Oxidation of Fatty Acids and Ketogenesis

BIOMEDICAL IMPORTANCE

Although fatty acids are broken down by oxidation to acetyl-CoA and also synthesized from acetyl-CoA, fatty acid oxidation is not the simple reverse of fatty acid biosynthesis but an entirely different process taking place in a **separate compartment** of the cell. The separation of **fatty acid oxidation in mitochondria** from **biosynthesis in the cytosol** allows each process to be individually controlled and integrated with tissue requirements. Each step in fatty acid oxidation involves acyl-CoA derivatives, is catalyzed by **separate** enzymes, utilizes NAD^+ and FAD as coenzymes, and generates ATP. It is an **aerobic** process, requiring the presence of oxygen.

Increased fatty acid oxidation is a characteristic of starvation and of diabetes mellitus, and leads to increased **ketone body** production by the liver **(ketosis)**. Ketone bodies are **acidic** and when produced in excess over long periods, as in diabetes, cause **ketoacidosis**, which is ultimately fatal. Because **gluconeogenesis is dependent upon 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 poisons, for example, **hypoglycin**.

OXIDATION OF FATTY ACIDS OCCURS IN MITOCHONDRIA

Fatty Acids Are Transported in the Blood as Free Fatty Acids

Free fatty acids (FFAs)—also called unesterified (UFA) or nonesterified (NEFA) fatty acids—are fatty acids that are in the **unesterified state**. In plasma, longer chain FFA are combined with **albumin**, and in the cell they are attached to a **fatty acid binding protein**, so that in fact they are never really "free." Shorter chain fatty acids are more water-soluble and exist as the unionized acid or as a fatty acid anion.

Fatty Acids Are Activated Before Being Catabolized

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**, using one high-energy phosphate and forming AMP and PPi (Figure 1). The PPi 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, peroxisomes, and inside and on the outer membrane of mitochondria.

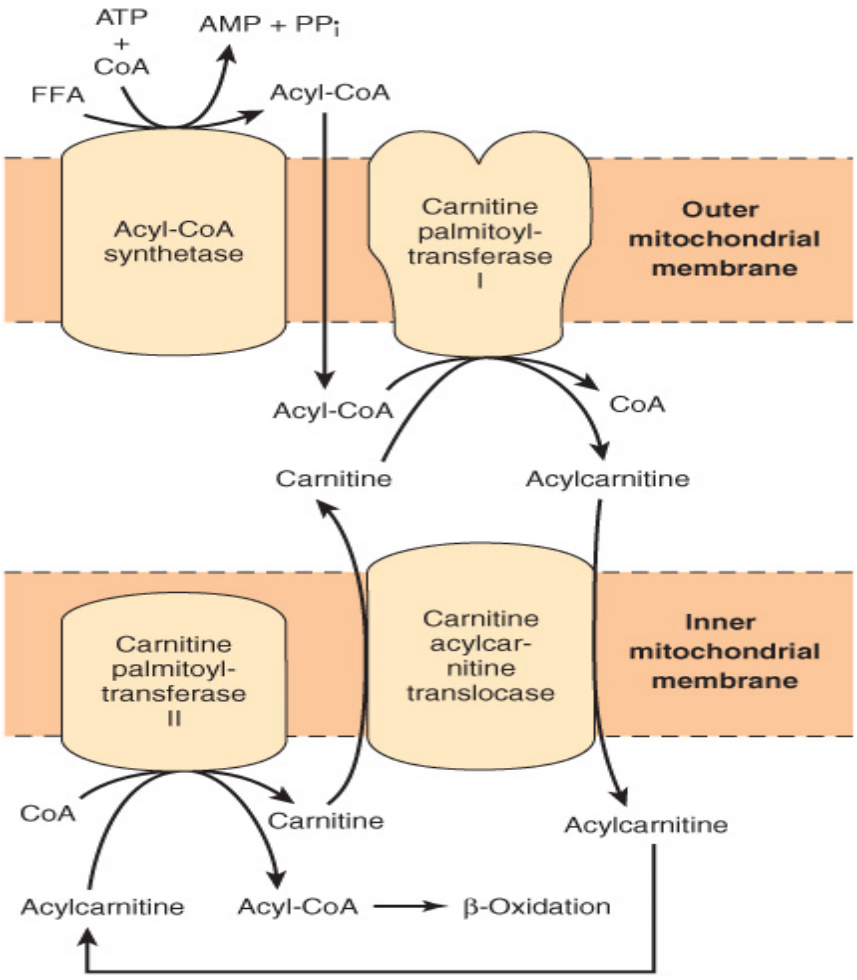

Figure 1 Role of carnitine in the transport of long chain fatty acids through the inner mitochondrial membrane. Long-chain acyl-CoA enters the intermembrane space after its formation by acyl-CoA synthetase, but cannot pass through the inner mitochondrial membrane. For transport across the membrane, therefore, acyl groups are transferred from CoA to carnitine by carnitine palmitoyl transferase I (embedded in the outer mitochondrial membrane). The acylcarnitine formed can then be carried into the mitochondrial matrix by carnitine acylcarnitine translocase (embedded in the inner mitochondrial membrane) in exchange for a free carnitine. The acyl group is then transferred back to CoA by carnitine palmitoyl transferase II, reforming acyl-CoA, and the carnitine released is transported back into the intermembrane space via the translocase enzyme.

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

Carnitine (β-hydroxy-γ-trimethylammonium butyrate), which has the formula $(CH_3)_3 N^+—CH_2—CH(OH)—CH_2—COO^-$, 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, transfers long-chain acyl group from CoA to carnitine, forming **acylcarnitine** and releasing CoA. Acylcarnitine is able to penetrate the inner membrane and gain access to the β-oxidation system of enzymes via the inner membrane exchange transporter **carnitine-acylcarnitine translocase.** The transporter binds acylcarnitine and transports it across the membrane in exchange for carnitine. The acyl group is then transferred to CoA so that acyl-CoA is reformed and carnitine is liberated. This reaction is catalyzed by **carnitine palmitoyltransferase-II**, which is located on the inside of the inner membrane (Figure 1).

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

In the **β-oxidation** (Figure 2) pathway, two carbons at a time are cleaved from acyl-CoA molecules, **starting at the carboxyl end**. The chain is broken between the α(2)- and β(3)-carbon atoms—hence the name β-oxidation. The two-carbon units formed are **acetyl-CoA**; thus, palmitoyl-CoA forms eight acetyl-CoA molecules.

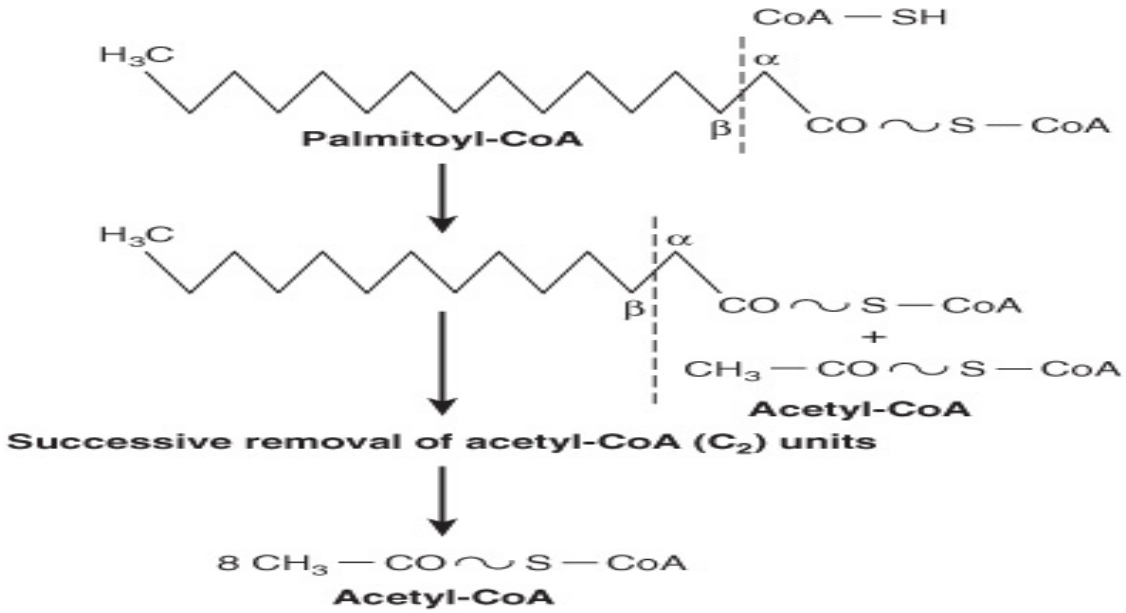

Figure 2 Overview of a-oxidation of fatty acids.

The β-Oxidation Cycle Generates $FADH_2$ & 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 via the β-oxidation pathway**. The system proceeds in cyclic fashion which results in the **degradation of long fatty acids to acetyl CoA**. In the process, large quantities of the reducing equivalents **$FADH_2$** and **NADH** are generated and are used to form ATP by oxidative phosphorylation (Figure 3).

The first step is the removal of two hydrogen atoms from the 2(α)- and 3(β)-carbon atoms, catalyzed by **acyl-CoA dehydrogenase** and requiring FAD. This results in the formation of Δ^2 - trans-enoyl-CoA and $FADH_2$. The reoxidation of $FADH_2$ by the respiratory chain requires the mediation of another flavoprotein, termed **electron-transferring flavoprotein**. Water is added to saturate the double bond and form 3-hydroxyacyl-CoA, catalyzed by **Δ^2 -enoyl-CoA hydratase.** The 3-hydroxy derivative undergoes further dehydrogenation on the 3-carbon catalyzed by L(+)-**3-hydroxyacyl-CoA dehydrogenase** to form the corresponding 3-ketoacyl-CoA compound. In this case, NAD^+ is the coenzyme involved. Finally, 3-ketoacyl-CoA is split at the 2,3-position by **thiolase** (3-ketoacyl-CoA-thiolase), forming acetyl-CoA and a new acyl-CoA two carbons shorter than the original acyl-CoA molecule. The shorter acyl-CoA formed in the cleavage reaction reenters the oxidative pathway at reaction 2 (Figure 3). In this way, a long-chain fatty acid with an **even number** of carbons may be degraded completely to acetyl-CoA (C_2 units). For example, **after seven cycles, the C16 fatty acid, palmitate, would be converted to eight acetyl CoA molecules**. Since acetyl-CoA can be oxidized to CO_2 and water via the citric acid cycle (which is also found within the mitochondria), the complete oxidation of fatty acids is achieved.

Oxidation of a Fatty Acid With an Odd Number of Carbon Atoms Yields Acetyl-CoA Plus a Molecule of Propionyl-CoA

Fatty acids with an odd number of carbon atoms are oxidized by the pathway of β-oxidation described above producing acetyl CoA until a three-carbon (propionyl-CoA) residue remains. This compound is converted to succinyl-CoA, a constituent of the citric acid cycle. Hence, **the propionyl residue from an odd-chain fatty acid is the only part of a fatty acid that is glucogenic.**

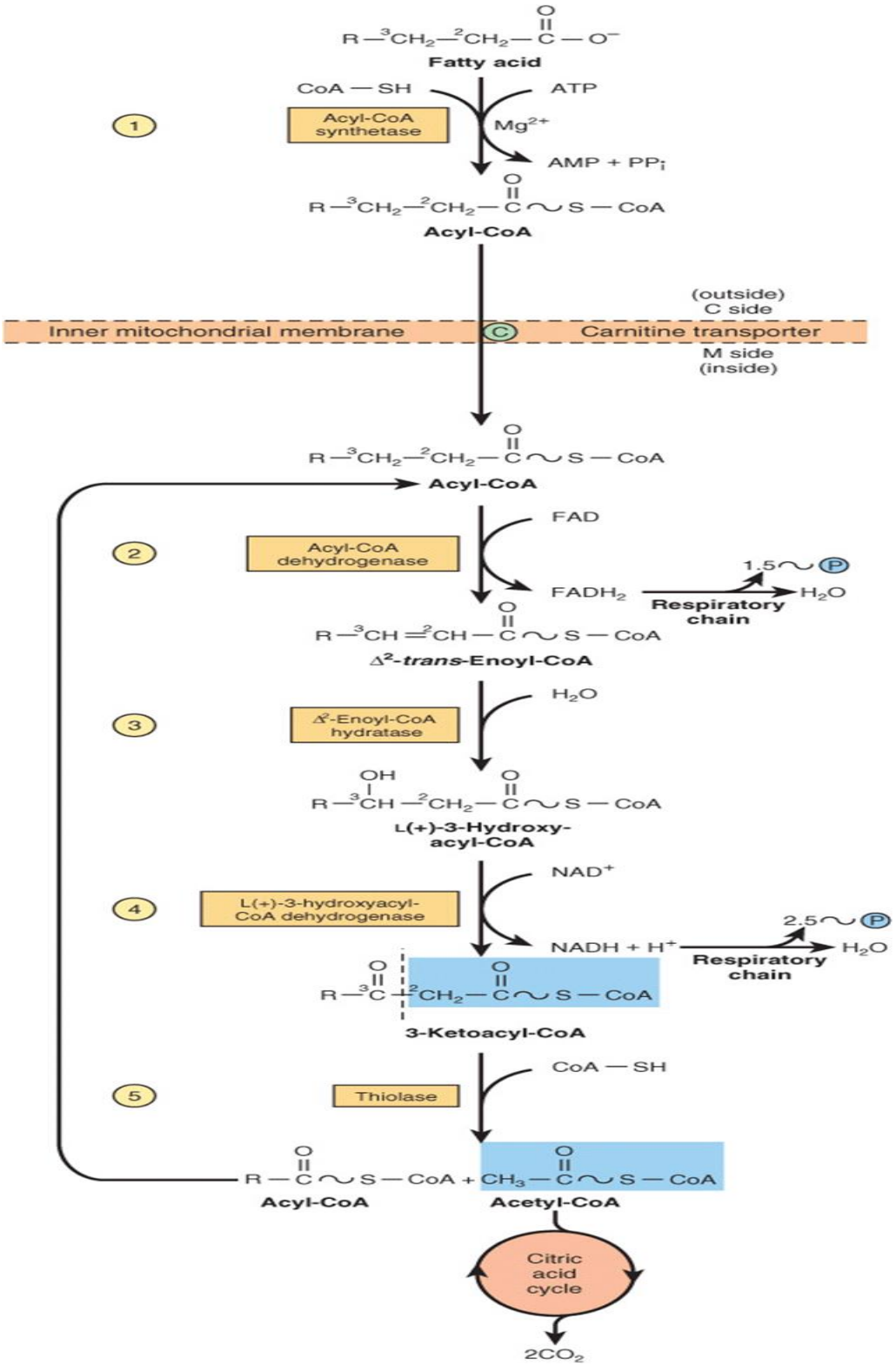

Figure 3 β-Oxidation of fatty acids. Long-chain acyl-CoA is cycled through reactions 2–5 , acetyl-CoA being split off, each cycle, by thiolase (reaction 5). When the acyl radical is only four carbon atoms in length, two acetyl-CoA molecules are formed in reaction 5.

Oxidation of Fatty Acids Produces a Large Quantity of ATP

Transport of electrons from $FADH_2$ 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 (7 × 4 = 28). 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, making 8 × 10 = 80 mol. 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 (Table 1), or 106 × 30.5 = 3233 kJ. This represents 33% of the free energy of combustion of palmitic acid.

TABLE 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).

Peroxisomes Oxidize Very Long Chain Fatty Acids

A **modified form of β-oxidation** is found in **peroxisomes** and leads to the formation of acetyl-CoA and H_2O_2 (from the flavoprotein-linked dehydrogenase step), which is broken down by catalase. Thus, the dehydrogenation in peroxisomes is not linked directly to phosphorylation and the generation of ATP. The system facilitates the oxidation of **very long chain fatty acids** (eg, C_{20}, C_{22}). The enzymes responsible are **induced** by high-fat diets and in some species by hypolipidemic drugs such as clofibrate.

The enzymes in peroxisomes **do not attack shorter chain fatty acids**; the β-oxidation sequence ends at octanoyl-CoA. Octanoyl and acetyl groups are both further oxidized in mitochondria. Another role of peroxisomal β-oxidation is to shorten the side chain of cholesterol in bile acid formation. Peroxisomes also take part in the synthesis of ether glycerolipids, cholesterol, and dolichol.

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

The CoA esters of unsaturated fatty acids are degraded by the enzymes normally responsible for β-oxidation until either a Δ^3 -cis-acyl-CoA compound or a Δ^4 -cis-acyl-CoA compound is formed, depending upon the position of the double bonds (Figure 4). The former compound is isomerized (Δ^3 cis → Δ^2-**trans-enoyl-CoA isomerase**) to the corresponding Δ^2-trans-CoA stage of β-oxidation for subsequent hydration and oxidation. Any Δ^4-cis-acyl-CoA either remaining, as in the case of linoleic acid, or entering the pathway at this point after conversion by acyl-CoA dehydrogenase to Δ^2 - trans - Δ^4 - cis-dienoyl-CoA, is then metabolized as indicated in Figure 4.

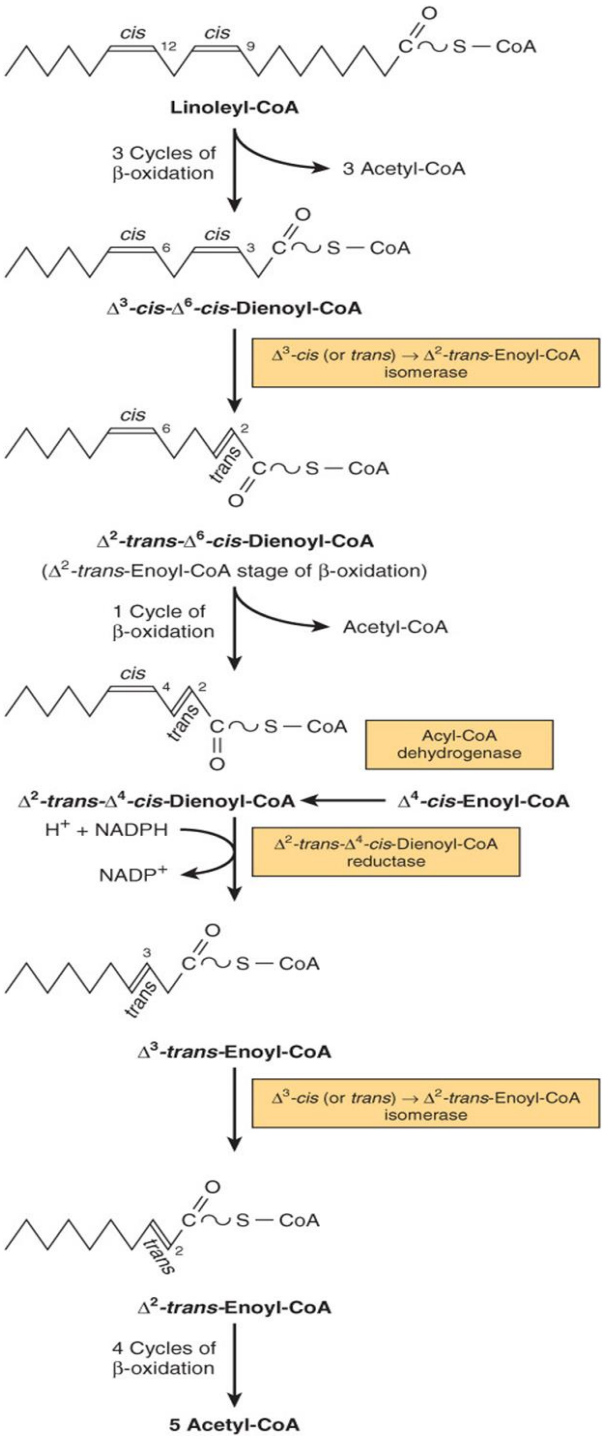

Figure 4 Sequence of reactions in the oxidation of unsaturated fatty acids, for example, linoleic acid. Δ^4 -cis-fatty acids or fatty acids forming Δ^4 -cis-enoyl-CoA enter the pathway at the position shown. NADPH for the dienoyl-CoA reductase step is supplied by intramitochondrial sources such as glutamate dehydrogenase, isocitrate dehydrogenase, and NAD(P)H transhydrogenase.

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** (also called acetone bodies or [incorrectly] "ketones"). The term ketones should not be used as there are ketones in blood that are not ketone bodies, for example, pyruvate and fructose. (Figure 5). Acetoacetate and 3-hydroxybutyrate are interconverted by the mitochondrial enzyme D(–)-**3-hydroxybutyrate dehydrogenase**; the equilibrium is controlled by the mitochondrial [NAD^+]/[NADH] ratio, that is, the **redox state**. The concentration of total ketone bodies in the blood of well-fed mammals does not normally exceed 0.2 mmol/L except in ruminants, where 3-hydroxybutyrate is formed continuously from butyric acid (a product of ruminal fermentation) in the rumen wall. In vivo, the liver appears to be the only organ in nonruminants to add significant quantities of ketone bodies to the blood. **Extrahepatic tissues utilize** acetoacetate and β-hydroxybutyrate as respiratory substrates. Acetone is a waste product which, as it is volatile, can be excreted via the lungs. Because there is active synthesis but little utilization of ketone bodies in the liver, while **they are used but not produced in extrahepatic tissues**, there is a net flow of the compounds to the extrahepatic tissues (Figure 6).

3-Hydroxy-3-Methylglutaryl-CoA (HMG-CoA) Is an Intermediate in the Pathway of Ketogenesis

Enzymes responsible for ketone body formation are associated mainly with the mitochondria. Two acetyl-CoA molecules formed in β-oxidation condense to form acetoacetyl-CoA by a reversal of the **thiolase** reaction. Acetoacetyl-CoA, which is the starting material for ketogenesis, also arises directly from the terminal four carbons of a fatty acid during β-oxidation (Figure 7). Condensation of acetoacetyl-CoA with another molecule of acetyl-CoA by 3-hydroxy-3-methylglutaryl-CoA synthase forms **3-hydroxy-3-methylglutaryl-CoA (HMGCoA). 3-Hydroxy-3-methylglutaryl-CoA lyase** then causes acetyl-CoA to split off from the HMG-CoA, leaving free acetoacetate. The carbon atoms split off in the acetyl-CoA molecule are derived from the original acetoacetyl-CoA molecule. **Both enzymes must be present in mitochondria for ketogenesis to take place.** This occurs **solely in liver and rumen epithelium**. D(−)-3-Hydroxybutyrate is quantitatively the **predominant** ketone body present in the blood and urine in ketosis.

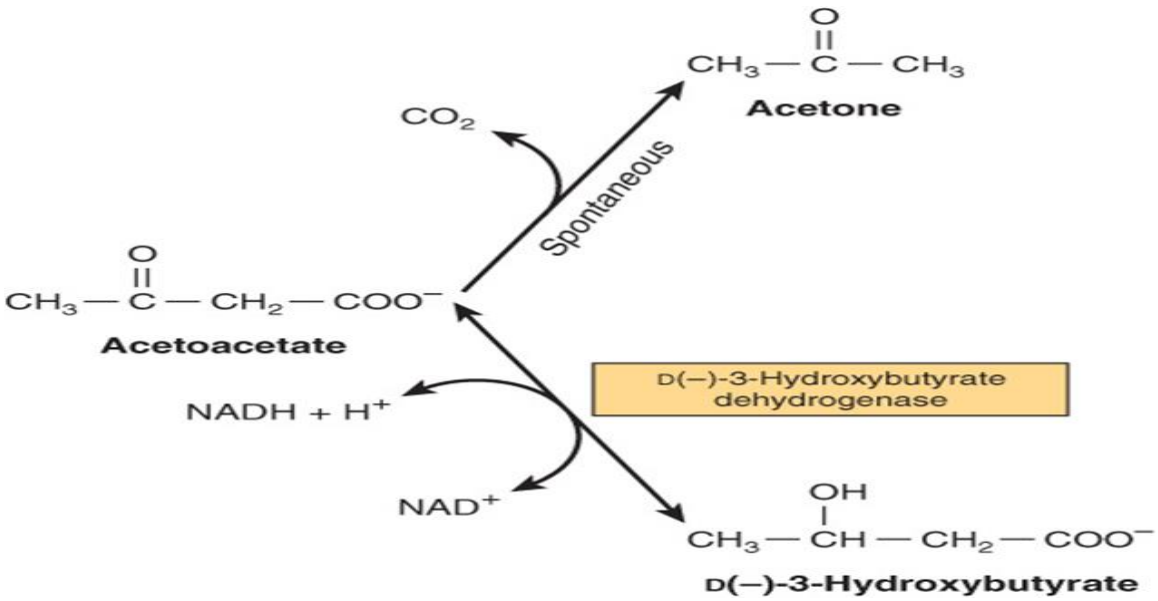

FIGURE 5 Interrelationships of the ketone bodies. D(−)-3-hydroxybutyrate dehydrogenase is a mitochondrial enzyme.

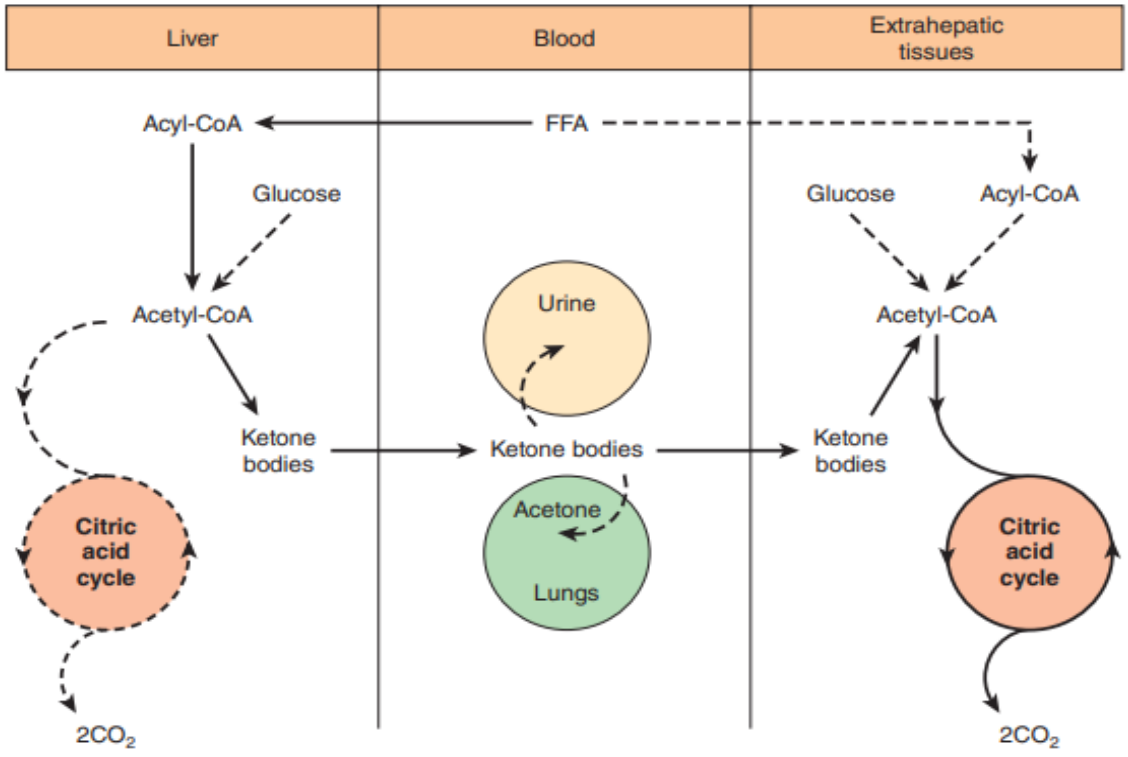

Figure 6 Formation, utilization, and excretion of ketone bodies. (The main pathway is indicated by the solid arrows.)

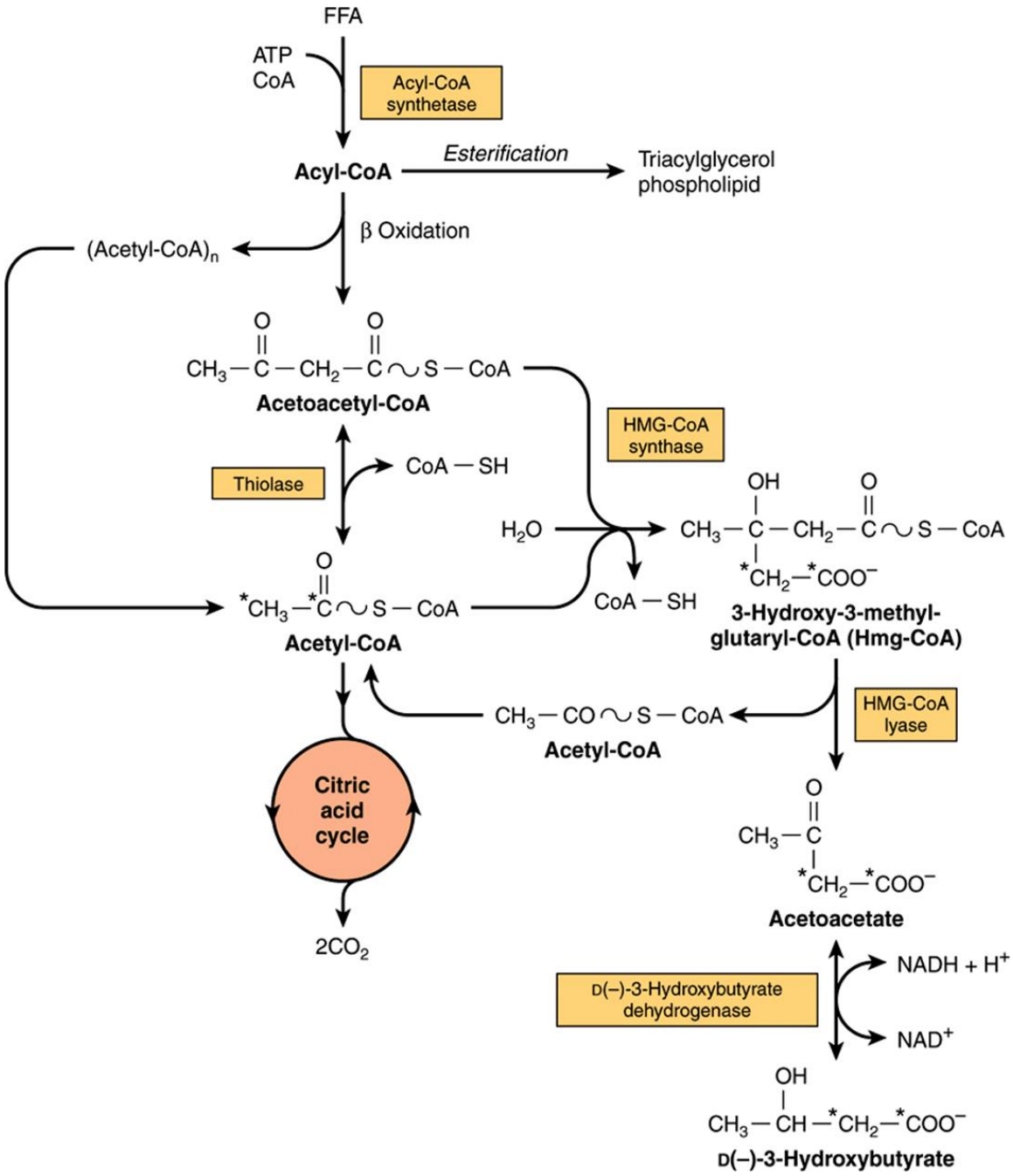

Figure 7 Pathways of ketogenesis in the liver. (FFA, free fatty acids.)

Ketone Bodies Serve as a Fuel for Extrahepatic Tissues

While an active enzymatic mechanism produces acetoacetate from acetoacetyl-CoA in the liver, acetoacetate once formed cannot be reactivated directly except in the cytosol, where it is used in a much less active pathway as a precursor in cholesterol synthesis. This accounts for the net production of ketone bodies by the liver.

In extrahepatic tissues, acetoacetate is activated to acetoacetyl-CoA by **succinyl-CoA-acetoacetate CoA transferase**. CoA is transferred from succinyl-CoA to form acetoacetyl-CoA (Figure 8). With the addition of a CoA, the acetoacetyl-CoA is split into two acetyl-CoAs by thiolase and oxidized in the citric acid cycle. If the blood level is raised, oxidation of ketone bodies increases until, at a concentration of ~12 mmol/L, the oxidative machinery is saturated. When this occurs, a large proportion of oxygen consumption may be accounted for by the oxidation of ketone bodies.

In most cases, **ketonemia is due to increased production of ketone bodies by the liver** rather than to a deficiency in their utilization by extrahepatic tissues. While acetoacetate and D(−)-3-hydroxybutyrate are **readily oxidized** by extrahepatic tissues, acetone is **difficult to oxidize** in vivo and to a large extent is volatilized in the lungs.

In moderate ketonemia, the loss of ketone bodies via the urine is only a few percent of the total ketone body production and utilization. Since there are renal threshold-like effects (there is not a true threshold) that vary between species and individuals, measurement of the ketonemia, not the ketonuria, is the preferred method of assessing the severity of ketosis.

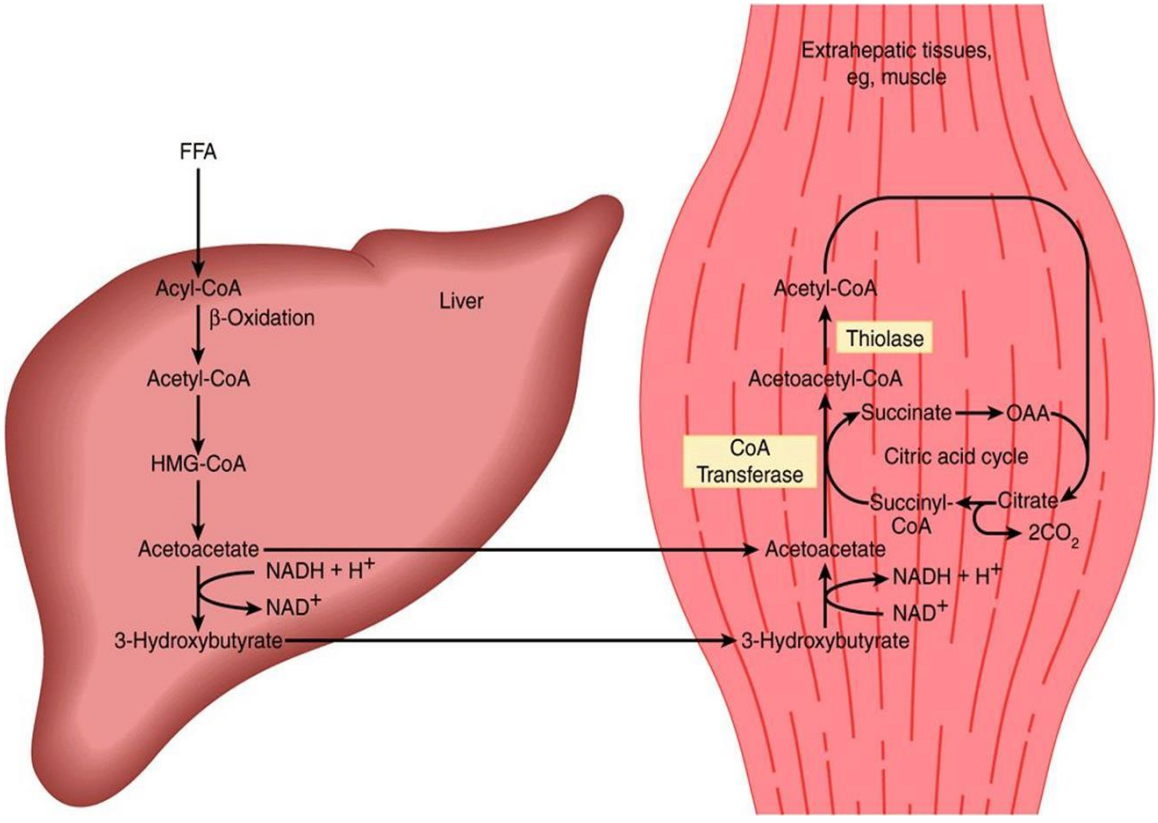

Figure 8 Transport of ketone bodies from the liver and pathways of utilization and oxidation in extrahepatic tissues.

KETOGENESIS IS REGULATED AT THREE CRUCIAL STEPS

1. Ketosis does not occur in vivo unless there is an increase in the level of circulating FFAs that arise from lipolysis of triacylglycerol in adipose tissue. **FFAs are the precursors of ketone bodies in the liver**. The liver, both in fed and in fasting conditions, extracts ~30% of the FFAs passing through it, so that at high concentrations the flux passing into the liver is substantial. **Therefore, the factors regulating mobilization of FFA from adipose tissue are important in controlling ketogenesis** (Figures 9).

2. After uptake by the liver, FFAs are either **β-oxidized** to CO_2 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 1), and the remainder of the fatty acid taken up is esterified. CPT-I activity is low in the fed state, leading to depression of fatty acid oxidation, and high in starvation, allowing fatty acid oxidation to increase.

Malonyl-CoA, the initial intermediate in fatty acid biosynthesis formed by acetyl-CoA carboxylase in the fed state, **is a potent inhibitor of CPT-I** (Figure 10). Under these conditions, FFA enter the liver cell in low concentrations and are nearly all esterified to acylglycerols and transported out of the liver in **very low density lipoproteins** (VLDL). However, as the concentration of FFA increases with the onset of starvation, acetyl-CoA carboxylase is inhibited directly by acyl-CoA, and (malonyl-CoA) decreases, releasing the inhibition of CPT-I and allowing more acyl-CoA to be β-oxidized. These events are reinforced in starvation by a decrease in the **(insulin)/(glucagon) ratio**. Thus, β-oxidation from FFA is controlled by the CPT-I gateway into the mitochondria, and the balance of the FFA uptake not oxidized is esterified.

3. In turn, the acetyl-CoA formed in β-oxidation is oxidized in the citric acid cycle, or it enters the pathway of ketogenesis to form ketone bodies. **As the level of serum FFA is raised, proportionately more FFA is converted to ketone bodies and less is oxidized via the citric acid cycle to CO_2** . The partition of acetyl-CoA between the ketogenic pathway and the pathway of oxidation to CO_2 is regulated so that the total free energy captured in ATP which results from the oxidation of FFA remains constant as their concentration in the serum changes. This may be appreciated when it is realized that complete oxidation of 1 mol of palmitate involves a net production of 106 mol of ATP via β-oxidation and CO_2 production in the citric acid cycle, whereas only 26 mol of ATP are produced when acetoacetate is the end product and only 21 mol when 3-hydroxybutyrate is the end product. Thus, ketogenesis may be regarded as a mechanism that allows the liver to oxidize increasing quantities of fatty acids within the constraints of a tightly coupled system of oxidative phosphorylation.

A fall in the concentration of oxaloacetate, particularly within the mitochondria, can impair the ability of the citric acid cycle to metabolize acetyl-CoA and divert fatty acid oxidation toward ketogenesis. Such a fall may occur because of an increase in the (NADH)/(NAD^+) ratio caused by increased β-oxidation of fatty acids affecting the equilibrium between oxaloacetate and malate, leading to a decrease in the concentration of oxaloacetate, and when gluconeogenesis is elevated, which occurs when blood glucose levels are low. The activation of pyruvate carboxylase, which catalyzes the conversion of pyruvate to oxaloacetate, by acetyl-CoA partially alleviates this problem, but in conditions such as starvation and untreated diabetes mellitus, ketone bodies are overproduced causing ketosis.

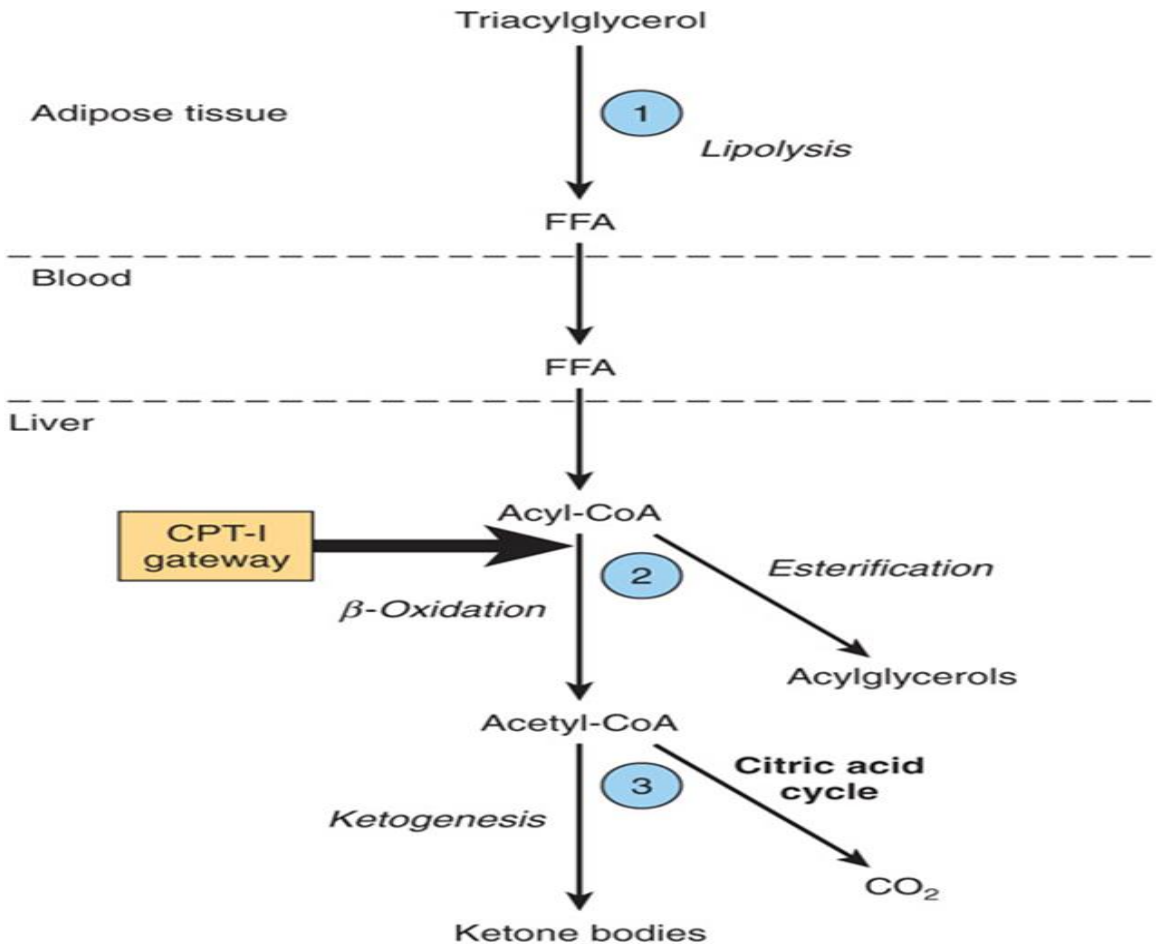

Figure 9 Regulation of ketogenesis. 1 to 3 show three crucial steps in the pathway of metabolism of free fatty acids (FFA) that determine the magnitude of ketogenesis. (CPT-I, carnitine palmitoyltransferase-I.)

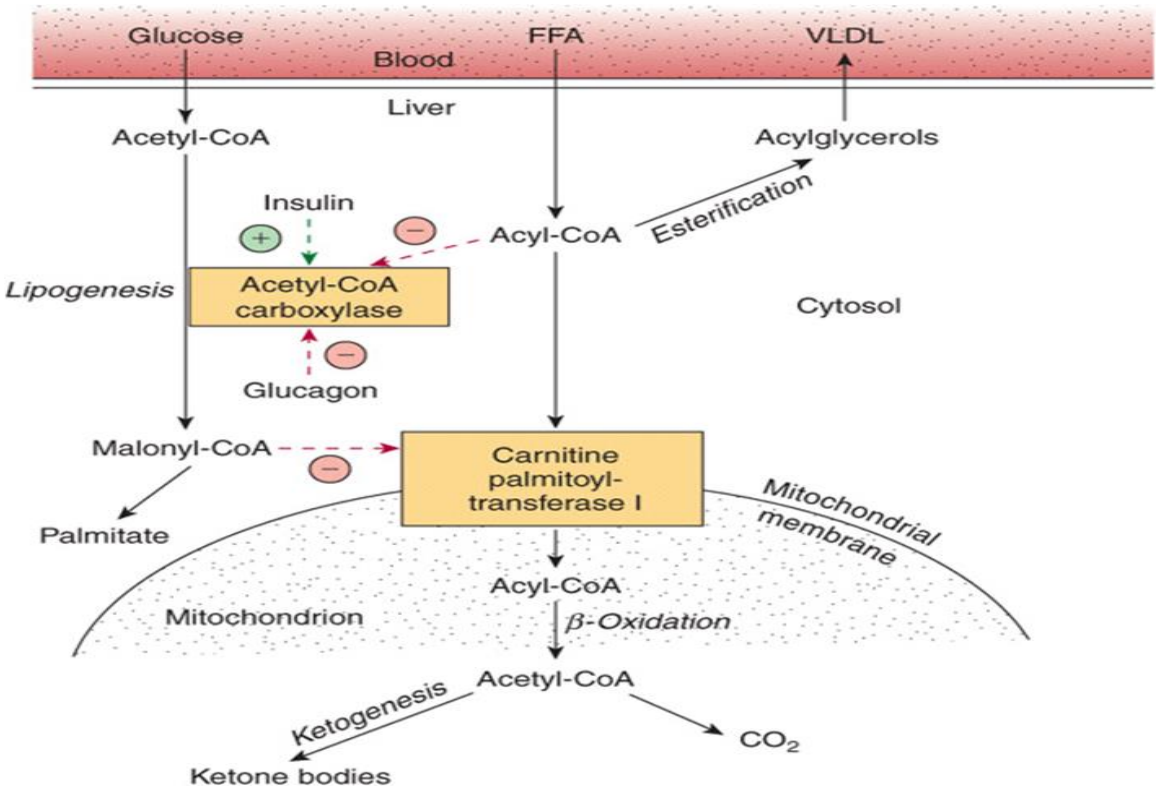

Figure 10 Regulation of long-chain fatty acid oxidation in the liver. (FFA, free fatty acids; VLDL, very low density lipoprotein.) Positive and negative regulatory effects are represented by broken arrows and substrate flow by solid arrows.

SUMMARY

■ Fatty acid oxidation in mitochondria leads to the generation of large quantities of ATP by a process called β-oxidation that cleaves acetyl-CoA units sequentially from fatty acyl chains. The acetyl-CoA is oxidized in the citric acid cycle, generating further ATP.

■ The ketone bodies (acetoacetate, 3-hydroxybutyrate, and acetone) are formed in **hepatic mitochondria when there is a high rate of fatty acid oxidation**. The pathway of ketogenesis involves synthesis and breakdown of 3-hydroxy-3- methylglutaryl-CoA (HMG-CoA) by two key enzymes, HMGCoA synthase, and HMG-CoA lyase.

■ Ketone bodies are important fuels in **extrahepatic tissues**.

■ Ketogenesis is regulated at three crucial steps: (1) control of FFA mobilization from adipose tissue; (2) the activity of carnitine palmitoyltransferase-I in liver, which determines the proportion of the fatty acid flux that is oxidized rather than esterified; and (3) partition of acetyl-CoA between the pathway of ketogenesis and the citric acid cycle.