MBMB 1224: ENZYMES, VITAMINS AND MINERALS

Instructor: Paul Sifuna (PhD)

Lecture schedule: **Tuesday** **7- 9 am**

Room **CT 1.2A**

INSTRUCTIONS FOR MBMB 1224 LAB REPORTS

Writing format

COVER PAGE:

TITLE OF EXPERIMENT:

AIM:

BACKGROUND:

MATERIALS/ REQUIREMENTS:

PROCEDURE:

OBSERVATIONS AND RESULTS:

DISCUSSION AND QUESTIONS

CONCLUSION:

REFERENCES:

- **Lab reports submitted in typed format.**

- **References must be cited (google, wikipedia are NOT valid references to be quoted).**

- **Lab report to be handed in one week from the day of experiment. N/B Late submissions will be penalised.**

- **If you copy or hand in someone else's work you score a ZERO for that particular lab practical.**

For each mineral:

- Classification
- Dietary sources
- RDA- recommended dietary allowance
- Biochemical functions in the body
- Metabolism (absorption)
- Deficiencies

Classification

- Macro or Major minerals
  - Sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P), sulfur (S), chloride (Cl)
- Present in body tissues at concentrations >50 mg/kg
- requirement of these is >100 mg/d

- Micro or Trace minerals
  - Manganese(Mg), iron(Fe), cobalt(Co), chromium(Cr), molybdenum(Mo), copper(Cu), zinc(Zn), fluoride(F), iodine(I), selenium(Se)
- Present in body tissues at concentrations <50 mg/kg
- requirement of these is < 100 mg/d

Functions of Minerals

- Some participate with enzymes in metabolic processes (cofactors, e.g. Mg, Mn, Cu, Zn, K)
- Some have structural functions (Ca, P in bone; S in keratin)
- Acid-base and water balance (Na, K, Cl)
- Nerve & muscle function (Ca, Na, K)
- Unique functions: hemoglobin (Fe), Vitamin B_{12} (Co), thyroxine (I)

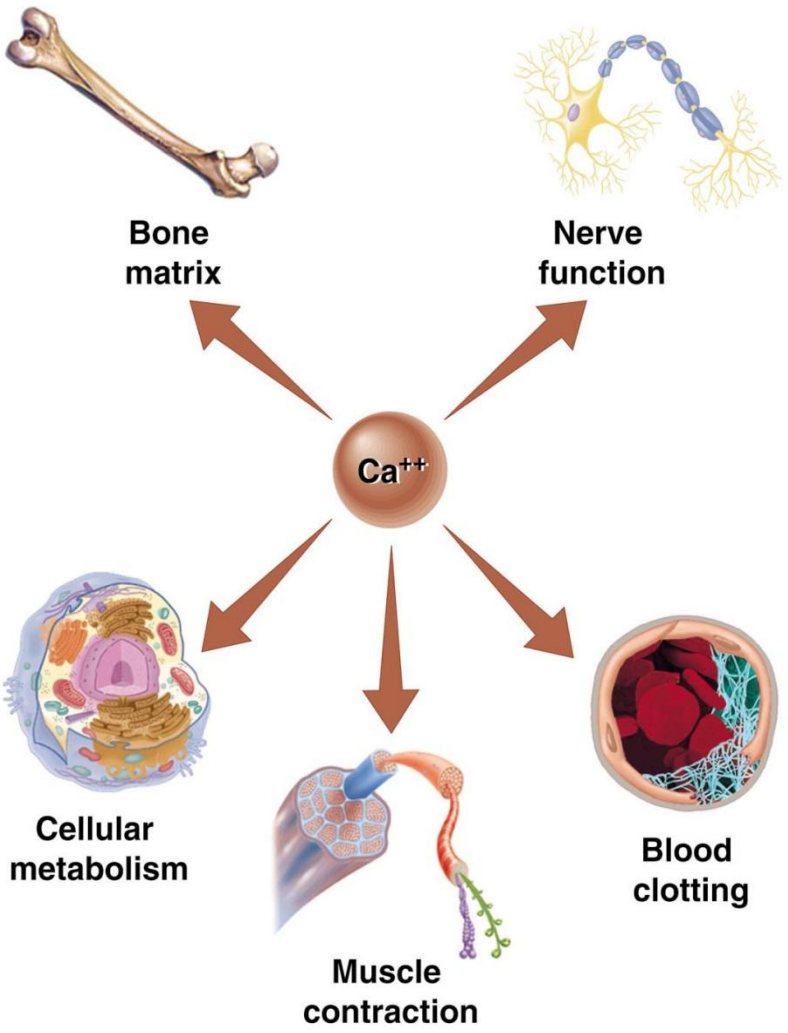
Characteristics of Biochemical Ion Complexes

	Na^+, K^+	Mg^{2+},Ca^{2+}	Zn^{2+}, Ni^{2+}	Fe, Cu, Co, Mo, Mn
Favored Oxidation state	+1	+2	+2	Variable, more than one state
Stability of complex	Very low	Low to medium	High	High (medium for Mn^{2+} and Fe^{2+})
Favored donor atoms	Oxygen	Oxygen	Sulfur or nitrogen	Sulfur or nitrogen (oxygen for Mn and Fe)
Mobility in biological media	Very mobile	Semi mobile	Static	Static, semi mobile for Mn^{2+} and Fe^{2+}

Calcium

Calcium (Ca)

- *Most abundant mineral* in animal tissues
  - 99% Ca in skeleton
  - 1% Present in: Blood & other tissues
- Food Sources:
  - Milk and milk products;
  - Beans, leafy vegetables, fish, cabbage, egg yolk
- Lots of functions
  - Bone structure
  - Nerve function
  - Blood clotting
  - Muscle contraction
  - Cellular metabolism

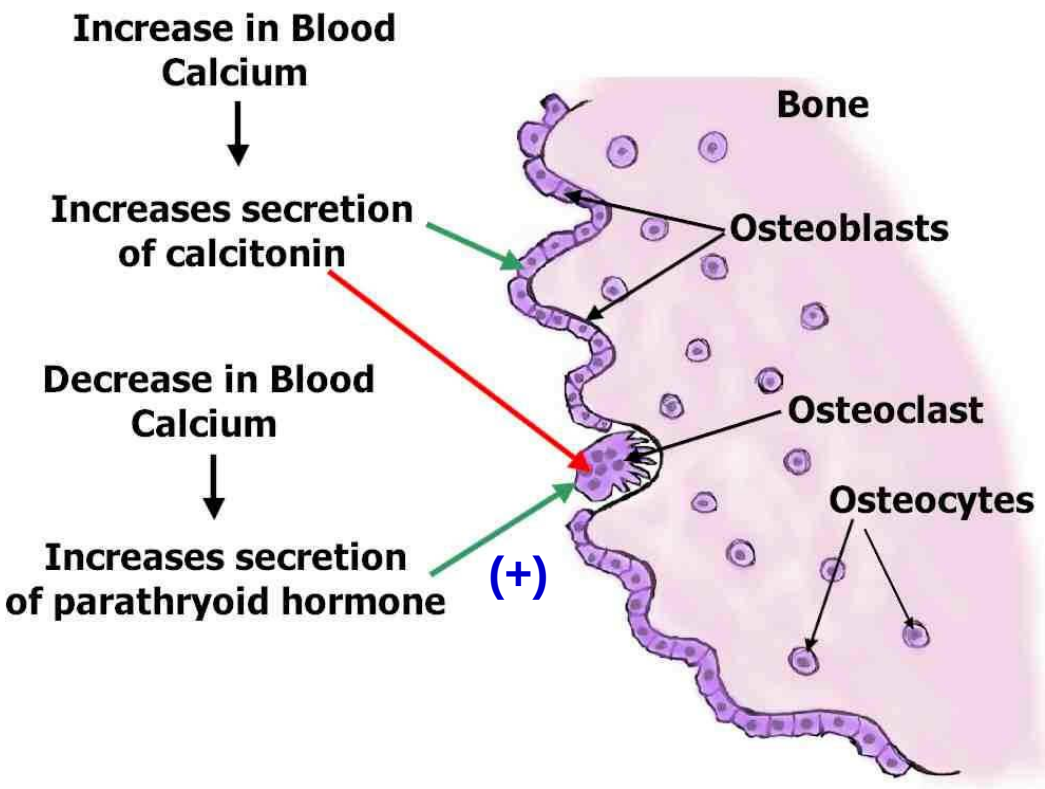


- Absorption of calcium:
  - in small intestine (duodenum), first half jejunum against electrical and concentration gradient, by an energy dependent active process, which influenced by several factors.

- **Mechanism** { **Simple diffusion**
  An active transport involving Ca pump

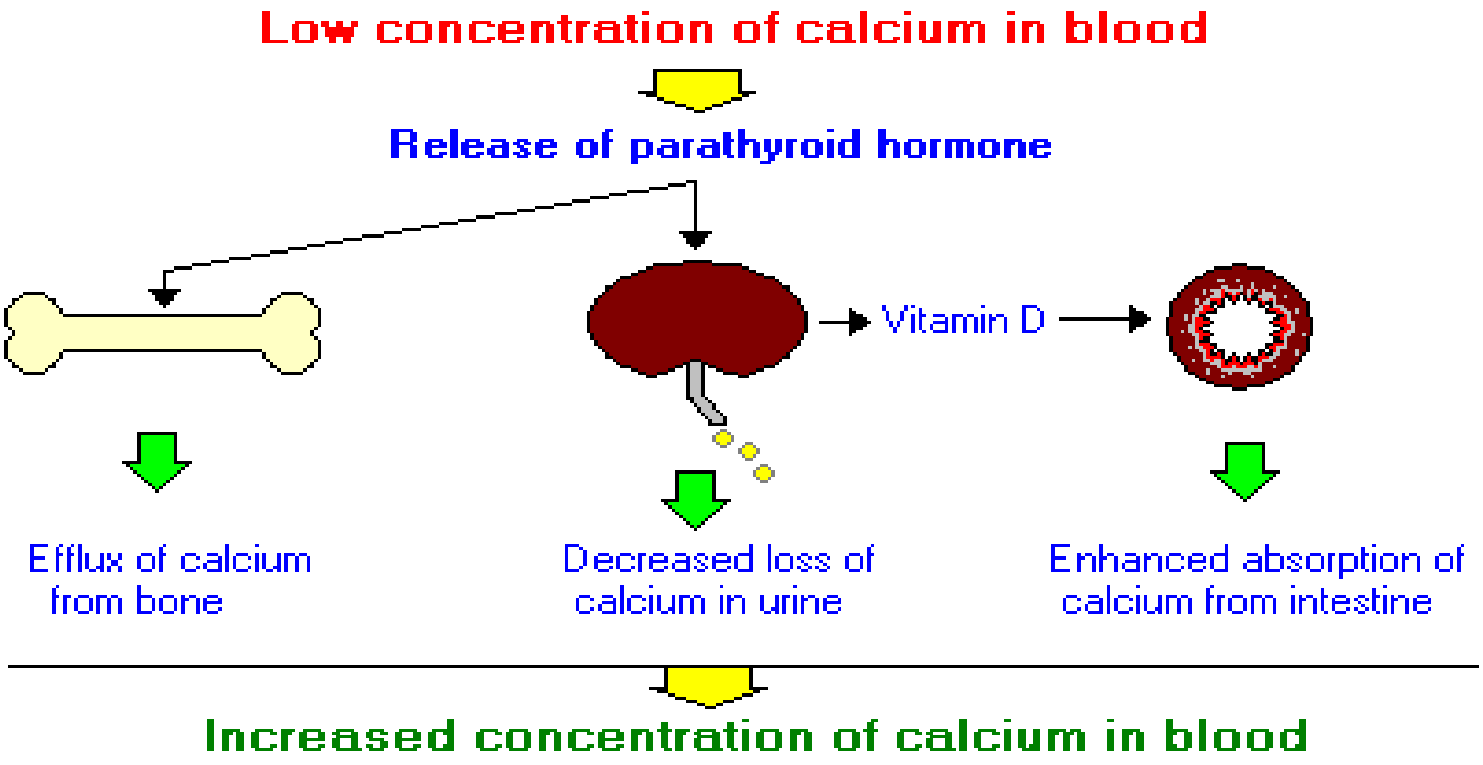
Action of Calcium on the bone

- Decalcification or demineralization of bone, carried out by osteoclasts.
- →blood Ca level ↑
- Note: this is being done at the expense of loss of Ca from bone, particularly in dietary Ca deficiency.



Action on the kidney and intestine

- Action on the kidney: increase the Ca reabsorption.
- Action on the intestine: indirect, increase the intestine absorption of Ca by promoting the synthesis of calcitriol.



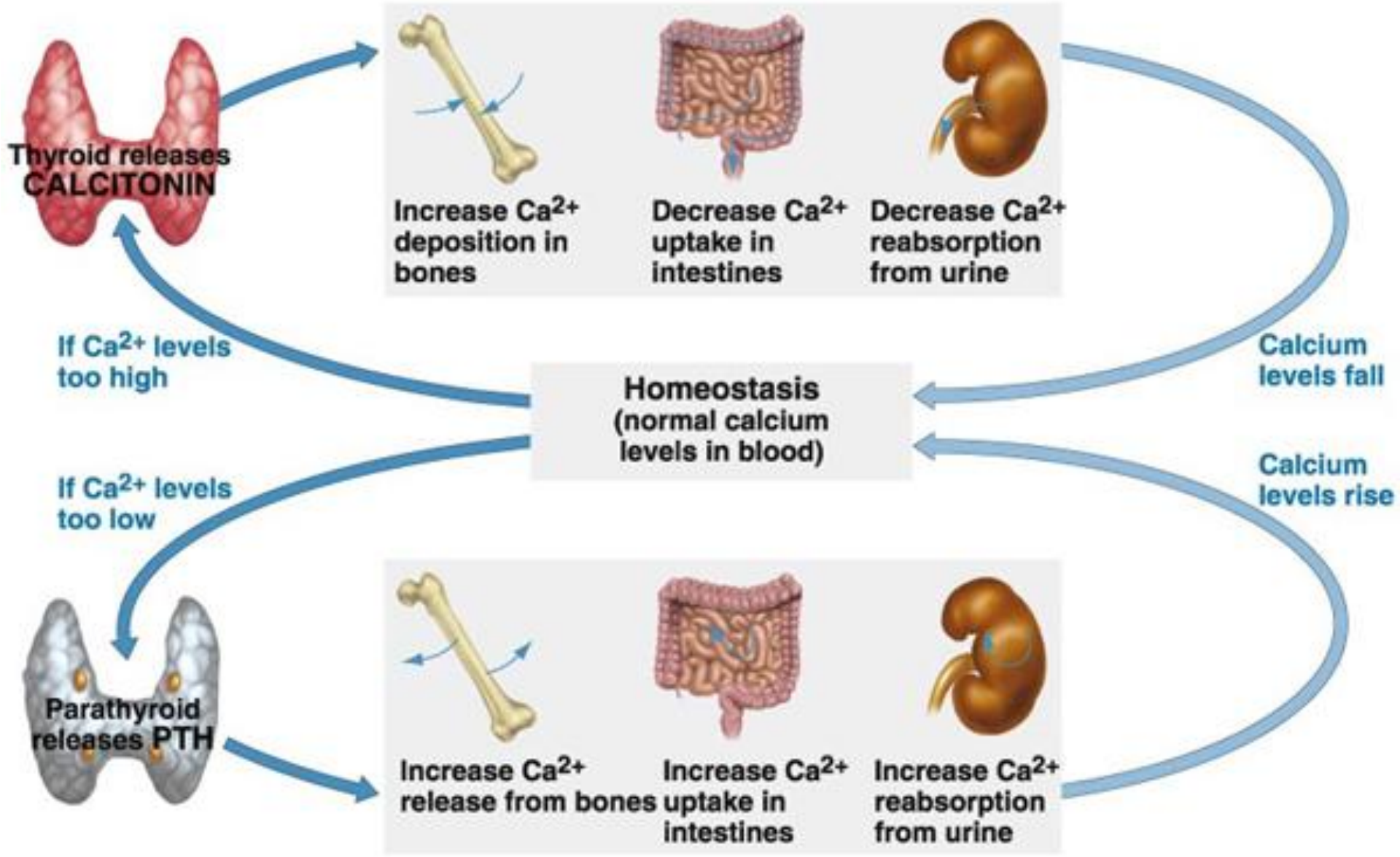
Calcitonin (CT)

- CT, 32aa, a hormone secreted by parafollicular cells of thyroid gland, is *opposite* to that of PTH.

- CT has the ability to decrease blood Ca and P levels and its major target cells also in bone, kidney and intestine.

1. bone: stimulate osteoclasts become osteoblasts, osteogenesis.
2. intestine: inhibit absorption of Ca.
3. kidney: enhance of Ca excretion from urine.

Regulation of Calcium Homeostasis



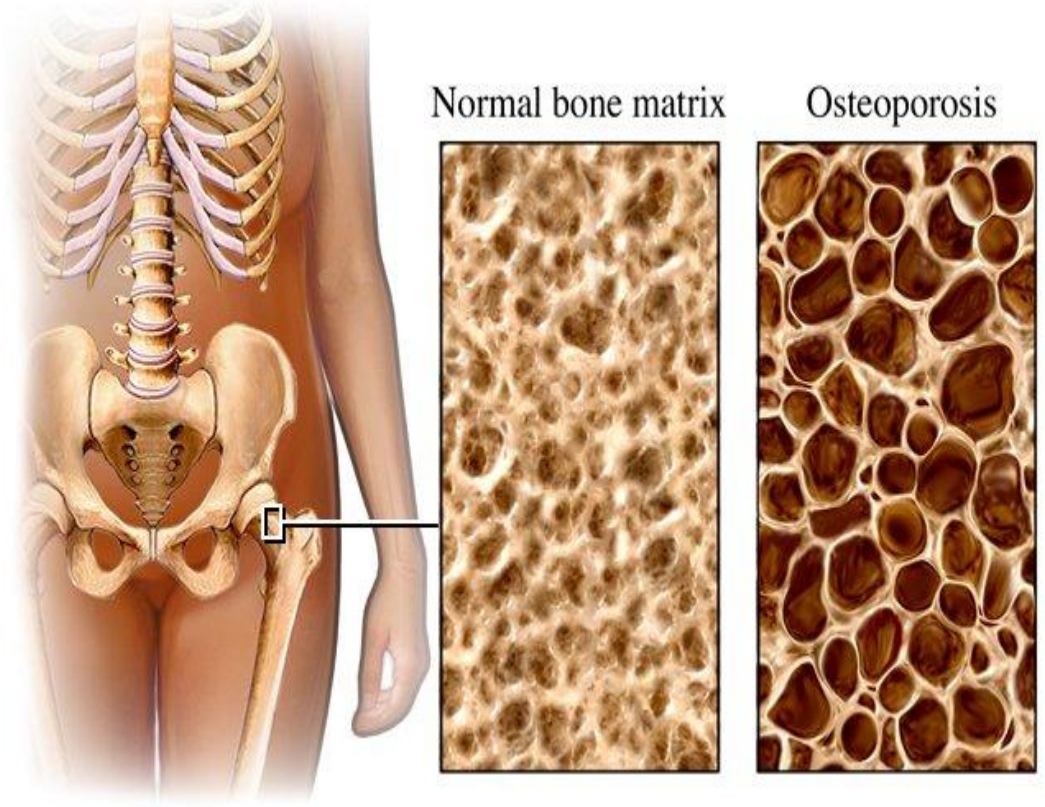
Excretion of Ca

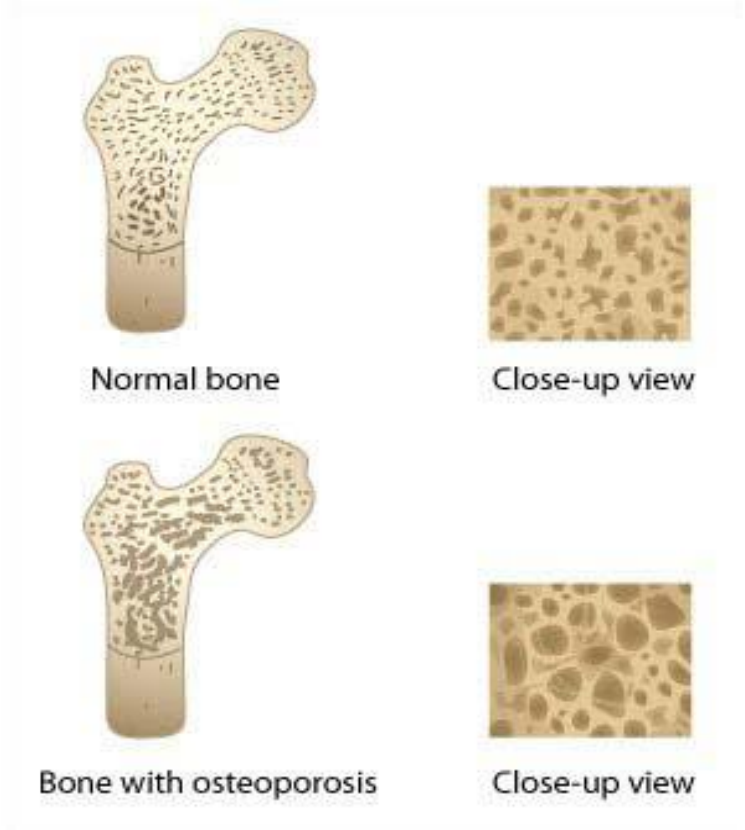
- Partly through the kidney.
- Mostly through the intestine.
- Notice: excretion of Ca into the feces is a continuous process and this is increased in vitamin D deficiency.

Calcium Deficiencies *-Rickets*

➢**weakness and deformity of the bones that occurs from vitamin D deficiency or dietary deficiency of Ca and P in a growing person or animal.**

Calcium Deficiencies -*Osteoporosis*





➢**progressive loss of bone density, thinning of bone tissue and increased vulnerability to fractures in the elderly people of both sexes.**

Phosphorus

Phosphorous (P)

- 80% of P occurs in combination with Ca in the bone and teeth
- About 10% is found in muscles and blood in association with proteins, carbohydrate and lipids
- The remaining 10% is widely distributed in various chemical compounds
- Sources:
  - milk, cereals, leafy vegetable, meat, eggs

Functions of Phosphorus

- Essential for the development of bones and teeth
- Phospholipids, Phosphoproteins
- Component of:
  - DNA & RNA
  - ATP, NAD^+, $NADP^+$
- Energy metabolism: ATP, GTP
- Maintenance of blood pH: phosphate buffer system

Absorption and Excretion

Absorption:

Phosphate absorption occur from jejunum

1. Calcitriol promotes phosphate uptake along with calcium.
2. absorption of P and Ca is optimum when the dietary Ca:P is 1:2-2:1.
3. acidity favors while phytate decreases phosphate uptake by intestinal cells.

Excretion:

About 500 mg phosphate is excreted in urine per day. The reabsorption of phosphate by renal tubules is inhibited by PTH.

Serum phosphate

blood: 40 mg/dl serum: 3-4 mg/dl

※ RBC and WBC have very high content of phosphate.

※ The serum P may exist as *free ions* (40%) or in a *complex form* (50%) with cation as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}. About 10% is *bound proteins*.

Importance of Ca:P ratio

– absorption of P and Ca is optimum when the dietary Ca:P is 1:2-2:1.

– The ratio of plasma Ca:P is important for calcification of bones.

- The product of Ca×P (in mg/dl)
  - Children ~ 50 mg/dl
  - Adults ~ 40 mg/dl
  - In rickets patient -> less than 30 mg/dl

Phosphorus Deficiency

– Rickets, osteomalacia, osteoporosis.

Factors Regulating Ca and P

hormone	1,25 DHCC	PTH	CT
Ca absorption in intestine	↑↑	↑	↓
osteolysis	↑	↑↑	↓
osteogenesis	↑	↓	↑
Ca excretion from kidney	↓	↓	↑
P excretion from kidney	↓	↑	↑
Blood calcium	↑	↑	↓
Blood phosphorus	↑	↓	↓

Iron

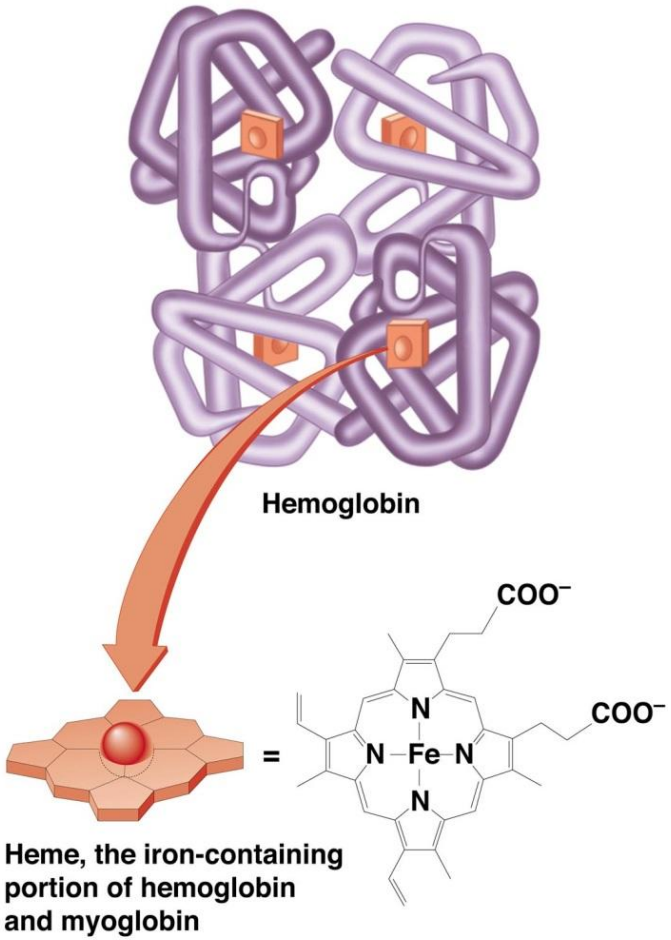
Iron

- Trace element
- The total content of iron in an adult body is 3-5 g

1. About 70%: in the erythrocytes of blood as a constituent of Hb
2. At least 5%: in Mb of muscle
3. Heme is the most predominant iron containing substance: e.g. Hb, Mb, cytochromes
4. Non-heme iron: e.g. transferrin, ferritin

Functions of Iron

- O_2 and CO_2 transport via hemoglobin
  - Thus, necessary for ATP production!
- Essential component of many enzymes
- Immune function
- Brain function
  - Iron deficiency/toxicity thought to slow mental development in kids.



Dietary requirements

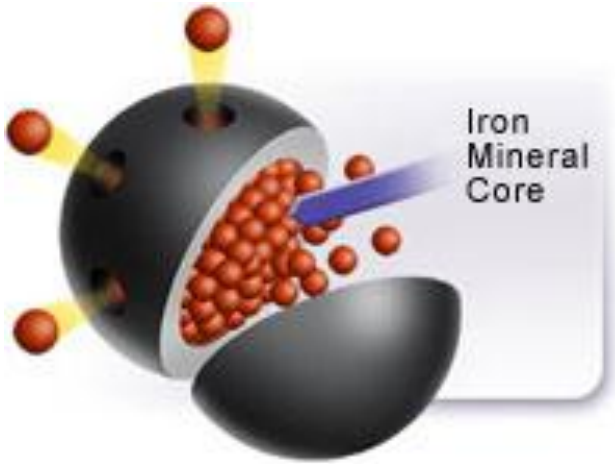
- Dietary requirements:
  - Adult man: 10 mg/day
  - Menstruating woman: 18 mg/day
  - Pregnant and lactating woman: 40 mg/dl

- Sources:
  - Rich source: organ meats (liver, heart, kidney).
  - Good source: leafy vegetables, pulses, cereals, fish, apple, dried fruits, molasses.
  - Poor sources: milk, wheat, polished rice.

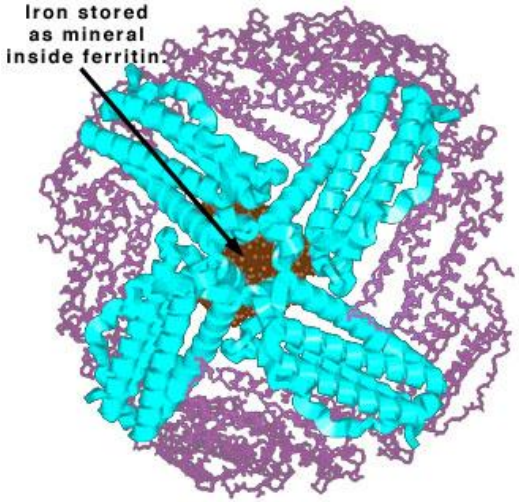
Iron absorption

- Iron is mainly absorbed in the stomach and duodenum.
  - mostly found in the food in ferric form (Fe^{3+}), bound to protein or organic acid.
  - In the acid medium provided by gastric HCl, the Fe^{3+} is released from food.
  - Reducing substances such as *ascorbate (Vitamin C)* and *cystein reduces ferric form (Fe^{3+}) to ferrous form (Fe^{2+}).*
  - Iron in ferrous form (Fe^{2+}) is soluble and readily absorbed.
- How much do we absorb?
  - We absorb iron from the diet only when we need it
  - In normal people, about 10% of dietary iron is usually absorbed.
  - Those with LOW stomach acid secretions absorb less.

- Iron storage
  - Iron can be stored by ferritin (a protein) or hemosiderin
    - Stored in liver, bone marrow (why here?), intestinal mucosa, and spleen
    - A apoferritin molecule can combine with 4,000 atoms of iron.



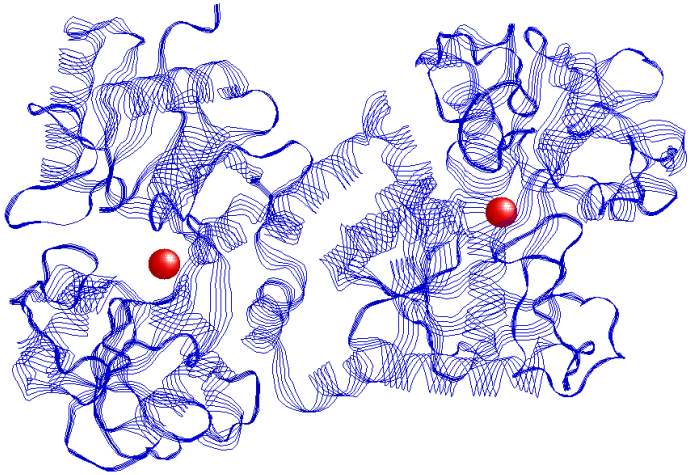
Ferritin



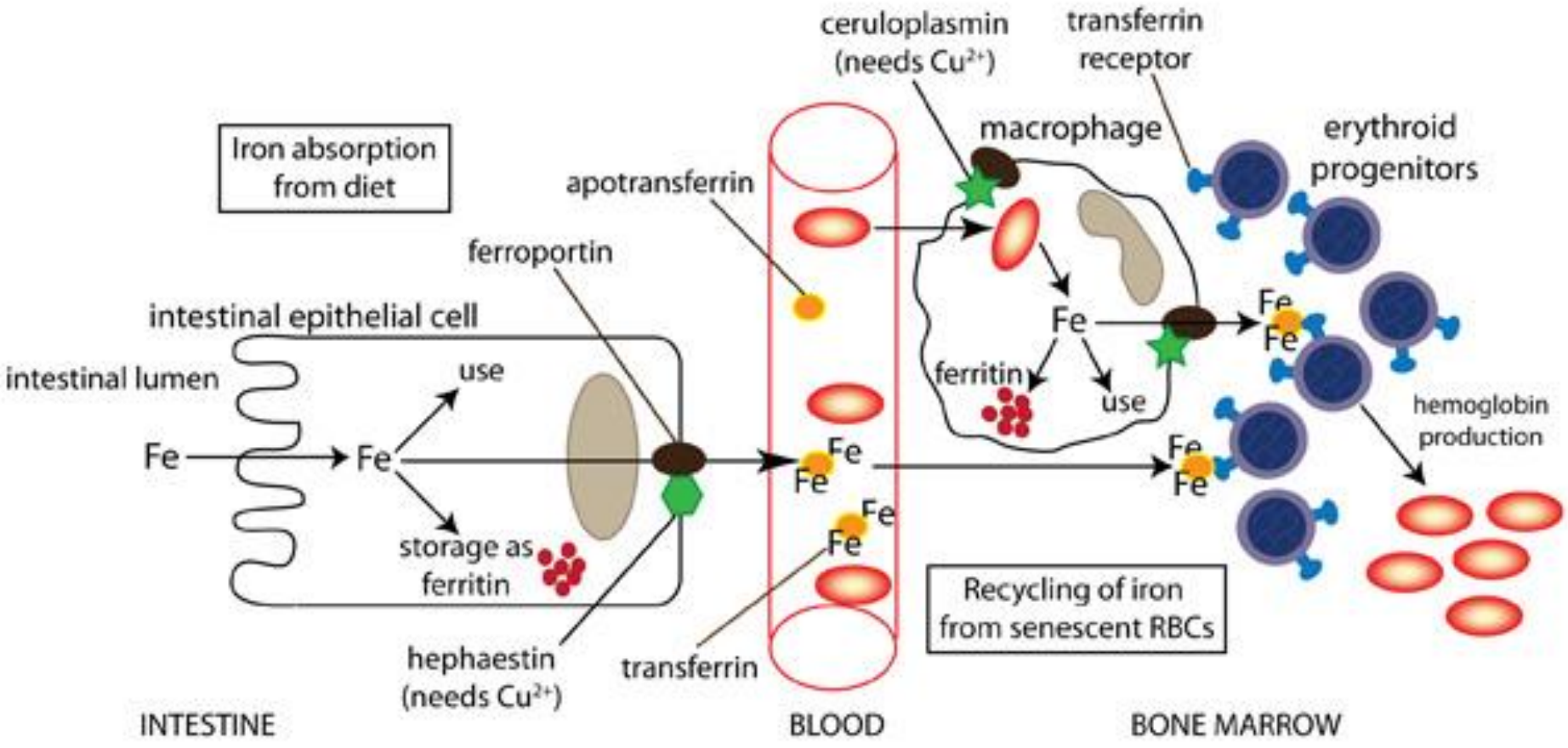
Ferritin

- Iron transport in the plasma
  - The iron enters the plasma in ferrous state (Fe^{2+}), then oxidized to ferric form (Fe^{3+}) by a copper-containing protein, ceruplasmin.
  - Fe^{3+} binds with a specific iron binding protein, namely transferrin. Each transferrin molecule can bind two atoms of ferric iron.

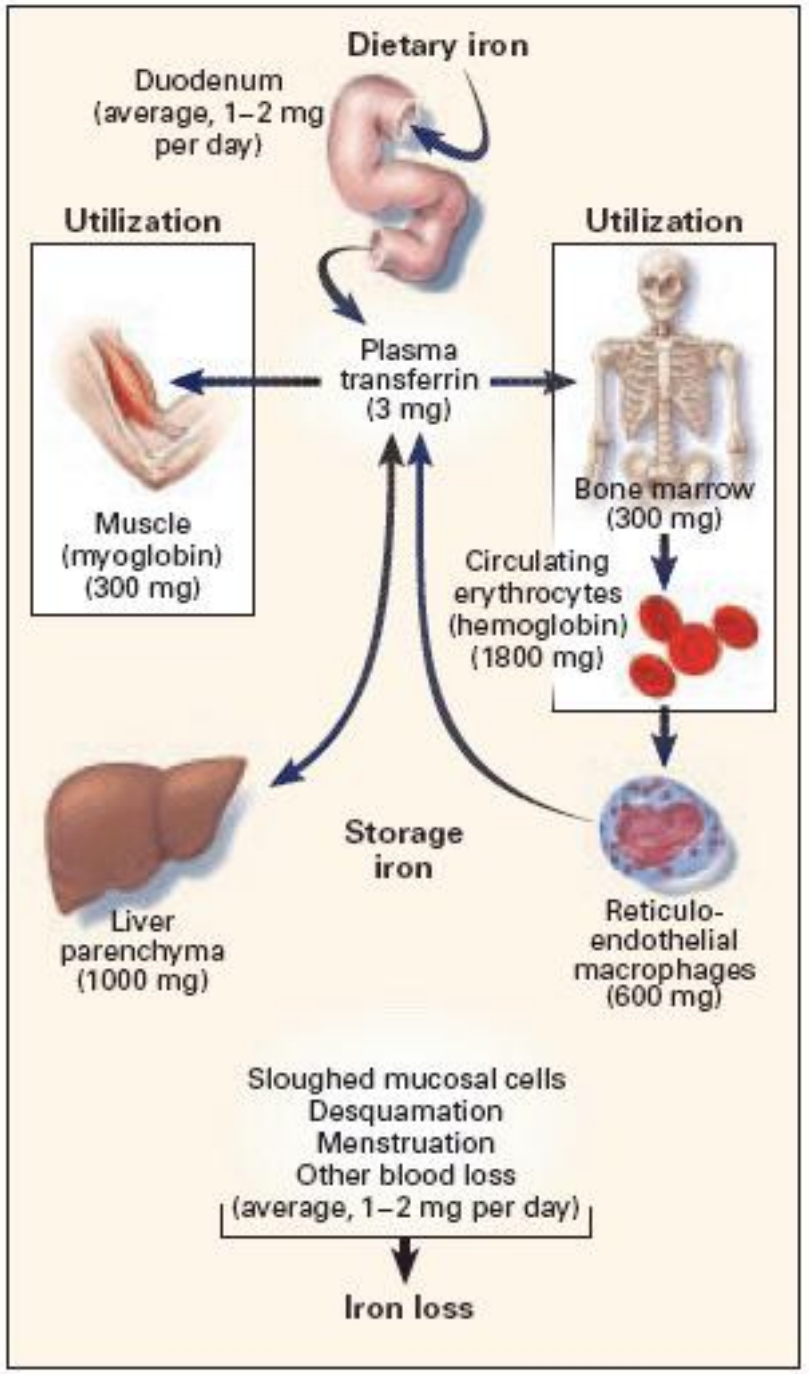
Transferrin



Basic Iron Metabolism



A general overview of **iron** metabolism



Disease states (iron)

1. Iron deficiency Anemia: The most common dietary deficiency worldwide is iron, affecting half a billion persons. However, *this problem affects women and children more*.

a) A growing child is increasing the RBC mass and needs additional iron.

b) Menstruating women require double the amount of iron that men do, but normally the efficiency of iron absorption from the gastrointestinal tract can increase to meet this demand.

c) A developing fetus draws iron from the mother, totaling 200-300 mg at term, so extra iron is needed in pregnancy.

2. Hemosiderosis: this is less common disorder and due to excessive iron in the body.

– It is commonly observed in subjects receiving repeated blood transfusions over the years, e.g. patients of hemolytic anemia, hemophilia.

3. Hemochromatosis: this is rare disease in which iron is directly deposited in the tissue (liver, spleen, pancreas and skin).

– Bronzed-pigmentation of skin, cirrhosis of liver. pancreatic fibrosis are the manifestations of this disorder.

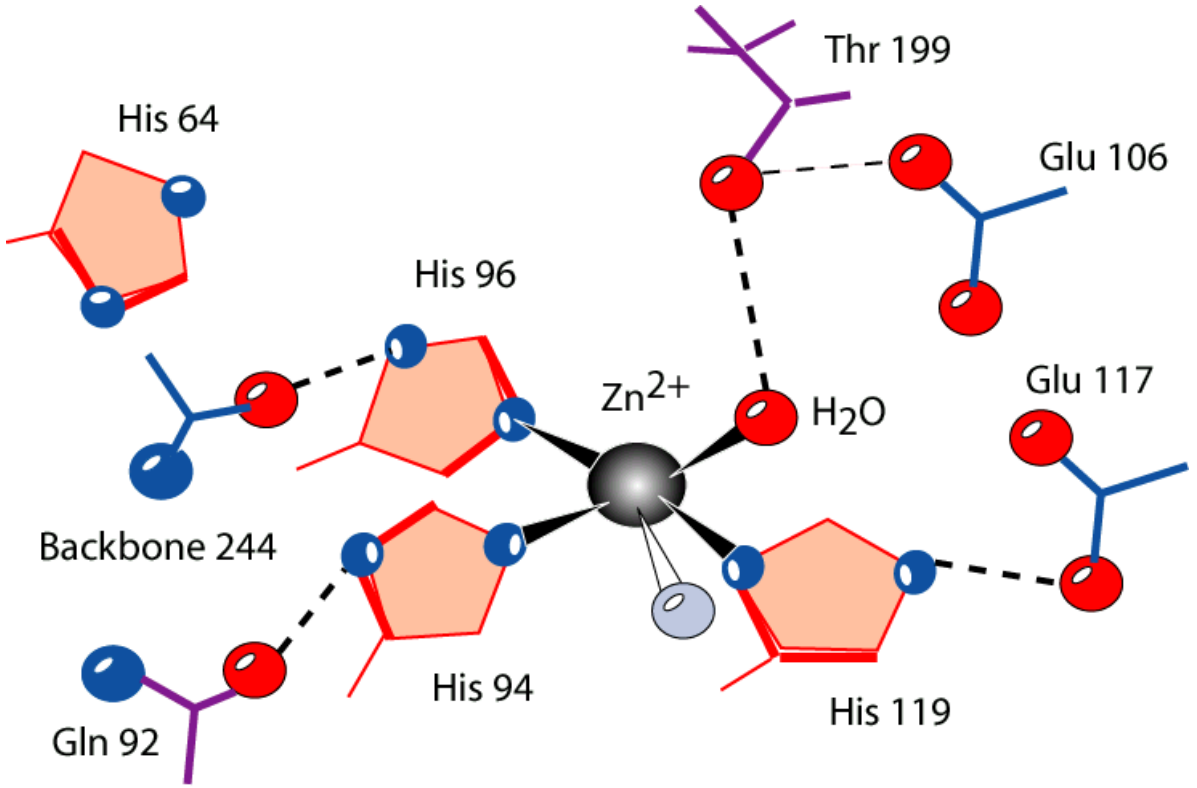
Zinc

Zinc

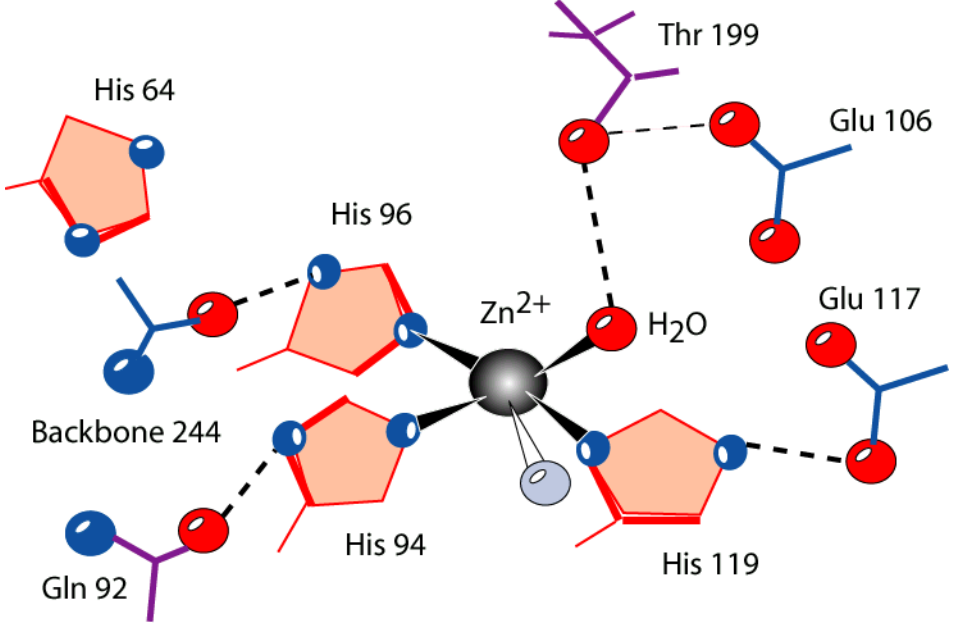
- 300 enzymes require zinc
  - DNA, RNA polymerases
- Numerous hormones require zinc
  - Insulin
  - Epidermal growth factor (EGF)
- Transcription factors (zinc finger proteins)
- Membrane stability
- Myelination
- Skeletal development

Metal Ions (Zinc) in Catalysis-

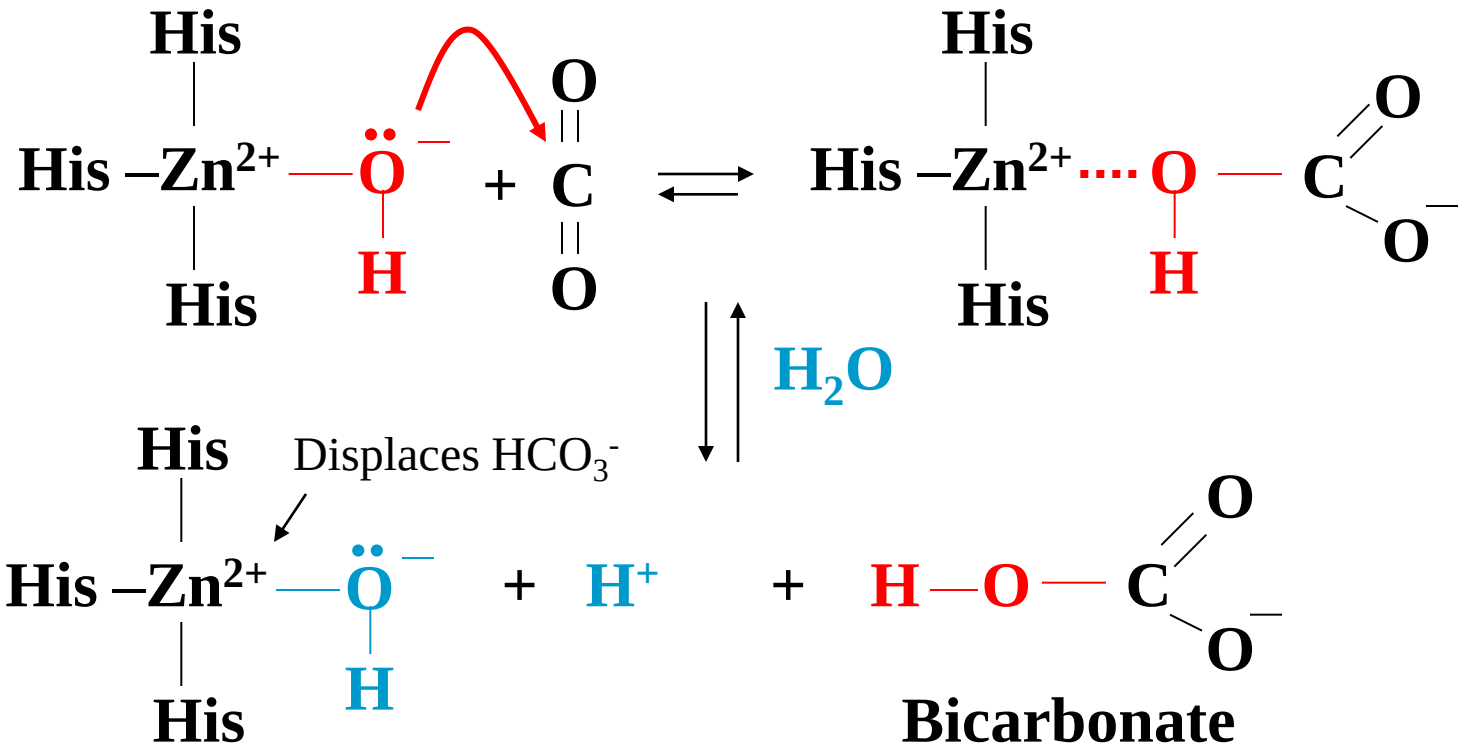
One third of all enzymes require a metal ion for catalysis



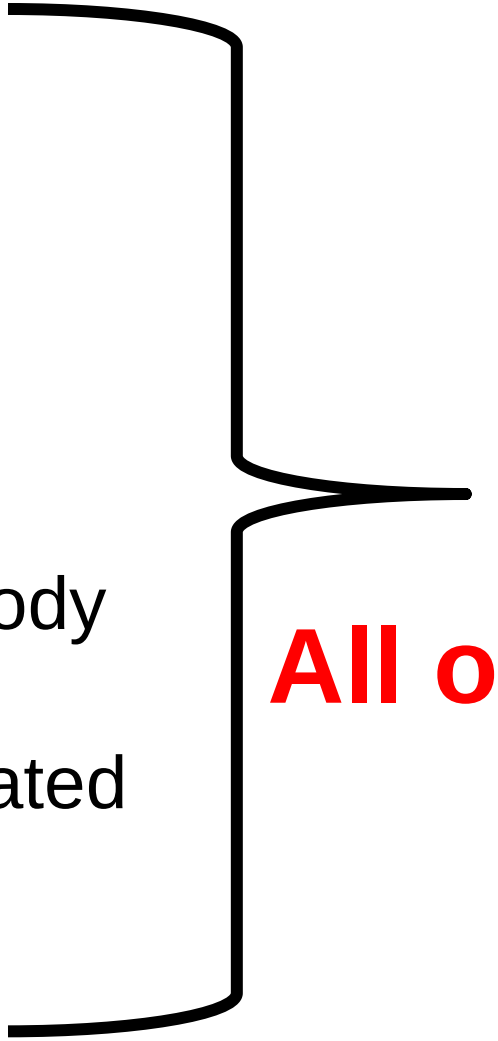
Zn at the Active Site of Carbonic Anhydrase



Zn $^{2+}$Polarizes H_2O, making it a better nucleophile



- Mineral
- Source
- RDA
- Function in the body
- Problems associated with deficiency



All other minerals

Mineral (Major)	Source	RDA	Function	Problems associated with Deficiency
Potassium	Meats, some fish, fruits, vegetables, legumes, dairy products	4700 mg	Nerve and muscle function; acts as an electrolyte	Hypokalemia: weakness, fatigue, muscle cramping, gastrointestinal problems, cardiac problems
Sodium	Table salt, milk, beets, celery, processed foods	2300 mg	Blood pressure, blood volume, muscle and nerve function	Rare
Calcium	Dairy products, dark green leafy vegetables, blackstrap molasses, nuts, brewer's yeast, some fish	1000 mg	Bone structure and health; nerve and muscle functions, especially cardiac function	Slow growth, weak and brittle bones
Phosphorous	Meat, milk	700 mg	Bone formation, metabolism, ATP production	Rare
Magnesium	Whole grains, nuts, leafy green vegetables	310–420 mg	Enzyme activation, production of energy, regulation of other nutrients	Agitation, anxiety, sleep problems, nausea and vomiting, abnormal heart rhythms, low blood pressure, muscular problems
Chloride	Most foods, salt, vegetables, especially seaweed, tomatoes, lettuce, celery, olives	2300 mg	Balance of body fluids, digestion	Loss of appetite, muscle cramps

Mineral (Trace)	Source	RDA	Function	Problems associated with Deficiency
Iron	Meat, poultry, fish, shellfish, legumes, nuts, seeds, whole grains, dark leafy green vegetables	8–18 mg	Transport of oxygen in blood, production of ATP	Anemia, weakness, fatigue
Zinc	Meat, fish, poultry, cheese, shellfish	8–11 mg	Immunity, reproduction, growth, blood clotting, insulin and thyroid function	Loss of appetite, poor growth, weight loss, skin problems, hair loss, vision problems, lack of taste or smell
Copper	Seafood, organ meats, nuts, legumes, chocolate, enriched breads and cereals, some fruits and vegetables	900 µg	Red blood cell production, nerve and immune system function, collagen formation, acts as an antioxidant	Anemia, low body temperature, bone fractures, low white blood cell concentration, irregular heartbeat, thyroid problems
Iodine	Fish, shellfish, garlic, lima beans, sesame seeds, soybeans, dark leafy green vegetables	150 µg	Thyroid function	Hypothyroidism: fatigue, weight gain, dry skin, temperature sensitivity
Sulfur	Eggs, meat, poultry, fish, legumes	None	Component of amino acids	Protein deficiency
Fluoride	Fluoridated water	3–4 mg	Maintenance of bone and tooth structure	Increased cavities, weak bones and teeth

Mineral (Trace)	Source	RDA	Function	Problems associated with Deficiency
Manganese	Nuts, seeds, whole grains, legumes	1.8–2.3 mg	Formation of connective tissue and bones, blood clotting, sex hormone development, metabolism, brain and nerve function	Infertility, bone malformation, weakness, seizures
Cobalt	Fish, nuts, leafy green vegetables, whole grains	None	Component of B_{12}	None
Selenium	Brewer's yeast, wheat germ, liver, butter, fish, shellfish, whole grains	55 µg	Antioxidant, thyroid function, immune system function	Muscle pain
Chromium	Whole grains, lean meats, cheese, black pepper, thyme, brewer's yeast	25–35 µg	Insulin function	High blood sugar, triglyceride, and cholesterol levels
Molybdenum	Legumes, whole grains, nuts	45 µg	Cofactor for enzymes	Rare

Next lecture

Vitamins