

23 Oxidation of Fatty Acids and Ketone Bodies

Fatty acids are a major fuel for humans and supply our energy needs between meals and during periods of increased demand, such as exercise. During overnight fasting, fatty acids become the major fuel for cardiac muscle, skeletal muscle, and liver. The liver converts fatty acids to ketone bodies (acetoacetate and β -hydroxybutyrate), which also serve as major fuels for tissues (e.g., the gut). The brain, which does not have a significant capacity for fatty acid oxidation, can use ketone bodies as a fuel during prolonged fasting.

*The route of metabolism for a fatty acid depends somewhat on its chain length. Fatty acids are generally classified as **very-long-chain length fatty acids** (greater than C20), **long-chain fatty acids** (C12–C20), **medium-chain fatty acids** (C6–C12), and **short-chain fatty acids** (C4).*

*ATP is generated from oxidation of fatty acids in the pathway of **β -oxidation**. Between meals and during overnight fasting, **long-chain fatty acids** are released from adipose tissue triacylglycerols. They circulate through blood bound to **albumin** (Fig. 23.1). In cells, they are converted to **fatty acyl CoA** derivatives by **acyl CoA synthetases**. The activated acyl group is transported into the mitochondrial matrix bound to **carnitine**, where fatty acyl CoA is regenerated. In the pathway of **β -oxidation**, the fatty acyl group is sequentially oxidized to yield FAD(2H), NADH, and acetyl CoA. Subsequent oxidation of NADH and FAD(2H) in the electron transport chain, and oxidation of acetyl CoA to CO₂ in the TCA cycle, generates ATP from oxidative phosphorylation.*

*Many fatty acids have structures that require variations of this basic pattern. Long-chain fatty acids that are **unsaturated fatty acids** generally require additional isomerization and oxidation–reduction reactions to rearrange their double bonds during β -oxidation. Metabolism of water-soluble **medium-chain-length fatty acids** does not require carnitine and occurs only in liver. **Odd-chain-length fatty acids** undergo β -oxidation to the terminal three-carbon **propionyl CoA**, which enters the TCA cycle as succinyl CoA.*

*Fatty acids that do not readily undergo mitochondrial β -oxidation are oxidized first by alternate routes that convert them to more suitable substrates or to urinary excretion products. **Excess fatty acids** may undergo microsomal ω -oxidation, which converts them to **dicarboxylic acids** that appear in urine. **Very-long-chain fatty acids** (both straight chain and **branched fatty acids** such as phytanic acid) are whittled down to size in peroxisomes. **Peroxisomal α - and β -oxidation** generates hydrogen peroxide (H₂O₂), NADH, acetyl CoA, or propionyl CoA and a short- to medium-chain-length acyl CoA. The acyl CoA products are transferred to mitochondria to complete their metabolism.*

*In the liver, much of the acetyl CoA generated from fatty acid oxidation is converted to the ketone bodies, **acetoacetate** and **β -hydroxybutyrate**, which enter the blood (see Fig. 23.1). In other tissues, these ketone bodies are converted to acetyl*

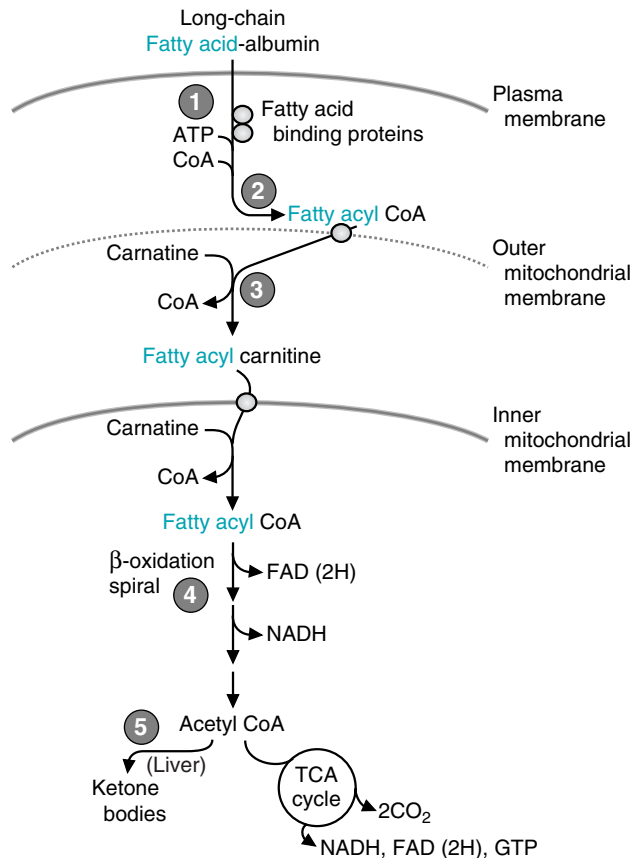


Fig. 23.1. Overview of mitochondrial long-chain fatty acid metabolism. (1) Fatty acid binding proteins (FaBP) transport fatty acids across the plasma membrane and bind them in the cytosol. (2) Fatty acyl CoA synthetase activates fatty acids to fatty acyl CoAs. (3) Carnitine transports the activated fatty acyl group into mitochondria. (4) β -oxidation generates NADH, FAD(2H), and acetyl CoA (5) In the liver, acetyl CoA is converted to ketone bodies

CoA, which is oxidized in the TCA cycle. The liver synthesizes ketone bodies but cannot use them as a fuel.

The **rate of fatty acid oxidation** is linked to the rate of NADH, FAD(2H), and acetyl CoA oxidation, and, thus, to the rate of oxidative phosphorylation and **ATP utilization**. Additional regulation occurs through **malonyl CoA**, which inhibits formation of the fatty acyl carnitine derivatives. Fatty acids and ketone bodies are used as a fuel when their level increases in the blood, which is determined by hormonal regulation of adipose tissue lipolysis.



THE WAITING ROOM



Otto Shape was disappointed that he did not place in his 5-km race and has decided that short-distance running is probably not right for him. After careful consideration, he decides to train for the marathon by running 12 miles three times per week. He is now 13 pounds over his ideal weight, and he plans on losing this weight while studying for his Pharmacology finals. He considers a variety of dietary supplements to increase his endurance and selects one containing carnitine, CoQ, pantothenate, riboflavin, and creatine.



The liver transaminases measured in the blood are aspartate aminotransferase (AST), which was formerly called serum glutamate-oxaloacetate transaminase (SGOT), and alanine aminotransferase (ALT), which was formerly called serum glutamate pyruvate transaminase (SGPT). Elevation of liver enzymes reflects damage of the liver plasma membrane.



During Otto's distance running (a moderate-intensity exercise), decreases in insulin and increases in insulin counterregulatory hormones, such as epinephrine and norepinephrine, increase adipose tissue lipolysis. Thus, his muscles are being provided with a supply of fatty acids in the blood that they can use as a fuel.



Lofata Burne developed symptoms during fasting, when adipose tissue lipolysis was elevated. Under these circumstances, muscle tissue, liver, and many other tissues are oxidizing fatty acids as a fuel. After overnight fasting, approximately 60 to 70% of our energy supply is derived from the oxidation of fatty acids.



Lofata Burne is a 16-year-old girl. Since age 14 months she has experienced recurrent episodes of profound fatigue associated with vomiting and increased perspiration, which required hospitalization. These episodes occurred only if she fasted for more than 8 hours. Because her mother gave her food late at night and woke her early in the morning for breakfast, Lofata's physical and mental development had progressed normally.

On the day of admission for this episode, Lofata had missed breakfast, and by noon she was extremely fatigued, nauseated, sweaty, and limp. She was unable to hold any food in her stomach and was rushed to the hospital, where an infusion of glucose was started intravenously. Her symptoms responded dramatically to this therapy.

Her initial serum glucose level was low at 38 mg/dL (reference range for fasting serum glucose levels = 70–100). Her blood urea nitrogen (BUN) level was slightly elevated at 26 mg/dL (reference range = 8–25) as a result of vomiting, which led to a degree of dehydration. Her blood levels of liver transaminases were slightly elevated, although her liver was not palpably enlarged. Despite elevated levels of free fatty acids (4.3 mM) in the blood, blood ketone bodies were below normal.



Di Abietes, a 27-year-old woman with type 1 diabetes mellitus, had been admitted to the hospital in a ketoacidotic coma a year ago (see Chapter 4). She had been feeling drowsy and had been vomiting for 24 hours before that admission. At the time of admission, she was clinically dehydrated, her blood pressure was low, and her breathing was deep and rapid (Kussmaul breathing). Her pulse was rapid, and her breath had the odor of acetone. Her arterial blood pH was 7.08 (reference range, 7.36–7.44), and her blood ketone body levels were 15 mM (normal is approximately 0.2 mM for a person on a normal diet).

I. FATTY ACIDS AS FUELS

The fatty acids oxidized as fuels are principally long-chain fatty acids released from adipose tissue triacylglycerol stores between meals, during overnight fasting, and during periods of increased fuel demand (e.g., during exercise). Adipose tissue triacylglycerols are derived from two sources; dietary lipids and triacylglycerols synthesized in the liver. The major fatty acids oxidized are the long-chain fatty acids, palmitate, oleate, and stearate, because they are highest in dietary lipids and are also synthesized in the human.

Between meals, a decreased insulin level and increased levels of insulin counterregulatory hormones (e.g., glucagon) activate lipolysis, and free fatty acids are transported to tissues bound to serum albumin. Within tissues, energy is derived from oxidation of fatty acids to acetyl CoA in the pathway of β -oxidation. Most of the enzymes involved in fatty acid oxidation are present as 2-3 isoenzymes, which have different but overlapping specificities for the chain length of the fatty acid. Metabolism of unsaturated fatty acids, odd-chain-length fatty acids, and medium-chain-length fatty acids requires variations of this basic pattern. The acetyl CoA produced from fatty acid oxidation is principally oxidized in the TCA cycle or converted to ketone bodies in the liver.

A. Characteristics of Fatty Acids Used as Fuels

Fat constitutes approximately 38% of the calories in the average North American diet. Of this, more than 95% of the calories are present as triacylglycerols (3 fatty acids esterified to a glycerol backbone). During ingestion and absorption, dietary triacylglycerols are broken down into their constituents and then reassembled for transport to adipose tissue in chylomicrons (see Chapter 2). Thus, the fatty acid composition of adipose triacylglycerols varies with the type of food consumed.

The most common dietary fatty acids are the saturated long-chain fatty acids palmitate (C16) and stearate (C18), the monounsaturated fatty acid oleate (C18:1), and the polyunsaturated essential fatty acid, linoleate (C18:2) (To review fatty acid nomenclature, consult Chapter 5). Animal fat contains principally saturated and monounsaturated long-chain fatty acids, whereas vegetable oils contain linoleate and some longer-chain and polyunsaturated fatty acids. They also contain smaller amounts of branched-chain and odd-chain-length fatty acids. Medium-chain-length fatty acids are present principally in dairy fat (e.g., milk and butter), maternal milk, and vegetable oils.

Adipose tissue triacylglycerols also contain fatty acids synthesized in the liver, principally from excess calories ingested as glucose. The pathway of fatty acid synthesis generates palmitate, which can be elongated to form stearate, and unsaturated to form oleate. These fatty acids are assembled into triacylglycerols and transported to adipose tissue as the lipoprotein VLDL (very-low-density lipoprotein).

B. Transport and Activation of Long-Chain Fatty Acids

Long-chain fatty acids are hydrophobic and water insoluble. In addition, they are toxic to cells because they can disrupt the hydrophobic bonding between amino acid side chains in proteins. Consequently, they are transported in the blood and in cells bound to proteins.

1. CELLULAR UPTAKE OF LONG-CHAIN FATTY ACIDS

During fasting and other conditions of metabolic need, long-chain fatty acids are released from adipose tissue triacylglycerols by lipases. They travel in the blood bound in the hydrophobic binding pocket of albumin, the major serum protein (see Fig. 23.1).

Fatty acids enter cells both by a saturable transport process and by diffusion through the lipid plasma membrane. A fatty acid binding protein in the plasma membrane facilitates transport. An additional fatty acid binding protein binds the fatty acid intracellularly and may facilitate its transport to the mitochondrion. The free fatty acid concentration in cells is, therefore, extremely low.

2. ACTIVATION OF LONG-CHAIN FATTY ACIDS

Fatty acids must be activated to acyl CoA derivatives before they can participate in β -oxidation and other metabolic pathways (Fig. 23.2). The process of activation involves an acyl CoA synthetase (also called a thiokinase) that uses ATP energy to form the fatty acyl CoA thioester bond. In this reaction, the β bond of ATP is cleaved to form a fatty acyl AMP intermediate and pyrophosphate (PPi). Subsequent cleavage of PPi helps to drive the reaction.

The acyl CoA synthetase that activates long-chain fatty acids, 12 to 20 carbons in length, is present in three locations in the cell: the endoplasmic reticulum, outer mitochondrial membranes, and peroxisomal membranes (Table 23.1). This enzyme has no activity toward C22 or longer fatty acids, and little activity below C12. In contrast, the synthetase for activation of very-long-chain fatty acids is present in peroxisomes, and the medium-chain-length fatty acid activating enzyme is present only in the mitochondrial matrix of liver and kidney cells.

3. FATES OF FATTY ACYL COAS

Fatty acyl CoA formation, like the phosphorylation of glucose, is a prerequisite to metabolism of the fatty acid in the cell (Fig. 23.3). The multiple locations of the long-chain acyl CoA synthetase reflects the location of different metabolic routes taken by fatty acyl CoA derivatives in the cell (e.g., triacylglycerol and phospholipid synthesis

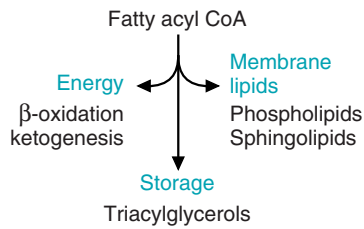


Fig. 23.3. Major metabolic routes for long-chain fatty acyl CoAs. Fatty acids are activated to acyl CoA compounds for degradation in mitochondrial β-oxidation, or incorporation into triacylglycerols or membrane lipids. When β-oxidation is blocked through an inherited enzyme deficiency, or metabolic regulation, excess fatty acids are diverted into triacylglycerol synthesis.

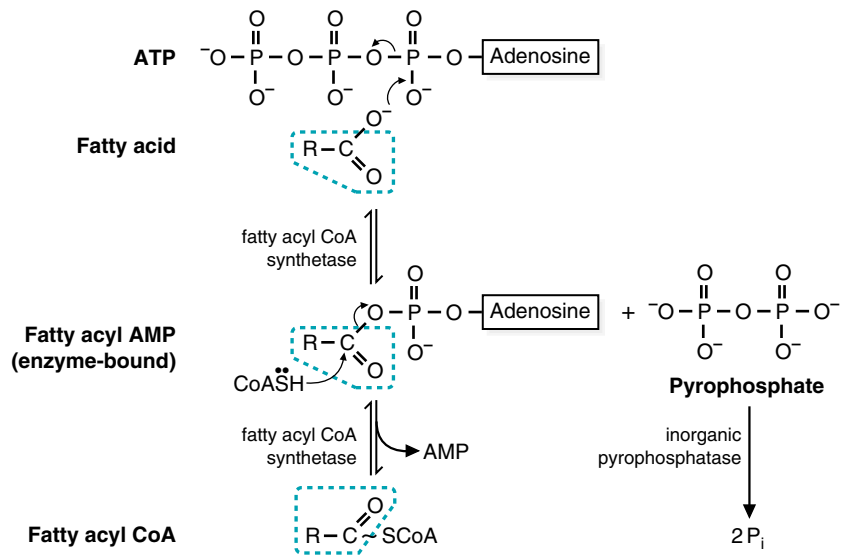


Fig. 23.2. Activation of a fatty acid by a fatty acyl CoA synthetase. The fatty acid is activated by reacting with ATP to form a high-energy fatty acyl AMP and pyrophosphate. The AMP is then exchanged for CoA. Pyrophosphate is cleaved by a pyrophosphatase.

Table 23.1. Chain-Length Specificity of Fatty Acid Activation and Oxidation Enzymes

Enzyme	Chain Length	Comments
Acyl CoA synthetases		
Very Long Chain	14–26	Only found in peroxisomes
Long Chain	12–20	Enzyme present in membranes of ER, mitochondria, and peroxisomes to facilitate different metabolic routes of acyl CoAs.
Medium Chain	6–12	Exists as many variants, present only in mitochondrial matrix of kidney and liver. Also involved in xenobiotic metabolism.
Acetyl	2–4	Present in cytoplasm and possibly mitochondrial matrix
Acyltransferases		
CPTI	12–16	Although maximum activity is for fatty acids 12–16 carbons long, it also acts on many smaller acyl CoA derivatives
Medium Chain (Octanoylcarnitine transferase)	6–12	Substrate is medium-chain acyl CoA derivatives generated during peroxisomal oxidation.
Carnitine:acetyl transferase	2	High level in skeletal muscle and heart to facilitate use of acetate as a fuel
Acyl CoA Dehydrogenases		
VLCAD	14–20	Present in inner mitochondrial membrane
LCAD	12–18	Members of same enzyme family, which also includes acyl CoA dehydrogenases for carbon skeleton of branched-chain amino acids.
MCAD	4–12	
SCAD	4–6	
Other enzymes		
Enoyl CoA hydratase, Short-chain	>4	Also called crotonase. Activity decreases with increasing chain length.
L-3-Hydroxyacyl CoA dehydrogenase, Short-Chain	4–16	Activity decreases with increasing chain length
Acetoacetyl CoA thiolase	4	Specific for acetoacetyl CoA
Trifunctional Protein	12–16	Complex of long-chain enoyl hydratase, acyl CoA dehydrogenase and a thiolase with broad specificity. Most active with longer chains.

in the endoplasmic reticulum, oxidation and plasmalogen synthesis in the peroxisome, and β -oxidation in mitochondria). In the liver and some other tissues, fatty acids that are not being used for energy generation are re-incorporated (re-esterified) into triacylglycerols.

4. TRANSPORT OF LONG-CHAIN FATTY ACIDS INTO MITOCHONDRIA

Carnitine serves as the carrier that transports activated long chain fatty acyl groups across the inner mitochondrial membrane (Fig. 23.4). Carnitine acyl transferases are able to reversibly transfer an activated fatty acyl group from CoA to the hydroxyl group of carnitine to form an acylcarnitine ester. The reaction is reversible, so that the fatty acyl CoA derivative can be regenerated from the carnitine ester.

Carnitine:palmitoyltransferase I (CPTI; also called carnitine acyltransferase I, CATI), the enzyme that transfers long-chain fatty acyl groups from CoA to carnitine, is located on the outer mitochondrial membrane (Fig. 23.5). Fatty acylcarnitine crosses the inner mitochondrial membrane with the aid of a translocase. The fatty acyl group is transferred back to CoA by a second enzyme, carnitine:palmitoyltransferase II (CPTII or CATII). The carnitine released in this reaction returns to the cytosolic side of the mitochondrial membrane by the same translocase that brings fatty acylcarnitine to the matrix side. Long-chain fatty acyl CoA, now located within the mitochondrial matrix, is a substrate for β -oxidation.

Carnitine is obtained from the diet or synthesized from the side chain of lysine by a pathway that begins in skeletal muscle, and is completed in the liver. The reactions use S-adenosylmethionine to donate methyl groups, and vitamin C (ascorbic acid) is also required for these reactions. Skeletal muscles have a

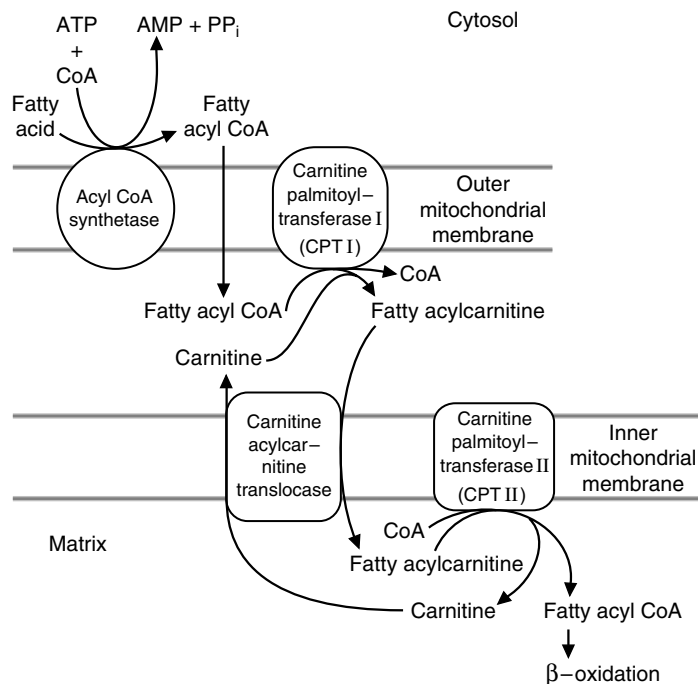
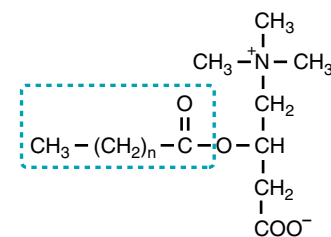


Fig. 23.5. Transport of long-chain fatty acids into mitochondria. The fatty acyl CoA crosses the outer mitochondrial membrane. Carnitine palmitoyl transferase I in the outer mitochondrial membrane transfers the fatty acyl group to carnitine and releases CoASH. The fatty acyl carnitine is translocated into the mitochondrial matrix as carnitine moves out. Carnitine palmitoyl transferase II on the inner mitochondrial membrane transfers the fatty acyl group back to CoASH, to form fatty acyl CoA in the matrix.



A number of inherited diseases in the metabolism of carnitine or acylcarnitines have been described. These include defects in the following enzymes or systems: the transporter for carnitine uptake into muscle; CPT I; carnitine:acylcarnitine translocase; and CPTII. Classical CPTII deficiency, the most common of these diseases, is characterized by adolescent to adult onset of recurrent episodes of acute myoglobinuria precipitated by prolonged exercise or fasting. During these episodes, the patient is weak, and may be somewhat hypoglycemic with diminished ketosis (hypoketosis), but metabolic decompensation is not severe. Lipid deposits are found in skeletal muscles. CPK levels, and long-chain acylcarnitines are elevated in the blood. CPTII levels in fibroblasts are approximately 25% of normal. The remaining CPTII activity probably accounts for the mild effect on liver metabolism. In contrast, when CPTII deficiency has presented in infants, CPT II levels are below 10% of normal, the hypoglycemia and hypoketosis are severe, hepatomegaly occurs from the triacylglycerol deposits, and cardiomyopathy is also present.



Fatty acylcarnitine

Fig. 23.4. Structure of fatty acylcarnitine. Carnitine: palmitoyl transferases catalyze the reversible transfer of a long-chain fatty acyl group from the fatty acyl CoA to the hydroxyl group of carnitine. The atoms in the dashed box originate from the fatty acyl CoA.



Otto Shape's power supplement contains carnitine. However, his body can synthesize enough carnitine to meet his needs, and his diet contains carnitine. Carnitine deficiency has been found only in infants fed a soy-based formula that was not supplemented with carnitine. His other supplements likewise probably provide no benefit, but are designed to facilitate fatty acid oxidation during exercise. Riboflavin is the vitamin precursor of FAD, which is required for acyl CoA dehydrogenases and ETFs. CoQ is synthesized in the body, but it is the recipient in the electron transport chain for electrons passed from complexes I and II and the ETFs. Some reports suggest that supplementation with pantothenate, the precursor of CoA, improves performance.

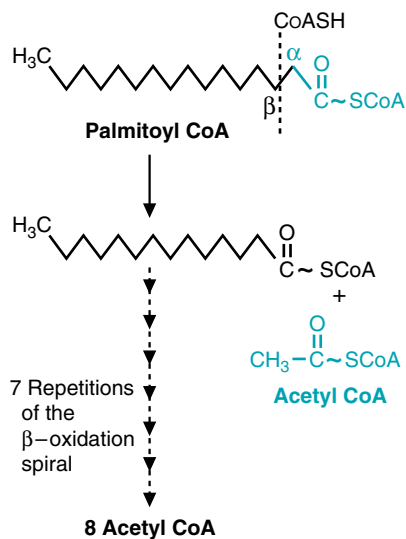


Fig. 23.6. Overview of β -oxidation. Oxidation at the β -carbon is followed by cleavage of the α — β bond, releasing acetyl CoA and a fatty acyl CoA that is two carbons shorter than the original. The carbons cleaved to form acetyl CoA are shown in blue. Successive spirals of β -oxidation completely cleave an even-chain fatty acyl CoA to acetyl CoA.

high-affinity uptake system for carnitine, and most of the carnitine in the body is stored in skeletal muscle.

C. β -Oxidation of Long-Chain Fatty Acids

The oxidation of fatty acids to acetyl CoA in the β -oxidation spiral conserves energy as FAD(2H) and NADH. FAD(2H) and NADH are oxidized in the electron transport chain, generating ATP from oxidative phosphorylation. Acetyl CoA is oxidized in the TCA cycle or converted to ketone bodies.

1. THE β -OXIDATION SPIRAL

The fatty acid β -oxidation pathway sequentially cleaves the fatty acyl group into 2-carbon acetyl CoA units, beginning with the carboxyl end attached to CoA (Fig. 23.6). Before cleavage, the β -carbon is oxidized to a keto group in two reactions that generate NADH and FAD(2H); thus, the pathway is called β -oxidation. As each acetyl group is released, the cycle of β -oxidation and cleavage begins again, but each time the fatty acyl group is 2 carbons shorter.

There are four types of reactions in the β -oxidation pathway (Fig. 23.7). In the first step, a double bond is formed between the β - and α -carbons by an acyl CoA dehydrogenase that transfers electrons to FAD. The double bond is in the trans

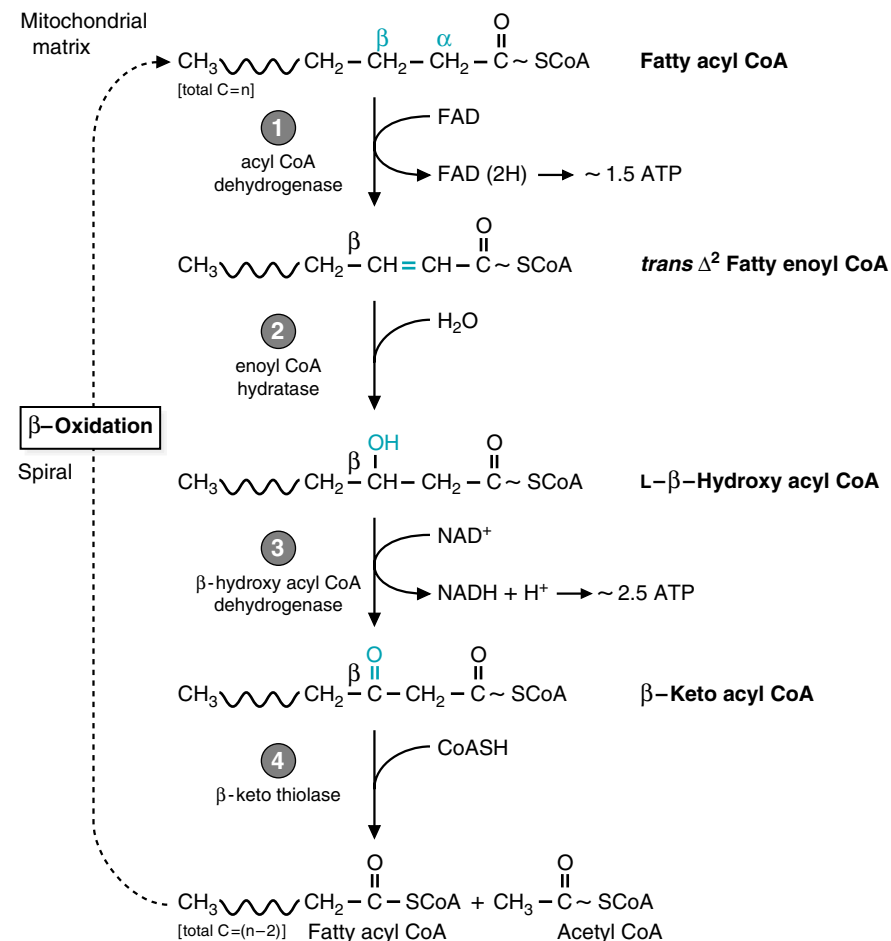


Fig. 23.7. Steps of β -oxidation. The four steps are repeated until an even-chain fatty acid is completely converted to acetyl CoA. The FAD(2H) and NADH are reoxidized by the electron transport chain, producing ATP.

configuration (a Δ^2 -*trans* double bond). In the next step, an OH from water is added to the β -carbon, and an H from water is added to the β -carbon. The enzyme is called an enoyl hydratase (hydratases add the elements of water, and “ene” in a name denotes a double bond). In the third step of β -oxidation, the hydroxyl group on the β -carbon is oxidized to a ketone by a hydroxyacyl CoA dehydrogenase. In this reaction, as in the conversion of most alcohols to ketones, the electrons are transferred to NAD^+ to form NADH. In the last reaction of the sequence, the bond between the β - and α -carbons is cleaved by a reaction that attaches CoASH to the β -carbon, and acetyl CoA is released. This is a thiolytic reaction (lysis refers to breakage of the bond, and thio refers to the sulfur), catalyzed by enzymes called β -ketothiolases. The release of two carbons from the carboxyl end of the original fatty acyl CoA produces acetyl CoA and a fatty acyl CoA that is two carbons shorter than the original.

The shortened fatty acyl CoA repeats these four steps until all of its carbons are converted to acetyl CoA. β -Oxidation is, thus, a spiral rather than a cycle. In the last spiral, cleavage of the four-carbon fatty acyl CoA (butyryl CoA) produces two acetyl CoA. Thus, an even chain fatty acid such as palmitoyl CoA, which has 16 carbons, is cleaved seven times, producing 7 FAD(2H), 7 NADH, and 8 acetyl CoA.

2. ENERGY YIELD OF β -OXIDATION

Like the FAD in all flavoproteins, FAD(2H) bound to the acyl CoA dehydrogenases is oxidized back to FAD without dissociating from the protein (Fig. 23.8). Electron transfer flavoproteins (ETF) in the mitochondrial matrix accept electrons from the enzyme-bound FAD(2H) and transfer these electrons to ETF-QO (electron transfer flavoprotein-CoQ oxidoreductase) in the inner mitochondrial membrane. ETF-QO, also a flavoprotein, transfers the electrons to CoQ in the electron transport chain. Oxidative phosphorylation thus generates approximately 1.5 ATP for each FAD(2H) produced in the β -oxidation spiral.

The total energy yield from the oxidation of 1 mole of palmitoyl CoA to 8 moles of acetyl CoA is therefore 28 moles of ATP: 1.5 for each of the 7 FAD(2H), and 2.5 for each of the 7 NADH. To calculate the energy yield from oxidation of 1 mole of palmitate, two ATP need to be subtracted from the total because two high-energy phosphate bonds are cleaved when palmitate is activated to palmitoyl CoA.

3. CHAIN LENGTH SPECIFICITY IN β -OXIDATION

The four reactions of β -oxidation are catalyzed by sets of enzymes that are each specific for fatty acids with different chain lengths (see Table 23.1). The acyl dehydrogenases, which catalyze the first step of the pathway, are part of an enzyme family that have four different ranges of specificity. The subsequent steps of the spiral use enzymes specific for long- or short-chain enoyl CoAs. Although these enzymes are structurally distinct, their specificity overlaps to some extent.



The β -oxidation spiral uses the same reaction types seen in the TCA cycle when succinate is converted to oxaloacetate.

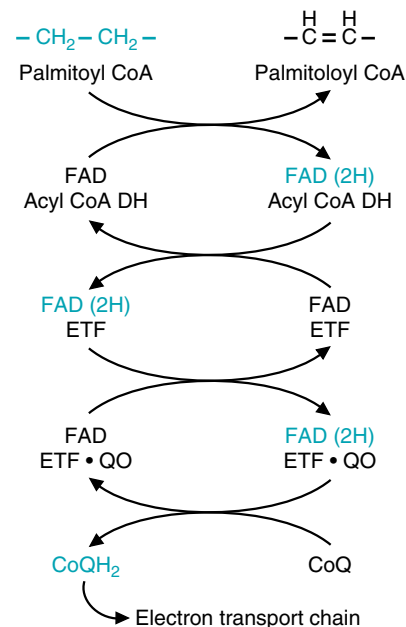


Fig. 23.8. Transfer of electrons from acyl CoA dehydrogenase to the electron transport chain. Abbreviations: ETF, electron-transferring flavoprotein; ETF-QO, electron-transferring flavoprotein-Coenzyme Q oxidoreductase.



What is the total ATP yield for the oxidation of 1 mole of palmitic acid to carbon dioxide and water?



After reviewing **Lofata Burne's** previous hospital records, a specialist suspected that Lofata's medical problems were caused by a disorder in fatty acid metabolism. A battery of tests showed that Lofata's blood contained elevated levels of several partially oxidized medium-chain fatty acids, such as octanoic acid (8:0) and 4-decenoic acid (10:1, Δ^4). A urine specimen showed an increase in organic acid metabolites of medium-chain fatty acids containing 6 to 10 carbons, including medium-chain acylcarnitine derivatives. The profile of acylcarnitine species in the urine was characteristic of a genetically determined medium-chain acyl CoA dehydrogenase (MCAD) deficiency. In this disease, long-chain fatty acids are metabolized by β -oxidation to a medium-chain-length acyl CoA, such as octanoyl CoA. Because further oxidation of this compound is blocked in MCAD deficiency, the medium chain acyl group is transferred back to carnitine. These acylcarnitines are water soluble and appear in blood and urine. The specific enzyme deficiency was demonstrated in cultured fibroblasts from Lofata's skin as well as in her circulating monocytic leukocytes.

In LCAD deficiency, fatty acylcarnitines accumulate in the blood. Those containing 14 carbons predominate. However, these do not appear in the urine.



Palmitic acid is 16 carbons long, with no double bonds, so it requires 7 oxidation spirals to be completely converted to acetyl-CoA. After 7 spirals, there are 7 FAD(2H), 7 NADH, and 8 acetyl-CoA. Each NADH yields 2.5 ATP, each FAD(2H) yields 1.5 ATP, and each acetyl-CoA yields 10 ATP as it is processed around the TCA cycle. This then yields $17.5 + 10.5 + 80.5 = 108$ ATP. However, activation of palmitic acid to palmitoyl-CoA requires two high-energy bonds, so the net yield is $108 - 2$, or 106 moles of ATP.



Linoleate, although high in the diet, cannot be synthesized in the human and is an essential fatty acid. It is required for formation of arachidonate, which is present in plasma lipids, and is used for eicosanoid synthesis. Therefore, only a portion of the linoleate pool is rapidly oxidized.



The medium-chain-length acyl CoA synthetase has a broad range of specificity for compounds of approximately the same size that contain a carboxyl group, such as drugs (salicylate, from aspirin metabolism, and valproate, which is used to treat epileptic seizures), or benzoate, a common component of plants. Once the drug acyl CoA is formed, the acyl group is conjugated with glycine to form a urinary excretion product. With certain disorders of fatty acid oxidation, medium- and short-chain fatty acylglycines may appear in the urine, together with acylcarnitines or dicarboxylic acids.

As the fatty acyl chains are shortened by consecutive cleavage of two acetyl units, they are transferred from enzymes that act on longer chains to those that act on shorter chains. Medium- or short-chain fatty acyl CoAs that may be formed from dietary fatty acids, or transferred from peroxisomes, enter the spiral at the enzyme most active for fatty acids of their chain length

4. OXIDATION OF UNSATURATED FATTY ACIDS

Approximately one half of the fatty acids in the human diet are unsaturated, containing *cis* double bonds, with oleate (C18:1, Δ^9) and linoleate (18:2, $\Delta^{9,12}$) being the most common. In β -oxidation of saturated fatty acids, a *trans* double bond is created between the 2nd and 3rd (α and β) carbons. For unsaturated fatty acids to undergo the β -oxidation spiral, their *cis* double bonds must be isomerized to *trans* double bonds that will end up between the 2nd and 3rd carbons during β -oxidation, or the double bond must be reduced. The process is illustrated for the polyunsaturated fatty acid linoleate in Fig. 23.9. Linoleate undergoes β -oxidation until one double bond is between carbons 3 and 4 near the carboxyl end of the fatty acyl chain, and the other is between carbons 6 and 7. An isomerase moves the double bond from the 3,4 position so that it is *trans* and in the 2,3 position, and β -oxidation continues. When a conjugated pair of double bonds is formed (two double bonds separated by one single bond) at positions 2 and 4, an NADPH-dependent reductase reduces the pair to one *trans* double bond at position 3. Then isomerization and β -oxidation resume.

In oleate (C18:1, Δ^9), there is only one double bond between carbons 9 and 10. It is handled by an isomerization reaction similar to that shown for the double bond at position 9 of linoleate.

5. ODD-CHAIN-LENGTH FATTY ACIDS

Fatty acids containing an odd number of carbon atoms undergo β -oxidation, producing acetyl CoA, until the last spiral, when five carbons remain in the fatty acyl CoA. In this case, cleavage by thiolase produces acetyl CoA and a three-carbon fatty acyl CoA, propionyl CoA (Fig. 23.10). Carboxylation of propionyl CoA yields methylmalonyl CoA, which is ultimately converted to succinyl CoA in a vitamin B12-dependent reaction (Fig. 23.11). Propionyl CoA also arises from the oxidation of branched chain amino acids.

The propionyl CoA to succinyl CoA pathway is a major anaplerotic route for the TCA cycle and is used in the degradation of valine, isoleucine, and a number of other compounds. In the liver, this route provides precursors of oxaloacetate, which is converted to glucose. Thus, this small proportion of the odd-carbon-number fatty acid chain can be converted to glucose. In contrast, the acetyl CoA formed from β -oxidation of even-chain-number fatty acids in the liver either enters the TCA cycle, where it is principally oxidized to CO_2 , or is converted to ketone bodies.

D. Oxidation of Medium-Chain-Length Fatty Acids

Dietary medium-chain-length fatty acids are more water soluble than long-chain fatty acids and are not stored in adipose triacylglyceride. After a meal, they enter the blood and pass into the portal vein to the liver. In the liver, they enter the mitochondrial matrix by the monocarboxylate transporter and are activated to acyl CoA derivatives in the mitochondrial matrix (see Fig. 23.1). Medium-chain-length acyl CoAs, like long-chain acyl CoAs, are oxidized to acetyl CoA via the β -oxidation spiral. Medium-chain acyl CoAs also can arise from the peroxisomal oxidation pathway.

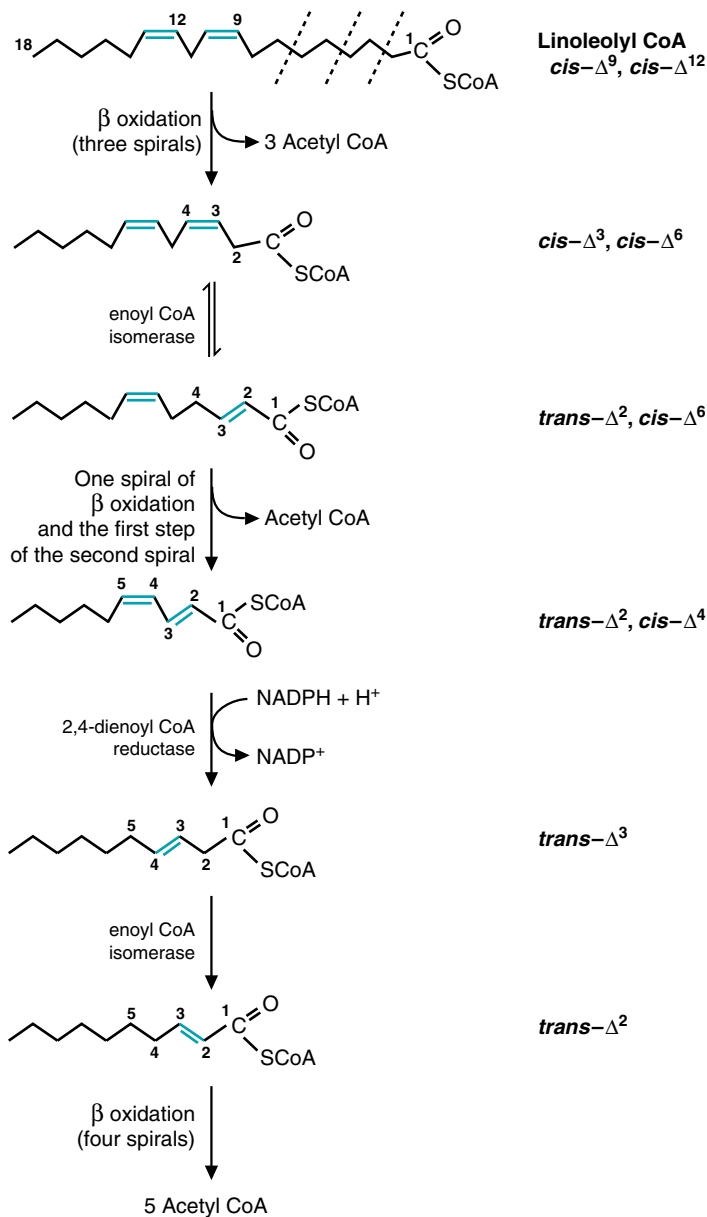


Fig. 23.9. Oxidation of linoleate. After three spirals of β -oxidation (dashed lines), there is now a 3,4 *cis* double bond and a 6,7 *cis* double bond. The 3,4 *cis* double bond is isomerized to a 2,3-*trans* double bond, which is in the proper configuration for the normal enzymes to act. One spiral of β -oxidation occurs, plus the first step of a second spiral. A reductase that uses NADPH now converts these two double bonds (between carbons 2 and 3 and carbons 4 and 5) to one double bond between carbons 3 and 4 in a *trans* configuration. The isomerase (which can act on double bonds that are in either the *cis* or the *trans* configuration) moves this double bond to the 2,3-*trans* position, and β -oxidation can resume.

E. Regulation of β -Oxidation

Fatty acids are used as fuels principally when they are released from adipose tissue triacylglycerols in response to hormones that signal fasting or increased demand. Many tissues, such as muscle and kidney, oxidize fatty acids completely to CO₂ and H₂O. In these tissues, the acetyl CoA produced by β -oxidation enters the TCA cycle. The FAD(2H) and the NADH from β -oxidation and the TCA cycle are

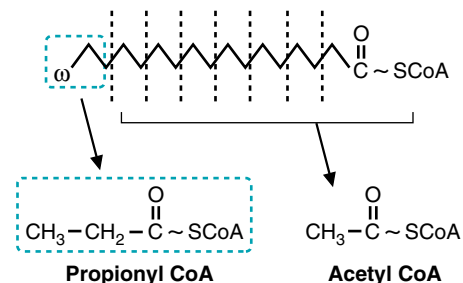


Fig. 23.10. Formation of propionyl CoA from odd-chain fatty acids. Successive spirals of β -oxidation cleave each of the bonds marked with dashed lines, producing acetyl CoA except for the three carbons at the ω -end, which produce propionyl CoA.

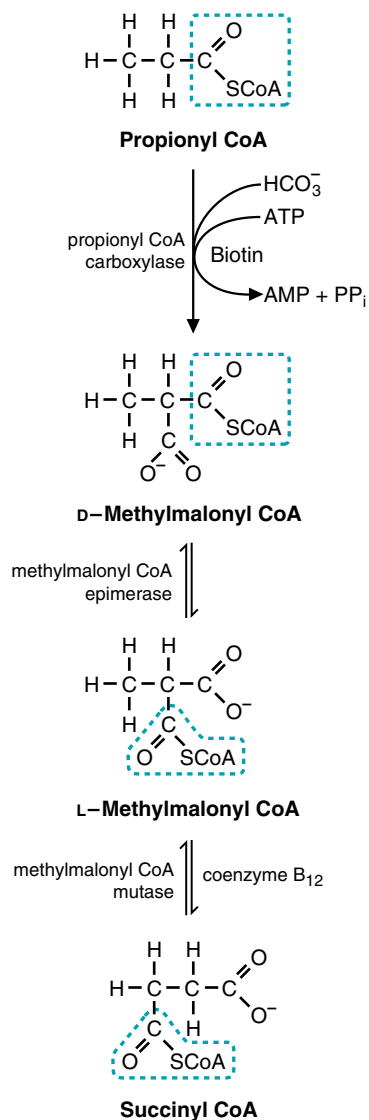


Fig. 23.11. Conversion of propionyl CoA to succinyl CoA. Succinyl CoA, an intermediate of the TCA cycle, can form malate, which can be converted to glucose in the liver through the process of gluconeogenesis. Certain amino acids also form glucose by this route (see Chapter 39).



As **Otto Shape** runs, his skeletal muscles increase their use of ATP and their rate of fuel oxidation. Fatty acid oxidation is accelerated by the increased rate of the electron transport chain. As ATP is used and AMP increases, an AMP-dependent protein kinase acts to facilitate fuel utilization and maintain ATP homeostasis. Phosphorylation of acetyl CoA carboxylase results in a decreased level of malonyl CoA and increased activity of carnitine: palmitoyl CoA transferase I. At the same time, AMP-dependent protein kinase facilitates the recruitment of glucose transporters into the plasma membrane of skeletal muscle, thereby increasing the rate of glucose uptake. AMP and hormonal signals also increase the supply of glucose 6-P from glycogenolysis. Thus, his muscles are supplied with more fuel, and all the oxidative pathways are accelerated.

reoxidized by the electron transport chain, and ATP is generated. The process of β -oxidation is regulated by the cells' requirements for energy (i.e., by the levels of ATP and NADH), because fatty acids cannot be oxidized any faster than NADH and FAD(2H) are reoxidized in the electron transport chain.

Fatty acid oxidation also may be restricted by the mitochondrial CoASH pool size. Acetyl CoASH units must enter the TCA cycle or another metabolic pathway to regenerate CoASH required for formation of the fatty acyl CoA derivative from fatty acyl carnitine.

An additional type of regulation occurs at carnitine:palmitoyltransferase I (CPTI). Carnitine:palmitoyltransferase I is inhibited by malonyl CoA, which is synthesized in the cytosol of many tissues by acetyl CoA carboxylase (Fig. 23.12). Acetyl CoA carboxylase is regulated by a number of different mechanisms, some of which are tissue dependent. In skeletal muscles and liver, it is inhibited when phosphorylated by protein kinase B, an AMP-dependent protein kinase. Thus, during exercise when AMP levels increase, AMP-dependent protein kinase phosphorylates acetyl CoA carboxylase, which becomes inactive. Consequently, malonyl CoA levels decrease, carnitine:palmitoyltransferase I is activated, and the β -oxidation of fatty acids is able to restore ATP homeostasis and decrease AMP levels. In liver, in addition to the regulation by the AMP-dependent protein kinase acetyl CoA carboxylase is activated by insulin-dependent mechanisms, which promotes the conversion of malonyl CoA to palmitate in the fatty acid synthesis pathway. Thus, in the liver, malonyl CoA inhibition of CPTI prevents newly synthesized fatty acids from being oxidized.

β -oxidation is strictly an aerobic pathway, dependent on oxygen, a good blood supply, and adequate levels of mitochondria. Tissues that lack mitochondria, such

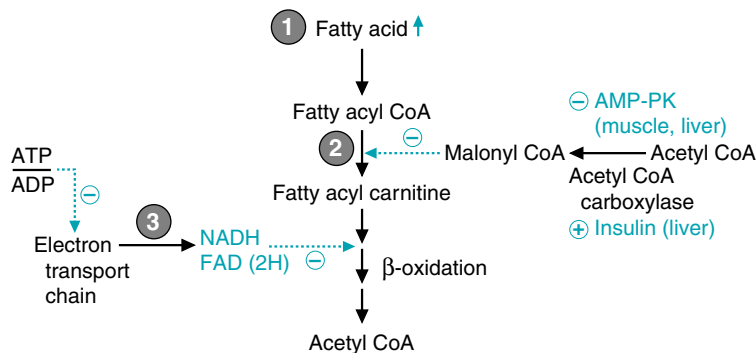


Fig. 23.12. Regulation of β -oxidation. (1) Hormones control the supply of fatty acids in the blood. (2) Carnitine:palmitoyl transferase I is inhibited by malonyl CoA, which is synthesized by acetyl CoA carboxylase (ACC). AMP-PK is the AMP-dependent protein kinase. (3) The rate of ATP utilization controls the rate of the electron transport chain, which regulates the oxidative enzymes of β -oxidation and the TCA cycle.

as red blood cells, cannot oxidize fatty acids by β -oxidation. Fatty acids also do not serve as a significant fuel for the brain. They are not used by adipocytes, whose function is to store triacylglycerols to provide a fuel for other tissues. Those tissues that do not use fatty acids as a fuel, or use them only to a limited extent, are able to use ketone bodies instead.

II. ALTERNATE ROUTES OF FATTY ACID OXIDATION

Fatty acids that are not readily oxidized by the enzymes of β -oxidation enter alternate pathways of oxidation, including peroxisomal β - and α -oxidation and microsomal ω -oxidation. The function of these pathways is to convert as much as possible of the unusual fatty acids to compounds that can be used as fuels or biosynthetic precursors, and to convert the remainder to compounds that can be excreted in bile or urine. During prolonged fasting, fatty acids released from adipose triacylglycerols may enter the ω -oxidation or peroxisomal β -oxidation pathway, even though they have a normal composition. These pathways not only use fatty acids, but they act on xenobiotic carboxylic acids that are large hydrophobic molecules resembling fatty acids.

A. Peroxisomal Oxidation of Fatty Acids

A small proportion of our diet consists of very-long-chain fatty acids (20 or more carbons) or branched-chain fatty acids arising from degradative products of chlorophyll. Very-long-chain fatty acid synthesis also occurs within the body, especially in cells of the brain and nervous system, which incorporate them into the sphingolipids of myelin. These fatty acids are oxidized by peroxisomal β - and α -oxidation pathways, which are essentially chain-shortening pathways.

1. VERY-LONG-CHAIN FATTY ACIDS

Very-long-chain fatty acids of 24 to 26 carbons are oxidized exclusively in peroxisomes by a sequence of reactions similar to mitochondrial β -oxidation in that they generate acetyl CoA and NADH. However, the peroxisomal oxidation of straight-chain fatty acids stops when the chain reaches 4 to 6 carbons in length. Some of the long-chain fatty acids also may be oxidized by this route.

The long-chain fatty acyl CoA synthetase is present in the peroxisomal membrane, and the acyl CoA derivatives enter the peroxisome by a transporter that does not require carnitine. The first enzyme of peroxisomal β -oxidation is an oxidase, which donates electrons directly to molecular oxygen and produces hydrogen peroxide (H_2O_2) (Fig. 23.13). (In contrast, the first enzyme of mitochondrial β -oxidation is a dehydrogenase that contains FAD and transfers the electrons to the electron transport chain via ETF.) Thus, the first enzyme of peroxisomal oxidation is not linked to energy production. The three remaining steps of β -oxidation are catalyzed by enoyl-CoA hydratase, hydroxyacyl CoA dehydrogenase, and thiolase, enzymes with activities similar to those found in mitochondrial β -oxidation, but coded for by different genes. Thus, one NADH and one acetyl CoA are generated for each turn of the spiral. The peroxisomal β -oxidation spiral continues generating acetyl CoA until a medium-chain acyl CoA, which may be as short as butyryl CoA, is produced (Fig. 23.14).

Within the peroxisome, the acetyl groups can be transferred from CoA to carnitine by an acylcarnitine transferase, or they can enter the cytosol. A similar reaction converts medium-chain-length acyl CoAs and the short-chain butyryl CoA to acyl carnitine derivatives. These acylcarnitines diffuse from the peroxisome to the mitochondria, pass through the outer mitochondrial membrane, and are transported through the inner mitochondrial membrane via the carnitine translocase system.



Xenobiotic: a term used to cover all organic compounds that are foreign to an organism. This can also include naturally occurring compounds that are administered by alternate routes or at unusual concentrations. Drugs can be considered xenobiotics.

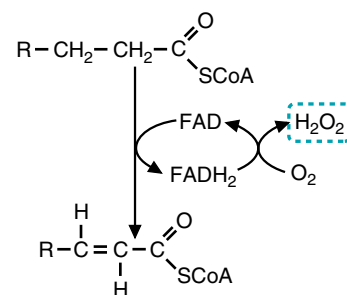


Fig. 23.13. Oxidation of fatty acids in peroxisomes. The first step of β -oxidation is catalyzed by an FAD-containing oxidase. The electrons are transferred from FAD(2H) to O_2 , which is reduced to hydrogen peroxide (H_2O_2).



A number of inherited deficiencies of peroxisomal enzymes have been described. Zellweger's syndrome, which results from defective peroxisomal biogenesis, leads to complex developmental and metabolic phenotypes affecting principally the liver and the brain. One of the metabolic characteristics of these diseases is an elevation of C26:0, and C26:1 fatty acid levels in plasma. Refsum's disease is caused by a deficiency in a single peroxisomal enzyme, the phytanoyl CoA hydroxylase that carries out α -oxidation of phytanic acid. Symptoms include retinitis pigmentosa, cerebellar ataxia, and chronic polyneuropathy. Because phytanic acid is obtained solely from the diet, placing patients on a low-phytanic acid diet has resulted in marked improvement.

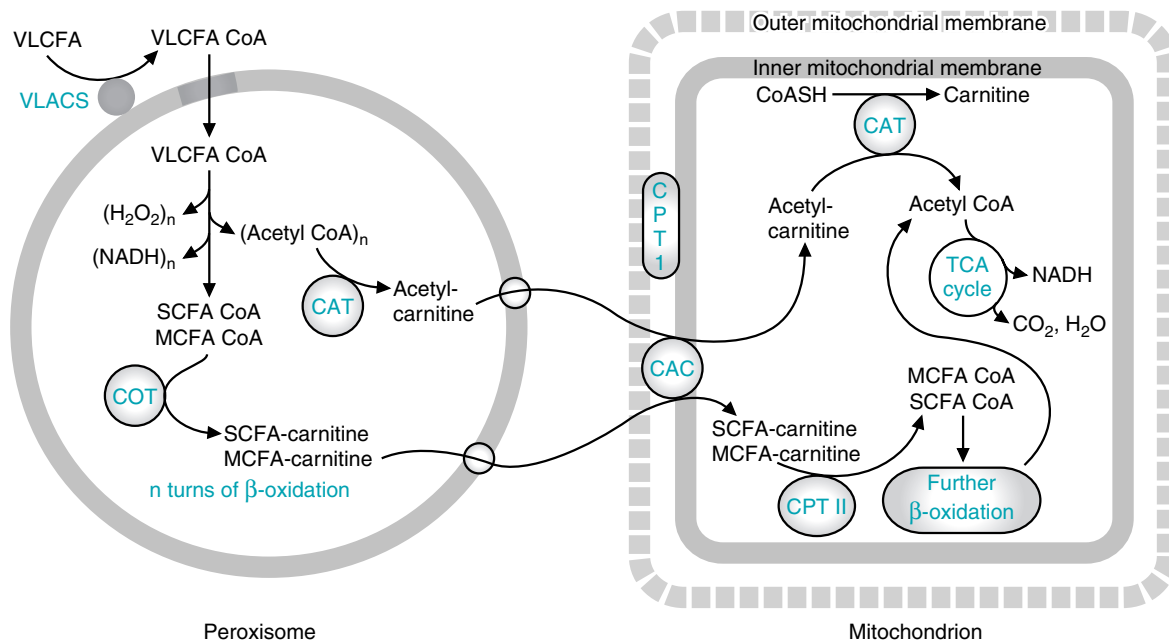


Fig. 23.14. Chain-shortening by peroxisomal β -oxidation. Abbreviations: VLCFA, very-long-chain fatty acyl; VLACS, very-long-chain acyl-CoA synthetase; MCFA, medium-chain fatty acyl; SCFA, short-chain fatty acyl; CAT, carnitine:acetyltransferase; COT, carnitine:octanoyltransferase; CAC, carnitine:acylcarnitine carrier; CPT1, carnitine: palmitoyltransferase 1; CPT2, carnitine: palmitoyltransferase 2; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane. Very-long-chain fatty acyl CoAs and some long-chain fatty acyl CoAs are oxidized in peroxisomes through n cycles of β -oxidation to the stage of a short- to medium-chain fatty acyl CoA. These short to medium fatty acyl CoAs are converted to carnitine derivatives by COT or CAT in the peroxisomes. In the mitochondria, SCFA-carnitine are converted back to acyl CoA derivatives by either CPT2 or CAT.

They are converted back to acyl CoAs by carnitine: acyltransferases appropriate for their chain length and enter the normal pathways for β -oxidation and acetyl CoA metabolism. The electrons from NADH and acetyl CoA can also pass from the peroxisome to the cytosol. The export of NADH-containing electrons occurs through use of a shuttle system similar to those described for NADH electron transfer into the mitochondria.

Peroxisomes are present in almost every cell type and contain many degradative enzymes, in addition to fatty acyl CoA oxidase, that generate hydrogen peroxide. H_2O_2 can generate toxic free radicals. Thus, these enzymes are confined to peroxisomes, where the H_2O_2 can be neutralized by the free radical defense enzyme, catalase. Catalase converts H_2O_2 to water and O_2 .

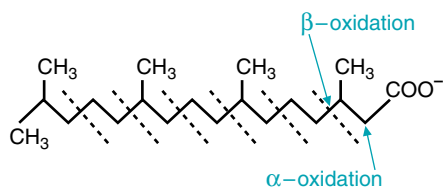


Fig. 23.15. Oxidation of phytanic acid. A peroxisomal α -hydroxylase oxidizes the α -carbon, and its subsequent oxidation to a carboxyl group releases the carboxyl carbon as CO_2 . Subsequent spirals of peroxisomal β -oxidation alternately release propionyl and acetyl CoA. At a chain length of approximately 8 carbons, the remaining branched fatty acid is transferred to mitochondria as a medium-chain carnitine derivative.

2. LONG-CHAIN BRANCHED-CHAIN FATTY ACIDS

Two of the most common branched-chain fatty acids in the diet are phytanic acid and pristanic acid, which are degradation products of chlorophyll and thus are consumed in green vegetables (Fig.23.15). Animals do not synthesize branched-chain fatty acids. These two multi-methylated fatty acids are oxidized in peroxisomes to the level of a branched C8 fatty acid, which is then transferred to mitochondria. The pathway thus is similar to that for the oxidation of straight very-long-chain fatty acids.

Phytanic acid, a multi-methylated C20 fatty acid, is first oxidized to pristanic acid using the α -oxidation pathway (see Fig.23.15). Phytanic acid hydroxylase introduces a hydroxyl group on the α -carbon, which is then oxidized to a carboxyl group with release of the original carboxyl group as CO_2 . By shortening the fatty acid by one carbon, the methyl groups will appear on the α -carbon rather than the

β -carbon during the β -oxidation spiral, and can no longer interfere with oxidation of the β -carbon. Peroxisomal β -oxidation thus can proceed normally, releasing propionyl CoA and acetyl CoA with alternate turns of the spiral. When a medium chain length of approximately eight carbons is reached, the fatty acid is transferred to the mitochondrion as a carnitine derivative, and β -oxidation is resumed.

B. ω -Oxidation of Fatty Acids

Fatty acids also may be oxidized at the ω -carbon of the chain (the terminal methyl group) by enzymes in the endoplasmic reticulum (Fig. 23.16). The ω -methyl group is first oxidized to an alcohol by an enzyme that uses cytochrome P450, molecular oxygen, and NADPH. Dehydrogenases convert the alcohol group to a carboxylic acid. The dicarboxylic acids produced by ω -oxidation can undergo β -oxidation, forming compounds with 6 to 10 carbons that are water-soluble. Such compounds may then enter blood, be oxidized as medium-chain fatty acids, or be excreted in urine as medium-chain dicarboxylic acids.

The pathways of peroxisomal α and β -oxidation, and microsomal ω -oxidation, are not feedback regulated. These pathways function to decrease levels of water-insoluble fatty acids or of xenobiotic compounds with a fatty acid-like structure that would become toxic to cells at high concentrations. Thus, their rate is regulated by the availability of substrate.

III. METABOLISM OF KETONE BODIES

Overall, fatty acids released from adipose triacylglycerols serve as the major fuel for the body during fasting. These fatty acids are completely oxidized to CO_2 and H_2O by some tissues. In the liver, much of the acetyl CoA generated from β -oxidation of fatty acids is used for synthesis of the ketone bodies acetoacetate and β -hydroxybutyrate, which enter the blood (Fig. 23.17). In skeletal muscles and other

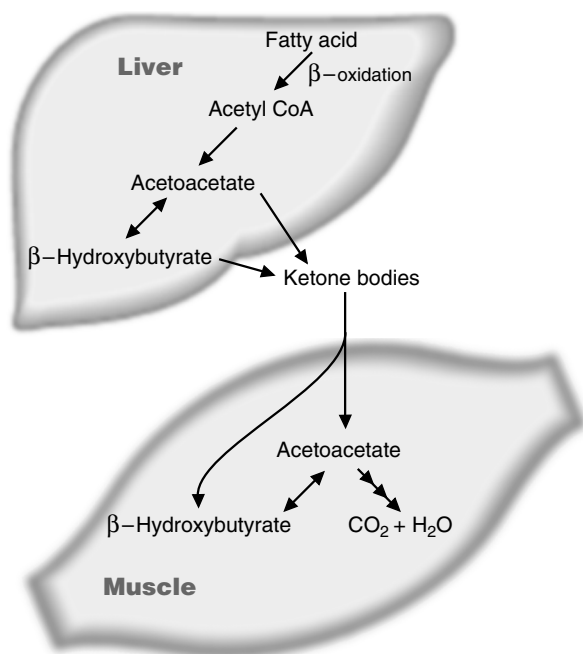


Fig. 23.17. The ketone bodies, acetoacetate and β -hydroxybutyrate, are synthesized in the liver. Their principle fate is conversion back to acetyl CoA and oxidation in the TCA cycle in other tissues.

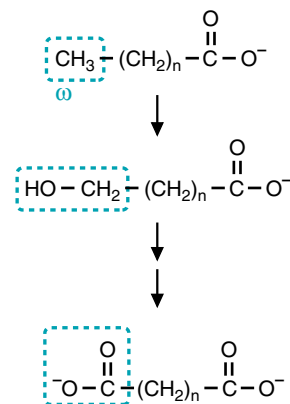
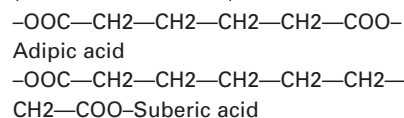


Fig. 23.16. ω -Oxidation of fatty acids converts them to dicarboxylic acids.



Normally, ω -oxidation is a minor process. However, in conditions that interfere with β -oxidation (such as carnitine deficiency or deficiency in an enzyme of β -oxidation), ω -oxidation produces dicarboxylic acids in increased amounts. These dicarboxylic acids are excreted in the urine.

Lofata Burne was excreting dicarboxylic acids in her urine, particularly adipic acid (which has 6 carbons) and suberic acid (which has 8 carbons).



tissues, these ketone bodies are converted back to acetyl CoA, which is oxidized in the TCA cycle with generation of ATP. An alternate fate of acetoacetate in tissues is the formation of cytosolic acetyl CoA.

A. Synthesis of Ketone Bodies

In the liver, ketone bodies are synthesized in the mitochondrial matrix from acetyl CoA generated from fatty acid oxidation (Fig. 23.18). The thiolase reaction of fatty acid oxidation, which converts acetoacetyl CoA to two molecules of acetyl CoA, is a reversible reaction, although formation of acetoacetyl-CoA is not the favored direction. It can, thus, when acetyl-CoA levels are high, generate acetoacetyl CoA

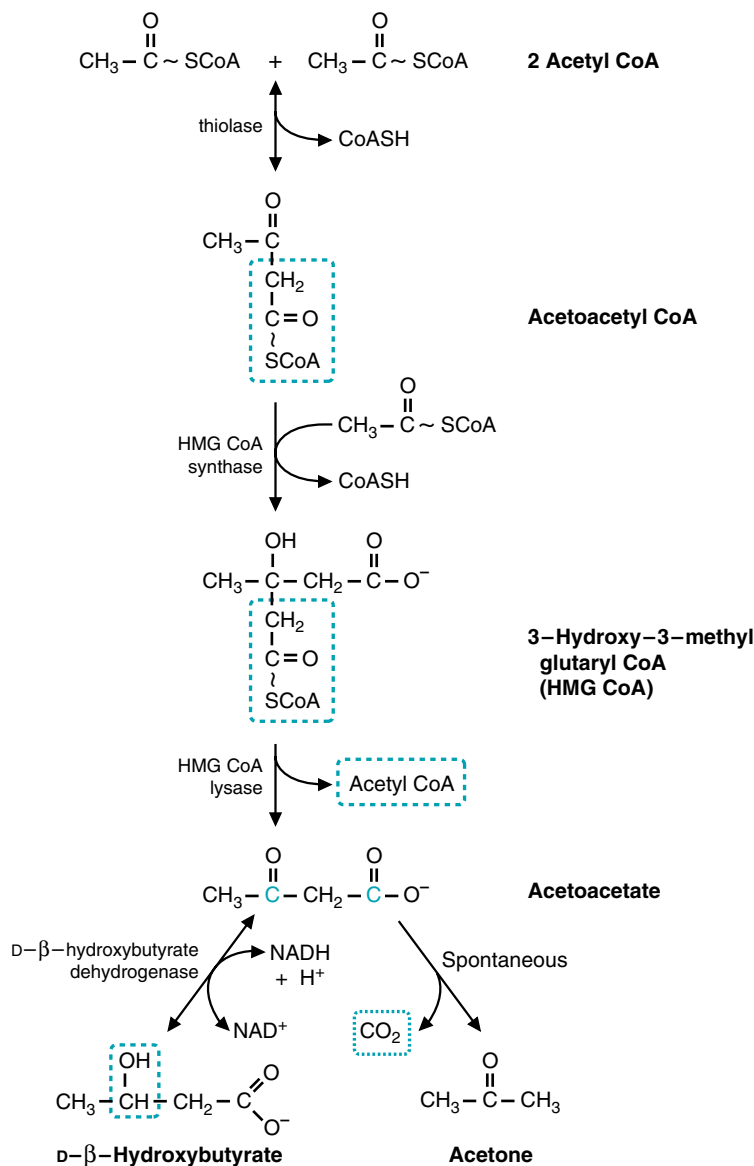


Fig. 23.18. Synthesis of the ketone bodies acetoacetate, β-hydroxybutyrate, and acetone. The portion of HMG-CoA shown in blue is released as acetyl CoA, and the remainder of the molecule forms acetoacetate. Acetoacetate is reduced to β-hydroxybutyrate or decarboxylated to acetone. Note that the dehydrogenase that interconverts acetoacetate and β-hydroxybutyrate is specific for the D-isomer. Thus, it differs from the dehydrogenases of β-oxidation, which act on 3-hydroxy acyl CoA derivatives and is specific for the L-isomer.

for ketone body synthesis. The acetoacetyl CoA will react with acetyl CoA to produce 3-hydroxy-3-methylglutaryl CoA (HMG-CoA). The enzyme that catalyzes this reaction is HMG-CoA synthase. In the next reaction of the pathway, HMG-CoA lyase catalyzes the cleavage of HMG-CoA to form acetyl CoA and acetoacetate.

Acetoacetate can directly enter the blood or it can be reduced by β -hydroxybutyrate dehydrogenase to β -hydroxybutyrate, which enters the blood (see Fig. 23.18). This dehydrogenase reaction is readily reversible and interconverts these two ketone bodies, which exist in an equilibrium ratio determined by the NADH/NAD⁺ ratio of the mitochondrial matrix. Under normal conditions, the ratio of β -hydroxybutyrate to acetoacetate in the blood is approximately 1:1.

An alternate fate of acetoacetate is spontaneous decarboxylation, a nonenzymatic reaction that cleaves acetoacetate into CO₂ and acetone (see Fig. 23.18). Because acetone is volatile, it is expired by the lungs. A small amount of acetone may be further metabolized in the body.

B. Oxidation of Ketone Bodies as Fuels

Acetoacetate and β -hydroxybutyrate can be oxidized as fuels in most tissues, including skeletal muscle, brain, certain cells of the kidney, and cells of the intestinal mucosa. Cells transport both acetoacetate and β -hydroxybutyrate from the circulating blood into the cytosol, and into the mitochondrial matrix. Here β -hydroxybutyrate is oxidized back to acetoacetate by β -hydroxybutyrate dehydrogenase. This reaction produces NADH. Subsequent steps convert acetoacetate to acetyl CoA (Fig. 23.19).

In mitochondria, acetoacetate is activated to acetoacetyl CoA by succinyl CoA:acetoacetate CoA transferase. As the name suggests, CoA is transferred from succinyl CoA, a TCA cycle intermediate, to acetoacetate. Although the liver produces ketone bodies, it does not use them, because this thiotransferase enzyme is not present in sufficient quantity.

Acetoacetyl CoA is cleaved to two molecules of acetyl CoA by acetoacetyl CoA thiolase, the same enzyme involved in β -oxidation. The principal fate of this acetyl CoA is oxidation in the TCA cycle.

The energy yield from oxidation of acetoacetate is equivalent to the yield for oxidation of 2 acetyl CoA in the TCA cycle (20 ATP) minus the energy for activation of acetoacetate (1 ATP). The energy of activation is calculated at one high-energy phosphate bond, because succinyl CoA is normally converted to succinate in the TCA cycle, with generation of one molecule of GTP (the energy equivalent of ATP). However, when the high-energy thioester bond of succinyl CoA is transferred to acetoacetate, succinate is produced without the generation of this GTP. Oxidation of β -hydroxybutyrate generates one additional NADH. Therefore the net energy yield from one molecule of β -hydroxybutyrate is approximately 21.5 molecules of ATP.

C. Alternate Pathways of Ketone Body Metabolism

Although fatty acid oxidation is usually the major source of ketone bodies, they also can be generated from the catabolism of certain amino acids: leucine, isoleucine, lysine, tryptophan, phenylalanine, and tyrosine. These amino acids are called ketogenic amino acids because their carbon skeleton is catabolized to acetyl CoA or acetoacetyl CoA, which may enter the pathway of ketone body synthesis in liver. Leucine and isoleucine also form acetyl CoA and acetoacetyl CoA in other tissues, as well as the liver.

Acetoacetate can be activated to acetoacetyl CoA in the cytosol by an enzyme similar to the acyl CoA synthetases. This acetoacetyl CoA can be used directly in cholesterol synthesis. It also can be cleaved to two molecules of acetyl CoA by a cytosolic thiolase. Cytosolic acetyl CoA is required for processes such as acetylcholine synthesis in neuronal cells.

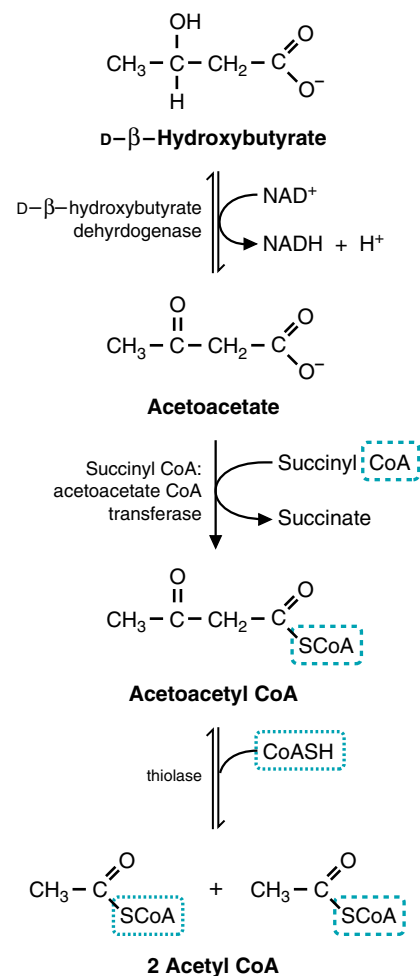


Fig. 23.19. Oxidation of ketone bodies. β -Hydroxybutyrate is oxidized to acetoacetate, which is activated by accepting a CoA group from succinyl CoA. Acetoacetyl CoA is cleaved to two acetyl CoA, which enter the TCA cycle and are oxidized.



Ketogenic diets, which are high-fat diets with a 3:1 ratio of lipid to carbohydrate, are being used to reduce the frequency of epileptic seizures in children. The reason for its effectiveness in the treatment of epilepsy is not known. Ketogenic diets are also used to treat children with pyruvate dehydrogenase deficiency. Ketone bodies can be used as a fuel by the brain in the absence of pyruvate dehydrogenase. They also can provide a source of cytosolic acetyl CoA for acetylcholine synthesis. They often contain medium-chain triglycerides, which induce ketosis more effectively than long-chain triglycerides.

IV. THE ROLE OF FATTY ACIDS AND KETONE BODIES IN FUEL HOMEOSTASIS

Fatty acids are used as fuels whenever fatty acid levels are elevated in the blood, that is, during fasting, starvation, as a result of a high-fat, low-carbohydrate diet, or during long-term low- to mild-intensity exercise. Under these conditions, a decrease in insulin and increased levels of glucagon, epinephrine, or other hormones stimulate adipose tissue lipolysis. Fatty acids begin to increase in the blood approximately 3 to 4 hours after a meal and progressively increase with time of fasting up to approximately 2 to 3 days (Fig. 23.20). In the liver, the rate of ketone body synthesis increases as the supply of fatty acids increases. However, the blood level of ketone bodies continues to increase, presumably because their utilization by skeletal muscles decreases.



Children are more prone to ketosis than adults because their body enters the fasting state more rapidly. Their bodies use more energy per unit mass (because their muscle-to-adipose-tissue ratio is higher), and liver glycogen stores are depleted faster (the ratio of their brain mass to liver mass is higher). In children, blood ketone body levels reach 2 mM in 24 hours; in adults, it takes more than 3 days to reach this level. Mild pediatric infections causing anorexia and vomiting are the commonest cause of ketosis in children. Mild ketosis is observed in children after prolonged exercise, perhaps attributable to an abrupt decrease in muscular use of fatty acids liberated during exercise. The liver then oxidizes these fatty acids and produces ketone bodies.

After 2 to 3 days of starvation, ketone bodies rise to a level in the blood that enables them to enter brain cells, where they are oxidized, thereby reducing the amount of glucose required by the brain. During prolonged fasting, they may supply as much as two thirds of the energy requirements of the brain. The reduction in glucose requirements spares skeletal muscle protein, which is a major source of amino acid precursors for hepatic glucose synthesis from gluconeogenesis.

A. Preferential Utilization of Fatty Acids

As fatty acids increase in the blood, they are used by skeletal muscles and certain other tissues in preference to glucose. Fatty acid oxidation generates NADH and FAD(2H) through both β -oxidation and the TCA cycle, resulting in relatively high NADH/NAD⁺ ratios, acetyl CoA concentration, and ATP/ADP or ATP/AMP levels. In skeletal muscles, AMP-dependent protein kinase (see Section I.E.) adjusts the concentration of malonyl CoA so that CPT1 and β -oxidation operate at a rate that is able to sustain ATP homeostasis. With adequate levels of ATP obtained from fatty acid (or ketone body) oxidation, the rate of glycolysis is decreased. The activity of the regulatory enzymes in glycolysis and the TCA cycle (pyruvate dehydrogenase and PFK-1) are decreased by the changes in concentration of their allosteric regulators (ADP, an activator of PDH,

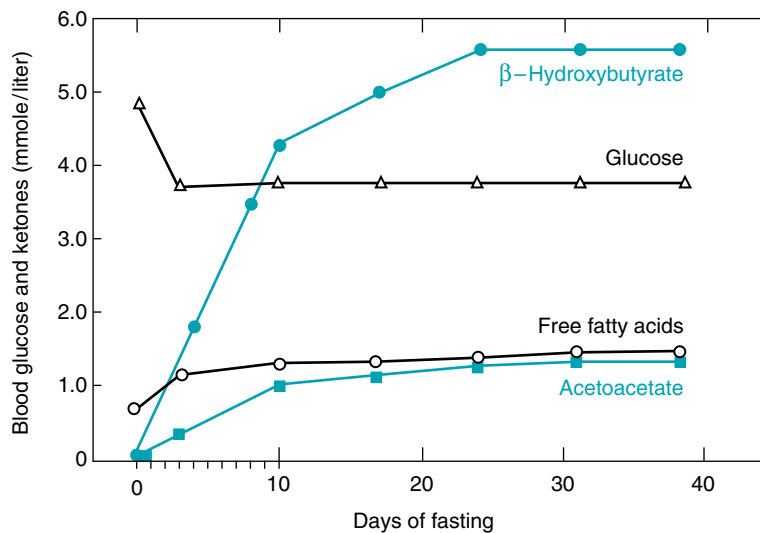


Fig. 23.20. Levels of ketone bodies in the blood at various times during fasting. Glucose levels remain relatively constant, as do levels of fatty acids. Ketone body levels, however, increase markedly, rising to levels at which they can be used by the brain and other nervous tissue. From Cahill GF Jr, Aoki TT. *Med Times* 1970;98:109.

decreases in concentration; NADH, and acetyl CoA, inhibitors of PDH, are increased in concentration under these conditions; and ATP and citrate, inhibitors of PFK-1, are increased in concentration). As a consequence, glucose-6-P accumulates. Glucose-6-P inhibits hexokinase, thereby decreasing the rate of entry of glucose into glycolysis, and its uptake from the blood. In skeletal muscles, this pattern of fuel metabolism is facilitated by the decrease in insulin concentration (see Chapter 36). Preferential utilization of fatty acids does not, however, restrict the ability of glycolysis to respond to an increase in AMP or ADP levels, such as might occur during exercise or oxygen limitation.

B. Tissues That Use Ketone Bodies

Skeletal muscles, the heart, the liver, and many other tissues use fatty acids as their major fuel during fasting and other conditions that increase fatty acids in the blood. However, a number of other tissues (or cell types), such as the brain, use ketone bodies to a greater extent. For example, cells of the intestinal mucosa, which transport fatty acids from the intestine to the blood, use ketone bodies and amino acids during starvation, rather than fatty acids. Adipocytes, which store fatty acids in triacylglycerols, do not use fatty acids as a fuel during fasting but can use ketone bodies. Ketone bodies cross the placenta, and can be used by the fetus. Almost all tissues and cell types, with the exception of liver and red blood cells, are able to use ketone bodies as fuels.

C. Regulation of Ketone Body Synthesis

A number of events, in addition to the increased supply of fatty acids from adipose triacylglycerols, promote hepatic ketone body synthesis during fasting. The decreased insulin/glucagon ratio results in inhibition of acetyl CoA carboxylase and decreased malonyl CoA levels, which activates CPTI, thereby allowing fatty acyl CoA to enter the pathway of β -oxidation. (Fig. 23.21). When oxidation of fatty acyl CoA to acetyl CoA generates enough NADH and FAD(2H) to supply the ATP needs of the liver, acetyl CoA is diverted from the TCA cycle into ketogenesis and oxaloacetate in the TCA cycle is diverted toward malate and into glucose synthesis (gluconeogenesis). This pattern is regulated by the NADH/NAD⁺ ratio, which is relatively high during β -oxidation. As the length of time of fasting continues, increased transcription of the gene for mitochondrial HMG-CoA synthase facilitates high rates of ketone body production. Although the liver has been described as “altruistic” because it provides ketone bodies for other tissues, it is simply getting rid of fuel that it does not need.



The level of total ketone bodies in **Lofata Burne's** blood greatly exceeds normal fasting levels and the mild ketosis produced during exercise. In a person on a normal mealtime schedule, total blood ketone bodies rarely exceed 0.2 mM. During prolonged fasting, they may rise to 4 to 5 mM. Levels above 7 mM are considered evidence of ketoacidosis, because the acid produced must reach this level to exceed the bicarbonate buffer system in the blood and compensatory respiration (Kussmaul's respiration) (see Chapter 4).



Q: Why can't red blood cells use ketone bodies for energy?

CLINICAL COMMENTS



As **Otto Shape** runs, he increases the rate at which his muscles oxidize all fuels. The increased rate of ATP utilization stimulates the electron transport chain, which oxidizes NADH and FAD(2H) much faster, thereby increasing the rate at which fatty acids are oxidized. During exercise, he also uses muscle glycogen stores, which contribute glucose to glycolysis. In some of the fibers, the glucose is used anaerobically, thereby producing lactate. Some of the lactate will be used by his heart, and some will be taken up by the liver to be converted to glucose. As he trains, he increases his mitochondrial capacity, as well as his oxygen delivery, resulting in an increased ability to oxidize fatty acids and ketone bodies. As he runs, he increases fatty acid release from adipose tissue triacylglycerols. In the liver, fatty acids are being converted to ketone bodies, providing his muscles with another fuel. As a consequence, he experiences mild ketosis after his 12-mile run.



Red blood cells lack mitochondria, which is the site of ketone body utilization.

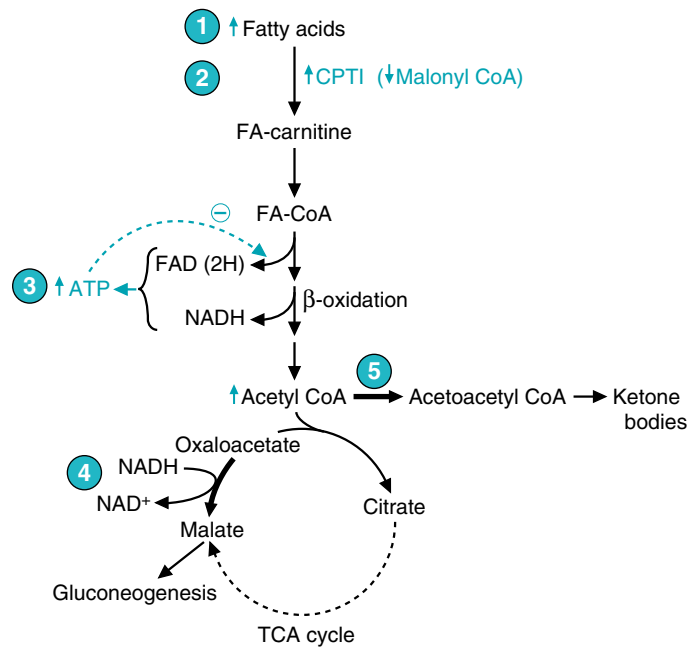


Fig. 23.21. Regulation of ketone body synthesis. (1) The supply of fatty acids is increased. (2) The malonyl CoA inhibition of CPTI is lifted by inactivation of acetyl CoA carboxylase. (3) β -Oxidation supplies NADH and FAD(2H), which are used by the electron transport chain for oxidative phosphorylation. As ATP levels increase, less NADH is oxidized, and the NADH/NAD⁺ ratio is increased. (4) Oxaloacetate is converted into malate because of the high NADH levels, and the malate enters the cytoplasm for gluconeogenesis. (5) Acetyl CoA is diverted from the TCA cycle into ketogenesis, in part because of low oxaloacetate levels, which reduces the rate of the citrate synthase reaction.



More than 25 enzymes and specific transport proteins participate in mitochondrial fatty acid metabolism. At least 15 of these have been implicated in inherited diseases in the human.



Recently, medium-chain acyl-CoA dehydrogenase (MCAD) deficiency, the cause of **Lofata Burne's** problems, has emerged as one of the most common of the inborn errors of metabolism, with a carrier frequency ranging from 1 in 40 in northern European populations to less than 1 in 100 in Asians. Overall, the predicted disease frequency for MCAD deficiency is 1 in 15,000 persons.

MCAD deficiency is an autosomal recessive disorder caused by the substitution of a T for an A at position 985 of the MCAD gene. This mutation causes a lysine to replace a glutamate residue in the protein, resulting in the production of an unstable dehydrogenase.

The most frequent manifestation of MCAD deficiency is intermittent hypoketotic hypoglycemia during fasting (low levels of ketone bodies and low levels of glucose in the blood). Fatty acids normally would be oxidized to CO₂ and H₂O under these conditions. In MCAD deficiency, however, fatty acids are oxidized only until they reach medium-chain length. As a result, the body must rely to a greater extent on oxidation of blood glucose to meet its energy needs.

However, hepatic gluconeogenesis appears to be impaired in MCAD. Inhibition of gluconeogenesis may be caused by the lack of hepatic fatty acid oxidation to supply the energy required for gluconeogenesis, or by the accumulation of unoxidized fatty acid metabolites that inhibit gluconeogenic enzymes. As a consequence, liver glycogen stores are depleted more rapidly, and hypoglycemia results. The decrease in hepatic fatty acid oxidation results in less acetyl CoA for ketone body synthesis, and consequently a hypoketotic hypoglycemia develops.

Some of the symptoms once ascribed to hypoglycemia are now believed to be caused by the accumulation of toxic fatty acid intermediates, especially in those

patients with only mild reductions in blood glucose levels. **Lofata Burne's** mild elevation in the blood of liver transaminases may reflect an infiltration of her liver cells with unoxidized medium-chain fatty acids.

The management of MCAD-deficient patients includes the intake of a relatively high-carbohydrate diet and the avoidance of prolonged fasting.



Di Abietes, a 26-year-old woman with type 1 diabetes mellitus, was admitted to the hospital in diabetic ketoacidosis. In this complication of diabetes mellitus, an acute deficiency of insulin, coupled with a relative excess of glucagon, results in a rapid mobilization of fuel stores from muscle (amino acids) and adipose tissue (fatty acids). Some of the amino acids are converted to glucose, and fatty acids are converted to ketones (acetoacetate, β -hydroxybutyrate, and acetone). The high glucagon: insulin ratio promotes the hepatic production of ketones. In response to the metabolic “stress,” the levels of insulin-antagonistic hormones, such as catecholamines, glucocorticoids, and growth hormone, are increased in the blood. The insulin deficiency further reduces the peripheral utilization of glucose and ketones. As a result of this interrelated dysmetabolism, plasma glucose levels reach 500 mg/dL (27.8 mmol/L) or more (normal fasting levels are 70–100 mg/dL, or 3.9–5.5 mmol/L), and plasma ketones rise to levels of 8 to 15 mmol/L or more (normal is in the range of 0.2–2 mmol/L, depending on the fed state of the individual).

The increased glucose presented to the renal glomeruli induces an osmotic diuresis, which further depletes intravascular volume, further reducing the renal excretion of hydrogen ions and glucose. As a result, the metabolic acidosis worsens, and the hyperosmolarity of the blood increases, at times exceeding 330 mOsm/kg (normal is in the range of 285–295 mOsm/kg). The severity of the hyperosmolar state correlates closely with the degree of central nervous system dysfunction and may end in coma and even death if left untreated.

BIOCHEMICAL COMMENTS



The unripe fruit of the akee tree produces a toxin, hypoglycin, which causes a condition known as Jamaican vomiting sickness. The victims of the toxin are usually unwary children who eat this unripe fruit and develop a severe hypoglycemia, which is often fatal.

Although hypoglycin causes hypoglycemia, it acts by inhibiting an acyl CoA dehydrogenase involved in β -oxidation that has specificity for short- and medium-chain fatty acids. Because more glucose must be oxidized to compensate for the decreased ability of fatty acids to serve as fuel, blood glucose levels may fall to extremely low levels. Fatty acid levels, however, rise because of decreased β -oxidation. As a result of the increased fatty acid levels, α -oxidation increases, and dicarboxylic acids are excreted in the urine. The diminished capacity to oxidize fatty acids in liver mitochondria results in decreased levels of acetyl CoA, the substrate for ketone body synthesis.

Suggested References

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**REVIEW QUESTIONS—CHAPTER 23**

- A lack of the enzyme ETF:CoQ oxidoreductase leads to death. This is due to which of the following reasons?
 - The energy yield from glucose utilization is dramatically reduced.
 - The energy yield from alcohol utilization is dramatically reduced.
 - The energy yield from ketone body utilization is dramatically reduced.
 - The energy yield from fatty acid utilization is dramatically reduced.
 - The energy yield from glycogen utilization is dramatically reduced.
- The ATP yield from the complete oxidation of 1 mole of a C_{18:0} fatty acid to carbon dioxide and water would be closest to which **ONE** of the following?
 - 105
 - 115
 - 120
 - 125
 - 130
- The oxidation of fatty acids is best described by which of the following sets of reactions?
 - Oxidation, hydration, oxidation, carbon-carbon bond breaking
 - Oxidation, dehydration, oxidation, carbon-carbon bond breaking
 - Oxidation, hydration, reduction, carbon-carbon bond breaking
 - Oxidation, dehydration, reduction, oxidation, carbon-carbon bond breaking
 - Reduction, hydration, oxidation, carbon-carbon bond breaking
- An individual with a deficiency of an enzyme in the pathway for carnitine synthesis is not eating adequate amounts of carnitine in the diet. Which of the following effects would you expect during fasting as compared with an individual with an adequate intake and synthesis of carnitine?
 - Fatty acid oxidation is increased.
 - Ketone body synthesis is increased.
 - Blood glucose levels are increased.
 - The levels of dicarboxylic acids in the blood would be increased.
 - The levels of very-long-chain fatty acids in the blood would be increased.
- At which one of the periods listed below will fatty acids be the major source of fuel for the tissues of the body?
 - Immediately after breakfast
 - Minutes after a snack
 - Immediately after dinner
 - While running the first mile of a marathon
 - While running the last mile of a marathon