Digestion of Lipids & Oxidation of Fatty Acids : Ketogenesis

University of Anbar/College of Pharmacy

Second semester 2020-2021 / Biochemistry II / 3rd stage

References :

1- Harper's Illustrated Biochemistry

2- Lehninger Principles of Biochemistry

By

Dr. Muthanna Owaid Hussein

BIOMEDICAL IMPORTANCE

The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, that are related more by their physical than by their chemical properties.

They have the common property of being

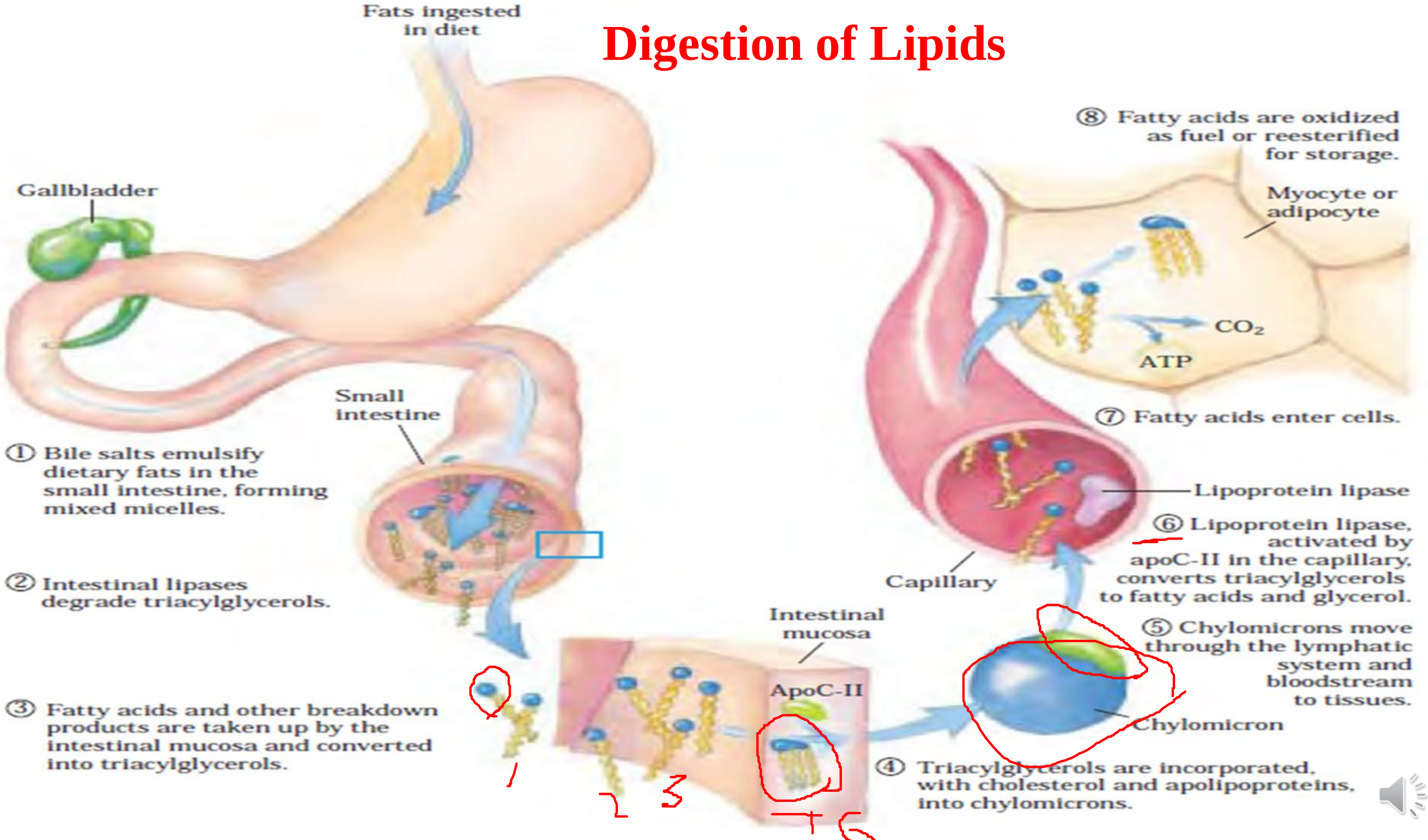
(1) Relatively insoluble in water

(2) Soluble in nonpolar solvents such as ether and chloroform.

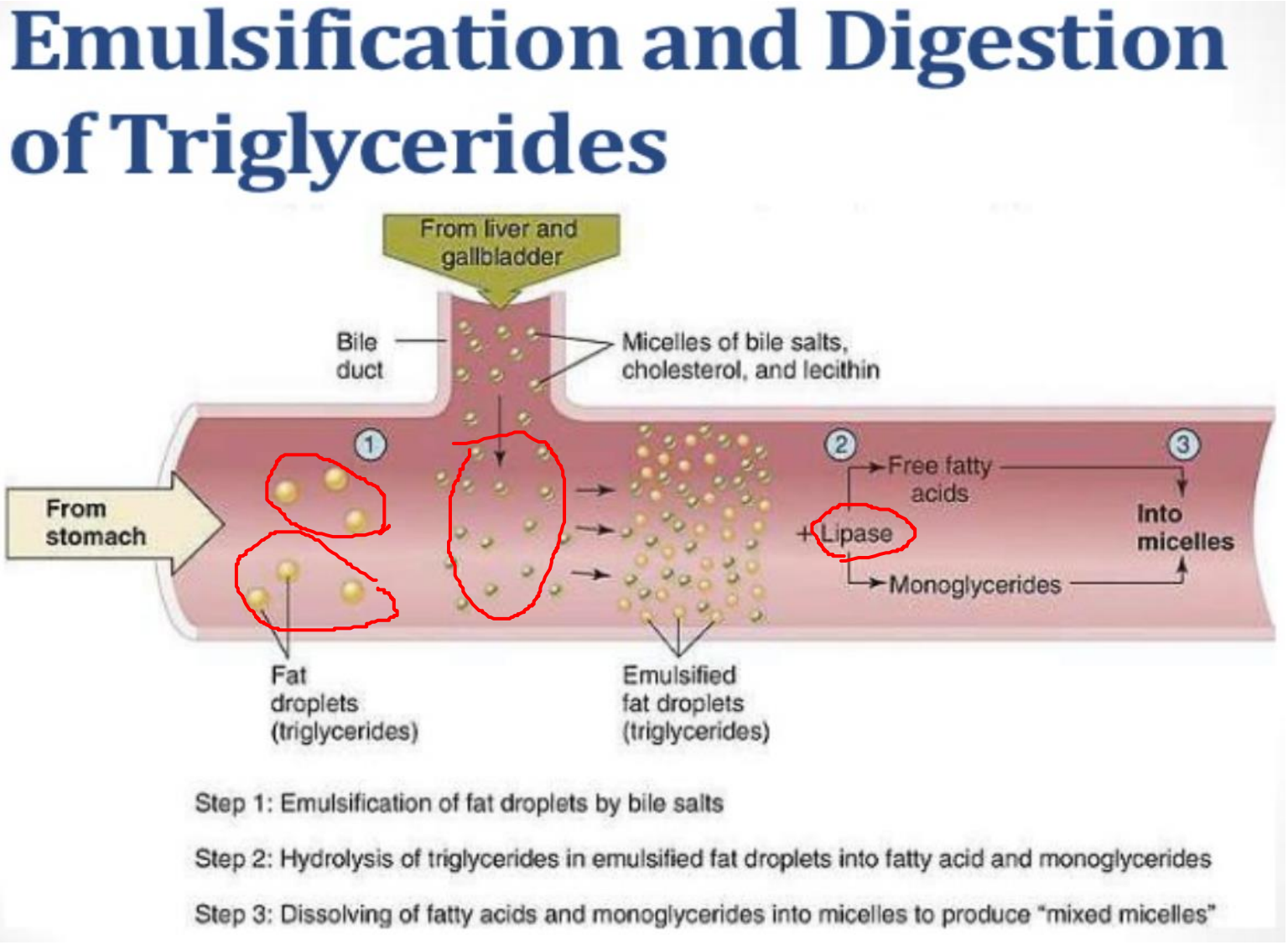
They are important dietary components not only because of the high energy value of fats but also because essential fatty acids, fat-soluble vitamins, and other lipophilic micronutrients are contained in the fat of natural foods. Dietary supplementation with long-chain ω3 fatty acids is believed to have beneficial effects in several chronic diseases, like cardiovascular disease.

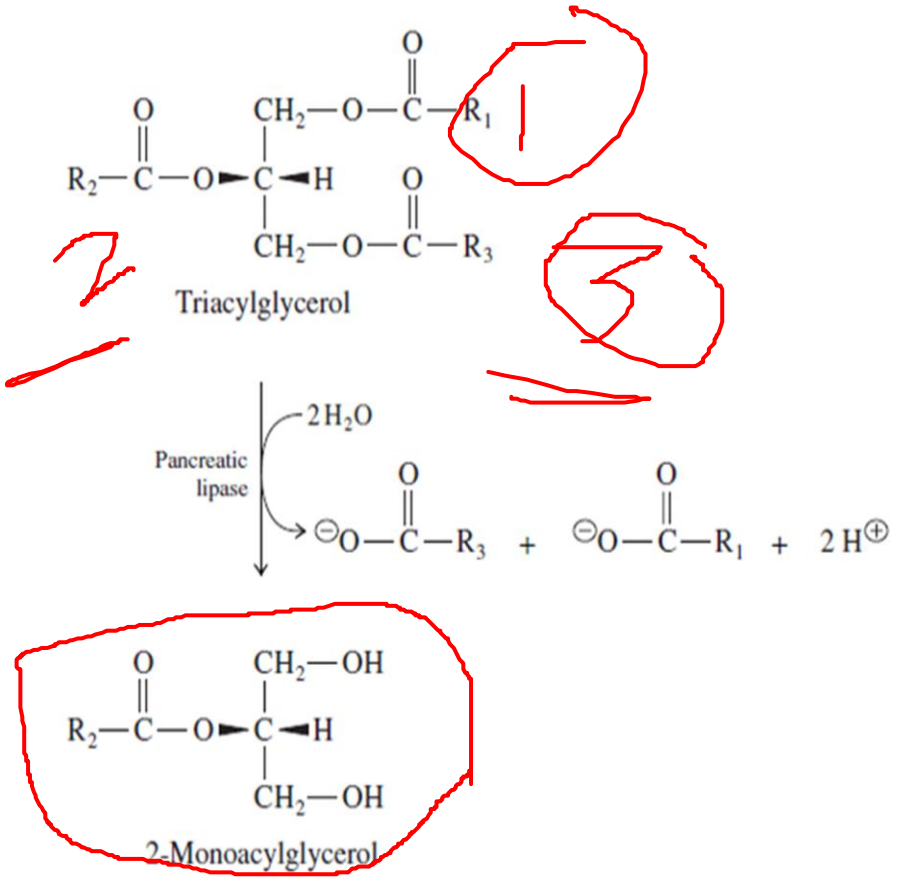
Fatty acids are broken down in mitochondria by oxidation to acetyl-CoA in a process that generates large amounts of energy. When this pathway is happening at a high rate, three compounds, acetoacetate, D-3- hydroxybutyrate, and acetone, known collectively as the ketone bodies, are produced by the liver. Acetoacetate and D-3-hydroxybutyrate are used as fuels by extrahepatic tissues in normal metabolism, but overproduction of ketone bodies causes ketosis. Increased fatty acid oxidation and consequently ketosis is a characteristic of starvation and of diabetes. Since ketone bodies are acidic, when they are produced in excess over long periods, as in diabetes, they cause ketoacidosis, which is ultimately fatal. Because gluconeogenesis is dependent on fatty acid oxidation, any impairment in fatty acid oxidation leads to hypoglycemia. This occurs in various states of carnitine deficiency or deficiency of essential enzymes in fatty acid oxidation, for example, carnitine palmitoyltransferase, or inhibition of fatty acid oxidation by toxins, for example, hypoglycin.

Digestion of Lipids



Pancreatic lipase acts to degrade triacylglycerols in the fat particles. This lipase catalyzes hydrolysis at the C-1 and C-3 positions of a triacylglycerol, producing free fatty acids and a 2-monoacylglycerol

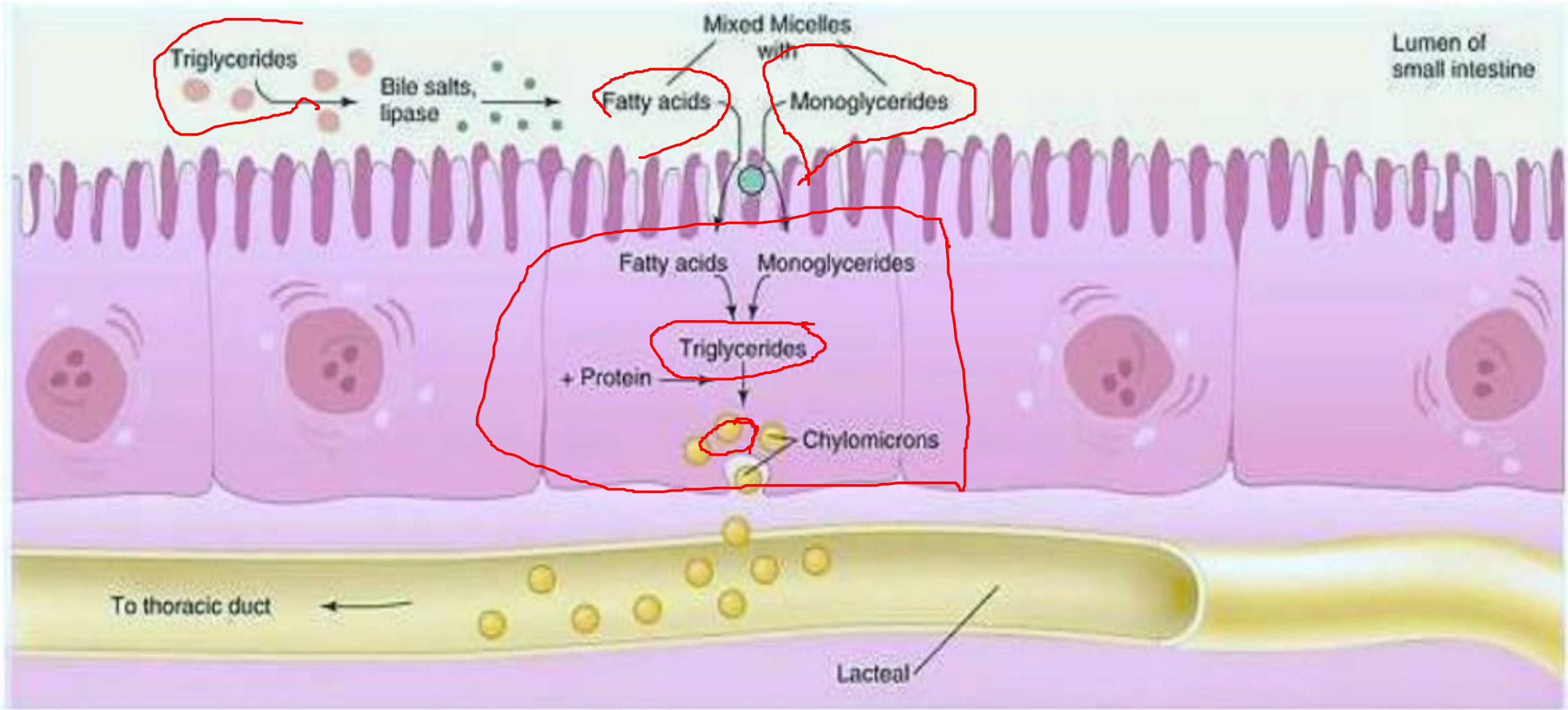




pancreatic lipases.

Note: The obesity-management / weightreduction prescription drug **Xenical** is an inhibitor of pancreatic lipase. It prevents absorption of dietary TG.

Formation and Transportation of Chylomicrons



Absorption of Dietary Lipids:

- On average, fat makes up 37% of calories in American diet
- Most diet lipids of mammals are TAGs
- 90% of the fat we eat is TAG; rest: cholesterol esters, phospholipids, essential unsaturated fatty acids (Linoleic acid (LA) (omega-6) $18{:}2\Delta^{9,12}$ and linolenic acid (omega-3) $18{:}3\Delta^{9,12,15}$), and fat soluble vitamins A, D, E, and K
- In normal individuals, 95% of fat consumed is absorbed and most transported to adipose for storage.
- General principle of dietary lipid assimilation is to hydrolyze large non-absorbable molecules into smaller units.

1. Emulsification starts in the stomach and continues in the lumen of the small Intestine.

2. In the small intestine, fat particles are coated with bile salts and digested

3. Free FA and cholesterol from bile salt mixed micelles are taken up through the intestinal wall

4. Once inside intestinal cells, cholesteryl acyl-CoA esters are formed and FA are resynthesized into triacylglycerols (TAGs).

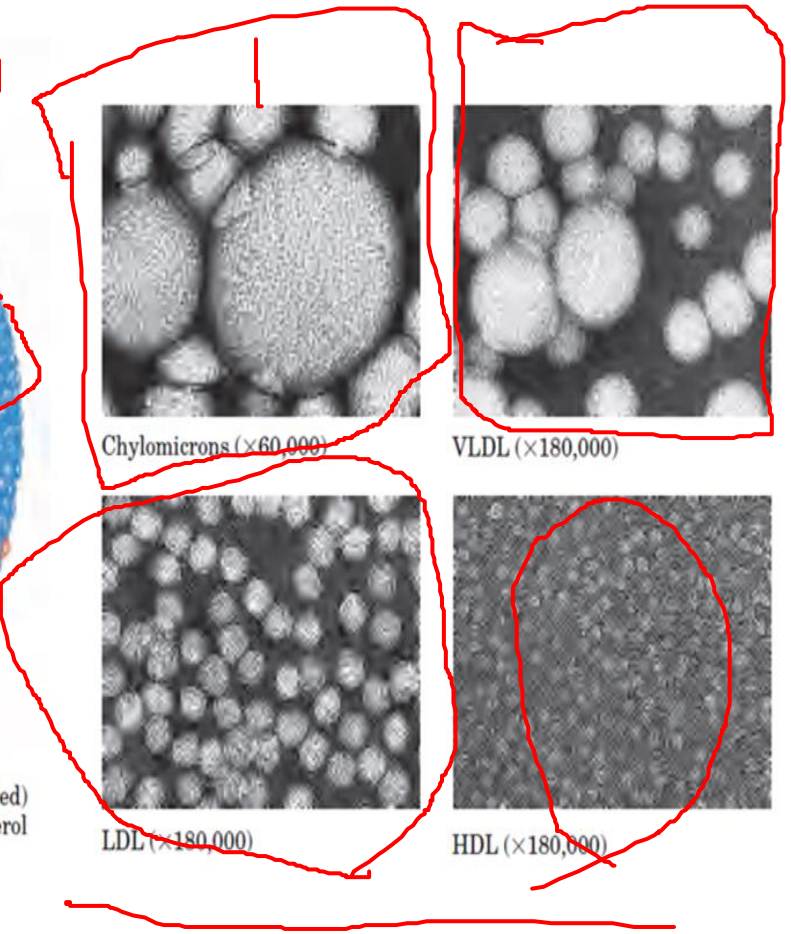
5. TAGs, cholesterol and apoproteins are packaged into a chylomicron (lipoprotein) and exported into blood

6. Lipoprotein lipase on cells lining capillary wall adjacent to ADIPOSE and MUSCLE tissue promotes release of fatty acids; The chylomicrons dock with lipoprotein lipases

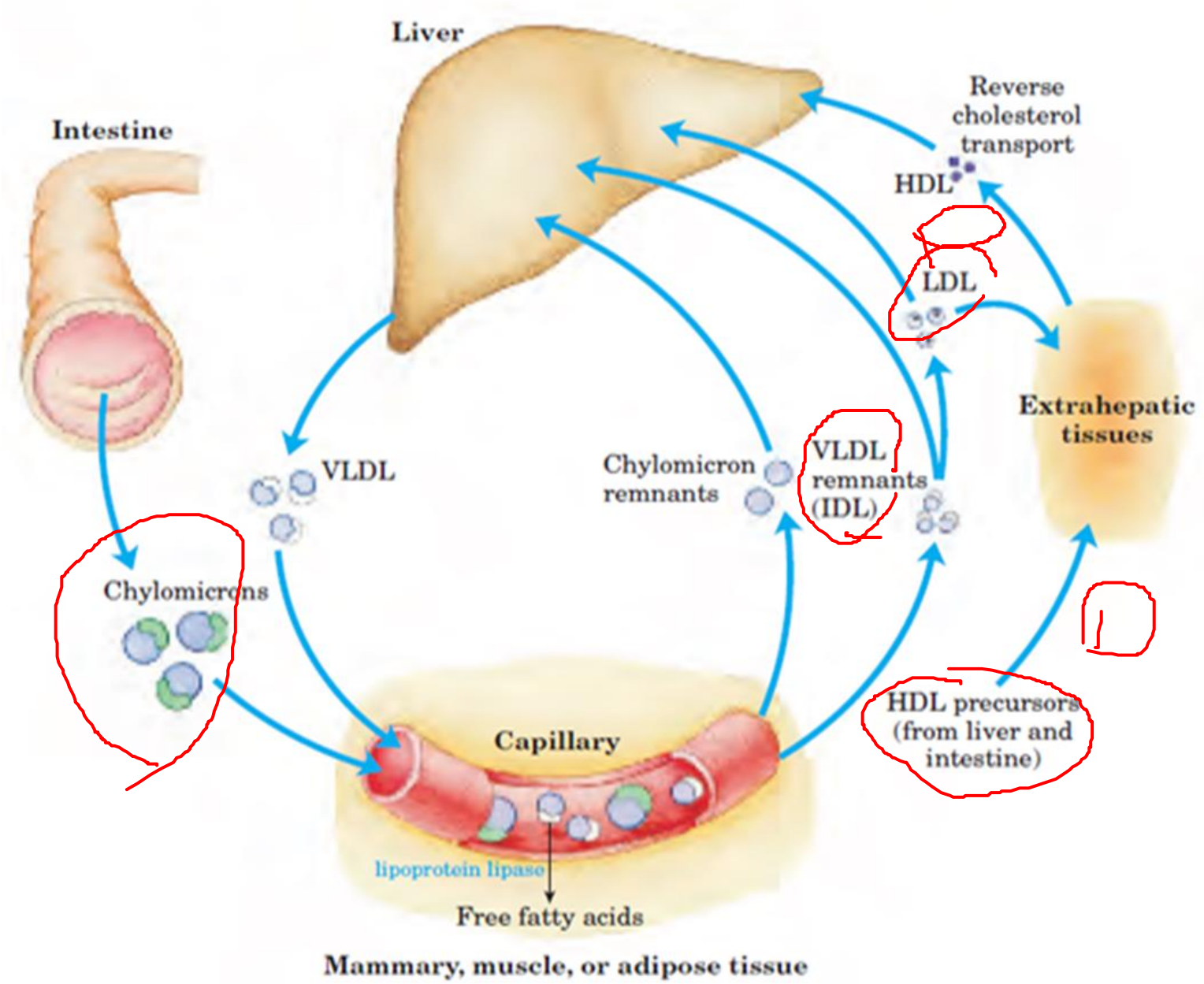
7. Fatty acids are taken up and degraded by β-oxidation in mitochondrial matrix

Lipoproteins

Triacylglycerols, cholesterol, and cholesteryl esters cannot be transported in blood or lymph as free molecules because they are insoluble in water. Instead, these lipids assemble with phospholipids and amphipathic lipid binding proteins to form macromolecular particles known as lipoproteins. A lipoprotein has a hydrophobic core containing triacylglycerols and cholesteryl esters and a hydrophilic surface consisting of a layer of amphipathic molecules such as cholesterol, phospholipids and proteins.



Blood plasma contains several other types of lipoproteins. They are classified according to their relative densities and types of lipid .



Bile salts: Biological Detergents

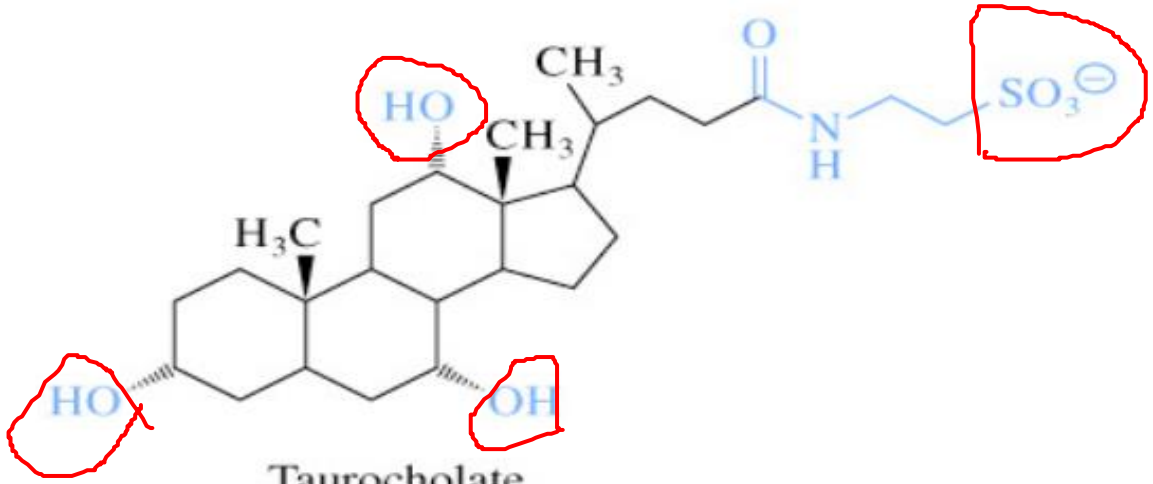
Bile salts are amphipathic: synthesized in liver, stored and secreted by gall bladder to intestine. Made from cholesterol: retain the ring structure but have more hydroxyl groups and a polar side chain – can act as DETERGENTS - Serve to convert water-insoluble lipids to dispersible micellar aggregates

• They emulsify fat globules into smaller micelles, increasing the surface area accessible to lipidhydrolyzing enzymes. Aid in lipid digestion and are essential for the absorption of lipid digestion products

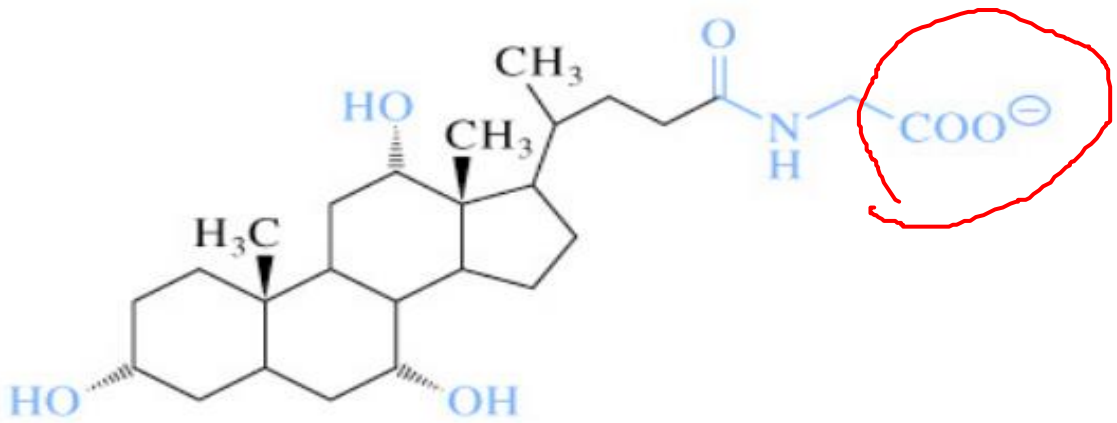
• Also required for efficient intestinal absorption of lipid-soluble vitamins A, D, E, and K

• Taurocholate and glycocholate (cholesterol derivatives) are the most abundant bile salts

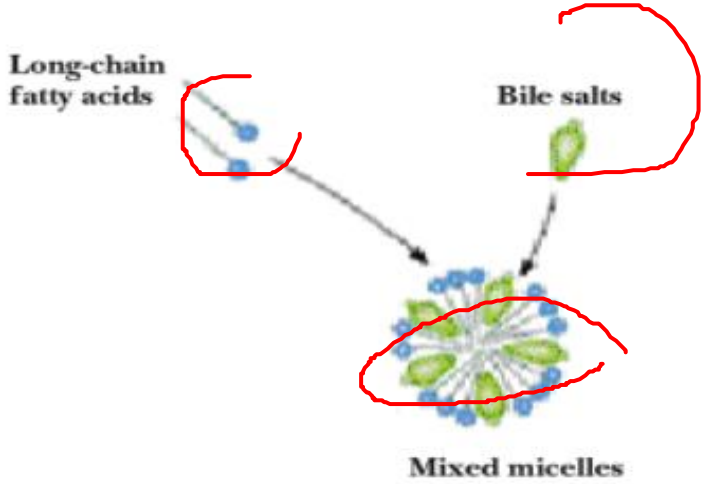
Amphipathic.



Taurocholate

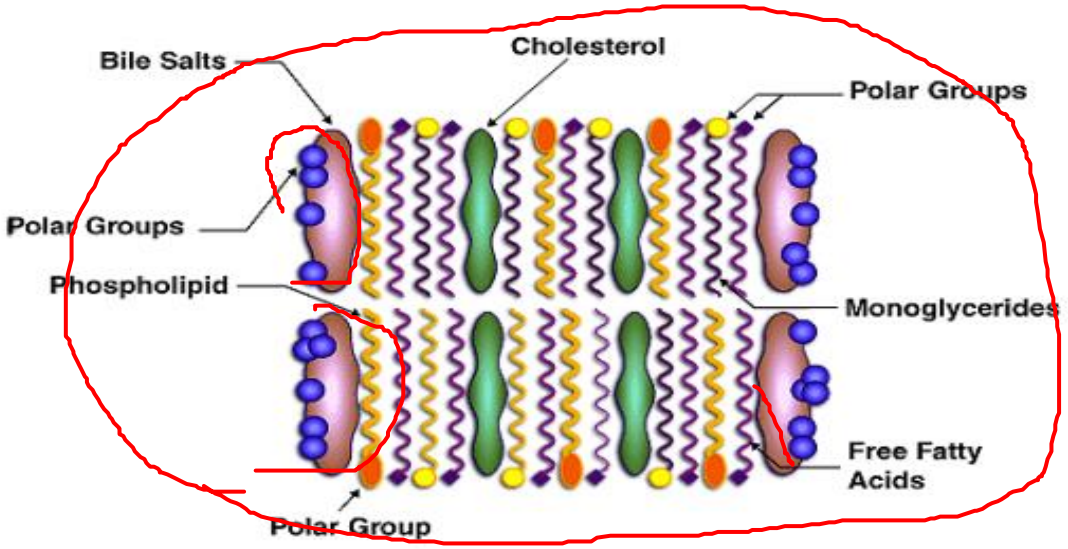


Glycocholate



Mixed micelles

Bile Salts Solubilize Products of Fat Digestion



Mixed Micelle
(bile salt + fat hydrolysis products)
longitudinal section

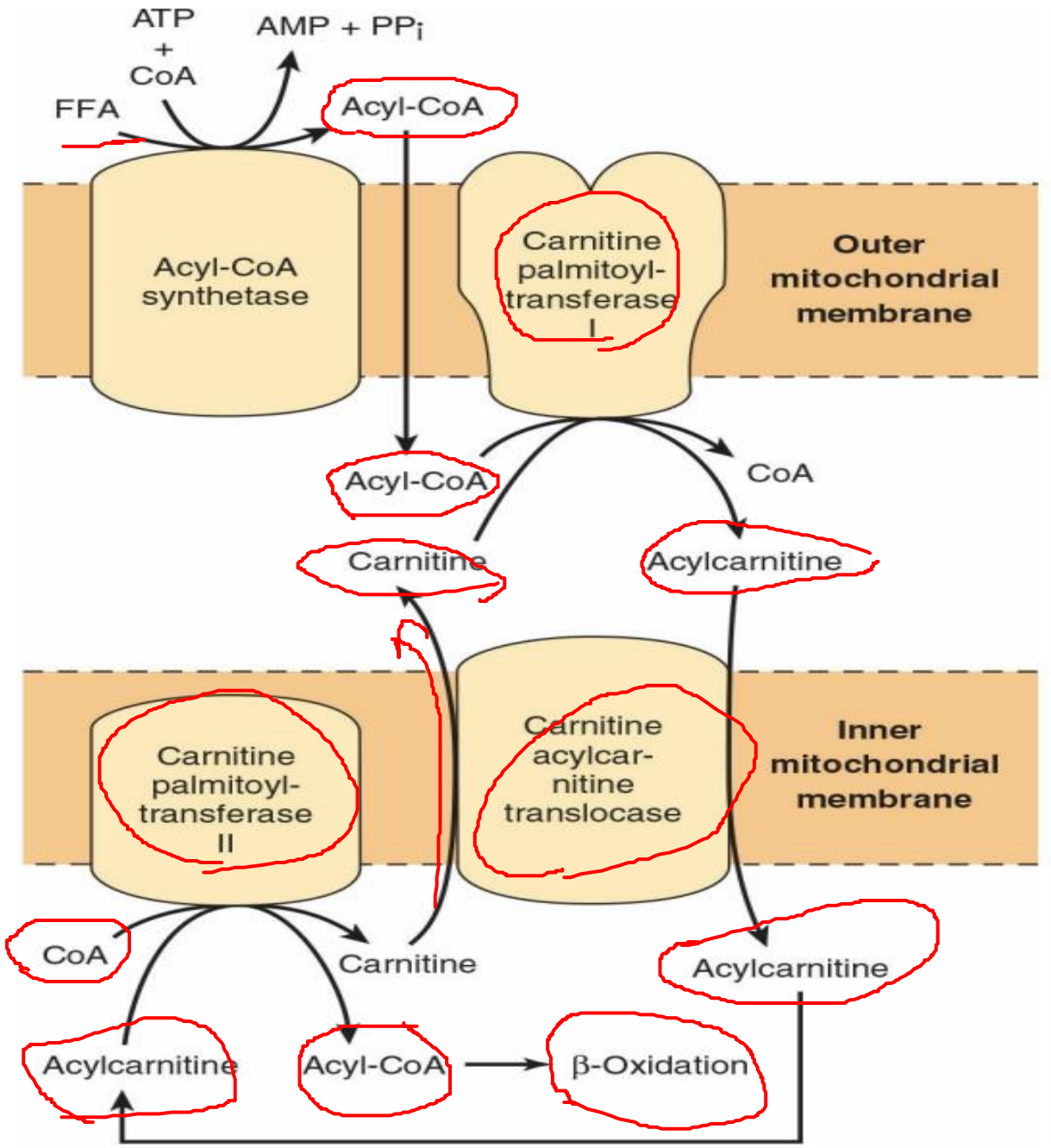
OXIDATION OF FATTY ACIDS OCCURS IN MITOCHONDRIA

Fatty acids must first be converted to an active intermediate before they can be catabolized. This is the only step in the complete degradation of a fatty acid that requires energy from ATP. In the presence of ATP and coenzyme A, the enzyme **acyl-CoA synthetase (thiokinase)** catalyzes the conversion of a fatty acid (or FFA) to an "active fatty acid" or acyl-CoA, which uses one high-energy phosphate with the formation of AMP and PP_i **(Figure 22–1).** The PP_i. is hydrolyzed by **inorganic pyrophosphatase** with the loss of a further high-energy phosphate, ensuring that the overall reaction goes to completion. Acyl-CoA synthetases are found in the endoplasmic reticulum and on the outer membrane of mitochondria.

Long-Chain Fatty Acids Penetrate the Inner Mitochondrial Membrane as Carnitine Derivatives

Carnitine (β-hydroxy-γ-trimethylammonium butyrate), $(CH_3)_3N^+—CH_2—CH(OH)—CH_2—COO^-$, is widely distributed and is particularly abundant in muscle. Long-chain acyl-CoA (or FFA) cannot penetrate the inner membrane of mitochondria. In the presence of carnitine, however, **carnitine palmitoyltransferase-I**, located in the outer mitochondrial membrane, converts long-chain acyl-CoA to **acylcarnitine**, which is able to penetrate the inner membrane and gain access to the β-oxidation system of enzymes (Figure 22–1). **Carnitine-acylcarnitine translocase** acts as an inner membrane exchange transporter. Acylcarnitine is transported in, coupled with the transport out of one molecule of carnitine. The acylcarnitine then reacts with CoA, catalyzed by **carnitine palmitoyltransferase-II**, located on the inside of the inner membrane, reforming acyl-CoA in the mitochondrial matrix, and carnitine is liberated.

FIGURE 22–1 Role of carnitine in the transport of long-chain fatty acids through the inner mitochondrial membrane. Long-chain acylCoA formed by acyl-CoA synthetase enters the intermembrane space. For transport across the inner membrane, acyl groups must be transferred from CoA to carnitine by carnitine palmitoyltransferase-I. The acylcarnitine formed is then carried into the matrix by a translocase enzyme in exchange for a free carnitine and acyl-CoA is reformed by carnitine palmitoyltransferase-II.



CARNITINE: -

Diet: red meat, dairy, poultry, fish :

made in liver and kidneys
- Enters cells by specific transporter
- Carnitine deficiencies: §

Symptoms:
- Poor muscle tone
- Muscle weakness
- Brain dysfunction
- Heart dysfunction

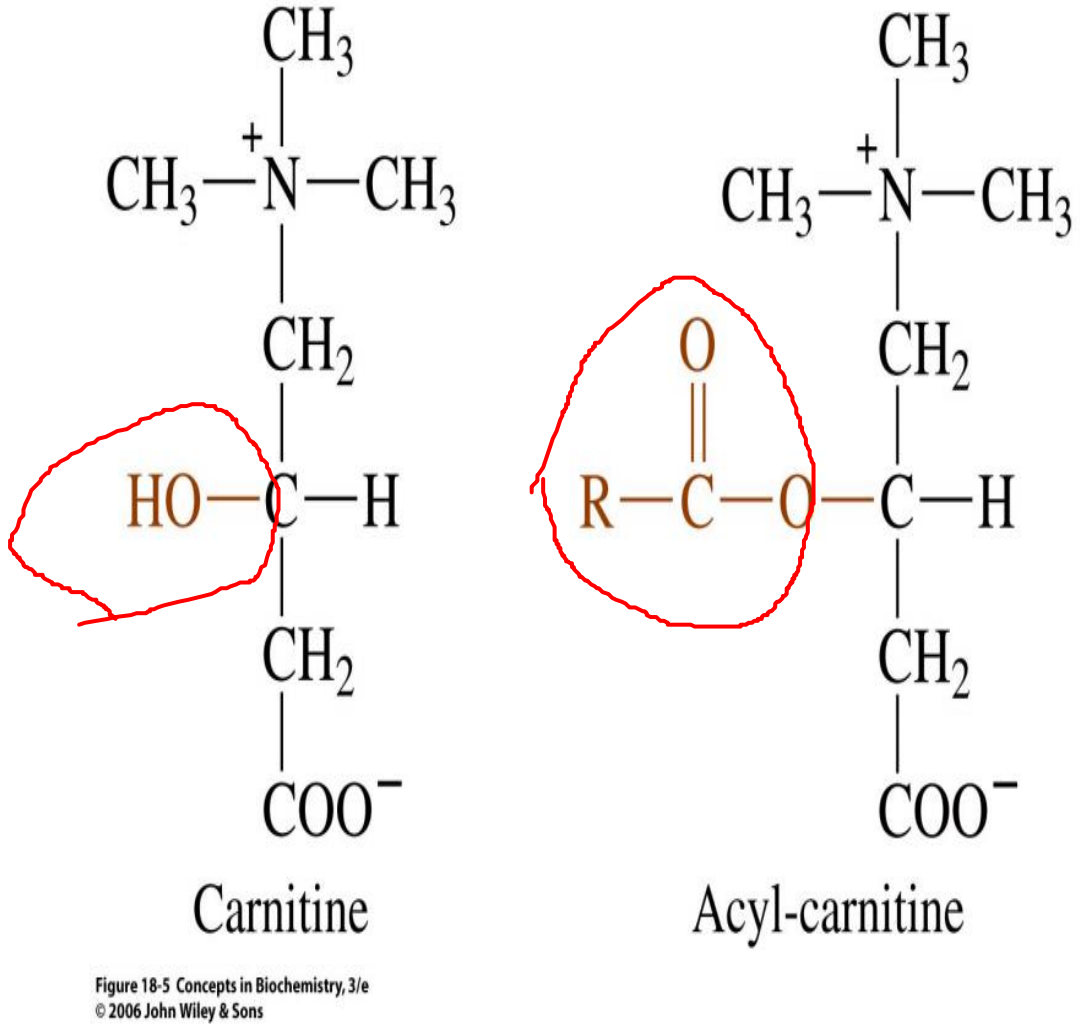


Figure 18-5 Concepts in Biochemistry, 3/e

β-OXIDATION OF FATTY ACIDS INVOLVES SUCCESSIVE CLEAVAGE WITH RELEASE OF ACETYL-CoA

1. Dehydrogenation of fatty acyl CoA to produce a trans double bond between the α and β carbons (or C-2 & C-3). the product is trans-Δ2-enoyl-CoA reaction catalyzed by acyl CoA dehydrogenase. the electron acceptor is FAD the reaction is analogous to succinate dehydrogenase.
2. Addition of water across double bond of trans-Δ2-enoyl-CoA. reaction catalyzed by enoyl-CoA hydratase product: L-β-hydroxyacyl CoA (3-hydroxyacyl CoA) reaction analogous to fumarase.
3. Dehydrogenation of L-β-hydroxyacyl CoA. product is to β-ketoacyl-CoA. enzyme: β-hydroxyacyl CoA dehydrogenase cofactor: NAD+ reduced to NADH + H+ reaction analogous to malate dehydrogenase.
4. The final step of β-oxidation cycle is thiolysis of C2-C3 (CαCβ) bond by nucleophilic attack on C2 (β carbon) by the –SH group of a new CoASH. products are acetyl CoA and acyl CoA (shorter by 2 C) Enzyme: Thiolase (acyl-CoA acetyltransferase) cofactor is CoASH.

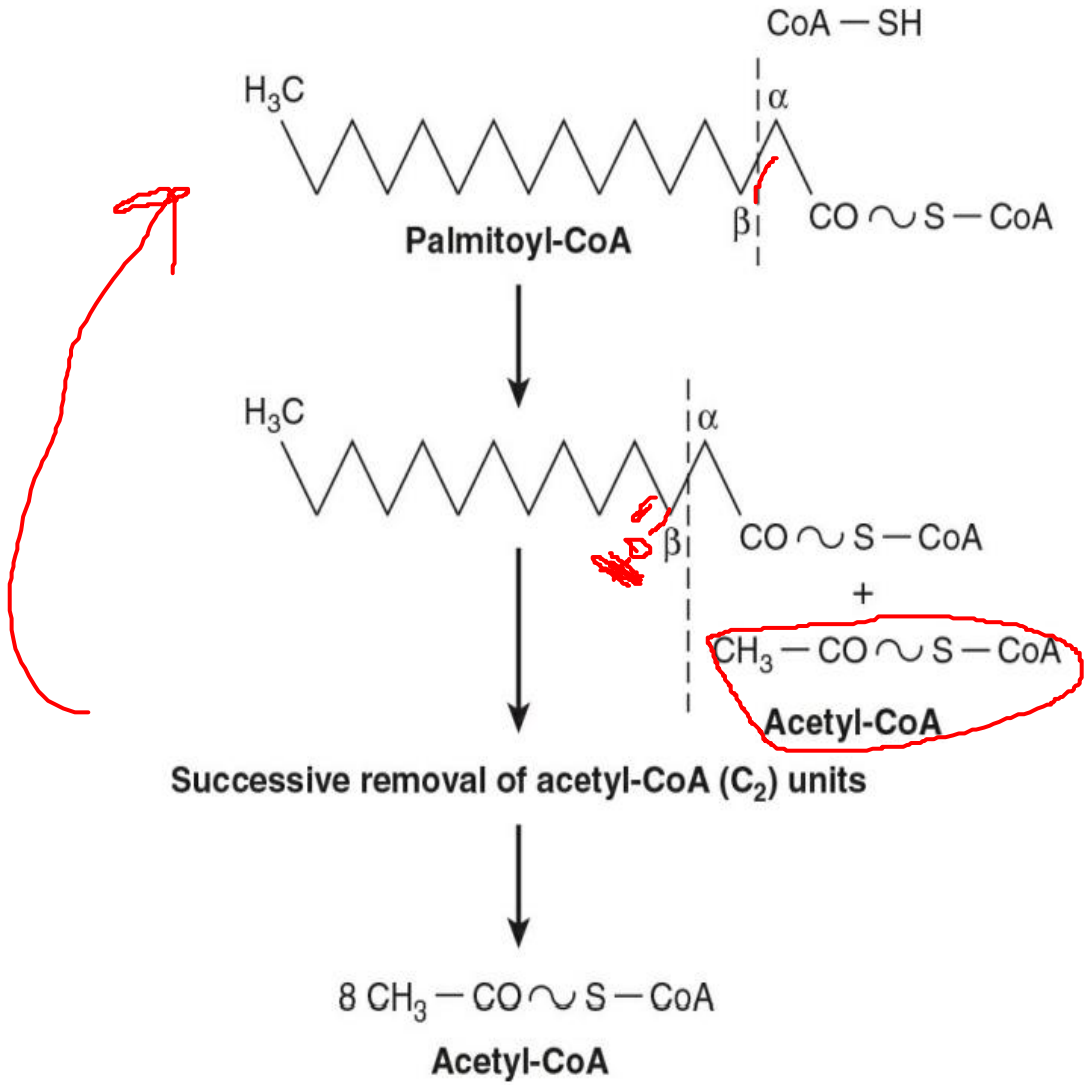


FIGURE 22–2 Overview of β-oxidation of fatty acids.

The β-Oxidation Cycle Generates FADH2 & NADH

Several enzymes, known collectively as “fatty acid oxidase,” are found in the mitochondrial matrix or inner membrane adjacent to the respiratory chain. These catalyze the oxidation of acyl-CoA to acetyl-CoA by the βoxidation pathway. The system proceeds in cyclic way which results in the degradation of long fatty acids to acetyl-CoA. In the process, large quantities of the reducing equivalents FADH2 and NADH are generated and are used to form ATP by oxidative phosphorylation (Figure 22–3).

Reactions for fatty acid activation, transport, and the β-oxidation spiral

Reaction Number	Reaction	Enzyme
1	Fatty acid + CoASH + ATP $\rightleftharpoons$ acyl-CoA + AMP + PP_i	Acyl-CoA synthetase
2	$PP_i + H_2O \rightleftharpoons 2\,P_i$	Pyrophosphatase
3	Carnitine + acyl-CoA $\rightleftharpoons$ acyl-carnitine + CoASH (intermembrane space)	Carnitine acyltransferase I
4	Acyl-carnitine + CoASH $\rightleftharpoons$ acyl-CoA + carnitine (mitochondria)	Carnitine acyltransferase II
5	Acyl-CoA + E—FAD $\rightleftharpoons$ *trans*-Δ^2-enoyl-CoA + E—$FADH_2$[b]	Acyl-CoA dehydrogenase
6	*trans*-Δ^2-Enoyl-CoA + $H_2O \rightleftharpoons$ L-3-hydroxyacyl-CoA	Enoyl-CoA hydratase
7	L-3-Hydroxyacyl-CoA + $NAD^+ \rightleftharpoons$ 3-ketoacyl-CoA + NADH + H^+	Hydroxyacyl-CoA dehydrogenase
8	3-Ketoacyl-CoA + CoASH $\rightleftharpoons$ acetyl-CoA + acyl-CoA[c]	β-Ketothiolase

[a] Reaction type: 1, oxidation–reduction; 2, group transfer; 3, hydrolysis; 4, nonhydrolytic cleavage (addition or elimination); 5, isomerization–rearrangement; 6, bond formation coupled to ATP cleavage.

[b] E—FAD and E—$FADH_2$ refer to the cofactor flavin adenine dinucleotide covalently linked to the enzyme.

[c] Acyl-CoA product is shortened by a C_2 unit.

The Reactions of β-oxidation

• One round of β-oxidation: 4 enzyme steps produce acetyl CoA from fatty acyl CoA

• Each round generates one molecule each of:

1 FADH2 – oxidative phosphorylation

1 NADH – oxidataive phosphorylation

1 Acetyl CoA – enters TCA cycle Fatty acyl CoA (2 carbons shorter each round)

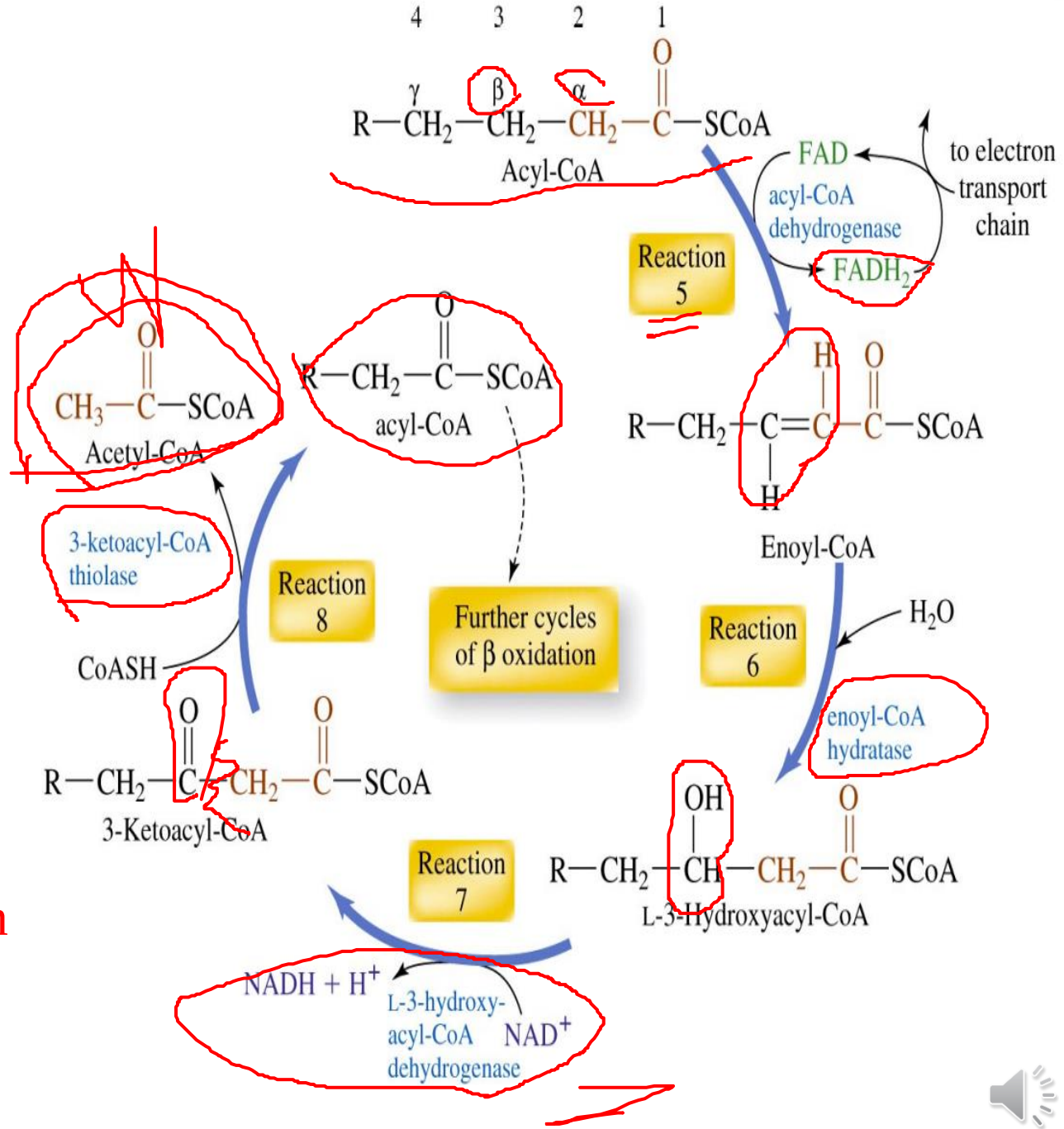
Example: §

Palmitic acid = 16:0

• 8 moles of acetyl-CoA (enter TCA cycle)

• 7 rounds of β-oxidation

Note: the propionyl residue from an odd-chain fatty acid is the only part of a fatty acid that is glucogenic.



Oxidation of Fatty Acids Produces a Large Quantity of ATP

Transport of electrons from FADH2 and NADH via the respiratory chain leads to the synthesis of four high-energy phosphates for each of the seven cycles needed for the breakdown of the C16 fatty acid, palmitate, to acetyl-CoA . A total of 8 mol of acetyl-CoA is formed, and each gives rise to 10 mol of ATP on oxidation in the citric acid cycle. Two must be subtracted for the initial activation of the fatty acid, yielding a net gain of 106 mol of ATP per mole of palmitate. This represents 68% of the free energy of combustion of palmitic acid.

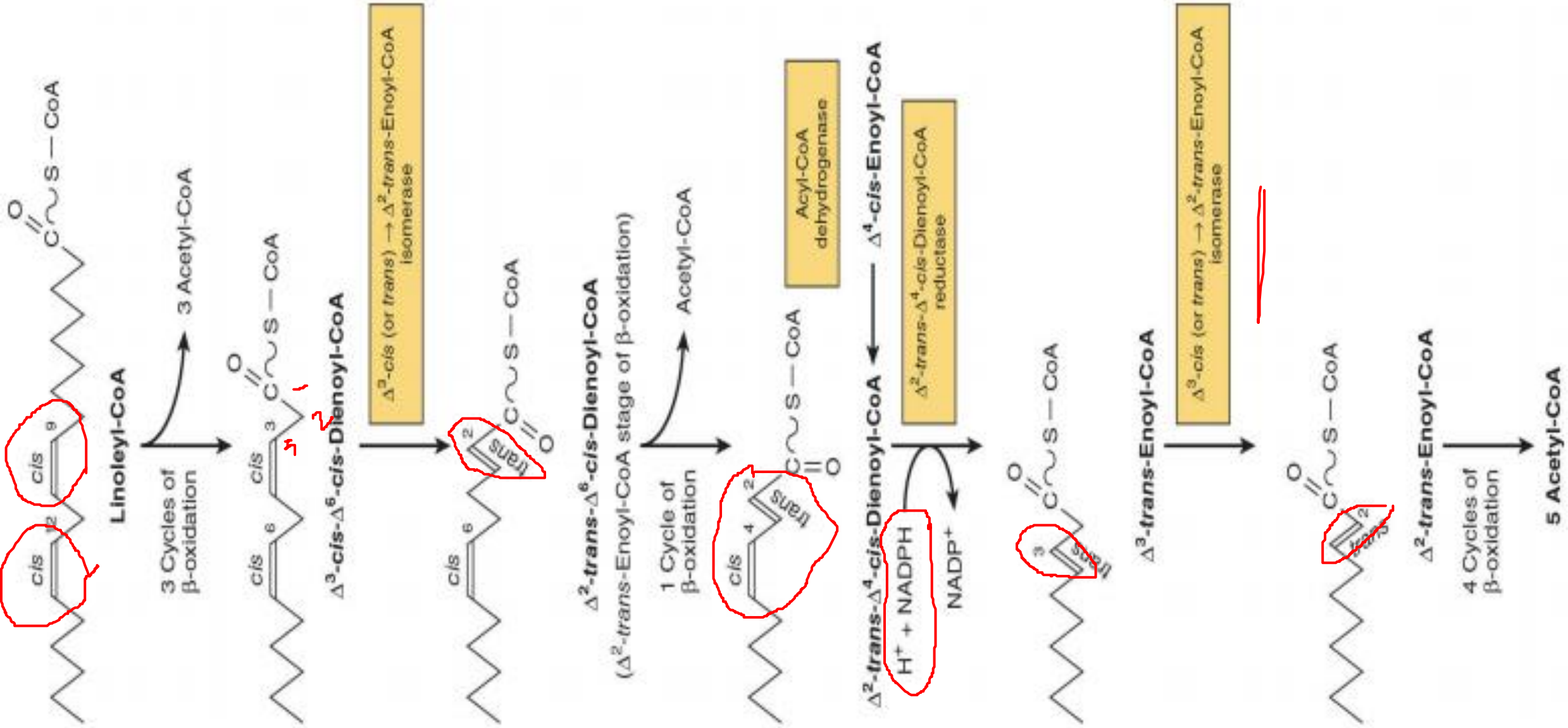
TABLE 22–1 Generation of ATP from the Complete Oxidation of a C16 Fatty Acid

Step	Product	Amount Product Formed (mol)/mol Palmitate	ATP Formed (mol)/ mol Product	Total ATP Formed (mol)/mol Palmitate	ATP Used (mol)/ mol Palmitate
Activation		–			2
β-Oxidation	$FADH_2$	7	1.5	10.5	–
β-Oxidation	NADH	7	2.5	17.5	–
Citric acid cycle	Acetyl-CoA	8	10	80	–
	Total ATP formed (mol)/mol palmitate			108	
	Total ATP used (mol)/mol palmitate				2

The table shows how the oxidation of 1 mol of the C16 fatty acid, palmitate, generates 106 mol of ATP (108 formed in total—2 used in the activation step).

Oxidation of Unsaturated Fatty Acids Occurs by a Modified β-Oxidation Pathway

Sequence of reactions in the oxidation of unsaturated fatty acids, for example, linoleic acid



KETOGENESIS OCCURS WHEN THERE IS A HIGH RATE OF FATTY ACID OXIDATION IN THE LIVER

Under metabolic conditions associated with a high rate of fatty acid oxidation, the liver produces considerable quantities of acetoacetate and D-3-hydroxybutyrate (β-hydroxybutyrate). Acetoacetate continually undergoes spontaneous decarboxylation to yield acetone. These three substances are collectively known as the ketone bodies (Figure 22–5).

the equilibrium is controlled by the mitochondrial [NAD+]/[NADH] ratio, that is, the redox stat

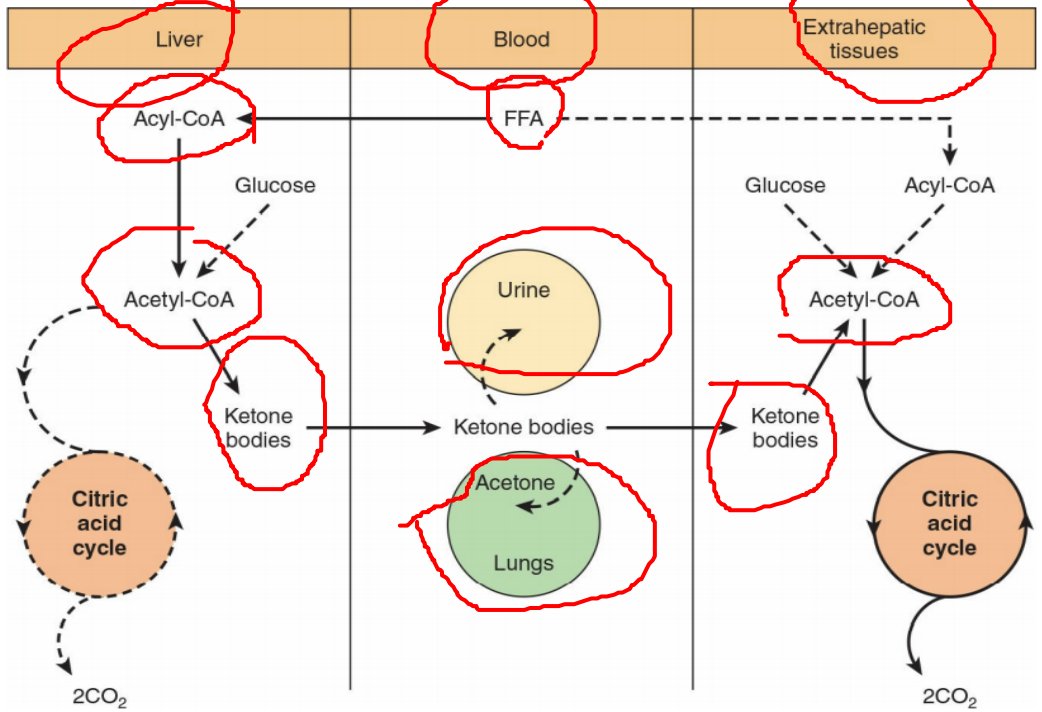


FIGURE 22–6 Formation, utilization, and excretion of ketone bodies. (The main pathway is indicated by the solid arrows.)

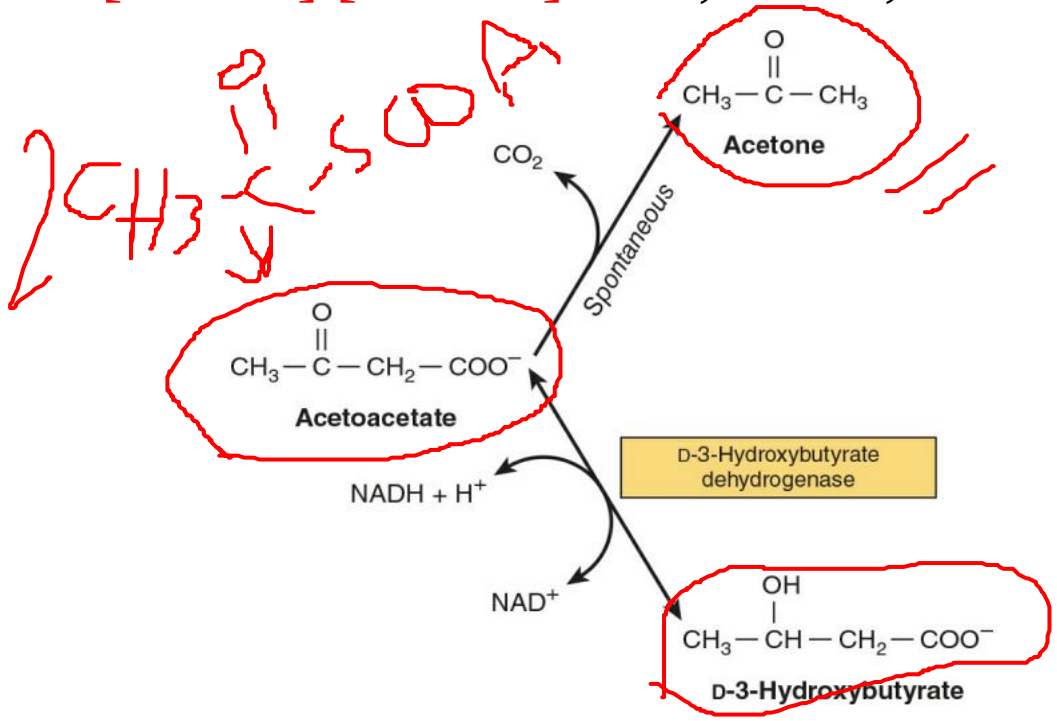


FIGURE 22–5 Interrelationships of the ketone bodies. D-3-Hydroxybutyrate dehydrogenase is a mitochondrial enzyme.

Acetoacetyl-CoA Is the Substrate for Ketogenesis & Use of Ketone Bodies as Fuel

- Ketone bodies are formed in the liver mitochondria
- Acetoacetate and β-hydroxybutyrate are transported by the blood to extrahepatic tissues
- Acetone is produced in smaller amounts and is exhaled.
- Ketone bodies are converted to acetyl CoA and oxidized by the TCA cycle for energy in skeletal and cardiac muscle and brain.
- The brain prefers glucose as fuel but can adapt to using ketones when glucose is not available.
- Oxidation of Ketones by other tissues facilitates the continued oxidation of FA in the liver.

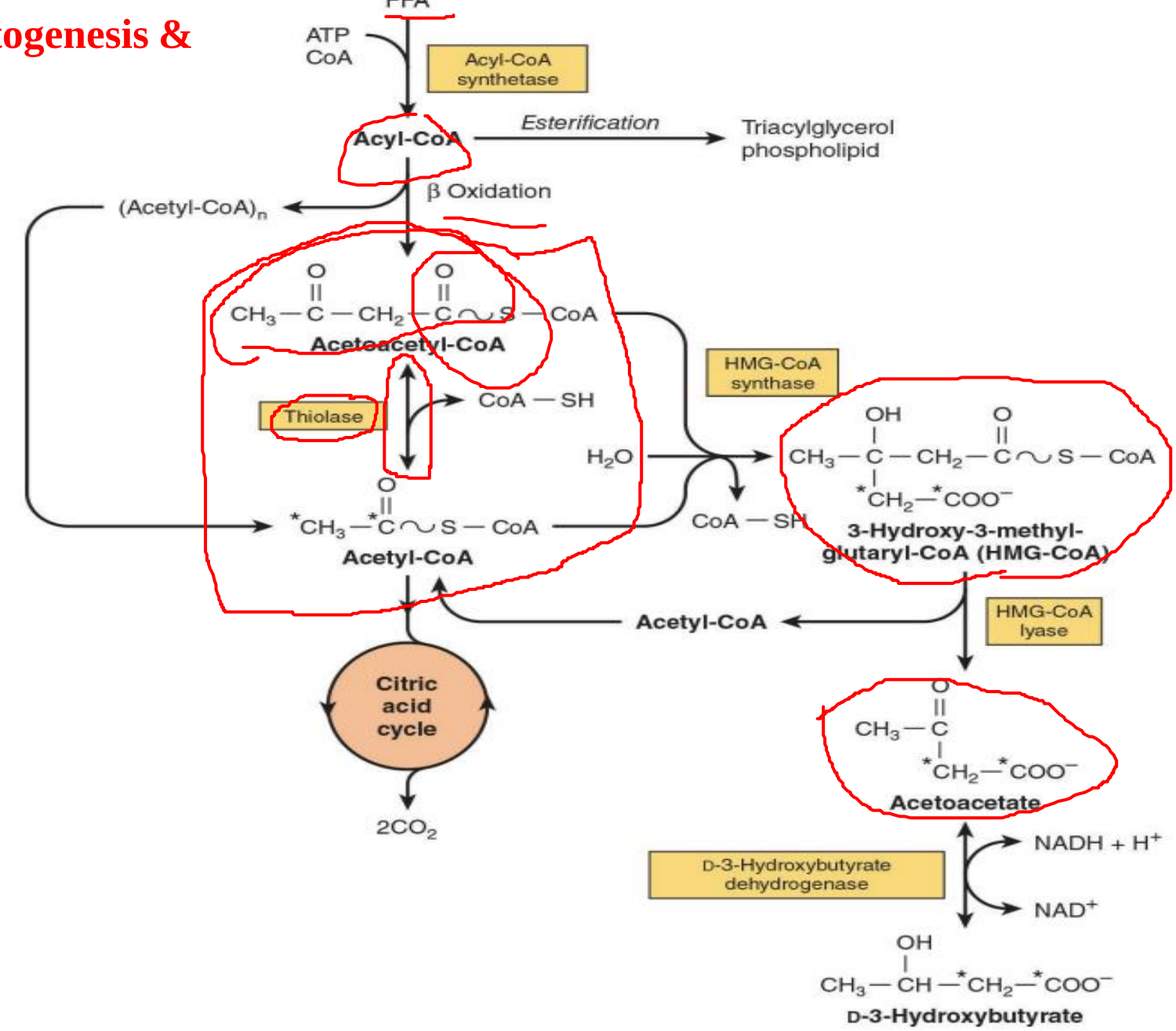


FIGURE 22–7 Pathways of ketogenesis in the liver. (FFA, free fatty

KETOGENESIS IS REGULATED AT THREE CRUCIAL STEPS

- Ketosis does not occur in vivo unless there is an increase in the level of circulating FFAs arising from lipolysis of triacylglycerol in adipose tissue. FFAs are the precursors of ketone bodies in the liver.

- After uptake by the liver, FFAs are either oxidized to CO2 or ketone bodies or esterified to triacylglycerol and phospholipid. There is regulation of entry of fatty acids into the oxidative pathway by carnitine palmitoyltransferase-I (CPT-I)

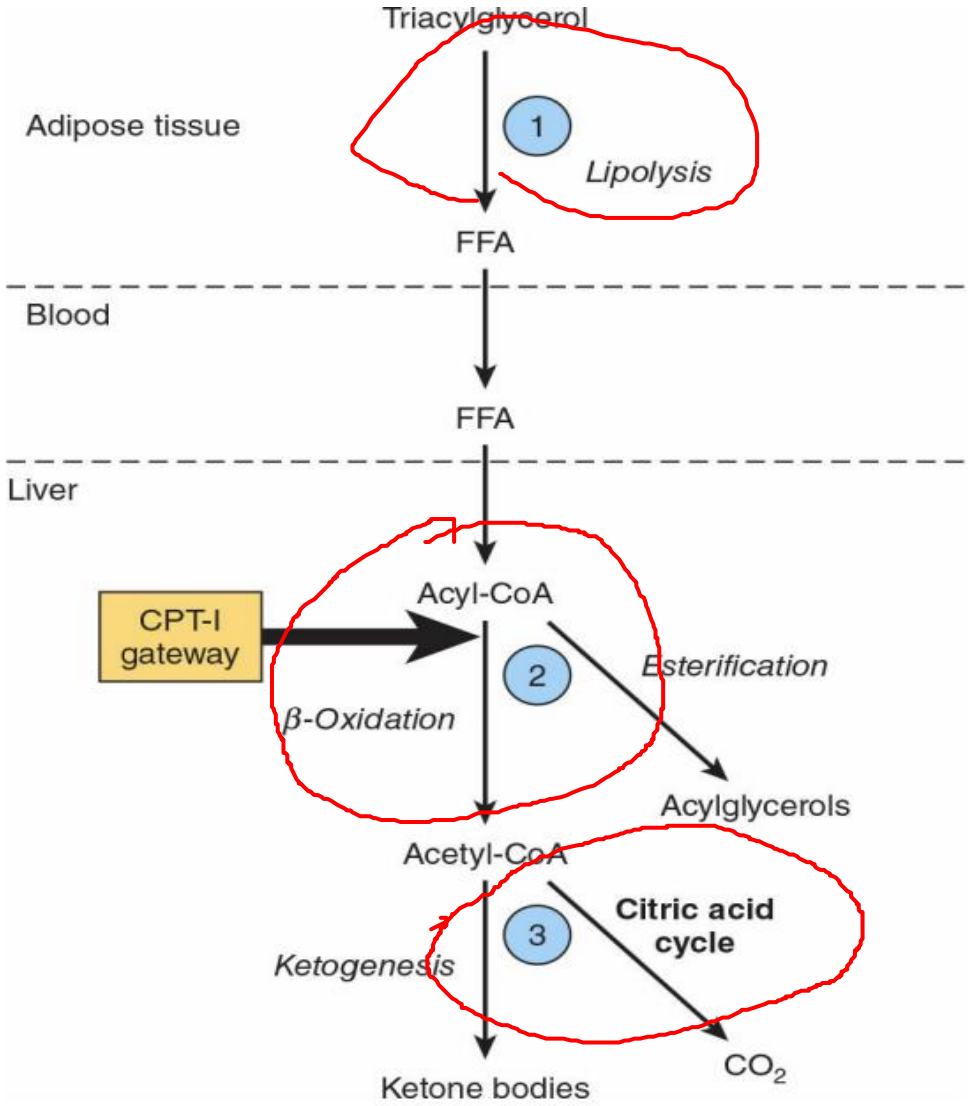


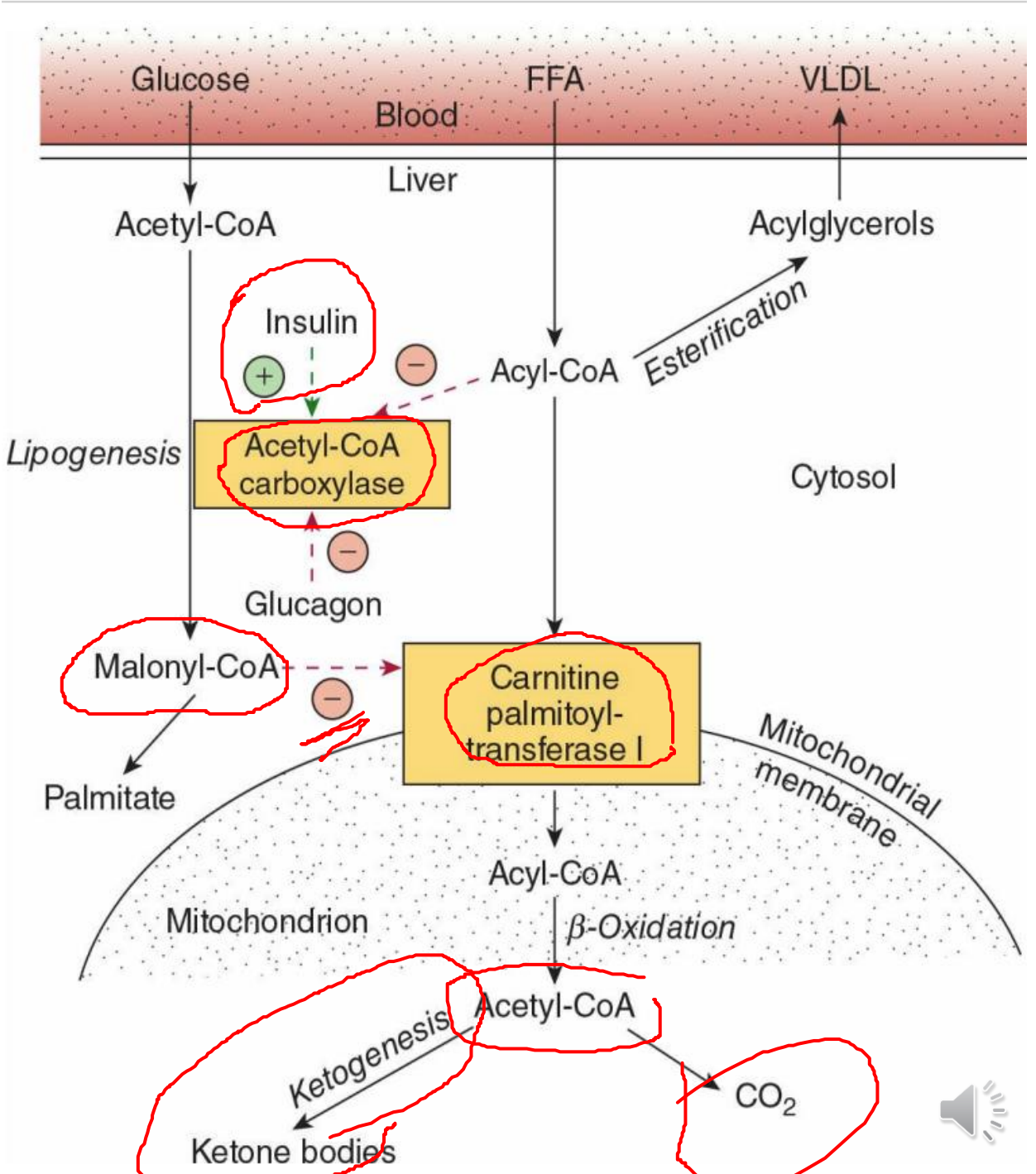
FIGURE 22–9 Regulation of ketogenesis. Symbol 1 to Symbol 3 show three crucial steps in the pathway of metabolism of free fatty acids (FFA) that determine the magnitude of ketogenesis. (CPT-I, carnitine palmitoyltransferase-I.)

Regulation of Fatty Acid Oxidation

FA metabolism is under hormonal regulation. When fuel levels are low, Epinephrine and Glucagon stimulate mobilization of fat and glycogen reserves. Insulin, which is secreted during the fed-state, is anti-lipolytic (it inhibits βoxidation).

The transport of FA into mitochondria is allosterically regulated. This is the rate-limiting step in β-oxidation. Carnitine Palmitoyl Transferases I and II are inhibited by malonyl-CoA, an intermediate of fatty acid synthesis. Thus fatty acid oxidation is reduced under conditions favoring fatty acid synthesis.

The two final steps in the β-oxidation cycle are also regulated. 3-hydroxyacyl-SCoA dehydrogenase is inhibited by NADH. Thiolase is regulated by feed-back inhibition by acetyl CoA.



THANK YOU