Biochemistry II third stage

Dr. Maytham Ahmed

Lecture 2

Oxidation of Fatty Acids: Ketogenesis

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Biomedical importance

Although fatty acids are both oxidized to acetyl-CoA and 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 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, leading to 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, eg, carnitine palmitoyltransferase, or **inhibition** of fatty acid oxidation by **poisons**, eg, **hypoglycin**.

Oxidation of Fatty Acids

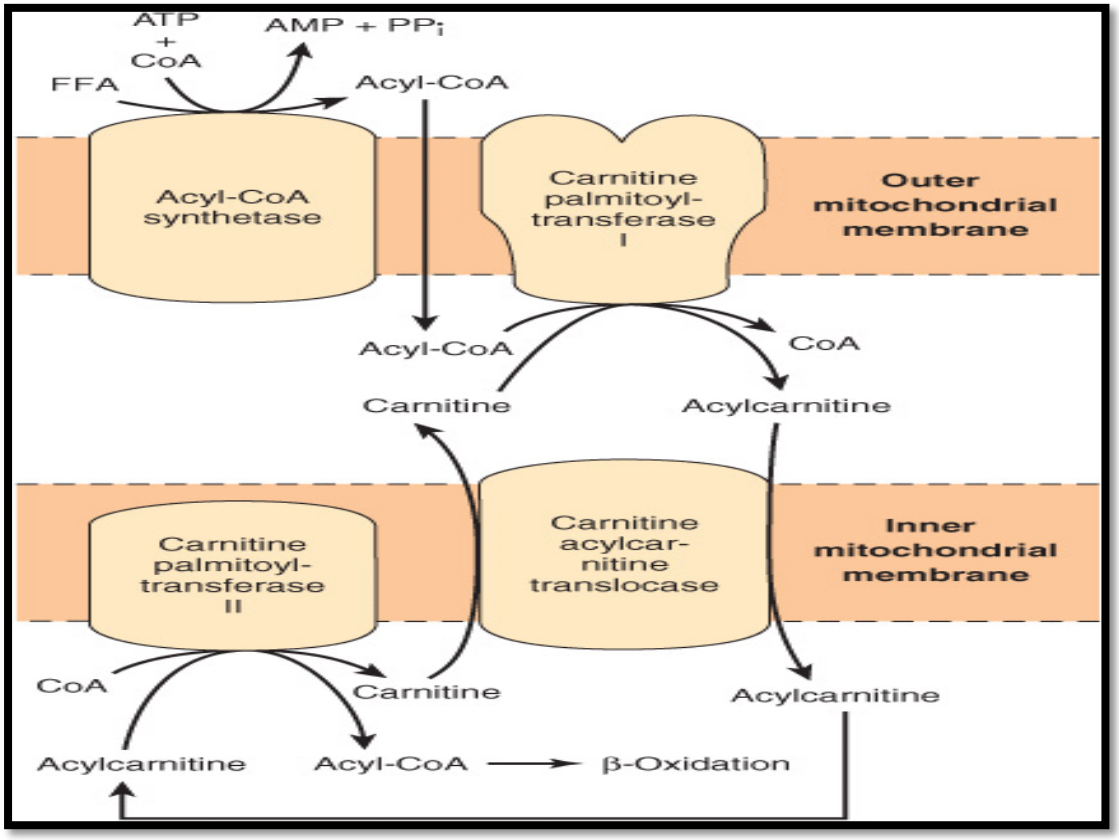
A. Fatty acids are activated before being catabolized

Fatty acids **must first** be converted to an **active intermediate** before they can be catabolized (β-oxidation of fatty acids in mitochondria). 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. The PPi is hydrolyzed by **inorganic pyrophosphatase** with the loss of a further high energy phosphate, ensuring that the

overall reaction goes to completion (**loss of two ATP**). **Acyl-CoA synthetases** are found inside and on the **outer membrane** of mitochondria.

Transport of long chain fatty acids into the mitochondria by carnitine

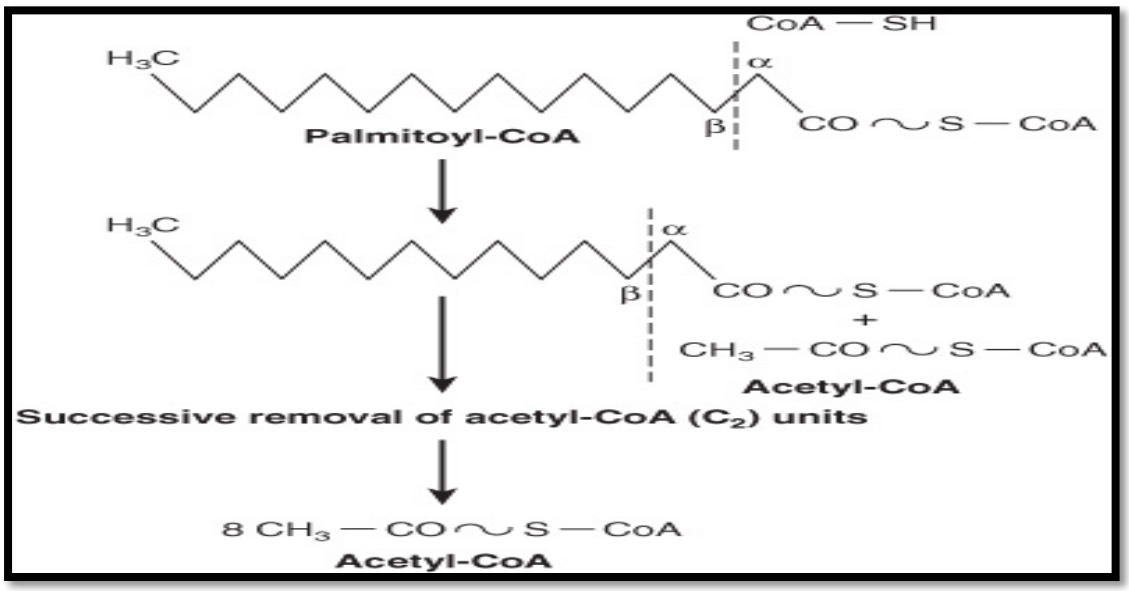
Carnitine (β-hydroxy-γ-trimethylammonium butyrate) is widely distributed and is particularly abundant in **muscle**. Long-chain acyl-CoA or FFA cannot penetrate the inner membrane of mitochondria. In the presence of **carnitine**, however, **carnitine palmitoyltransferase-I**, located in the **outer** mitochondrial membrane, transfers long-chain acyl group from CoA to **carnitine**, forming acylcarnitine and releasing CoA. Acylcarnitine is able to penetrate the inner membrane 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.



Role of carnitine in the transport of long chain fatty acids

β-oxidation of fatty acids

In the β-oxidation 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.



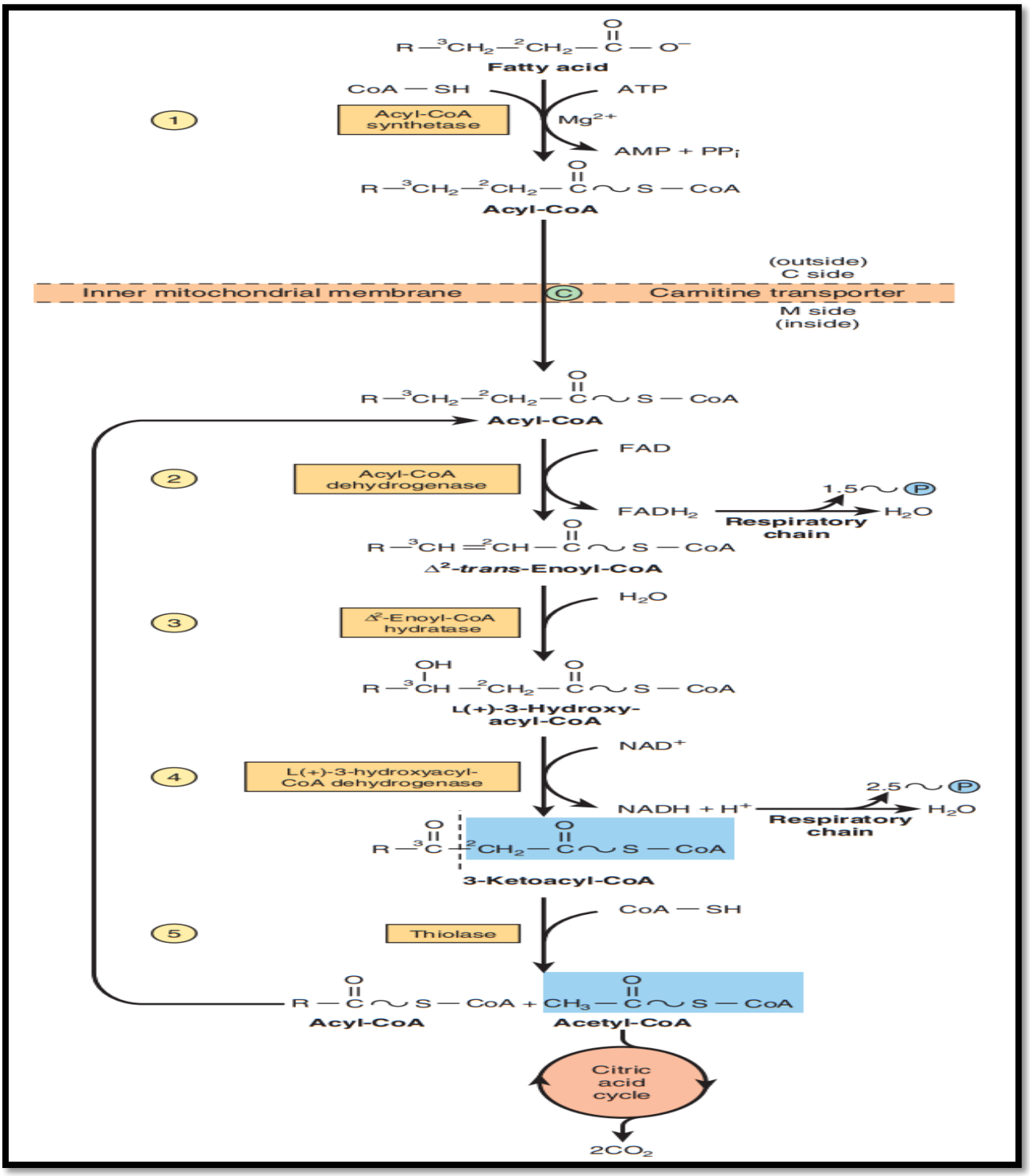
Overview of β-oxidation of fatty acids

The β-oxidation cycle

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.

The β-oxidation cycle is a sequence of four reactions involving the β-carbon (carbon 3) that results in shortening the fatty acid chain by two carbons. The steps include an oxidation step that produces $FADH_2$, a hydration step, an oxidation step that produces NADH, and a thiolytic cleavage that releases a molecule of 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.

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.



β-Oxidation of fatty acids

Oxidation of a fatty acid with an odd number of carbon atoms

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 (**synthesis of glucose** from fatty acid with an **odd number** of carbon atoms).

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 ($7 \times 4 = 28$). A total of 8 mol of acetyl-CoA is formed, and each acetyl-CoA gives rise to 10 mol of ATP on oxidation in the citric acid cycle, making $8 \times 10 = 80$ mol. **Two ATP** must be subtracted for the initial activation of the fatty acid, yielding a **net gain of 106 mol** of ATP per mole of palmitate, or $106 \times 30.5 = 3233$ kJ. This represents 33% of the free energy of combustion of palmitic 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

The oxidation of unsaturated fatty acids provides less energy than that of saturated fatty acids because unsaturated fatty acids are less highly reduced and, therefore,

fewer reducing equivalents can be produced from these structures. Oxidation of monounsaturated fatty acids, such as 18:1 (9) (oleic acid) requires one additional enzyme, **isomerase**. Oxidation of polyunsaturated fatty acids, as 18:2 (9,12) (linoleic acid), requires an **NADPH-dependent reductase** in addition to the **isomerase**.

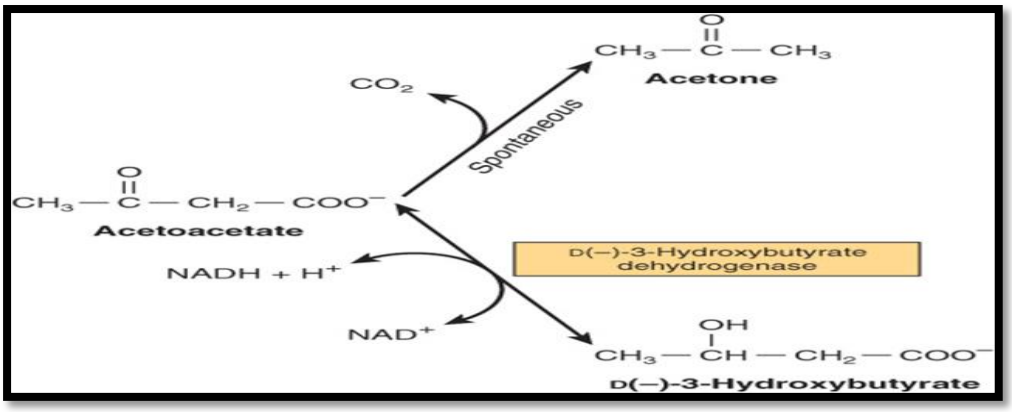
Ketogenesis

During a fast, the low insulin/glucagon ratio stimulate **hormone sensitive lipase** which initiate the lipolysis and release a fatty acid from triglycerides in adipose tissue. The free (unesterified) fatty acids move through the cell membrane of the adipocyte, and bind to plasma albumin which transported to liver. The liver is flooded with fatty acids mobilized from adipose tissue. The resulting elevated hepatic acetyl CoA produced primarily by 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).

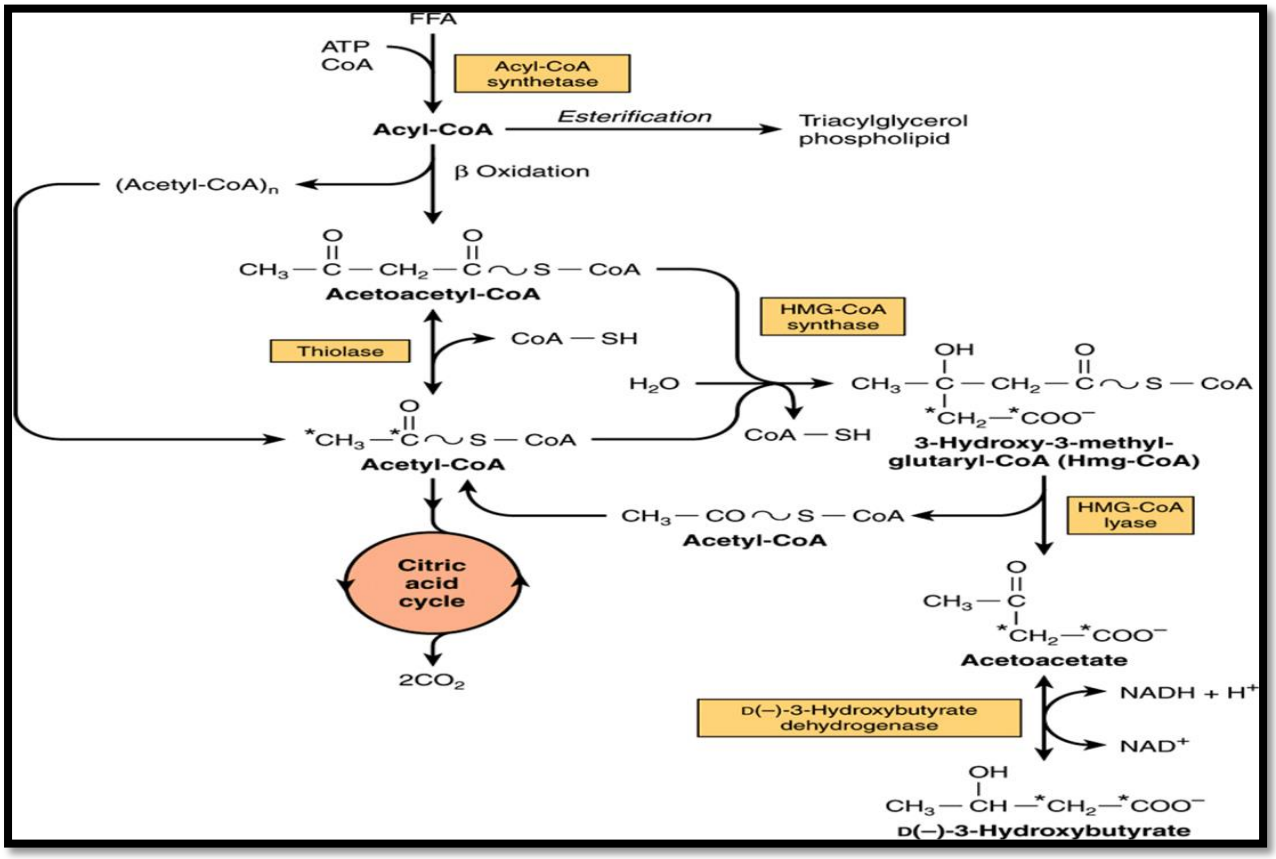
Acetoacetate and 3-hydroxybutyrate are interconverted by the mitochondrial enzyme **D(–)-3-hydroxybutyrate dehydrogenase**; the equilibrium is controlled by the mitochondrial $[NAD^+]/[NADH]$ ratio, that is, the redox state. The concentration of total ketone bodies in the blood of well-fed mammals does not normally exceed 0.2 mmol/L. 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.

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.

Condensation of acetoacetyl-CoA with another molecule of acetyl-CoA by **3-hydroxy-3-methylglutaryl-CoA synthase** (**HMGCoA synthase**) forms 3-hydroxy-3-methylglutaryl-CoA (**HMGCoA**). **3-Hydroxy-3-methylglutaryl-CoA lyase** (**HMGCoA 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.



Interrelationships of the ketone bodies

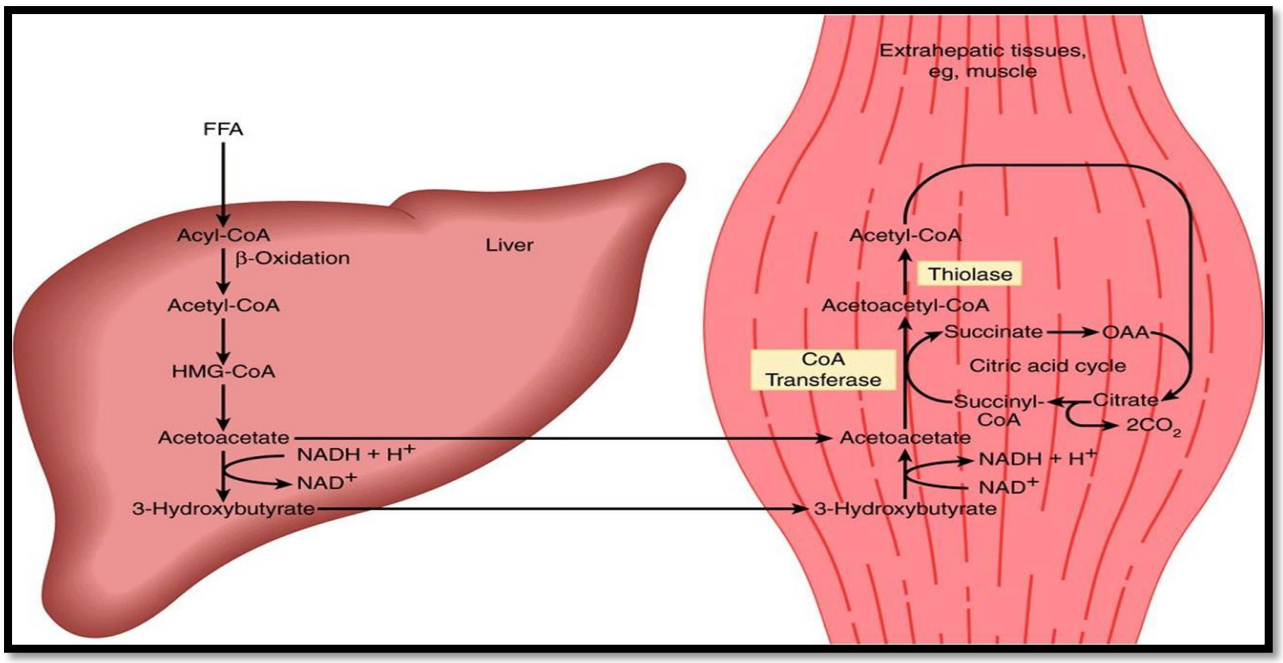


Pathways of ketogenesis in the liver (FFA, free fatty acids)

Ketone bodies serve as a fuel for extrahepatic tissues (Ketolysis)

The production of ketone bodies by the liver becomes much more significant during fasting when ketone bodies are needed to provide energy to the peripheral tissues. In extrahepatic tissues, 3-Hydroxybutyrate is oxidized to acetoacetate by **3-hydroxy butyrate dehydrogenase**, producing NADH. Acetoacetate is then provided with a CoA molecule taken from succinyl CoA by **succinyl CoA: acetoacetate CoA transferase (thiophorase)**. This reaction is reversible, but the product, acetoacetyl CoA, is actively removed by its conversion to two acetyl CoA by **thiolase** enzyme. Extrahepatic tissues, including the brain but excluding cells lacking mitochondria (for example, red blood cells), efficiently oxidize acetoacetate and 3-hydroxybutyrate in this manner. In contrast, although the liver actively produces ketone bodies, it lacks **thiophorase** and, therefore, is unable to use ketone bodies as fuel.

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. 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.



Ketone body synthesis in the liver and use 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.

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)**, 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**. 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**. The following figure displays comparison of the synthesis and degradation of long-chain, even-numbered, saturated fatty acids.

	SYNTHESIS	DEGRADATION
Greatest flux through pathway	After carbohydrate-rich meal	In starvation
Hormonal state favoring pathway	High insulin/glucagon ratio	Low insulin/glucagon ratio
Major tissue site	Primarily liver	Muscle, liver
Subcellular location	Primarily cytosol	Primarily mitochondria
Carriers of acyl/acetyl groups between mitochondria and cytosol	Citrate (mitochondria to cytosol)	Carnitine (cytosol to mitochondria)
Phosphopantetheine-containing active carriers	Acyl carrier protein domain, coenzyme A	Coenzyme A
Oxidation/reduction coenzymers	NADPH (reduction)	NAD^+, FAD (oxidation)
Two-carbon donor/product	Malonyl CoA: donor of one acetyl group	Acetyl CoA: product of β-oxidation
Activator	Citrate	
Inhibitor	Long-chain fatty acyl CoA (inhibits *acetyl CoA carboxylase*)	Malonyl CoA (inhibits *carnitine palmitoyltransferase-I*)
Product of pathway	Palmitate	Acetyl CoA
Repetitive four-step process	Condensation, reduction dehydration, reduction	Dehydrogenation, hydration dehydrogenation, thiolysis