c0050 # Fatty Acid and Triglyceride Metabolism

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s0010 ●●● **FATTY ACID METABOLISM**

p0090 Fatty acid chains are polymerized in the cytoplasm and oxidized in the mitochondrial matrix. This prevents competing side reactions between pathway intermediates and allows separate regulation of both pathways. However, since the precursor for fat synthesis, acetyl-coenzyme A (CoA), arises in the matrix, it must first be transported to the cytoplasm for incorporation into a fatty acid. Likewise, free fatty acids (FFAs) mobilized for oxidation must be transported into the mitochondrion to undergo oxidation. Each of the fatty acid metabolic pathways must therefore be preceded by a transport process. (Note: The synthetic and oxidative pathways are treated separately to facilitate comparisons.)

HISTOLOGY

b0010

Red Blood Cell Metabolism

s0355

p0105 Red blood cells have no mitochondria and therefore cannot use FFAs for energy. They are totally reliant on anaerobic glycolysis for their energy source.

Pathway Reaction Steps in Fatty Acid Synthesis—Acetyl-Coenzyme A to Palmitate

s0015

Acetyl-Coenzyme A Shuttle

s0020

p0110 Four reactions shuttle acetyl-CoA from mitochondrial matrix to cytoplasm (Fig. 10-1):

Citrate synthase

s0025

p0115 Acetyl-CoA (e.g., from glucose following a meal) is condensed with oxaloacetate to form citrate. Citrate is then transported through the mitochondrial membrane to the cytoplasm.

Citrate cleavage enzyme (citrate lyase)

s0030

p0120 Acetyl-CoA and oxaloacetate are regenerated from citrate in the cytoplasm in a reaction that requires adenosine triphosphate (ATP) and CoA.
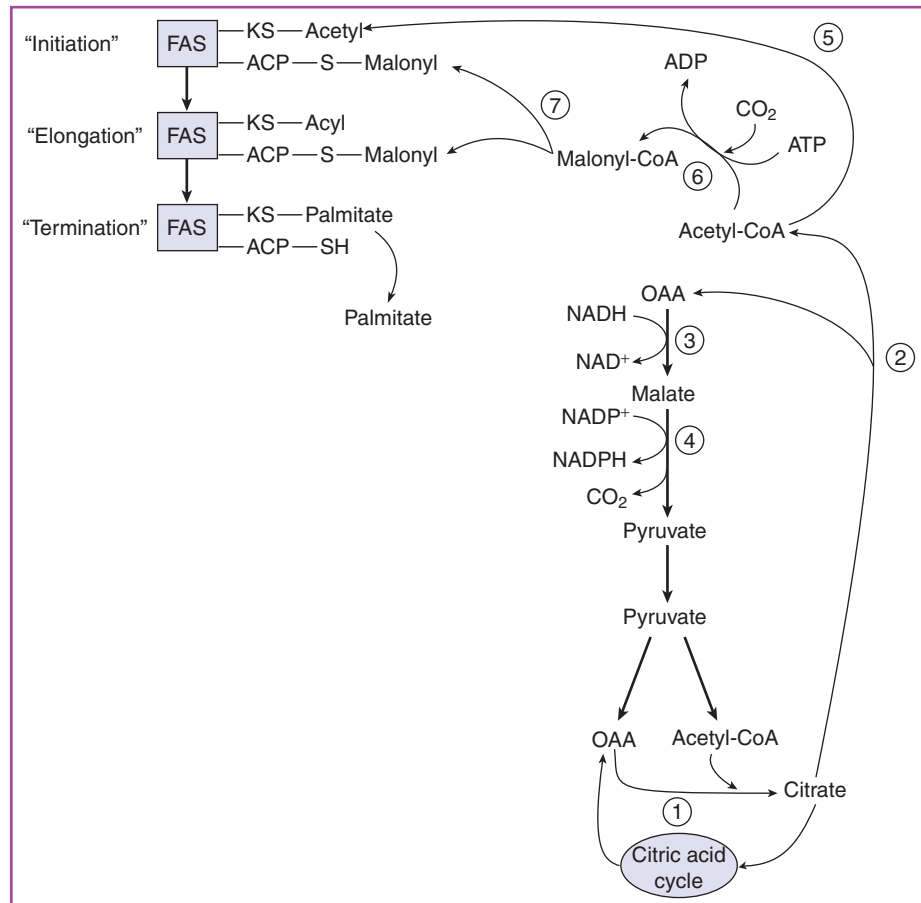
Malate dehydrogenase

s0035

p0125 Oxaloacetate is reduced with nicotine adenine dinucleotide (NADH) to produce malate. Malate can be transported directly back into the mitochondrion, or it can undergo oxidative decarboxylation with malic enzyme.

Malic enzyme

s0040

p0130 Oxidative decarboxylation of malate produces pyruvate, CO_2, and nicotinamide adenine dinucleotide phosphate (NADPH). The pyruvate is transported back into the mitochondrion and converted back to oxaloacetate with pyruvate carboxylase.

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f0010 **Figure 10-1.** Metabolic steps in the synthesis of fatty acids. Ketoacyl site contains an acetyl group during initiation, an acyl group during elongation, and palmitate before release as free palmitate. Step 1, citrate synthase; 2, citrate cleavage enzyme (citrate lyase); 3, malate dehydrogenase; 4, malic enzyme; 5, acetyl-coenzyme A (CoA)–acyl carrier protein (ACP) transacylase; 6, acetyl-CoA carboxylase; 7, malonyl-CoA-ACP transacylase. *FAS,* fatty acid synthesis.

b0015

s0360   **Fat Oxidation in Mitochondria**

p0145   The mitochondrion contains not only the enzymes for aerobic production of energy from glucose but also the enzymes necessary for β-oxidation of fats. Because there is no alternative pathway for fats to be metabolized, any condition that impairs mitochondrial function will also impair fat oxidation. This will result in an accumulation of fat in the tissues (steatosis), generally as neutral triglyceride.

s0045   *Fatty Acid Polymerization Initiation*

p0150   Four reactions initiate fatty acid polymerization with condensation of acetyl and malonyl groups (Fig. 10-2) to produce an acetoacetyl group. Each enzyme function is catalyzed by individual domains of the fatty acid synthase multienzyme complex, which is a single polypeptide.

s0050   **Acetyl–coenzyme A–acyl carrier protein transacylase**

p0155   The 2-carbon acetyl group is transferred from the phosphopantetheine group of acetyl-CoA to the phosphopantetheine group of acyl carrier protein (ACP). The ACP then transfers the acetyl group to the cysteine thiol group of 3-ketoacyl synthase (KS).

Acetyl-coenzyme A carboxylase   s0060

CO_2 is attached to acetyl-CoA to produce malonyl-CoA. ATP p0160 provides the energy input. Note that this same CO_2 will be removed when the malonyl group condenses with the growing acyl chain. Like all carboxylases, acetyl-CoA carboxylase requires biotin as a cofactor.

Malonyl-coenzyme A–acyl carrier protein transacylase   s0065

The malonyl group of malonyl-CoA is transferred from phosphopantetheine in the CoA to the phosphopantetheine in the p0165 active site of the ACP.

3-Ketoacyl synthase   s0070

The acetyl group (or a longer acyl group) in the KS site is condensed with malonyl-ACP, accompanied by release of the p0170 terminal CO_2 of the malonyl group and producing a 4-carbon 3-ketoacyl chain attached to the ACP. The loss of CO_2 drives the reaction to completion. (Note: All further 2-carbon additions to the acyl chain are also from malonyl-CoA.)
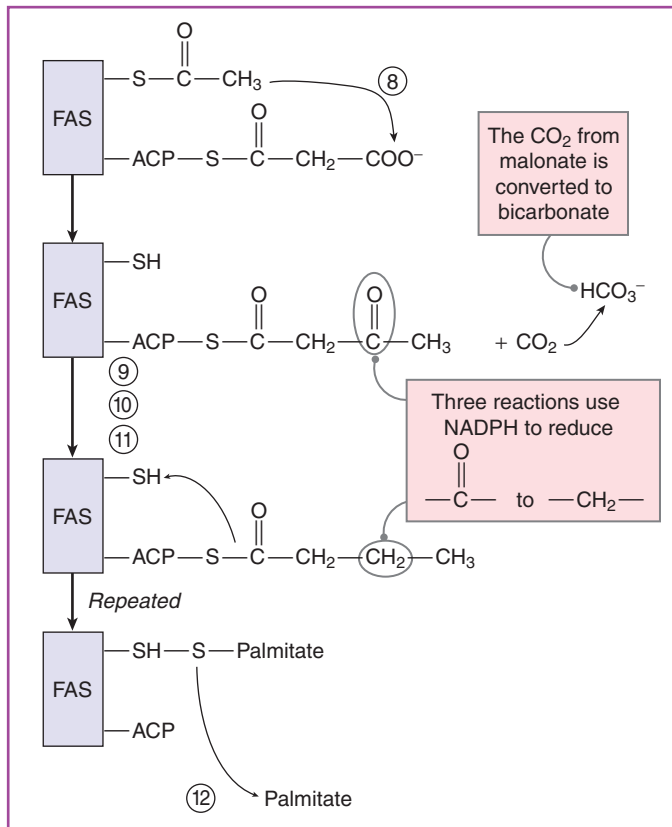
Fatty acid metabolism • **83**

Figure 10-2. Elongation of fatty acid chain. Step 8, 3-ketoacyl synthase; 9, 3-ketoacyl reductase; 10, dehydratase; 11, enoyl reductase; 12, thioesterase.

β-Carbonyl Reduction
Three reactions reduce the β-carbonyl on acyl-ACP:

3-Ketoacyl reductase
The 3-ketoacyl group is reduced to a 3-hydroxyacyl group by NADPH.

Dehydratase
An unsaturated bond is created by removal of water; this is similar to the enolase reaction in glycolysis.

Enoyl reductase
The unsaturated bond is reduced with NADPH. This reduced acyl intermediate is then transferred to the free cysteine at the KS active site, and the cycle begins again.

Elongation Cycle
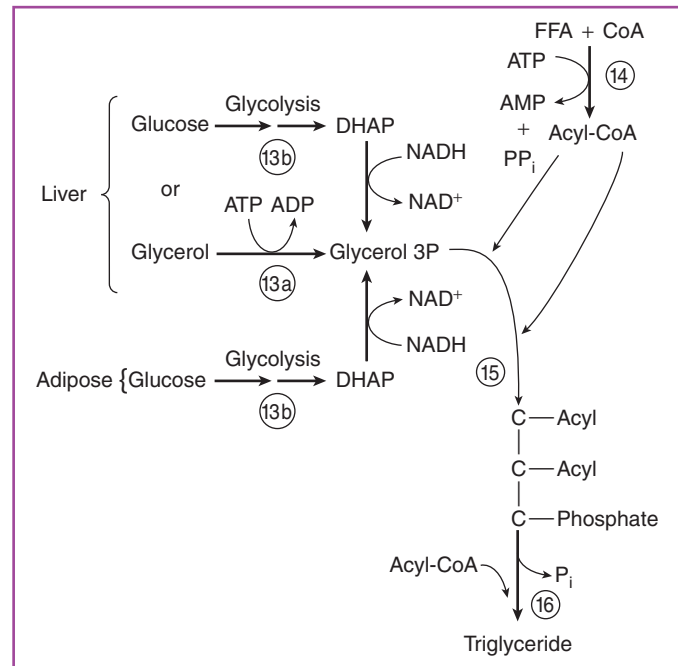Repetitive condensation and reduction of malonyl-CoA units continues to produce palmitic acid.

Thioesterase
When the growing acyl chain reaches a length of 16 carbons, it is released from ACP as free palmitic acid.

Triglyceride Synthesis *(Fig. 10-3)*
Glycerol kinase
In liver, glycerol is phosphorylated with ATP.

Figure 10-3. Assembly of a triglyceride. Step 13a, glycerol kinase; 13b, glycerol-3-phosphate dehydrogenase; 14, acetyl-coenzyme A synthase; 15 and 16, acyltransferase.

Glycerol-3-phosphate dehydrogenase
In both liver and adipose tissue, glyceraldehyde 3-phosphate produced during glycolysis is reduced to glycerol 3-phosphate.

Acyl-coenzyme A synthase (fatty acid thiokinase)
Fatty acids are activated with CoA to acyl-CoA in an ATP-dependent reaction; adenosine monophosphate (AMP) and pyrophosphate are produced instead of ADP. The pyrophosphate is hydrolyzed to phosphate by pyrophosphatase, so that, in effect, two high-energy bonds are expended for production of each acyl-CoA.

Two acyl-CoA molecules are then esterified to glycerol 3-phosphate to produce a diacylphosphoglycerate.

The phosphate is then removed, and the third acyl group is added to form a triglyceride.

Regulated Reactions in Fatty Acid Synthesis—Acetyl-Coenzyme A Carboxylase

The irreversible step in fatty acid synthesis, acetyl-CoA carboxylase, is controlled by two mechanisms (Fig. 10-4).

Covalent Modification
The active dephospho- form of acetyl-CoA carboxylase is inactivated by phosphorylation catalyzed by an AMP-activated protein kinase (Note: AMP, not cyclic AMP). This ensures that under circumstances of low energy charge no acetyl-CoA will be diverted away from the citric acid cycle.
- Protein phosphatase 2A (PP2A) reactivates acetyl-CoA carboxylase.
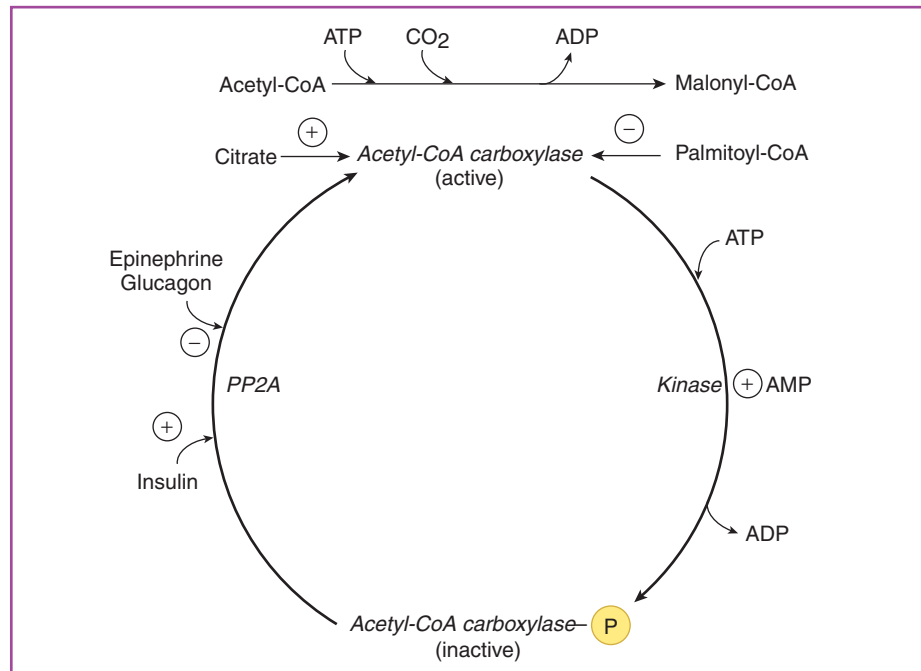- Insulin reactivates acetyl-CoA carboxylase through stimulation of PP2A.

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f0025 **Figure 10-4.** Regulation of acetyl-coenzyme A carboxylase by allosteric feedback and covalent modification.

u0100 ● Epinephrine and glucagon inhibit fatty acid synthesis by inhibiting PP2A.

s0135 *Allosteric Regulation*

p0255 The active dephospho- form of acetyl-CoA carboxylase is regulated by citrate and palmitoyl-CoA.

u0105 ● Stimulation by citrate assures fatty acid synthesis when 2-carbon units are plentiful.

u0110 ● Inhibition by palmitoyl-CoA coordinates palmitate synthesis with triglyceride assembly. (Note: Palmitate is the product of fatty acid synthesis [FAS] complex.)

Au2

s0140 **Unique Characteristics of Fatty Acid Synthesis**

s0145 *Multienzyme Complex*

p0270 In humans, the enzymes for fatty acid biosynthesis exist as a single polypeptide consisting of eight catalytic domains. Thus the multiple enzymatic activities form a structurally organized complex that binds to the growing acyl chain until it is completed and released. The acyl carrier protein domain contains the same phosphopantetheine group as in CoA. The phosphopantetheine is attached by a long, flexible arm, allowing contact with the multiple active sites in the multienzyme complex. Note that the fatty acid synthase complex is not subject to regulation, except by the availability of malonyl-CoA.

s0150 *Compartmentation*

p0275 FAS does not compete with fatty acid oxidation because they occur in separate compartments of the cell. Cytoplasmic synthesis ensures that NADPH will be available and that the product, palmitate, will not undergo β-oxidation.

Adipose Tissue Versus Liver   s0155

p0280 Adipose tissue does not contain glycerol kinase, an enzyme found in liver. Thus the glycerol backbone for triglyceride assembly in adipose tissue must come from dihydroxyacetone phosphate (DHAP) in the glycolytic pathway. In other words, uptake of glucose is essential for adipose synthesis of triglycerides.   Au3

Interface with Other Pathways   s0160

Elongation of Palmitate   s0165

p0285 When longer fatty acids are needed (e.g., in the synthesis of myelin in the brain), palmitate is elongated by enzymes in the endoplasmic reticulum. The palmitate elongation reactions also use malonyl-CoA as the 2-carbon donor and NADPH as the redox coenzyme. These extensions are carried out by enzymes in the endoplasmic reticulum, not by the fatty acid synthase complex.

Desaturation of Fatty Acids   s0170

p0290 Unsaturated fatty acids are a component of the phospholipids in cell membranes and help maintain membrane fluidity. Phospholipids contain a variety of unsaturated fatty acids, but not all of these can be synthesized in the body.

● Fatty acid desaturase, an enzyme in the endoplasmic reticulum, introduces double bonds between carbons 9 and 10 in palmitate and in stearate, producing palmitoleic acid (16:1:Δ9) and oleic acid (18:1:Δ9), respectively.   u0115

● Fatty acid desaturase requires O_2 and either NAD^+ or NADPH.   u0120

p0305 Humans lack the enzymes necessary to introduce double bonds beyond carbon 9. Thus linoleic acid (18:2:Δ9,Δ12) and linolenic acid (18:2:Δ9,Δ12,Δ15) cannot be synthesized. These are essential fatty acids. Linoleic acid can serve as a precursor for arachidonate, sparing it as an essential fatty acid.   Au4

Fatty acid mobilization and oxidation   **85**

Arachidonate is an important component of membrane lipids and, together with linoleic and linolenic acid, serves as a precursor for the synthesis of prostaglandins, thromboxanes, leukotrienes, and lipoxins.

KEY POINTS ABOUT FATTY ACID METABOLISM

- Fatty acid chains are polymerized in the cytoplasm and oxidized in the mitochondrial matrix.
- The precursor for fat synthesis, acetyl-CoA, arises in the matrix and must first be transported to the cytoplasm for incorporation into a fatty acid.
- FFAs that have been mobilized for oxidation must be transported into the mitochondrion to undergo oxidation.
- FAS in eukaryotes occurs on a multifunctional enzyme complex contained within a single polypeptide.
- Humans lack the enzymes necessary to introduce double bonds beyond carbon 9, thus making linoleic acid (18:2:Δ9,Δ12) and linolenic acid (18:2:Δ9,Δ12,Δ15) essential fatty acids in the diet.
- Malonyl-CoA synthesis from acetyl-CoA by acetyl-CoA carboxylase is regulated by both covalent modification and by allosteric feedback.

●●● FATTY ACID MOBILIZATION AND OXIDATION

Pathway Reaction Steps in Fatty Acid Oxidation—Palmitate to Acetyl-Coenzyme A and Ketone Bodies

Fatty Acid Transport into Mitochondria

Fatty acids are transported across the mitochondrial membrane by the carnitine cycle (Fig. 10-5). Fatty acids are first activated to an acyl-CoA in the cytoplasm.

Carnitine acyltransferase I

The acyl group is transferred to carnitine by the cytoplasmic form of the enzyme. The acylcarnitine then diffuses across the outer mitochondrial membrane.

Carnitine-acylcarnitine translocase

This membrane transporter (antiporter) exchanges cytoplasmic acylcarnitine for mitochondrial carnitine.

Carnitine acyltransferase II

The mitochondrial form of this enzyme then transfers the acyl group back to CoA. Medium-chain (6 to 12 carbons) and short-chain fatty acids (acetate propionate and butyrate) enter the mitochondrion directly and therefore bypass the carnitine cycle. They are activated in the mitochondrial matrix by acyl-CoA synthetases.

β-Oxidation of an Acyl-Coenzyme A (Fig. 10-6)
Acyl-coenzyme A dehydrogenase

1. Oxidation at the β-carbon of the fatty acid occurs with reduction of flavin adenine dinucleotide (FAD) (creates a trans double bond) at the Δ2 position to produce Δ2-trans-enoyl-CoA. The electrons from $FADH_2$ are subsequently transferred to ubiquinone in the electron transport chain. A separate acyl-CoA dehydrogenase exists for long-, medium-, and short-chain fatty acids. This reaction is analogous to the succinate dehydrogenase reaction in the citric acid cycle.

Enoyl-coenzyme A reductase

The Δ2-trans-enoyl double bond is then hydrated to create a 3-hydroxyl group. This reaction is analogous to that of fumarase.

#### 3-Hydroxyacyl–coenzyme A dehydrogenase

The 3-hydroxyl group is then oxidized with reduction of NAD^+ to NADH to produce a β-keto group. This reaction is analogous to that of malate dehydrogenase.

β-Ketothiolase

Acetyl-CoA is cleaved at the β-keto group and CoA is attached to the shortened acyl chain to reenter the β-oxidation cycle. The acetyl-CoA is in the matrix and available as a substrate for the citric acid cycle for further oxidation.
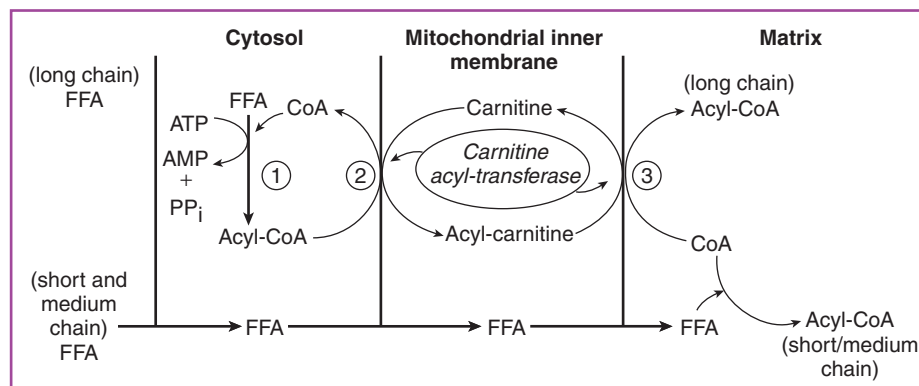
Figure 10-5. Transport of acetyl-coenzyme A by the carnitine cycle. Step 1, carnitine acyltransferase I; 2, carnitine acyl–carnitine translocase; 3, carnitine acyltransferase II.

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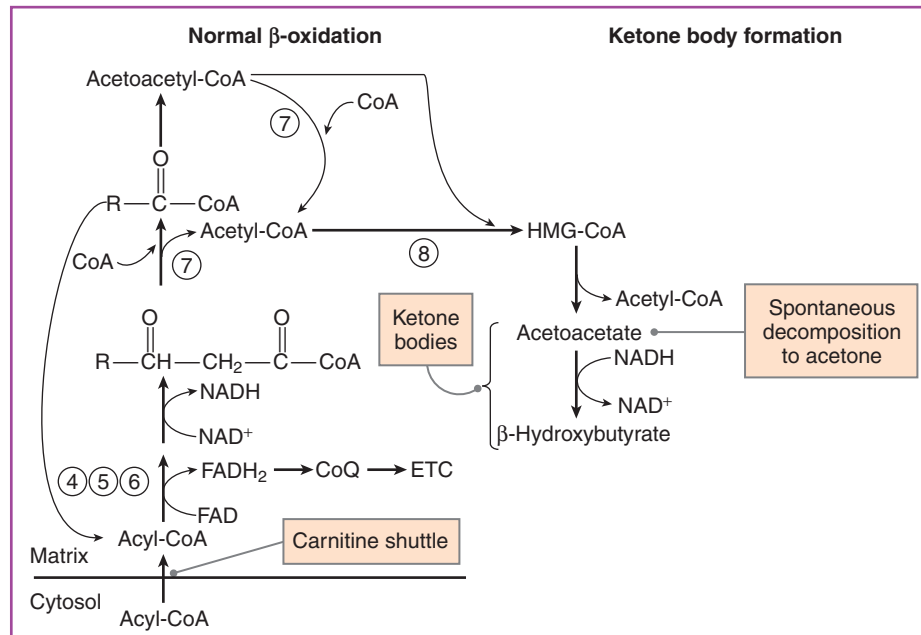
f0035   **Figure 10-6.** β-Oxidation of fatty acids. Acyl-coenzyme A (CoA) in the matrix is oxidized by a reversal of the steps involved in fatty acid synthesis, but with different enzymes and with nicotinamide adenine dinucleotide cofactor. Step 4, acyl-CoA dehydrogenase; 5, enoyl-CoA reductase; 6, 3-hydroxyacyl-CoA dehydrogenase; 7, β-ketothiolase.

s0235   *Formation and Degradation of Ketone Bodies*

s0240   **β-Hydroxy-β-methylglutaryl–coenzyme A synthase**

p0390   A third molecule of acetyl-CoA is condensed with acetoacetyl-CoA to form β-hydroxy-β-methylglutaryl-CoA (HMG-CoA).

s0245   **β-Hydroxy-β-methylglutaryl–coenzyme A lyase**

p0395   HMG-CoA is hydrolyzed to produce acetyl-CoA and acetoacetate, a ketone body.

s0250   **β-Hydroxybutyrate dehydrogenase**

p0400   Acetoacetate is further reduced to form β-hydroxybutyrate.

s0255   **Acetone formation**

p0405   Acetoacetate spontaneously degrades in a nonenzymatic reaction to produce acetone. When acetone accumulates in the blood, it imparts a fruity odor to the breath.

s0260   **Succinyl-coenzyme A:acetoacetate-coenzyme A transferase**

p0410   In peripheral tissues, acetoacetate is converted to acetyl-CoA by reaction with succinyl-CoA. Since acetoacetate is metabolized in the mitochondrial matrix, the succinate produced is metabolized as a citric acid cycle intermediate.

$$2 \text{ succinyl-CoA} + \text{acetoacetate}$$
$$\rightarrow 2 \text{ acetyl-CoA} + 2 \text{ succinate}$$
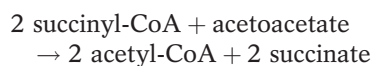
s0265   ## Regulated Reactions in Fatty Acid Oxidation—Hormone-Sensitive Lipase

p0415   The only site for regulation of fatty acid oxidation is mobilization that occurs at the level of hormone-sensitive lipase in
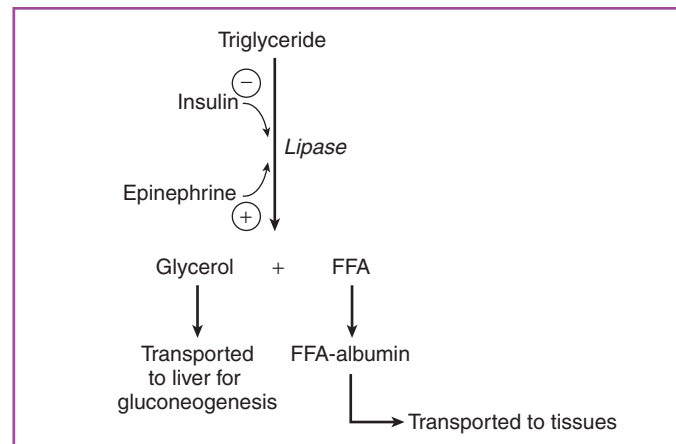
Figure 10-7. Activation of hormone-sensitive lipases. Specialized lipases remove free fatty acids from the respective glycerides.   f0040

adipose tissue (Fig. 10-7). This is the underlying reason for the runaway fat mobilization that leads to ketosis in conditions such as starvation and untreated type 1 diabetes. Under fasting conditions, with minimal insulin in the blood, glucagon promotes formation of the phosphorylated, active form of hormone-sensitive lipase. When epinephrine is present, it further shifts the equilibrium to active hormone-sensitive lipase, increasing the hydrolysis of triglycerides to produce FFAs and glycerol. The glycerol is carried to the liver, where it enters gluconeogenesis, while FFAs are carried on serum albumin to the tissues where they are catabolized for energy. The liver uses some of the energy from fat mobilization to support gluconeogenesis.

p0420   The oxidation of newly synthesized FFAs is prevented by malonyl-CoA, which is present in high amounts during fatty acid synthesis. Carnitine acyltransferase is inhibited by malonyl-CoA, preventing transport and β-oxidation of the newly synthesized fatty acids.

s0270 Unique Characteristics of Fatty Acid Oxidation

s0275 *Energy Gained from Fatty Acid Oxidation*

p0425 The caloric value of neutral fat is approximately 9 kcal/g; this compares with the caloric value of carbohydrate and protein of approximately 4 kcal/g. More than half of the oxidative energy requirement of the liver, kidneys, heart, and resting skeletal muscle is provided by fatty acid oxidation. The NADH, FADH$_2$, and acetyl-CoA produced from β-oxidation create a net 129 moles of ATP for each palmitate oxidized.

s0280 *Compartmentation of Ketone Body Formation and Use*

p0430 The liver cannot metabolize the ketone bodies that it produces because it lacks the enzyme succinyl-CoA:acetoacetate-CoA transferase that is needed to convert acetoacetate to acetyl-CoA. This enzyme is found only in the peripheral tissues, where the energy from ketone bodies is used. Thus when acetyl-CoA produced from excessive fatty acid oxidation saturates the capacity of the citric acid cycle in the liver, it is shunted into the formation of ketone bodies that flow unidirectionally from the liver to the peripheral tissues.

s0285 Interface with Other Pathways

s0290 *β-Oxidation of Dietary Unsaturated Fatty Acids*

p0435 Unsaturated bonds in unsaturated fatty acids may be out of position and not recognized by β-oxidation enzymes. Any double bonds that are out of position are corrected by an isomerase, which shifts their position and configuration to produce the normal Δ2-trans-enoyl-CoA intermediate that is recognized by enoyl-CoA reductase in normal β-oxidation (see Fig. 10-6, step 5).

s0295 *β-Oxidation of Odd-Chain Fatty Acids* (Fig. 10-8)

p0440 Odd-numbered fatty acids yield propionyl-CoA (3 carbons) as the last intermediate in β-oxidation. (Note: Propionyl-CoA is also formed from catabolism of methionine, valine, and isoleucine.) Propionyl-CoA cannot be catabolized further, so it is converted to succinyl-CoA by the following short pathway.

s0300 Propionyl-coenzyme A carboxylase

p0445 Propionyl-CoA is first converted to methylmalonyl-CoA.
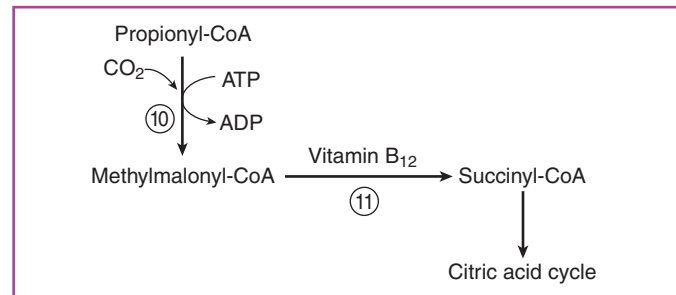
Figure 10-8. Conversion of propionyl-coenzyme A (CoA) to succinyl-CoA. Step 10, propionyl-CoA carboxylase; 11, methylmalonyl-CoA mutase.

s0305 Methylmalonyl-coenzyme A mutase

p0450 Methylmalonyl-CoA is then converted to succinyl-CoA by a vitamin B$_{12}$–dependent reaction. Succinyl-CoA enters the citric acid cycle.

s0310 Peroxisomal Oxidation of Fatty Acids

p0455 Very long chain fatty acids (20 to 26 carbons) can be degraded in peroxisomes. The process is similar to β-oxidation for fatty acids except that no NADH or FADH$_2$ is produced; instead H$_2$O$_2$ is produced and then degraded by catalase. Final products of this process are octanoyl-CoA and acetyl-CoA, which are then metabolized normally in mitochondria.

s0315 *ω-Oxidation of Fatty Acids*

p0460 Oxidation at the terminal carbon (ω-carbon) can be carried out by enzymes in the endoplasmic reticulum, creating a dicarboxylic acid. This process requires cytochrome p450, NADPH, and molecular O$_2$. Normal β-oxidation can then occur at both ends of the fatty acid.

s0320 *α-Oxidation of Fatty Acids*

p0465 Very long (>20 carbons) fatty acids and branched-chain fatty acids (e.g., phytanic acid in the diet) are metabolized by α-oxidation, which releases a terminal carboxyl as CO$_2$ one at a time. This occurs mainly in brain and nervous tissue. (Note: Few fatty acids are metabolized one carbon at a time. For example, branched-chain phytanic acids release one CO$_2$, followed by equal amounts of acetyl- and propionyl-CoA.)

PATHOLOGY   b0025

Adrenoleukodystrophy   s0365

p0480 The neurologic disorder adrenoleukodystrophy is due to defective peroxisomal oxidation of very long chain fatty acids. This syndrome demonstrates a marked reduction in plasmalogens (see Chapter 11), adrenocortical insufficiency, and abnormalities in the white matter of the cerebrum.   Au5

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b0030 **KEY POINTS ABOUT FATTY ACID MOBILIZATION AND OXIDATION**

u0155 ■ To be oxidized, fatty acids are transported across the mitochondrial membrane by the carnitine cycle.

u0160 ■ β-Oxidation oxidizes the β-carbon of an acyl-CoA to form a carbonyl group, followed by release of acetyl-CoA.

u0165 ■ The only point for regulation of fatty acid oxidation is at the level of hormone-sensitive lipase in adipose tissue.

u0170 ■ Odd-numbered fatty acids yield propionyl-CoA (3 carbons) as the last intermediate in β-oxidation after which it is converted to succinyl-CoA.

s0325 ●●● **RELATED DISEASES OF FATTY ACID METABOLISM**

s0330 **Medium-Chain Acyl-Coenzyme A Dehydrogenase Deficiency**

p0510 Long-chain fatty acids are oxidized until reaching a chain length of about 16 carbons. Because of the inability to use fatty acids to support gluconeogenesis, this deficiency produces a nonketotic hypoglycemia. It is normally dangerous only in cases of extreme or frequent fasting.

Jamaican Vomiting Sickness   s0335

p0515 The unripe fruit of the Jamaican ackee tree contains a toxin, hypoglycin, that inhibits both the medium- and short-chain acyl-CoA dehydrogenases. This inhibits β-oxidation and leads to nonketotic hypoglycemia.

Zellweger Syndrome   s0340

p0520 Associated with the absence of peroxisomes in the liver and kidneys, Zellweger syndrome results in accumulation of very long chain fatty acids, especially in the brain.

Carnitine Deficiency   s0345

p0525 Carnitine deficiency produces muscle aches and weakness following exercise, elevated blood FFAs, and low fasting ketone production. Nonketotic hypoglycemia results because gluconeogenesis cannot be supported by fat oxidation.

Refsum Disease   s0350

p0530 Also referred to as deficient α-oxidation, Refsum disease results in accumulation of phytanic acid in the brain, producing neurologic symptoms. Phytanic acid is a branched-chain fatty acid found in plants and in dairy products.

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