MACQUARIE University

Lipid metabolism - 1

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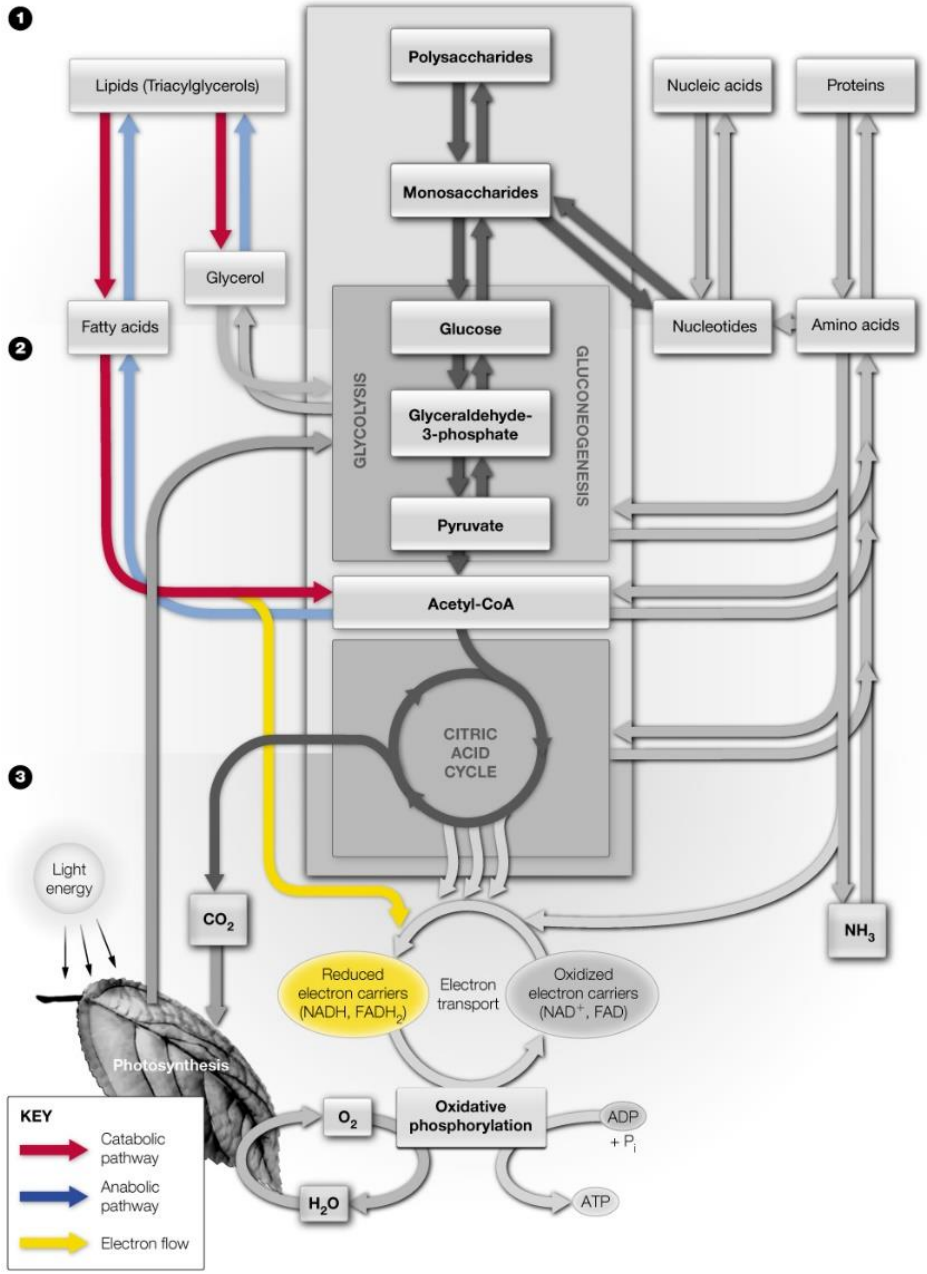
Objectives

- Lipids for energy
  1. Digestion and storage
  2. Mobilization
  3. Transport
- Fat burning: beta (β-)oxidation

Textbook Chapter 16

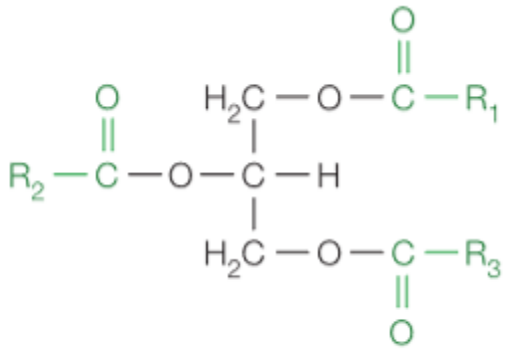
Intermediary Metabolism of Fatty Acids and Triacylglycerols (TAGs)

- The catabolic pathways of lipid metabolism are shown in red, while the anabolic pathways are indicated in blue.
- The electron flows from lipid oxidation is highlighted in yellow



We need lipids

- Major source of energy - 5% to 25% of body weight: 90% of this is triacylglycerols (aka triglycerides)
- Three sources of fatty acids
  - diet, adipose tissue and biosynthesized fatty acids.
- Major source is diet (around 40%)
- The role of fatty acid oxidation varies with the organism or tissue
  - Mammals - fuel, excess can be stored in adipose tissue
  - Plants - biosynthetic precursors and as a fuel
- Regardless of the organism, the reactions are the same.



Triacylglycerol (TG)

Fatty acid degradation (aka beta oxidation)

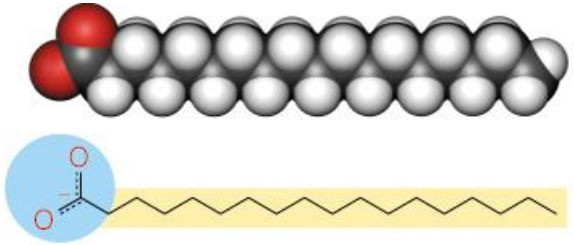
- Why are fatty acids good fuels?
  - Fatty acids are highly reduced - energy of complete oxidation is >2 x carbohydrates or proteins (37kJ/g)

Tissue Fuel Stores for Average 70 kg Human

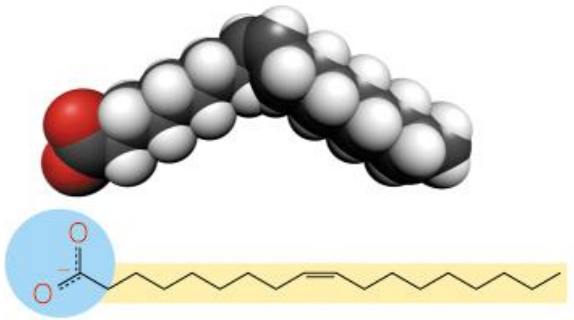
Fuel	Weight (g)	Energy Content (kJ / g)	Total Energy (kJ)
Triacylglycerols	~15,000	37	555,000
Protein	~6,000	17	100,000
Glycogen	~400	17	6,800
Glucose	~20	17	340
Total fuel stores			662,140

  - **However, triacylglycerols** are anhydrous
  - Segregate into lipid droplets leading to no increase in osmotic pressure
  - They are chemically inert and therefore do not undergo any other biochemical reactions
- These properties cause problems in aqueous environments such as blood and plasma …….

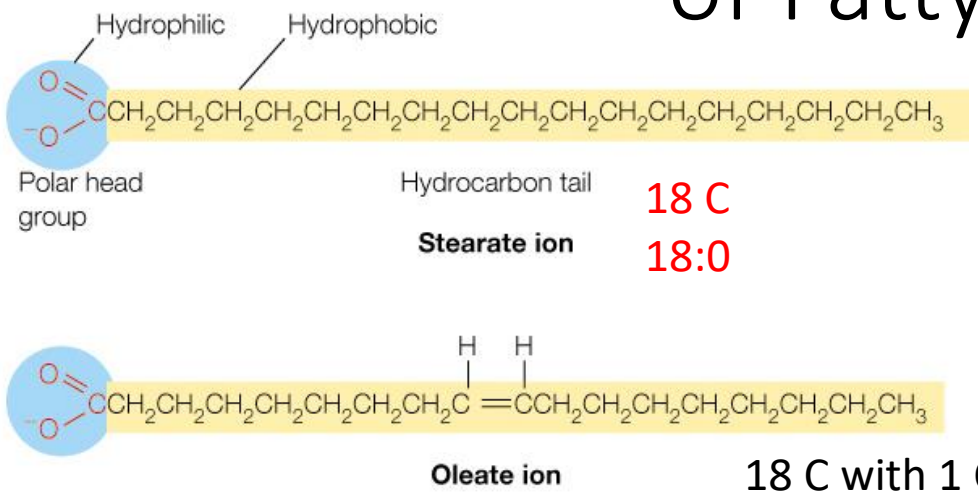
Lipids are made up of Fatty Acids (recap)



(a) **Stearate ion.** Stearate (the anionic, deprotonated form of stearic acid) is a saturated fatty acid.



(b) **Oleate ion.** Oleate is an unsaturated fatty acid with one *cis* double bond.



(c) **Formulas.** Alternate representations for (a) and (b).

18 C

18:0

18 C with 1 C=C

18:1*n*-9

FIGURE 10.1 Structures of the ionized forms of two representative fatty acids.

- Fatty acids are major constituents of lipids
- In a fatty acid, a hydrophilic carboxylate group is attached to one end of a hydrocarbon chain (containing typically between 12 and 24 carbons)
- In their hydrocarbon chains, unsaturated fatty acids contain one or more *cis* C=C bonds, whereas saturated fatty acids contain none
- The fluidity of fatty acids decreases as the chain length increases and the number of *cis* double bonds decreases

Example Fatty Acids (recap)

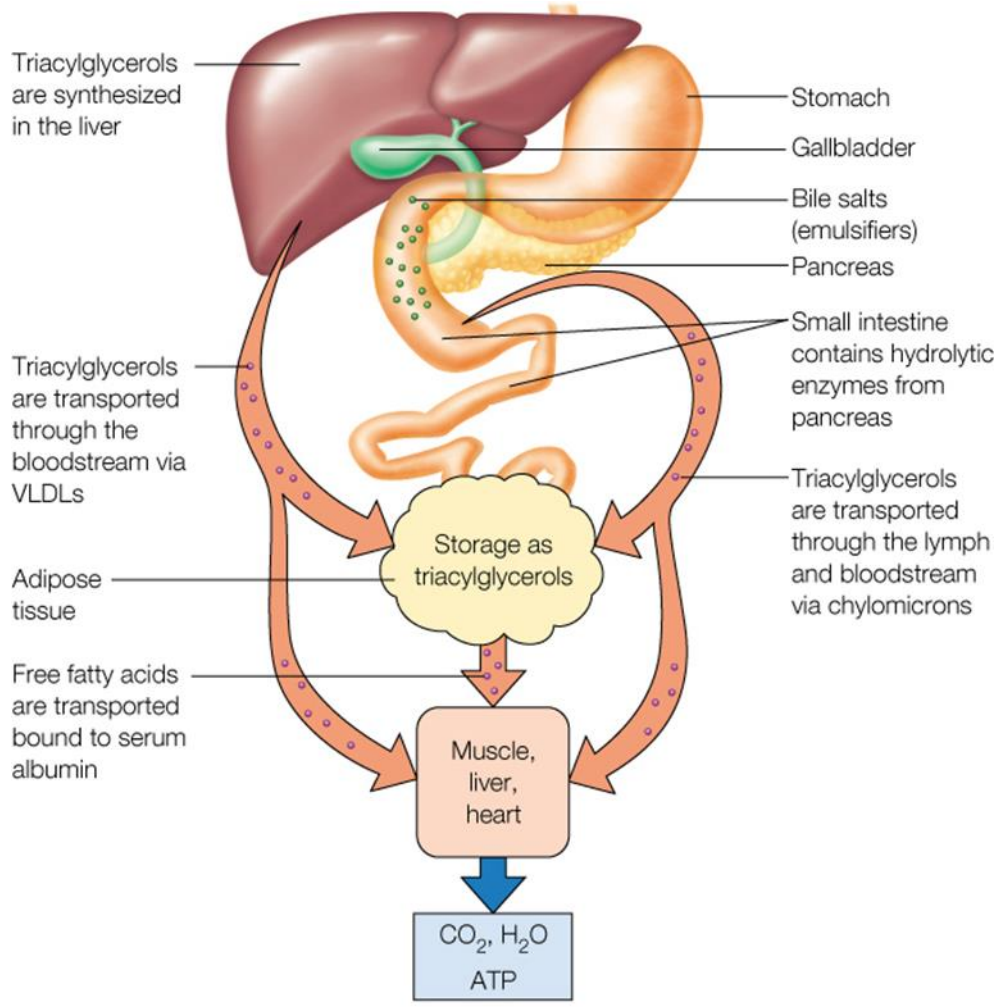
TABLE 10.1 Some biologically important fatty acids

Common Name	Systematic Name	Abbreviation	Structure	Melting Point (°C)
Saturated Fatty Acids				
Capric acid	Decanoic acid	10:0	$CH_3(CH_2)_8COOH$	31.6
Lauric acid	Dodecanoic acid	12:0	$CH_3(CH_2)_{10}COOH$	44.2
Myristic acid	Tetradecanoic acid	14:0	$CH_3(CH_2)_{12}COOH$	53.9
Palmitic acid	Hexadecanoic acid	16:0	$CH_3(CH_2)_{14}COOH$	63.1
Stearic acid	Octadecanoic acid	18:0	$CH_3(CH_2)_{16}COOH$	69.6
Arachidic acid	Eicosanoic acid	20:0	$CH_3(CH_2)_{18}COOH$	76.5
Behenic acid	Docosanoic acid	22:0	$CH_3(CH_2)_{20}COOH$	81.5
Lignoceric acid	Tetracosanoic acid	24:0	$CH_3(CH_2)_{22}COOH$	86.0
Cerotic acid	Hexacosanoic acid	26:0	$CH_3(CH_2)_{24}COOH$	88.5
Unsaturated Fatty Acids				
Palmitoleic acid	*cis*-9-Hexadecenoic acid	16:1cΔ9	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	0
Oleic acid	*cis*-9-Octadecenoic acid	18:1cΔ9	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	16
Linoleic acid	*cis,cis*-9,12-Octadecenoic acid	18:2cΔ9,12	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	5
Linolenic acid	all-*cis*-9,12,15-Octadecenoic acid	18:3cΔ9,12,15	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	−11
Arachidonic acid	all-*cis*-5,8,11,14-Eicosatetraenoic acid	20:4cΔ5,8,11,14	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_3COOH$	−50

- Fatty acid double bonds are almost always in the *cis* form.
- This puts a rigid 30° bend in the chain which prevents tight packing of chains and lowers the transition (liquid↔gel) temperature of the membrane lipids.

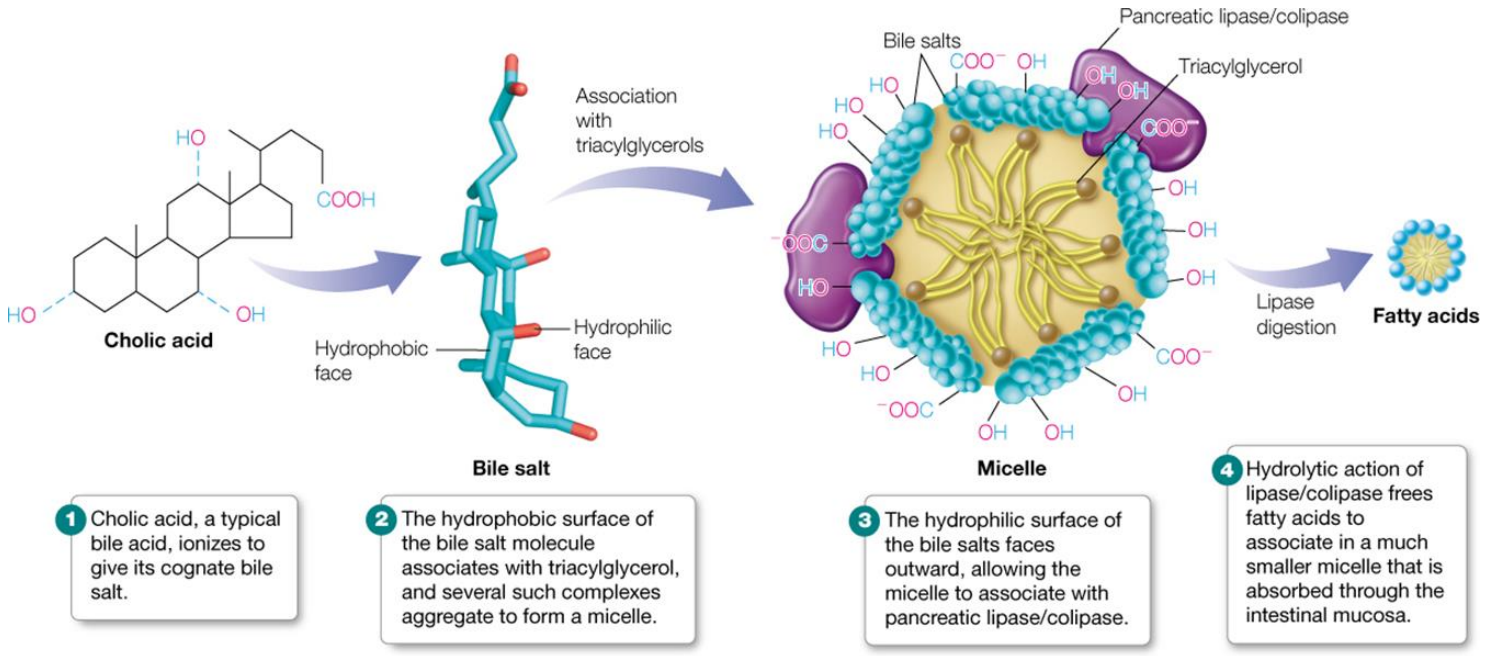
1. Triacylglycerols are digested

1. **<u>Dietary fats are dispersed by bile detergents</u>**
    - **Bile acids/salts** (eg taurocholic acid) are synthesized from **cholesterol** in the **liver** and stored in **the gall bladder.** These are **secreted** into the **small intestine** where ***lipid digestion and absorption mainly occurs***.
    - These acids form **micelles** (see lecture 10) with fatty acids (b,c,d in the image below)
    - This increases the available surface area of the fatty acids to that of solution-based enzymes.



Triacylglycerols are then absorbed

2. **Triglycerides are degraded by lipases in the intestine at the lipid-water interface**
   - Products include monoglycerides, diglycerides, free fatty acids and glycerol.
3. **Bile acids and fatty acid-binding protein assist intestinal absorption of lipids**
   - **Bile acids** are **also required** for **absorption** of the **lipid-soluble vitamins A, D, E and K**.



Lipids are transported as lipoproteins

Complexes of lipid and protein, globular micelle-like particles (*in general, micelles*)

- Nonpolar core of triacylglyerols and cholesteryl esters
- Amphiphilic coating protein, phospholipid and cholesterol
- 5 classes of lipoproteins: different composition and function

TABLE 16.1 Properties of major human plasma lipoprotein classes

	Chylomicron	VLDL	IDL	LDL	HDL
Density (g/mL)	< 0.95	0.950–1.006	1.006–1.019	1.019–1.063	1.063–1.210
Diameter (Å)	10^3–10^4	300–800	250–350	180–250	50–120
Components (% dry weight)					
Protein	2	8	15	22	40–55
Triacylglycerol	86	55	31	6	4
Free cholesterol	2	7	7	8	4
Cholesterol esters	3	12	23	42	12–20
Phospholipids	7	18	22	22	25–30
Apolipoprotein composition	A-I, A-II,	B-100,	B-100,	B-100, E	A-I, A-II,
	A-IV, B-48,	C-I, C-II,	C-I, C-II,		C-I,
	C-I, C-II,	C-III, E	C-III, E		C-II,
	C-III, E				C-III,
					D, E

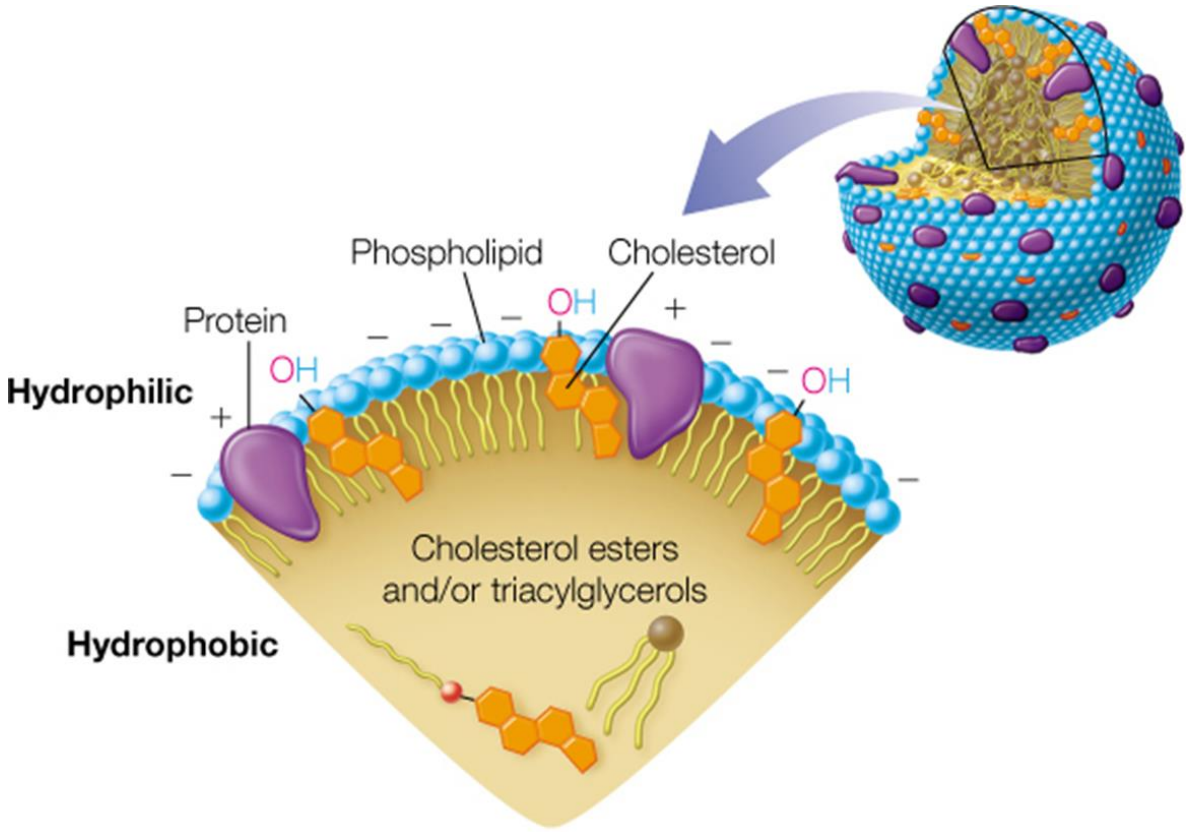
Data from A. Jonas (2002) Lipoprotein structure. In *Biochemistry of lipids, lipoproteins and membranes*, 4th ed., D. E. Vance and J. E. Vance, eds., Ch. 18, pp. 483–504, Elsevier, Amsterdam; and R. J. Havel and J. P. Kane (2001) Introduction: Structure and metabolism of plasma lipoproteins. In *The Metabolic and Molecular Bases of Inherited Disease*, C. R. Scriver, A. L. Beaudet, W. S. Sly, D. Valle, B. Childs, K. W. Kinzler, and B. Vogelstein, eds., Vol. II, Ch. 114, pp. 2705-2716, McGraw-Hill, New York.

From food: in intestines

From endogenous lipid synthesis: in the liver

From tissue: back to liver

Lipoproteins in human plasma



- Apolipoproteins combine to form particles of different density e.g. **VLDL, VHDL** (very low / very high density lipoproteins)
- The nature of these proteins determines the site of delivery of the lipids
- Each lipoprotein contains just enough protein, phospholipid and cholesterol to form a monolayer (blue part) as thick as a biological membrane.
- The most dense particles are HDL, which are also the smallest.

Lipids are transported as lipoproteins

Chylomicrons enter the blood stream for transport

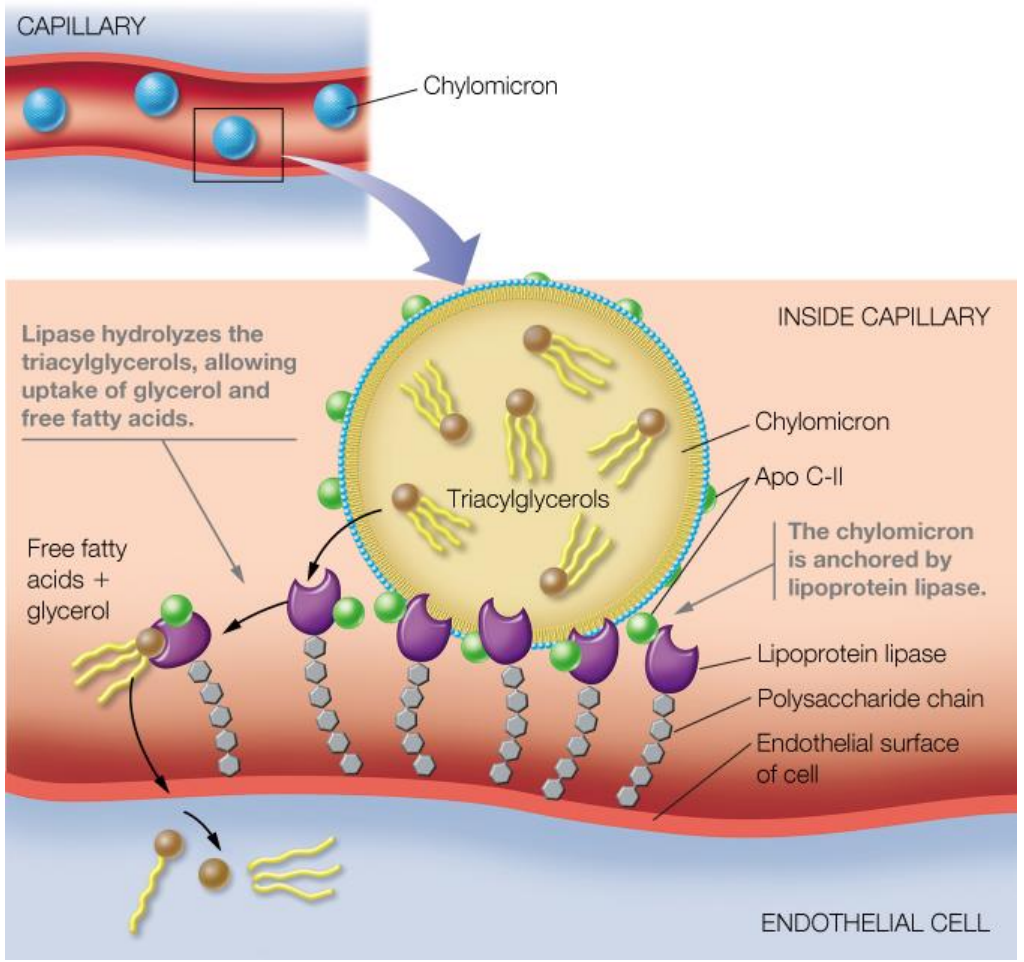
- Intestinal mucosal cells convert dietary fatty acids into triacylglycerols (TAGs) and package then, with dietary cholesterol, into large lipoproteins called **chylomicrons**
- These are released into the intestinal lymph and transported through the lymphatic vessels, draining into large veins.
- The bloodstream then delivers chylomicrons everywhere.
- Chylomicrons provide TAGs to tissues at the capillaries of peripheral tissues with the remnants providing dietary cholesterol to the liver.

Other lipoproteins (VLDL, IDL, LDL) are synthesized by the liver: take endogenous TAGs and cholesterol to tissues

HDL transports cholesterol and other lipids from the tissues back to the liver

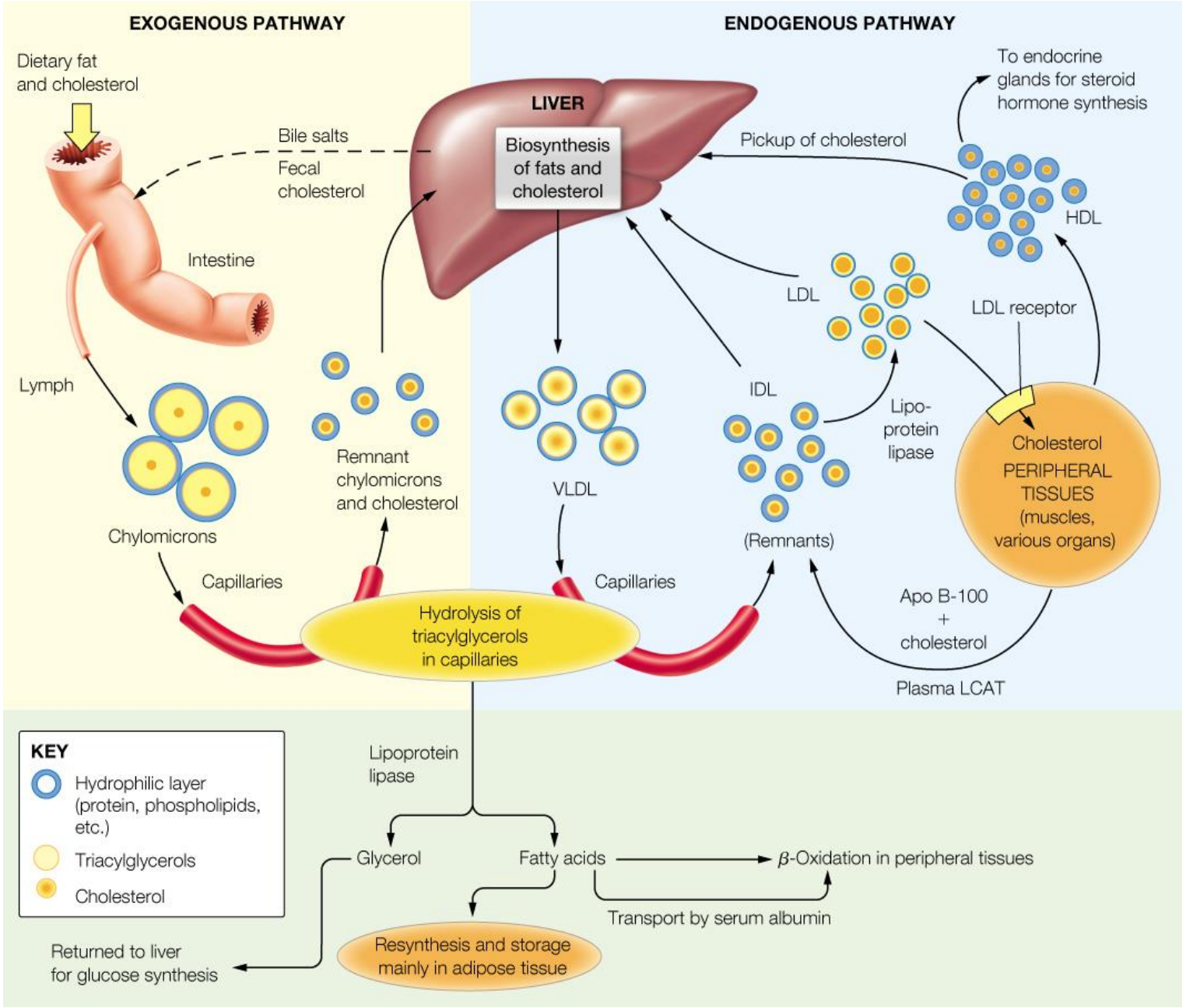
Chylomicron Binding to Lipoprotein Lipase on the Inner Capillary Surface

- Lipoprotein lipase binds chylomicron (and VLDL) at the capillaries and hydrolyzes TAGs to glycerol and free fatty acids for uptake into cells
- Lipoprotein lipase like other lipases is a member of the serine esterase family (like trypsin) with active site serine, histidine, and aspartate

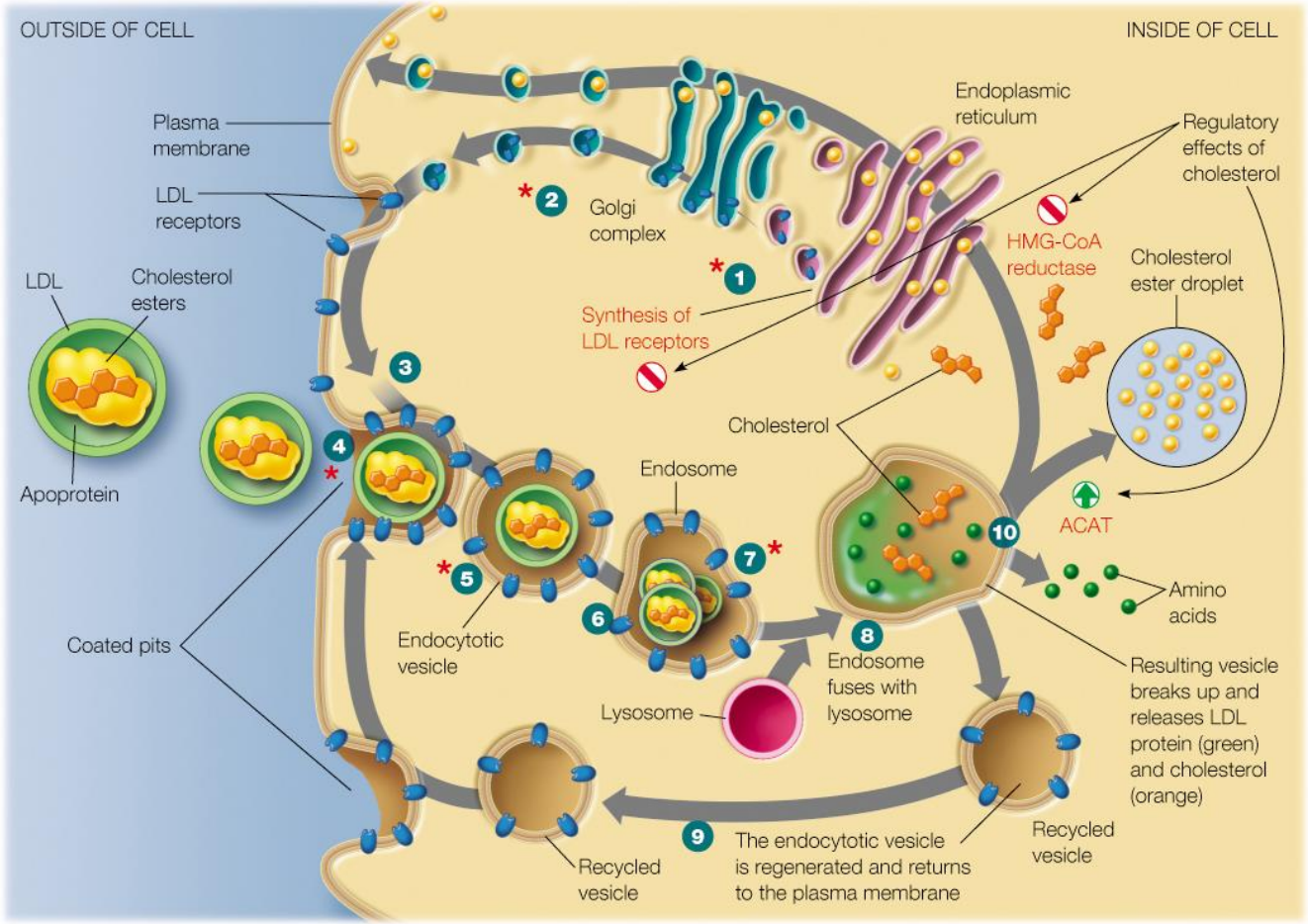


Lipoprotein Transport Pathways starting in the Liver to Redistribute Fat and Cholesterol

- Cholesterol is both free and as cholesteryl esters
- Total plasma cholesterol (cholesterol plus cholesterol esters) above 200 mg/100 mL is **a major risk factor for coronary artery disease**
- Prolonged **hypercholesterolemia** hastens the formation of **atherosclerotic plaque**



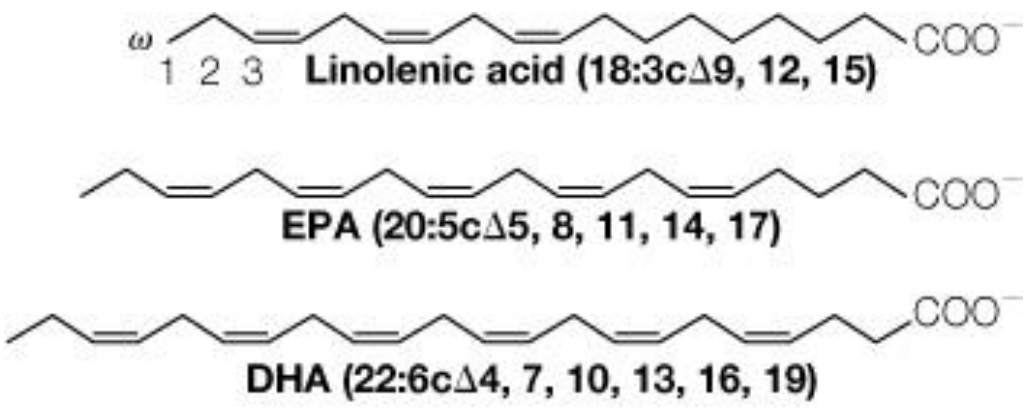
LDL Receptors Involved in Cholesterol Metabolism



▲ **FIGURE 16.9 Involvement of LDL receptors in cholesterol uptake and metabolism.** LDL receptors are synthesized in the endoplasmic reticulum 1 and mature in the Golgi complex 2. They then migrate to the cell surface, where they cluster in clathrin-coated pits 3. LDL, made up of cholesterol esters and apolipoprotein, binds to the LDL receptors 4 and is internalized in endocytotic vesicles 5. Several such vesicles fuse to form an organelle called an endosome 6. Proton pumping in the endosome membrane causes the pH to drop, which in turn causes LDL to dissociate from the receptors 7. The endosome fuses with a lysosome, 8 and the receptor-bearing clathrin coat dissociates and returns to the membrane 9. The LDL particle is degraded in the lysosomes, 10 and cholesterol has various fates. Regulatory targets of cholesterol are shown in red. ACAT, acyl-CoA:cholesterol acyltransferase. Red asterisks indicate steps affected by mutations in the LDL receptor gene.

Cholesterol, LDL, and Atherosclerosis

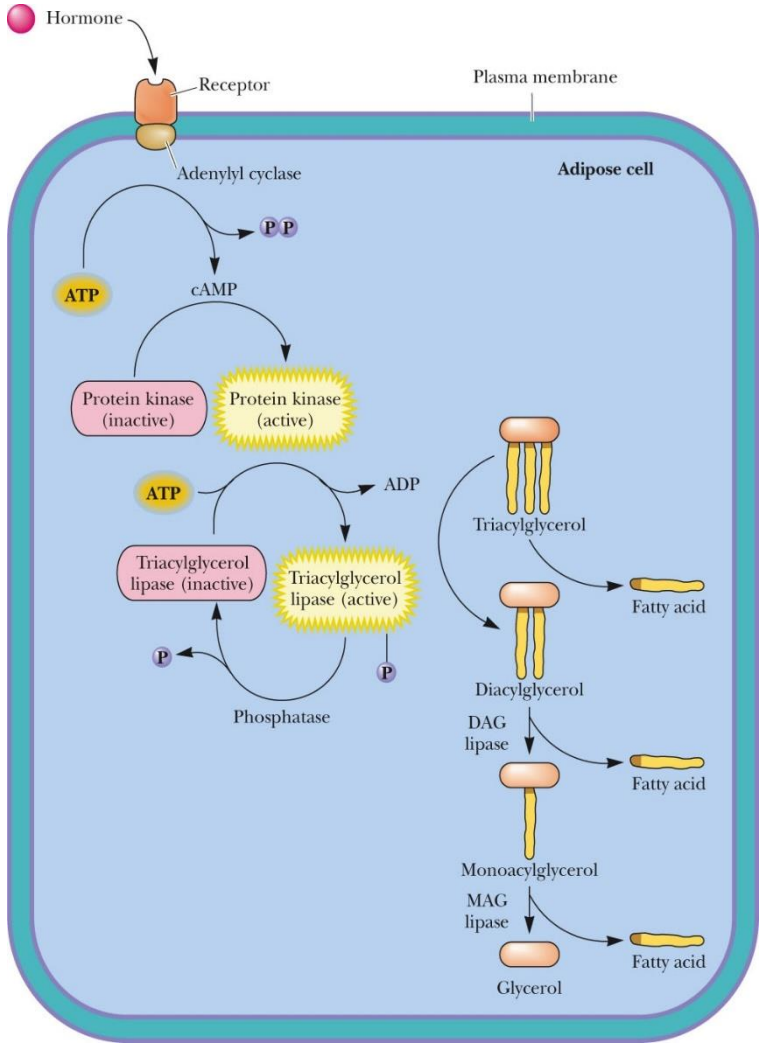
- Why do diets rich in saturated fatty acids elevate LDL?
- Why do polyunsaturated fatty acids, particularly omega-3 fatty acids (like linolenic acid) lower LDL?



Omega-3 PUFAs
(linolenic acid labeled using nutritionist nomenclature)

- LDL is subject to oxidation—oxidized LDL can be taken up by scavenger receptors. Uptake of oxidized LDL by scavenger receptors on macrophages is a key event in atherogenesis.
- This process (called atherogenesis) yields almost unlimited uptake of cholesterol into a cell and produces a cholesterol-gorged foam cell, which is a precursor to atherosclerotic plaque
- There are **no degradative pathways for cholesterol** within the cell
- Omega-3 fatty acids possibly compete with LDL for oxidation and provide protection against coronary heart disease
- HDL can return excess cholesterol to the liver for passage through the bile duct to the intestine, leading to excretion

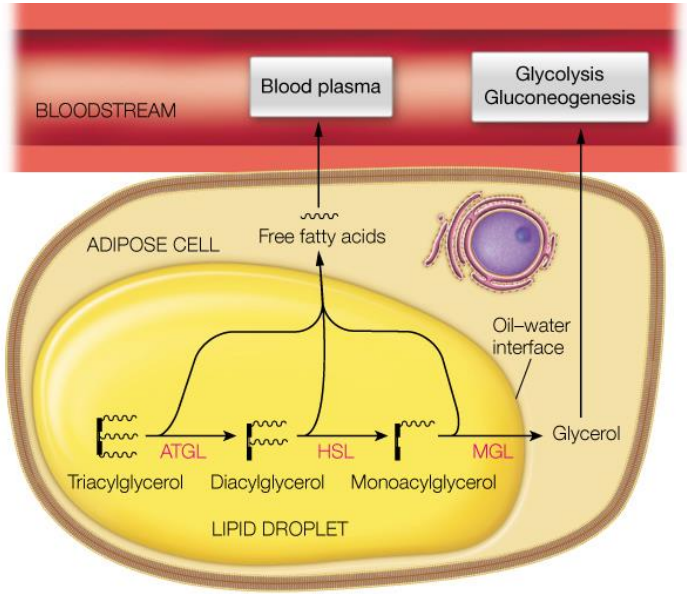
Mobilization of Stored Fat



- Synthesis of TAG (triacylglycerol) and its deposition in adipose cells is unlimited
- Mobilization of stored fat (lipolysis) is hormonally controlled via cAMP
- Mediated by **epinephrine during stress situations** and by **glucagon during fasting**
- Other hormones regulate the process under different conditions
- 2 ATPs required for lipase activation (similar to glycolysis)
- Fatty acid release from triacylglycerols

❖ Fatty acids are oxidized (burnt) or re-esterified

- chylomicrons are recycled back through the liver



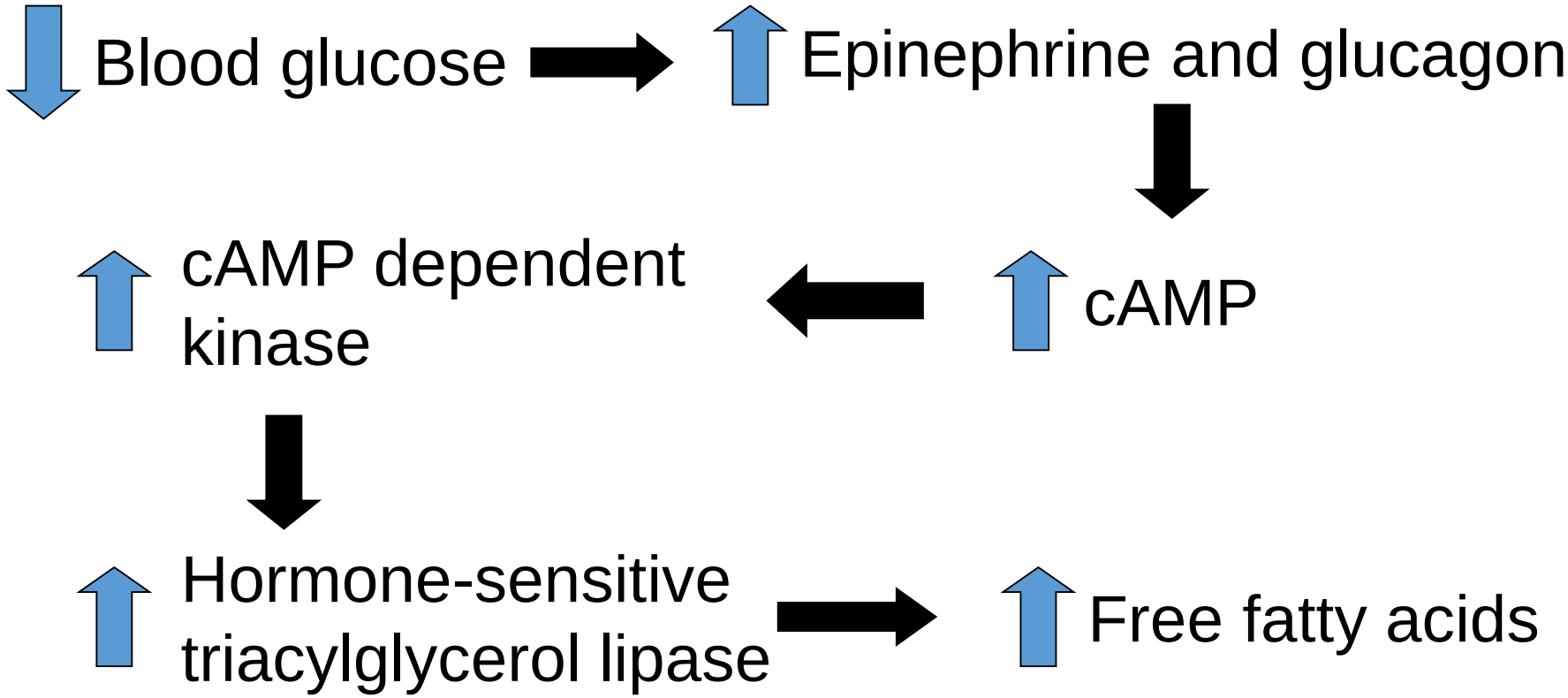
▲ **FIGURE 16.10 Mobilization of adipose cell triacylglycerols by lipolysis.** Three lipases act sequentially to hydrolyze triacylglycerol (TG) to glycerol and free fatty acids (FFA). These enzymes act at the oil–water interface of the lipid droplet. FFA are exported to the blood plasma, where they are bound to albumin for transport to liver and other tissues for subsequent oxidation. Glycerol is released to the blood to be taken up by liver cells, where it serves as a gluconeogenic substrate. ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase; MGL, monoacylglycerol lipase.

Summary

- Lipid Digestion, Absorption, and Transport
  - Triacylglycerol digestion depends on solubilisation by bile acids and the activation of lipases at the lipid-water interface.
  - Lipoproteins are complexes of nonpolar lipids surrounded by a coat of amphipathic lipids and apolipoproteins, that transport lipids in the bloodstream.
  - Cells take up cholesterol and other lipids by the receptor-mediated endocytosis of LDL.

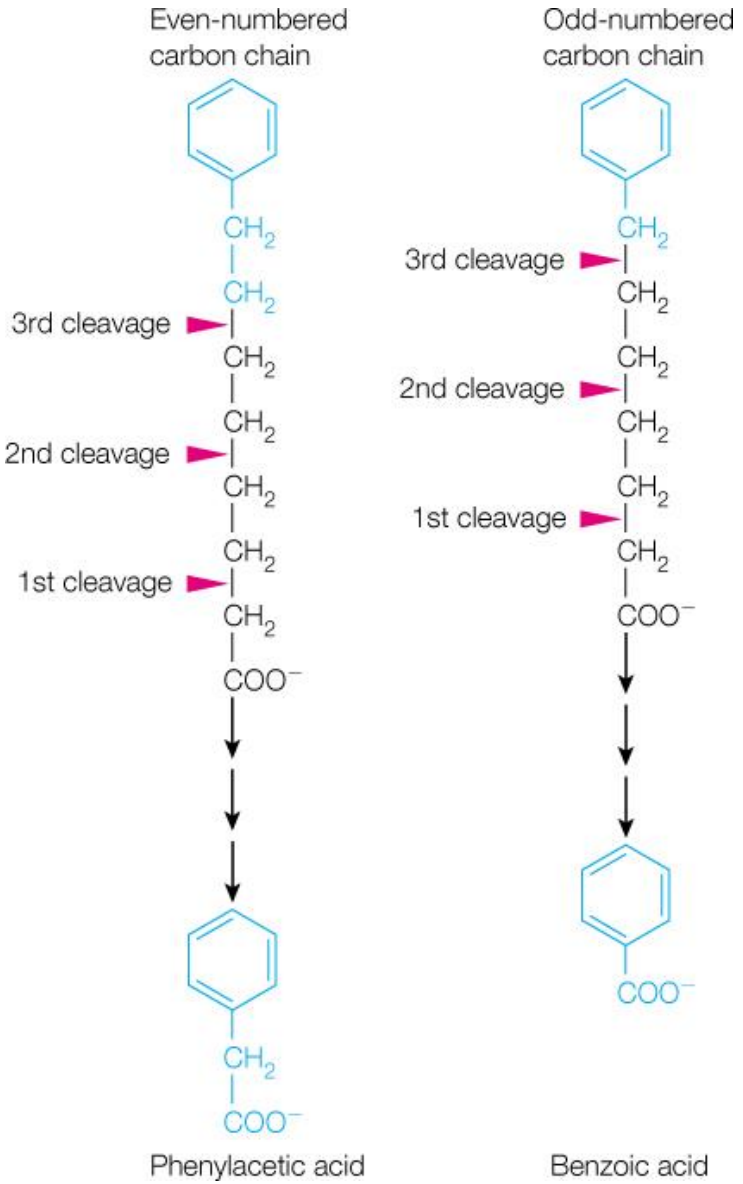
Mobilization of fat stores

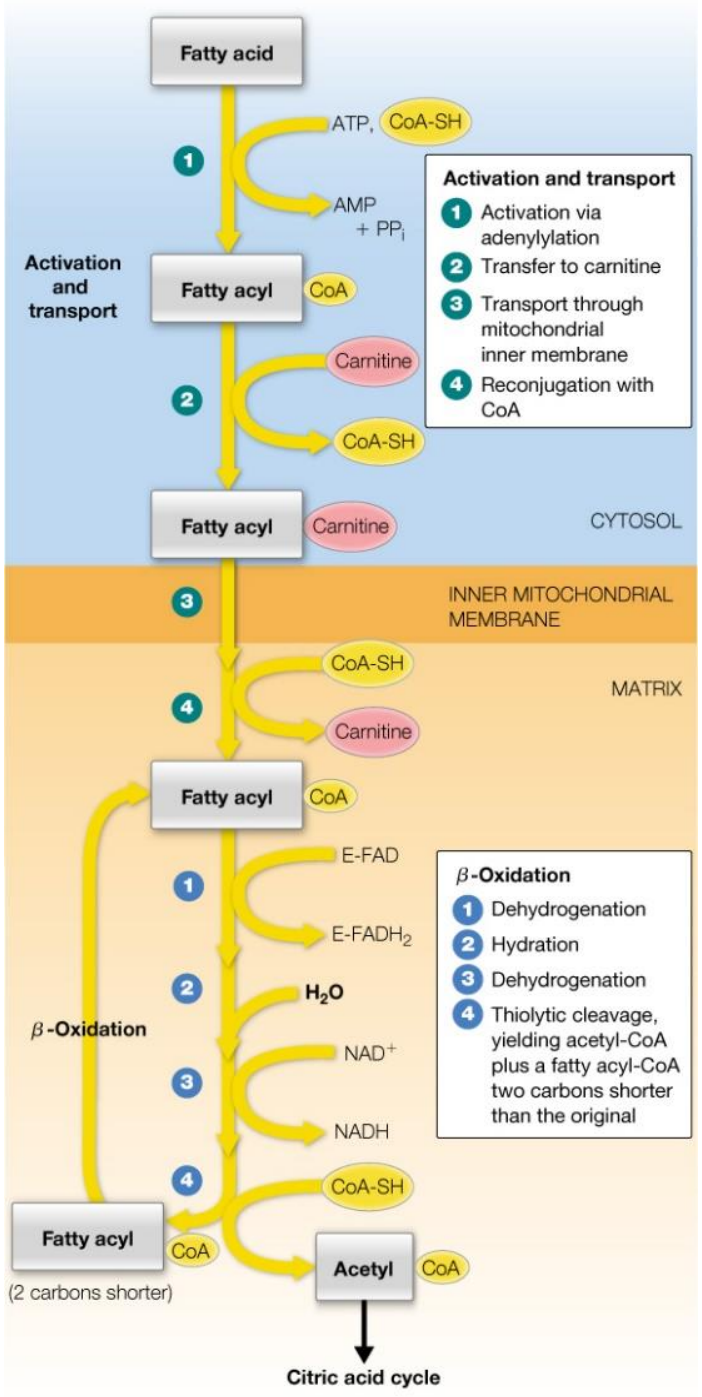
- Hormones signal the need for fuel by tissues
  - skeletal muscle, heart, renal cortex



Early Experiments on Fatty Acid Oxidation

- Early insight into fatty acid oxidation by Knoop in 1904 using phenyl derivatives of fatty acids
- First use of metabolic tracers (long before radioisotopes)
- Knoop proposed the oxidation of fatty acids occurred in a stepwise fashion at carbon 3, releasing a two-carbon fragment
- Release of a two-carbon fragment would occur in each step of oxidation



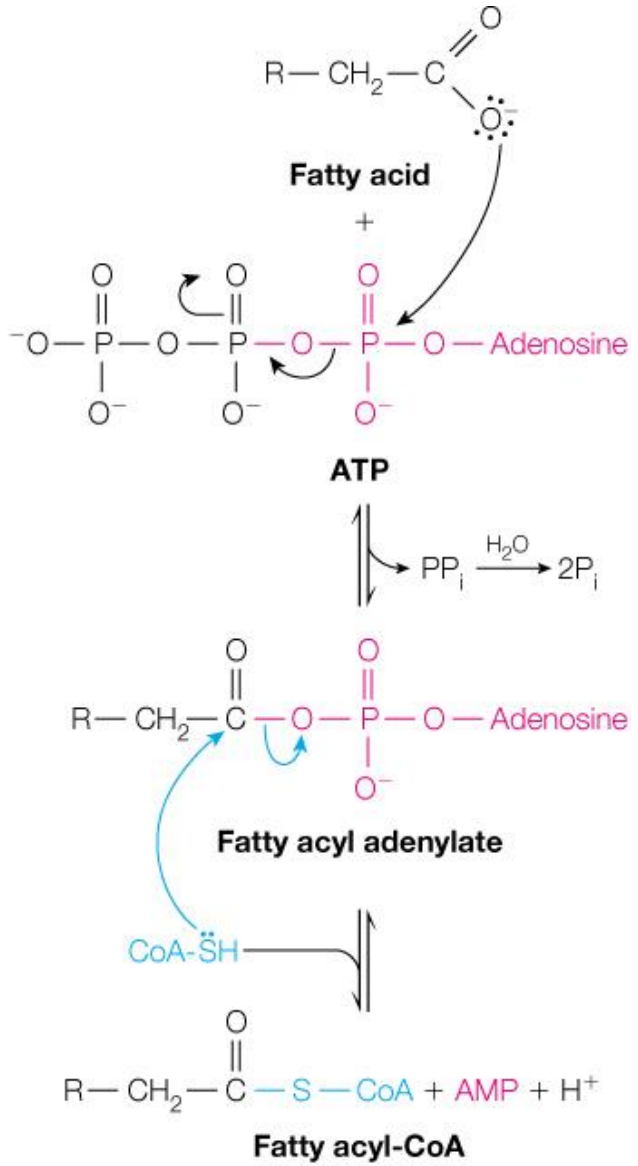


Overview of the Fatty Acid Oxidation Pathway

- Fatty acids are converted (activated) in the cytosol to fatty acyl-CoA (~2 ATPs)
- Burn only in the mitochondria!
- Short-chain and medium-chain fatty acyls (up to 10C) can enter mitochondria via nonmediated diffusion
- Long-chain (12C or more) fatty acyls are transported into mitochondria by the carnitine-acyltransferase system.
- In mitochondria, fatty acyls are catabolized to acetyl-CoA via beta-oxidation

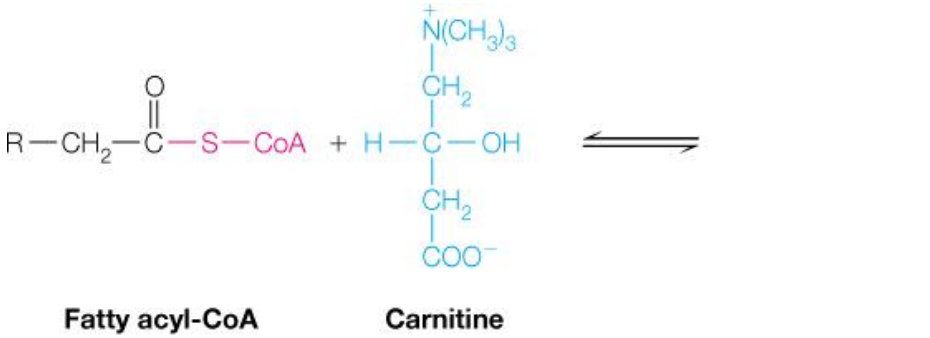
Fatty Acid Activation in the Cytosol

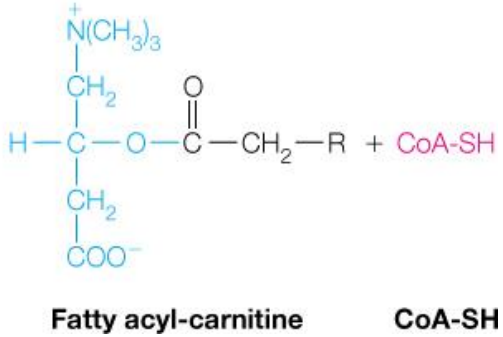
- A series of **acyl-CoA synthetases** specific for short-chain (2-3 C), medium-chain (4-12 C), or long-chain (10-20 C) fatty acids catalyzes the formation of a fatty acid thioester conjugate with coenzyme A (activated fatty acid)
- Equivalent of 2 ATPs used up!



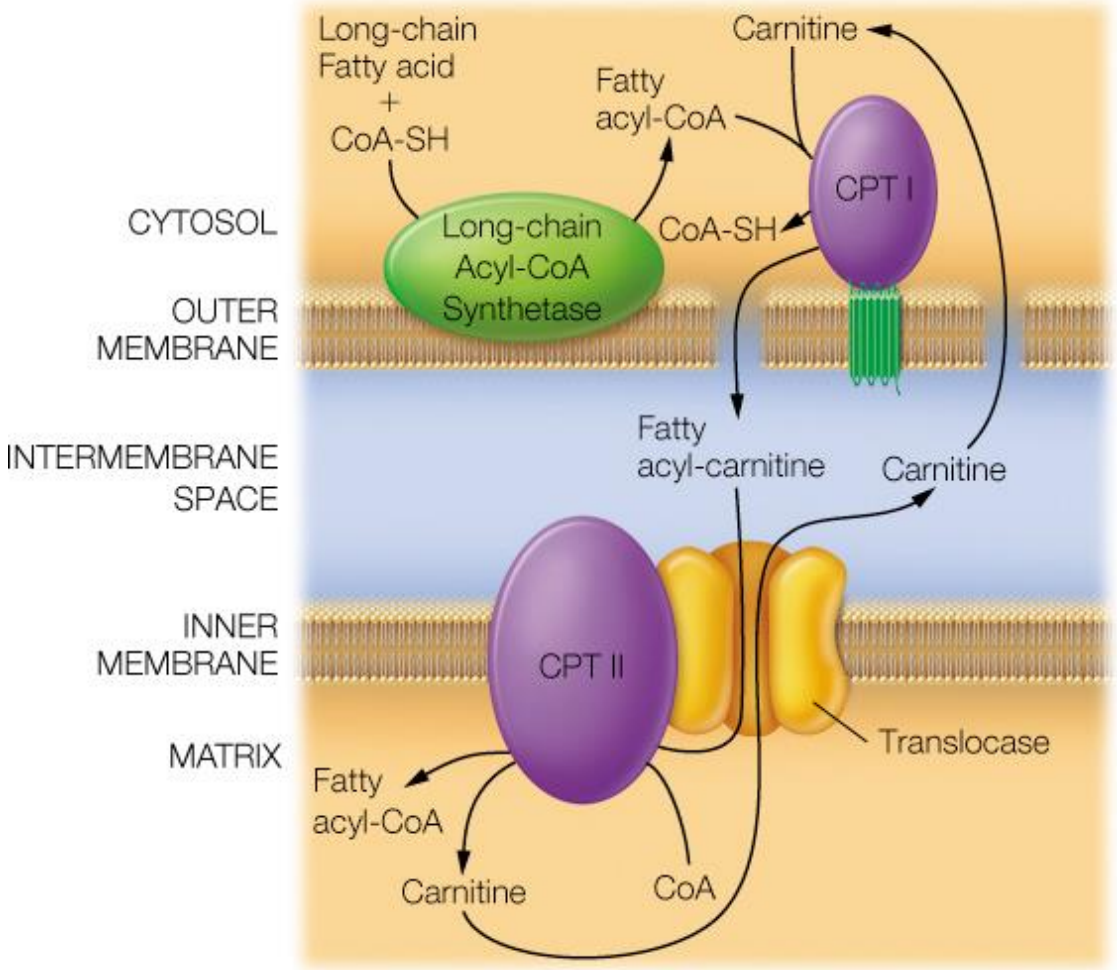
Fatty Acid Transport into Mitochondria – the Carnitine Acyltransferase System

- While short-chain and medium-chain fatty acyls (up to 10C) can diffuse into mitochondria without the need of a specific transport system, long-chain (12C or more) fatty acyls require the carnitine-acyltransferase system to enter mitochondria

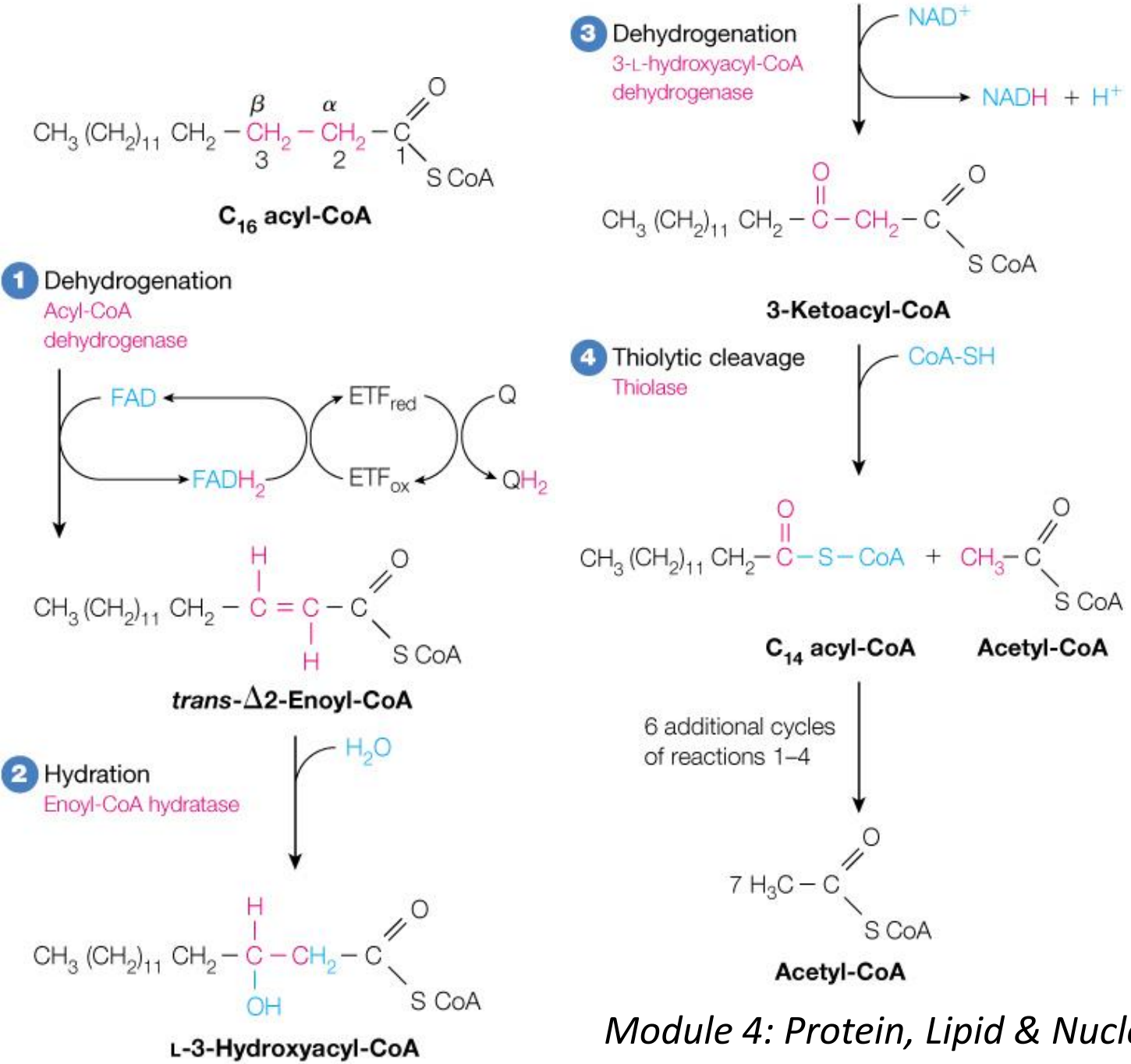




CPT: carnitine palmitoyl transferase

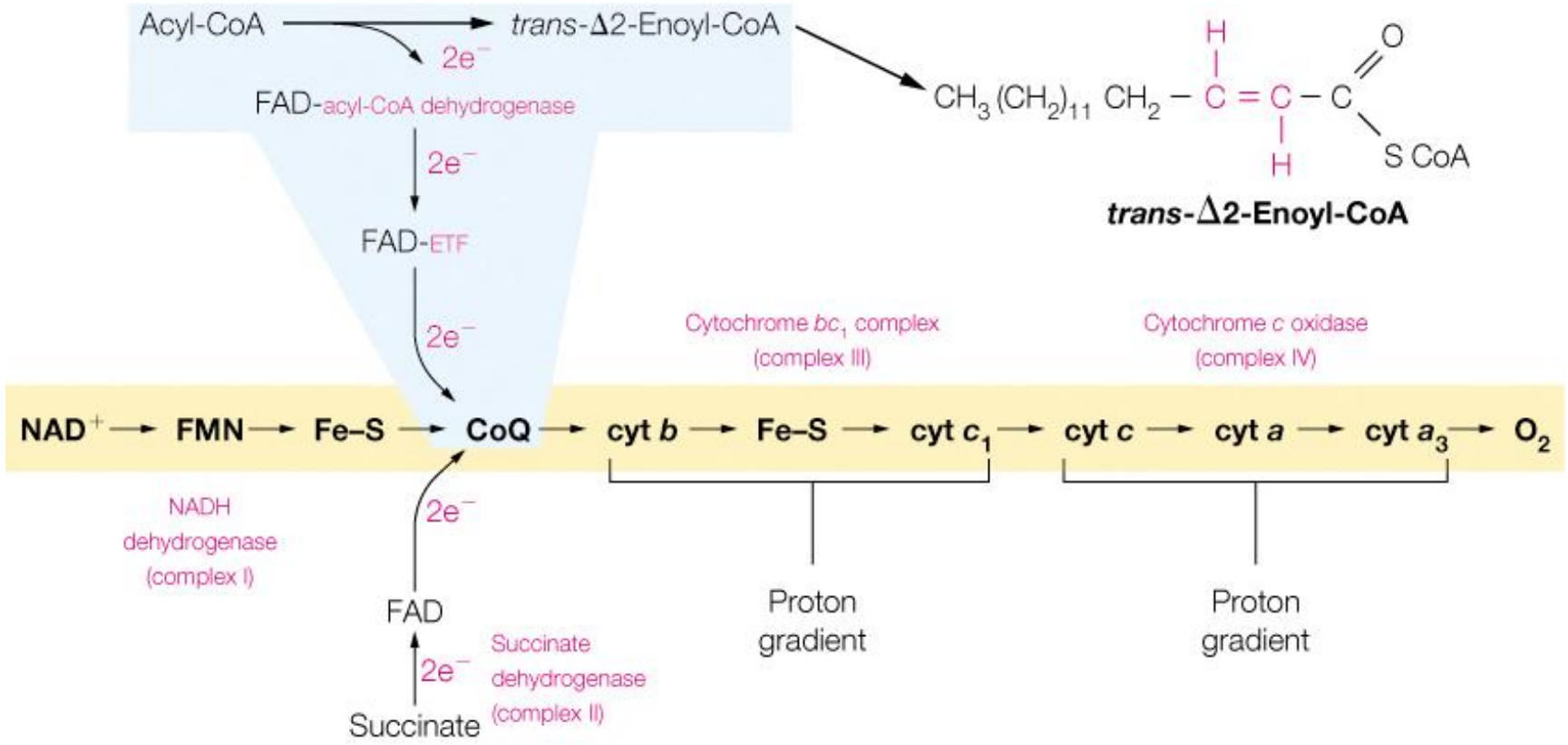


Overview of β-Oxidation



- In the mitochondrial matrix, fatty acyls are oxidized in a series of four enzymatic steps (first dehydrogenation, hydration, second dehydrogenation, and thiolytic cleavage)
- The fatty acyl chain is shortened by two carbons at a time
- The released two-carbon fragment is acetyl-CoA

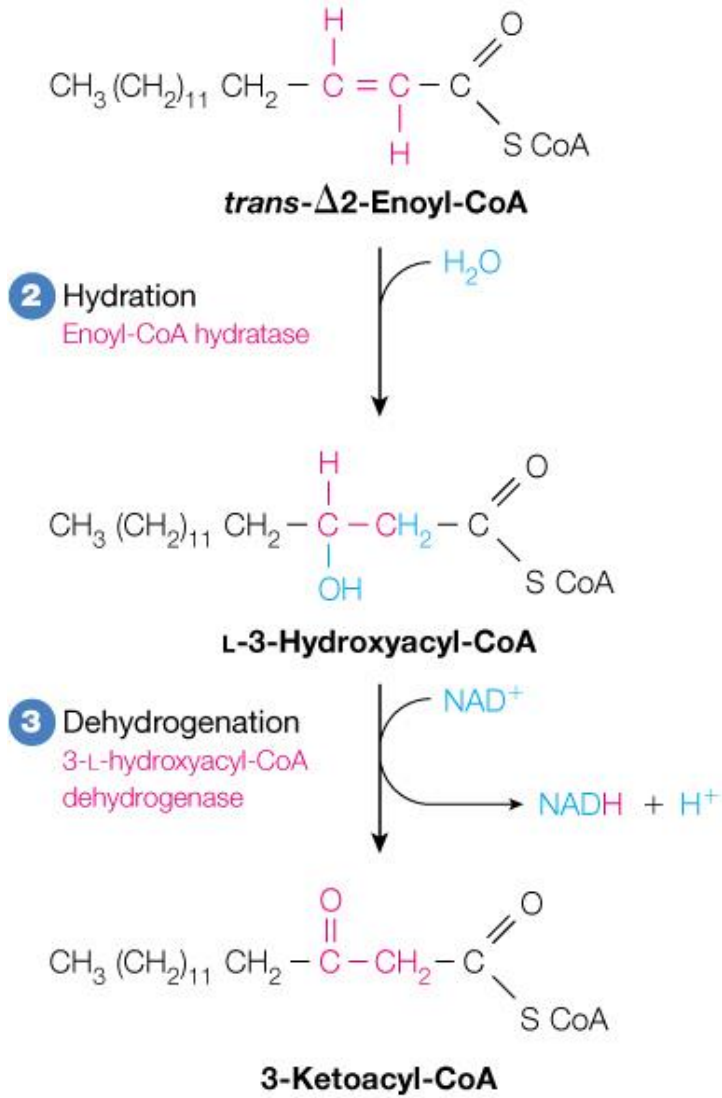
Reaction 1 – Acyl-CoA Dehydrogenase



ETF = electron-transferring flavoprotein

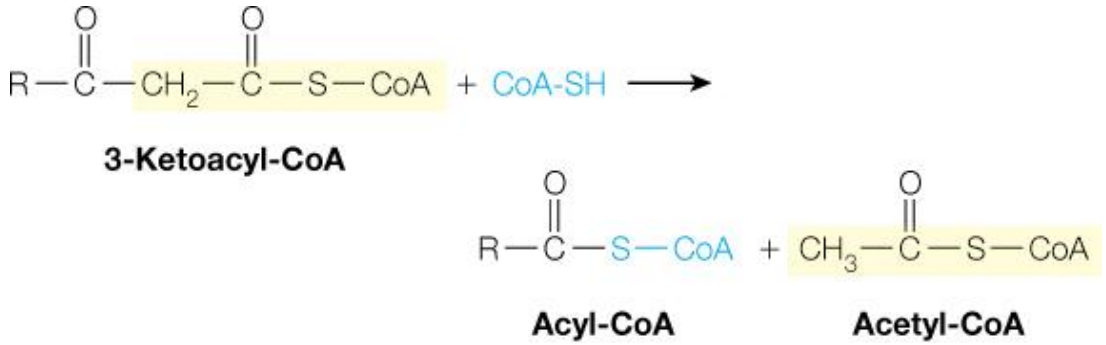
Reactions 2 and 3 – Enoyl-CoA Hydratase and L-3-Hydroxyacyl-CoA Dehydrogenase

- Both reactions are stereospecific
- Because carbon 3 is β with respect to the carboxyl carbon, this pathway has been called β-oxidation

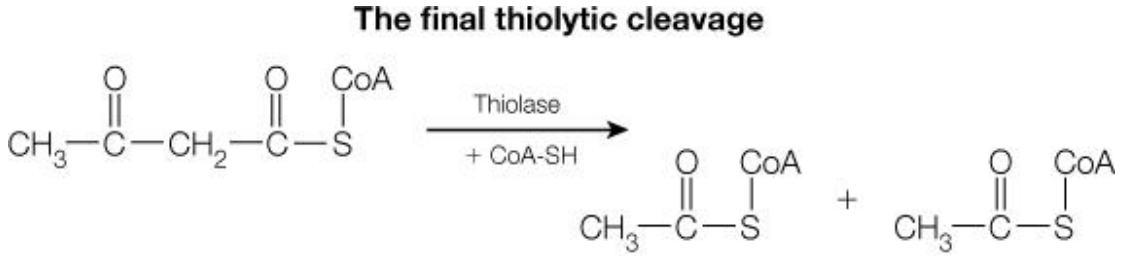


Reaction 4 – Thiolase

- Catalyzes the thiolytic cleavage of 3-ketoacyl-CoA



- Each cycle shortens the acyl chain by two carbons
- The final thiolytic cleavage generates two molecules of acetyl-CoA



Energy Yield from Fatty Acid Oxidation

- Equation for the overall degradation of palmitoyl-CoA (a 16 carbon saturated fatty acid) to 8 acetyl-CoA:

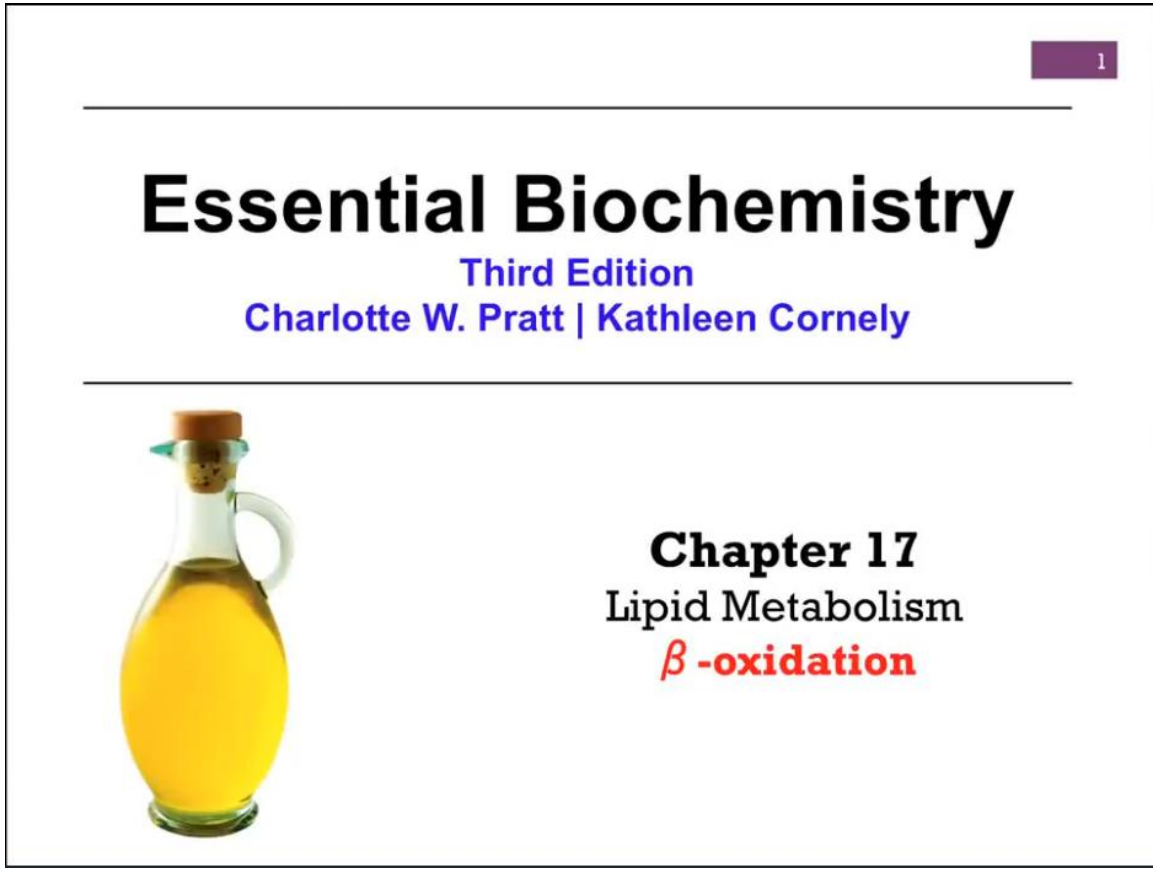
$$\text{Palmitoyl-CoA} + 7\ \text{CoA-SH} + 7\ \text{FAD} + 7\ \text{NAD}^+ + 7\ \text{H}_2\text{O} \rightarrow$$
$$8\ \text{acetyl-CoA} + 7\ \text{FADH}_2 + 7\ \text{NADH} + 7\ \text{H}^+$$

- From 1 mole of palmitate, the energy yield in ATP is:

Reaction	ATP Yield
Activation of palmitate to palmitoyl-CoA	**–2**
Oxidation of 8 acetyl-CoA (by CAC)	8 × 10 = **80**
Oxidation of 7 $FADH_2$	7 × 1.5 = **10.5**
Oxidation of 7 NADH	7 × 2.5 = **17.5**
Net: Palmitate → CO_2 + H_2O	**108 - 2 = 106**

Youtube video

https://www.youtube.com/watch?v=L5QSebY176k



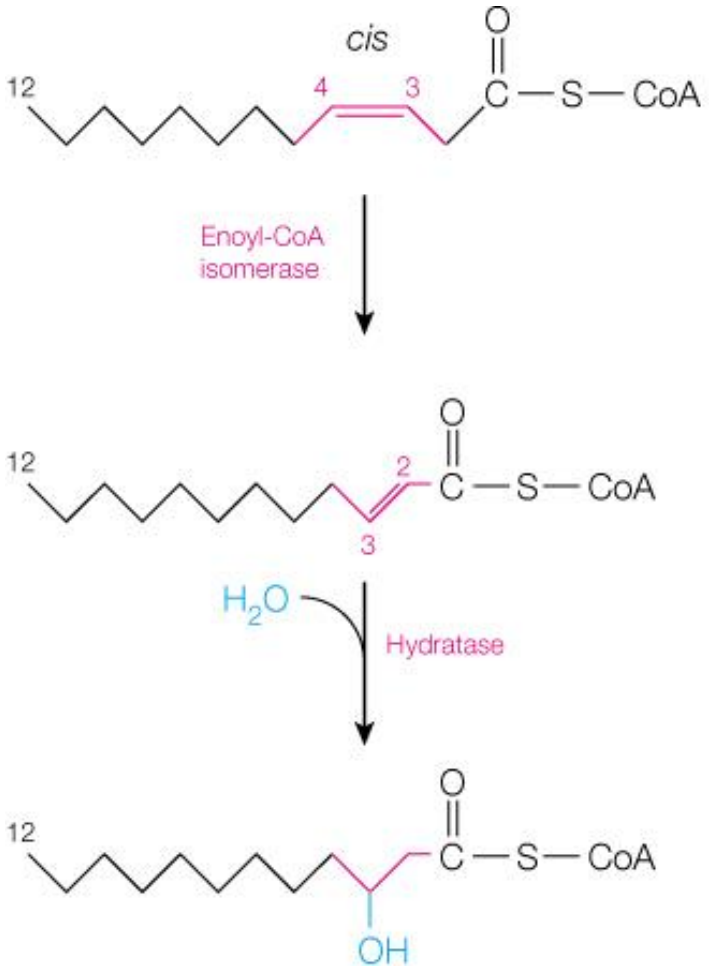
Total ATP using the video calculation

- Note: QH_2 is equivalent to $FADH_2$

1. $NADH+FADH_2$ = 4 ATPs
2. ($NADH+FADH_2$) from 7 rounds of β-oxidation = 28 ATPs
3. 1 Ac-CoA = 10 ATPs (from CAC+ETC+OxPhos)
4. 1 palmitate (16 C) produces 8 Ac-CoA (2 C)
5. 8 Ac-CoA = 80 ATPs
6. Total = 28 + 80 = 108 ATPs.
7. Net = 106 ATPs still (same as in slide 24)

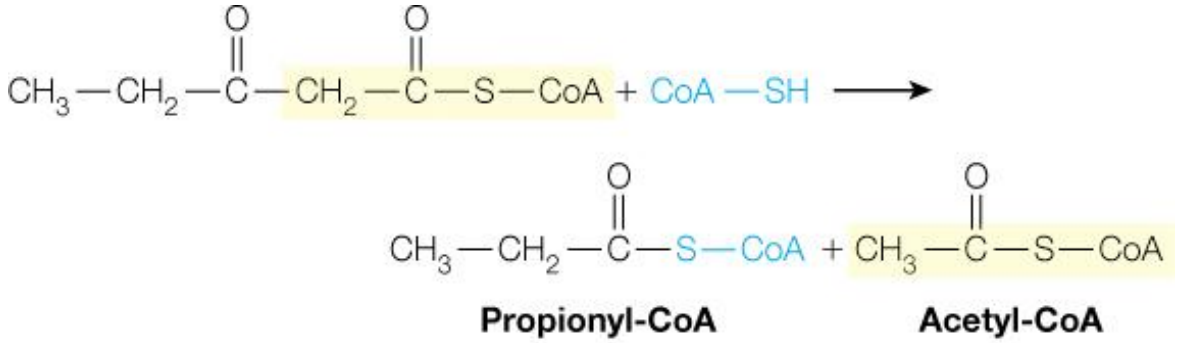
Oxidation of Even Number Unsaturated Fatty Acids

- Two reactions prepare unsaturated acyl-CoA for β-oxidation:
  1) Enoyl-CoA isomerase converts the ***cis* to *trans*** double bond, which is then acted on by enoyl-CoA hydratase (at right)
  2) 2,4-Dienoyl-CoA reductase is used in the **oxidation** of poly unsaturated fatty acids (same from Step 2 on Slide 24)

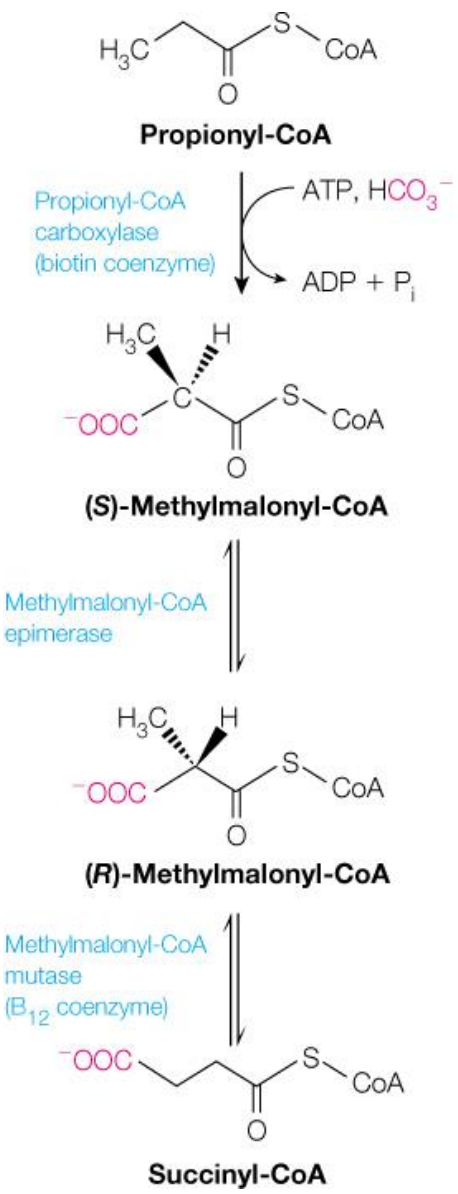


Oxidation of Odd-Numbered Fatty Acids

- Odd-numbered fatty acids can be degraded via β-oxidation to propionyl-CoA and acetyl-CoA



  - Metabolism of propionyl-CoA (to succinyl-CoA) requires three different enzymes (a carboxylase, an epimerase and a mutase) and a coenzyme (deoxyadenosylcobalamin) derived from vitamin B_{12}
  - A *deficiency* of methylmalonyl-CoA mutase or synthesis of the coenzyme from vitamin B_{12} causes methylmalonic acid to accumulate resulting in severe acidosis and nervous system damage (**methylmalonic acidemia**)



Ketogenesis

- During fasting or starvation, oxaloacetate levels fall, limiting flux through citric acid cycle
- Acetate (produced by the liver) is converted back to acetyl-CoA:

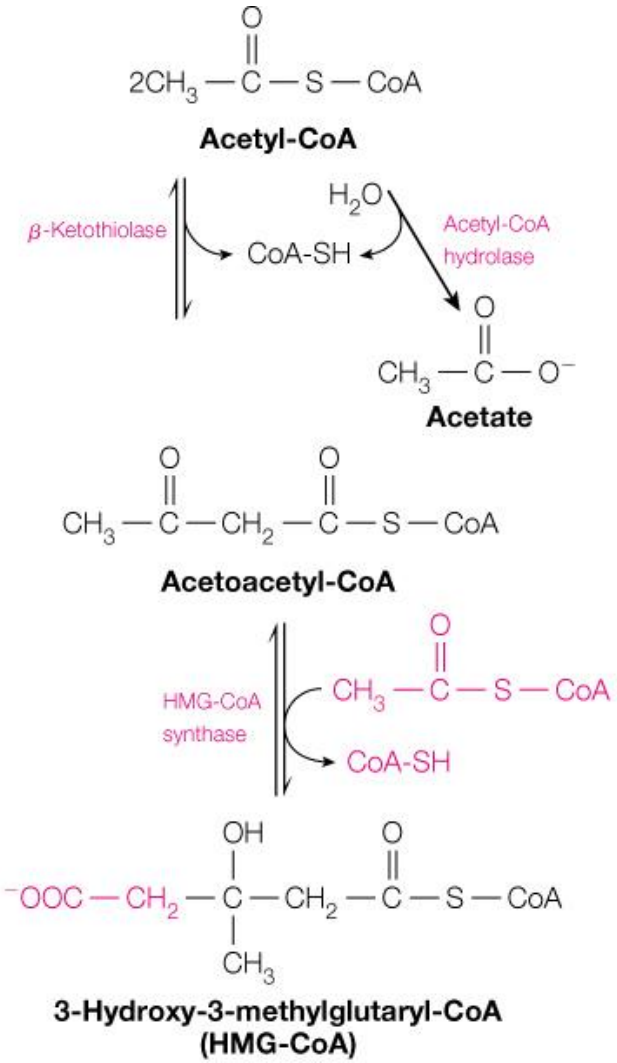
$$\text{acetate} + \text{ATP} + \text{CoA-SH} \rightleftharpoons \text{acetyl-CoA} + \text{AMP} + \text{PP}_\text{i}$$

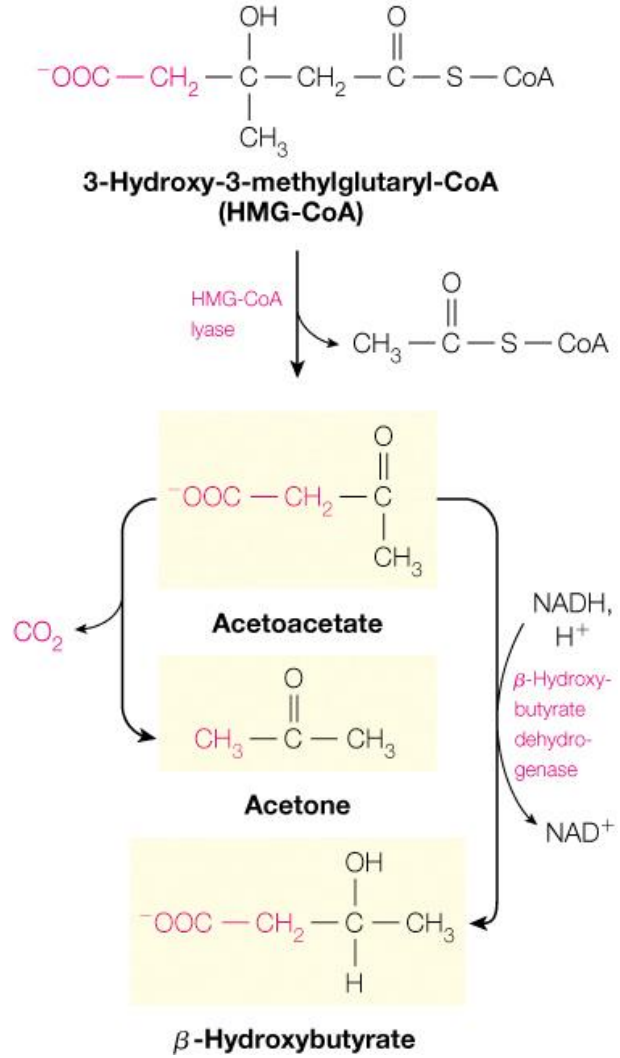
- Acetyl-CoA is converted to **ketone bodies** (i.e., acetoacetate, acetone, and β-hydroxybutyrate) by three to five different enzymes
- Ketogenesis occurs primarily in the liver, and it can be considered an "overflow pathway"
- Ketone bodies can be transported from liver to other tissues for energy generation.

Ketogenesis reactions

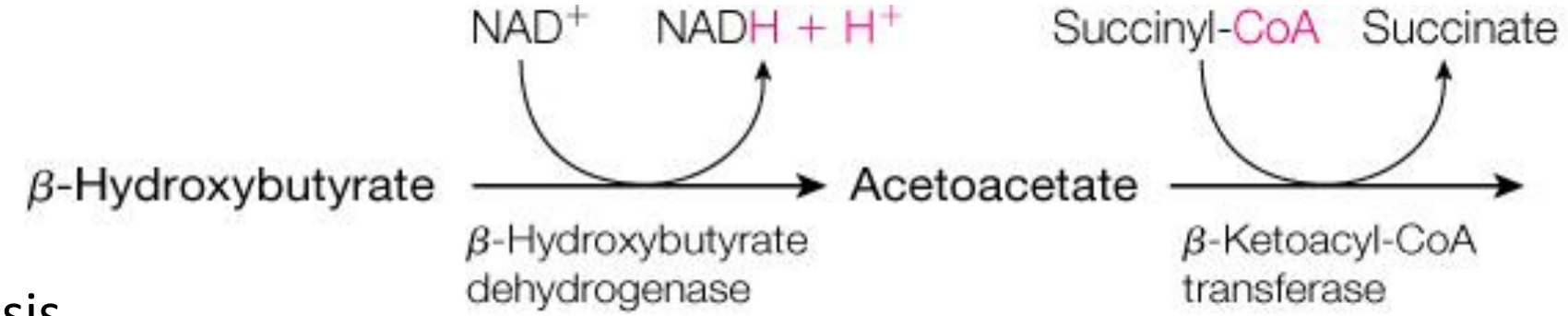
- Ketone bodies are important fuels for peripheral tissues: heart and skeletal muscle
- Main source of fuel for the brain *under starvation conditions* as these molecules can cross the blood-brain barrier (note: the brain uses glucose under normal conditions)

- In diabetes, where glucose utilization is impaired, ketogenesis is stimulated
- Ketone bodies are responsible for the ketoacidosis in diabetes; accumulation of acetoacetate and β-hydroxybutyrate leads to ionization of their carboxyl groups

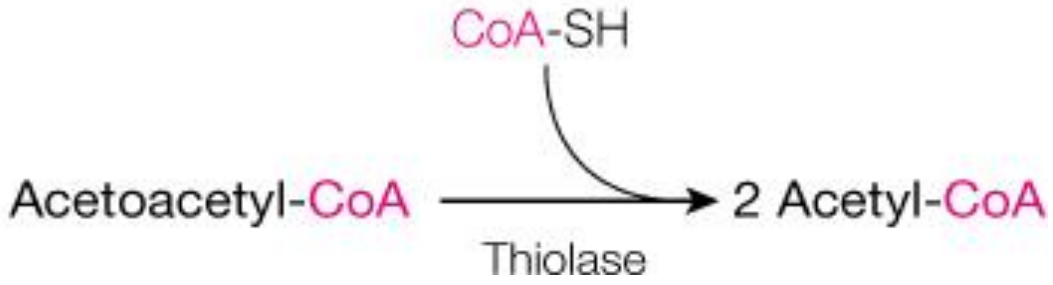




Ketolysis

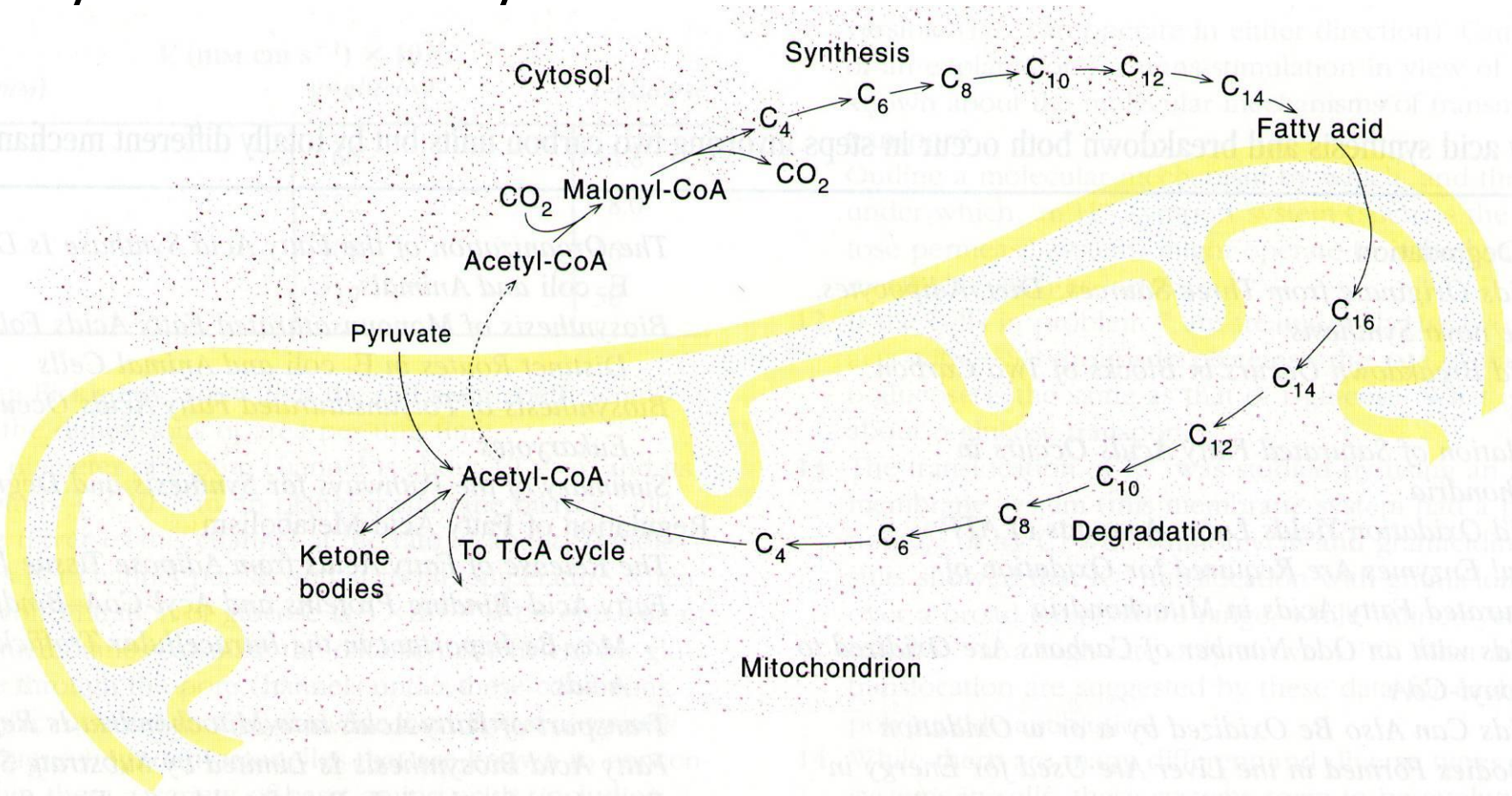


- Reverse of ketogenesis
- Ketone bodies to acetyl-CoA (ketolysis)
- Uses in part the same enzymes as ketogenesis



- However, HMG-CoA synthase and HMG-CoA lyase are replaced by β-ketoacyl-CoA transferase (KCT)
- KCT transfers a CoA moiety from succinyl-CoA (and not acetyl-CoA) to acetoacetate, yielding acetoacetyl-CoA and succinate
- KCT is present in all tissues except the liver, the main ketogenic organ

Degradation – mitochondria;
Synthesis – cytosol



Summary

Fatty Acid Oxidation

- Fatty acid oxidation begins with the activation of the acyl group by formation of a thioester with CoA. The acyl group is transferred to carnitine for transport into the mitochondria, where it is converted back to CoA.
- β-oxidation occurs in four reactions:
  1. formation of an double bond,
  2. hydration of the double bond,
  3. dehydrogenation to form α-ketoacyl-CoA, and
  4. thiolysis by CoA to produce acetyl-CoA and an acyl-CoA shortened by two carbons.
     - This process is repeated until fatty acids with even numbers of carbon atoms are converted to acetyl-CoA and the fatty acids with odd numbers of carbon atoms are converted to acetyl-CoA and one molecule of propionyl-CoA.
- The acetyl-CoA is oxidized by the citric acid cycle and oxidative phosphorylation to generate ATP. Propionyl-CoA is converted to the citric acid cycle intermediate succinyl-CoA.

Summary

Fatty Acid Oxidation

- The oxidation of unsaturated fatty acids requires an isomerase to convert double bonds to double bonds and a reductase to remove double bonds. The oxidation of odd-chain fatty acids yields propionyl-CoA, which is converted to succinyl-CoA through a cobalamin (B_{12})-dependent pathway.

Ketone Bodies

- The liver uses acetyl-CoA to synthesize the ketone bodies acetoacetate and -hydroxybutyrate, which are released into the bloodstream. Tissues that use the ketone bodies for fuel convert them back to acetyl-CoA.