carbon dioxide to increase pH and hypoventilation (or a slowing of respiration) retaining carbon dioxide and therefore the carbonic acid level to decrease pH.

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MACROMINERALS AND HYPERTENSION

Hypertension (high blood pressure) affects over 29% (70 million) of adults in the United States [1]. Hypertension is diagnosed when systolic and/or diastolic blood pressure values are ≥140/90 mm Hg, respectively. Causes of hypertension are often unknown, and designated as primary (also called essential). In other cases, hypertension is secondary, resulting from conditions such as kidney, endocrine, or neurological diseases. Whether it is essential or secondary, hypertension is a risk factor for heart disease, stroke, and renal disease. In fact, the risk for heart disease increases progressively as blood pressure increases above 115/75 mm Hg [2,3]. In adults, a 2 mm Hg reduction in blood pressure can reduce mortality secondary to heart disease by 4% and secondary to stroke by 6% [4].

Although some risk factors for hypertension are not controllable (e.g., genetic predisposition, race, aging), others can be modified by a person's commitment to lifestyle changes that include diet modifications. Yet, because hypertension is a heterogeneous disease with a variety of precipitating factors, dietary modifications work for some but not all hypertensive individuals. This Perspective focuses on some of the major minerals (sodium, potassium, calcium, and magnesium) and blood pressure, and briefly addresses some other dietary factors that may also be associated with the condition.

SODIUM

Sodium, as salt—sodium chloride—was one of the first nutrients directly linked to hypertension. A plethora of studies has been conducted over several decades examining salt and/or sodium and blood pressure across and within population groups. While study designs, time periods, and subjects included and excluded have varied, as have the means used to assess sodium intake (analysis of diet or urinary sodium excretion), overall evidence shows relatively consistently that reductions in sodium intake are associated with reductions in blood pressure, especially in those with hypertension. The Dietary Approaches to Stop Hypertension (DASH) sodium trials provided some of the most convincing evidence. The studies, which compared the effects of the DASH diet with three levels of sodium intake (3.3 g, 2.4 g, and 1.5 g) with those of a control diet, showed that additional blood pressure reduction could be achieved through reduction in dietary sodium to 1.5 g/day [5,6]. A progressive and direct dose–response effect was observed between sodium intake and blood pressure especially in hypertensive individuals [7–9]. Decreasing sodium intake by 2,300 mg (5.8 g of salt) per day predicted a reduction in blood pressure of 7.11/3.88 mm Hg in hypertensive individuals and of 3.57/1.66 mm Hg in normotensive individuals [8].

The relationship between sodium intake and blood pressure varies with some exhibiting a more pronounced direct response than others. Individuals who are frequently "sensitive to sodium," exhibiting an increase in blood pressure in response to sodium ingestion, include those who are obese, African American, have diabetes, and are older than 65 years of age. Generally, about 30–50% of individuals are thought to be "salt sensitive". The Prospective Urban Rural Epidemiology (PURE) study, which included more than 100,000 adults from 17 countries, found a direct association between sodium intake and blood pressure; the association was greatest typically in those consuming high sodium diets, in older adults, and in those with hypertension [10]. The study also found that those with higher or lower urinary sodium excretion (serving as an indicator of intake) versus those with moderate urinary sodium excretion had an increased risk of cardiovascular disease outcomes [10,11]. This J-shaped curve, linking especially high (>6 g), and to a lesser extent low (<3 g), sodium intakes (based on urinary sodium excretion) with adverse outcomes, has been documented in other studies [12]. An expert panel of the Institute of Medicine, after evaluating research addressing sodium and its relationship with health outcomes, reported that data were of insufficient quantity and quality to conclude whether sodium intakes of less than 2.3 g/day was associated with risk (increased or decreased) of cardiovascular disease in the general population [13]. The committee also concluded from a review of published studies that there was a positive relationship between higher sodium intakes and risk of cardiovascular disease (consistent with known effects on blood pressure) in the general population [13].

No one mechanism is thought to be responsible for salt-induced elevations in blood pressure. Many reviews are available on the subject, with some focusing on both excessive sodium and insufficient potassium intakes [14,15]. Policy recommendations for sodium intake continue to be controversial. Some experts are passionate in their promotion of reductions in salt intake for the general population, while others believe such recommendations go beyond available data. Other dietary factors, however, impact sodium's effects. These factors include intakes of potassium, calcium, and magnesium, which are discussed in subsequent sections of this Perspective.

POTASSIUM

While deficits in potassium augment the effects of excess sodium in the pathogenesis of hypertension, the relationship between potassium and blood pressure has also been shown to be independent of sodium. Higher potassium intakes typically reduce blood pressure, especially in those with hypertension, those ingesting large amounts of sodium, and those who are salt sensitive [10,11,14–18]. Systolic and diastolic blood pressure reductions of 4.4 mm Hg and 2.5 mm Hg, respectively, have been reported in those with hypertension excreting at least 2 g (50 mmol) of potassium/per day in the urine, and systolic and diastolic blood pressure reductions of 1.8 mm Hg and 1.0 mm Hg, respectively, have been reported in normotensive individuals excreting at least 2 g of potassium per day in the urine [14].

The mechanisms by which potassium affects blood pressure are multiple and are the subject of many reviews [17,18]. The Adequate Intake recommendation for potassium is 4,700 mg (120 mmol) per day [7]; however, raising potassium intakes from current amounts at less than 3,000 mg up to 3,600–3,800 mg/day may also reduce blood pressure and cardiovascular morbidity and mortality, especially if coupled with reductions in sodium intake [4,17,18]. Increases in potassium intake can be accomplished easily with the inclusion of additional fruits and vegetables into the diet plan. Adding more legumes, nuts, seeds, and dairy products to the diet can also boost dietary potassium intakes.

OTHER DIETARY AND LIFESTYLE FACTORS

Calcium

While an inverse relationship between calcium and blood pressure has been confirmed in numerous studies, the effect of calcium supplementation (400 mg–2 g) trials on blood pressure has been disappointing, with no or only very small reductions in systolic blood pressure, and typically no effect on diastolic blood pressure. Thus, evidence has been considered weak and insufficient to recommend calcium supplementation as an approach to lower blood pressure [3,19]. Nonetheless, given many adults fail to consume recommended amounts of calcium and given inadequate calcium intakes appear to potentiate the rise in blood pressure in response to sodium ingestion, individuals are strongly advised to increase intakes of foods rich in calcium to meet recommended intakes.

Magnesium

Similar to calcium, an inverse relationship between blood pressure and magnesium (intake and/or serum) has also been documented, but the effects of magnesium supplementation on blood pressure in clinical trials are inconsistent and small (less than 3 mm Hg in diastolic blood pressure) [3,20,21]. An additional meta-analysis examining trials providing

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combinations of magnesium, calcium, and potassium also found a lack of robust evidence in favor of supplementation of any combination of these three major minerals [22]. However, given the inverse relationship between magnesium and blood pressure and that most Americans fail to meet recommended magnesium intakes, dietary approaches to include more whole grains, nuts, seeds, legumes, and green leafy vegetables should be employed. The use of magnesium supplements in the prevention and treatment of hypertension is not recommended at this time. Supplementation of magnesium above the Recommended Dietary Allowance is suggested only for those with magnesium deficiency or those with increased urinary magnesium losses secondary to diuretic use [22].

Alcohol

A direct, dose-dependent relationship exists between alcohol consumption (especially ingesting three or more drinks per day) and blood pressure [3,23]. Recommendations to lower blood pressure suggest moderation of alcohol intake (among those who drink); moderation is defined as ≥1 drink/day for women and ≥2 drinks/day for men [3]. A drink is defined as 12 oz beer, 5 oz wine (12% alcohol), or 1.5 oz of 80-proof distilled spirits.

Omega-3 Fatty Acids

The effectiveness of fish oil supplements, rich in omega-3 fatty acids eicosapentaenoic acid (EPA) and decosahexanoic acid (DHA), on blood pressure has been examined in several clinical trials and meta-analyses of studies. Small, but significant, reductions in systolic and diastolic blood pressure (about 3–5 mm Hg systolic and 2–4 mm Hg diastolic) have been typically observed in those with hypertension (but not in those who are normotensive) with the daily consumption of at least 2–3 g of omega-3 fatty acids [24,25]. For those who choose to ingest supplements, the Food and Drug Administration recommends the consumption of no more than 3 g of EPA and DHA per day, with not more than 2 of the 3 g derived from supplements [26].

Lifestyle

Lifestyle approaches have also been investigated in the prevention and treatment of hypertension. The randomized trial Dietary Approaches to Stop Hypertension (DASH) found that low-fat diets rich in fruits, vegetables, and low-fat dairy products were more effective in reducing blood pressure than a control diet low in fruits and vegetables and average in fat (~36% of kcal). The DASH diet provides about 4,500 mg of potassium, 8–10 servings of fruits and vegetables, and 2–3 servings of low-fat dairy products daily; limits red meat, fats, and sugar-sweetened foods and beverages; and emphasizes nuts, seeds, and legumes. The sodium content of the initial diet was about 3.3 g, but lowering dietary sodium further provided greater benefits.

Other lifestyle modification approaches have been investigated. The PREMIER Trial, which included weight loss, increased physical activity, sodium reduction, and the DASH diet, was found to be beneficial to those with hypertension not on medications (−14.2 mm Hg systolic and −7.4 mm Hg diastolic) and to those without hypertension (−9.2 mm Hg systolic and −5.8 mm Hg diastolic) [27]. The effectiveness of lifestyle interventions for hypertension, investigated in a meta-analysis, also showed that weight reduction, regular exercise, alcohol restriction, and salt restriction were associated with significant reductions in systolic and diastolic blood pressure; however, evidence did not support the use of relaxation therapies or potassium, calcium, or magnesium supplements to reduce blood pressure [28].

In summary, several dietary modifications can be made to help to lower blood pressure, including reducing sodium and increasing potassium intake; consuming enough dietary calcium and magnesium to meet recommendations; consuming alcohol in moderation (for those who drink); and following a healthy diet plan such as DASH. Lifestyle management recommendations for the reduction of heart disease, including hypertension [29] (and that are endorsed by the Joint National Committee for High Blood Pressure [30]) include consumption of a DASH-style diet that is rich in vegetables, fruits, and whole grains; includes low-fat dairy, poultry and lean meats, legumes, nuts, and nontropical oils; and limits sweets, sugar-sweetened beverages, red meats, and sodium (at most 2,400 mg with intakes of 1,500 mg more desirable).

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Web Sites

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www.nlm.nih.gov/medlineplus/highbloodpressure.html
http://www.cdc.gov/nchs/fastats/hypertension.htm

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13 ESSENTIAL TRACE AND ULTRATRACE MINERALS

IRON
ZINC
COPPER
SELENIUM
CHROMIUM
IODINE
MANGANESE
MOLYBDENUM
For each of the minerals listed, the following subtopics (if known and when applicable) are discussed:
Sources
Digestion, Absorption, Transport, and Storage
Functions and Mechanisms of Action
Interactions with Other Nutrients
Excretion
Recommended Dietary Allowance or Adequate Intake
Deficiency
Toxicity
Assessment of Nutriture
PERSPECTIVE
NUTRIENT–DRUG INTERACTIONS

THE TRACE MINERALS OR TRACE ELEMENTS (also called microminerals) initially gained the description "trace" because their concentrations in tissue were not easily quantified by early analytical methods. Today, however, trace minerals can be analyzed by a variety of techniques. Trace minerals or elements are now typically defined as minerals that are needed by the body in small amounts, that is, less than 100 mg/day. However, some of the trace elements may be categorized as ultratrace if required in amounts of less than 1 mg/day.

Figure 13.1 shows the periodic table, highlighting some of the essential trace and ultratrace elements. This chapter discusses the sources, digestion, absorption, transport, storage, functions, interactions with other nutrients, excretion, recommended intakes, deficiency, toxicity, and assessment of nutriture for the trace minerals iron, zinc, and copper, and the ultratrace minerals selenium, iodine, and molybdenum, for which Recommended Dietary Allowances have been established by the Food and Nutrition Board. In addition, these same topics are covered for the trace mineral manganese and the ultratrace mineral chromium, for which Adequate Intakes have been established by the Food and Nutrition Board.

Very little is known about the need for many other trace and ultratrace elements, including nickel, silicon, vanadium, arsenic, and boron; therefore, no recommendations for intake exist. Chapter 14 addresses these elements, as well as fluoride, a nonessential element with established Adequate Intakes.

Table 13.1 provides an overview of the minerals addressed in the chapter, including information on the approximate mineral quantities in the body, which range from <1 mg to about 4 g. Keeping in mind that an ounce weighs about 28.4 g, contrast these amounts with the body's concentrations of the major minerals, which range from about 35 to 1,200 g.

IRON

The human body contains about 2–4 g of iron, or ~38 mg iron/kg body weight for women and ~50 mg iron/kg body weight for men. Over 65% (~1.3–2.6 g) of body iron is found in hemoglobin, up to about 10% (~0.2–0.4 g) is found as myoglobin, about 1–5% (up to 0.1–0.2 g) is found as part of enzymes, and the remaining body iron (about 20% or 0.4–0.8 g) is found in the blood and in storage. While the metal exists in several oxidation states varying from Fe^{6+} to Fe^{2-}, depending on its chemical environment, the only states that are stable in the body's aqueous environment and in food are the ferric (Fe^{3+}) and ferrous (Fe^{2+}) forms.

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Figure 13.1 The periodic table highlighting some of the essential trace and ultratrace elements.
Source: Derived from Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

Sources

Iron is found in foods in two forms: heme and nonheme. Heme iron represents iron that is contained within the porphyrin ring structure shown in Figure 13.2. Heme iron is derived mainly from hemoglobin and myoglobin and thus is found in animal products, especially meat, fish, and poultry; dairy products, however, are a relative poor source of iron. About 50–60% of the iron in meat, fish, and poultry is heme iron; the rest is nonheme iron. Nonheme iron is the main form of iron in plant foods, especially whole grains, nuts, legumes including lentils and peas, some fruits and vegetables, and tofu.

Foods particularly high in iron include liver and other organ meats; however, these foods are not popular items in most American diets. More popular foods that are relatively good sources of iron include meats, especially red meats, and seafoods. Legumes, dark green leafy vegetables, and some dried fruits, like raisins, among other foods shown in Table 13.2 also contribute dietary iron. Although iron is fairly widely distributed in food, its content in an average American diet is estimated at 5–7 mg iron per 1,000 kcal.

Figure 13.2 Heme iron, a metalloporphyrin.

In addition to the amount of iron found naturally in foods, foods such as breads, rolls, pasta, cereals, grits, and flour are often fortified with iron. Fortified flour, for example, contains 20 mg of iron per lb, and corn grits, corn meal, and rice contain from 13 to 26 mg/lb. Pasta has 13–16.5 mg/lb, and bread, rolls, and buns contain 12.5 mg iron per lb. Elemental iron, ferrous ascorbate, ferrous carbonate, ferrous citrate, ferrous fumarate, ferrous gluconate, ferrous lactate, ferric ammonium citrate, ferric chloride, ferric citrate, ferric pyrophosphate, and ferric sulfate are approved and used for food fortification.

Oral iron supplements, providing 30 mg or more of ferrous iron, are available in complexes with sulfate, succinate, citrate, lactate, tartrate, fumarate, and gluconate. These supplements provide nonheme iron and are used typically to treat iron deficiency. Amino acid–iron chelates, such as iron glycine, are also available; however, iron administered as a chelate has not been shown to be absorbed better than iron given as ferrous sulfate or ferrous ascorbate. Use of iron supplements is often associated with gastrointestinal problems including abdominal pain, nausea, and constipation. Iron dextrans also can be administered intravenously to correct iron deficiency if oral supplementation is not effective or contraindicated. Multivitamin/mineral supplements generally contain similar forms of iron as those found in iron supplements; the iron

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Table 13.1 Approximate Body Content, Selected Functions, Deficiency Symptoms, Food Sources, and Recommended Dietary Allowance (RDA) or Adequate Intake (AI) for the Essential Trace and Ultratrace Minerals

Mineral	Approximate Body Content	Selected Physiological Roles	Selected Enzyme Cofactor Roles	Selected Deficiency Symptoms	Selected Food Sources	RDA or AI* (Adults)
Chromium	4–6 mg	Possibly potentiates insulin signaling		Glucose intolerance, glucose and lipid metabolism abnormalities	Grape juice, broccoli, meats, cereals, Brewer's yeast	35 µg* male; 25 µg* female
Copper	50–150 mg	Iron use; synthesis of collagen, pigment, neurotransmitters	Superoxide dismutase, lysyl oxidase, cytochrome c oxidase, dopamine monooxygenase, amine oxidase, peptidylglycine α-amidating monooxygenase	Anemia, neutropenia, bone and blood vessel abnormalities, impaired immune function	Liver, shellfish, nuts, seeds, legumes, meat, fish	900 µg
Iodine	15–20 mg	Thyroid hormone synthesis		Enlarged thyroid gland (goiter)	Iodized salt, salt-water seafood, dairy products, liver, eggs, legumes	150 µg
Iron	2–4 g	O_2 transport and use; amino acid metabolism; antioxidant; carnitine, collagen, and thyroid hormone synthesis	Catalase, cytochromes, myeloperoxidase, thyroperoxidase, lysine and proline dioxygenases, phosphoenolpyruvate (PEP) carboxykinase	Anemia, fatigue, impaired work performance, decreased resistance to infection	Organ meats (liver), meat, molasses, clams, oysters, nuts, legumes, green leafy vegetables, dried fruits, enriched/whole grains	8 mg male; 18 mg female
Manganese	10–20 mg	Brain and CNS function, collagen, bone, growth, urea synthesis, glucose and lipid metabolism	Glucosyltransferase, arginase, pyruvate carboxylase, PEP carboxykinase, superoxide dismutase, glutamine synthetase	In animals, possibly humans: impaired growth, skeletal abnormalities, impaired CNS function	Wheat bran, legumes, hazelnuts, blueberries, pineapple, seafood, poultry, meat, nuts	2.3 mg* male; 1.8 mg* female
Molybdenum	2 mg	Metabolism of purines, pyrimidines, pteridines, aldehydes; oxidation	Xanthine oxidoreductase, aldehyde oxidase, sulfite oxidase, amidoxime reductase	Hypermethioninemia, urinary xanthine, sulfite excretion, urinary sulfate and urate excretion	Legumes, meat, poultry, fish, grains	45 µg
Selenium	20 mg	Protection against hydrogen peroxide and free radicals, thyroid hormone production	Glutathione peroxidase, 5′-deiodinase, thioredoxin reductase, selenoprotein P, selenophosphate synthesis, among other selenoproteins	Myalgia, cardiac myopathy, poor growth, abnormal sulfur metabolism	Oysters, tuna, meat, poultry, fish, cereals, Brazil nuts	55 µg
Zinc	1.5–3.0 g	Nutrient metabolism, collagen formation, alcohol detoxification, carbon dioxide elimination, sexual maturation, cell replication and growth	DNA-RNA polymerase, carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, alkaline phosphatase, deoxythymidine kinase, superoxide dismutase	Poor wound healing, subnormal growth, anorexia, abnormal taste/smell; impaired reproductive system development	Oysters, beef, liver, poultry, whole grains, legumes	11 mg male; 8 mg female

* Indicates Adequate Intake values.

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Table 13.2 Iron Content of Selected Foods*

Selected Foods/Food Group	Iron (mg)
Meat	
Liver, beef (3 oz)	5
Beef, eye of the round (3 oz)	2
Ground beef (3 oz)	2
Turkey, dark meat (3 oz)	2
Chicken or turkey, white meat (3 oz)	1
Pork, loin (3 oz)	1
Seafood	
Clams, mixed species (3 oz)	9
Oysters, Eastern (3 oz)	6
Cod, Atlantic (3 oz)	0.7
Salmon, Atlantic (3 oz)	1
Grouper (3 oz)	1
Legumes, variety, cooked (1/2 c)	1.8–2.2
Vegetables	
Spinach, cooked (1/2 c)	3.2
Peas, green (1/2 c)	1.2
Collards (other greens), cooked (1 c)	2.2
Broccoli, cooked (1 c)	1.1
Potato, baked with skin (1 medium)	2
Fruits	
Raisins, seedless (1/4 c)	0.7
Apricots, dried (1/4 c)	1.0
Peaches, dried (1/4 c)	0.8
Raspberries (1/2 c)	0.4
Blueberries (1/2 c)	0.2
Apple (1 c sliced)	<0.1
Tomato (1 medium)	0.3
Nuts	
Almonds, pistachio, and walnuts (1 oz)	0.8–1
Cashews (1 oz)	1.9
Grains	
Pasta, enriched (1 c)	1.5
Quinoa (1/2 c)	1.3
Rice, brown, enriched (1 c)	0.6
Rice, enriched (1/2 c)	1.4
Bread, whole wheat (1 slice)	0.7
Bread, white, enriched (1 slice)	1.1
Other	
Milk, 1% fat (1 c)	0.4
Egg, hard boiled (1)	0.6
Cheese, cheddar (1 oz)	0.2

* The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

content of most multinutrient supplements is, however, lower, ranging from about 8 to 18 mg. The Daily Value, provided on foods and supplement labels, for iron is 18 mg.

Digestion, Absorption, Transport, and Storage

Figure 13.3 provides an overview of iron digestion, absorption, and transport, as well as some of iron's fates in the enterocyte.

Heme Iron Digestion and Absorption

Heme iron must be hydrolyzed from the globin portion of hemoglobin and myoglobin before absorption. This digestion is accomplished by proteases in both the stomach and the small intestine and results in the release of heme from the globin. Heme, containing the iron bound to the porphyrin ring (also called a metalloporphyrin), remains soluble in the presence of the degradation products (amino acids and peptides) of globin, and is readily absorbed intact in the duodenum and proximal jejunum. A heme carrier protein (hcp1) transports the heme across the brush border membrane. A proton-coupled folate transporter (PCFT) also has been identified as a heme carrier but is not thought to contribute substantially to heme absorption. About 25% (range 15–35%) of heme iron from foods is absorbed. Within the enterocyte, heme's porphyrin ring is hydrolyzed by heme oxygenase into ferrous iron and protoporphyrin. This released iron is thought to associate with cytosolic proteins. The iron is either used by the enterocyte, stored as ferritin, excreted with the sloughing of the enterocytes, or, following transport out of the enterocyte, used by other body tissues.

Nonheme Iron Digestion and Absorption

Nonheme iron, which is typically bound to components of foods, must be freed (hydrolyzed) in the gastrointestinal tract to be absorbed. Hydrochloric acid in the stomach and proteases in the stomach and small intestine aid in the release of nonheme iron, mostly as Fe^{3+}, from some food components. These actions in the stomach also result in the reduction of some, but not all, ferric iron (Fe^{3+}) to the ferrous state (Fe^{2+}). Ferrous iron remains fairly soluble in both the acidic environment of the stomach and the more alkaline environment of the small intestine.

Ferric iron that passes from the stomach into the small intestine mixes with alkaline juices secreted into the intestine from the pancreas. In this more alkaline environment, some ferric iron may complex to produce ferric hydroxide ($Fe(OH)_3$), a relatively insoluble compound that tends to aggregate and precipitate, making the iron less available for absorption. Alternately, ferric iron may undergo reduction to a ferrous state by one or more reductases found primarily on the enterocyte's (duodenum's) brush border membrane. These reductases include cytochrome b reductase 1, cytochrome b (558) ferric cupric reductase, and six-transmembrane epithelial antigen of the prostate (steap) 2. Vitamin C may be needed for the activity of ferric cupric reductase, which also has the ability to reduce copper from the cupric (Cu^{2+}) to the cuprous state (Cu^{1+}) [1].

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1. Iron is released from bound food components. Some HCl in the stomach may reduce Fe^{3+} to Fe^{2+}.
2. Free heme is absorbed intact by heme carrier protein 1(hcp1) , located primarily in the proximal small intestine.
3. Within the enterocyte, heme is catabolized by heme oxygenase to protoporphyrin and Fe^{2+}.
4. Nonheme iron in the small intestine may react with one or more inhibitors, which promote the fecal excretion of iron.
5. Any of three reductases, cytochrome b reductase 1, cytochrome b (558) ferric cupric reductase, and six-transmembrane epithelial antigen of the prostate (steap) 2, may reduce Fe^{3+} to Fe^{2+}.
6. Divalent metal transporter 1(DMT1) carries Fe^{2+} across the brush border membrane into the cytosol of the enterocyte, although endocytosis of DMT1 as part of transcytosis may also enable iron absorption.
7. Fe^{2+} may bind to poly (rC)-binding protein or a yet unidentified protein for transport in the cytosol; iron may also be used within the cell or stored as part of ferritin.
8. Ferroportin transports iron across the basolateral membrane. Iron transport is coupled with its oxidation to Fe^{3+} by hephaestin.
9. Fe^{3+} attaches to transferrin for transport in the blood.

Figure 13.3 Iron digestion, absorption, enterocyte use, and transport.

The main transporter for ferrous iron in the brush border membrane of intestinal cells is divalent cation (also called mineral) transporter 1 (abbreviated DCT or DMT); hereafter, the transporter is abbreviated DMT1. In the gastrointestinal tract, DMT1 transporters are found primarily in the duodenum and transport primarily iron (but only in its ferrous state) and to a lesser extent, other minerals such as zinc, manganese, copper, nickel, and lead. Mineral transport using DMT1 is coupled with H^+ transport (symport) into the enterocyte. DMT1 operates at acidic pH (about 5.5). Synthesis of DMT1 is affected by iron status, with increased transporter synthesis associated with the presence of low cellular iron, and decreased expression of DMT1 associated with increased enterocyte iron concentrations. Hypoxia (low blood oxygen) also triggers an increase in DMT1 gene expression via the production of specific transcription factors (hypoxia-inducible factor 2α).

The mechanism by which ferric iron is absorbed is not clearly delineated and likely plays only a minor role in iron uptake. A membrane protein called integrin may facilitate ferric iron absorption across the enterocyte's brush border membrane. Integrin is thought to exist as part of the paraferritin complex, which includes an intracellular iron transport protein, mobilferrin, and a flavin-dependent ferrireductase.

Factors Influencing Iron Absorption

Several chelators/ligands may bind with nonheme iron to either inhibit or enhance its absorption. **Chelators** are small organic compounds that form a complex with a metal ion. Ligands are compounds that also bind or complex

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with minerals. Whether chelated iron or iron attached to a ligand is absorbed or not absorbed depends in part on the nature of the iron chelate ligand complex. If the iron chelate ligand complex maintains solubility and the iron is loosely bonded, the iron typically can be released at the enterocyte and absorption is enhanced. However, if the iron chelate ligand is strongly bonded and insoluble, iron is not absorbed; this iron is then excreted in the feces as part of the chelate ligand.

Enhancers of Nonheme Iron Absorption Some dietary factors known to enhance nonheme iron absorption include:

- sugars, especially fructose and sorbitol
- acids, such as ascorbic, citric, lactic, and tartaric
- meat, poultry, and fish or their digestion products
- mucin.

Sugars, such as fructose and sorbitol, are thought to form chelates or serve as ligands for iron. Ascorbic acid (vitamin C), along with citric, lactic, and tartaric acids, for example, acts as a reducing agent and forms a chelate with nonheme ferric iron at an acidic pH. Meat, poultry, and fish factors that enhance nonheme iron absorption have not been clearly identified, although digestion products from animal tissues high in the contractile proteins actin and myosin and the amino acids cysteine and histidine have been shown to promote iron absorption [2].

The amount of iron available for absorption can be estimated from the quantity of vitamin C and meat, fish, or poultry that is ingested with the nonheme iron source, assuming ~500 mg body iron stores. Seventy-five units of ascorbic acid or meat, fish, or poultry (MFP) factor (one unit = 1.3 g of raw or 1 g of cooked meat, fish, or poultry or 1 mg of ascorbic acid) has been shown to maximize iron absorption when consumed with the iron source [3]. Units in excess of 75 seem to have no further benefit. The absence of enhancing factors predicts a nonheme iron absorption of only 2–3%, but 75 units of these factors can increase absorption of nonheme iron to 8% (some suggest up to 20% if the person is also iron deficient) [4].

Mucin, an endogenously synthesized chelator, is a small protein made in both gastric and intestinal cells. Gastric mucin (sometimes called gastroferrin) is released into the lumen of the gastrointestinal tract, although some mucin is also found on the brush border membrane of intestinal cells. Mucin binds multiple ferric iron atoms (as well as possibly zinc and chromium) at an acid pH and maintains ferric iron solubility in the alkaline pH of the small intestine to enhance iron absorption. Histidine, ascorbic acid, and fructose, other chelators of iron, are thought to donate the iron to mucin in the small intestine. In addition to the aforementioned compounds, a more acidic environmental pH such as that found in the upper duodenum favors iron absorption.

Inhibitors of Nonheme Iron Absorption Many dietary factors inhibit iron absorption, including:

- polyphenols such as tannin derivatives of gallic acid, chlorogenic acids, monomeric flavinoids, and polyphenolic polymerization products (found in tea and coffee)
- oxalic acid (found in spinach, chard, berries, chocolate, and tea, among other sources)
- phytic acid, also referred to as phytate, inositol hexaphosphate, or polyphosphate (found in whole grains, legumes, lentils, nuts, and seeds)
- phosvitin, a protein containing phosphorylated serine residues (found in egg yolks)
- divalent cations such as calcium, zinc, and manganese.

Polyphenols or polyphenolic compounds, when consumed with a source of nonheme iron, can reduce iron absorption by over 50%. Coffee consumption, with or just after a meal, may reduce iron absorption by 40%.

Phytic and oxalic acids complex with iron, as well as zinc and copper, among other minerals. The phytic acid–mineral and oxalic acid–mineral complexes are insoluble and poorly absorbed. Fermentation of bread dough reduces the phytic acid content and improves the absorption of some minerals, but, in general, mineral absorption is better without the presence of phytic or oxalic acids. (Figure 13.11, in the section on zinc, shows the structures of both phytic acid and oxalic acid.) Individuals in the United States consuming primarily a plant-based, high-phytic-acid diet may not absorb sufficient iron and be at increased risk for deficiency.

Some minerals reduce iron absorption. Calcium in amounts of 300–600 mg and in many forms (such as calcium phosphate, calcium citrate, calcium carbonate, and calcium chloride and in milk), when given with up to 18 mg of iron as ferrous sulfate or when incorporated into food, substantially decreases iron (both heme and nonheme) absorption. The inhibitory effect, however, appears to be of short duration, and adaptation results such that iron status is not negatively impacted [5]. Specifically, calcium transiently (for about 4 hours) inhibits iron absorption by causing the iron transporter ferroportin to relocate temporarily from the enterocyte's basolateral membrane to the cytosol; DMT1 availability and changes in membrane fluidity, however, may also play roles in reducing iron absorption [5]. (See the next section for a discussion on the role of ferroportin.) In addition to calcium, zinc and manganese also interact with nonheme iron and may negatively affect absorption by an undefined mechanism. Inhibition of absorption (often by greater than 50%) of iron (usually as ferrous sulfate) has been demonstrated with the coingestion of zinc, usually as zinc sulfate, in solution, and in amounts greater than those of iron [6]. However, such interactions are not thought to occur with typical ingestion of the nutrients from food sources.

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Other intraluminal factors inhibitory to iron absorption include rapid transit time, malabsorption syndromes, achylia (absence of digestive juices), and excess alkalinization (i.e., increased pH) of the gastrointestinal tract. The alkalinization of the gastrointestinal tract is frequently associated with the use of medications (antacids, H_2 receptor blockers [such as Zantac (ranitidine), Tagamet (cimetidine), or Pepcid (famotidine)] and proton pump blockers [such as Prevacid (lansoprazole) or Prilosec (omeprazole)]) that are commonly taken to treat heartburn, gastroesophageal reflux disease (GERD), and ulcers. A more alkaline environment in the gastrointestinal tract may also occur with aging due to age-related reductions in gastric acid production.

Overall absorption of iron from the U.S. diet is estimated at about 14–18%, but a person's iron status also affects iron absorption. Absorption, for example, may range from about 10% (for persons with normal iron status) up to about 35% (for persons who are iron deficient). In other words, iron absorption can rise to 3–6 mg daily when the body has low iron stores and can fall to 0.5 mg or less daily when iron stores are high. Additional information on the regulation of iron absorption follows the section about intestinal cell iron use. A review of algorithms used to predict iron availability reports that the effects of iron status on iron absorption can be estimated based on selected biochemical indicators of iron status [7]. Absorption of about 1–2 mg of iron each day is needed to balance out the body's usual daily iron losses.

Intestinal Cell Iron Use

The preceding sections have reviewed digestion and absorption, including factors inhibiting and enhancing iron absorption into the enterocyte. Following absorption across the enterocyte's brush border membrane, iron enters the cytosol of the cell. Because of the potential for free iron to initiate oxidative damage, little iron is thought to exist unbound within the enterocyte (or any cell). However, at present, it is not clear what substance(s) iron attaches to within cells. Possible cytosolic carriers include amino acids such as cysteine and histidine, organic anions such as phosphate, citrate, and carboxylate, and protein(s). One identified protein, poly (rC)-binding protein 1 (PCBP1), which binds up to three iron atoms with high affinity, delivers iron to ferritin for storage. Iron transport into the mitochondria is mediated by proteins known as mitoferrins. Another possibility for iron transport within cells is transcytosis, whereby DMT1-bound ferrous iron is endocytosed into the cell and carried through the cytosol. Additionally, ferric iron has been reported to interact with a membrane receptor, integrin, and then bind to a cytosolic protein, mobilferrin. However, studies using DMT1 knock-out mice suggest that DMT1 mediates most iron absorption into enterocytes.

As depicted in Figure 13.3, once in the enterocyte, iron is either:

- used by the intestinal cell in a functional capacity (see "Functions and Mechanisms of Action")
- stored in ferritin
- transported across the enterocyte's basolateral membrane, entering circulation for transport to body tissues.

Iron that is not needed for functional uses may be incorporated into (apo)ferritin in the intestinal cell for short-term storage. Stored iron may be released over the next few days should it be needed by the enterocyte or other (nonintestinal) cells. If not needed, the iron remains attached as part of ferritin and is excreted when the short-lived (3 days) enterocytes are sloughed off into the lumen of the gastrointestinal tract.

Iron transport across the enterocyte's basolateral membrane requires the iron to bind to the membrane transport protein ferroportin (fp or fpn). This transport of iron is also coupled with iron's oxidation to Fe^{3+} by a copper-containing protein called hephaestin (or, in the absence of hephaestin, by the copper-containing protein ceruloplasmin). Hephaestin is found on the enterocyte's basolateral membrane near ferroportin. This role of copper as part of hephaestin and ceruloplasmin in the oxidation of iron is crucial to iron metabolism. In fact, copper deficiency results in decreased ceruloplasmin and hephaestin, iron accumulation in cells, and reduced iron transport to tissues. The copper-dependent oxidation of iron to its ferric state allows the transport of iron in the blood as part of the protein transferrin. Specifically, transferrin carries one or two ferric iron atoms (becoming monoferric transferrin or diferric transferrin, respectively) for delivery to body tissues.

Regulation of Iron Absorption

Iron absorption is regulated by body iron and cellular iron. Some of these regulatory mechanisms are reviewed hereafter and are shown later in Figure 13.4.

Body Iron Regulation The main regulator of iron absorption and body iron metabolism is a small protein called hepcidin. This protein is released primarily from the liver when iron stores are high. Hepcidin functions to reduce intestinal iron absorption and to reduce cellular iron release. Specifically, hepcidin (upon release from the liver and travel in the blood attached primarily to α-2-macroglobulin) targets ferroportin on the basolateral cell membranes of enterocytes and on the cell membranes of macrophages. Hepcidin binds ferroportin, resulting in internalization and degradation (via ubiquitin and lysosomes) of both the hepcidin and the ferroportin. With the hepcidin-induced loss of ferroportin from the cell membranes, iron cannot be transported out of enterocytes or out of macrophages and thus cannot be released into the blood for use by other

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tissues. Consequently, increased hepcidin concentrations result in increased enterocyte and macrophage iron concentrations. In the case of the enterocyte, the availability of newly absorbed iron to the body is decreased. Conversely, when little iron is present, little hepcidin is produced, and ferroportin concentrations on enterocyte and macrophage cell membranes remain sufficient for iron export out of the cells. This situation enables iron release into the blood for distribution and use by body tissues.

The liver recognizes the body's iron status based on transferrin saturation, or more specifically by the binding of diferric transferrin to transferrin receptors 1 (TfR1) on its cell membranes. This binding in turn releases a high-iron (HFE) protein that is normally attached to hepatocyte TfR1; this released HFE protein then binds to TfR2, another type of transferrin receptor present on hepatocyte membranes (Figure 13.4). The HFE–TfR2 complex is thought to serve as a "sensor" or "signal" of the body's iron status and in turn stimulates hepcidin synthesis at the transcription level through mitogen-activated protein kinase (MAPK) intracellular signaling. In contrast, in the presence of low plasma iron (i.e., reduced transferrin saturation), TfR1 remains attached to HFE and prevents its interaction with TfR2. Without the intracellular signaling cascade, hepcidin transcription is repressed.

Another pathway, referred to as BMP6/SMAD, also affects hepcidin synthesis and is shown in Figure 13.4. BMP (bone morphogenic protein) 6 is secreted from liver cells as its iron content increases. In this pathway, BMP6 binds to hepatic membrane receptors for BMP6 and hemojuvelin (HJV); hemojuvelin serves as a coreceptor and is required for BMP6 receptor signaling for hepcin production. The formed complex promotes phosphorylation of several SMAD (an abbreviation for small mothers against decapentaplegic) proteins, and upon subsequent binding of SMAD4, the complex translocates into the nucleus and interacts with response elements in the promoter region of the gene for hepcidin. This interaction enhances hepcidin gene transcription. In contrast, with low cellular iron, BMP6 is not released from the liver and signaling through SMAD is reduced.

Defects in any of the genes coding for proteins that are involved in the signaling pathways modulate hepcidin synthesis, and thus impact iron status. Transmembrane serine protease s6 (TMPRSS6; also called matriptase-2), for example, degrades hemojuvelin and thus reduces hepcidin synthesis. An absence of hepcidin (e.g., due to a genetic defect) enables the sustained presence of ferroportin on cell membranes and thus continued iron absorption/uptake, cellular accumulation, and toxicity (see the "Toxicity" section). In contrast, mutations in the matriptase-2 gene cause overproduction of hepcidin and result in iron-refractory iron-deficiency anemia. Iron deficiency is refractory when after about 4–6 weeks of treatment with iron, hematological parameters fail to rise (normally, hemoglobin concentrations should rise at least 1 g with iron therapy).

Hepcidin and Inflammation/Infection Other triggers of hepcidin synthesis include inflammation and infection. In fact, under such conditions, macrophages and neutrophils also produce hepcidin. As discussed in Chapter 6's Perspective, macrophages detect pathogen-associated, as well as damage-associated, molecular patterns

*See text for abbreviations.

Figure 13.4 Overview of some pathways stimulating hepcidin synthesis.

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(PAMPs and DAMPs). These products of damaged and necrotic cells interact with pattern-recognition receptors, such as Toll-like receptors, among others, on macrophages (and other immune system cells) and promote the production and secretion of cytokines such as tumor necrosis factor α and interleukin (IL)6. IL6, upon binding to IL6 receptors on hepatocytes, triggers "Janus kinase (JAK)" and "signal transducer and activator of transcription" (STAT) 3 signaling. STAT, following phosphorylation, translocates into the nucleus and interacts with STAT-sites in the promoter region of the hepcidin gene to enhance transcription. Because hepcidin inhibits iron release from enterocytes as well as from macrophages, these cells accumulate iron. If the infection or inflammation is prolonged, serum/blood iron concentrations may drop (hypoferremia), creating a condition commonly referred to as the anemia of chronic disease. Other factors, such as tumor necrosis factor α, however, also contribute to reductions in iron absorption and reduce serum iron concentrations, especially during the initial stages of inflammation.

Tissue Oxygen and Erythopoietic Activity In contrast to inflammation and infection, conditions such as hypoxia decrease hepcidin synthesis. Hypoxia is detected initially by *hypoxia inducible factor* in the kidneys and stimulates erythropoietin release. Erythropoietin, in turn, stimulates hematopoiesis in the bone marrow. While the mechanism(s) has/have not been clearly elucidated, hypoxia inducible factor appears to increase the hepatic expression of matriptase-2, which disrupts signaling needed for hepcidin gene transcription. Without hepcidin, iron is released from intestinal cells and from macrophages and used functionally in erythroblasts for the synthesis of heme.

Cellular Iron Regulation In addition to hepcidin's role in the regulation of iron absorption and body iron utilization, a cell's iron content affects iron absorption/uptake and utilization. The regulation involves cytosolic iron regulatory proteins (IRPs, also called binding proteins), which interact with mRNA to alter the translation of proteins needed for the body's use of iron (Figure 13.5).

When little cellular iron is present, IRPs exist as 3Fe-4S (iron sulfur) clusters (ISC) and function as binding proteins. IRPs bind to iron response elements (IREs), which are stem loop structures of about 30 nucleotides located in specific 3′ and 5′ untranslated regions of mature mRNAs. These IREs are present in the mRNA for several proteins involved with iron, including transferrin receptors, ferritin, cytochrome b reductase, ferroportin, and DMT1, among others. In low-iron situations, IRPs bind to an IRE in the 5′ untranslated region of ferritin mRNA and act as a repressor to inhibit ribosome assembly

Figure 13.5 The influence of intracellular iron on the translation of ferritin mRNA and transferrin receptor mRNA.

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and translation. Thus, less ferritin is made in cells when the cellular iron content is low. From a physiological standpoint, this inhibition makes sense because ferritin stores iron, and not much ferritin would be needed if the cell's iron content was low. Simultaneously, with low cellular iron, the translation of mRNAs for ferroportin, transferrin receptors, duodenal cytochrome b reductase, and DMT1 is enhanced with IRPs binding to specific IREs in the 3′ untranslated region of mRNA for these proteins. These actions increase the synthesis of proteins, which enhance iron absorption by enterocytes and iron uptake by extraintestinal cells including erythroblasts. From a physiological standpoint, this too makes sense since under conditions of low iron, such changes facilitate iron absorption/uptake and utilization.

Under the opposite conditions, in which enterocytes (and other cells) have relatively high iron contents, IRPs contain 4Fe-4S clusters, are unable to bind to IREs, and exhibit aconitase (a TCA cycle enzyme) activity. In the case of ferritin, without interactions between IRPs and IREs in the mRNA for ferritin, the ferritin mRNA undergoes translation and more ferritin is made. Without interactions between IRPs and IREs in the mRNAs for ferroportin, transferrin receptors, duodenal cytochrome b reductase, and DMT1, the translation of these mRNAs is reduced and less iron is absorbed into enterocytes and taken up by extraintestinal cells.

Further regulation occurs under conditions of hypoxia. Reductions in oxygen tension within enterocytes increase iron absorption. The increase is mediated by the production of a transcription factor called hypoxia inducible factor 2α, which enhances the translation of mRNA for both DMT1 and ferroportin and promotes iron absorption.

Transport

Iron in its oxidized ferric state is transported in the blood attached to the protein transferrin. Transferrin, a glycoprotein made primarily in the liver, has two binding sites for minerals, one near its carboxy (C)–terminal end and the other near its amino (N)–terminal end. Both binding sites have a high affinity for ferric iron, but the one near the amino (N)-terminal end also binds other minerals, such as chromium, followed in descending order by copper, manganese, cadmium, zinc, and nickel. The binding of ferric iron to transferrin requires the presence of an anion, usually bicarbonate, at each binding site. The plasma iron pool typically contains about 3–4 mg of iron bound to transferrin. Transferrin is typically one-third (33%, range about 30–40%) saturated with ferric iron and nontransferrin-bound iron (NTBI) is usually undetectable in the blood under normal physiological conditions. (Note: If all of transferrin's binding sites were occupied [e.g., as in a toxicity situation], then the transferrin would be fully [100%] saturated; it is also under toxicity situations that NTBI increases.)

The role of proteins in the transport as well as storage of iron is important because of iron's redox activity. The binding of iron by proteins serves as a protective mechanism. When iron is left unbound, its redox activity can lead to the generation of harmful free radicals. Free ferrous iron (Fe^{2+}), for example, readily reacts with hydrogen peroxide (H_2O_2) in what is known as the Fenton reaction: $Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$. This reaction generates a hydroxyl anion and a free hydroxyl radical (${}^{\bullet}OH$), which is extremely reactive and damaging to cells. In addition, the binding of iron by protein is important to ensure that bacteria that may be present in the body, as with an infection, are unable to use the iron for their own (bacterial) growth. Free iron—but not protein-bound iron—is readily used by bacteria for proliferation and growth. Bacteria cannot multiply without nutrients, such as iron, acquired from the host. Thus, keeping iron attached to proteins in the body diminishes bacterial replication.

Transferrin binds and transports not only newly absorbed dietary iron that has crossed the basolateral membrane of the enterocyte, but also iron that has been released following the degradation of iron-containing compounds in the body. In fact, the overwhelming majority of iron (about 20–25 mg) entering the plasma for distribution by transferrin is contributed from hemoglobin degradation. The transferrin iron pool turns over five to eight times each day. Transferrin has a half-life of about 7–10 days.

Cellular Iron Uptake The amount of iron taken up by tissues depends in part upon transferrin's saturation level and the presence of transferrin receptors on cell membranes. For example, iron delivery is greater from diferric transferrin (transferrin containing two bound iron atoms) than from monoferric transferrin (transferrin containing only one bound iron atom). The mono- and diferric transferrin bind to transferrin receptors (TfRs) on cell membranes. Most cell membranes contain a form of transferrin receptors abbreviated TfR1; liver and intestinal cells, however, also contain an isoform known as TfR2, which preferentially binds diferric transferrin. Transferrin receptors consist of two subunits; each subunit binds one transferrin molecule.

Once the transferrin molecule with its bound iron attaches to the transferrin receptor, a complex is formed, as shown in Figure 13.6. The transferrin receptor–transferrin complex is next internalized by endocytosis and forms a vesicle (also called an endosome) within the cell's cytosol. Next, in an ATP-dependent process, protons are pumped into the endosome and reduce the pH from about 7.4 to about 5.5. In the presence of the acidic pH and possibly other factors, ferric iron atoms are released from the transferrin molecule. The apotransferrin then returns to the plasma and receptor returns to the membrane. Use of

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1. Transferrin with its bound $Fe3^+$ atoms attaches to transferrin receptors on the cell membranes and following attachment, the complex is endocytosed into the cell cytosol where it forms an endosome.
2. A drop in pH in the endosome helps initiate the release of $Fe3^+$ which is then reduced by steap3 and transported out of the endosome by a transporter such as DMT1.
3. The Fe released from the endosome may be oxidized and stored as part of ferritin, or
4. The Fe may be used within the cell functionally such as for heme synthesis, or
5. The Fe may be oxidized by ceruloplasmin (Cp) and exported from the cell via ferroportin (Fpn).

Figure 13.6 Overview of cellular iron uptake.

the released ferric iron requires reduction and transport across the endosomal membrane; however, in some cells such as reticulocytes, the endosomes appear to provide the iron directly to the mitochondria to enable heme synthesis [8]. In other cells, reduction of ferric iron to a ferrous state is thought to be accomplished by the ferrireductase activity of steap3. DMT1 transports the ferrous iron out of the endosome and into the cytosol. Iron released from the endosome is typically transported to other sites for use, although it may be stored in ferritin.

The number of transferrin receptors on cell membranes increases or decreases depending on intracellular iron concentrations. In other words, intracellular iron affects the genetic expression of transferrin receptors. As discussed in the previous section, in a low-cellular-iron situation, IRPs interact with the IRE and stabilize the transferrin receptor mRNA. This stabilized transferrin receptor mRNA exhibits a longer half-life, and consequently more transferrin receptors are synthesized. Once made, these transferrin receptors become embedded in the cell's plasma membrane to promote cellular iron uptake. Thus, in conditions of low cellular iron, transferrin receptor synthesis is increased. If the intracellular iron concentration is adequate or relatively high, fewer transferrin receptors are translated and less iron is brought into the cell. Thus, transferrin receptor expression indicates the cell's need for iron uptake.

Storage

Iron not needed in a functional capacity is stored in three main sites: the liver, bone marrow, and spleen. Transferrin delivers iron to these sites, especially the liver, which stores about 60% of the body's iron in both reticuloendothelial (RE) cells and hepatocytes. The remaining 40% is found in reticuloendothelial cells within the spleen and bone marrow (and possibly between muscle fibers). Most of the stored iron is derived from phagocytosis of red blood cells and subsequent degradation of the hemoglobin within those cells. Stored iron may be released for subsequent cellular use during times of increased needs; the released iron requires ferroportin for transport across the cell membrane and ceruloplasmin for oxidation to its ferric state and thus binding to transferrin.

$$Fe^{2+} \longrightarrow Fe^{3+} \text{(which can now bind to transferrin)}$$
$$\text{Ceruloplasmin-}Cu^{2+} \longrightarrow \text{Ceruloplasmin-}Cu^{1+}$$

Ferritin is the primary storage form of iron in cells; the role of iron in the control of ferritin synthesis was previously described in the "Regulation of Iron Absorption" section. The protein is initially synthesized as apoferritin, containing no iron atoms; it has a hollow spherelike shape

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and is composed of 24 protein subunits. Once iron enters apoferritin, the protein is referred to as ferritin. Ferritin's subunits are classified based on molecular mass as heavy (H) or light (L), and the proportions of H and L subunits within ferritin vary among tissues. The L form, for example, predominates in the liver and spleen and takes up iron rather slowly, compared with the H form, which is found in higher amounts in the heart and red blood cells. Iron enters apoferritin through channels or pores. The pores serve as the site of the oxidation of the ferrous iron and generate ferric oxyhydroxide crystals ($4Fe^{2+} + O_2 + 6H_2O \longrightarrow 4FeOOH + 8H^+$) and ferrihydrite ($5Fe_2O_3 + 9H_2O$); molecular oxygen functions as the electron acceptor. Ferric oxyhydroxide and ferrihydrite are deposited in the interior of the protein shell. As many as 4,500 iron atoms can be stored in ferritin; however, the protein more commonly stores about 800–1,500 atoms.

Ferritin is constantly being degraded and resynthesized, providing an available intracellular iron pool. Equilibration occurs between tissue ferritin and serum ferritin. Thus, serum ferritin is used as an index of body iron stores: 1 ng of ferritin/mL serum equals ~10 mg of body iron stores. Normal serum ferritin concentrations (for adults) typically range from about 18 to 300 ng/mL; however, ferritin concentrations increase secondary to inflammation and infection/illness and under these conditions do not reflect iron stores. In other words, serum ferritin concentrations may be elevated or within the normal range in the blood, despite an individual's having little iron stores. Methods of assessing iron status are described further in the "Assessment of Nutriture" section.

Hemosiderin is another iron storage protein. Hemosiderin is thought to be a degradation product of ferritin, representing, for example, aggregated ferritin or a deposit of degraded apoferritin and coalesced iron atoms. The content of iron in hemosiderin may be as high as 50%. The ratio of ferritin to hemosiderin in the liver varies according to the level of iron stored in the organ, with ferritin predominating at lower iron concentrations, and hemosiderin predominating at higher concentrations (iron overload). Although iron in hemosiderin can be labilized to supply free iron, iron is released at a slower rate from hemosiderin than from ferritin. The release of iron from its stores involves both proteosomal and lysosomal-mediated degradation mechanisms. Lysosomal-mediated degradation requires nuclear receptor coactivator 4 (NCOA4), which binds and transports the ferritin to the lysosomes. The released iron is then used within the cell or exported via ferroportin (and oxidized by ceruloplasmin) for transport in the blood by transferrin (see Figure 13.8).

Functions and Mechanisms of Action

Iron functions in the body as part of several proteins, including the dozens of enzymes for which it serves as a cofactor. These iron-dependent proteins are involved in diverse body processes (Table 13.3), as described in this section. Moreover, the iron in the proteins is found in different forms. For example, in many proteins, iron is present as part of heme, while in others, iron is found in a cluster with sulfur (2Fe-2S, 4Fe-4S, or 3Fe-4S), by itself as a single atom, or as part of a bridge with oxygen. Heme proteins represent the largest group and include hemoglobin, myoglobin, and cytochromes, as well as some enzymes. Iron-sulfur proteins also include several enzymes with diverse roles that participate in electron transport, the TCA cycle, and heme synthesis. Proteins that contain single iron atoms are mostly mono- and dioxygenase enzymes, and the one iron-oxygen bridge protein is found in the enzyme ribonucleotide reductase.

Table 13.3 Selected Functions of Iron

Hemoglobin and myoglobin
Electron transport: ATP production
Amino acid metabolism: phenylalanine, tyrosine, tryptophan, and arginine
Niacin synthesis
Carnitine synthesis
Procollagen synthesis
Nitric oxide synthesis
Antioxidant: protection
Destruction of bacteria, viruses, and microbes
Thyroid hormone synthesis
Sulfite oxidation
DNA purine base catabolism
Carbohydrate metabolism
DNA synthesis

Heme: Hemoglobin and Myoglobin — Oxygen Delivery

The essentiality of iron is due in part to its presence in heme, which functions as a prosthetic group for many proteins. The atom of iron in the center of the heme molecule enables oxygen transport to tissues (hemoglobin); transitional storage of oxygen in tissues, particularly muscle (myoglobin); and transport of electrons through the respiratory chain (cytochromes).

Hemoglobin is synthesized in red blood cells and carries about 98.5% of the total oxygen found in the blood. Hemoglobin, a conjugated tetrameric protein, consists of four heme groups and globin, which is made up of four polypeptide chains (see Figure 6.20). Each polypeptide chain is associated with one of the heme molecules. Heme is an iron-containing derivative of porphyrin. Porphyrins, in turn, are cyclic compounds made up of four pyrrole rings joined together by methenyl bridges. Nitrogen atoms in each of the four pyrrole rings bind to the iron atom, and these bonds hold the iron atom in the plane of the porphyrin ring. The iron atom in the center of the heme has two remaining coordinate bonds available for binding. One is with an amino acid (often the nitrogen atom of histidine) of the protein to which the heme is attached. For example, in hemoglobin, the iron in the heme binds to the nitrogen

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of an amino acid in the protein globin; heme is found in a hydrophobic pocket of the protein. The sixth and last coordinate bond in heme proteins that bind oxygen—namely, hemoglobin and myoglobin—is positioned between the iron and oxygen. The oxygen is held quite loosely so that transfer to tissues can be rapid. In heme proteins that do not bind oxygen, the sixth coordinate bond is with atoms of amino acid groups in the protein (such as an enzyme) with which the heme group is associated. Heme synthesis accounts for the largest use of functional iron in the body. In fact, each red blood cell is thought to contain millions of hemoglobin molecules, and all the red blood cells in the body together contain about two-thirds of total body iron. Any one red blood cell may carry up to a billion oxygen atoms.

To synthesize heme for hemoglobin, iron is needed in the erythropoietic cells in the bone marrow. These cells possess transferrin receptors on their plasma membranes; transferrin delivers the iron for heme synthesis to the erythropoietic cells. Mitoferrin transports the iron (following cellular transferrin uptake and degradation and iron release from the endosome) across the mitochondrial membrane, and a mitochondrial matrix chaperone, frataxin, is thought to further transport the iron within the mitochondria, the site where heme synthesis begins. Alternately, degradation of old red blood cells within the bone marrow can directly provide the necessary iron for hemoglobin synthesis without the use of transferrin (see the "Turnover" section).

Briefly, the synthesis of heme (shown in Figure 13.7) occurs as follows:

- Glycine and succinyl-CoA condense to form Δ (also called 5)-amino-levulinic acid (ALA) in the cell's mitochondria. The reaction is catalyzed by **Δ**-aminolevulinic acid synthase, a vitamin B_6–dependent enzyme that is inhibited by the final end product (heme) and whose synthesis is enhanced by an IRP interaction with the IRE in the mRNA for the enzyme when the cell contains ample iron.
- Next, ALA enters the cytosol, where a zinc-dependent dehydratase catalyzes the condensation of two ALA molecules to form porphobilinogen. This enzyme is sensitive to lead, which binds to its sulfhydryl groups to inactivate the enzyme.
- Next, in a series of cytosolic reactions involving a deaminase, a synthase, and a decarboxylase, four porphobilinogens condense to form a tetrapyrrole that cyclizes. Side chains are modified, and coproporphyrinogen III is formed and enters the mitochondria through ATP-binding cassette B6.
- Coproporphyrinogen is converted in the mitochondria to protoporphyrinogen.
- Protoporphyrinogen is oxidized to form protoporphyrin IX.
- Last, an iron (Fe^{2+}) atom is inserted into protoporphyrin IX to yield heme. The insertion of iron into the heme is catalyzed by ferrochelatase, a 2Fe-2S cluster mitochondrial protein. The transcription of the ferrochelatase gene appears to be regulated by iron. Globin chains are added to the heme after it moves out of the mitochondria and into the cytosol.

Unlike the tetrameric hemoglobin, myoglobin consists of a single hemoprotein chain. Myoglobin, which is found in the cytosol of the muscle cells, facilitates the diffusion rate of dioxygen from hemoglobin in capillary red blood cells to the cytosol and mitochondria of muscle cells.

Cytochromes and Other Enzymes Involved in Electron Transport: Energy Production

Heme-containing cytochromes in the electron transport chain, such as cytochromes b and c, pass along single electrons. The transfer of electrons along the chain is made possible by the change in the oxidation state of iron. In the reduced cytochromes, the iron atom is in the ferrous state. The iron atom of the reduced cytochrome becomes oxidized to the ferric state when a single electron is transferred to the next cytochrome. The iron atom of the cytochrome receiving the electron then becomes reduced. Nonheme iron–sulfur enzymes involved in electron transport include NADH dehydrogenase, succinate dehydrogenase, and ubiquinone–cytochrome c reductase. See Chapter 3 for a more thorough discussion of electron transport. Whether iron is carrying oxygen or transporting electrons, its essentiality in energy transformation is without question.

Monooxygenases and Dioxygenases: Amino Acid Metabolism, Carnitine Synthesis, and Procollagen Synthesis

Many additional enzymes involved in a variety of processes besides energy production also require iron. Some monooxygenases, which insert one of two oxygen atoms into a substrate, require a single iron atom to function. Similarly, many dioxygenases, which catalyze the insertion of two oxygen atoms into a substrate, also require iron. Some examples include:

- phenylalanine monooxygenase (needed for amino acid metabolism)
- tyrosine monooxygenase (needed for amino acid metabolism)
- tryptophan monooxygenase (needed for amino acid metabolism)
- tryptophan dioxygenase (needed for amino acid metabolism)
- parahydroxyphenylpyruvate hydroxylase and homogentisate dioxygenase (needed for amino acid metabolism)

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Figure 13.7 Heme biosynthesis. Vinyl group: $CH{=}CH_2$; propionic acid group: $(CH_2)_2COO^-$; acetate group: CH_2COO^-.

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- trimethyllysine dioxygenase and 4-butyrobetaine dioxygenase (needed for carnitine synthesis)
- lysine dioxygenase and proline dioxygenase (needed for procollagen synthesis)
- nitric oxide synthase (needed for amino acid metabolism and involved in signal transduction).

Specifically, the monoxygenases insert an oxygen atom into the aromatic amino acids phenylalanine, tyrosine, and tryptophan, respectively, and use cosubstrates to furnish the hydrogen atoms that reduce the second oxygen atom to water (see Figures 6.10 and 6.11). Tetrahydrobiopterin is frequently used as a cosubstrate, and during the reactions tetrahydrobiopterin is oxidized to dihydrobiopterin. The reactions catalyzed by phenylalanine, tyrosine, and tryptophan monooxygenases are important for amino acid metabolism. In other words, the use of these three amino acids for functions other than protein synthesis (including catecholamine, serotonin, and melatonin synthesis) depends on iron-dependent enzymes.

Heme-containing tryptophan dioxygenase (also called a pyrrolase) converts the amino acid tryptophan to N-formylkynurenine (see Figure 6.11), representing the first step of tryptophan catabolism for the generation of energy and the B-vitamin niacin. Iron deficiency reduces niacin synthesis from tryptophan.

In tyrosine catabolism for energy production (see Figure 6.10), two reactions require iron-dependent enzymes. The conversion of p-hydroxyphenylpyruvate to homogentisate (homogentisic acid) requires the nonheme iron–dependent enzyme p-hydroxyphenylpyruvate hydroxylase and vitamin C. Next, homogentisate dioxygenase, a single-atom iron-dependent enzyme, converts homogentisate to (4-) maleylacetoacetate. Defects in this enzyme result in the genetic disorder alkaptonuria, which is characterized by high concentrations of homogentisic acid in the urine. When this urine is excreted and the homogentisic acid is exposed to air, the compound turns a very dark color, causing the urine to appear almost black. In those with alkaptonuria, the homogentisic acid also accumulates in joints, causing arthritis.

Two of the four steps required for carnitine synthesis involve iron-dependent dioxygenases. Recall that carnitine is an important nitrogen-containing compound necessary for the transport of long-chain fatty acids into the mitochondria for oxidation. The first step in carnitine synthesis (see Figure 6.23), in which trimethyl lysine is converted to 3-OH trimethyl lysine, requires a single iron–containing trimethyl lysine dioxygenase, and the final step, in which 4-butyrobetaine is converted to carnitine, requires 4-butyrobetaine dioxygenase, another single iron–containing enzyme.

Hydroxylation reactions for procollagen synthesis are shown in Figure 9.4. Both lysine and proline dioxygenases contain single iron atoms. These reactions are important for the synthesis of the protein collagen, a component of bone, cartilage, skin, and blood vessels, among other body structures.

Two isoforms of nitric oxide synthase, a dioxygenase needed for the synthesis of nitric oxide from the amino acid arginine, contain heme iron. Nitric oxide is a potent biological effector molecule that is involved in a variety of physiological processes including regulation of blood pressure (relaxation of vascular smooth muscle) and intestinal motility, inhibition of platelet aggregation, macrophage function, and signal transduction, to name a few.

Peroxidases: Antioxidant Roles and Thyroid Hormone Synthesis

Other important reactions required to protect the body also involve iron-containing enzymes.

- Catalase, with four heme groups, converts hydrogen peroxide to water and molecular oxygen: $2H_2O_2 \longrightarrow 2H_2O + O_2$. Catalase is one of the major antioxidant enzymes of the body and thus helps prevent cellular damage that can be induced by hydrogen peroxide (see the Perspective in Chapter 10).
- Myeloperoxidase (also called chloroperoxidase), another heme-containing enzyme, is found in the plasma as well as in neutrophils (white blood cells). During phagocytosis, myeloperoxidase is released into the phagocytic vesicle within the neutrophil. The phagocytic vesicle contains a variety of destructive compounds, including hydrogen peroxide (H_2O_2), free hydroxyl radicals ($^{\bullet}OH$), and other ions such as chloride (Cl^-). Myeloperoxidase catalyzes the following reaction: $H_2O_2 + Cl^- \longrightarrow H_2O + OCl^-$. The OCl^- (hypochlorite) formed in the reaction is a strong cytotoxic oxidant responsible for the destruction of foreign substances, such as bacteria. The activity of myeloperoxidase may be impaired with iron deficiency, resulting in increased susceptibility to or severity of infection.
- Peroxidases are also important in producing the thyroid hormones T_3 and T_4. Thyroperoxidase (also called thyroid peroxidase), a heme-dependent enzyme, is necessary for organification of iodide (a process in which two iodides are added to tyrosine residues on thyroglobulin). This same enzyme also conjugates the thyroglobulins to form the thyroid hormones (see the "Functions and Mechanisms of Action" section in the "Iodine" portion of this chapter). Iron deficiency decreases thyroperoxidase activity, resulting in decreased T_3 and T_4 synthesis [9].

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Oxidoreductases

Some oxidoreductases that are iron- (and also molybdenum-) dependent include:

- aldehyde oxidase, which converts aldehydes (RCOH) to alcohols (RCOOH)
- sulfite oxidase, an iron- and sulfur-containing enzyme, converts sulfite (SO_3) to sulfate (SO_4)
- xanthine oxidoreductase, an iron-sulfur cluster enzyme, metabolizes hypoxanthine generated from DNA purine base catabolism to uric acid.

These reactions are discussed in detail in the "Functions and Mechanisms of Action" section for molybdenum.

Other Iron-Containing Enzymes: Carbohydrate Metabolism, DNA Synthesis, and Lipid, Steroid, and Drug Metabolism

Two enzymes involved in carbohydrate oxidation require iron as a cofactor. In glycolysis, the flavoenzyme glycerol phosphate dehydrogenase has a nonheme iron component. In addition, phosphoenolpyruvate (PEP) carboxykinase, important in gluconeogenesis, requires iron for its functioning.

Another iron-dependent enzyme involved in DNA synthesis, and thus cell replication, is ribonucleotide reductase, which converts ribonucleotides into deoxyribonucleotides. This enzyme contains nonheme iron as part of a bridge with oxygen (Fe^{3+}—O_2— Fe^{3+}).

A few additional heme iron–containing cytochromes include cytochrome b5 involved in lipid metabolism, and the cytochrome P450 family involved in drug metabolism and steroid hormone synthesis.

Iron as a Pro-oxidant

As a pro-oxidant, free ferrous iron may catalyze the nonenzymatic Fenton reaction: $Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$. In this reaction, ferrous iron reacts with hydrogen peroxide to generate ferric iron and the free hydroxyl radical (${}^{\bullet}OH$). In a reaction known as the Haber-Weiss reaction, the superoxide radical, $O_2^{\bullet}$, reacts with hydrogen peroxide to generate molecular oxygen and free hydroxyl radicals: $O_2^{\bullet} + H_2O_2 \longrightarrow O_2 + {}^{\bullet}OH + OH^-$. Hydroxyl radicals (${}^{\bullet}OH$) are dangerous membrane oxidants.

Turnover

Although dietary iron is important in maintaining the long-term adequacy of body iron for its various roles, the amount of iron absorbed (about 0.06% of the total body iron content) cannot meet the daily iron needs of the body. Rather, avid conservation and constant recycling (turnover) of body iron ensure an adequate supply.

Most iron that enters the plasma for distribution or redistribution by transferrin results from hemoglobin and ferritin (also hemosiderin) degradation, a process that occurs primarily in the liver, spleen, and bone marrow. (See the section on "Storage" for ferritin degradation.) Briefly, the initial step in hemoglobin degradation involves the uptake of old (senescent) (and also damaged) red blood cells, which normally circulate in the blood for about 120 days. Degradation via phagocytosis occurs primarily by macrophages present in the spleen; however, reticuloendothelial cells/macrophages in bone marrow and Kupffer cells (macrophages) in the liver also degrade the red blood cells. Once the old red blood cells are engulfed, an erythrophagosome is formed and degradation follows fusion with lysosomes. The heme portion of the hemoglobin molecule is carried by heme-responsive gene 1 protein (HRG1) across the phagolysosomal membrane into the cytosol where it is catabolized by heme oxygenase to release iron and protoporphyrin. Protoporphyrin is subsequently degraded to biliverdin, which is then converted into bilirubin; the bilirubin is secreted into the bile for excretion from the body. With this heme degradation, about 20–25 mg of iron per day is released for reuse. Ferroportin, the same protein responsible for iron efflux from intestinal cells, enables the transport of iron out of the macrophages and reticuloendothelial cells. Specifically, ferroportin facilitates the transport of iron into vesicles, from which it is subsequently secreted into the blood. Oxidation of the iron by ceruloplasmin is also required as the iron is transported out of the cell by ferroportin; as stated previously, this oxidation of iron enables its transport in the blood by transferrin.

The release of iron from the reticuloendothelial cells and macrophages is facilitated by low hepcidin concentrations. Iron released from these cells may be reused, for example, for erythropoiesis, or for incorporation into iron-dependent enzymes, or the iron may be deposited for storage. In situations with increased hepcidin (as occurs with increased body iron as well as with inflammation and infection), ferroportin is degraded, and iron is retained within cells.

Although most red blood cells are degraded in the reticuloendothelial system within the spleen, liver, or bone marrow, some (up to ~10%) red blood cell lysis occurs within the blood. Two proteins synthesized in the liver, haptoglobin and hemopexin, remove any released free hemoglobin and heme, respectively, from the blood. Haptoglobin forms complexes with free hemoglobin, and hemopexin forms a complex with free heme in the blood. The proteins then deliver the iron-containing compounds to the liver, where further degradation occurs to enable reuse of the iron. Macrophages, however, also bind haptoglobin–hemoglobin complexes and hemopexin, and thus scavenge iron under conditions of hemolysis and tissue damage. With significant hemolysis, the quantity of iron passing through the plasma can expand to six to eight times the normal amount. In contrast, should erythropoiesis decline dramatically, as occurs on descent from high altitudes, the quantity of iron in the plasma pool may decrease to as little as one-third of normal. Figure 13.8 represents schematically the internal iron exchange in the body.

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Figure 13.8 Internal iron exchange.

Interactions with Other Nutrients

You have read that iron and ascorbic acid interact, with vitamin C enhancing iron absorption and maintaining iron in the appropriate valence state for enzyme function. The potential also may exist for vitamin C–induced release of ferric iron from ferritin, with subsequent reduction of iron to the ferrous form [10]. Whether such reactions result in Fenton reactions and occur *in vivo* is unclear.

An interaction also occurs between iron and copper, with a copper deficiency resulting in iron-deficiency anemia. In the 1920s, studies revealed that iron supplements were unable to cure anemia in rats; however, ashed foodstuffs containing copper replenished blood hemoglobin concentrations [11]. Specifically, the copper was needed for the ferroxidase activity of hephaestin and ceruloplasmin, which in turn enabled iron to be mobilized and used for hemoglobin synthesis.

Another interaction involves iron and zinc, and may occur if the two minerals are ingested together in the absence of food and if iron is present as nonheme iron in a ratio with zinc of 2 (or higher) to 1 [6]. While the interaction is not likely to occur with consumption of foods, care should be taken to avoid simultaneous coingestion of these minerals in supplement forms, as perhaps done when treating a deficiency.

Vitamin A and iron also interact. Reduced vitamin A status causes iron accumulation in the spleen and liver. Inadequate vitamin A status is also associated with altered red blood cell morphology and decreased plasma iron and blood hemoglobin and hematocrit. The interaction between the nutrients appears to be mediated at least in part through erythropoietin, a hormone made in the kidneys that stimulates erythropoiesis (red blood cell production). Specifically, vitamin A as retinoic acid binds to a response element in the erythropoietin gene and stimulates its transcription. Thus, with insufficient vitamin A, the erythropoietin gene is not transcribed adequately, red blood cell synthesis is diminished, and iron remains in stores. Supplementation of vitamin A in people with poor vitamin A and iron status increases erythropoietin synthesis and increases iron release from stores to provide the iron that is needed for erythropoiesis [12]. Another possible means by which vitamin A may influence iron is through interactions between retinoic acid and the transferrin receptor gene.

Iron and lead also interact. Lead inhibits the activity of Δ-aminolevulinic acid dehydratase, which is required for heme synthesis. Lead also inhibits the activity of ferrochelatase, the enzyme that incorporates iron into heme. Thus, lead poisoning is associated with iron-deficiency anemia secondary to decreased hemoglobin production. In addition, increased absorption of lead occurs with iron deficiency and could be problematic for children, who are often iron deficient and may have increased exposure to lead. The mechanism by which lead absorption is enhanced in situations of iron deficiency is unknown, but it may involve increased lead uptake through the enterocyte transporter DMT1.

Excretion

Total daily iron losses for an adult male are ~0.9–1.2 mg/day. Iron losses for women (postmenopausal) are a bit lower, ~0.7–0.9 mg/day because of women's smaller surface area [13].

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Most (0.6 mg) iron losses occur through the gastrointestinal tract. Of this 0.6 mg, about 0.45 mg is lost through minute (~1 mL) blood loss (which occurs even in healthy people), and another 0.15 mg through losses in bile and desquamated mucosal cells. Skin losses of ~0.2–0.3 mg of iron occur with desquamation of surface cells from the skin. Finally, a very small amount, about 0.08 mg, is excreted in the urine. Losses of iron, however, may be greater in people with gastrointestinal ulcers or intestinal parasites, or with hemorrhage induced by surgery or injury.

In contrast to postmenopausal women and men, total iron losses in premenopausal women are higher, estimated at ~1.3–1.4 mg/day because of iron loss in menses. The average loss of blood during a menstrual cycle is ~35 mL, with an upper limit of ~80 mL. The iron content of blood is ~0.5 mg/mL, which translates into a loss of nearly 17.5 mg of iron per period. Averaged out over a month, iron loss attributable to menses is ~0.4–0.5 mg/day; in some women, however, iron loss during menses alone may exceed 1.4 mg/day. Balancing iron losses from the body with iron absorption is important to health. Iron deficiency remains one of the most common nutritional deficiencies worldwide.

Recommended Dietary Allowance

For adult men, the requirement and Recommended Dietary Allowance (RDA) for iron are 6 mg/day and 8 mg/day, respectively. For postmenopausal women, the requirement and RDA for iron are 5 mg/day and 8 mg/day, respectively [13]. Because of the greater losses associated with menses, premenopausal women require 8.1 mg of iron/day; the recommended intake is 18 mg/day [13]. During pregnancy, though no menstrual losses occur, iron is needed for the fetus, for expanding blood volume, and for tissue and storage such that the RDA for iron is 27 mg/day. The RDA for iron is 9 mg/day during lactation [13]. The inside front cover of the book provides additional RDAs for iron for other age groups.

Deficiency

Iron deficiency occurs most often due to inadequate iron intake. Iron intake is frequently inadequate in four population groups:

- infants and young children (6 months to about 4 years) because of the low iron content of milk and other preferred foods, rapid growth rate, and insufficient body reserves of iron to meet needs beyond about 6 months
- adolescents in their early growth spurt because of rapid growth and the needs of expanding red blood cell mass
- females during childbearing years because of menstrual iron losses
- pregnant women because of their expanding blood volume, the demands of the fetus and placenta, and blood losses that are incurred in childbirth.

In addition, many nonpregnant females in their childbearing years fall short of the RDA for iron because of restricted energy (caloric) intake and inadequate consumption of iron-rich foods. Furthermore, individuals with renal disease develop iron-deficiency anemia because of an impaired ability to synthesize red blood cells due to reductions in erythropoietin synthesis by the diseased kidney.

The need for iron may also be increased secondary to other iron losses or impaired iron absorption. Other conditions associated with increased iron losses include hemorrhage, hemodialysis (a form of renal replacement therapy that results in the premature destruction of some red blood cells), decreased (faster than normal) gastrointestinal transit time associated with diarrhea, and infection (such as with parasites like the hookworm). Gastrointestinal tract conditions as well as surgical procedures (such as Roux-en-Y gastric bypass) associated with damage to or resection of the duodenum and proximal jejunum diminish iron absorption and frequently cause anemia. Impaired iron absorption may also occur with protein-energy malnutrition, renal disease, achlorhydria (the absence of hydrochloric acid in gastric juice), and prolonged use of medications, which increase the pH of the digestive tract, such as antacids and proton pump inhibitors used in the treatment of heartburn, gastroesophageal reflux disease, and ulcers. Individuals who rely on a plant-based diet, in which the iron may be less bioavailable, are also more likely to develop iron deficiency.

Figure 13.9 depicts the gradual depletion of the body's iron content. Iron deficiency is associated with suboptimal health, and, if not treated, usually progresses to iron-deficiency anemia. Iron-deficiency anemia impairs the oxygen-carrying capacity of the blood. Signs and symptoms of iron deficiency, more commonly seen in children, include pallor, listlessness, behavioral disturbances, impaired performance in some cognitive tasks, some irreversible impairment of learning ability, and short attention span. In adults, work performance and productivity are commonly impaired with iron deficiency. Further details about iron deficiency with and without anemia as it relates to changes that occur in indices of iron status are covered in the "Assessment of Nutriture" section.

The treatment of iron-deficiency anemia usually requires the use of iron supplements providing 30–65 mg (but sometimes as high as 120 mg) of iron. The initial effects of oral iron supplements on red blood cell counts

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	Normal	Early Negative Iron Balance	Iron Depletion	Iron-Deficient Erythropoiesis	Iron-Deficiency Anemia
Reticuloendothelial marrow iron	2–3+	1+	0–1+	0	0
Transferrin iron-binding capacity (µg/dL)	330±30	330–360	360	390	410
Plasma ferritin (µg/L)	100±60	<25	20	10	<10
Iron absorption (%)	5–10	10–15	10–15	10–20	10–20
Plasma iron (µg/dL)	115±50	<120	115	<60	<40
Transferrin saturation (%)	35±15	30	30	<15	<15
Sideroblasts (%)	40–60	40–60	40–60	<10	<10
Erythrocyte protoporphyrin (µg/dL)	30	30	30	100	200
Erythrocytes	Normal	Normal	Normal	Normal	Microcytic Hypochromic
Serum transferrin receptors	Normal	Normal–high	High	Very high	Very high
Ferritin iron	Normal	Normal–low	Low	Very low	Very low

* Iron within circulating erythrocytes and their precursors.

Figure 13.9 Sequential changes in iron status associated with iron depletion.
Source: Adapted from Victor Herbert, "Recommended dietary intakes (RDI) of iron in humans," *American Journal of Clinical Nutrition*, 1987; 45:679–86. Copyright © American Society for Clinical Nutrition. Reprinted by permission.

and hemoglobin concentrations take about 2 weeks. Iron therapy to increase body stores of iron may be needed for 3 months to 1 year. Side effects associated with the use of iron supplements frequently include constipation, dark stools, nausea, and/or stomach pain. Parenteral (intravenous) iron therapy is available as iron dextran, sodium ferric gluconate, and iron sucrose should oral supplements not be tolerated or not adequately improve iron status.

Supplemental iron is also recommended for specific population groups due to higher risks of deficiency. For example, pregnant women are typically advised to take a prenatal supplement providing iron (usually at least 30 mg) and, if iron deficient, a supplement providing higher amounts of iron is typically advised. In addition, the use of an iron-fortified formula is recommended for infants less than 1 year of age who are being primarily breastfed. Iron may also be recommended for breastfed infants who are not consuming iron-fortified cereals by about 4-6 months of age.

Toxicity

The Tolerable Upper Intake Level for iron for adults is 45 mg [13]. Acute iron toxicity, which can be lethal, is observed mostly with accidental iron overload, as may occur with the ingestion of excessive numbers of iron pills or iron-containing multivitamin/mineral supplements. With acute toxicity, the excessive presence of an overload of iron atoms is thought to exceed the transport carrying capacity of transferrin. The unbound iron in turn behaves in a free radical manner, damaging the gastrointestinal tract and other tissues. Manifestations of acute toxicity initially include abdominal pain, vomiting, and diarrhea. The stool may become darker secondary to the presence of iron; however, iron-induced damage to the digestive tract mucosa also causes hemorrhage, resulting in blood in the emesis and feces. Excessive blood loss may result in hypovolemic shock. Some additional manifestations that may develop include acidosis, coma, and liver failure.

Chronic iron toxicity is generally associated with the genetic disorder hemochromatosis, a condition most often seen in Caucasian males and that becomes evident around 20 years of age. An estimated 50 per 10,000 people in the United States are homozygous for the disorder. Hemochromatosis is characterized by increased (at least two times normal) iron absorption. Mutations in one of several genes that result in diminished hepcidin synthesis cause the condition and prevent the body from accurately sensing iron stores and downregulating intestinal iron absorption. For example, in the C282Y mutation in the

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HFE protein, tyrosine is substituted for cysteine because of a single base change; this alteration inhibits HFE's ability to stimulate the synthesis of hepcidin, which tells the intestinal cells to downregulate iron absorption. Similarly, the H63D mutation also reduces hepcidin synthesis, whereas a mutation (Q248H) in ferroportin promotes hemochromatosis due to continued ferroportin expression.

Although several mutations can cause hemochromatosis, in most people with the condition, iron absorption generally continues unregulated despite high iron stores. The absorbed iron is progressively deposited throughout the body (including within joints and tissues, especially the liver, heart, and pancreas), causing extensive organ damage and ultimately organ failure. Iron deposition in the liver, for example, leads to cirrhosis, usually by about 50 years of age. Heterozygotes for the condition do not develop as severe organ dysfunction but exhibit abnormal iron status. For example, if untreated, serum ferritin concentrations in hemochromatosis continue to rise in the blood and may exceed 1,000 μg/L (normal: < 300 μg/L).

Treatment of hemochromatosis requires frequent phlebotomy (removal of blood), usually the weekly removal of about 1 unit (~400–500 mL), which contains about 200–250 mg of iron. In addition, deferoxamine may be given. Deferoxamine works by chelating (binding to) iron in the body and increasing urinary iron excretion. Treatment of hemochromatosis usually continues as described until serum ferritin concentrations are less than about 50 μg/L, and transferrin saturation is less than about 30% [14]. Once these levels are achieved, the frequency with which the person undergoes phlebotomy can be diminished.

Other people at particularly high risk for iron overload are those with iron-loading anemias, thalassemia, and sideroblastic anemia. The elevated erythropoiesis in the bone marrow in people so affected causes increased iron absorption.

Assessment of Nutriture

The most common indices used to diagnose iron-deficiency anemia are hemoglobin (amount of iron-containing protein found in red blood cells per unit, usually deciliter or liter, of blood) and hematocrit (that proportion of the total blood volume that is red blood cells). However, although these indices indicate the presence of anemia, they are among the last to change as iron deficiency develops.

In the first stages of iron deficiency, iron stores in the liver, spleen, and bone marrow are diminished. Although iron stores can be aspirated and measured from bone marrow, the routine test involves measurement of plasma (or serum) ferritin. Decreases in plasma ferritin concentration are thought to parallel the decrease in the amount of iron found in stores. Plasma ferritin concentrations less than about 15 μg/L are associated with iron deficiency and with bone marrow iron deficits. However, if inflammation or infection is present, the plasma ferritin concentration rises, an occurrence unrelated to iron stores. Thus, plasma ferritin may appear within normal range or high while the body's iron status is quite low; measurement of the levels of C-reactive protein or alpha-1 acid glycoprotein in the blood can be used to detect the presence of inflammation or infection. Once iron stores are depleted, plasma ferritin concentrations no longer reflect the tissue iron pool.

As iron deficiency progresses into the second stage, iron stores typically remain low, and transport iron decreases. Thus, plasma ferritin concentrations continue to be diminished, and circulating iron begins to decrease. Because iron circulates in the blood bound to transferrin, with iron deficiency, transferrin saturation decreases; levels less than 16% are diagnostic of deficiency (remember normal saturation is about 30–40%). Transferrin saturation can be calculated by multiplying the serum iron concentration by 100 and then dividing by the total iron-binding capacity (TIBC). TIBC represents the amount of iron that plasma transferrin can bind and normally ranges from ~250 to 400 μg/dL. Levels greater than 400 μg/dL suggest iron deficiency. Serum iron concentrations, which represent the amount of iron bound to transferrin and other blood proteins, are also affected with iron deficiency, decreasing to <~50 μg/dL (normal range ~50–180 μg/dL).

As circulating iron diminishes, functional or cellular iron also becomes limited. With diminished iron, free protoporphyrin concentrations in erythrocytes rise. Protoporphyrin is a precursor of heme (for hemoglobin) and accumulates within red blood cells when iron is not available. Erythrocyte protoporphyrin levels greater than 70 μg/dL red blood cells are associated with iron deficiency. In iron deficiency, the number of transferrin receptors on the cell surface, especially of immature red cells, also increases. The increased receptor number represents an upregulation to enable cells to better compete for transferrin-bound iron. With iron deficiency, concentrations of serum transferrin receptors (sTfR), truncated forms of the membrane receptor protein, increase to greater than 8.0 mg/L and are thought to be directly proportional to the functional tissue (i.e., cellular) iron deficit after depletion of iron stores.

In the final stages of iron deficiency, anemia occurs. With anemia, blood hemoglobin concentrations drop below the lower limit of normal, which is typically 12 g/dL and 13 g/dL for females and males, respectively. Hematocrit concentrations with anemia also decrease below the lower limit of normal, less than about 37% and 40% for women and men, respectively. Hematocrit values are normally about three times higher than hemoglobin values. Characterization of red blood cells with respect

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to size (mean corpuscular volume, or MCV) and amount of hemoglobin they contain (mean corpuscular hemoglobin, or MCH, and mean corpuscular hemoglobin concentration, or MCHC) typically shows that they are smaller and lower in hemoglobin than normal in the final stages of iron-deficiency anemia. Descriptions of these assessments follow.

- MCV (fL) represents the size of the red blood cell. It is calculated by dividing hematocrit by red blood cells and then multiplying by 10.
- MCH (pg/rbc) represents the average hemoglobin content of each individual red blood cell. It is calculated by dividing hemoglobin by number of red blood cells and then multiplying by 10.
- MCHC represents the amount of hemoglobin in grams per deciliter (%) of red blood cells. It is calculated by dividing hemoglobin by hematocrit and then multiplying by 100.

Thus, red blood cells are pale (hypochromic) and small (microcytic) with iron-deficiency anemia. Figure 13.8 illustrates the changes that occur in the various measurements.

Other assessments that can be useful in assessing iron status, especially with inflammatory conditions, include the ratio of serum soluble transferrin receptors to the log of ferritin, with derived values greater than 2 suggesting anemia of chronic disease with iron deficiency. A ratio less than 1 is indicative of anemia of chronic disease without iron deficiency. The presence of inflammation is most often determined by elevations in C-reactive protein (CRP) concentrations (> about 0.5 or 1 mg/dL). A functional iron deficit is also suggested by low reticulocyte hemoglobin concentrations (less than about 28 pg).

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Web Site for Nutrient Composition Information

www.ars.usda.gov/Services/docs.htm?docid=18877

ZINC

The human body contains about 1.5–3.0 g of zinc. Zinc is found in all organs, tissues (especially muscle and bone), and body fluids. Zinc, a metal, can exist in several different valence states, but it is almost universally found as the divalent ion (Zn^{2+}) in the human body.

Sources

Zinc is found in foods complexed with nucleic acids and with amino acids that are part of peptides and proteins. The zinc content of foods varies widely (Table 13.4). Very good sources of zinc are red meats (especially organ meats) and seafood (especially oysters and mollusks). Other relatively good animal sources of zinc include poultry, pork, and dairy products. Animal products are thought to provide 40–70% of zinc consumed by most people in the United States. Whole grains and legumes also provide moderate amounts of zinc. Cereals, some of which may be fortified, are thought to provide about 30% of the zinc in the U.S. diet. Fruits contain little zinc. Plant sources, however, not only have lower zinc contents, but zinc from plants is also absorbed to a lesser extent than zinc from animal sources (e.g., meat). The Daily Value for zinc (used on food and supplement labels) is 15 mg.

Processing of certain foods may affect zinc availability. Heat treatment can cause zinc in food to form complexes that resist hydrolysis, thereby making zinc less available for absorption. Maillard reaction products—that is, amino

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Table 13.4 Zinc Content of Selected Foods*

Selected Foods/Food Group	Zinc (mg)
Seafood	
Oyster (1)	12.8
Crabmeat (3 oz)	3.8
Shrimp (3 oz)	1.8
Flounder/sole (3 oz)	0.5
Meat and poultry	
Liver, beef (3 oz)	4.3
Chicken, dark meat (3 oz)	2.4
Beef, ground (3 oz)	3.8
Veal (3 oz)	3.5
Pork (3 oz)	1.8
Eggs and dairy products	
Egg (1)	0.5
Milk (1 cup)	1.0
Cheeses (1 oz)	0.5–1.2
Legumes, cooked (1/2 c)	1.0–2.7
Nuts (1 oz)	0.9–1.5
Grains and cereals	
Rice and pasta (1 c)	0.7–1.2
Bread, whole wheat (1 slice)	0.3
Bread, white (1 slice)	0.1
Vegetables, cooked (1 c)	0.1–0.7
Fruits	< 0.1

*The United States Department of Agriculture publishes extensive information of nutrient contents on foods. See http://ndb.nal.usda.gov.

acid–carbohydrate complexes resulting from browning, for example—are particularly notable for inhibiting zinc's availability for absorption.

Zinc supplements are available in several forms including oral tablets and lozenges, throat or nasal sprays, and nasal gels. Zinc is typically found in supplements as zinc oxide, zinc sulfate, zinc acetate, zinc chloride, and zinc gluconate. These various forms provide differing amounts of zinc. Zinc gluconate, for example, is approximately 14.3% zinc, whereas zinc sulfate is 23% zinc, zinc acetate is 30% zinc, and zinc chloride is 48% zinc. Zinc chloride and zinc sulfate are very soluble, as is zinc acetate. In contrast, zinc carbonate and zinc oxide are fairly insoluble. Multivitamin/mineral supplements contain up to about 15 mg of zinc, while single-nutrient supplements provide about 10–100 mg of zinc. Zinc supplements for oral consumption should be consumed on an empty stomach, without simultaneously ingesting other mineral supplements such as iron or calcium. Abdominal pain (e.g., gastric irritation), dyspepsia, nausea, vomiting, and diarrhea are commonly reported side effects with the use of zinc supplements.

Topical zinc products, which usually provide zinc as zinc oxide or zinc chloride, may be used in wound management and include paste bandages, occlusive adhesive dressings, alginates, stockings, and zinc-saline dressings. The percutaneous absorption of the zinc depends on the skin's integrity and requires (in the case of zinc oxide or chloride) the acidic moisture on the skin's surface to release the zinc. If skin is not intact (as may occur with burns or other injury), much more zinc is absorbed. In the presence of an intact skin barrier, zinc binds to the sulfhydryl groups in epidermal keratin upon release from the zinc oxide or chloride salt in the acidic environment. A small amount of the zinc will then penetrate beyond the superficial keratinocytes and enter circulation in a process that takes about 60 minutes from the time of topical application. The rest of the zinc (not making it into circulation) binds to metallothionein (which is induced with topical zinc application) in epidermal keratinocytes and will eventually be sloughed off with skin cell turnover.

Endogenous zinc (up to about 4.5 mg), from pancreatic, intestinal, and biliary secretions, is released into the gastrointestinal tract with food ingestion and augments the zinc present from dietary food sources and any consumed supplements. The secreted zinc can be reabsorbed and represents an important means for body zinc homeostasis.

Digestion, Absorption, Transport, and Storage

Figure 13.10 provides an overview of zinc digestion, absorption, and transport as well as some of zinc's fates in the enterocyte.

Digestion

Zinc needs to be hydrolyzed from amino acids and nucleic acids before it can be absorbed. Zinc is believed to be liberated from these food constituents during the digestive process, most likely by the acidic environment of the stomach and upper duodenum and by proteases and nucleases in the stomach and small intestine.

Absorption

Zinc absorption occurs primarily in the proximal small intestine, that is, the duodenum and upper jejunum. Two mechanisms (carrier-mediated transport and diffusion) are responsible for intestinal zinc absorption (Figure 13.9). The primary means of absorption with usual intakes, which is up to about 7–9 mg of zinc, is saturable and carrier mediated [1]. The protein carrier Zrt- and Irt-like protein 4 (ZIP4) is the major transporter of zinc across the enterocyte's brush border membrane and is expressed throughout the gastrointestinal tract. Zinc intake influences ZIP4. With high zinc intakes, ZIP4 is degraded more rapidly to downregulate absorption, whereas zinc restriction enhances ZIP4 mRNA stability, rapidly induces ZIP4 synthesis, and shifts ZIP4 proteins to the brush border membrane. The enhanced ZIP4 synthesis is mediated by upregulation of the transcription factor Kruppel-like factor 4 (KLF4), which binds to the promoter region on the ZIP4 gene.

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1. Bound zinc is released from food components, primarily proteins and nucleic acids.
2. Most zinc is absorbed by Zrt- and Irt-like protein 4 (ZIP4) across the brush border membrane.
3. Divalent mineral transporter 1 (DMT1) and amino acids may play a minor role in zinc absorption across the brush border membrane.
4. Some zinc may be directed into the feces if bound to inhibitors, or absorption may be enhanced by organic acids, ↓pH, or chelators.
5. With high zinc intakes, zinc may be absorbed between cells (i.e., paracellularly).
6. Within cells, zinc may be used functionally or stored in vesicles, in the trans-Golgi network, or as part of metallothionein.
7. Zinc may be transported across the basolateral membrane by ZnT1.
8. Zinc binds to proteins for transport in the blood.

Figure 13.10 Digestion, absorption, enterocyte use, and transport of zinc.

In addition to carrier-mediated transport, paracellular (meaning between cells) diffusion of zinc through the tight junctions of the enterocytes enables absorption. This process is thought to contribute to the absorption of zinc when zinc intakes (typically 20 mg or more) exceed the capacity of the ZIP4 carriers.

A mutation in ZIP4 causes the disorder acrodermatitis enteropathica. The condition is characterized by poor zinc absorption and is clinically manifested by skin lesions (which often become infected), especially on the face, knees, and buttocks; impaired growth; and low plasma zinc concentrations, representing signs and symptoms of zinc deficiency. If untreated, the condition can be fatal. The provision of high doses of zinc (30–150 mg/day) that can be absorbed by paracellular diffusion typically helps compensate for the impaired ZIP4 transporters.

Although other carrier proteins, such as DMT1, present in the gastrointestinal tract bind zinc, they are not thought to contribute except in a minor capacity to zinc absorption. Similarly, some zinc may bind to amino acids such as histidine and perhaps enter the enterocyte using amino acid transporters; however, this contribution to zinc absorption is minor. ZIP11 is also found on the brush border membrane, and its synthesis is upregulated with zinc restriction; however, at this time, its role in zinc absorption is thought to be small, if any [1].

Overall, about 20–50% of zinc is absorbed from the typical U.S. diet. However, fractional zinc absorption varies from approximately 10 to 80%; at higher intakes absorption diminishes, whereas at lower intakes absorption increases. For example, 100% of zinc may be absorbed at an intake less than 1 mg, whereas about 40% may be absorbed with a zinc intake of 12 mg [1]. This up- and downregulation of absorption as well as the ability to increase and decrease zinc excretion are important for maintaining zinc homeostasis in the body.

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Factors Influencing Zinc Absorption

As is the case with iron, chelators or ligands bind to zinc. Whether these substances are enhancers or inhibitors of zinc absorption depends on the digestibility and absorbability of the zinc chelates or ligands formed.

Enhancers of Zinc Absorption Ligands or chelators including organic acids (like citric acid and picolinic acid) and prostaglandins may bind and promote zinc absorption. Pancreatic secretions are thought to contain an unidentified constituent that enhances zinc absorption. In addition, glutathione (a tripeptide composed of cysteine, glutamate, and glycine) and products of protein digestion, such as tripeptides and amino acids, are purported to serve as ligands. Zinc typically binds to sulfur (e.g., cysteine alone, or as part of glutathione) and nitrogen (e.g., histidine) within these ligands. Amino acids serving as ligands help maintain zinc's solubility in the gastrointestinal tract; whether zinc bound to amino acid ligands can be absorbed using amino acid transporters is unclear.

Absorption of zinc is also enhanced by an acidic environment. Thus, the use of medication such as antacids, H_2 receptor blockers (such as Zantac [ranitidine], Tagamet [cimetidine], or Pepcid [famotidine]), and proton pump blockers (such as Prevacid [lansoprazole] or Prilosec [omeprazole]), which are commonly taken to treat heartburn, gastroesophageal reflux disease, and ulcers, increases gastric and proximal intestinal pH and decreases zinc absorption.

Inhibitors of Zinc Absorption In addition to a more alkaline environment, which diminishes zinc absorption, many compounds in food complex with zinc to inhibit its absorption. Some examples of inhibitors include:

- *Phytic acid* found in plant foods, particularly legumes, lentils, nuts, seeds, and whole-grain cereals. It binds to zinc (as well as other minerals) via oxygen within the compound's phosphate groups. The zinc–phytic acid complex is large, insoluble, and poorly absorbed. Reductions in zinc bioavailability are greatest when the phytate-to-zinc molar ratio is greater than 15 to 1; the aforementioned plant foods typically contain such phytate-to-zinc ratios [2]. Calculation of the ratio is done as follows: "phytic acid content of the food" / "molecular weight of phytic acid, which is 660" ÷ "zinc content of the food" / "atomic weight of zinc, which is 64.5" [2]. The fermentation of foods, such as bread, reduces its phytic acid content and improves zinc absorption. Figure 13.11 depicts the binding of zinc by phytic acid. Individuals in the United States consuming primarily plant-based high-phytate diets may absorb less than adequate amounts of dietary zinc.
- *Oxalic acid* (oxalate), found in a variety of foods, most notably spinach, chard, berries, chocolate, and tea. The binding of zinc by oxalic acid is shown in Figure 13.11.

Oxalic acid

Phytic acid

Figure 13.11 The binding of zinc by oxalic acid and phytic acid.

- *Polyphenols* (such as tannins and gallic acid), found in tea and coffee, and some *fibers*, found in whole grains, fruits, and vegetables. The impact of these substances on an individual's zinc status varies with the amount consumed and other sources of zinc in the diet. Most individuals in the United States consume well under the recommended amount of dietary fiber.
- *Other minerals such as iron and calcium*. Iron and calcium have not been consistently shown to negatively impact zinc absorption. Iron in amounts of 20–25 mg or more, or in a ratio with zinc of 2 (or higher) to 1, impairs zinc absorption when ingested in solution but not as part of a meal [3]. Thus, in practice, to maximize zinc absorption, a zinc supplement should not be consumed at the same time as a nonheme iron supplement on an empty stomach. The effects of calcium on zinc absorption and balance are equivocal. Some studies have shown that ingestion of calcium (500 mg–2 g) as calcium carbonate, hydroxyapatite, or calcium citrate malate has no effect on zinc absorption, whereas other studies providing similar amounts of calcium as milk, calcium phosphate, and calcium carbonate found reductions in net zinc absorption and zinc balance. Results appear to vary with the forms and amounts of the nutrients provided, the study populations, and the simultaneous presence/absence of other inhibitors such as phytate in the diets. To minimize the likelihood of interactions, it is prudent to not take mineral supplements at the same time, to avoid the ingestion of calcium supplements at meals providing significant amounts of zinc, and to ensure adequate dietary intake of zinc from bioavailable foods.

Intestinal Cell Zinc Use

Zinc entering the enterocyte has several possible fates. The zinc may be:

- used functionally within the enterocyte (see the section "Functions and Mechanisms of Action")

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- stored or sequestered in the enterocyte
- transported through the cytosol and across the basolateral membrane for entry into the blood and thus use by other tissues.

If not used functionally within cells such as enterocytes, zinc is largely sequestered or bound to proteins; in other words, little zinc is found free. Zinc transporters function to remove zinc from the environment; that is, they lower intracellular (cytosolic) zinc concentrations, mediating both zinc efflux from cells and movement of zinc into intracellular compartments. Intracellularly, zinc is sequestered in vesicles, secretory granules, endosomes, or the trans-Golgi network (complex). The intracellular trafficking protein ZnT7 in the enterocyte is thought to be involved in zinc movement into the trans-Golgi network, while ZIP7 may transport the mineral out of the trans-Golgi network; however, the exact roles of these two transporters have not been clearly established. ZnT2 appears to sequester zinc in endosome-like vesicles within enterocytes. Overall, however, how zinc is transported within the cytosol (i.e., intracellular trafficking) is not well delineated.

The protein metallothionein (sometimes called thionein if free of metals) serves as zinc's main short-term storage protein and as an intracellular binding ligand. Metallothionein contains an unusually high content (30%) of cysteine, which functions in metal binding. Metallothionein is thought to both transport zinc to zinc-requiring enzymes and store zinc transiently. The metallothionein-bound zinc can be released for cellular use, but if not used, it is lost into the feces with the sloughing of enterocytes that is part of normal intestinal cell turnover. Metallothionein is covered in further detail in the "Storage" section.

Zinc needed for extraintestinal use is carried across the basolateral membrane of the enterocyte for release into the blood. Zinc transporter 1 (ZnT1), which does not require sodium or ATP, preferentially transports zinc out of duodenal and jejunal cells, likely in exchange for H^+ or K^+; ZnT1 is also found on several other cell membranes. The synthesis of ZnT1 is increased with high dietary zinc intake but does not appear to be affected with decreased zinc intake. In addition to 1 (ZnT1), a sodium-zinc exchanger has been identified that appears to direct sodium-dependent active extrusion of zinc from some cells. DMT1, found on the basolateral membrane of enterocytes, may also play a minor role in intestinal cell zinc efflux into the blood.

Transport

Zinc passing into portal blood from the intestinal cell is mainly transported loosely bound to albumin. Most zinc is then taken up by the liver, where the mineral is initially concentrated. Zinc leaving the liver binds mainly (about 70–75%) to albumin, with the remaining zinc bound more tightly to α-2-macroglobulin. Two amino acids, histidine and cysteine, also loosely bind and transport (in a ternary complex as histidine-zinc-cysteine) less than 1% of zinc in the blood. Plasma zinc concentrations range from about 70 to 120 μg/dL (10–18 μmol/L), with plasma containing about 3 mg of zinc. Plasma zinc concentrations decrease after eating, as well as under conditions of infection and trauma.

Multiple transporters, including at least 14 ZIPs and 10 ZnTs, facilitate cellular zinc uptake and release, yet many of the mechanisms remain unclear. ZIP carriers 1, 2, 4, 5, 6, 7, 8, and 14 appear to be involved in cellular zinc uptake from extracellular locations and the release of zinc from intracellular stores, both to effect increased cytosolic zinc concentrations. ZIP14, for example, transports zinc into hepatocytes, and its activity appears to be increased as part of the acute-phase (reactant) response (as occurs with infections and trauma). The transporter ZIP5 is expressed in the intestinal cell as well as the pancreas, liver, and kidneys. In the intestine, ZIP5 is found on the basolateral membrane, where it facilitates serosal to mucosal zinc transport. In other words, ZIP5 moves zinc out of the circulation, that is, from the blood into the intestinal cell. The zinc transporter ZnT6, found on the enterocyte's brush border membrane, is thought to mediate the exocytosis of zinc from the intestinal cell back into the lumen for ultimate excretion in the feces. Not all ZIP carriers, however, solely transport zinc; many, such as ZIP14 which transports both Fe^{2+} and Zn^{2+}, carry other minerals as well.

Storage

Zinc is found in all body tissues/organs, most notably the liver, kidneys, muscle, skin, and bones. Within cells, about 30–40% of zinc is bound to proteins in the nucleus, about 50% is in the cytosol, and the remaining zinc is found in cell membranes. The zinc content of most soft tissues (including muscle, brain, heart, and lungs) is relatively stable. This soft-tissue zinc does not respond to or equilibrate with other zinc pools to release zinc if dietary zinc intake is low. Similarly, although zinc is found in bones as part of apatite, bones release the mineral very slowly and cannot be depended on to supply zinc during dietary deprivation. Instead, when dietary zinc intake is insufficient, catabolism of selected "less essential" zinc-containing metalloproteins (enzymes) and liver metallothionein occurs to enable the release and redistribution of zinc to meet particularly crucial needs for the mineral.

Zinc is stored attached to metallothionein, which contains a high proportion of cysteine residues that bind metals, including not only zinc (seven atoms/molecule), but also copper, cadmium, and mercury. Metallothionein is found in most body tissues, including the liver, pancreas, kidneys, intestine, keratinocytes, and red blood cells. Various forms of the protein exist and

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are designated by number as metallothionein (MT)-1 through MT-4. MT-1 and MT-2 appear to be the most common tissue forms. Although metallothionein is thought to provide a short-term storage pool of zinc, other roles have also been attributed to the protein. Metallothionein may regulate the distribution (serving like a transporter or chaperone) and transfer of zinc to enzymes, gene-regulatory molecules, or other acceptor proteins. Metallothionein also exhibits antioxidant-type functions. For example, the protein is known to scavenge free hydroxyl radicals. In times of cell injury/stress, metallothionein synthesis increases and helps control free radical concentrations. This response is part of the body's acute-phase response, and the enhanced thionein gene expression results in part from the increased release of glucagon and the cytokine interleukin 1 (which is synthesized and secreted by monocytes and activated macrophages). The induction in thionein gene transcription during infection promotes zinc storage and prevents bacterial use of the mineral. Should zinc be needed within cells, it may be released from metallothionein by the action of lysosomal proteases. At an acidic pH, these proteases degrade metallothionein to release the zinc for use by cells.

Zinc regulates the gene expression of thionein. More specifically, metal regulatory (also called response) elements (MREs), consisting of particular nucleotide sequences, are found in the promoter region of the thionein gene. A metal transcription factor dependent on zinc interacts with the MRE to induce thionein synthesis. See the "Gene Expression" section for a more complete description of how zinc affects gene transcription.

Functions and Mechanisms of Action

Zinc plays a vital role facilitating hundreds of biochemical reactions. Because of its expansive enzymatic functions, zinc impacts most metabolic pathways including carbohydrate, protein, nucleic acid, and lipid metabolism. As a structural component of thousands of transcription factors, zinc has profound effects on gene expression and in turn impacts numerous physiologic processes in the body.

Zinc-Dependent Enzymes

As a component of metalloenzymes, zinc (1) provides structural integrity to the enzyme by binding directly to amino acid residues and thereby stabilizing the enzyme's tertiary structure and/or (2) participates in the reaction at the catalytic site. Zinc appears to be part of more enzyme systems than all the rest of the trace minerals combined; over 300 enzymes from every enzyme class (oxidoreductases, hydrolases, lyases, isomerases, transferases, and ligases) require zinc. A few of these zinc-dependent enzymes are listed in Table 13.5 and are described hereafter.

Table 13.5 Selected Functions of Zinc

Metalloenzyme component
Carbonic anhydrase
Alkaline phosphatase
Alcohol dehydrogenase
Carboxypeptidases
Aminopeptidases
Delta aminolevulinic acid dehydratase
Superoxide dismutase
Phospholipase C
Polyglutamate hydrolase
Matrix metalloproteinases
Polymerases
Kinases
Nucleases
Transcriptases
Gene expression: zinc fingers
Membrane/cytoskeletal stabilization
Immune function
Sexual maturation
Fertility and reproduction

Carbonic Anhydrase: Acid-Base Balance Carbonic anhydrase has a very high affinity for zinc, which plays a catalytic role. The enzyme, found primarily in erythrocytes and in renal tubule cells, is essential for acid-base balance/buffering and respiration. More zinc (about eight to nine times) is found associated with this enzyme in red blood cells than is found in the plasma. The enzyme catalyzes the following reaction, thereby allowing the rapid disposal of carbon dioxide: $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$. The H^+ dissociated from carbonic acid reduces oxyhemoglobin as oxygen is released to the tissues; the bicarbonate passes into the plasma to participate in buffering reactions. Concentrations of the enzyme are not significantly affected by zinc deprivation, but activity in red blood cells diminishes with low-zinc (3.8 mg/day for several weeks) diets [4]. (See also "Acid-Base Balance" in Chapter 12.)

Alkaline Phosphatase: Phosphate Release Alkaline phosphatase contains four zinc atoms per enzyme molecule. Two of the four atoms are required for enzyme activity. The other two are needed for structural purposes (protein conformation). The enzyme, found mainly in bones and in the liver (with small amounts in the plasma), lacks substrate specificity, hydrolyzing monoesters of phosphates from various compounds. Enzyme activity decreases with zinc deficiency.

Alcohol Dehydrogenase: Nonspecific Aldehyde Synthesis Alcohol dehydrogenase also contains four zinc atoms per enzyme molecule, with two of the four required for catalytic activity and two required for structural purposes (protein conformation). This enzyme, which generally lacks specificity, is important in the NADH-dependent conversion of

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alcohols to aldehydes. For example, the enzyme converts a form of vitamin A, retinol, to retinal; retinal is needed for the visual cycle and especially night vision. In addition, the enzyme converts ethanol to acetyl aldehyde, a reaction important in alcohol metabolism.

Carboxypeptidases A and B and Aminopeptidases: Protein Digestion Carboxypeptidases A and B, exopeptidases secreted by the pancreas into the duodenum, are necessary for protein digestion. Zinc is bound tightly to carboxypeptidases and is essential for enzymatic activity; in fact, enzyme activity decreases with zinc deficiency. Figure 13.12 shows the zinc-containing portion of the carboxypeptidase A enzyme.

Aminopeptidases consist of a group of enzymes also involved in protein digestion. Aminopeptidases typically contain one or two zinc atoms, needed for catalytic activity. The enzymes cleave amino acids from the amino (N)–terminal end of proteins or polypeptides that are being digested in the intestinal tract.

Delta (Δ)-Aminolevulinic Acid Dehydratase: Heme Synthesis Δ-aminolevulinic acid dehydratase, needed for heme synthesis, is also zinc dependent. This enzyme is made up of eight subunits, each of which binds one zinc atom. Zinc is essential for the maintenance of free thiols (–SH) in the enzyme because zinc prevents the oxidation of thiol groups and consequently disulfide bond formation within the enzyme. The enzyme catalyzes the condensation of two Δ-aminolevulinic acids to form porphobilinogen (see Figure 13.7). Lead, if present in the body in high concentrations (as occurs with lead poisoning), replaces zinc in the dehydratase and diminishes heme synthesis.

Superoxide Dismutase: Antioxidant Superoxide dismutase (SOD1) found in the cell cytosol requires two atoms each of zinc and copper for function; zinc appears to have a structural role in the enzyme. An extracellular form of the enzyme (SOD3) that is also zinc and copper dependent has been characterized and appears to be more sensitive to zinc than is the cytosolic form of the enzyme. The extracellular form is found in the plasma, lymph, synovial fluid, and lungs; it exists in equilibrium between cell surfaces and the plasma. Both the cytosolic and extracellular forms of superoxide dismutase serve important antioxidant defense roles in the body by catalyzing the removal of superoxide radicals, $O_2^{\bullet}$.

$$2O_2^{\bullet} + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2$$

Further information on this enzyme is found in the section on copper, in the "Functions and Mechanisms of Action" subsection.

Phospholipase C: Phospholipid Metabolism Phospholipase C requires three zinc atoms for catalytic activity. This enzyme hydrolyzes the glycerophosphate bond in phospholipids.

Polyglutamate Hydrolase: Folate Digestion Polyglutamate hydrolase, also called γ-glutamylhydrolase or pteroylglutamate hydrolase, is a zinc-dependent enzyme necessary for folate digestion in the gastrointestinal tract. Folate, a B-vitamin, is found in foods bound to several (i.e., poly) glutamic acid residues. For folate to be absorbed, all but one of the glutamic acids must be removed. The enzyme polyglutamate hydrolase catalyzes the removal of glutamic acids from folate. Poor zinc status can diminish folate absorption.

Figure 13.12 Partial structure of carboxypeptidase A.

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Matrix Metalloproteinases: Wound Repair Matrix metalloproteinases include a group of zinc-containing endopeptidases (with zinc located at the catalytic site where the substrate binds) that are found in keratinocytes, macrophages, fibroblasts, and endothelial cells. The enzymes are synthesized as inactive zymogens and become active in the presence of several soluble mediators and extracellular matrix compounds that are generated with cell injury. The matrix metalloproteinases generally function in wound healing, degrading components of the extracellular matrix (among other roles) to allow for remodeling of extracellular matrix proteins and tissue repair. Based on structural elements, the enzymes may be categorized, for example, as collagenases (important for wound debriding), gelatinases, matrilysins, or stromelysins (needed for wound contraction).

Polymerases, Kinases, Nucleases, Transferases, Phosphorylases, and Transcriptases: Nucleic Acid Synthesis and Cell Replication and Growth Polymerases, kinases, nucleases, transferases, phosphorylases, and transcriptases all require zinc. Paramount in nucleic acid synthesis and in cell replication and growth is the zinc metalloenzymes DNA and RNA polymerase and deoxythymidine kinase. Deoxythymidine kinase is necessary for the conservation or salvaging of thymine, the pyrimidine unique to DNA. Additionally, catabolism of RNA appears to be regulated by zinc because of zinc's influence on ribonuclease activity. Enzymes such as deoxynucleotidyl transferase, nucleoside phosphorylase, and reverse transcriptase also depend on zinc.

Gene Expression

Zinc plays a major structural role in regulating gene transcription. To perform this function, zinc binds to transcription factors (proteins). The binding of zinc to transcription factors results in a conformational change in the shape of the transcription factor protein such that it resembles a "finger." *Zinc fingers* is the term used to indicate the secondary shape (configuration) of the transcription factor proteins when bound to zinc. About 30 amino acids held together by one zinc atom are thought to make up a zinc finger; the zinc, attached to four of the amino acids through cysteine residues or a combination of cysteine and histidine residues, stabilizes the structure. These zinc fingers, once formed, interact with specific DNA sequences, called metal response or regulatory elements (MREs), located in the promoter region of selected genes to either enhance or repress transcription (Figure 13.13). Thousands of transcription factors have been identified with over 2,000 requiring zinc. In addition, some zinc finger proteins interact with mRNA to repress translation and some may facilitate protein to protein interactions.

1 Zinc fingers are proteins with a secondary structure or shape like a finger due, in part, to the presence of a zinc atom linked through cysteinyl or histidyl residues in the protein.

2 Zinc fingers are found within many transcription factors, which bind to the metal response/regulatory elements in the promoter regions of genes to enhance or inhibit transcription.

Figure 13.13 The role of zinc in gene expression.

Other Roles

Zinc plays several additional roles in the body. Zinc helps maintain cell membranes through multiple actions on membrane proteins including direct effects on membrane proteins' conformation, on protein-to-protein interactions, and on other membrane components. Zinc may affect the activity of enzymes attached to plasma membranes, including alkaline phosphatase and superoxide dismutase, among others. Zinc itself also is believed to stabilize membrane structure by stabilizing phospholipids and thiol (SH) groups in enzymes and membrane proteins that need to be maintained in a reduced state. Zinc may also stabilize membranes by quenching free radicals as part of metallothionein and by promoting associations between membrane skeletal and cytoskeletal proteins. Additionally, zinc in cells is found bound to tubulin, a protein that makes up the microtubules. Microtubules are thought to act as a framework for structural support of the cell as well as enable movement.

Zinc is also involved with insulin and thus influences carbohydrate metabolism. Zinc is transported into pancreatic β-cells by zinc transporter ZnT8, which also enables uptake into secretory vesicles. Pancreatic β-cells are responsible for insulin production and secretion. Once synthesized, insulin is stored with zinc in granules within the cells until it is released into the blood. Zinc deficiency decreases the insulin response, resulting in impaired glucose tolerance. Zinc also appears to regulate the protein kinase mammalian target of rapamycin (mTor), thereby affecting insulin-signaling and protein-synthesis pathways that occur when insulin binds to cell receptors.

Some of zinc's other diverse roles include effects on basal metabolic rate. A decrease in thyroid hormones and

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basal metabolic rate has been observed with consumption of a zinc-restricted diet. Zinc is also important for taste; it is a component of gustin, a protein involved in taste acuity. Cell survival and immune function (both cell-mediated and humoral) are also influenced by zinc. The literature in this area is extensive, but an example of one of the effects of zinc is illustrated through its actions on thymulin, a zinc-dependent hormone peptide that binds to T-cells and promotes their differentiation and functions (including cytokine release). T-cells are critical to immune system function and with zinc deficiency, thymulin activity diminishes and profoundly affects T-cell numbers and functions, and pre-T-cell apoptosis (programmed cell death) [5].

Selected Pharmacological Uses

Colds The relationship between immunity and zinc has led to many studies examining the use of zinc to treat colds. Zinc supplementation (orally as a lozenge or as a nasal spray or gel) appears in some, but not all, studies to reduce the duration and severity of cold symptoms. The benefits, if found, have been more likely if supplementation started within the first 24 hours of the onset of symptoms. Typical zinc dosages used to treat colds approach 75–80 mg/day, with supplementation recommended for up to 1 week. Caution, however, should be exercised if taking these products, given the possibility of side effects (see the "Toxicity" section) and unanswered questions regarding appropriate dosing and treatment duration.

Eye Health Zinc's presence in the eye and its cofactor roles in antioxidant enzymes have prompted its inclusion in studies examining nutritional supplementation and eye health, primarily age-related macular degeneration (see Chapter 9 under "Vitamin C" for a description of the condition). The benefits of multinutrient supplementation —including zinc (initially 80 mg and later 25 mg), vitamin C (500 mg), vitamin E (273 mg/400 IU), beta-carotene (15 mg), and copper (2 mg)—on age-related macular degeneration was examined as part of a large, randomized, placebo-controlled 5-year clinical trial known as the Age-Related Eye Disease Study (AREDS) and a subsequent trial, AREDS2. In the AREDS trial the inclusion of zinc along with vitamins C and E and beta-carotene produced the greatest benefits [6]. The AREDS2 trial involved individuals with intermediate or advanced age-related macular degeneration and provided nutrient supplements containing reduced zinc (25 mg), no beta-carotene, and added lutein (110 mg), zeaxanthin (2 mg), eicosapentaenoic acid (660 mg), and docosahexaenoic acid (330 mg). AREDS2 reported no further reductions (versus the first AREDS) in the risk of progression to advanced age-related macular degeneration with the changes in nutrient supplementation [7].

Interactions with Other Nutrients

Substances that interact with zinc in the gastrointestinal tract to inhibit its absorption have been addressed in the section on absorption. Other types of interactions between zinc and selected nutrients are presented in this section. Zinc and vitamin A interact in a couple of ways. From the discussion on zinc functions, you may remember that zinc is required for alcohol dehydrogenase structure and activity. Retinol (the alcohol form of vitamin A) serves as a substrate for this enzyme, which converts retinol to retinal (retinaldehyde), the aldehyde form of vitamin A. This metabolism of the vitamin is necessary for its function in the body. In addition, zinc is necessary for the hepatic synthesis of retinol-binding protein, which transports vitamin A in the blood. Zinc deficiency is associated with both decreased mobilization of retinol from the liver (even with adequate liver vitamin A stores) as well as decreased plasma retinol-binding protein concentrations.

The detrimental effect of excessive zinc intake on copper absorption is thought to be attributable to zinc's stimulation of the synthesis of metallothionein, which has a higher affinity for copper than for zinc. With increased intestinal concentrations of metallothionein induced by high zinc levels, ingested copper readily binds to the metallothionein within the enterocyte and becomes "trapped," preventing its passage into the plasma. The increased risk of copper deficiency precipitated by zinc supplementation led to the Tolerable Upper Intake Level for elemental zinc of 40 mg daily [8].

Diminished calcium absorption has been observed with the ingestion of zinc supplements when calcium intake is low (<300 mg/day of calcium) [9]. However, calcium absorption appears to be unaffected by zinc when calcium intake is at adequate (recommended) levels.

Cadmium, if present in high concentrations in the body, appears to bind to sites to which zinc would normally bind and thus disrupts normal zinc functions. For example, cadmium can replace zinc in zinc fingers, preventing the fingers from functioning as they would with zinc present.

Excretion

Zinc is lost from the body primarily via the gastrointestinal tract, kidneys, and skin. Most zinc is excreted through the gastrointestinal tract in the feces; the amount lost increases or decreases depending on body zinc concentrations, although absorption is also regulated to control body zinc homeostasis. Zinc in the feces comes from unabsorbed dietary zinc, sloughed intestinal cells, and unabsorbed endogenous zinc from digestive tract secretions. Additionally, the purposeful intestinal excretion of zinc is accomplished by ZIP5, which facilitates the movement of zinc from the blood across the basolateral membrane and into the enterocyte, followed by the action of ZnT6 on the enterocyte's brush border membrane, which mediates the

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exocytosis of zinc from the intestinal cell into the lumen for excretion in the feces. Fecal zinc losses range from 3 mg/day among adults ingesting very low dietary zinc up to about 4.6 mg with dietary zinc intakes of 15 mg; higher dietary zinc intakes promote greater fecal losses of zinc [10].

Small amounts of zinc are also lost via the kidneys and skin (dermal) as well as in semen and menses. Most zinc filtered by the kidneys is reabsorbed by the tubules. ZnT1 is thought to control renal zinc resorption. About 0.3–0.7 mg of zinc/day is typically excreted in the urine. The zinc appearing in the urine is believed to be derived from the small percentage of plasma zinc that is complexed with histidine and cysteine. It is not affected by dietary zinc except with severe deficiency. Zinc losses of ~0.4–0.7 mg/day occur with exfoliation of skin and with sweating. Other minor routes of zinc loss include (for men) semen (1 mg/ejaculate) and (for women) menses (0.1–0.5 mg total). Hair contains ~0.1–0.2 mg zinc/g of hair [8].

Recommended Dietary Allowance

Zinc recommendations are based on the intake needed to maintain balance as well as on estimates of zinc absorption and body losses. Total daily zinc losses for adult men and women were calculated at 3.84 mg and 3.3 mg, respectively [8]. Zinc losses for men consisted of 0.63 mg urinary zinc, 0.54 mg integumental and sweat zinc, 0.1 mg semen zinc, and 2.57 mg endogenous intestinal zinc; for women, urinary zinc losses were 0.44 mg, integumental and sweat zinc losses were 0.46 mg, menses zinc losses were 0.1 mg, and endogenous intestinal zinc losses were 2.3 mg [8]. To account for absorption, the daily requirements for zinc for adult men and women were set at 9.4 mg and 6.8 mg, respectively, and the RDAs were set at 11 mg and 8 mg, respectively. The RDA for zinc during pregnancy is 11 mg/day to cover the calculated need for growth of the fetus and placenta [8]. The zinc recommendation for lactating women is 12 mg/day [8]. The inside front cover of the book provides additional RDAs for zinc for other age groups.

Deficiency

Signs and symptoms of zinc deficiency observed in children are growth retardation, skeletal abnormalities, poor wound healing, diarrhea, skin rash/lesions/dermatitis (especially around body orifices), and delayed sexual maturation. Some signs and symptoms of deficiency in adults include anorexia, diarrhea, lethargy, depression, skin rash/lesions/dermatitis, hypogeusia (blunting of sense of taste), alopecia (some hair loss; remaining hair make take on a reddish hue), vision problems, and impaired immune function, protein synthesis, and wound healing. Treatment of deficiency in adults typically requires oral zinc supplementation; doses of 10–20 mg/day are recommended. Although higher doses (often up to 50 mg given two to three times per day) may be prescribed, use of such doses is more likely to impair copper status.

Some population groups—especially older adults, vegetarians, and those with alcoholism and with limited income—have been found to consume less than adequate amounts of zinc. Alcohol ingestion additionally reduces intestinal zinc absorption and increases urinary zinc excretion. Thiazide diuretics increase urinary excretion of zinc; consequently, the use of such medications for prolonged periods and in high doses may contribute to deficiency and require increased intakes to maintain zinc status. Additional conditions associated with an increased need for intake include trauma, sickle-cell anemia, and disorders causing malabsorption such as Crohn's disease, short bowel syndrome, celiac disease, and liver failure, as well as surgical bariatric procedures, especially Roux-en-Y gastric bypass and duodenal switch, used to treat obesity. Diarrhea and intestinal fistulas also substantially increase fecal zinc losses; supplementation with up to 20 mg of zinc/day may be needed under such conditions.

Toxicity

Excessive intakes of zinc cause toxicity. An acute zinc toxicity (such as from 570 mg of elemental zinc) produces some of the following symptoms: metallic taste, headache, nausea, vomiting, epigastric pain, abdominal cramps, and bloody diarrhea. In addition, reduced immune function and alterations in copper and iron status may occur. Chronic ingestion of zinc in amounts of about 40 mg (lower for some people) results in a copper deficiency (see the "Interactions with Other Nutrients" section) as well as neurologic problems such as numbness, weakness, ataxia, and spastic gait [8]. Excessive zinc absorbed from the overuse of zinc-containing denture creams has also been associated with toxicity. Additionally, zinc used intranasally (spray or gel) has been reported to cause anosmia (permanent loss of smell) in some individuals. The Tolerable Upper Intake Level for zinc has been set at 40 mg daily based on its interaction with copper [8].

Assessment of Nutriture

Evaluating zinc nutriture is difficult, owing to homeostatic control of body zinc. A variety of indices have been used to assess zinc status, including measurements of zinc in red blood cells, leukocytes, neutrophils, and plasma or serum. The most common basis for assessment is serum or plasma zinc, with fasting concentrations less than about 70 μg/dL (10 μmol/L) suggesting deficiency. A cutoff of 50 μg/dL, however, may better predict clinical signs of zinc deficiency [11]. Low fasting plasma zinc concentrations indicate that little zinc is present in the exchangeable zinc pool and may reflect a loss of tissue zinc (especially from the liver). Plasma zinc concentrations, however, must be interpreted with caution because concentrations are influenced by

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many factors unrelated to zinc depletion, including meals, time of day (diurnal variation), stress, infection, and medications such as steroid therapy. In fact, postprandial (after eating) plasma zinc concentrations have been found to be more sensitive to low dietary zinc intake than fasting plasma zinc concentrations.

Metallothionein has also been used to assess zinc status. Concentrations of metallothionein respond to changes in dietary zinc. For example, liver and red blood cell metallothionein concentrations diminish as dietary zinc intake decreases and are thought to reflect zinc status or stores. Serum zinc and serum metallothionein concentrations can be used to indicate poor zinc status if both are low. Elevations in serum metallothionein coupled with low serum zinc, however, usually suggest an acute-phase response, and in such conditions these indices are not reliable.

Urinary zinc excretion remains fairly constant over a range of intakes and is thought to be a useful marker of status in those with moderate to severe zinc deficiency [8]. Low hair zinc may be associated with chronic intake of dietary zinc in suboptimal amounts; however, the concentration of zinc in hair depends not only on delivery of zinc to the root but also on the rate of hair growth, which is affected by other conditions (including protein status).

Measurement of the activity of zinc-dependent enzymes has also been employed as an index of zinc status. Studies using enzymes as indicators typically have measured carbonic anhydrase or alkaline phosphatase, which "hold" zinc less securely than other zinc metalloenzymes. Ideally, measurements of activity should be taken before and after zinc supplementation.

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Web Site for Nutrient Composition Information

www.ars.usda.gov/Services/docs.htm?docid=18877

COPPER

The copper content of the human body ranges from about 50 to 150 mg. Copper is found in all body tissues and most secretions. In the body, the mineral is found in two valence states, the cuprous state (Cu^{1+}) and cupric state (Cu^{2+}).

Sources

The copper content of food varies widely, reflecting the origin of the food and the conditions under which the food was produced, handled, and prepared for use. The richest sources of copper are meats (especially organ meats like liver) and shellfish (especially oysters and lobster), as shown in Table 13.6. Plant food sources rich in copper include nuts (especially cashews), seeds, legumes, and dried fruits. Potatoes, whole grains, and cocoa (chocolate) are also good sources. In contrast, milk and dairy products are poor sources of the mineral. In the United States, the median copper intake from foods by adults ranges from about 1,000 to 1,600 μg/day [1]. The Daily Value for copper (used on food and supplement labels) is 2 mg (over twice the recommended intake of the mineral).

The main form of copper found in mineral-fortified food products and supplements is copper sulfate; however, cupric oxide is also found in some supplements. The use of cupric oxide as a source of copper is discouraged because the copper has been shown to be unavailable for absorption from the gastrointestinal tract of animals; in fact, it is no longer used as a copper supplement in animal nutrition [2]. In addition to copper sulfate (~25% copper), other bioavailable forms of copper include cupric chloride (~47% copper), cupric acetate (~35% copper), and copper carbonate (~57% copper) [2]. Multivitamin/mineral supplements typically provide about 0.5–2 mg of copper. Single-nutrient supplements contain about 2–3 mg of copper.

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Table 13.6 Copper Content of Selected Foods*

Selected Foods/Food Group	Copper (mg)
Seafood	
Oysters (3 oz)	6.4
Crabmeat (3 oz)	1.0
Lobster (3 oz)	1.6
Salmon (3 oz)	0.05
Eggs and dairy products	
Egg (1)	0.007
Milk (1 cup)	0.02–0.06
Cheeses (1 oz)	0.007–0.12
Grains and cereals	
Rice and pasta, cooked (1 c)	0.14–0.20
Bread white/whole wheat (1 slice)	0.06/0.11
Meat	
Liver, beef (3 oz)	12.4
Chicken (3 oz)	0.04–0.07
Beef, ground (3 oz)	0.07
Pork (3 oz)	0.06–0.14
Legumes, cooked (1 c)	0.35–0.61
Nuts (1 oz)	0.11–0.45
Fruits	0.02–0.09
Vegetables, cooked (1 c)	0.02–0.06
Potato, baked (1 medium)	0.33
Other, cocoa powder (1 Tbsp)	0.2

*The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

Copper from endogenous sources (up to about 4.5 mg) is also released with the secretion of digestive tract juices. For example, the copper contents of saliva and gastric juice are ~400 µg and 1 mg, respectively; pancreatic juice contains up to 2 mg, and bile released into the duodenum contributes another 2.5 mg of copper [3]. The reabsorption of secreted copper is important for body copper homeostasis.

Digestion, Absorption, Transport, and Storage

Figure 13.14 provides an overview of copper digestion, absorption, and transport as well as some of copper's fates in the enterocyte.

Digestion

Most copper in foods is found as Cu^{2+} and is bound to organic components, especially amino acids that make up food proteins. Thus, digestion is needed to free the bound copper before absorption can occur. Hydrochloric acid and pepsin facilitate the release and reduction of bound copper in the stomach. Additional proteolytic enzymes in the small intestine hydrolyze proteins further to release copper.

Absorption

Copper is absorbed primarily in its reduced (cuprous/Cu^{1+}) state from the proximal small intestine, especially the duodenum. While a small amount may be absorbed from the stomach, gastric copper absorption is thought to contribute relatively little to the overall absorption of the mineral.

The reduction of copper (from Cu^{2+} to Cu^{1+}) may occur in the acidic environment of the stomach, but more likely results from the action of one or more reductases, including cytochrome b ferric/cupric reductase, six-transmembrane epithelial antigen of the prostate (steap) 2, and/or cytochrome b reductase 1 found on intestinal cell membranes. Vitamin C may also facilitate the reduction.

Copper absorption across the enterocyte's brush border membrane is accomplished by one or more carrier proteins. One such carrier is copper transporter 1 Ctr 1 (also designated hCtr1), which enables copper (in its Cu^{1+} state) absorption through a gated channel. Ctr1 is found not only on the enterocyte's brush border membrane, but it is also associated with vesicles in the cytosol and is found on most extraintestinal cell membranes to facilitate copper uptake. Synthesis of Ctr1 appears to be regulated by transcription factor Sp1 and is responsive to the body's copper status. However, the mechanism by which body copper influences Sp1 and Ctr1 synthesis is not clear.

In addition to Ctr1, divalent mineral (cation) transporter 1 (DMT1 or DCT1) also serves as a copper transporter, specifically cotransporting (symporting) Cu^{1+} and H^+ across the enterocyte's brush border membrane. Additional, but as of yet unidentified, copper transporters may also facilitate intestinal copper absorption.

The gastrointestinal tract absorbs about 50–80% of ingested copper. Fractional absorption of copper increases as copper intake decreases, and vice versa. Absorption, for example, may average about 20–25% when copper intake is high, such as >5 mg/day, but increases to over 50% when intake is <1 mg/day [4]. Copper absorption was calculated at 75% with an intake of 350 µg of copper, an amount that is about one-half of the adult requirement for the mineral [1].

Factors Influencing Copper Absorption

Copper transport across the enterocyte's brush border membrane may be influenced by a variety of dietary components, with some having a positive effect and others exerting a negative influence on absorption.

Enhancers of Copper Absorption Examples of ligands or chelators that facilitate copper absorption include amino acids, especially histidine and cysteine. Whether copper bound to these amino acids can be absorbed through amino acid carrier systems is not clear. Copper also forms ligands with sulfhydryl groups in cysteine residues that are found within the tripeptide glutathione. Glutathione

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1. Cu^{2+} is released from food components.
2. Copper is reduced to Cu^{1+}, most likely by cytochrome b ferric/cupric reductase, cytochrome b reductase 1, and/or steap2.
3. Cu^{1+} crosses the brush border membrane by a high-affinity Ctr1 transporter, and to a lesser extent by DMT1. Amino acid transporters (not shown) may play a minor role.
4. Within the cytosol, copper binds to one of several chaperones for transport and delivery to target enzymes. Atox1 transports Cu^{1+} to the trans-Golgi network (TGN) from which ATP7A relocates to the basolateral membrane where it functions to export copper from the enterocyte.
5. Copper is delivered to enzymes by chaperones to enable its use in the cells, or it binds to metallothionein for storage.
6. ATP7A transports Cu^{1+} across the basolateral membrane. A defect in this ATPase causes Menkes disease.
7. Copper attaches to proteins for transport in the blood. Following hepatic uptake of copper, most copper is found in the blood as ceruloplasmin.

Figure 13.14 Overview of copper digestion, absorption, enterocyte use, and transport.

is found both within the lumen of the gastrointestinal tract and intracellularly.

The presence of organic acids in foods also improves copper absorption. Citric, gluconic, lactic, acetic, and malic acids act as binding ligands to improve solubilization and thus absorption of copper. Citric acid, for example, forms a stable complex with copper and improves its absorption. A more acidic environment, as found in the proximal intestine versus the distal intestine, also favors copper absorption.

Inhibitors of Copper Absorption Just as an acidic environment facilitates copper absorption, the opposite is true of an alkaline environment. Excessive antacid ingestion or the use of medications such as H_2 receptor blockers (such as Zantac [ranitidine], Tagamet [cimetidine], or Pepcid [famotidine]) and proton pump blockers (such as Prevacid [lansoprazole] or Prilosec [omeprazole]), which are commonly taken to treat heartburn, gastroesophageal reflux disease, and ulcers, results in a high-pH (more alkaline) environment and can diminish copper absorption. Copper atoms in an alkaline medium often bind to hydroxides (OH), forming insoluble compounds that are not readily absorbable. In addition to these pH effects, substances found in foods may bind to copper to diminish its absorption. Phytic acid (see Figure 13.11), found mainly in plant foods (cereals, legumes, etc.), is a known inhibitor of the absorption of copper (among other minerals, including iron, zinc, and calcium).

Zinc also impedes copper absorption. The use of zinc supplements, typically in amounts of about 40 mg or more (but sometimes less), impairs copper absorption

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and diminishes copper status. The detrimental effect of excessive zinc intake on copper absorption is thought to result from zinc's stimulation of metallothionein synthesis in intestinal cells. Although its synthesis is stimulated by zinc, metallothionein more avidly binds copper than zinc, and thus reduces copper's luminal-to-serosal flux (i.e., from the lumen of the gastrointestinal tract across the basolateral membrane) and entry into the blood. Copper deficiency induced by high zinc intake can be difficult to correct. For example, when zinc (110–165 mg) supplements were taken for 10 months, discontinuation of the zinc and 2 months of oral copper supplementation failed to correct the copper deficiency. Intravenous administration of cupric chloride for 5 days (total dose of 10 mg) was needed to bypass the intestinal cells and correct the deficiency, suggesting that the correction of a zinc-induced copper deficiency is a slow process [5]. The effects of supplemental iron and/or iron fortification on copper absorption remain unclear, with conflicting reports depending on the forms and amounts of the nutrients provided, and the populations being studied.

Intestinal Cell Copper Use

Once within the enterocyte, copper is bound to amino acids (especially histidine and cysteine), glutathione (a tripeptide composed of glycine, cysteine, and glutamate, which transports Cu^{1+}), and/or proteins/chaperones. Like iron, free copper ions may damage cells through nonenzymatic reactions (see the "Other Roles" section). Consequently, copper is usually found attached to compounds within the body.

Copper entering the enterocyte has several possible fates. The copper may be:

- stored or sequestered in the enterocyte
- used functionally within the enterocyte
- transported through the cytosol and across the basolateral membrane for entry into the blood and thus use by other tissues.

Copper can be stored temporarily within enterocytes (and other cells) in cytosolic vesicles; the protein Ctr2 serves to transport copper out of the cell cytosol and into cytosolic vesicles for temporary storage. Storage of copper also occurs attached to glutathione (short term) or as part of the protein metallothionein (discussed further in the "Storage" section). While metallothionein may in some cases only temporarily hold onto the copper, the copper, if not released from metallothionein, will be lost into the feces with intestinal cell turnover, approximately every 3 days. Copper's uses in the intestinal cell are similar to its uses in the body and are discussed in the "Functions and Mechanisms of Action" section.

Copper transport across the intestinal cell's basolateral membrane occurs primarily via active transport as Cu^{1+} by the ATPase ATP7A. ATP7A is expressed in most body cells with the exception of hepatocytes, which express a similar transporter, ATP7B. Mutations in the ATP7A gene result in Menkes disease, an X-linked disorder characterized by defective copper efflux from cells. Menkes disease, with an estimated prevalence of 1 in 40,000–350,000, results in increased intestinal cell copper concentrations and impaired delivery of copper to peripheral tissues. Symptoms become apparent at about 2–3 months of age. The condition is characterized by laxity of joints, "steely or kinky" hair, impaired growth, vascular problems, and neurological problems (including progressive neurodegeneration, severe mental retardation, and seizures). While some symptoms may be partially alleviated by intravenous administration of copper, the condition is usually fatal within the first few years of life.

Transport

From the intestinal cells, copper is transported in portal blood to the liver bound loosely to the proteins albumin (specifically the amino [N]–terminus of albumin) and α-2-macroglobulin (which binds two copper atoms and may have a higher affinity for the mineral than albumin) [8]. Following the uptake of copper into the liver (and other tissues), which occurs by multiple carrier proteins, including Ctr1, Ctr2 (which has been identified on the plasma membrane of some cells), DMT1, and likely unidentified carriers, copper binds to glutathione and/or metallothionein or directly to chaperones. Copper chaperones are soluble intracellular proteins that bind intracellular copper and deliver it to various, but specific, locations following cellular copper uptake. The chaperone atox1 transports Cu^{1+} to the trans-Golgi network, where ATP7B directs the copper for insertion into ceruloplasmin as well as other cuproenzymes. In the liver, most copper is used for the synthesis of ceruloplasmin, which contains six copper atoms (giving the protein a blue color). Although copper does not appear to influence ceruloplasmin synthesis, ceruloplasmin activity is diminished without sufficient copper, and ceruloplasmin's half-life is shortened.

Ceruloplasmin is released from the liver and constitutes about 60–70% of circulating copper in the blood; the remaining copper circulates loosely bound to albumin and α-2-macroglobulin for delivery to tissues. Plasma ceruloplasmin concentrations typically range from about 20 to 50 mg/dL; serum copper concentrations normally range from about 70 to 150 μg/dL (10–24 μmol/L).

Storage

The liver is the main organ that stores copper and that controls copper homeostasis in the body. However, compared to other trace minerals, relatively little copper (up to about 150 mg) is found in the body. On a percentage basis, the skeleton, followed by muscles and then the liver, contains the most copper; on an absolute basis, the organs with the most copper per gram are the kidneys, liver, brain,

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and skeleton. Within cells, the protein metallothionein serves as a copper storage pool. Metallothionein binds 12 copper atoms (as well as zinc atoms) per molecule; it is also thought to regulate copper uptake and cellular zinc distribution and to scavenge free hydroxyl radicals, thereby acting in an antioxidant capacity. Copper positively influences hepatic, renal, and brain, but not intestinal, metallothionein synthesis. The mechanism by which copper influences metallothionein synthesis is not well defined.

Functions and Mechanisms of Action

The essentiality of copper is due, in part, to its participation as an enzyme cofactor, either at the enzyme's active site (perhaps as an intermediate in electron transfer) or at the enzyme's allosteric regulatory site. Before addressing the coenzyme roles of copper, a brief discussion of copper chaperones is needed because it is the chaperones that function within cells to transport the copper to the specific enzymes in need of copper for their function. In other words, these chaperones direct intracellular trafficking of the mineral to sites where the copper is needed. Some of the identified chaperones for copper include:

- cyclooxygenase (cox) 17, cox 19, cox 23, and cox 11
- Sco1 and Sco2
- CCS (copper chaperone for superoxide dismutase)
- atox1 (also called hAtx or Hah1).

Six chaperone proteins are involved in the transport of Cu^{1+} to cytochrome c oxidase; these proteins include cox 17, cox 19, and cox 23, as well as the mitochondrial membrane proteins cox 11, Sco1, and Sco2. CCS, which is found in the mitochondria and cytosol, delivers Cu^{1+} to superoxide dismutase. Atox1 ferries Cu^{1+} to ATPases ATP7A and ATP7B, which are necessary for cellular export of copper. More specifically, atox1 transports Cu^{1+} to the trans-Golgi network, where ATP7B then directs it for insertion into cuproenzymes such as ceruloplasmin. This section addresses a few of the body's cuproenzymes (copper-requiring metalloenzymes; Table 13.7).

Table 13.7 Selected Functions of Copper

Ceruloplasmin and hephaestin: iron oxidation
Superoxide dismutase: antioxidant
Cytochrome c oxidase: ATP production
Amine oxidases: oxidation of biogenic mono- and diamines
Lysyl oxidase: collagen and elastin cross-linking
Dopamine monooxygenase/hydroxylase: norepinephrine (catecholamine) synthesis
Tyrosinase: melanin pigment production
Peptidylglycine α-amidating monooxygenase: activation of selected hormones/peptides
Factors V and VIII: blood clotting
Immune function

Ceruloplasmin: Iron Utilization

Ceruloplasmin, also known as ferroxidase, is critical for iron utilization in the body. This oxidase is found in the blood and bound to receptors on the plasma membranes of cells, where it oxidizes minerals, most notably ferrous (Fe^{2+}) iron but also manganese (Mn^{2+}). Its role in the oxidation of iron (Fe^{2+} to Fe^{3+}) is critical for cellular iron release and binding to transferrin for transport to the body's tissues.

An absence of functioning ceruloplasmin due to genetic mutations impairs iron utilization and is characterized by increased iron deposition in tissues, especially the liver, pancreas, and brain, and by low serum iron concentrations. Copper metabolism is not affected.

Another proposed function of ceruloplasmin is as an antioxidant or modulator of the inflammatory process. As modulators of the inflammatory process, acute-phase (also called reactant) proteins like ceruloplasmin increase in the blood in response to severe infections and other inflammatory events (such as injury). This rise in blood levels is important because during infections, for example, phagocytosis of invading organisms by white blood cells generates superoxide radicals, among other damaging compounds. These compounds, while normally produced by cells, are generated in larger amounts with infections and inflammation and must be eliminated (by ceruloplasmin, superoxide dismutase, or other enzymes) to prevent excessive damage to body cells.

Hephaestin: Iron Utilization

Hephaestin, like ceruloplasmin, oxidizes iron. However, this copper-containing ferroxidase protein is present on the basolateral membrane of enterocytes where it oxidizes iron to its ferric state to enable transport attached to transferrin. The protein may also be present in other tissues.

Superoxide Dismutase: Antioxidant

Superoxide dismutase (SOD) is a copper- and zinc-dependent enzyme (although another form in the mitochondria is manganese dependent). In the enzyme, copper and zinc are linked to the enzyme by an imidiazole group, and histidine and aspartate residues. Copper (Cu^{2+}) is found at the enzyme's active site, where the superoxide substrate binds to the enzyme. Removal of copper, but not zinc, results in reduced cytosolic superoxide dismutase activity. Specifically, superoxide dismutase catalyzes the removal (dismutation) of the superoxide radicals ($O_2^{\bullet}$). During the reaction, copper is reduced along with the oxygen radical

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to initially generate molecular oxygen (O_2) and then, by reoxidation, hydrogen peroxide (H_2O_2).

$$2O_2^{\bullet} + 2H^+ \xrightarrow{\text{Superoxide dismutase}} O_2 + H_2O_2$$

Superoxide radicals along with other free radicals can cause peroxidative damage to the phospholipid component of cell membranes, especially disrupting unsaturated double bonds in fatty acids, as well as damaging other cellular components. Superoxide dismutase therefore assumes an important protective function. The enzyme is found in the cytosol of most cells (SOD1). Additionally, an extracellular form (SOD3) is found in the blood and other body fluids (e.g., lymph, synovial fluid, and lungs); it is also secreted and bound to heparan sulfate on the surface of cells and is found in relatively large concentrations in the arterial wall. Increased peroxidation of cell membranes is observed with copper deficiency.

Cytochrome c Oxidase: Energy Production

Cytochrome c oxidase contains three copper atoms per molecule. One subunit of the enzyme contains two copper atoms and functions to receive electrons from cytochrome c and then transfer the electrons to the second subunit. The second subunit contains another copper atom and is involved in reducing molecular oxygen and ultimately in energy production. Cytochrome c oxidase (which also requires iron) functions in the terminal oxidative step in mitochondrial electron transport. It is when the enzyme transfers an electron so that molecular oxygen (O_2) is reduced to form water molecules that enough free energy is generated to permit ATP production. Severe copper deficiency impairs the activity of this enzyme.

Lysyl Oxidase: Collagen Synthesis

Lysyl oxidase, secreted by connective tissue cells (bone, blood vessels, etc.), generates cross-links between connective tissue proteins, including collagen and elastin. Specifically, lysyl oxidase catalyzes the removal (oxidative deamination) of the epsilon (**ε**)-amino group of lysyl and hydroxylysyl residues of a collagen and elastin polypeptide and the oxidation of the terminal carbon atom of an aldehyde to form cross-links. The cross-linking is needed to stabilize the extracellular matrix. Lysyl oxidase activity decreases with inadequate copper intake, negatively affecting the strength of connective tissues [6]. While lysyl oxidase is an amine oxidase, it is listed here, separate from the other section addressing amine oxidases because of its different physiological role in the body.

Dopamine β-Monooxygenase/Hydroxylase, Tyrosinase, and Peptidylglycine α-amidating Monooxygenase: Catecholamine, Pigment, and Neurotransmitter/Neuropeptide Activation

Catecholamine and Pigment Synthesis In tyrosine metabolism (see Figure 6.10), the production of the catecholamine norepinephrine and of melanin pigments requires copper-dependent enzymes. Norepinephrine synthesis, which occurs mainly in the adrenal medulla and neurons, begins with tyrosine, which is converted in an iron-dependent reaction to 3,4-dihydroxyphenylalanine (also called L-dopa). The L-dopa is then decarboxylated to form dopamine. To synthesize norepinephrine from dopamine, the enzyme dopamine β-monooxygenase/hydroxylase, which contains up to eight copper atoms per molecule, is required for the hydroxylation reaction as shown. Vitamin C serves as a cosubstrate and reduces the copper. Norepinephrine functions in the body as both a hormone and neurotransmitter, affecting a wide range of physiological processes.

While in some cells L-dopa is used to form dopamine, in melanocytes, which are found in the epidermal layer of skin and in the eyes and hair, L-dopa can be oxidized by the copper-dependent enzyme tyrosinase (also called catechol oxidase) to produce dopaquinones. The dopaquinones then polymerize to form pigments called melanin. Melanin provides color to the iris of the eye, skin, and hair. A mutation in the tyrosinase enzyme causes the condition called albinism, which is characterized by lighter than normal skin color, white hair, and light-colored eyes.

Neurotransmitter/Neuropeptide Activation The amidation of some peptide hormones, such as bombesin, calcitonin, gastrin, and cholecystokinin, is necessary for hormone function. The amidation requires the copper-dependent enzyme peptidylglycine α-amidating monooxygenase, which is found mostly in the brain. This enzyme cleaves a carboxy terminal glycine residue off peptides that have a C-terminal glycine. The amino group

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of glycine is retained by the peptide as a terminal amide. The oxidized residue is released as glyoxylate. Peptidylglycine α-amidating monooxygenase also requires vitamin C to reduce Cu^{2+} back to Cu^{1+}, and is shown in Figure 9.5. Because of the divergent roles that these hormones play in the body once activated by amidation, copper indirectly has wide-ranging effects on multiple body processes.

Amine (Monoamine and Diamine) Oxidases: Biogenic Amine Degradation

Amine oxidases, including mono- and diamine, are also copper dependent and found in the blood and body tissues. The enzymes catalyze the oxidation of biogenic amines, such as tyramine, histamine, and dopamine, as well as serotonin (5-hydroxytryptamine), norepinephrine, and polyamines, to form aldehydes and ammonium ions (NH_4 is generated from the cleaved amine group). In the reaction, oxygen (O_2) is reduced to form hydrogen peroxide (H_2O_2). Because of the wide range of biogenic amines oxidized by copper-dependent amine oxidases, suboptimal copper status may result in a broad range of neurological and physiological manifestations.

Other Roles

Copper also plays a variety of other roles in the body, some of which are not well characterized. The blood clotting proteins (factors) V and VIII both contain copper atoms. Copper is also involved in angiogenesis, immune system function, nerve myelination, and endorphin action. Copper is thought to influence gene expression by binding to specific transcription factors, which in turn bind to promoter sequences on DNA. Once the copper-bound transcription factors interact with DNA, transcription may be enhanced or suppressed. These interactions, however, are not well characterized.

Pro-Oxidant Role

As a pro-oxidant, copper (if free) behaves similarly to iron. Copper reacts with superoxide radicals and catalyzes the formation of hydroxyl radicals through the Fenton reaction: $Cu^{1+} + H_2O_2 \rightarrow Cu^{2+} + OH^- + {}^{\bullet}OH$. Associated with the generation of reactive oxygen species is increased oxidative damage to DNA (base oxidation and strand breaks), proteins, and lipids (peroxidation), especially membrane lipids.

Interactions with Other Nutrients

Copper is known to interact with a number of dietary constituents. Those that affect copper absorption have been described previously. Additional interactions with iron and molybdenum that are unrelated to intestinal copper absorption are discussed here.

The importance of copper in normal iron metabolism is evidenced by the microcytic anemia that results from prolonged copper deficiency. This anemia is caused by impaired mobilization and use of iron, stemming from the reduced ferroxidase activity of hephaestin and ceruloplasmin, which oxidize iron to its trivalent (Fe^{3+}) state. With copper deficiency, the activity of ceruloplasmin and the expression of hephaestin are reduced, and iron remains mostly trapped within cells. Thus, copper deficiency results in a secondary iron-deficiency anemia, and treatment with copper (and not iron) is required to correct the problem.

Another nutrient that interacts with copper is molybdenum. The mineral, when given as tetrathiomolybdate, interferes with copper utilization and consequently has been shown to be beneficial in the management of Wilson's disease (see "Toxicity") [7].

Excretion

Copper is excreted primarily (~95%) through the bile into the feces. In fact, biliary copper excretion is regulated by the liver to maintain copper balance (homeostasis). Thus, with high dietary copper intake, biliary copper excretion via the feces increases, and with low dietary copper intake, fecal copper excretion decreases. Fecal copper losses range from about 0.5 to 2.5 mg/day [3].

The ATPase ATP7B, which is positioned usually in the trans-Golgi network, in hepatocytes plays a major role in copper excretion. Concentrations of ATP7B are controlled, at least in part, by "copper metabolism (Murr1) domain containing 1," abbreviated COMMD1, and X-linked inhibitor of apoptosis (XIAP). When the copper content of hepatic cells is low or normal, atox1 takes copper to the trans-Golgi network where it is directed into the secretory pathway for incorporation into ceruloplasmin and other apocuproenzymes. In the presence of high copper, the copper gets "stored" likely in cytosolic vesicles. Additionally, ATP7B translocates from the trans-Golgi network and facilitates the fusion and exocytosis of the copper-containing vesicles and the exocytosis of lysosomal copper across the hepatic canalicular membrane for excretion into the bile (Figure 13.15). This bile containing the excess copper is secreted into the duodenum; however, the copper in the bile is bound to bile components and thus cannot be reabsorbed from the small intestine. Wilson's

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1. Ctr1 transports copper into the hepatocytes from the blood.
2. Atox1 functions as a chaperone to take Cu^{1+} to the trans-Golgi network.
3. Within the trans-Golgi network (TGN), copper is incorporated into ceruloplasmin (Cp) and other cuproenzymes. ATP7B transports the proteins into the secretory pathway.
4. In the presence of excess copper, ATP7B moves from the TGN to copper-containing cytosolic vesicles (and lysosomes—not shown) to direct the secretion of the copper from these sites into the bile duct.

Figure 13.15 The role of ATP7B in copper use and excretion in the liver.

disease, an inherited disorder of copper metabolism, is characterized by defective biliary copper excretion and thus copper toxicity. The disorder results from a mutation(s) in ATP7B and is discussed further in the "Toxicity" section. Other conditions such as cholestasis, however, can impair bile flow, diminish copper excretion, and increase the likelihood of copper toxicity.

Other minor routes of copper excretion/losses include the urine, sweat and skin cell desquamation, menses (for women), semen (for men), hair, and nails. Copper loss via the urine is small (<60 μg). Urinary copper excretion does not typically change with changes in copper intake except under extreme conditions [8]. Similarly, only small amounts (<50 μg) of copper are lost in sweat and with desquamation of skin cells. Women experience trace losses of copper in normal menstrual flow; however, a woman's copper status, unlike her iron status, is not compromised by menstruation. Together, losses from menses or semen, along with losses from hair and nails, are not thought to exceed surface losses [1].

Recommended Dietary Allowance

The results of depletion and repletion studies, along with other studies permitting factorial analysis of obligatory losses over a range of intakes, have enabled estimates of copper requirements. Based on a copper requirement for adults of 700 μg, a 30% coefficient of variation of the requirement, and rounding to the nearest 100 μg, an RDA for copper was set at 900 μg/day [1]. Recommendations during pregnancy and lactation are 1,000 μg and 1,300 μg, respectively [1]. RDAs for copper for other age groups are found on the inside front cover of the book.

Deficiency

Various clinical manifestations are associated with copper deficiency. Most commonly recognized signs and symptoms include hypochromic, microcytic anemia (reduced red blood cell counts and hemoglobin) that does not respond (is refractory) to supplemental iron and leukopenia (specifically neutropenia, a lower-than-normal number of neutrophils). Other manifestations may include hypopigmentation or depigmentation of skin and hair, impaired immune function, blood vessel/connective tissue and bone abnormalities, altered cholesterol metabolism, hypotonia, and cardiovascular and pulmonary dysfunction.

The likelihood of copper deficiency increases in persons consuming excessive amounts of zinc (especially greater than 40 mg/day). In addition, deficiency is more likely with prolonged use of medications (such as proton pump inhibitors) that increase the intestinal pH and thus diminish copper absorption. Additionally, persons with conditions

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that promote increased loss of copper from the body, such as nephrosis, or that decrease the copper absorption, such as gastrointestinal malabsorptive disorders (e.g., celiac disease, Crohn's disease, and ulcerative colitis), are at greater risk of deficiency. Bariatric surgical procedures, such as Roux-en-Y, also increase the likelihood of deficiency secondary to decreased absorption.

Copper deficiency usually responds to oral supplementation with copper. Ingestion of doses providing about 1.5–3 mg of copper, taken once or twice daily for about 1–3 months, is usually sufficient to correct deficiency. However, higher doses or intravenous copper administration is sometimes needed, especially if the copper deficiency is zinc induced.

Toxicity

Copper toxicity is fairly rare in the United States, although acute poisonings have occurred because of water contamination and accidental ingestion. A Tolerable Upper Intake Level for copper was set at 10 mg/day by the Food and Nutrition Board [1], although intakes below this level, such as 5 mg, may cause gastrointestinal discomfort. Ingestion of a large dose of copper (such as 64 mg elemental copper provided by 250 mg of copper sulfate) may cause acute toxicity, characterized typically by epigastric pain, nausea, vomiting, diarrhea, weakness, lethargy, and anorexia. Other symptoms of toxicity with prolonged intake of high amounts of copper include hematuria (blood in urine), liver damage resulting in jaundice, and kidney damage resulting in oliguria (little urine production) or anuria (no urine production) [9]. Chronic copper ingestion of 30 mg daily for 2 years followed by 60 mg daily for a year resulted in liver failure in a young man who self-prescribed copper supplements [10].

Wilson's disease, a genetic disorder resulting from a mutation(s) in the gene coding for ATP7B, is characterized by copper toxicity and is lethal if not treated. The estimated prevalence worldwide is between 1 in 30,000 and 1 in 100,000 individuals. The most common mutation in Caucasians is C3207A, which disrupts the binding of ATP to the transporter. The absence or dysfunction of ATP7B in turn disrupts copper excretion into the bile and into the secretory pathway for incorporation into cuproenzymes such as ceruloplasmin. Thus, in Wilson's disease, copper accumulates in the liver but also leaks out (unbound, i.e., not as part of ceruloplasmin) into the blood and is deposited in joints and other organs, especially the brain, kidneys, heart, and eyes (cornea). Symptoms are usually not apparent until at least 7 years of age. Deposition of copper in the liver leads to cirrhosis, inflammation and liver failure, and in the kidneys causes renal failure. The copper deposition in the corneas results in Kayser-Fleischer (greenish or brownish gold) rings visible in the eyes. Osteoarthritis occurs with copper deposits in joints. Neurologic dysfunction (such as seizures and movement disorders) and/or psychiatric problems (including personality changes) may also occur secondary to copper deposition in the brain. At present, Wilson's disease is treated primarily with systemic chelation medications, such as D-penicillamine or triethylenetetramine therapy, to bind body copper and increase its urinary excretion. Avoidance of high-copper foods is recommended. Additionally, zinc supplements (such as 50 mg zinc as zinc acetate or zinc sulfate given three times a day) along with molybdenum supplements (such as thiomolybdate given in six doses of 20 mg each) may also be recommended to decrease intestinal copper absorption and to enhance urinary copper excretion, respectively [11].

Assessment of Nutriture

Copper status is best assessed using multiple indicators. Serum, plasma, or red blood cell copper is frequently used, but these indicators are likely inadequate to assess short-term changes in copper status. The lower end of the normal range for serum copper concentrations is about 70 μg/dL (10 μmol/L). The change in plasma/serum copper concentration that occurs when subjects consume inadequate copper varies considerably between individuals and is further affected by several factors unrelated to diet. An extremely low copper intake (~0.38 mg/day), however, appears to be sufficient to significantly decrease not only plasma copper but also ceruloplasmin concentration and activity as well as urinary copper excretion [12]. Changes in serum ceruloplasmin concentration and activity are also used to assess copper status. In fact, decreased concentrations of serum ceruloplasmin (<20 mg/dL) are often an early manifestation of copper deficiency.

Response of serum ceruloplasmin to copper supplements may also be used to assess copper status; however, because ceruloplasmin is an acute-phase reactant protein, it may be elevated secondary to inflammation and not representative of copper status. Typically, provision of supplemental copper first normalizes serum copper and the neutrophil count, then serum ceruloplasmin [13]. Ceruloplasmin concentration increases following supplementation only in copper-deficient subjects. Another useful indicator of copper status is measurement of the activity of copper-dependent enzymes such as superoxide dismutase in the red blood cell. Superoxide dismutase activity is sensitive to longer-term copper deficiency [14]. Platelet copper concentrations, along with platelet or leukocyte cytochrome c oxidase and skin lysyl oxidase activity, are responsive to changes in copper status.

Copper concentrations in hair have not been correlated with either serum or organ copper, even though they are reduced with a prolonged period of copper deficiency. Hair copper concentrations are not, however, thought to be useful as an indicator of copper status. Similarly, urinary copper excretion, which is normally very low, is responsive to change, but only when intake is so low that other indicators have already declined [12,14].

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Web Site for Nutrient Composition Information

www.ars.usda.gov/Services/docs.htm?docid=18877

SELENIUM

Selenium, which is a nonmetal but which has some metallic properties, exists in several oxidation states, including Se^{2-}, Se^{4+}, and Se^{6+}. The chemistry of selenium is similar to that of sulfur; consequently, selenium can often substitute for sulfur. The total body selenium content is about 20 mg [1].

Sources

Perhaps more than any other essential element, selenium varies greatly in its soil concentration throughout the regions of the world, with low concentrations especially prevalent in New Zealand, Finland, Denmark, and parts of China and Russia. Consequently, the selenium content of plant foods and products is extremely variable. Generally, the richest sources of selenium include organ meats and seafoods, followed in descending order by muscle meats, cereals and grains, eggs, and milk/dairy products (Table 13.8). Fruits and most vegetables are typically poor sources of the element; however, some plants, such as wheat, broccoli, onions, asparagus, cabbage, and garlic, hyperaccumulate selenium from the soil, and thus may provide significant amounts. The selenium content of cereals varies from less

Table 13.8 Selenium Content of Selected Foods*

Selected Foods/Food Group	Selenium (µg)
Meats (3 oz)	**~15–40**
Beef, bottom round (3 oz)	33
Beef, ground (3 oz)	18
Chicken, white meat (3 oz)	26
Turkey, white meat (3 oz)	31
Pork, tenderloin (3 oz)	32
Seafood	
Snapper (3 oz)	41
Swordfish (3 oz)	58
Grouper (3 oz)	40
Salmon, Atlantic, farmed (3 oz)	20
Halibut (3 oz)	47
Tuna, yellowfin (3 oz)	92
Tuna, canned, light (1/2 c)	55
Shrimp (3 oz)	42
Nuts and Seeds	
Almonds (1 oz)	<1
Cashews (1 oz)	3
Brazil (1 nut)	90
Sunflower seeds (1/4 c)	25
Dairy	
Milk, 1% fat (1 c)	8
Yogurt, Greek (5.3 oz)	14
Cottage cheese, 2% fat (1/4 c)	13
Feta (1/4 c)	5
Cheese, mozzarella/swiss (1 oz)	4
Grains	
Bread, white (1 slice)	6
Bread, whole wheat (1 slice)	13
Cereals, fortified (1 c)	15
Pasta, enriched (1/2 c)	16
Rice, brown or white (1/2 c)	7
Couscous (1/2 c)	2
Quinoa (1/2 c)	3
Other	
Egg, poached (1)	15
Peanut butter (2 Tbsp)	2
Spinach, cooked (1/2 c)	5
Lentils, cooked (1/2 c)	3
Beans, variety (1/2 c)	1

*The United States Department of Agriculture publishes extensive information on nutrient contents of foods. See http://ndb.nal.usda.gov.

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than 10 μg to over 80 μg/100 g based on the selenium content of the soil in which the cereals were grown. Additionally, although fish is generally an excellent source of selenium, the mercury content, if high, in some fish limits selenium's bioavailability secondary to the formation of unabsorbable mercury–selenium complexes. Another very rich source of selenium is the Brazil nut, which provides up to ~90 μg of selenium (as low as 10 μg) per nut [2]; however, regular consumption of even an ounce (about six to eight nuts, which can contain about 540 μg of selenium) increases the likelihood of toxicity (see the section on "Toxicity"). Selenium intakes in the United States are typically adequate.

Selenium occurs naturally in foods and in the body in both organic and inorganic forms. The organic forms, selenomethionine and selenocysteine, represent selenium analogues of the sulfur-containing amino acids methionine and cysteine, respectively (Figure 13.16). The element substitution is made possible due to the chemical similarity between selenium and sulfur. In plants, these selenium analogues become incorporated into plant proteins. Consumption of plant foods provides mostly selenomethionine, with lesser amounts of selenocysteine and selenium methyl selenocysteine, and some inorganic forms of selenium such as selenite (H_2SeO_3) and selenate (H_2SeO_4). Animal products tend to contain selenium primarily as selenocysteine. Another major inorganic form of selenium is selenide (H_2Se or HSe^-), which serves as an important metabolite in the body, as discussed in the section "Metabolism."

Supplements provide selenium as selenomethionine, selenium methyl selenocysteine, sodium selenate, sodium selenite, and/or selenium-enriched yeast (which usually contains a mixture of different forms of selenium). Multivitamin/mineral supplements typically provide 50–200 μg of selenium. Selenium supplements sold as a single nutrient contain similar amounts and forms as is used in the multivitamin/mineral preparations. The various forms of selenium, however, do not appear to equally increase the body's selenoproteins; selenium as selenomethionine is about two times more available than selenite [3,4]. The Daily Value for selenium is 70 μg.

Figure 13.16 Selenomethionine and selenocysteine.

Digestion, Absorption, Transport, and Storage

Digestion and Absorption

Selenium, in organic and inorganic forms, is efficiently absorbed throughout the small intestine, including the duodenum, jejunum, and ileum, and does not require digestion prior to its absorption into enterocytes. Selenoamino acid absorption, which occurs through amino acid transport systems, is estimated to be over 80%, with selenomethionine typically better absorbed (at about 97%) than selenocysteine. Selenate is absorbed by active, sodium-dependent transport, primarily in the distal small intestine. Selenite absorption occurs by passive diffusion, primarily in the jejunum. Usually greater than 90% of dietary selenate and greater than 50% of dietary selenite are absorbed into enterocytes. Selenium absorption does not appear to be regulated and thus does not play a role in body selenium homeostasis. Within the intestinal cell, some selenite may be reduced using glutathione or other thiols to form selenide.

Factors Influencing Selenium Absorption Factors enhancing selenium absorption include vitamins C, A, and E, as well as the presence of reduced glutathione in the intestinal lumen. Heavy metals (such as mercury) and phytic acid are thought to inhibit selenium absorption through chelation and precipitation.

Transport

Selenoamino acids are likely transported across the basolateral membrane using amino acid transporters; however, the mechanism(s) by which inorganic selenium crosses the enterocyte's basolateral membrane to enter portal blood is largely unknown. Within portal blood, selenoamino acids travel free (unattached) to the liver and other tissues. Selenite entering portal blood is thought to be taken up by red blood cells and reduced to selenide, which is then released back into portal blood for transport to the liver bound to albumin. Some selenite, and likely other inorganic forms of selenium, upon leaving the intestinal cells, may also attach to the sulfhydryl groups of apoproteins found on the surface of lipoproteins for travel to the liver. The liver takes up about 50% of absorbed selenium on first pass [1].

Once in the liver, organic and inorganic forms of selenium are metabolized. Selenium is found in the blood mainly as part of selenoprotein P, which contains 50–80% of selenium in the plasma; other transporters including albumin, however, also likely provide selenium to tissues for use. Selenoprotein P has a half-life of about 3 hours. Receptors for selenoprotein P are found on the cell membranes of some body tissues. An apoprotein E receptor (ApoER2) governs selenoprotein P uptake into the brain and testes. In the kidneys, a megalin receptor mediates selenoprotein P uptake, most likely by receptor-mediated endocytosis. Selenoprotein P is described further

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in the "Functions and Mechanisms of Action" section. Plasma selenium concentrations typically range from about 60 to 150 μg/L (Note μg/L = ng/mL).

Storage

Tissue selenium concentrations vary to some extent, with amounts typically higher in the kidneys, liver, and spleen, and lower in the pancreas, heart, brain, and lungs. Skeletal muscle, bones, and red blood cells also contain fairly large quantities of the mineral. The form(s) in which selenium is found in these tissues is not clear.

Metabolism

Within tissues such as the liver and to some extent in other tissues such as the kidneys, selenoamino acids and inorganic forms of selenium undergo metabolism (Figure 13.17). Selenate ingested from the diet is reduced to selenite. Selenite, either obtained directly from the diet or produced from selenate, is further reduced to selenide. The reduction of selenite (but not selenate) is facilitated by thioredoxin reductase and glutathione; how selenate is reduced to selenite remains unclear [5].

Selenomethionine, which is derived from the diet, may be either "stored" in tissues as selenomethionine in an amino acid pool, used for protein synthesis (just as the amino acid methionine is used), catabolized in multiple reactions (referred to as trans-selenation) to yield selenocysteine, or directly lysed at the γ-position to generate selenide. This latter reaction is thought to be induced with excessive selenium intakes. Selenocysteine, which is derived either from selenomethionine metabolism or from the diet, is degraded primarily by selenocysteine β-lyase; it also yields selenide.

Selenide, derived from selenite, selenate, and selenomethionine as well as selenocysteine, in turn, can be methylated and excreted in the urine or it can be converted by selenophosphate synthase into selenophosphate. Selenophosphate is an important intermediate in the synthesis of the body's selenium-dependent enzymes (selenoenzymes), as shown in Figure 13.17.

Interestingly, although selenocysteine is required for selenium-dependent enzyme function, selenocysteine obtained directly from the diet or selenomethionine degradation cannot be utilized. Instead, the selenocysteine must be synthesized in the body from the amino acid serine, while the serine is attached to transfer (t) RNA, and from selenophosphate (Figure 13.17). It is through the activity of the selenocysteine-containing enzyme selenophosphate synthetase 2 that selenophosphate is made. Thus, while attached to tRNA, serine is converted, through a series of reactions involving selenophosphate, into selenocysteine (abbreviated $tRNA^{SEC}$). Should selenophosphate not be produced in sufficient quantities, the subsequent generation of all selenoproteins becomes diminished. A total abolishment of selenoprotein synthesis is lethal. Furthermore, diminished activity of even some selenoproteins also results in massive cellular damage due to the reduction of the antioxidant/cell redox services normally provided by the selenoproteins.

The incorporation of selenocysteine that is made while attached to the tRNA involves a novel process whereby the UGA stop codon is reprogrammed to be read as a sense codon. This reprogramming requires (1) $tRNA^{SEC}$, (2) a selenocysteine insertion sequence (SECIS) in the 3′ untranslated section of the mRNA of selenoproteins, (3) SEC insertion sequence binding protein 2 (SBP2), and (4) elongation factor selenocysteine (EFsec). The selenocysteine insertion sequence is found in the 3′ untranslated part of the mRNA of selenoproteins. The attachment of the SEC insertion sequence binding protein 2 to the insertion sequence on the mRNA enables interaction with a specific elongation factor and with the $tRNA^{SEC}$. In the presence of these elements, selenocysteine is incorporated at the UGA codon, and UGA is not read as a stop codon. Because selenocysteine is encoded by a codon and is incorporated into proteins by specific tRNA, it is widely considered as the 21st naturally occurring amino acid in the genetic code. The amino acid's three-letter abbreviation is SEC and one-letter abbreviation is U.

Functions and Mechanisms of Action

The better-characterized roles of selenium are related to its functions as an integral part of specific proteins/enzymes (e.g., selenoproteins) in the body. Over 25 genes encoding these selenoproteins have been identified in humans. These proteins primarily function in antioxidant capacities, and thus regulate cell redox status. The next sections describe some of the selenium-dependent enzymes and their metabolic roles.

Selenophosphate Synthetase 2: Selenoprotein Synthesis

Selenophosphate is a key compound needed in the body to synthesize the other selenocysteine-containing proteins/enzymes such as glutathione peroxidase, deiodinase, thioredoxin reductase, and selenoprotein P, among others. At least two forms of selenophosphate synthetase have been identified. One form (designated SPS1) does not contain selenocysteine and is thought to recycle selenium from selenocysteine. The selenophosphate synthetase 2 isoform contains selenocysteine and catalyzes the synthesis of selenophosphate from selenide, as shown here:

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1 Synthesis of selenocysteine-containing proteins begins with selenophosphate and the amino acid serine, which is esterified to a specific transfer (t) RNA to form seryl $tRNA^{SEC}$. Selenocysteine synthase replaces the hydroxy group of serine with a HSe^- from selenophosphate to form SEC-$tRNA^{SEC}$ (or SEC-tRNA for short).

2 SEC-tRNA delivers selenocysteine to the growing peptide chains of the various SEC-containing proteins like glutathione peroxidase, iodothyronine 5′-deiodinase, and thioredoxin reductase, among others.

Figure 13.17 Selenium metabolism in the liver.

Mutations in selenophosphate synthetase 2 or defective selenophosphate synthetase 2 activity significantly reduces selenoprotein synthesis. This in turn leads to increased reactive oxygen species generation and cellular apoptosis.

Glutathione Peroxidase (GPX): Antioxidant

One of the most clearly established functions of selenium is as an integral part of the enzyme glutathione peroxidase. Several glutathione peroxidase enzymes (designated GPX followed by a number) have been characterized, and each catalyzes the same basic reaction but in different tissues. Most GPXs (of which seven have been identified) are selenium dependent, containing selenium as selenocysteine. Within cells, glutathione peroxidase is found mainly (~70%) in the cytosol and to a lesser extent (~30%) in the mitochondrial matrix; however, GPX4 is found predominantly associated with cell membranes. GPX1 is found in most body tissues but most notably in the liver, kidneys, and red blood cells. GPX1 concentrations are reduced with selenium deficiency to a greater extent than other GPXs. GPX2 is found mainly in the gastrointestinal tract and liver. GPX6 is found in olfactory epithelium. GPX3 originates in the kidney but is released into the plasma (extracellular); GPX3 accounts for 10–30% of selenium in the plasma and has a half-life of about 12 hours. Serum selenium concentrations of about 70 or 80 ng/mL and a selenium intake of about 37 μg as selenomethionine or 66 μg as selenite/day are needed to optimize GPX3 activity [3].

Glutathione peroxidase catalyzes the removal of hydrogen peroxides (H_2O_2) and organic (including lipid) hydroperoxides (designated ROOH). GPX4 functions in a similar capacity, removing phospholipid hydroperoxides (designated LOOH) associated with membranes. Organic peroxides are derived from nucleic acids and other molecules, including unsaturated fatty acids; however, a peroxide derived from fatty acids may be designated as a lipid peroxide. Hydrogen peroxides are generated in many cells throughout the body as part of normal metabolism and may be generated in large amounts by activated white

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blood cells as they phagocytize foreign substances. If not removed, these peroxides typically damage cellular membranes and other cell components including proteins and DNA. Glutathione, a tripeptide of glycine, cysteine, and glutamate found in most body cells, is needed in its reduced form (GSH) for the glutathione peroxidase–catalyzed reaction. Reduced glutathione, which is thought to be the most abundant antioxidant found in cells, furnishes the reducing equivalents, as shown in the following reactions.

The oxidized glutathione (GSSG) that is formed as a result of glutathione peroxidase activity must be regenerated back to its reduced form (GSH). This regeneration is imperative for cells to maintain appropriate redox states. Glutathione reductase, a flavoenzyme, catalyzes this reduction in a reaction dependent on $NADPH + H^+$, which is derived from the pentose phosphate pathway (hexose monophosphate shunt). The regeneration of reduced glutathione is shown here:

Selenoprotein P: Antioxidant

Selenoprotein P, a glycoprotein, is synthesized mostly in the liver but also in the brain; it is found throughout the body, including in association with capillary endothelial cells. The protein transports selenium from the liver to some tissues for use, but also exhibits antioxidant functions such as peroxynitrite (ONOO) radical disposal. Peroxynitrite is synthesized by activated white blood cells (e.g., at infection sites) from superoxide radicals ($O_2^{\bullet}$) and nitrogen monoxide (NO). If not inactivated, peroxynitrite causes DNA single-strand breaks and lipid peroxidation, among other damage. Selenoprotein P may also catalyze the reduction of membrane phospholipid hydroperoxides to alcohols, similar to the function of GPX4.

Selenoprotein P, unlike most selenoenzymes (which contain one to four selenium atoms as selenocysteine), contains up to 10 selenocysteine residues. However, under conditions in which selenium is limited, selenoprotein P may be synthesized with fewer selenocysteine residues. In other words, instead of having 10 selenocysteines, selenoprotein P may only have two or three or so selenocysteines if sufficient selenium is not available in the cells. Moreover, when selenium is limited, selenoprotein P appears to preferentially receive selenium over other selenoenzymes such as glutathione peroxidases [6]. Selenoprotein P is optimized at a serum selenium concentration of about 120 ng/mL; a selenium intake (as selenomethionine) of about 37 μg/day was not sufficient to optimize selenoprotein P activity [3].

Thioredoxin Reductase: Antioxidant

Thioredoxin reductase is a flavoenzyme (containing FAD) that, like glutathione peroxidase, selenoprotein P, and selenophosphate synthetase 2, contains selenocysteine at its active site. The enzyme is found in three forms designated by numbers: form 1 is typically located in the cytosol, form 2 is present in the mitochondria, and form 3 is found primarily in the testes. Within cells, thioredoxin reductase helps maintain the redox state (i.e., prevents oxidative stress) by reducing oxidized thioredoxin (TrxS-SxrT) and oxidized glutaredoxin, as well as a multitude of other oxidized molecules and protein substrates. Some of these protein substrates include transcription factors, receptors, and enzymes. An example of an enzyme substrate is ribonucleotide reductase, needed in the conversion of ribonucleotides to deoxyribonucleotides in DNA synthesis.

Specifically, thioredoxin reductase transfers reducing equivalents from NADPH through its bound FAD to reduce disulfide bonds (S-S) within the oxidized substrate, as shown next.

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Some of the transcription factors, which contain cysteine in their DNA binding region and which rely on the thioredoxin-thioreductase system (composed of thioredoxin, thioredoxin reductase, and NADPH), include activator protein (AP) 1, p53, and nuclear factor κβ (NFκβ). Additionally, thioredoxin and glutaredoxin are thought to modulate intracellular signaling cascades that in turn affect other cellular processes. Thioredoxin, for example, inhibits apoptosis through interactions with apoptosis signal-regulating kinase (ASK) 1 and thus affects cell division, proliferation, and longevity.

Iodothyronine 5′-Deiodinases (IDI or DI): Thyroid Hormone Synthesis

Selenium is also necessary for iodine metabolism, including the generation of thyroid hormones, as a constituent of a group of enzymes called deiodinases. The deiodinases are selenocysteine-containing enzymes with the selenocysteine present at the active site. Three types of deiodinases have been characterized. Type 1 is found mainly in the thyroid gland, pituitary gland, liver, and kidneys. Type 2 is found in a larger group of tissues including the thyroid gland, brain/central nervous system, heart, pituitary gland, skeletal muscle, and brown adipose tissue. Type 3 is found mainly in the brain and a few other tissues such as skin. Deiodinase activity is optimized at serum selenium concentrations of about 60 ng/mL [7].

5′-deiodinases catalyze the deiodination (removal of iodine) from the 5 or 5′ positions of thyroid hormones and some of their metabolites. Deiodinases 1 and 2 remove one of four iodines in the thyroid hormone thyroxine (T_4) to produce 3,5,3′-triiodothyronine (T_3). T_3 is the body's primary regulator of metabolism as well as of normal growth and development; a selenium deficiency decreases T_3 concentrations and increases T_4 concentrations. Deiodinase 2 provides for the production and use of T_3 within specific tissues. Such function in individualized tissues enables specific adaptations; for example, expression of deiodinase 2 in brown adipose tissue increases in response to cold, resulting in increased heat production. Surplus T_3 is degraded to inactive T_2 (also called 3,3′-diiodothyronine), and T_4 is degraded to inactive reverse (r)T_3 by deiodinases 1 and 3.

For further information regarding thyroid hormone metabolism, see the "Iodine" section.

Methionine R-Sulfoxide Reductase (formerly Selenoprotein R): Antioxidant

Methionine sulfoxide reductases catalyze the reduction of R- and S-forms of methionine sulfoxide; however, it is the R-form of the enzyme that contains selenocysteine. The enzyme, found in both the cytosol and nucleus of cells, regulates cellular redox levels. Specifically, the enzyme reduces methionine R-sulfoxides (oxidized methionines) using reduced thioredoxin as well as GSH-dependent glutaredoxin. Methionine (met) sulfoxides (met-R-O) are generated in proteins when free radicals cause oxidation of methionine residues. The reaction catalyzed by methionine R-sulfoxide reductase is as follows:

$$\text{Protein} - \text{met-R-O} + \text{Trx(SH)}_2 \longrightarrow \text{protein} - \text{met} + \text{Trx-S}_2 + \text{H}_2\text{O}$$

The presence of the sulfoxide within the protein damages the protein so that it is unable to perform its normal function; however, methionine repair and thus return of normal protein function occurs with the action of methionine sulfoxide reductase.

Other Selenoproteins

Several other selenoproteins containing at least one selenocysteine have been identified, but little is known about their functions. Table 13.9 provides an overview of some of the characteristics and proposed functions of these selenoproteins. Reference [8] provides an excellent review of selenoproteins.

Other Roles: Disease Prevention

Low serum selenium concentrations or impaired selenium status have been inversely associated with increased risk of heart disease and some cancers. Yet, selenium supplementation studies for the prevention of these diseases report inconsistent findings and, if benefits are reported, they are mostly confined to those with low baseline serum selenium concentrations. A U-shaped curve between serum selenium concentrations and disease risk has been demonstrated such that plasma selenium concentrations less than about 120 (or 130) μg/L as well as above about 160 (or 150) μg/L are associated with increased disease risk whereas concentrations in between these cutoff values are associated with reduced disease risk [7,9]. Higher serum selenium concentrations in some studies have been associated with an increased risk for diabetes, hypertension, and some cancers as well as overt selenium toxicity. Reductions in the risk of cardiovascular diseases also have been reported with serum selenium concentrations ranging from 55 to 145 μg/L [10]. At present, however, scientific studies do not support the use of selenium supplements for the prevention of cancer or heart disease. Increased dietary selenium intakes are recommended for those with poor selenium status or low serum selenium concentrations.

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Table 13.9 Proposed Functions/Characteristics of Selected Selenoproteins

Well-established/better-characterized selenoproteins
Selenophosphate synthetase 2: selenoprotein synthesis
Glutathione peroxidase: antioxidant
Selenoprotein P: antioxidant
Thioredoxin reductase: cellular redox state maintenance
Iodothyronine 5′-deiodinases: thyroid hormone synthesis and metabolism
Methionine R-sulfoxide reductases: oxidative damage repair
Lesser-characterized selenoproteins and their possible roles
W: in skeletal and heart muscles, binds glutathion–antioxidant role and muscle growth
N: in muscle cells, endoplasmic reticulum–calcium regulation and antioxidant role
H: antioxidant role and transcription factor to upregulate selenoprotein expression
M: in neuronal cells and in endoplasmic reticulum–antioxidant, regulator of calcium release and protein folding roles
K and 15: role in process and/or folding of proteins in the endoplasmic reticulum
S: in endoplasmic reticulum–removal of misfolded proteins and antioxidant function
T: in endoplasmic reticulum–calcium mobilization and neuroendocrine function
O, I, and V: unclear roles

Interactions with Other Nutrients

Selenium may help prevent some toxic effects associated with some metals. Selenium, for example, may reduce arsenic-associated skin lesions and arsenic-associated oxidative tissue damage [11].

Excretion

Urinary excretion of selenium enables body selenium homeostasis with urinary excretion directly proportional to selenium intake when intake is adequate. The major urinary metabolites are methylated forms of selenium, with the most prevalent form being the selenosugar methylseleno-N-acetylgalactosamine (CH_3Se-GalN). Other urinary metabolites, especially with higher intakes of the mineral, include methylselenol (CH_3SeH), dimethylselenide [$(CH_3)_2Se$], and trimethylselenonium [$(CH_3)_3Se^+$]. Selenium losses through the lungs and skin also contribute to selenium excretion to a small extent. Pulmonary elimination (i.e., exhalation in the breath) of selenium increases when selenium is consumed in large amounts. The main form of selenium that is exhaled is dimethylselenide, which is quite volatile and has a garlicky odor.

Recommended Dietary Allowance

The Food and Nutrition Board set a Recommended Dietary Allowance for selenium for adults of 55 μg/day [12]. Based mostly on balance studies as well as on repletion studies of men with selenium deficiency in regions of China, the adult requirement for selenium was determined to be 45 μg. The requirement was based on calculation of the amount of selenium necessary to plateau concentrations of selected selenoproteins, primarily GPX, in the plasma. To set the RDA, a 20% coefficient of variation was added, and the final number was rounded to the nearest five. RDAs for selenium for pregnancy and lactation were set at 60 μg and 70 μg, respectively [12]. The inside front cover of the book provides RDAs for selenium for other age groups. Studies, however, suggest that the RDA for selenium for adults may be suboptimal, with some recommending as much as about 200 μg of selenium/day [3,13].

Deficiency

Selenium deficiency occurs primarily in selected regions of the world such as China, central Africa, and parts of Europe. The deficiency is linked to Keshan disease and Kashin-Beck disease. Keshan disease is characterized by cardiomyopathy involving cardiogenic shock, congestive heart failure, or both, along with multifocal necrosis of heart tissue, which becomes replaced with fibrous tissue. Infection by coxsackie virus appears to be a cofactor in the development of Keshan disease. In the absence of sufficient selenium, mutations occur in benign strains of the virus. These mutations cause the virus to become virulent; the presence of the virus is thought to account for some of the symptoms of Keshan disease. Kashin-Beck disease is characterized by osteoarthropathy involving chronic degeneration and necrosis of the joints and of epiphyseal-plate cartilages of the legs (primarily knees and ankles) and arms (mostly fingers, hands, and elbows). Several factors, including selenium deficiency, are thought to contribute to the development of Kashin-Beck disease.

In the United States, selenium deficiency has been observed in people receiving total parenteral nutrition. Some deficiency symptoms included poor growth, muscle pain and weakness, loss of pigmentation of hair and skin, and whitening of nail beds. The observed poor growth may be associated selenium's role in thyroid hormone metabolism. Individuals with HIV and cirrhosis are at increased risk of deficiency secondary to malabsorption and increased diarrhea-induced losses of selenium. Selenium deficiency is typically treated with supplements providing 100–200 μg of selenium/day. Absorption of selenomethionine is typically greater than that of selenite. However, those with impaired liver function (and thus possibly an impaired ability to metabolize selenomethionine) may respond better to supplementation with selenite.

Impaired or low selenium status is also found in those with critical illness and with some inflammatory conditions. Selenium deficiency is sometimes associated with reductions in immune system function and the body's anti-inflammatory response. Selenium is thought to perhaps modulate both processes through some of its redox functions. The thioredoxin-thioreductase system's control of gene expression via transcription factors enables the blocking of proinflammatory cytokine production. Additionally, the mineral provides transient pro-oxidant actions by inducing apoptosis and/or cytotoxic effects against proinflammatory or hyperactivated cells (such as polymorphonuclear neutrophils), among other actions [14,15]. Selenium supplementation,

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however, in those with a variety of inflammatory conditions has not consistently yielded benefits. Supplementation with high doses of selenium in those with critical illness has in some, but not all, studies been associated with reductions in the inflammation and/or lower mortality, but current evidence for supplementation is considered disputable [14,16].

Toxicity

Selenium toxicity, called selenosis, has been observed both in miners and in people who consumed excess selenium from supplements. Signs and symptoms of toxicity include nausea, vomiting, fatigue, diarrhea, hair brittleness and loss, brittle and thickened nails, muscle cramps, paresthesia, interference in sulfur metabolism (primarily oxidation of sulfhydryl groups), and inhibition of protein synthesis [17]. Acute poisoning from ingestion of gram amounts of selenium is lethal, with damage occurring to most organ systems [17]. A Tolerable Upper Intake Level of 400 μg/day has been set by the Food and Nutrition Board [12].

Assessment of Nutriture

Serum or plasma selenium concentrations are widely used for assessment with concentrations less than about 70 ng/mL suggestive of inadequate recent intake [12]. Urinary selenium excretion is also used as an indicator, with concentrations less than 15 μg/L indicative of inadequate recent intakes and those greater than about 50 μg/L suggestive of excessive selenium ingestion. Whole-blood selenium concentrations represent longer-term intake (about 120 days, the lifespan of the red blood cell). Toe nails can be used as an indicator of even longer-term (months to a year) intakes, and are considered better than hair concentrations due to more consistent growth rate and less contamination (from shampoo, etc.)

The activities and concentrations of some selenoproteins have also been used to assess selenium status. Selenoprotein P and glutathione peroxidase in tissues (GPX1) and in the plasma (GPX3) are commonly used. Selenoprotein P and glutathione peroxidase concentrations decrease in the plasma as selenium deficiency worsens, thus serving as an index of selenium status in populations with low intake [17,18]. However, selenoprotein P is thought to be a better indicator of selenium status than GPX; GPX achieves maximal activity at a selenium intake considerably lower than selenoprotein P [3].

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Suggested Readings

National Institutes of Health, Office of Dietary Supplements. Selenium Health Professional Fact Sheet. https://ods.od.nih.gov/factsheets/Selenium-HealthProfessional/

Pillai R, Uyehara-Lock JH, Bellinger FP. Selenium and selenoprotein function in brain disorders. IUBMB Life. 2014; 66:229–39.

Web Site for Nutrient Composition Information

www.ars.usda.gov/Services/docs.htm?docid=18877

CHROMIUM

Chromium, a metal with a ubiquitous presence in air, water, and soil, exists in several oxidation states from Cr^{2-} to Cr^{6+}. Trivalent chromium, Cr^{3+}, is the stablest of the oxidation states and is often found attached to ligands containing nitrogen, oxygen, or sulfur to form hexacoordinate or octahedral complexes. It is this trivalent form of chromium that is most important in humans.

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Sources

In foods, chromium exists in the trivalent form. Few foods, however, have been analyzed to accurately quantify chromium content; this task is further complicated by the variability of chromium within foods. Good dietary sources of chromium are generally thought to include meats and grains (especially whole grains) along with some vegetables and fruits. Some examples of the approximate chromium content of some foods include beef and turkey breast (3 oz), 2 μg; whole-wheat bread (1 slice), 1 μg; orange juice (1 c), 2 μg; grape juice (1 c), 7 μg; banana and apple (1 medium), 1 μg; potatoes, mashed (1 c), 3 μg; broccoli (1/2 c), 11 μg; and green beans (1/2 c), 1 μg. Relatively large amounts of chromium may be present in some red wines. Additionally, chromium is found in selected spices (such as cinnamon, cloves, bay leaves, and turmeric), in tea and beer, and in some yeast preparations. Most adults in the United States are thought to ingest 23–54 μg of chromium daily [1].

Chromium is available in multivitamin/mineral supplements and in single-nutrient supplements as inorganic salts (such as with chloride) and as an organic complex (such as with acetate, nicotinic acid alone, or with amino acids, or picolinic acid). The multinutrient supplements usually contain about 25–120 μg, whereas the individual-nutrient supplements provide about 50–200 μg of chromium. Although all forms appear to be absorbed and used, different forms of the supplement appear to affect tissue concentrations differently. For example, chromium picolinate has been touted as superior to other forms of chromium; however, urinary chromium excretion from chromium picolinate is also higher than other supplemental forms, suggesting poor tissue uptake [2]. The Daily Value for chromium is 120 μg.

Digestion, Absorption, Transport, and Storage

Digestion and Absorption

Chromium, Cr^{3+}, may be released from food components in acidic solutions, as would be found in the stomach. Chromium is absorbed throughout the small intestine, especially in the jejunum. Although the mode of absorption in humans is not known, chromium is thought to be absorbed by passive diffusion (as shown in rats), by a carrier-mediated transporter, and/or perhaps by endocytosis. About 0.4–2.5% of dietary chromium (with intakes of 40 μg and 10 μg, respectively) is absorbed from the gastrointestinal tract [1]. If, for example, absorption averaged 0.5%, a chromium intake of 30 μg would provide 0.15 μg of absorbed chromium.

Factors Influencing Chromium Absorption Like that of other trace minerals, the absorption of chromium may be influenced by dietary factors. Within the stomach, amino acids or other ligands may chelate chromium. Amino acids such as phenylalanine, methionine, and histidine as well as picolinic acid (picolinate) act as ligands to improve chromium absorption [3]. These chelations typically help chromium remain soluble and prevent olation (see next paragraph) once it reaches the alkaline pH of the small intestine. Lipophilic compounds such as picolinate may enhance Cr^{3+} absorption through the cell's lipid membranes. Vitamin C and niacin may also enhance chromium absorption.

Chromium in a neutral or alkaline environment may react with hydroxyl ions (OH^-), which readily polymerize to form high-molecular-weight compounds in a process called olation. This reaction, which occurs more readily with use of antacids, proton pump inhibitors, or H_2 receptor blocker medications that increase gastric pH (e.g., are used to relieve heartburn), may result in chromium precipitation and thus reduced absorption. Phytic acid, found mostly in grains, legumes, nuts, and seeds, also binds to and diminishes chromium absorption.

Transport

In the blood, Cr^{3+}, like Fe^{3+}, binds to transferrin. If transferrin sites are unavailable (due to occupation by iron, for example), albumin is thought to transport chromium. Globulins and possibly lipoproteins may also transport the mineral if present in very high concentrations. Some chromium may also circulate unbound in the blood. Plasma chromium concentrations are typically low, ranging from 1 to 5 μg/L. The uptake of transferrin-bound chromium into cells is thought to occur like that of iron (see Figure 13.6).

Storage

The body contains ~4–6 mg of chromium. Tissues especially high in chromium include the liver, spleen, and bone and to a lesser extent the kidneys, heart, and pancreas. Tissue chromium concentrations appear to decline with age.

Functions and Mechanisms of Action

Chromium is thought to potentiate the action of insulin; however, the mechanism by which potentiation occurs is still unclear. For decades, this biological action of chromium was believed to be attributable to its complexing with nicotinic acid and amino acids to form the organic compound **glucose tolerance factor** (GTF). GTF was first identified in brewer's yeast, but this factor has never been purified, nor has its exact structure been characterized.

While it is still thought to potentiate the action of insulin, more recent studies have suggested that the biologically active form of chromium is a low-molecular-weight chromium-binding substance called chromodulin. Chromodulin is thought to be produced in response to insulin secretion, which stimulates chromium uptake by cells. Once within the cell, chromium atoms (four) bind to apochromodulin, an oligopeptide that is thought to be composed of glycine, cysteine, aspartate, and one or more glutamates. Once the four chromium atoms bind to the apochromodulin, the complex is called holochromodulin (Cr^4-chromodulin) or chromodulin. This proposed process is shown in Figure 13.18.

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1. Transferrin delivers Cr^{3+} to transferrin receptors (TfR) on cell membranes.
2. Cr^{3+} is released inside the cell.
3. Four Cr^{3+} atoms complex with chromodulin to form holo chromodulin or Cr_4-chromodulin.
4. Cr_4-chromodulin functions to increase the kinase activity of the beta subunit of the insulin receptor and other cytosolic tyrosine kinases.

Figure 13.18 Proposed role of chromium (Cr^{3+}) as part of chromodulin in potentiating insulin's reactions.

Insulin signaling within cells is regulated (in part) by the phosphorylation of tyrosine residues on selected proteins. More specifically, insulin initially binds to the alpha subunit of an insulin receptor; this binding leads to autophosphorylation of specific tyrosine residues on the beta subunit of the insulin receptor and stimulates tyrosine kinase activity of the receptor and of other intracellular (cytosolic) protein substrates (such as insulin receptor substrate 1) involved in signal transduction. For example, the activation of phosphatidylinositol-3-kinase results from the binding of insulin receptor substrates to the enzyme's regulatory subunit; this in turn enables a variety of insulin-dependent cellular activities such as GLUT4 translocation for cellular glucose uptake and protein synthesis, among others. Chromodulin is thought to bind to the cytosolic beta subunit of the insulin receptor, where it stimulates (or amplifies) the kinase activity of the beta subunit of the insulin receptor. Chromodulin may also stimulate the tyrosine kinase activity of other enzymes involved in insulin signaling to effect GLUT4 translocation and improved cellular glucose uptake [4,5]. Cells treated with selected forms of chromium have also been shown to exhibit increased insulin receptor gene expression, improved protein anabolism, decreased protein degradation, and improved insulin sensitivity, although the results of studies on the effects of chromium supplementation on insulin sensitivity have been conflicting [4,6,7].

Another possibly biologically active form of chromium in the body is chromate. Chromate or chromic acid (CrO_3), which contains the hexavalent form of chromium, can be produced within the body from oxidation of Cr^{3+} by oxidants such as hydrogen peroxide and free radicals. Chromate, similarly to vanadate, may inhibit the activity of phosphotyrosine phosphatase to prolong or enhance insulin signaling; however, the methodological approaches used in chromate studies as well as in some of the studies examining chromodulin's mechanism(s) of action have been questioned [4].

In addition to its proposed association with insulin, Cr^{3+} may also affect gene expression and/or maintain the structural integrity of nuclear strands [8]. The expression of a handful of genes in adipose tissue of diabetic mice has shown to be altered in response to niacin-bound Cr^{3+} [4,8].

Chromium and Health

Diabetes A plethora of studies and meta-analyses of studies continue to document mixed findings regarding the effectiveness of chromium supplements on improvements in measures of glucose control (primarily glycosylated hemoglobin and fasting glucose concentrations) in individuals with type 2 diabetes. Similar mixed results have been demonstrated in studies examining the effectiveness of chromium supplementation on blood lipid concentrations in those with hyperlipidemias. Consequently, unless a person is chromium deficient (which is difficult to assess; see the section "Assessment of Nutriture"), supplementation is not currently recommended.

Weight Loss/Body Composition Changes Chromium as a supplement has also been purported to effect changes in body composition, weight, and strength performance. However, most well-controlled studies providing chromium supplementation have shown no significant effects on strength gains, muscle accretion, or fat loss and have shown conflicting results on weight loss; in fact, the Federal Trade Commission ordered the discontinuation of such claims [9].

Excretion

Most chromium (~95%) is excreted from the body in the urine. In absolute terms, urinary chromium excretion ranges from ~0.2 to 0.4 μg/day [2]. In addition to urinary losses, small amounts of chromium are lost with desquamation of skin cells. Fecal chromium represents mostly unabsorbed dietary chromium, not endogenous chromium excreted via the bile into the feces.

Adequate Intake

The Adequate Intakes (AIs) for chromium for adult men and women through age 50 years are 35 μg and 25 μg, respectively; these values drop to 30 μg and 20 μg for men and women, respectively, over 50 years of age [1]. During

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pregnancy and lactation, intakes of 30 μg and 45 μg of chromium, respectively, are recommended [1]. AIs for chromium for other age groups are provided on the inside front cover of the book.

Deficiency

Chromium deficiency was originally described in individuals receiving intravenous nutrition (total parenteral nutrition). Signs and symptoms of deficiency included weight loss, peripheral neuropathy, elevated plasma glucose concentrations or impaired glucose use (also called insulin resistance, which may be characterized by hyperinsulinemia), and high plasma free fatty acid concentrations. While the reversal of these symptoms with chromium supplementation was viewed as evidence of chromium's essentiality, it is now apparent that the parenteral nutrition solution given to correct the deficiency provided pharmacological amounts of chromium, 2–6 μg/day (i.e., more than would typically be absorbed from the diet) [5,10].

Toxicity

Oral supplementation of up to about 1,000 μg of chromium as Cr^{3+} appears to be safe [1]. However, the use of chromium (Cr^{3+}) picolinate has been associated with chromosomal and organ damage. Documented chromium-induced DNA damage includes adducts, single- and double-strand DNA breaks, and inter- and intrastrand cross-links, among other types of destruction [11]. Organ damage, specifically renal failure and hepatic dysfunction, has been reported in those ingesting chromium picolinate supplements providing 600–2,400 μg of chromium [1]. Renal problems were also documented in some individuals with higher than normal concentrations of chromium in their blood and urine due to long-term receipt of parenteral nutrition contaminated with high amounts of chromium [10]. No Tolerable Upper Intake Level for chromium has been established by the Food and Nutrition Board to date [1].

Assessment of Nutriture

No specific tests are currently available to determine chromium status. Fasting plasma chromium is not in equilibrium with tissue chromium. Responses of plasma chromium to an oral glucose load are inconsistent. Urinary chromium appears to reflect only recent intake, not status.

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Suggested Reading

National Institutes of Health, Office of Dietary Supplements. Chromium. Health Professional Fact Sheet. https://ods.od.nih.gov/factsheets/Chromium-HealthProfessional/

IODINE

Iodine, a nonmetal, is typically found and functions in its ionic form, iodide (I^-). Hence, the term *iodide* is used throughout this section. The human body contains about 15–20 mg of iodide, most (70–80%) of which is found in the thyroid gland. The mineral, however, is also a component of topical agents used as antiseptics. Iodine tincture and iodine compounds, such as povidone iodine, have broad-spectrum anti-infective actions against yeast, fungi, bacteria, viruses, spores, and protozoa.

Sources

The iodide concentration in foods is extremely variable because, as is so often the case, it reflects the regionally variable soil concentrations of the element and the amount and nature of fertilizer used in plant cultivation. Thus, the iodide content of grains, vegetables, and fruits varies with the iodide content of the soil, and the iodide content of meats depends on the iodide of the soil and plants that the animals ate. The amount of iodide in drinking water is an indication of the iodide content of the rocks and soils of a region and closely parallels the incidence of iodine deficiency among the inhabitants of that region. For example, the iodide content of water from goitrous areas in India, Nepal, and Ceylon ranged from 0.1 to 1.2 mg/L, compared to 9.0 mg/L found in nongoitrous Delhi [1]. In the United States, before salt was fortified with the mineral in the 1920s, people living in the Great Lakes and Rocky Mountain areas had iodine-poor diets. Iodized salt was

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Table 13.10 Iodine Content of Selected Foods*

Selected Foods/Food Group	Iodine (μg)
Seafood	
Cod (3 oz)	99
Shrimp (3 oz)	35
Kelp (1/4 c)	415
Meats, variety	25–35
Eggs and Dairy	55–85
Milk, reduced fat (1 c)	56
Cheese, cheddar (1 oz)	12
Egg, hard boiled (1)	28
Grains	
Bread, white (1 slice)	23
Macaroni, enriched (1 c)	27
Other	
Legumes, variety (1/2 c)	35

* See https://ods.od.nih.gov/factsheets/Iodine-HealthProfessional/#h3

introduced in 1924 in the state of Michigan [2]. Yet, in 2007, 29–50% of the world's population was thought to be iodine deficient, especially those living in mountainous regions (e.g., the Himalayas, European Alps, and the Andes) and lowland noncoastal regions (such as central Africa and Eastern Europe) [3].

Iodide is found in seafoods; however, large differences in iodide content exist between marine fish and freshwater fish. Edible marine (sea) fish contain about 30–300 μg of iodide/100 g, in contrast to only 2–4 μg of iodide/100 g in freshwater fish. In addition to seafoods, dairy products represent a major source of iodide in the American diet. Breads (due to the addition of iodates, IO^{3-}, to improve gluten cross-linking) also serve as a source of dietary iodide. Table 13.10 provides the approximate iodide content of selected foods.

Individuals needing to restrict dietary iodide intakes (as may be needed in the treatment of thyroid cancer) are typically instructed to limit dietary intake to less than about 50 μg of iodide/day. Most commonly restricted foods include all seafoods, iodized salt, eggs and dairy products, and soybean/soybean products such as tofu, soy milk, and soy sauce.

Iodized salt (¼ teaspoon) supplies about 70 μg of iodide. The mineral is usually added to salt as potassium iodate or iodide. About 90% of households in North and South America use iodized salt; in contrast, less than half of households in Europe do. In the United States, processed foods are typically manufactured with noniodized salt. Restricting salt intake (as may be necessary for people being treated for hypertension) may negatively affect iodine status [4].

Iodine in multivitamin/mineral supplements is provided usually as potassium or sodium iodide and in quantities similar to the Daily Value, which for iodine is 150 μg. However, the use of iodine derived from kelp, which is present in some supplements, can result in significantly more or less supplemental iodide than the amount stated on the label [5]. The use of the medication amiodarone (for some types of arrhythmias) also provides pharmacological amounts of iodide. Iodine is further provided in large amounts as a contrast agent for some radiological procedures such as computerized tomography (CT) scans. The use of the iodinated contrast agents during a single X-ray procedure can result in well over 10,000 μg of infused iodine, an amount several thousand times higher than recommended intakes and Tolerable Upper Intake Levels [5].

Digestion, Absorption, Transport, and Storage

Dietary iodine (I) is either bound to amino acids (sometimes termed organified) or found free, primarily as iodate (IO^{3-}) or iodide (I^-). During digestion, organic bound iodine is released and converted to iodide. Iodate, for example from breads or iodized salt, is usually reduced to iodide by glutathione within the gastrointestinal tract. Small quantities of iodinated amino acids and other organic forms of iodide that escape digestion may be absorbed, but not as efficiently as the iodide ion. The thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) are also absorbed unchanged, with a bioavailability of about 70%, which allows T_4 medication to be administered orally.

Iodide is absorbed rapidly, mostly from the stomach and to a lesser extent from the duodenum. Overall, absorption of iodide is greater than 90%. Following absorption, free iodide appears in the blood, from which it is capable of permeating all tissues. The mineral selectively concentrates, however, in the thyroid gland, with lesser amounts found in the ovaries, placenta, skin, and salivary, gastric, and mammary glands. Absorption of iodine applied topically is thought to be negligible, except in cases where the skin is not intact (as may occur in individuals with burns) and in cases of prolonged, repeated exposure [5].

The thyroid gland traps iodide most aggressively, by way of an active transport system against an iodide gradient that is often 20–50 times the plasma concentration. Specifically, iodide is taken up into the thyroid gland by a Na^+/I^- symporter located in the basolateral membrane of the thyroid gland; the transporter carries two sodiums and one iodide, with the sodium gradient generated by the Na^+/K^+ ATPase serving as the driving force. The thyroid gland contains 70–80% of the total body iodide and takes up about 120 μg of iodide/day. Because the thyroid gland and its synthesis of the thyroid hormones are the focal points of iodide metabolism, information on the transport of iodide into nonthyroidal tissue is sparse. However, iodide uptake by other tissues, such as salivary glands, likely occurs by an active transport mechanism.

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Functions and Mechanisms of Action

The main function of iodide is in the synthesis of the thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3). As long as the daily iodide intake is above about 50 µg, uptake of iodide by the thyroid gland is usually sufficient for hormone production [6]. Thyroid-stimulating hormone (also called thyrotropin), released from the pituitary gland, helps to regulate thyroid hormone production and secretion. Four atoms of iodide are needed for each T_4, and three atoms of iodide for each T_3. Amino acids are also needed for the synthesis of the thyroid hormones.

Thyroid Hormone Synthesis

The thyroid gland is made of multiple acini, also called follicles. The follicles are spherical in shape and are surrounded by a single layer of thyroid cells. The follicles are filled with

* Structures:

1. I^- is actively transported into the thyroid cell.
2. I is bound to a tyrosine residue on thyroglobulin to form thyroglobulin-3-monoiodotyrosine (Thg-MIT).
3. Thg-MIT is iodinated to form Thg-DIT, thyroglobulin-3, 5-diodotyrosine, which 4 condenses with another Thg-DIT in the colloid to form Thg-T_4.
5. Thg-DIT also can condense with Thg-MIT to form Thg-T_3 and reverse (r)T_3.
6. T_4 and T_3, active thyroid hormones, are released into the blood following endocytosis of Thg-T_3 and Thg-T_4 back into the thyroid cell and hydrolysis of the Thg by proteases.

Figure 13.19 Overview of iodine intrathyroidal metabolism and hormonogenesis.

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colloid, a proteinaceous material. The events in thyroid hormone synthesis are shown in Figure 13.19 and described here:

- The thyroid cells actively (through an Na^+/K^+-ATPase pump) take up iodide from the blood.
- Once within the cell, iodide (I^-) is oxidized to iodine (I), which is then bound to the number 3 position of tyrosyl residues of the glycoprotein thyroglobulin (a process called organification of the iodine). This binding of iodine to the tyrosyl residues is catalyzed by the heme iron–dependent enzyme thyroperoxidase and generates thyroglobulin-3-monoiodotyrosine (Thg-MIT). Hydrogen peroxide acts as the electron acceptor.
- Next, MIT is iodinated in the number 5 position by thyroperoxidase to form thyroglobulin-3,5-diiodotyrosine (Thg-DIT).
- In the colloid, two DITs condense or couple to form Thg-3,5,3′,5′-tetraiodothyronine (Thg-T_4), with the elimination of an alanine side chain. Thyroperoxidase also catalyzes this coupling reaction. DIT also condenses or couples with MIT to form 3,5,3′-triiodothyronine (T_3) and reverse T_3 (rT_3).

DIT and MIT not used for thyroid hormone synthesis in the thyroid cells are deiodinated, and the iodine is made available for recycling.

To release the thyroid hormones into the blood, iodothyroglobulin must be resorbed in colloid droplets by endocytosis back into the thyroid cell. Within the thyroid cell, the iodothyroglobulin (Thg-T_4) and (Thg-T_3) is hydrolyzed by lysosomal proteases, and T_4 and T_3 are released into the blood.

Transport of Thyroid Hormones in the Blood

Three transport proteins bind and transport T_4 and T_3 in the blood. The protein thyroxine-binding globulin has the smallest capacity but the greatest affinity for T_4 and T_3. Albumin and transthyretin (also called prealbumin) also transport the thyroid hormones. A very small fraction (<0.1%) of T_4 and T_3 in the blood is not bound to transport proteins; it is this free form that binds to cell receptors and affects physiological processes. The plasma concentration of T_4 is nearly 50 times that of T_3, but T_3 is many times (~20–100) more potent on an equal molar basis. Several tissues—the liver, kidneys, brain, pituitary, and brown adipose, to name a few—deiodinate T_4 to generate T_3, although most T_3 in the blood has been synthesized in the liver from T_4. The conversion of T_4 to T_3 is catalyzed by the selenium-dependent enzyme 5′-deiodinase; this conversion is impaired with selenium deficiency.

Table 13.11 Selected Physiological Effects of Thyroid Hormones

Adipose tissue: enhances lipolysis
Muscle: enhances contraction
Bone: promotes anabolism (growth and development)
Cardiovascular system: increases heart rate
Gastrointestinal tract: stimulates nutrient digestion and absorption
Metabolism: stimulates metabolic rate and cellular oxygen consumption in metabolically active tissues

For a more in-depth description of thyroid hormone synthesis, see the reviews by Visser [7] and Vanderpas [8].

Thyroid Hormone Functions

The multiple effects of the thyroid hormones result from the hormones' occupancy of nuclear receptors, with subsequent effects on gene expression. The receptors appear to be the same in all tissues, binding T_3 more avidly than T_4 and requiring fivefold to sevenfold higher concentrations of T_4 to achieve comparable physiological effects. Thyroid hormones bind to DNA as monomers, homodimers, and heterodimers, such as with retinoid X receptors (discussed under "Vitamin A" in Chapter 10). Additionally, the hormone receptor complex may interact with the DNA through zinc fingers. Although the mechanisms of action of the thyroid hormones are unclear, biological effects occur in response to increased mRNA and protein synthesis triggered by the thyroid hormone receptor complex interactions with DNA.

The effects of thyroid hormones on metabolism are many and varied. Thyroid hormones stimulate oxygen consumption, the basal rate of metabolism, and body heat production, and are necessary for normal nervous system development and linear growth. Directly or indirectly, most organ systems are under the influence of these hormones (Table 13.11).

Prophylactic Use of Iodide

Iodide supplementation, as potassium iodide, is recommended in large (pharmacologic) amounts by the Centers for Disease Control and Prevention upon exposure or risk of exposure to radioactive iodine (as may occur during nuclear accidents) [9]. Ingestion of doses of up to 130 mg of iodide (depending on age) is suggested as quickly as possible and enables "saturation" of the thyroid gland with mineral. The uptake of this supplemental iodide by the thyroid gland (which occurs to a greater extent in those that are deficient) reduces the likelihood that the gland takes up the released radioactive iodine and reduces the person's subsequent risk of thyroid cancer [9].

Interactions with Other Nutrients

The metabolism of the thyroid hormones is largely dependent upon a group of three selenium-dependent iodothyronine 5′-deiodinases. Impaired selenium status results in altered thyroid hormone metabolism and function. The roles of these deiodinases are described further under the

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section of this chapter addressing selenium, specifically "Iodothyronine 5′-Deiodinases (IDI or DI)."

Like selenium deficiency, iron and vitamin A deficiencies may magnify the effects of inadequate iodine. Heme iron is a component of the enzyme thyroperoxidase, which attaches iodine to tyrosine residues on thyroglobulin, and which then conjugates the thyroglobulins for the production of the thyroid hormones. Iron may also be involved in the binding of T_3 to nuclear receptors. Thus, iron deficiency impairs thyroid hormone synthesis and functions. Similarly, vitamin A deficiency reduces iodine uptake by the thyroid gland and decreases the synthesis of thyroglobulin and the coupling of iodotyrosine residues to form T_4 [10].

Another well-established interaction is that between iodide and goitrogens. Substances that interfere with iodide metabolism to inhibit thyroid hormonogenesis are called goitrogens because their effect is to secondarily augment thyroid-stimulating hormone release and consequently thyroid gland enlargement. Goitrogens may affect iodide uptake by the gland, organification of the iodide, or hormone release from the thyroid cells. That some natural foods are goitrogenic was evidenced many years ago when it was discovered that rabbits fed a fresh cabbage diet developed goiters that could be reversed by iodine supplementation. It was later shown that cruciferous vegetables, including cabbage, kale, cauliflower, broccoli, rutabaga, turnips, Brussels sprouts, and mustard greens, contain glucosinolates, which compete with iodide for uptake into the thyroid gland. Perhaps the only food to be identified directly with goiter etiology is cassava, which is consumed in large quantities in many developing countries. Cassava contains the cyanogenic glucoside linamarin that later became known as goitrin (Figure 13.20). The linamarin, once hydrolyzed within the gastrointestinal tract, releases cyanide, which is then metabolized to thiocyanate (SCN^-). Thiocyanate competes with iodide for uptake into the thyroid gland. Cyanogenic glucosides are also found in lima beans, flaxseed, linseed, sorghum, and sweet potatoes.

Other goitrogenic compounds that compete with iodide's active transport into the thyroid cells include halide ions such as bromide (Br^-) and astatide (At^-). Perchlorate (ClO_4^-), along with perrhenate (ReO_4^-) and pertechnetate (Tc_4^-), interferes with organification as well as iodide uptake; perchlorate is a known contaminant in drinking water. Lithium (Li^-), used to treat some psychiatric disorders, inhibits thyroid hormone release from the gland. Some other classes of goitrogens that interfere with iodide metabolism include polycyclic hydrocarbons and phenol compounds derived from coal.

Figure 13.20 Goitrin.

Excretion

The kidneys have no mechanism to conserve iodide, and they therefore provide the major route (~80–90%) for iodide excretion. Usual urinary iodine excretion exceeds 100 μg/L if intake is adequate [11]. Fecal excretion of iodide is relatively low, ranging from 6.7 to 42.1 μg/day [12]. Some iodide is also lost in sweat, a loss that can be of consequence in hot, tropical regions where iodide intake may be only marginally adequate.

Recommended Dietary Allowance

Because of its important link to thyroid function, iodide nutriture has been investigated thoroughly for over half a century. Dating as far back as the 1930s, requirements have been published based on results of balance studies and on calculations of average daily urinary losses. Adult daily requirements established by those early studies ranged from 100 to 200 μg. The amount of iodide to prevent goiter is estimated at 50–75 μg/day or ~1 μg/kg of body weight, and has not changed significantly over the years. The RDA for iodine is 150 μg/day for adults [11]. Although the recommendations apply equally to both sexes, iodide needs are higher during pregnancy and lactation: 220 μg and 290 μg, respectively [11]. The inside front cover of this book provides additional RDAs for iodine for other age groups.

Deficiency

Thyroid Hormone Release as Related to Iodide Deficiency

The release of thyroid hormones by the thyroid gland is controlled. Thyrotropin-releasing hormone secreted by the hypothalamus acts on the pituitary gland to stimulate thyroid-stimulating hormone (TSH). TSH, in response to thyrotropin-releasing hormone, is secreted from the anterior pituitary and increases the uptake of iodide from the blood into the thyroid gland to enhance T_4 production. TSH output is regulated by T_4 through negative feedback to the pituitary. A decline in the blood level of T_4 triggers the release of TSH, whereas elevated T_4 inhibits the release of TSH and thyrotropin-releasing hormone.

Iodine Deficiency and Iodine Deficiency Disorders

Iodine deficiency, known as goiter, prevails in many areas of the world and is associated most often with dietary insufficiency of iodine (typically less than about 10–20 μg of iodine/day), although high goitrogen intakes may also be a contributing factor. About 200–300 million people worldwide are iodine deficient [13]. The condition is characterized by enlargement (hyperplasia) of the thyroid gland. This enlargement is caused by overstimulation by TSH. Iodide deficiency depletes the thyroid gland's iodide stores and therefore reduces the output of T_4 and T_3. The

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reduced blood T_4 concentrations trigger continuous release of TSH, resulting in hyperplasia of the thyroid gland. The growth of the gland is self-restricting, however, because in its enlarged state it traps and processes available iodide more efficiently. The gland can return to normal size over time (months to years) as dietary iodide is increased to adequate amounts.

Because iodide deficiency also affects growth, development, and other health indices, the term *iodide deficiency disorders (IDDs)* has been coined. Iodine deficiency in a fetus results from iodide deficiency of the mother; two types of cretinism can result. Neurological cretinism in the infant is characterized by mental deficiency, hearing loss or deaf mutism, and motor disorders such as spasticity and muscular rigidity [6]. Hypothyroid cretinism results in thyroid failure. Early treatment of cretinism with iodine can often correct the condition.

Defects in the Na^+/I^- symporter have been characterized and result in the absence of thyroid hormone production. Hypothyroidism, goiter, and mental retardation, among other signs and symptoms of iodide deficiency, develop if the condition goes untreated.

The addition of iodide to table salt and the administration of iodized oil, potassium iodide or iodine, and iron salts have done much to alleviate the problem of endemic goiter in some goitrous regions of the world. Yet iodide deficiency continues to be a major health problem in many underdeveloped countries and, in many countries, may be coupled with selenium and iron deficiencies. In the United States, the restriction of salt intake (as may be necessary for people being treated for hypertension) or use of salt that is not iodized may increase a person's risk for developing iodine deficiency [4]; this risk is further increased if the diet does not include dairy products and seafood [4]. Additionally, in the United States, pregnant women may not consume sufficient iodide to meet the increased needs of the mineral during pregnancy. See Patrick [14] for a review of the history of iodine deficiency.

Toxicity

A Tolerable Upper Intake Level for iodine has been set at 1,100 μg (1.1 mg)/day [11]. Poor monitoring and over-supplementation of iodine in several countries with supplementation programs have resulted in excessive intakes. Excessive intakes, however, may also result from the over-consumption of foods, such as kelp, that are naturally very high in iodine. Some signs of acute iodide toxicity include burning of the mouth, throat, and stomach; nausea; vomiting; diarrhea; and fever. High iodine intake or infusion of large amounts of iodine secondary to radiological procedures results in excess iodine stores in the body and potentially impaired function of the thyroid gland, including both hyper- and hypothyroidism as well as inflammation of the thyroid (thyroiditis) (see [5] for a review).

Assessment of Nutriture

Assessment of iodide status is generally directed at populations living in areas suspected of being iodide deficient, although individuals may be assessed if thyroid problems are suspected. Several methods are used for iodine assessment. Urinary iodine excretion represents an indicator of recent iodine intake; in fact, daily urinary iodine can be used to calculate iodine intake using the following formula: Daily iodine intake = urinary iodine × 0.0235 × body weight, with urinary iodine measured in μg/L and weight measured in kg [11]. Usual urinary iodine excretion exceeds 100 μg/L if intake is adequate. Urinary iodide excretion of 100 μg/L corresponds to an iodide intake of about 150 μg [6]. Median urinary iodine concentrations of <100 μg/L suggest inadequate iodine intake and iodine insufficiency (or mild iodine deficiency) in a population. Urinary iodine concentrations less than 50 μg/L are usually associated with insufficient thyroid hormone secretion and are indicative of moderate iodine deficiency, while concentrations less than 20 μg/L are considered severe [6]. As dietary iodine intake increases, urinary iodine concentrations also rise. Urinary iodine concentrations of 300 μg/L or higher have been associated with increased thyroid volume, which in turn indicates thyroid dysfunction [5].

Thyroid size, measured by ultrasonography or by palpation, is also used to assess goiter, and indirectly iodine status. Enlargement of the gland is associated with suboptimal iodide status; however, the size of the gland may take months to years to return to normal in response to treatment (iodine supplementation) [6]. Thus, this indicator is typically used along with urinary iodine excretion. In addition to urinary iodide excretion and measurement of the thyroid gland, serum TSH concentrations are an especially sensitive indicator of iodine status in newborn infants from at-risk populations. Serum TSH concentrations greater than about 5 μU/L in a population suggest deficiency. Finally, serum concentrations of thyroglobulin, which rise with iodide deficiency as the size of the thyroid gland enlarges, may be used to assess iodine status. Serum thyroglobulin concentrations greater than 10 μg/L suggest inadequate iodine intake.

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Suggested Reading

Molecular and Cellular Endocrinology 2010, volume 322, is devoted to disorders involving iodine utilization.

Web Site for Nutrient Composition Information

www.ars.usda.gov/Services/docs.htm?docid=18877

MANGANESE

Although widely distributed in nature, manganese exists primarily as Mn^{2+} or Mn^{3+} in only trace amounts (about 10–20 mg) in the body.

Sources

Whole-grain cereals, nuts, and leafy vegetables are considered manganese-rich foods. Wheat bran–based breakfast cereals provide about 1.7 mg of manganese/cup. Wheat germ contains about 2.0 mg of manganese/quarter cup, and 1 cup of oatmeal provides about 1.3 mg. Nuts (almonds, pecans, cashews, hazelnuts) provide about 0.5–1.8 mg of manganese/oz. Leafy green vegetables (cooked), such as spinach, collard greens, and turnip greens, contain about 0.8–1.7 mg of manganese/half cup. Pineapple is particularly rich in manganese, with the fruit providing 2.3 mg/cup and pineapple juice (1/2 cup) containing about 1.3 mg. Blueberries also provide fairly good amounts at about 0.9 mg of manganese/cup. Tea contains relatively large amounts of manganese (about 0.4–1.6 mg/cup). Legumes (such as black, kidney, pinto, and navy) provide some manganese, about 0.7–1 mg/cup. The manganese content of grain products varies widely, due partly to plant species differences and partly to the efficiency with which the milling process separates the manganese-rich and manganese-poor parts of the grain. White flour, for example, has a much lower manganese concentration than the wheat grain from which it was produced. A slice of whole-wheat bread provides 0.30 mg of manganese, whereas a slice of white bread contains only 0.15 mg. The usual intake of manganese among Americans ranges from about 2 to 9 mg/day. The Daily Value for manganese is 2 mg. Supplements provide managanese as manganese gluconate, manganese sulfate, manganese ascorbate, and amino acid chelates of manganese. Multivitamin/mineral supplements typically contain about 2–5 mg of manganese.

Digestion, Absorption, Transport, and Storage

Whether manganese is bound to food components that need to undergo digestion to release manganese prior to its absorption is not clear. Manganese absorption, likely as Mn^{2+}, is typically less than about 5%, with absorption decreasing as intake increases and vice versa. Females may absorb greater amounts than males for unclear reasons. Manganese absorption appears to be quickly saturable and is thought to involve a low-capacity, high-affinity, active carrier protein such as divalent mineral transporter (DMT) 1 and/or Zrt- and Irt-like protein (ZIP) 14. Diffusion may contribute to absorption at higher manganese intakes. Absorption likely occurs throughout the length of the small intestine. Both regulation of intestinal absorption and control of excretion enable manganese homeostasis in the body.

Relative to many of the other trace minerals, little information is available on factors influencing manganese absorption. Low-molecular-weight ligands, such as histidine and citrate, enhance manganese absorption. In contrast, but as with other divalent cations, fiber, phytic acid, and oxalic acid may precipitate manganese in the gastrointestinal tract, making it unavailable for absorption. Iron also appears to compete with manganese for absorption, likely using DMT1. Copper also decreases manganese absorption and retention.

Manganese entering into portal circulation from the gastrointestinal tract may either remain free or become bound as Mn^{2+} to α-2-macroglobulin before traversing the liver, where it is almost totally removed. Upon release from the liver, some manganese in the blood may (1) remain free (as Mn^{2+}); (2) be bound (as Mn^{2+}) to albumin, α-2-macroglobulin, β-globulin, or γ-globulin; or (3) be oxidized by ceruloplasmin to Mn^{3+} and complexed with transferrin. Serum manganese concentrations normally range from about 0.4 to 1.1 ng/mL [1]. The mineral has a half-life of about 10–42 days.

Manganese is cleared rapidly from the blood. Zrt- and Irt-like protein (ZIP) 8 (expressed on the membranes of cells of the liver, kidneys, lungs, and testes), along with ZIP14 (expressed primarily in the intestine and liver), and DMT1 (ubiquitiously expressed) are thought to transport manganese into cells. In addition, uptake of manganese bound to transferrin occurs through transferrin receptors (see the "Cellular Iron Uptake" section).

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Within cells, manganese is found primarily as Mn^{2+} in the mitochondria; unlike iron and copper, manganese is not readily oxidized within tissues. Manganese is found in most organs and tissues (including hair) and does not tend to concentrate significantly in any particular one, although its concentration is highest in the bones, liver, pancreas, and kidneys. In bones (which may contain 25–40% of total body stores), manganese is found as part of the apatite.

Functions and Mechanisms of Action

Manganese, like some other trace elements, functions both as an enzyme activator and as a constituent of metalloenzymes. In the activation of enzyme-catalyzed reactions, manganese may bind to the substrate, to ATP, or to the enzyme directly, inducing conformational changes. Enzymes from nearly every class (including transferases, kinases, hydrolases, oxido-reductases, ligases, and lyases) can be activated by manganese in this manner and are numerous and diverse in function. However, largely because the activation of many enzymes is not manganese specific, a manganese deficiency does not impair the activity of most of these enzymes. The metal is typically replaced by other divalent cations like magnesium. An exception to this apparent lack of specificity is the manganese-specific activation of the glycosyl transferases. Examples of roles that manganese-dependent enzymes perform in the body are described in the next section.

Bone, Cartilage, and Connective Tissue Synthesis

Two manganese-dependent transferases important for connective tissue synthesis are xylosyl transferase and glycosyl (also called galactosyl or galacto) transferase. Glycosyl transferase is especially important for the synthesis of glycosaminoglycans, such as chondroitin sulfate, which attach to proteins to form proteoglycans. Remember that proteoglycans are important structural components of connective tissues such as cartilage and bone. Specifically, glycosyl transferase catalyzes the transfer of a sugar moiety (galactose) from uridine diphosphate (UDP) to an acceptor molecule, as shown by the general reaction:

$$\begin{array}{c} \text{UDP-sugar} \\ + \\ \text{acceptor} \end{array} \xrightarrow{\text{Glycosyl transferase}} \begin{array}{c} \text{UDP} \\ + \\ \text{acceptor-sugar} \end{array}$$

Manganese deficiency is associated with impaired glycosyl transferase activity.

Manganese also activates the hydrolase prolidase, a dipeptidase with specificity for dipeptides. Prolidase is found in dermal fibroblasts and is important for collagen formation.

Urea Synthesis

Arginase, a hydrolase that requires four manganese atoms per molecule, is a cytosolic enzyme responsible for urea formation. The enzyme is found in high concentrations in the liver, the site of the urea cycle. The enzyme cleaves arginine to generate urea and ornithine. Low-manganese diets in animals have been shown to decrease arginase activity.

Carbohydrate/Nutrient Metabolism

Pyruvate carboxylase, a ligase/synthetase that contains four manganese atoms, converts pyruvate to the TCA cycle intermediate oxaloacetate. Because magnesium can replace manganese in pyruvate carboxylase, minimal changes in pyruvate carboxylase activity occur with manganese deficiency.

Phosphoenolpyruvate carboxykinase (PEPCK), a lyase activated by manganese, converts oxaloacetate to phosphoenolpyruvate and carbon dioxide. This reaction is important in gluconeogenesis. The activity of phosphoenolpyruvate carboxykinase decreases in animals with manganese deficiency.

The enzyme isocitrate dehydrogenase requires either manganese or magnesium. This enzyme catalyzes the conversion of isocitrate to alpha-ketoglutarate in the TCA cycle; $NADP^+$ is needed for the reaction.

Amino Acid Metabolism

Glutamine synthetase, activated by manganese or magnesium, is important in the synthesis of glutamine from glutamate.

Antioxidant Roles

Superoxide dismutase, a manganese-dependent (Mn^{3+}-SOD) oxido-reductase metalloenzyme (not manganese activated), functions in a manner similar to copper- and zinc-dependent superoxide dismutase to prevent lipid peroxidation by superoxide radicals. However, manganese-SOD is found in the mitochondria, whereas copper-zinc-SOD is found extracellularly and in the cell cytosol. Thus, Mn-SOD likely eliminates superoxides before they damage mitochondrial function. The activity of the electron transport/respiratory chain generates large amounts of superoxide radicals, necessitating substantial Mn-SOD activity. Cellular ultrastructural abnormalities associated with manganese deficiency are likely caused by uncontrolled lipid peroxidation in the membranes because of reduced Mn-SOD activity or simply by reduced availability of manganese to directly scavenge free radicals. Manganese, one of a few minerals able to scavenge free radicals, quenches peroxyl radicals, as shown in this equation: $Mn^{2+} + ROO^{\bullet} \rightarrow Mn^{3+} + ROOH$. Low-manganese diets in animals have been shown to decrease Mn-SOD activity. Moreover, Mn-SOD knockout mice die shortly after birth, an indication of Mn-SOD's essential protective role in the body.

Other Roles

Manganese may also act as a modulator of second-messenger pathways in tissues. For example, manganese increases cAMP accumulation through binding to ATP and ADP. Manganese can activate guanylate cyclase, and manganese may affect cytosolic calcium levels and thus regulate calcium-dependent processes.

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Interactions with Other Nutrients

Only a few interactions between manganese and other trace elements are thought to be of significance nutritionally. One such relationship—that between manganese and iron—is detailed in the section on absorption. However, the interaction is reciprocal; that is, iron in excess inhibits manganese absorption, and manganese, when ingested in amounts about four to eight times the recommended intake, decreases iron absorption up to about 40%. Interactions may also occur between manganese and calcium and between manganese and zinc; however, because of the paucity of information and the divergent results from various studies, the nature of such interactions remains undefined.

Excretion

Manganese is excreted primarily (~90%) via the bile in the feces. Excess absorbed manganese from the diet is quickly released by the liver into the bile to maintain homeostasis. Very little manganese is excreted in the urine, less than 1 μg/L. Small losses also occur through sweat and skin desquamation [2].

Adequate Intake

The recommendation for manganese intake is based on median intake because data are insufficient to calculate requirements for the mineral. Recommended Adequate Intakes of manganese are 2.3 mg for adult men and 1.8 mg for adult women daily [3]. With pregnancy and lactation, recommendations increase to 2 mg and 2.6 mg, respectively [3]. The inside front cover of the book gives additional recommendations for manganese for other age groups.

Deficiency

Manganese deficiency is associated with diverse physiological malfunctions. In humans, manganese deficiency generally does not develop unless the mineral is deliberately eliminated from the diet. Studies in which men received either 0.11 mg manganese/day for 39 days (the diet was also devoid of vitamin K, making it difficult to separate the effects of the manganese and vitamin K deficiencies) or 0.35 mg manganese/day resulted in negative manganese balance [2]. Symptoms and signs of deficiency include nausea; vomiting; dermatitis; decreased serum manganese; decreased fecal manganese excretion; increased serum calcium, phosphorus, and alkaline phosphatase (thought to be associated with skeletal bone changes); decreased growth of hair and nails; changes in hair and beard color; poor bone formation and skeletal defects; decreased clotting proteins; and altered carbohydrate and lipid metabolism [2,3]. Other problems associated with deficiency include ataxia, loss of equilibrium, cell ultrastructure abnormalities, compromised reproductive function, abnormal glucose tolerance, and impaired lipid metabolism [2,3].

Toxicity

The Tolerable Upper Intake Level for manganese is 11 mg/day [3]. Exposure to high levels of oral, parenteral, and air manganese may result in toxicity. Additionally, individuals with liver failure are at greater risk for toxicity because manganese homeostasis is maintained largely by the liver with excretion of excess manganese occurring via the bile. Manganese toxicity secondary to liver failure is characterized by manganese accumulation within the liver and other organs such as the brain; accumulation in the brain results in neurologic abnormalities [4,5]. Motor and cognitive dysfunction also occurs with manganese toxicity. Neonates receiving parenteral nutrition are thought to be at higher risk for manganese toxicity because of diminished biliary manganese excretion [1]. Symptoms of manganese toxicity with chronic exposure to airborne manganese include cough, bronchitis, pneumonitis, and reduced lung function, as well as symptoms resembling Parkinson's disease including tremors, diminished memory capacity, and loss of coordination/difficulty walking [4,5]. Other signs and symptoms of toxicity include insomnia, headache, increased forgetfulness, anxiety, mood changes, compulsive behaviors, reduced response speed/reaction time, rapid hand movements, and gait disturbance [4,5].

Assessment of Nutriture

Assessment of manganese status is typically based on concentrations of manganese in the plasma/serum and whole blood. Serum concentrations have been found to be somewhat sensitive to large variations in intake but do not necessarily correlate with intake or status [1]. Enzyme activity, primarily Mn-SOD and arginase, has also been used but has not been shown to be a good indicator of manganese status [1].

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Suggested Reading

Roth JA. Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination. Biol Res. 2006; 39:45–57.

Web Site for Nutrient Composition Information

www.ars.usda.gov/Services/docs.htm?docid=18877

MOLYBDENUM

The need for molybdenum was established in humans through the observation that a genetic defect in enzymes that required molybdenum as a cofactor resulted in severe pathology. In the body, molybdenum, a metal, is found primarily in two valence states, Mo^{4+} and Mo^{6+}.

Sources

Molybdenum is widespread among foods, but as with many other minerals, the molybdenum content of a plant food varies depending on the concentration of molybdenum in the soil. Better dietary sources of molybdenum are legumes, which can provide up to 184 μg/100 g; meat, fish, and poultry, which contain up to ~129 μg/100 g; and grains and grain products, which provide up to ~117 μg/100 g [1,2]. Nuts and vegetables usually contain less than 50 μg/100 g, but fruits and dairy products are especially low in molybdenum, providing less than 12 μg/100 g [1,2].

The molybdenum content of multivitamin/mineral supplements ranges from about 25 to 45 μg and is provided as ammonium or sodium molybdate. Individual-nutrient supplements provide varying amounts, up to about 1,000 μg (1 mg) of molybdenum. The form of molybdenum in single-nutrient supplements is usually as sodium or ammonium molybdate or as an amino acid chelate. The Daily Value for molybdenum is 75 μg.

Digestion, Absorption, Transport, and Storage

Molybdenum, which is found in foods primarily as molybdate (MoO_4^{2-}), does not appear to require digestion prior to its absorption from the small intestine, which occurs mostly in the proximal region. The mechanism by which molybdenum is absorbed is thought to be passive, although carrier-mediated uptake may also occur. Molybdenum absorption increases with increasing dietary intake over a range of 22–1,490 μg/day [3]. Molybdenum absorption from foods ranges from ~50 to 85% and, from the supplement ammonium molybdate, absorption is over 90% [3]. Sulfates (SO_4^{2-}), if present in large quantities, may compete with molybdate for absorption. Transport of molybdenum in the blood is thought to occur as molybdate, which binds to albumin and α-2-macroglobulin.

The molybdenum content of human tissues is quite low, averaging 0.1–1.0 μg/g of wet weight; the human body contains about 2 mg of the mineral. Molybdate uptake by tissues is thought to occur by one or more carriers, including one that perhaps also transports sulfate. The liver, kidneys, and bones contain the most molybdenum in terms of both absolute amount and concentration. Other tissues, such as the small intestine, lungs, spleen, brain, thyroid and adrenal glands, and muscle, also contain the element. In tissues, molybdenum is found as molybdate, free molybdopterin, and/or molybdopterin that is bound to enzymes.

Functions and Mechanisms of Action

The biochemical role of molybdenum centers around the redox function of the element and its necessity as a cofactor in the form of molybdopterin for four metalloenzymes (sulfite oxidase, aldehyde oxidase, xanthine oxidoreductase, and amidoxime reductase), all of which catalyze oxidation-reduction reactions. Each of the reactions involves a transfer of two electrons, which results in a change in the molybdenum oxidation state (from 4+ to 6+ and vice versa). Molybdenum is attached to enzymes as a part of molybdopterin, an alkylphosphate-substituted pterin to which molybdenum is coordinated through two sulfur atoms. Molybdopterin anchors the molybdenum to the apoenzyme at its catalytic site. The molybdenum is further bonded either to two oxygen molecules (called dioxomolybdopterin) or to one oxygen and one sulfur (called oxosulfidomolybdopterin), as shown in Figure 13.21.

Figure 13.21 Molybdopterin structures.

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The inability to synthesize molybdopterin because of a genetic defect is usually lethal, as discussed in the section "Deficiency."

Sulfite Oxidase: Sulfur Metabolism

Sulfite oxidase, a mitochondrial intermembrane enzyme found in many body tissues—especially the liver, heart, and kidneys—has iron-sulfur clusters, two molybdopterins (dioxo cofactor form), and two cytochrome residues. The enzyme catalyzes the terminal step in the metabolism of the sulfur-containing amino acids (methionine and cysteine), in which sulfite (SO_3^{2-}) is converted to sulfate (SO_4^{2-}), as shown here:

Cytochrome c is the physiological electron acceptor for the reaction.

In addition to originating from the catabolism of methionine and cysteine, sulfites are found in the diet, where they are added to some foods as an antimicrobial agent. Sulfate generated from this reaction in the body typically is excreted in the urine or used for the synthesis of sulfoproteins, sulfolipids, and mucopolysaccharides (a component of mucus).

Aldehyde Oxidase: Various Roles

Aldehyde oxidase is a molybdoenzyme (oxosulfido form) that is similar to xanthine oxidoreductase (see the next section) in size, cofactor composition, and substrate specificity. It presumably functions in the cytosol of liver cells as a true oxidase, using exclusively molecular oxygen as its physiological electron acceptor. The enzyme's primary substrates are thought to include a variety of aldehydes, including, for example, the retinal form of vitamin A and the pyridoxal form of vitamin B_6, as well as acetylaldehyde and other drugs. Other enzymes, however, such as an NADH-dependent aldehyde dehydrogenase also found in the liver, are thought to catalyze reactions similar to those catalyzed by this aldehyde oxidase.

Xanthine Oxidoreductase: Hydroxylation of Purines, Pteridines, and Pyrimidines, among Other Compounds

Xanthine oxidoreductase is an iron-dependent (containing two iron–two sulfur centers) enzyme that exists as a homodimer of two identical subunits and that also requires FAD and molybdopterin in the oxosulfido cofactor form. The molybdenum is necessary for the oxidative capacity of the enzyme; inactivation of the enzyme results if the mineral is not present. The enzyme catalyzes the conversion of hypoxanthine to xanthine and the conversion of xanthine to uric acid, as part of purine degradation as shown in Figure 13.22. Both reactions are oxidative hydroxylations with two electrons passed from the substrate to the molybdenum in the enzyme. Electrons are then transferred to FAD via the iron-sulfur centers, and then are typically passed onto NAD^+, forming NADH. However, when conformational changes occur in the enzyme, the enzyme becomes an oxidase (xanthine oxidase) and molecular oxygen becomes the electron acceptor (instead of NAD^+) and hydrogen peroxide (H_2O_2), or a superoxide radical ($O_2^{\bullet}$), is formed.

Xanthine oxidoreductase is found in a variety of tissues, including the liver, lungs, kidneys, and intestine, and is also capable of hydroxylating other purines as well as pteridines, pyrimidines, and other heterocyclic nitrogen-containing compounds. Conversion to xanthine oxidase occurs following the oxidation of sulfhydryl groups or by proteolysis. Healthy tissues may contain about 10% of their total xanthine enzymes in the oxidase form [4].

Reductions in xanthine oxidoreductase activity cause no apparent clinical effects. The genetic disorder xanthinuria, in which large amounts of xanthine are excreted in the urine, provides evidence of the body's ability to tolerate low xanthine oxidoreductase activity. The condition is essentially free of clinical manifestations, except for the possible development of kidney calculi (stones) caused by the high urinary xanthine concentration.

The effects of xanthine oxidase activity, however, can be damaging with administration of oxygen for ischemia (a local or temporary deficient blood supply and thus relative oxygen deprivation). Xanthine oxidase activity generates large amounts of hydrogen peroxide, which further induces damage (called reperfusion injury) in the ischemic tissues.

Amidoxime Reductase: Drug Metabolism

Amidoxime reductase is found attached to the outer mitochondrial membrane, where it complexes with cytochrome b5 and molybdopterin [5]. The enzyme complex reduces N-hydroxylated amidines and hydroxyamines. N-hydroxylated drugs, for example, are synthesized by drug companies to improve the solubility and absorption

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Figure 13.22 Molybdenum's role in hypoxanthine and xanthine degradation.

of drugs into intestinal cells. Within the body, however, the drug must be reduced to exert its desired effects. Amidoxime reductase enables such a reaction (Figure 13.23).

Interactions with Other Nutrients

The most notable interaction in humans involving molybdenum is with copper. Ingestion of molybdenum as tetrathiomolybdate inhibits copper utilization, and use of the mineral has been shown to be of benefit in the treatment of Wilson's disease [6,7]. Molybdenum's ability to induce copper deficiency also inhibits angiogenesis through downregulating the expression of vascular growth factors involved in angiogenesis signal pathways [6–10]. Because angiogenesis provides blood to tumors and enables their growth, substances like molybdenum that can prevent copper-induced angiogenesis have exhibited promising results such as inhibition of tumor growth in some animal studies [9,10].

Excretion

Most molybdenum is excreted as molybdate in the urine. Urinary excretion of molybdenum increases as dietary molybdenum intake increases. In other words, little molybdenum is retained in the body when dietary intake is high, and the kidneys are responsible for molybdenum homeostasis. Small amounts of molybdenum are excreted from the body in the feces by way of the bile. Small amounts of the mineral can also be lost in sweat (20 μg) and in hair (0.01 μg/g of hair).

Figure 13.23 The reduction of N-hydroxylated compounds such as benzamidoxine by the molybdenum-dependent enzyme amidoxime reductase.

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Recommended Dietary Allowance

Based on balance studies as well as depletion and repletion studies providing varying amounts of dietary molybdenum, an Estimated Average Requirement for molybdenum for adults was set at 34 μg [11]. The RDA for molybdenum for adults (men and women) is 45 μg (130% of the requirement), with 50 μg suggested during pregnancy and lactation [11]. The inside front cover of the book provides additional RDAs for molybdenum for other age groups.

Deficiency

Molybdenum deficiency is rarely encountered, although the condition has been documented in a patient maintained for 18 months on total parenteral (intravenous) nutrition. Molybdenum deficiency was associated with high blood concentrations of methionine, hypoxanthine, and xanthine, as well as low blood concentrations of uric acid. Additionally, urinary concentrations of sulfate were low, and those of sulfite were high. Treatment with 300 μg of ammonium molybdate (providing 163 μg of molybdenum) resulted in clinical improvement and normalized sulfur amino acid metabolism and uric acid production.

Genetic defects in enzymes involved in molybdopterin synthesis disrupt both cofactor production and molybdenum use in the molybdenum-dependent enzymes. Defects in a few of the enzymes involved in the four-step set of reactions required for molybdopterin synthesis have been characterized along with a review of cases [12]. The condition, which is evident at birth, results in severe and progressive neurological deterioration. It is further characterized by microcephaly, progressive encephalopathy, intractable seizures, facial dysmorphic features, and poor feeding [12]. Isolated sulfite oxidase deficiency presents similarly to the cofactor deficiency. The genetic disorder is characterized by poor feeding, hypoactivity, dyspnea, dislocation of the ocular lenses, attenuated brain growth, seizures, severe neurological damage, and death in childhood. Elevated levels of urinary sulfite and thiosulfate, along with biochemical manifestations reflecting aberrant sulfur amino acid metabolism and sulfite oxidation, are also present.

Toxicity

Molybdenum appears to be relatively nontoxic with intakes up to about 1,500 μg/day [11,13]. However, symptoms such as gout (inflammation of the joints caused by the accumulation of uric acid) have appeared in some people living in regions that contain high soil molybdenum levels and in those with occupational exposure to molybdenum [14]. A Tolerable Upper Intake Level for molybdenum has been set at 2 mg [11].

Assessment of Nutriture

Molybdenum appears to distribute itself fairly equally between the plasma and red blood cells; however, the use of these as indicators of molybdenum status has not been validated. Similarly, while urinary molybdenum concentrations increase with increased molybdenum intake, urinary molybdenum is not necessarily reflective of molybdenum status.

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Suggested Reading

Mendel RR. The molybdenum cofactor. J Biol Chem. 2013; 288:13165–72.

Web Site for Nutrient Composition Information

www.ars.usda.gov/Services/docs.htm?docid=18877

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NUTRIENT–DRUG INTERACTIONS

Nutrient–drug interactions represent nutrient-induced changes in the kinetics of a drug or drug-induced changes in nutrient metabolism or nutritional status. Such interactions can be extremely detrimental. Some nutrient interactions can lead to failure of the drug to perform its desired actions or to drug toxicity. Alternately, some drug interactions can promote nutrient deficiencies or toxicities. In addition to these direct interactions, drugs may also influence nutrition status indirectly by, for instance, diminishing appetite, altering taste, or promoting nausea, vomiting, or diarrhea. This Perspective reviews some examples of foods and nutrients that affect the absorption, distribution, metabolism, actions (functions), or excretion of drugs, as well as some examples of drugs that affect the absorption, metabolism, or excretion of nutrients. Table 1 provides an overview of some of the interactions presented in this Perspective.

EFFECTS OF FOODS AND NUTRIENTS ON DRUG ABSORPTION

Foods or nutrients in foods can alter drug absorption by serving as a physical barrier or through effects on transit time (i.e., motility of the gastrointestinal tract), secretions, drug dissolution, chelation, or carrier uptake, among other effects. Many drugs should be taken without food or beverages to prevent interference with drug absorption. For example, the absorption of Fosamax (alendronate), used to treat osteoporosis, is greatly diminished with concurrent ingestion of food or beverages (other than water). Similarly, the antibiotics erythromycin and penicillin (ampicillin), along with the antihypertensive drugs Capoten (captopril) and Univasc or Uniretic (moexipril), should be ingested only with water; food should not be consumed for at least 1 hour. On the other hand, the absorption of many other drugs is enhanced with coingestion of food or even specific dietary nutrients such as those provided by a high-fat meal.

Foods or antacids that contain relatively large amounts of magnesium, calcium, zinc, iron, and aluminum need to be avoided or should be ingested separately (by several hours) from antibiotics such as Achromycin and Sumycin (tetracycline antibiotics), Cipro (ciprofloxacin), Maxaquin (lomefloxacin), and Levaquin (levofloxacin), and from other groups of antibiotics and antifungals such as Nizoral (ketoconazole). The divalent and trivalent minerals in the antacids or from the foods chelate (bind to) and decrease the absorption of the drugs. An appropriate gastrointestinal tract pH is important for dissolving or absorbing some drugs. Thus, ingesting foods or antacids that can promote gastrointestinal secretions or alter pH may be detrimental.

Table 1 An Overview of Some Selected Drug–Nutrient/Food Interactions

Drug(s)	Nutrient(s)/Food(s)
Antibiotics: tetracycline, Achromycin, Sumycin, Cipro, Maxaquin, and Levaquin; **Antifungal:** Nizoral	**Calcium, magnesium, zinc, iron, and aluminum**
Immunosupressant: Neoral; some **HMG-CoA reductase inhibitors:** Zocor, Mevacor, and Lipitor; **Anti-intermittent claudication:** Pletal; **Antimigraine:** Relpax	**Grapefruit juice**
Anti-Parkinson's: Dopar, Larodopa, Sinemet, and Parcopa	**Protein and vitamin B_6**
Monoamine oxidase inhibitors: Parnate and Nardil; **Antituberculosis:** Isoniazid	**Amine-containing foods:** aged cheeses; smoked, salted, and pickled fish; sausage; salami; pepperoni; corned beef; bologna; meat extracts; wines; and chocolate, among others
Anticoagulants: Coumadin	**Vitamin K**
Bronchodilators: Theo-24, Theolair, Uniphyl, and Elixophyllin	**Caffeine and vitamin B_6**
Antimanic: Eskalith, Lithobid, and Lithotabs	**Sodium**
Bile-acid sequestrants: Questran	**Fat, fat-soluble vitamins A, D, E, and K; folate; iron; magnesium; calcium; and zinc**
Antituberculosis: Isoniazid	**Vitamin B_6**
Anticonvulsants: Phenobarbital, Dilantin, and Phenytek	**Vitamin D and folate**
H_2 receptors blockers: Tagament and Zantac; **Proton pump inhibitors:** Prilosec and Prevacid	**Iron, zinc, calcium, magnesium, and vitamin B_{12}**
Loop diuretics: Lasix and Bumex	**Potassium, chloride, magnesium, thiamin, and sodium**

EFFECTS OF FOODS ON DRUG METABOLISM

Many drugs that undergo substantial first-pass metabolism in the gastrointestinal tract are affected by coingestion of grapefruit juice. Some of these medications include the immunosuppressants Neoral and Sandimmune (cyclosporine); some HMG-CoA reductase inhibitors (used to treat high blood cholesterol) such as Zocor (simvastatin), Mevacor (lovastatin), and Lipitor (atorvastatin); Pletal (cilostazol), which is used to treat intermittent claudication and peripheral vascular disease; VePesid (etoposide), which is used to treat some cancers; and Relpax (eletriptan), which is used to treat migraines. The exact compound or compounds in grapefruit juice that cause the interaction are not clear. Grapefruit juice is rich in many phytochemicals, especially flavonoids such as the flavanone naringenin and its glycoside naringin and the flavonol kaempferol. Ingesting grapefruit juice is thought to decrease (downregulate) the isozyme of cytochrome P450 known as CYP 3A4, which is found in the intestine. This enzyme normally begins intestinal cell metabolism of many drugs. Consequently, the downregulation of this enzyme by ingestion of grapefruit juice causes the drugs to be absorbed without any metabolism and causes blood concentrations of the drugs to be much higher than desired. The high blood concentrations of the drug, in turn, can result in undesirable side effects, including toxicity. Similarly, inhibition of intestinal non-CYP3A enzymes as well as enterocyte transport proteins has been demonstrated *in vitro* and may alter drug metabolism.

In addition to alterations in drug distribution caused by grapefruit juice, a high protein intake (two to three times

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recommendations) alters the distribution of the anti-Parkinson drugs Dopar and Larodopa (levodopa) and Sinemet and Parcopa (levodopa and carbidopa). The effects are thought to result from competition for carriers at the blood–brain barrier between the drug and large neutral amino acids (such as phenylalanine, tyrosine, and tryptophan). These large neutral amino acids appear in the blood following consumption of large amounts of protein. Vitamin B_6 can also increase the metabolism of levodopa by enhancing its conversion to dopamine before the drug crosses the blood–brain barrier. Vitamin B_6 is found in liver and other protein-rich foods, such as meats and legumes, as well as seeds and whole grains. Thus, ingesting levodopa with large amounts of protein or vitamin B_6, or regularly consuming a diet high in protein or vitamin B_6 while taking levodopa, is contraindicated.

EFFECTS OF FOODS AND NUTRIENTS ON THE ACTIONS OF DRUGS

Some foods or nutrients can enhance or oppose the actions of drugs. Foods that contain amines, especially tyramine, dopamine, or histamine, are known to interact with a group of drugs known as monoamine oxidase inhibitors (MAOIs), which are used mostly to treat some forms of depression. MAOIs such as Parnate (tranylcypromine sulfate) and Nardil (phenelzine sulfate) prevent the enzyme monoamine oxidase from catabolizing amines in the diet as well as amines made endogenously. Amines consist of vasoactive or pressor amines (e.g., tyramine, serotonin, and histamine) and neurotransmitters or psychoactive amines (e.g., dopamine and norepinephrine). The antituberculosis drug INH (isoniazid) exhibits MAOI-like activity. The problem arises when people on MAOIs or INH eat foods high in amines, especially tyramine or histamine, which may be found in fairly large quantities in some foods. Consuming these foods ordinarily presents no problem because the amines can quickly be inactivated by monoamine oxidase (MAO). However, in people taking MAOIs or isoniazid, these reactions do not occur. Consequently, high dietary amine intake coupled with high endogenous norepinephrine may result in excessive vasoconstriction, manifested as severe headache, acute hypertension or a hypertensive crisis, and cardiac dysrhythmia. People taking MAOIs are counseled against ingesting foods high in amines, such as aged cheeses (cheddar, Camembert, Stilton, and Boursault), yeast extracts (e.g., Marmite), and brewer's yeast. Smoked, salted, or pickled fish such as herring or cod, as well as sausage, salami, pepperoni, corned beef, and bologna, are also high in tyramine. Foods moderately high to high in tyramine include meat extracts; tenderizers; red wines, including Chianti, vermouth, sherry, and burgundy; and cheeses such as blue, natural brick, Brie, Gruyère, mozzarella, Parmesan, Romano, and Roquefort. Broad beans (fava, and Chinese pea pods), chocolate, large amounts of caffeine, liver (chicken or beef), and selected fruits may also contain large amounts of tyramine. Histamine is not typically found in large quantities in foods, with the exception of improperly stored or spoiled fish. Dopamine is found in fava and broad beans and snow peas.

A nutrient known to antagonize the action of the anticoagulant Coumadin (warfarin) is vitamin K. Coumadin works by inhibiting reactions in the vitamin K cycle that generate the active form of vitamin K needed for blood clotting. By inhibiting production of active vitamin K, the drug prolongs the clotting time of blood. Large amounts of vitamin K oppose the actions of the drug, promoting blood clotting and leading to drug resistance. Ingesting very large quantities of foods rich in vitamin K, including green vegetables, some legumes (soybeans, garbanzo beans), and liver, should be avoided.

Caffeine, a component of coffee, tea, many soft drinks, and chocolate, counters the actions of tranquilizers and may exacerbate some adverse effects of the bronchodilators Theo-24, Theolair, Uniphyl, and Elixophyllin (theophylline). Specifically, large amounts of caffeine coupled with use of theophylline promote increased nervousness, insomnia, and tremors. (These drugs also interfere with vitamin B_6 metabolism.)

EFFECTS OF FOODS AND NUTRIENTS ON DRUG EXCRETION

Sodium and the mineral lithium in the antimanic drugs Eskalith, Lithobid, and Lithotabs (lithium carbonate) are known to interact in the kidneys. Specifically, the sodium and lithium compete with each other for reabsorption into the tubules of the kidneys. Thus, a high intake of sodium promotes lithium excretion and thereby diminishes the effects of the drug, whereas a low intake of sodium promotes reabsorption of lithium and thereby enhances the likelihood of drug toxicity.

EFFECTS OF DRUGS ON NUTRIENT ABSORPTION

Drugs may alter the absorption of nutrients through several mechanisms. For example, drugs may alter the transit time of nutrients through the gastrointestinal tract, speeding up or slowing down the passage of its contents. Typically, when contents move quickly through the gastrointestinal tract, fewer nutrients are absorbed; when contents move slowly, more nutrients are absorbed. Changes in the pH of the gastrointestinal tract also may alter nutrient absorption. For example, H_2 receptor blockers such as Tagamet (cimetidine) and Zantac (ranitidine) and proton pump inhibitors such as Prilosec (omeprazole) and Prevacid (lansoprazole), which decrease hydrochloric acid secretion into the stomach and thus increase gastric pH, diminish the absorption of several nutrients, especially iron. H_2 receptor blockers and proton pump inhibitors are used to treat ulcers and gastroesophageal reflux disease (GERD).

The ability of some drugs to chelate or adsorb nutrients or gastrointestinal secretions such as bile also diminishes nutrient absorption. For example, bile acid sequestrants such as Questran (cholestyramine) adsorb bile and thus decrease the absorption of fat and the fat-soluble vitamins A, D, E, and K and the carotenoids. In addition, the drug chelates other nutrients (including folate) and some divalent minerals (including iron, magnesium, calcium, and zinc). Bile acid sequestrant drugs are used to treat high blood cholesterol concentrations, a risk factor for heart disease.

EFFECTS OF DRUGS ON NUTRIENT METABOLISM

In addition to altering nutrient absorption, drugs may alter the metabolism of nutrients in body tissues. Isoniazid, used in the treatment of tuberculosis, for example, diminishes the conversion of pyridoxine (vitamin B_6) to its functional coenzyme form in the liver and thus can cause a vitamin B_6 deficiency. Another group of drugs known to alter vitamin metabolism includes the anticonvulsants phenobarbital and phenytoin (Dilantin and Phenytek). These drugs alter the metabolism of vitamin D, leading to (in severe cases) the deficiency conditions rickets and osteomalacia if vitamin supplements (as 25-OH cholecalciferol) are not taken. Specifically, the anticonvulsants are thought to diminish the hepatic conversion of vitamin D as cholecalciferol to 25-OH cholecalciferol. Interestingly, these anticonvulsants also affect the vitamin folate by diminishing its absorption in the intestine.

EFFECTS OF DRUGS ON NUTRIENT EXCRETION

Drugs may increase or decrease the excretion of nutrients from the body. Loop diuretics used to treat high blood pressure such as Lasix (furosemide) and Bumex (bumetanide) promote the urinary excretion of sodium and water (important in lowering blood pressure); however, the drugs may also increase the urinary losses of potassium, chloride, thiamin, zinc, and magnesium. Dietary replacement of the minerals, especially potassium, is important to prevent low blood potassium concentrations.

SUMMARY

Nutrient–drug interactions can severely affect both nutritional status and the effectiveness of pharmacological treatment. Although accredited health care facilities are mandated to educate patients about interactions between foods and drugs, many individuals remain unaware of such interactions and their consequences.

Suggested Readings

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14 NONESSENTIAL TRACE AND ULTRATRACE MINERALS

FLUORIDE
ARSENIC
BORON
NICKEL
SILICON
VANADIUM
COBALT
For each of the minerals listed, the following subtopics (if known and when applicable) are discussed:
Sources
Absorption, Transport, Storage, and Excretion
Functions and Deficiency
Interactions with Other Nutrients
Recommended Intake, Toxicity, and Assessment of Nutriture

PERSPECTIVE
NO, SILVER IS *NOT* ANOTHER ESSENTIAL ULTRATRACE MINERAL: TIPS TO IDENTIFYING BOGUS CLAIMS ABOUT DIETARY SUPPLEMENTS

AS CHAPTER 13 EXPLAINED, TRACE ELEMENTS are those minerals that are needed by the body in amounts less than 100 mg/day, and ultratrace elements are those elements with estimated, established, or suspected requirements of less than 1 mg/day. Based on these definitions, at least 18 elements may be classified as ultratrace elements: aluminum, arsenic, boron, bromine, cadmium, chromium, copper, germanium, iodine, lead, lithium, molybdenum, nickel, rubidium, selenium, silicon, tin, and vanadium. However, while copper, chromium, iodine, molybdenum, and selenium are considered ultratrace elements by the preceding definition, they have been included in Chapter 13 based on their established essentiality and Adequate Intake or Recommended Dietary Allowance set by the Food and Nutrition Board. The elements that are addressed in this chapter—fluoride, arsenic, boron, nickel, silicon, and vanadium—are not, at present, considered essential, although for each, some evidence suggests a possible need. Figure 14.1 shows the location of these elements in the periodic table. Table 14.1 provides an overview of selected functions, food sources, and deficiency symptoms for these nutrients. A brief discussion is also provided for the element cobalt, which is needed but only as part of vitamin B_{12} (cobalamin).

FLUORIDE

Whereas fluorine (F) is a gaseous chemical element, fluoride (F^-) is typically found bound to a metal, nonmetal, or organic compound. The term *fluoride* is used throughout this section (analogous to the use of the terms *iodide* and *chloride* previously). Fluoride, although present in the body in trace amounts, is not considered an essential nutrient since the element has not been shown to be essential for life and no biochemical role has been identified for it. Fluoride is included in this chapter because it has been shown to exert beneficial effects as discussed in the "Functions and Deficiency" section.

Sources

Community drinking water has been fluoridated (with about 1 ppm or 1 mg of fluoride in a liter of drinking water) since about 1945 in the United States, due to the discovery of the inverse relationship between fluoride intake and the incidence of dental caries. Several beverages, including ready-to-use infant formulas, are made with fluoridated water, but others vary greatly in their fluoride content depending on the use or nonuse of fluoridated water in their processing.

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Figure 14.1 The periodic table highlighting important nonessential trace and ultratrace elements.

The fluoride content in most food groups is low, usually less than about 0.05 mg/100 g. However, a few foods contain higher amounts of fluoride, including some grains and cereal products, some fish (if consumed with the bones), and tea. Fish contain from ~0.01 to 0.17 mg/3.5 oz; canned sardines (with bones) have about 0.2–0.4 mg of fluoride/3.5 oz. Other seafood such as clams, lobster, crab, and shrimp are also good sources of fluoride. Tea, both caffeinated and decaffeinated, is rich in fluoride because tea leaves accumulate the element in fairly high amounts. Brewed tea contains from 1 to 6 mg of fluoride/L, with decaffeinated forms higher in fluoride than caffeinated. Table 14.2 provides the fluoride content of various food groups.

Most Americans obtain the majority of dietary fluoride from drinking water, which provides about 0.24 mg of fluoride/cup. Fluoridation of water continues to be recommended by various public health associations in amounts of about 0.7–1.2 mg of fluoride/L. Usual fluoride intake by Americans is up to about 3.4 mg/day.

Fluoride as sodium fluorosilicate is typically added to water, while sodium fluoride or monofluorophosphate is used in toothpaste or tablets. If swallowed, fluoride-containing toothpaste can be a significant source of the mineral; however, ingesting even small amounts of fluoride-containing toothpaste can result in ingestion of fluoride in amounts exceeding recommended intakes and thus can be dangerous, especially to young children. Consequently, many manufacturers recommend that toothpaste be kept out of reach of children and that only a "pea-sized" amount be used for toothbrushing.

Table 14.1 Nonessential Trace and Ultratrace Elements: Selected Functions, Deficiency Symptoms, and Food Sources

Mineral	Possible Physiological Roles	Selected Deficiency Symptoms in Animals	Food Sources
Fluoride	Reductions in dental caries	Growth and infertility	Fish, legumes, grains, tea, and drinking water (variable)
Arsenic	Methyl group use	Impaired growth and reproduction	Seafood, meat, grains, grain products, and water
Boron	Bone development, immune system function, and brain function	Impaired bone health, cognitive/brain function, and immune response	Fruits, vegetables, legumes, and nuts
Nickel	Possibly involved in hormonal membrane or enzyme activity	Depressed reproduction and growth, and impaired hematopoiesis	Nuts, legumes, grains, and cocoa products (chocolate)
Silicon	Connective tissue and bone formation	Decreased collagen, and long bone and skull abnormalities	Beer, whole grains, lentils, and root vegetables
Vanadium	Mimics insulin action (pharmacologic effect)	Impaired fertility, reduced survival, growth, and development	Fish, shellfish, grains, black pepper, parsley, mushrooms, and dill seed

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Table 14.2 Fluoride Content of Selected Food Groups

Food Group	Fluoride Content Range (mg/100 g)
Dairy products	0.002–0.082
Meat and poultry	0.004–0.092
Grain products	0.008–0.201
Potatoes	0.008–0.084
Green leafy vegetables	0.008–0.070
Legumes	0.015–0.057
Root vegetables	0.009–0.048
Other vegetables	0.006–0.017
Fruits	0.002–0.013
Fats and oils	0.002–0.044

Source: Taves, DR. Dietary intake of fluoride ashed (total fluoride) v. unashed (inorganic fluoride) analysis of individual foods. *Brit J Nutr*. 1983; 49:295–301. Reprinted with the permission of Cambridge University Press.

Absorption, Transport, Storage, and Excretion

In foods, fluoride may be bound to proteins, and must be hydrolyzed by pepsin or other proteases before absorption. Additionally, some minerals in foods, especially calcium and magnesium, may form insoluble complexes with fluoride to decrease its absorption. Fluoride absorption occurs in both the stomach and small intestine by passive diffusion. Absorption is nearly 100% and quite rapid (within 90 minutes of ingestion) when it is consumed as fluoridated water or toothpaste. Within the low pH environment of the stomach, fluoride is converted to hydrogen fluoride, also called hydrofluoric acid—a conversion that facilitates its rapid absorption. When consumed with food, fluoride absorption diminishes to about 50–80%.

Fluoride is transported in the blood free as ionic fluoride and hydrofluoric acid, as well as being bound to plasma proteins. From the blood, absorbed fluoride is distributed rapidly throughout body tissues. Most fluoride is found in bones and teeth but it can exert effects on several body systems. As the amount of absorbed fluoride increases, so does the quantity taken up by tissues. However, the percentage retained at high absorption rates becomes lower because of accelerated urinary excretion. Skeletal growth rate influences fluoride balance, exemplified by the fact that young, growing people incorporate more fluoride into the skeleton and excrete less in the urine than adults.

Ionic fluoride is rapidly excreted in the urine, which accounts for approximately 90% of total fluoride excretion. Urinary fluoride excretion normally ranges from about 0.2 to 1.1 mg of fluoride/L urine. Fecal elimination accounts for most of the remaining (up to about 10%) losses, with only minor losses occurring in sweat.

Functions and Deficiency

Hydroxyapatite, with the theoretic formula $Ca_{10}(PO_4)_6(OH)_2$, is found in teeth and bone. Tooth enamel is susceptible to decay (cavities) especially by acid-forming bacteria. Fluoride replaces some of the hydroxide ions in hydroxyapatite to form fluorohydroxyapatite. Ingested fluoride interacts with teeth mainly through its presence in saliva, which has a fluoride content of about 0.01–0.05 ppm. Ions in hydroxyapatite can be replaced during initial crystal formation or by displacement from previously deposited mineral, according to the following equation: $Ca_{10}(PO_4)_6(OH)_2 + xF^- \longrightarrow Ca_{10}(PO_4)_6(OH)_2 - xF^-_x$. In bone and dental enamel, the ratio of substitution of F^- for OH^- is from about 1:20 to 1:40.

Fluorohydroxyapatite increases the mineralization of the crystalline structure and is less acid soluble than hydroxyapatite and thus increases the resistance of tooth enamel to acid demineralization. Topical fluoride also accelerates the growth of a new surface on partially demineralized subsurface crystals in dental cavities (lesions); such effects enhance remineralization of the enamel. Additionally, the presence of fluoride inhibits both acid production (by buffering the hydrogen ions) and dental plaque formation by oral bacteria. Reductions in caries in the pits and fissure surfaces of permanent teeth are more pronounced when fluoride is provided at pre-eruptive stages of tooth development versus post-eruptive stages. Several meta-analyses of studies over the years have shown that water fluoridation reduces the prevalence of dental caries (cavities) by about 20–40% [1]. Fluoride deficiency conditions have not been documented in humans, although problems with growth among other conditions occur in fluoride-deficient animals.

Recommended Intake, Toxicity, and Assessment of Nutriture

With the goal of minimizing risk for dental caries in the population without causing side effects, the Food and Nutrition Board established Adequate Intakes of 4 and 3 mg of fluoride/day for adult males and females, respectively [2]. The inside front cover of the book provides additional AIs for fluoride for other age groups. Yet, given the Adequate Intake recommendations are based on ingested fluoride preventing tooth decay and given it is now known that many of fluoride's effects are largely topical, it is thought that intake guidelines may need to be reevaluated [1]. Arguments against water fluoridation as a means to prevent dental caries are increasing given the toxic effects of the element.

Acute toxicity (usually from accidental ingestion of fluoride supplements or excessive amounts of toothpaste) manifests as nausea, vomiting, diarrhea, acidosis, and cardiac arrhythmias. Death has been reported following ingestion of 5–10 g of sodium fluoride or about 32–64 mg of fluoride/kg body weight, although it may occur with an intake as low as 5 mg of fluoride/kg body weight [3].

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Chronic fluoride toxicity, called fluorosis, is characterized by changes in the teeth (dental fluorosis), skeleton (including bone deformities, restricted joint movement, abnormal/excessive bone formation and mineralization, and increased bone fracture risk), and nonskeletal tissues (including impaired skeletal muscle, brain, nerve, and kidney function) [3]. Dental fluorosis results from prolonged intake of fluoride in excessive amounts during pre-eruptive development of teeth. It is characterized by mottling of the tooth surface with white spots appearing on the teeth; however, more severe toxicity causes subsurface porosity, weakening of the teeth, and permanent brown spots. With skeletal fluorosis, fluoride accumulates in the joints, making movement difficult. Excessive fluoride intake has also been associated with increased risks of cancers of the bone, skin, and oral cavity.

The Tolerable Upper Intake Levels for fluoride range from 1.3 mg/day for children age 1–3 years to 10 mg/day for children older than 8 years and adults [2]. Removal of excess fluoride from drinking water such that concentrations do not exceed 4.0 mg/L is required by the Environmental Protection Agency.

Plasma and urinary fluoride can be monitored to determine toxicity and fluoride exposure, respectively, but not the body's fluoride status. Normal plasma concentrations for ionic fluoride range from about 0.01 to 0.2 μg of fluoride/mL. Serum fluoride concentrations of 190 ng/mL have been associated with toxic effects on bone (abnormal bone formation and mineralization) [4]. Urinary fluoride excretion of 15 mg/L is associated with a fluoride exposure of about 20–30 mg; however, this level of exposure is likely to occur over one or more decades [4].

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Suggested Readings

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Website providing information on fluoride in foods

http://www.nal.usda.gov/fnic/foodcomp/Data/Fluoride/Fluoride.html

ARSENIC

More than any other ultratrace mineral, arsenic, which is colorless and odorless, conjures an image of toxicity as a poison rather than of nutritional essentiality. The malevolent aspect of arsenic continues to attract attention because the majority of the arsenic literature addresses its toxicological, including its designation as a group 1 human carcinogen, rather than its nutritional properties. At present, proof of arsenic's essentiality is lacking, but the element appears to be nutritionally bioactive.

Sources

Arsenic is present throughout the earth's continental crust at an estimated concentration of 1.5–2.0 μg/g, with some of the heaviest concentrations found in Asia. Arsenic is present in water, rocks, and soils, in concentrations that vary considerably based on the geological history of the soil as well as on pollution. Fallout sources such as pesticides, smelters, and coal-fired power plants can, through aerosols and floating dust, enrich a particular area with arsenic, which then affects humans when it is incorporated into water and foods. Arsenic-contaminated ground water is a major problem in many countries.

Seafood is rich in arsenic, with fish containing up to 80 μg/g and oysters up to 10 μg/g [1,2]. Arsenic is also present in meats (0.005–0.1 μg/g) and cereal and grain products (0.05–0.4 μg/g) as well as eggs and dairy products (milk, 0.01–0.05 μg/g; eggs, 0.01–0.1 μg/g), among other foods and beverages [2]. Relatively high amounts of arsenic have sometimes been detected in samples of foods, especially rice, and/or beverages, such as apple and grape juices and wine. In accordance with the U.S. Environmental Protection Agency, the U.S. Food and Drug Administration set a ≤10 parts per billion (ppb) standard for arsenic in bottled drinking water. The Codex Alimentarius Commission of the World Health Organization set a maximum of 0.2 mg arsenic per kg polished rice. In the United States, there are no federal limits for arsenic in foods or juices. Dietary intake of arsenic in the United States is estimated at about 30–50 μg/day, with less than 4 μg usually coming from drinking water [1,2].

Arsenic is found in water and foods in organic and inorganic forms, and exists mostly in its trivalent [As^{3+}] and pentavalent [As^{5+}] states. The major inorganic forms of arsenic found in water and foods are pentavalent arsenate ($H_2AsO_4^-$ or $HAsO_4^{2-}$) and trivalent arsenite (H_3AsO_3 and $H_2AsO_3^-$). Seafood contains mostly organic forms of arsenic—arsenobetaine and arsenocholine, with seaweed also rich in arsenosugars. Some foods also may contain organic arsenic as arsenolipids as well as some methylated forms of the element.

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Absorption, Transport, Storage, and Excretion

Absorption of arsenic varies with its chemical form and solubility. Small amounts of arsenic from the environment may be absorbed through inhalation and via the skin. Greater than 90% of arsenate and arsenite is absorbed in the small intestine from water, and when consumed with food, absorption decreases to about 60% and 75%. Of the organic forms, greater than 90% of arsenobetaine and about 70–80% of arsenocholine are absorbed. Arsenosugars, on the other hand, are poorly absorbed. Several transporters (phosphate, glucose, organic anion transporter polypeptide, aquaporins) appear to transport inorganic arsenic into cells. From the intestine, some arsenic is transported in the blood bound to transferrin. The liver takes up both inorganic and organic forms of the element from portal blood.

In the liver, the organic forms (Figure 14.2) arsenobetaine and arsenocholine undergo little metabolism, although some arsenocholine may be converted to arsenobetaine or may be incorporated into phospholipids similar to choline. In contrast, inorganic arsenic is extensively metabolized—reduced and methylated—as shown in Figure 14.3, or reduced, conjugated, and methylated; the reactions occur primarily in the liver. The initial step in metabolism is the reduction of arsenate^{5+} to arsenite^{3+}; the reduction is facilitated by the presence of glutathione or other thiols. Methylation reactions that may follow are dependent upon methyl groups generated mainly from the catabolism of choline and the amino acid methionine. From the chapter on proteins, you may remember that methionine catabolism produces S-adenosylmethionine (abbreviated SAM); SAM is thought to provide most of the methyl groups needed for arsenic methylation. It has been estimated that 1 g of liver, which has about 70 million liver cells, can methylate up to 14.8 μmoles (1,108 μg) of inorganic trivalent arsenic in an hour [3]. Methylation of arsenite^{3+} generates various arsenicals including monomethylarsonic acid (MMA^{5+}), dimethylarsinic acid (DMA^{5+}), monomethylarsonous acid (MMA^{3+}), and dimethylarsinous acid (DMA^{3+}). These methylation reactions are catalyzed by arsenite methyltransferase, with arsenite^{3+} as its preferred substrate. As concentration of the element increases, methylation efficiency decreases with resulting arsenic accumulation in the liver and other soft tissues. Polymorphisms in arsenite methyltransferase have been demonstrated and shown to affect the formation of the various metabolites, with some mutations associated with greater risks of cancer and other health problems than other mutations [4–7].

Figure 14.2 Two forms of arsenic commonly found in seafoods.

Another pathway of arsenic metabolism involves conjugation of arsenite^{3+} with glutathione to form arsenic triglutathione [$As^{3+}(SG)_3$]. This compound can then be methylated by arsenite methyltransferase to produce monomethylarsenic diglutathione and dimethylarsenic glutathione. These conjugated forms of arsenic are then excreted into the bile via a subclass of ATP-binding cassette transporters. Reviews of arsenic metabolism are found in several references [4–6].

The general order of toxicity of the arsenicals is dimethylarsenic acid$^{(3+)}$ > monomethylarsonous acid$^{(3+)}$ > arsenite^{3+} > arsenate^{5+} > (mono)methylarsonic acid$^{(5+)}$ > dimethylarsinic acid$^{(5+)}$ [8]. Arsenobetaine and arsenocholine are generally considered nontoxic. Tissues that contain the most arsenic are those that contain

Figure 14.3 The metabolism (reductive methylations) of arsenic.

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thiol-rich proteins such as the skin, hair, and nails. Within tissues, inorganic arsenic, especially As^{3+}, is found bound to these thiols (i.e., sulfhydryl [SH] groups). Methylated organic (as opposed to unmethylated inorganic) forms of the element are less likely to bind to tissues. Deposition of arsenic in soft tissues—including the liver, kidneys, heart, pancreas, lungs, brain, and muscles, among others—is associated with high intakes of the element and reductions in the methylation reactions needed for its excretion.

Arsenic is excreted rapidly by the kidneys, in amounts less than about 50 μg/day. The primary urinary metabolite is dimethylarsinic acid along with lesser quantities of monomethylarsonic acid and trimethylated arsenic. Other organic forms of arsenic such as arsenobetaine, arsenocholine, and arsenosugars are also excreted in the urine. The conjugated forms of arsenic are generally excreted from the body through the feces.

Functions and Deficiency

No biological function for arsenic has been demonstrated in humans, although it has been suggested that arsenic exerts its biological activity via the body's use of methyl groups, such as from SAM [1]. SAM provides for the methylation of several important compounds in the body including DNA and histones. Arsenic deficiency in animals is associated with diminished growth, reduced conception rate, abnormal reproduction, and increased neonatal mortality.

A derivative of arsenic, arsenic trioxide (As_2O_3), is approved by the U.S. Food and Drug Administration for the treatment of acute promyelocytic leukemia, a relatively rare cancer characterized by leukopenia and coagulopathy. Arsenic trioxide–based treatment of this cancer is often combined with all-*trans* retinoic acid (a form of vitamin A) and chemotherapeutic agents; the cure rate for acute promyelocytic leukemia now exceeds 80% [9,10].

Recommended Intake, Toxicity, and Assessment of Nutriture

No recommendation for arsenic intake has been established by the Food and Nutrition Board [11]. Acute toxicity results in abdominal pain, vomiting and diarrhea (leading to dehydration and electrolyte imbalance), muscle cramping, numbness and tingling in the extremities, and liver damage. Chronic toxicity leads to arsenic deposition in organs (soft tissues), and affects most body systems, with reported dermatologic problems (hyperpigmentation, skin lesions, and hyperkeratosis [hard patches on the palms of the hands and soles of the feet]); muscle weakness; peripheral neuropathy; excessive sweating; hematological disorders; liver, renal, and respiratory system damage; delirium; encephalopathy; and cancers of the oral cavity, skin, lungs, liver, prostate, colon, bladder, and kidneys. High arsenic exposure, mostly from arsenic-contaminated drinking water, has also been linked with cardiovascular disease and, in some regions, black-foot disease, a peripheral vascular condition that can lead to gangrene. In blood vessels, arsenic reduces vasorelaxation through inhibition of endothelial nitric oxide synthase; it also increases platelet aggregation and reduces fibrinolysis, which enhance atherosclerosis [12].

Arsenic's toxicity relates in part to its interactions with sulfhydryl groups found in proteins (including enzymes); this interaction in turn disrupts normal function of the protein. The trivalent form of arsenic also directly promotes cellular apoptosis. Arsenic's inhibition of enzymes, for example, affects several metabolic pathways and results in the formation of free radicals, which further damage cells. Pyruvate dehydrogenase, which converts pyruvate to the TCA cycle intermediate acetyl-CoA, is one example of an enzyme inhibited by trivalent arsenic; other metabolic pathways affected by arsenic include gluconeogenesis and fatty acid oxidation [13]. The pentavalent forms of arsenic also cause damage. They can substitute for phosphate in glycolysis and the electron transport chain; this substitution in the latter set of reactions uncouples oxidative phosphorylation and thus diminishes ATP formation [13].

Chronic or acute exposure to arsenic elevates blood, hair, and urine concentrations of the element. The usual range of arsenic is about 2–62 ng/mL in whole blood and 1–20 ng/mL in plasma or serum; however, blood levels are not a good indicator of long-term arsenic exposure. Arsenic levels in hair range from about 0.1 to 1.1 μg/g. Hair analysis is particularly useful in toxicity situations because hair arsenic content, unlike that of the fluids, represents an average content over an extended period and does not fluctuate if exposure to the element is intermittent. Because arsenic is excreted primarily in the urine, urinary arsenic excretion is considered a reliable marker of acute arsenic exposure [13].

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BORON

Boron, as boric acid and sodium borate ($Na_2B_4O_7 \cdot H_2O$, called borax), was used to preserve foods such as fish, meat, cream, butter, and margarine for over 50 years—that is, until the 1920s, when it was decided that its use was dangerous. Today, boron is considered a beneficial bioactive element for humans, but is not considered essential as no biochemical function has been identified.

Sources

Foods of plant origin including fruits, vegetables, nuts, and legumes represent good sources of boron. In addition, wine, cider, and beer contribute to dietary intake. Specific foods particularly rich in boron include avocados, peanuts, peanut butter, pecans, raisins, grapes, and wine. Generally, raisins, legumes, nuts, and avocados provide about 1.0–4.5 mg of boron/100 g, and fruits and vegetables contain 0.1–0.6 mg of boron/100 g [1-4]. Animal foods such as meat, fish, and dairy products are poor sources of the element, usually providing less than about 0.6 mg of boron/100 g [3]. Boron appears in foods as sodium borate or as organic borate esters. Dietary boron intake is estimated at 0.8–1.5 mg/day, with many Americans consuming less than 1 mg/day [1,4,5]. Boron is also found as a contaminant/ingredient in some antibiotics, gastric antacids, lipsticks, lotions, creams, and soaps; it is not, however, typically absorbed through the skin. Some multivitamin/mineral supplements provide boron in amounts of up to about 9 mg [6].

Absorption, Transport, Storage, and Excretion

Greater than 85% of ingested boron is absorbed as boric acid (also called orthoboric acid; $[B(OH)_3]$) by passive diffusion from the gastrointestinal tract [4]. Boron is also found in the blood primarily as boric acid, with uptake occurring by soft tissues as well as bones, nails, and hair. The borate monovalent anion $B(OH)_4^-$ may also be present in human tissues, dependent on pH. The body is thought to contain about 3–20 mg of boron. Boron concentrations are thought to be under homeostatic control. A sodium-dependent transporter for boron, NaBCl, is present in the basolateral membrane of epithelial cells. The element is excreted primarily (greater than 70%) in the urine as boric acid, with less than 13% usually lost in the feces and only small amounts lost in sweat [4].

Functions and Deficiency

Beneficial effects of boron on bone and on immune and brain functions have been observed. Bone composition, structure (especially trabecular bone), and strength (especially cortical bone) are positively influenced by boron, possibly through modulation of osteoblast and/or osteoclast activity as well as extracellular matrix turnover [7,8]. Defects in bone growth and development occur with boron deficiency. Boron also promotes anti-inflammatory actions in response to injury or infection, with boron deprivation in animals associated with diminished production of lymphocytes and several cytokines [5]. Brain (central nervous system) function is also influenced by boron. Boron deprivation results in changes in electroencephalograms and selected cognitive processes affecting attention, mental alertness, and memory, among other skills [8,9].

Boron is thought to exert its influence on selected body functions through effects on and/or interactions with biomolecules such as S-adenosylmethionine (SAM) and nicotinamide adenine dinucleotide (NAD^+). For example, the binding of boron to NAD^+ affects intracellular signaling pathways that may inhibit calcium release from the endoplasmic reticulum into the cell cytosol [8]. Additionally, boron may form complexes with cell membrane components such as glycoproteins, glycolipids, and phosphoinositides (a subfraction of phospholipids that function as cell signaling molecules). Such interactions suggest that boron may alter the ability of some hormones to exert their effects on target cells [8,10].

Recommended Intake, Toxicity, and Assessment of Nutriture

Recommendations for intakes of boron have not been established. Intakes of 1–3 mg/day are thought to be beneficial for brain and bone health [5].

Acute boron toxicity results in nausea, vomiting, diarrhea, dermatitis, and lethargy. Chronic boron toxicity is associated with nausea, poor appetite, anemia, dermatitis, and seizures [11]. A Tolerable Upper Intake Level of 20 mg of boron/day has been established for adults based on animal studies [12].

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Urinary boron excretion is thought to be a good indicator of recent boron intake within an intake range of 0.35–10 mg of boron/day [4,5]. Plasma boron concentrations, which usually range from about 20–95 ng/mL, may be helpful in assessing boron status [9]. Plasma concentrations appear to rise in response to increased dietary intake of the element and may be representative of status if intake is low [9].

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Suggested Reading

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NICKEL

Nickel is used industrially in various capacities, such as in the production of stainless steel and nickel-cadmium batteries, and is released into the environment when nickel-containing products are burned. The weathering of rocks and volcanic emissions release nickel into the environment, including into the water and ultimately the food supply. Insoluble forms of nickel such as nickel sulfides, silicates, and oxides are found in dust and fumes. Nickel acetate, sulfate, and chloride represent water-soluble forms of the element. To date, no role for nickel has been established, but the element is considered as "possibly essential."

Sources

Foods of plant origin have higher nickel contents than foods of animal origin. Nuts, legumes, grains and grain products, and chocolate are particularly rich in nickel, providing up to ~228 μg/100 g [1]. Fruits and vegetables generally have intermediate nickel content, providing up to ~48 μg/100 g [1]. The nickel content of foods of animal origin, such as fish, milk, and eggs, is generally low. The total dietary intake of nickel by adults typically ranges from about 70 to 260 μg/day [2].

Absorption, Transport, Storage, and Excretion

Nickel absorption from foods is thought to be less than 10%. Absorption of nickel is higher (~20%, but it can be up to 50%) from water than from other beverages (such as coffee, tea, cow's milk, and orange juice) to which nickel has been added. The element is thought to be absorbed across the enterocyte's brush border membrane by both a carrier and passive diffusion. Nickel likely competes with iron for carrier transport on divalent mineral transporter 1 (DMT1) in the proximal small intestine; consequently, nickel absorption increases with iron deficiency. Transport across the basolateral membrane is thought to occur by diffusion or as part of a complex with an amino acid or other binding ligand.

In the blood, nickel binds mainly to albumin and to a lesser extent to small ligands like peptides and amino acids, including histidine, cysteine, and aspartic acid. Uptake of nickel into cells may occur with amino acids, with transferrin, or through a divalent cation channel. Nickel is widely distributed in the body. The highest concentrations of nickel are found in the thyroid and adrenal glands as well as in hair, bones, and soft tissues such as the lungs, heart, kidneys, and liver. Total body nickel is estimated at about 10 mg [3].

Most nickel is excreted in the urine in amounts less than about 13 μg/L [2]. Within the renal cells, nickel complexes with low-molecular-weight compounds such as uronic acid. In addition to urinary losses, small amounts (1.5–3.3 μg/day) of nickel are excreted in the bile [4]. Nickel can also be lost through sweat in fairly high (up to 69.9 μg/L) concentrations associated with active secretion of the element by sweat glands, even in acclimatized individuals [5].

Functions and Deficiency

No specific role for nickel in humans has been identified. Nickel may be involved in the activity of cyclic nucleotide-gated (CNG) channels and/or with

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guanylate cyclase activity to potentiate the action of CNG channels [6]. CNG channels play a role in renal function and are found in several organ systems including the central nervous system and the reproductive and urogenital systems [6].

Signs of nickel deprivation in animals include decreased reproduction; depressed growth; and altered iron (which in turn may impair hematopoiesis), carbohydrate, and lipid metabolism. Effects on bone and thyroid hormone metabolism may also be present.

Recommended Intake, Toxicity, and Assessment of Nutriture

Extrapolation from animal studies suggests that humans probably need less than 100 μg of nickel/day [2]. A Tolerable Upper Intake Level for adults for nickel is 1.0 mg/day in the form of soluble nickel salts [2].

Signs of acute nickel toxicity include headache, nausea, vomiting, insomnia, and irritability. Delayed symptoms, which may occur up to 5 days after ingestion, include tightness of the chest, cough, difficulty breathing, tachycardia (rapid heart rate), palpitations, sweating, weakness, and possibly death [3]. Nickel exposure, such as through skin contact, may induce an allergic reaction with an associated dermatitis and/or inflammatory response. Chronic toxicity, especially from occupational exposure via inhalation of nickel dust or vapors, causes respiratory, cardiovascular, and renal disorders as well as cancer [7]. Many of nickel's toxic effects are thought to be mediated, at least in part, from reactive oxygen species generation and damage to DNA (hypermethylation of DNA, inhibition of acetylation of histones, condensation of chromatin, and gene silencing) [7]. Additionally, nickel decreases absorption and tissue concentrations of several essential minerals, including iron, magnesium, manganese, zinc, and copper. The article by Zambelli and Ciurli [7] provides an excellent review of nickel toxicity.

Serum or plasma nickel concentrations normally range from less than 1 to 23 ng/mL. However, the analysis of plasma or serum is not considered, at present, a valid method to assess nickel status [3].

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SILICON

Silicon is second only to oxygen in earth-wide abundance. Silicon in nature, however, is usually found as silica, also known as silicon dioxide (SiO_2), because of its attraction for oxygen. Quartz is perhaps one of the most well-known of the silicates found in nature.

Whereas early investigations concentrated on silicon's toxic effects, such as silicon-related urolithiasis (stones in the urinary tract) and particularly silicosis (a respiratory condition caused by the inhalation of dust), since about the mid-1970s research has focused on the possible functional roles of silicon. The element has not yet, however, been found to be essential as no specific biochemical function for silicon has been identified.

Sources

Plant foods are typically richer in silicon than those of animal origin. Whole cereal grains (especially oat bran), lentils, soybeans, selected fruits (bananas, pineapple, mango, and dried fruits) and vegetables (green beans, spinach, and root vegetables), nuts, as well as sugar cane are especially rich sources of the element, providing from about 0.1 to 23 mg/100 g [1]. The element is also found in seafood (especially mussels), water, and beverages (tea, coffee, juices, wine, etc.) [1]. It is present in beer in relatively high amounts because the silicon in the hops and barley is solubilized in the beer-making process. Dietary silicon intake by adults in the United States is thought to range from about 24 to 33 mg/day [2,3].

Silicon is found in some medications (added often as inert ingredient), usually as magnesium silicate or magnesium trisilicate; silicon-containing medications include antacids, antidiarrheal agents, and analgesics. Silicon, as silicate, is also used as an additive in various foods, for example, to prevent caking or as a thickening, stabilizing, and/or clarifying agent. As an additive, it may be present as calcium silicate, sodium aluminosilicate, or magnesium trisilicate, for example. Implants, cosmetics (creams, lipsticks, etc.), powder (talcum), and toothpaste also may contain silicon as silica, silicates, silicone, and/or magnesium hydrogen silicate. These forms of silicon are not thought to be absorbable via the skin, with the exception of silicones (found in some creams and in implants).

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Absorption, Transport, Storage, and Excretion

Most silicon found in water and beverages is found as orthosilicic acid, also called monosilicic acid, $Si(OH)_4$. This form of silicon is readily absorbed (about 50%) from the proximal small intestine either paracellularly or via small pore transcellular pathways [2]. However, the chemical form of the element (such as polymerization of the orthosilicic acid), among other factors (such as pH and the fiber and cation contents of the meal), may decrease absorption to less than 1% [4]. For example, in higher concentrations and at neutral pH, orthosilicic acid condenses/polymerizes, creating less bioavailable larger polysilicic acid polymers, oligomers, or colloidal species; these forms of silicon are poorly absorbed. In plant foods, silicon is present mostly as phytolithic silica, which requires digestion. Absorption of this form of the element varies considerably, from less than 2% up to about 41%. The extent of silicon absorption from foods with silicate-containing additives is not clear; however, studies suggest at least some of the silicon may be digested to form the absorbable orthosilicic acid.

Once absorbed into the blood, orthosilicic acid is almost entirely free (i.e., not bound to proteins). The element is taken up by the red blood cells, liver, lungs, skin, and bones, with slower entry occurring into the heart, muscles, spleen, and testes. Generally, silicon concentrates in the body's connective tissues, including bone, skin, blood vessels, cartilage, and tendons. Total body silicon is estimated at about 1–2 g [3]. Most silicon is excreted from the body in the urine as orthosilicic acid and as magnesium orthosilicate. Urinary silicon excretion is significantly correlated with dietary silicon intake [4].

Functions and Deficiency

The physiological role of silicon centers on the normal formation, growth, and development of bones and connective tissues, including effects on bone mineralization, crystallization, and calcification and on collagen synthesis. Silicon is thought to play both metabolic and structural/binding roles in connective tissues. Collagen is present in high concentrations in bone and, along with elastin, is found in numerous connective tissues throughout the body including the skin, blood vessels, tendons, and cartilage, among others.

Silicon deficiency results in smaller, less flexible long bones and in skull deformation characterized by reductions in collagen in the connective tissue matrix. Silicon deficiency also weakens blood vessels and negatively impacts skin integrity and wound healing. Jugdaohsingh [2], Martin [3] and Rodella et al. [5] provide excellent reviews of silicon's potential roles in bone health, among other conditions.

Recommended Intake, Toxicity, and Assessment of Nutriture

The requirement for silicon is largely unknown, although estimates range from about 10 to 25 mg/day [6,7]. No Tolerable Upper Intake Level has been established for silicon, although a safe upper level of 700 mg/day has been suggested [3].

Toxicity from silicon has been associated with the formation of silicon-containing kidney stones; however, it is chronic (years) use of large amounts of silicon-containing antacids (e.g., magnesium trisilicate, which provides up to about 6.5 mg of elemental silicon per tablet) that appears to contribute to the rare development of kidney stones [2,3]. Silicosis occurs from the inhalation of particulate crystalline silica and silicates, including quartz, and man-made silicates, such as asbestos. Silicosis is characterized by a progressive fibrosis or scarring of the lungs leading to severe respiratory problems and increased risk of lung cancer. Based on inhalation as the means of exposure (not dietary intake), silica is classified as a "known human carcinogen." See the reference by Leung and coworkers [8] for a review of silicosis.

As in the case of most of the ultratrace elements, levels of silicon in biological fluids of healthy adults have been reported but may not accurately represent nutriture. Serum silicon concentrations usually range from about 11 to 31 μg/dL [9].

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VANADIUM

Vanadium was discovered in the early 1800s and named for a Swedish goddess of beauty and fertility, Vanadis. The element occurs in several oxidation states from V^{2+} to V^{5+}.

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In solution, vanadium produces a range of colors from yellowish orange in its pentavalent state to blue in its divalent state. In the human body, vanadium is found primarily in its pentavalent V^{5+} state as vanadate, also called monovanadate, (VO_3^-, VO_4^{3-}, or $H_2VO_4^-$), or in its less toxic tetravalent V^{4+} state as vanadyl (VO^{2+}).

Sources

The vanadium content of foods, which contain vanadium primarily as tetravalent vanadyl and pentavalent vanadate, is very low and consequently so is the average dietary intake. The richest sources of the element are fish and shellfish, such as oysters, which contain up to about 12 μg/100 g, and cereals and grain products with up to 15 μg/100 g [1,2]. Sweeteners provide up to 4.7 μg/100 g [1,2]. Other vanadium-rich foods include mushrooms, black pepper, parsley, dill seed, and canned apple juice. Most fats and oils contain particularly low levels of vanadium, less than 0.3 μg/100 g [1]. Beer and wine also provide some vanadium. Vanadium intake in the U.S. diet is thought to range from about 5 to 30 μg/day. Supplements providing vanadium as vanadyl sulfate, sodium or ammonium metavanadate, and sodium orthovanadate are available.

Absorption, Transport, Storage, and Excretion

Metabolism and absorption of vanadium vary with its oxidation states. Vanadate may be reduced to the tetravalent vanadyl in the acidic stomach before being absorbed by diffusion in the proximal small intestine. Alternately, vanadate may be absorbed directly via the same anion transport carrier system as used by phosphate and then reduced intracellularly by glutathione. Compared to vanadyl, vanadate is three to five times more efficiently absorbed. Overall, vanadium absorption is generally less than 5%; less than 1% is absorbed with ingestion of pharmacological doses of the element.

In blood cells, the plasma, and other body fluids, vanadium may be present as pentavalent vanadate or it may be converted to vanadyl using glutathione, NADH, and ascorbic acid as reducing agents. In the plasma, vanadyl and vanadate bind mostly to the iron-containing proteins transferrin and ferritin. Lesser amounts may be bound to albumin or lower-molecular-weight compounds such as citrate, lactate, or phosphate.

Vanadium enters cells as vanadate through anion transport systems including channels used by phosphate. Similarly to its metabolism elsewhere, intracellular vanadate is reduced primarily by glutathione to tetravalent vanadyl, which is then almost exclusively bound to ligands, primarily phosphates and iron-containing proteins.

The total body pool of vanadium is about 100–200 μg, with most tissues containing less than 10 ng of vanadium/g. The kidneys retain most of the absorbed mineral; however, accumulation is also found in the liver, lungs, spleen, and thyroid gland, with longer-term storage in muscles, adipose tissue, and bone.

Renal excretion is the major route for the elimination of absorbed vanadium, with urinary vanadium excretion occurring in amounts generally less than 0.8 μg/day [3]. In addition, small amounts of vanadium are excreted in the bile.

Functions and Deficiency

No specific biochemical function has been identified for vanadium. Vanadium's effects *in vivo*, however, are predictable from a consideration of its aqueous chemistry. First, as vanadate, it competes with phosphate at the active sites of phosphate transport proteins, phosphohydrolases, and phosphotransferases. Second, as vanadyl, it competes with other transition metals for binding sites on metalloproteins and small ligands such as adenosine tri- and diphosphate (ATP and ADP) and nicotinamide adenine dinucleotide (NAD). Vanadium's interaction with enzyme systems such as ATPases, protein kinases, and phosphatases in turn affects numerous physiological processes. For example, when vanadate binds to the ATP hydrolysis site on Na^+/K^+-ATPase, an enzyme involved in the transport of ions against a concentration gradient, enzyme's activity is inhibited. In muscle, vanadate forms ternary complexes with myosin and ADP and thus inhibits interactions with actin to affect muscle function. Third, it participates in redox reactions resulting in the generation of reactive oxygen species. The resulting reactive oxygen species initiate damage to DNA, chromosomes, and other cellular components.

Vanadium, in pharmacological doses, affects numerous processes. However, it is important to note that pharmacological activity is generally manifested at a concentration threshold that is considerably greater than that required to fulfill the need for essentiality. Vanadium, in pharmacological amounts, mimics the action of insulin. Vanadate is thought to exert its action not via the insulin receptor, but through interactions with cytosolic and plasma membrane protein kinases to affect intracellular insulin signaling pathways. This activation of cytosolic protein kinases enhances glucose and lipid metabolism, while activation of the plasma membrane protein kinases triggers phosphatidylinositol-3-kinase (PI3K). Vanadate activates Akt signaling through inhibition of protein tyrosine phosphatases, thereby prolonging the activity of the phosphorylated enzymes and enhancing the insulin-signaling pathway (Akt is a protein involved in insulin signaling; some of the other signaling and transcription factors affected by vanadium include mitogen-activated

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protein kinases [MAPKs] and extracellular nuclear factors–kappa B [NF-κb]). Among other effects, activation of Akt by vanadium promotes translocation of the glucose transporter GLUT4 to cell membranes to facilitate glucose uptake into cells. Vanadium also stimulates hepatic glycogen and lipid synthesis and inhibits gluconeogenesis and lipolysis. Vanadium is being tested in clinical trials for its blood glucose lowering effects in individuals with type 2 diabetes. Doses provided range from about 33 to 50 mg of elemental vanadium, mostly as vanadyl sulfate or sodium metavanadate. Studies-to-date have shown that vanadium improved insulin sensitivity and reduced serum glucose concentrations and hemoglobin A1c. See the article by Clark and associates [4] for a review of the use of vanadium compounds in diabetes treatment.

Antitumoral as well as carcinogenic properties have been observed with vanadium, depending on the type of cell and dose. In some cells, vanadium promotes selected phases of the cell cycle and decreases apoptosis. The observed effects may result, for example, from production of reactive oxygen species with resulting damage to DNA and other cellular components, direct damage to DNA bases, changes to enzyme systems impacting the cell cycle, and regulation of genes such as for interleukin 8, activator protein 1, and nuclear factor kappa B, among others [5,6]. The article by Ulbricht and coworkers [6] provides an evidence-based systematic review of vanadium, including information on other proposed effects of vanadium on organ/body systems. Deficiency of vanadium in animals impairs fertility/reproduction as well as survival, growth, and development.

Recommended Intake, Toxicity, and Assessment of Nutriture

The human requirement for vanadium is not established. Daily intakes of up to 100 μg of vanadium are considered safe. A Tolerable Upper Intake Level of 1.8 mg of vanadium/day has been established [7]. Side effects have been observed in humans with vanadium intakes above about 200 μg/kg body weight [6]. Mild toxic manifestations include green tongue syndrome (from deposition of green-colored vanadium in the tongue), diarrhea, and abdominal cramps. Chronic toxicity of vanadium as seen in miners manifests in hypertension, neurological disorders, respiratory tract irritation (characterized by rhinitis, wheezing, cough, etc.), and hepatic, cardiac, and renal damage [6].

Methods to assess vanadium status have not been established. Blood vanadium concentrations typically range from about 0.4 to 2.8 ng/mL but may be greater than 500 ng/mL in those ingesting vanadium supplements [1,8]. Urinary excretion of vanadium averages about 8 μg/day [3].

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Suggested Readings

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COBALT

Little evidence exists that cobalt plays a role in human nutrition other than its being a part of vitamin B_{12} (cobalamin). Although ionic cobalt can substitute for other metals in metalloenzyme activity *in vitro*, no evidence exists that it acts in that capacity *in vivo*. In this respect, the metal is unique among the elements, in that the requirement in humans is not for an ionic form of the metal but for a preformed metallovitamin that cannot be synthesized from dietary metal. Therefore, it is the vitamin B_{12} content of foods and the diet, rather than the cobalt present, that is important in human nutrition.

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NO, SILVER IS *NOT* ANOTHER ESSENTIAL ULTRATRACE MINERAL: TIPS TO IDENTIFYING BOGUS CLAIMS ABOUT DIETARY SUPPLEMENTS

Although found in the environment (and thus natural) and often worn as jewelry, the mineral silver is nonessential for human life. Silver has no known biochemical role or physiological function in the body. Yet, a quick search of "silver supplements" on the Internet will yield close to 20 million related Web sites. Many of these sites proclaim the benefits from ingesting supplements providing silver, either colloidal silver (a liquid suspension of tiny silver particles), ionic silver, native silver, or silver protein, among other forms of the element, as a cure for viral and bacterial infections, arthritis, gastrointestinal problems (such as gastroesophageal reflux), and various skin ailments including dry skin, rashes, and dermatitis. What the manufacturers of the oral silver supplements *do not* tell you is often more important—that is, the side effects or health hazards of silver supplements.

The 2009 television appearances (on *The Today Show*, *Oprah*, etc.) of the "man who turned blue" from the regular consumption of a liquid colloidal silver supplement provided visual proof of one of the supplement's detrimental effects—argyria. Argyria is a permanent (i.e., irreversible) bluish or grayish discoloration of skin, nails, gums, and conjunctiva (the membrane that covers the white part of the eye) that results from the ingestion of silver. The condition is not reversed upon discontinuation of the product or by medical interventions. The exact amount of silver that induces argyria is not clear; however, based on a review of cases of argyria associated with silver consumption, the Environmental Protection Agency established an oral reference dose of 5 µg of silver/kg body weight/day [1]. This corresponds to a daily intake of 341 µg of silver for a person weighing 150 lb (68.2 kg). Other health hazards associated with consumption of silver—all unlikely to be disclosed by the manufacturer of the product—include brain, nerve, liver, and kidney damage and gastrointestinal problems, as well as headaches and seizures. Silver is a heavy metal; thus, with oral ingestion, the mineral accumulates in organs, causing destruction, and in arteries, which can lead to atherosclerosis (heart disease).

Silver does, however, have a few approved medical uses. The mineral is used in *topical* ointments and in bandages/gauze dressings for the treatment of some burns, wounds, and infections of the skin; none of these silver-containing medical products, however, is ingested orally.

Silver supplements are just one example of the thousands of dietary supplements marketed to Americans to improve well-being and appearance and to prevent or cure diseases or ailments. The advertisements for supplements appear in stores, newspapers, and magazines and on television; they also "pop up" on computers and other electronic devices while a user browses the Internet. Added to the harmful effects that many of these supplements have on the body is their economic impact: These products are not cheap. Health fraud is estimated to cost consumers over $25 billion each year.

The Dietary Supplement Health and Education Act (DSHEA) of 1994 considers dietary supplements, including minerals, vitamins, herbs or other botanicals, amino acids, dietary substances (such as enzymes), metabolites, constituents, and extracts to be products ingested orally that contain dietary ingredient(s) intended to supplement the diet (not medications) [2]. As a consequence, the manufacturers of dietary supplements do not have to secure approval to sell products, nor do they have to demonstrate to any regulatory agency that the supplements are safe (unless they contain a dietary ingredient that was not sold prior to 1994). Moreover, manufacturers of supplements can list claims about their product on the label; specifically allowed are claims of general well-being, of structure/function (i.e., how the nutrient affects human body structure or function), and of benefit related to a classical nutrient deficiency disease [3]. Along with such claims, the supplement manufacturer must provide a disclaimer on the label stating that "this statement has not been evaluated by the Food and Drug Administration" and that "this product is not intended to diagnose, treat, cure, or prevent any disease." In addition, the manufacturer must have substantiation that the claim is truthful and not misleading, and must notify the U.S. Food and Drug Administration (FDA) that its product bears such a claim within 30 days of marketing the product [3].

Unfortunately, many consumers assume all dietary supplements are safe, and many fraudulent supplements remain on the market for a long time, causing injury or adverse reactions among users before the FDA accumulates enough evidence that a particular supplement is unsafe and removes it from the market. Consequently, the buyer must beware. Consumers must learn to identify bogus dietary supplement (as well as weight-loss diet) claims to avoid being scammed and potentially harmed. The Food and Nutrition Science Alliance (a coalition of four professional organizations: the Academy of Nutrition and Dietetics [formerly the American Dietetic Association], the American Society for Clinical Nutrition, the American Society for Nutritional Sciences, and the Institute of Food Technologists) developed "Ten Red Flags of Junk Science" to help consumers identify nutrition misinformation [4].

The Ten Red Flags of Junk Science include:

1. Recommendations that promise a quick fix
2. Dire warnings of danger from a single product or regimen
3. Claims that sound too good to be true
4. Simplistic conclusions drawn from a single study
5. Recommendations based on a single study
6. Dramatic statements that are refuted by reputable scientific organizations
7. Lists of "good" and "bad" foods
8. Recommendations made to help sell a product
9. Recommendations based on studies published without peer review
10. Recommendations from studies that ignore individual or group differences.

Many of these "red flags" are apparent in advertisements for dietary supplements, such as colloidal silver, which are frequently found on the Internet. (Remember: There are no rules to posting information on the Internet; anyone can create a Web site and post content.) Manufacturers' Web sites often *guarantee a quick fix* to problems, and it is not uncommon to see *promises of dramatic or even miraculous results*, with *cures to one or more diseases* (often those for which medical science has no cure). These claims, of course, are *"too good to be true"* and incorrectly suggest "one product can do it all." In addition, the products are often marketed as being *"natural"* (implying natural is "better," when in reality many "natural" substances are dangerous and many artificial or synthetic ones are not) and as containing "specialized formulas," making them superior to other products. Colloidal silver, for example, may be marketed as being better absorbed than other forms of silver because it contains nanoparticles (meaning it has a particle size between 1 and 100 nm) and because it is mined from secret (nondisclosed) natural sources. Finally, *proof* of the dietary supplement's effectiveness is usually provided by the manufacturer in the *form of testimonials or anecdotes from satisfied consumers*. Lacking are scientific data regarding the supplement's benefits provided by multiple well-designed, **double-blind**, placebo-controlled studies.

Each year hundreds of manufacturers of dietary supplements receive letters from the FDA warning against the promotion of products with unsubstantiated (false or misleading) claims. Products that present a direct health threat to consumers are usually targeted first by the FDA and

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Federal Trade Commission (which regulates the advertising of products), followed by those that present indirect health hazards. To facilitate the identification of harmful products, adverse events associated with the use of dietary supplements should be reported to the FDA's MedWatch program [5]. In addition, for reassurance that a product is of sufficient quality and potency, consumers can look for a U.S.P. (U.S. Pharmacopoeia) symbol or designation on the product's label. This designation indicates that the manufacturer followed established U.S.P. standards for quality, purity, strength, packaging, labeling, and storage. Nonetheless, the ultimate decision on whether to purchase and consume the supplement should be made with caution and considerable research. Remember the "Ten Red Flags of Junk Science," and remember to be skeptical and rely on reputable information sources in your evaluation of dietary supplements before spending your money.

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4. Position of the American Dietetic Association: Food and Nutrition Misinformation. J Am Diet Assoc. 2006; 106:601–7.
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Recommended Web Sites

National Council Against Health Fraud
www.ncahf.org

National Institutes of Health, Office of Dietary Supplements
https://ods.od.nih.gov

Quackwatch: Your Guide to Quackery, Health Fraud, and Intelligent Decisions
www.quackwatch.org

U.S. Food and Drug Administration
www.fda.gov

U. S. Food and Drug Administration, "How to Spot Health Fraud"
www.fda.gov/Drugs/EmergencyPreparedness/BioterrorismandDrugPreparedness/ucm137284.htm

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GLOSSARY

Achlorhydria Lack of hydrochloric acid in gastric juice.

Activation energy Energy introduced into the reactant molecules to activate them to the transition state so that an exothermic reaction can take place.

Acute Having a rapid or sudden onset.

Adequate Intake (AI) A recommended daily dietary nutrient intake based on the nutrient intake levels of healthy people; an AI is thought to exceed the daily requirement for a given nutrient.

Alkalosis A condition in which the pH of the blood is higher than approximately 7.45, the upper end of the normal range.

Alkoxyl radical ($RO^{\bullet}$ or $LO^{\bullet}$) A monovalent radical consisting of an alkyl group united with oxygen. Alkyl groups are derived from alkanes (a class of hydrocarbons in which the molecule contains only carbon and hydrogen atoms that are joined by single covalent bonds) by the removal of one hydrogen atom and have the general formula C_nH_{2n+1}.

Amenorrhea The absence of at least three consecutive menstrual cycles.

Amphibolic pathway A pathway that is involved in both the catabolism and the biosynthesis (anabolism) of carbohydrates, fatty acids, and/or amino acids.

Amphipathic Refers to a molecule that has a polar (hydrophilic) region at one location and a nonpolar (hydrophobic) region at another.

Amphoteric Capable of reacting as either an acid or a base.

Anaplerotic reaction A reaction that involves replenishing or restoring a substrate (e.g., the conversion of pyruvate to oxaloacetic acid).

Android obesity Excess body fat that accumulates centrally around the trunk. Also called apple-shaped obesity.

Anomeric carbon The carbon that comprises the carbonyl function that is capable of forming a ring structure with the OH group on the highest-numbered chiral carbon of a monosaccharide.

Anorexigenic Capable of producing anorexia or diminishing appetite.

Anticodons Three-base sequences of nucleotides within transfer RNA (tRNA) molecules.

Antral Pertaining to the antrum, the lower or distal portion of the stomach.

Apical At or near the apex; pertaining to the intestinal lumen side of an enterocyte.

Apolipoprotein The protein component of a lipoprotein particle; also called *apoprotein*.

Apoptosis An organized series of events that, once triggered, leads to cell death.

Arcuate nucleus The subcortical region of the brain that secretes appetite-enhancing neuropeptide Y and appetite-suppressing melanocortins.

Aromatic compound An organic compound that contains a benzene ring.

Ataxia Impaired muscle coordination, especially when trying to perform voluntary muscular movements.

Atheroma A mass of plaque consisting of degenerated, thickened arterial intima, occurring in atherosclerosis.

Autolysis The digestion of intracellular components (including organelles) by lysosomes.

Autophagy The breakdown or digestion of the body's proteins, such as those found in the blood or within cells.

Beriberi A condition resulting from a thiamin deficiency.

Bile A body fluid made in the liver and stored in the gallbladder that participates in emulsifying fat and forming micelles for fat absorption.

Bitot's spots Small, white, foamy-looking accumulations of sloughed cells and secretions in the eye that are associated with a vitamin A deficiency.

Buffer A compound that ameliorates a change in pH.

Calpain A calcium-dependent protease involved in protein turnover in the body.

Caspases A family of cysteine proteases involved in the degradative events during apoptosis (cell death).

Carboxylation The addition of a carboxyl group to a molecule.

Catabolism The process by which organic molecules are broken down.

Cathepsins A group of enzymes involved in breaking down or digesting the body's proteins.

Cells The basic units for all organisms that arise from pre-existing cells.

Cerebrosides Sphingolipids containing a single galactose or glucose unit at the terminal hydroxyl.

Ceremide The simplest sphingolipid containing no attached group at the terminal hydroxyl.

Chaperones Soluble intracellular proteins that bind to and deliver minerals to specific intracellular locations.

Chelators Small organic compounds that form a complex with another compound, such as a mineral.

Chemiosmotic theory The theory that most ATP synthesis occurs in a process whereby protons move down an electrochemical gradient, and the energy generated is used to phosphorylate ADP to make ATP.

Chiral carbon A carbon atom with four different atoms or groups covalently attached to it.

Chronic Long and drawn out in duration or recurring over a long period of time.

Chylomicron A type of lipoprotein that transports lipids and lipid-soluble vitamins from the intestine into the lymph and then the blood for use by body cells.

Chylomicron remnant The portion of a chylomicron that is left after blood lipoprotein lipase removes part of its triglycerides.

Chyme Partially digested food.

Codon A three-base sequence in a DNA or mRNA molecule that specifies the location of a single, particular amino acid in a polypeptide chain.

Cohort A group of individuals that share common characteristics.

Colloids Substances comprised of very small particles that are suspended uniformly in a medium.

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Complementary base pairing The pairing of nucleotide bases in two strands of nucleic acids; A pairs with T or U, while G pairs with C.

Complete protein A protein that contains all the essential (indispensable) amino acids in the approximate amounts needed by humans.

Cori cycle Metabolic pathway in which lactate (produced by anaerobic glycolysis in muscle and red blood cells) is released into the bloodstream and transported to the liver where the lactate is converted to glucose by gluconeogenesis. The glucose is released into the bloodstream and taken up by muscle and red blood cells were it can enter glycolysis, thus completing the cycle.

Cross-over study A longitudinal study in which subjects receive a sequence of different treatments. Each subject receives all treatments (including placebos), usually in a random order.

Cytochromes Heme-containing proteins that serve as electron carriers (e.g., in oxidative phosphorylation or the cytochrome P450 system).

Cytokines A generic term for nonantibody protein messengers released from a macrophage or lymphocyte that is part of an intracellular immune response.

Cytoskeleton Microtubules and microfilaments in the cell that provide internal reinforcement and communication.

Cytosol (cytoplasm) The continuous aqueous solution of the cell and the organelles contained in it.

Deamination The removal of an amino (NH_2) group from an amino acid.

Dehydrogenases Enzymes that catalyze reactions in which hydrogens and electrons are removed from a reactant.

Desaturation The process of converting a saturated compound to an unsaturated one.

Dietary fiber Nondigestible (by human digestive enzymes) carbohydrates and lignin that are intact and intrinsic in plants.

Dipeptidylaminopeptidase A protein-digesting enzyme that breaks apart dipeptides.

Diphosphatidylglycerol A phosphatidylglycerol esterified through the C-1 hydroxyl group of the glycerol moiety to the head phosphoryl group of another phosphatidic acid molecule; also called *cardiolipin*.

Direct calorimetry A method of measuring the dissipation of heat from the body.

Disaccharides Sugars formed by combining two monosaccharides through a glycosidic bond between the hydroxyl group of one monosaccharide and the hydroxyl group of another.

Double blind study An experiment in which neither the person administering the treatment nor the subject knows which treatment (placebo or experimental) the subject is receiving.

Dowager's hump A deformity of the spine characterized by a humpback or being bent forward; also called *kyphosis*.

Eicosanoids Biologically active substances derived from linoleic and α-linolenic (n-6 and n-3) essential fatty acids.

Electron transport chain The sequential transfer of electrons from reduced coenzymes to oxygen that is coupled with ATP formation and occurs within the mitochondria.

Elongation (1) The extension of the polypeptide chain of the protein product during protein synthesis. (2) The addition of carbons (in two-carbon increments) to a fatty acid chain.

Endocrine All of the body's hormone-secreting glands.

Endocytosis Uptake of a substance into a cell through the formation of vesicles derived from the plasma membrane.

Endopeptidase An enzyme that hydrolyzes amino acids linked to other amino acids in the interior of a peptide or protein.

Endoplasmic reticulum (ER) A network of membranous channels pervading the cytosol and providing continuity between the nuclear envelope, the Golgi apparatus, and the plasma membrane.

Endothermic reaction A reaction in which the products have more free energy than the reactants; it therefore requires energy.

Enkephalins Peptides that bind to opioid receptors found in the brain and gastrointestinal tract.

Enterocyte An intestinal cell.

Enterohepatic circulation The movement of a substance, such as bile, from the liver to the intestine and then back to the liver.

Enzymes Protein catalysts that increase the rate of a chemical reaction in the body.

Epidemiology (Epidemiological studies) The science concerned with studying those factors that influence the frequency and distribution of disease in a defined human population.

Estimated Average Requirement (EAR) The amount of a nutrient thought to meet the requirements of 50% of healthy individuals in a specified age and gender group.

Eukaryotic cells Cells with a defined nucleus surrounded by a nuclear membrane.

Exercise Planned, structured physical activity to enhance physical fitness.

Exocytosis A process by which compounds may be released from cells.

Exons The segments of a gene that code for a sequence of nucleotides in a specific molecule of mRNA.

Exopeptidase An enzyme that hydrolyzes amino acids off the terminal end of a peptide or protein.

Exothermic reaction A reaction in which the reactants have more free energy than the products; it therefore gives off energy as heat.

Exudate Fluids that have exuded (been forced or pressed) out of a tissue or its capillaries.

Ferment To break down substrates anaerobically to yield reduced products and energy.

Fermentation An anaerobic breakdown of carbohydrates and protein by bacteria.

Free energy The potential energy inherent in the chemical bonds of nutrients.

Free radical An atom or molecule that has one or more unpaired electrons.

Functional fiber Nondigestible carbohydrates that have been isolated, extracted, or manufactured and have been shown to have beneficial physiological effects in humans.

Gangliosides Sphingolipids containing an oligosaccharide at the terminal hydroxyl, with sialic acid attached to the oligosaccharide chain.

Gap junctions Channels between cells.

Gene A section of chromosomal DNA that codes for a single protein.

Genome The sum of all the chromosomal genes of a cell.

Ghrelin A hormone secreted by the stomach and duodenum that signals hunger.

Glucagon Hormone secreted by the pancreas in response to decreasing blood glucose concentration. Promotes glucose secretion by the liver, thus normalizing blood glucose concentration.

Gluconeogenesis The formation of glucose by the liver or kidney from noncarbohydrate precursors.

Glucose tolerance factor (GTF) A chromium-containing compound whose structure has yet to be characterized but may potentiate the action of insulin in the body.

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Glycemic index The relative number assigned to a particular food indicating its effect on blood glucose concentration above baseline (fasting level) compared to a reference food, usually pure glucose.

Glycemic load The glycemic load equals the glycemic index times the grams of carbohydrate in a typical portion of the food.

Glycocalyx The layer of glycoprotein and polysaccharide that surrounds many cells.

Glycogenesis The pathway by which glucose is converted to glycogen.

Glycogenolysis The pathway by which glycogen is enzymatically broken down to glucose.

Glycolysis The pathway by which glucose is converted to pyruvate.

Glycoproteins Proteins covalently bound to a carbohydrate.

Glycosaminoglycan An unbranched polysaccharide consisting of alternate units of two different sugars.

Glycosidases/carbohydrases Digestive enzymes that hydrolyze polysaccharides to their constituent monosaccharide units.

Golgi apparatus (network) The part of the cell responsible for modifying macromolecules synthesized in the endoplasmic reticulum and packaging them to be transported to the cell surface or cytosol.

Gynoid obesity Excess body fat that accumulates around the hips and thighs. Also called pear-shaped obesity, occurring mostly in women.

Hartnup disease A hereditary disorder in which tryptophan absorption and excretion are abnormal.

Heat stress Metabolic response to heat exposure, resulting in heat rash, heat cramps, heat exhaustion, and heat stroke.

Hemochromatosis An inherited disorder characterized by excessive iron absorption and iron overload in the body.

Heterodimers Complexes formed between two or more different receptors or molecules.

Hexose monophosphate shunt See *pentose phosphate pathway*.

Homeostasis The tendency toward stability in the internal environment of the body.

Homodimers Complexes formed between two of the same receptors or molecules.

Hormones Chemical messengers synthesized and secreted by endocrine tissue (glands) and transported in the blood to target tissues or organs.

Hydride ion Anion of hydrogen containing one proton and two electrons. Hydride ions participate in the reduction-oxidation reaction, $NAD^+ \leftrightarrow NADH$.

Hydrogen atom Chemical element of hydrogen containing one proton and one electron (electrically neutral), often abbreviated as $\frac{1}{2}H_2$.

Hydrogen ion Cation of hydrogen containing a single proton and no electrons, referred to simply as a proton and abbreviated H^+.

Hydrolases Enzymes that catalyze cleavage of bonds between carbon atoms and some other kind of atom by the addition of water.

Hydroperoxyl (perhydroxyl) radical ($HO_2^{\bullet}$ or $H{-}O{-}O^{\bullet}$) A protonated superoxide radical.

Hydrophilic Refers to a molecule or part of a molecule having a strong affinity for water and other polar substances. See *amphipathic*.

Hydrophobic Refers to a molecule or part of a molecule that repels water but has strong affinity for nonpolar substances. See *amphipathic*.

Hydroxyapatite A crystal-lattice-like substance with the formula $Ca_{10}(PO_4)_6OH_2$, found in bones and teeth.

Hydroxyl radical ($^{\bullet}OH$) An oxygen-centered radical that can be generated in the body when it is exposed to γ rays, low-wavelength electromagnetic radiation.

Hypercalciuria Excessive urinary calcium excretion.

Hyperglycemia An above-normal blood glucose level.

Hyperkalemia High concentrations of potassium in the blood.

Hyperlipidemia A general term for an elevated blood level of any lipid.

Hyperphosphatemia High concentrations of phosphorus in the blood.

Hyperplasia Abnormal cell proliferation.

Hyperpnea An abnormal increase in the rate and depth of breathing.

Hypertrophy Enlargement of the size of cells to increase the size of an organ or tissue.

Hypocalcemia Low concentrations of calcium in the blood.

Hypochondriasis Abnormal anxiety about one's own health.

Hypoglycemia A below-normal blood glucose level.

Hypokalemia Low concentrations of potassium in the blood.

Hyponatremia Low concentrations of sodium in the blood.

Immunoproteins Proteins made by plasma cells that help destroy foreign substances in the body; also called *immunoglobulins* or *antibodies*.

In vitro In a test tube or culture (outside the body).

In vivo Within the body.

Incomplete protein A dietary protein source that is missing or contains insufficient amounts of one or more indispensable amino acids needed for protein synthesis in the body. Incomplete proteins may also be called *low-quality proteins* and are generally derived from plants.

Indirect calorimetry Measurement of the consumption of oxygen and the expiration of carbon dioxide by the body, used to estimate metabolic rate.

Insulin Hormone secreted by the pancreas in response to rising blood glucose derived from food. Promotes glucose uptake into muscle and adipose tissue, thus normalizing blood glucose concentration.

Intermediate filaments Strong, ropelike cytoskeletal fibers that are made of protein and that function to provide mechanical stability for cells.

International Unit (IU) The quantity of a nutrient that produces a particular biologic effect. The amount varies among different nutrients. IUs are sometimes used with vitamins A, D, and E.

Introns Noncoding regions of a gene.

Ion An electrically charged atom or group of atoms; positively charged ions are called *cations*, and negatively charged ions are called *anions*.

Ischemia Deficiency of blood in a tissue.

Isomer One of two or more different chemical compounds that have the same molecular formula.

Isomerases Enzymes that catalyze the interconversion of optical or geometric isomers.

Isoprenoid Refers to the structure of the side chains of five-carbon units, as found in vitamins E and K.

Keratinocytes Cells that produce the protein keratin.

Ketoacidosis Blood acidosis due to excessive ketone bodies.

Ketogenesis The process of producing ketone bodies.

Ketone bodies Compounds (acetoacetate, β-hydroxybutyrate, and acetone) formed during the oxidation of fatty acids in the absence of adequate four-carbon intermediates.

Ketosis Condition resulting in elevated ketone body concentration in the blood.

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Krebs cycle See *tricarboxylic acid (TCA) cycle*.

Kyphosis A deformity of the spine characterized by a humpback or being bent forward; also called *dowager's hump*.

Lanugo Fine, soft, lightly pigmented hair that usually is found on a fetus toward the end of pregnancy but may appear on malnourished individuals.

Leptin A polypeptide hormone secreted by adipose tissue that reduces hunger through hypothalamic mechanisms.

Leptin resistance The concept that, despite increased circulating leptin levels and adequate leptin receptors, the feeling of hunger persists and food intake does not diminish.

Leukotrienes Biologically active compounds derived from linoleic or α-linolenic acids (n-6 and n-3 essential fatty acids).

Ligands Small molecules or minerals that bind to a larger molecule.

Ligases Enzymes that catalyze the formation of bonds between carbon and other atoms.

Limiting amino acid The amino acid within a protein with the lowest amino acid or chemical score; it is the amino acid present in a protein in the lowest amount, compared with a reference amount.

Lingual Pertaining to the tongue.

Lipophilic The state of being attracted to lipids and thus repelled by water.

Lipoproteins Complexes of lipids and proteins that play a role in the transport and distribution of lipids.

Lyases Enzymes that catalyze cleavage of carbon-carbon, carbon-sulfur, and certain carbon-nitrogen bonds without hydrolysis or oxidation-reduction.

Lysosomes Cell organelles that contain digestive enzymes.

Macronutrient The dietary nutrients that supply energy, including fats, carbohydrates, and proteins.

Marasmus Malnutrition caused by prolonged intake of a diet deficient in energy (kcal).

Metabolic syndrome A clustering of risk factors for cardiovascular disease and type 2 diabetes, including elevated blood pressure and obesity.

Microfilament A solid cytoskeletal structure made of a double-helix polymer of the protein actin that plays a role in cell motility.

Microflora Bacteria adapted to living in a specific environment, such as the intestines.

MicroRNAs Small non-coding RNAs that silence gene expression by binding to mRNA to inhibit its translation and/or promote its degradation.

Microtubules Hollow, cylindrical cytoskeletal structures composed of the protein tubulin that act to support the cell structure.

Microvilli Extensions of intestinal epithelial cells designed to present a large surface area for absorbing dietary nutrients.

Mitochondria Cellular organelles that are the site of energy production by oxidative phosphorylation and the site of the tricarboxylic acid (Krebs) cycle; they are surrounded by an outer membrane that is very permeable and an inner membrane that is only selectively permeable.

Monosaccharides The simplest form of carbohydrates, which cannot be reduced in size to smaller carbohydrate units.

Motility Movement.

Mucins Glycoproteins found in some body secretions, such as saliva.

Natriuresis The excretion of large amounts of sodium in the urine.

Nervous system The system of nervous tissue made up of neurons and glial cells.

Nitrogen dioxide ($^{\bullet}NO_2$ or $^{\bullet}ONO$) A nitrogen- and oxygen-containing radical, formed from a reaction between nitric oxide and molecular oxygen, in which one of the two oxygen atoms possesses an unpaired electron.

Nitrosation The substitution of a hydrogen atom in an organic compound with a nitroso group (—N=O)

Nitrosothiol (RSNO) A compound, which is typically organic, that contains a nitroso group (—N=O) attached to a sulfur atom of a thiol.

Nuclear envelope A set of two membranes that contain nuclear pores and surround the cell nucleus.

Nucleoli Regions of the nucleus containing condensed chromatin and sites for synthesizing ribosomal RNA.

Nucleotides A phosphate ester of the 5′-phosphate of a purine or pyrimidine in N-glycosidic linkage with ribose or deoxyribose, occurring in nucleic acids.

Nystagmus Constant, involuntary movement of the eyeball.

Obesogens Endocrine-disrupting chemicals in the environment, including the diet, that alter lipid metabolism by binding to hormone receptors.

Observational study An epidemiological study in which the assignments of subjects into control or treated groups is outside of the investigator's control. Inferences about the possible effects of the treatment are drawn from the differences between the two groups.

Oligosaccharides Short chains of monosaccharide units joined by covalent bonds.

Oncogenes Genes capable of causing a normal cell to convert to a cancerous cell.

Oncosis A pre-lethal pathway accompanied by cellular swelling, organelle swelling, and increased membrane permeability that lead to cell death.

Ophthalmoplegia Paralysis of the ocular muscles.

Orexigenic Pertaining to increasing or stimulating the appetite.

Osmolality A measure of solute particle numbers expressed as osmoles of solute per kg of solvent (osm/kg).

Osmolarity A measure of the solute particle numbers expressed as osmoles of solute particles in 1 L of solution (osm/L). In dilute aqueous solutions as found in the human body, only a small numerical difference exists between osmolarity and osmolality.

Osmoles (osm) The number of moles of each particle in solution.

Osmosis The net movement of the solvent (such as water) from a solution of lesser concentration to one of greater when the two solutions are separated by a membrane that selectively prevents passage of solute molecules but is permeable to the solvent.

Osmotic pressure A property of a solution that is proportional to the nondiffusible solute concentration.

Osteoblasts Bone-forming cells.

Osteoclasts Cells that break down or resorb bone.

Osteomalacia A disorder characterized by bone-mineralization defects that may occur in adults because of inadequate vitamin D intake.

Oxidation An enzymatic reaction in which oxygen is added to, or hydrogen and its electrons are removed from, the reactant.

Oxidative phosphorylation The pathway in the mitochondria that makes ATP from ADP and P_i.

Oxidoreductases Enzymes that catalyze all reactions in which one compound is oxidized and another is reduced.

Parenchymal cells The functional cells of an organ such as the liver.

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Paresthesia A sensation of burning, tingling, or pricking (like pins and needles) associated with peripheral nerve damage.

Pellagra A condition that results from niacin deficiency.

Pentose phosphate pathway The pathway that metabolizes glucose-6-phosphate to pentose phosphate, producing NADPH.

Peroxisomes Cell organelles containing enzymes that perform oxidative catabolic reactions.

Peroxyl radical (O_2^{2-}) A radical that contains a peroxyl $(-O-O-)$ group.

Peroxynitrate $(O_2NOO^{\bullet})$ A nitrogen- and oxygen-containing radical that is generated from a reaction between nitrogen dioxide and a superoxide radical and that typically decomposes to form singlet oxygen and nitrogen dioxide.

Peroxynitrite $(ONOO^-)$ A nitrogen- and oxygen-containing radical, formed by a reaction between nitric oxide and superoxide radicals, that can decompose to generate hydroxyl and nitrogen dioxide radicals or react with carbon dioxide to produce carbonate and nitrogen dioxide radicals.

Petechiae Skin discolorations caused by ruptured small blood vessels.

Phagocytosis An endocytotic process in which material is engulfed into a cell.

Phospholipids Lipids that belong to a class of lipids containing phosphate and one or more fatty acid residues.

Phosphorolysis Cleavage of a chemical bond with the addition of phosphoric acid, analogous to hydrolysis (an example is the sequential release of individual glucose units from glycogen).

Phosphorylation The metabolic process of adding a phosphate group to an organic molecule.

Phytochemical A biologically active, nonnutritive substance that is found in plants.

Phytyl tail Refers to the structure of the side chains of vitamins E and K.

Pinocytosis Uptake of a substance into a cell through the formation of vesicles derived from the plasma membrane.

Plasma The liquid portion of blood that has been separated from the particulate portion (through the removal of cells and platelets).

Plasma membrane The phospholipid bilayer encapsulating a cell.

Polymer A substance with a high molecular weight, made up of a chain of repeating units.

Polysaccharides Long chains of monosaccharide units that may number from several into the hundreds or thousands.

Porphyrin The nitrogen- and iron-containing nonprotein portion of hemoglobin.

Postprandial Occurring after a meal.

Prebiotics Nondigestible food ingredients that serve as substrates to promote the colonic growth and/or activity of selected health-promoting species of bacteria.

Preprandial Occurring before a meal.

Probiotics Products that contain specific strains of microorganisms in sufficient numbers to alter the microflora of the gastrointestinal tract, ideally to exert beneficial health effects.

Prokaryotic cells Primitive cells that do not contain a defined nucleus.

Propagation The ongoing generation of free radicals following the initiation stage of free radical formation.

Prophylactic A substance or regime that helps to prevent disease or illness.

Prospective study An epidemiological study in which subjects are selected on the basis of factors that are to be examined in the future for possible effects on some outcome.

Prostaglandins Biologically active compounds derived from linoleic or α-linolenic acids (n-6 or n-3 essential fatty acids).

Proteases Enzymes that digest (break down) proteins.

Protein kinases A family of enzymes that transfers a phosphate group to another protein from ATP.

Proteoglycans Large molecules made up of proteins and glycosaminoglycans.

Proteolytic Pertaining to the breakdown of protein.

Quenching A process by which electronically excited molecules, such as singlet molecular oxygen, are inactivated.

Receptors Macromolecules (usually proteins) that bind a signal molecule with a high degree of specificity that triggers intracellular events.

Recommended Dietary Allowance (RDA) The average daily dietary intake level of a nutrient that is thought to be sufficient to meet the nutrient requirements of about 97% of healthy individuals.

Reflex An involuntary response to a stimulus.

Reperfusion The resupply of an organ or tissue with oxygen, nutrients, or both.

Replication The synthesis of a daughter duplex DNA molecule identical to the parental duplex DNA.

Resin A compound that is usually solid or semisolid and usually exists as a polymer.

Respiratory quotient (RQ) The ratio of the volume of CO_2 expired to the volume of O_2 consumed.

Rhodopsin A vitamin A–containing protein found in the eye.

Rickets A condition in infants and children that results from vitamin D deficiency.

Ryanodine receptor A calcium channel in the sarcoplasmic reticulum of muscle that opens to permit the release of calcium.

Sarcoplasmic reticulum The smooth endoplasmic reticulum that is found in muscle cells and is the site of the calcium pump.

Scurvy A condition resulting from vitamin C deficiency.

Seborrheic dermatitis An inflammatory skin condition.

Sense strand The strand of DNA that serves as a template for mRNA.

Serum The pale yellowish, clear fluid portion of blood from which the clotting factors (fibrinogen) have been removed.

Short-chain fatty acids Fatty acids typically containing two to four carbons.

Sideroblastic anemia An inherited disorder that affects red blood cell production and function.

Signal-lipidomics A branch of the emerging field of lipidomics, which studies the pathways and networks of cellular lipids. Signal-lipidomics studies the lipidomics of various signaling sites of cell membranes, which often involve polyunsaturated fatty acids such as docosahexaenoic acid.

Signal transduction Cascade of intracellular reactions resulting from the binding of an extracellular molecule to its cell-surface receptor, resulting in a genetic or metabolic response.

Singlet (molecular) oxygen An electronically excited radical in which one of oxygen's electrons is excited to an orbital above the one it normally occupies.

Sphingolipids Class of lipids that contain the amino alcohol sphingosine as a backbone structure, with a fatty acid attached to the amino group.

Sphingomyelin Sphingolipid containing phosphocholine at the terminal hydroxyl.

Splanchnic Pertaining to the internal organs (viscera). The splanchnic organs or portal-drained viscera include the liver, stomach, intestines, and spleen.

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Standard reduction potential The tendency of a molecule to donate or receive electrons.

Steatorrhea The presence of an excessive amount of fat in the feces.

Stellate cells Storage cells of the liver.

Stereoisomers A group of compounds that have the same structure but different configurations.

Sterols A subclass of lipids that contain a cyclopentanoperhydrophenanthrene ring system, a hydroxyl group, and a side chain.

Substrate-level phosphorylation The process of transferring a phosphate group from one organic molecule to another.

Superoxide radical An oxygen-centered free radical, $O_2^{\bullet}$.

Teratogenic Capable of causing birth defects in a fetus.

Tetany A condition resulting from inadequate blood calcium concentrations, characterized by prolonged muscle contraction.

Thalassemia A hereditary form of anemia associated with defective synthesis of hemoglobin.

Thermogenesis The production of heat within the body.

Thermoregulation The process whereby a regulatory mechanism keeps heat production and loss about equal.

Thromboxanes Biologically active compounds derived from linoleic or α-linolenic acids (n-6 or n-3 essential fatty acids).

Tolerable Upper Intake Level The highest daily-intake level that is likely to cause no risk of adverse health effects to most individuals in the general population.

Total fiber The sum of dietary fiber plus functional fiber that is in a food.

Transcaltachia Rapid intestinal calcium absorption stimulated by the active form of vitamin D.

Transcription The process by which the genetic information (base sequence) in a single strand of DNA is used to specify a complementary sequence of bases in an mRNA chain.

Transcription factors Auxiliary proteins that bind to specific sites in the DNA and alter the transcription of nearby genes.

Transducin A G-protein found in the eye that responds to changes in opsin and is involved in the visual cycle.

Transferases Enzymes that catalyze reactions not involving oxidation and reduction in which a functional group is transferred from one substrate to another.

Transition state Energy level at which reactant molecules have been activated and can undergo an exothermic reaction.

Translation The process by which genetic information in an mRNA molecule specifies the sequence of amino acids in the protein product.

Translocation Movement of a compound or agent across a cell membrane, such as the intestinal cell, and into the blood.

Transport proteins Proteins that transport nutrients in blood or into and out of cells or cell organelles.

Tricarboxylic acid cycle An aerobic metabolic cycle in the mitochondria that produces ATP; also called the *citric acid cycle* or *Krebs cycle*.

Tumor necrosis factor A cytokine released by immune cells and mast cells that causes destruction of tumors and migration of neutrophils toward the site of bacterial infections.

Ubiquinol The alcohol form of ubiquinone, a fat-soluble molecule that functions in electron transport and ultimately ATP generation; also called *coenzyme* Q_{10} or CoQ_{10} .

Ubiquitin A protein that attaches to other proteins within cells or tissues to promote the degradation of the protein.

VO_2 max The maximal uptake of oxygen, as measured during a test with increasing work intensity.

Xenobiotics Foreign chemicals such as drugs, carcinogens, pesticides, food additives, pollutants, or other noxious compounds.

Xerophthalmia Dryness of the conjunctiva and keratinization of the epithelium of the eye following inflammation of the conjunctiva associated with vitamin A deficiency.

Zwitterion A dipolar ion that has both negatively and positively charged regions, such as an amino acid. The ion has no net charge in solution.

Zymogen An inactive form of an enzyme, also referred to as a *proenzyme*.

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