Lipid Metabolism (Muhammad Imran)

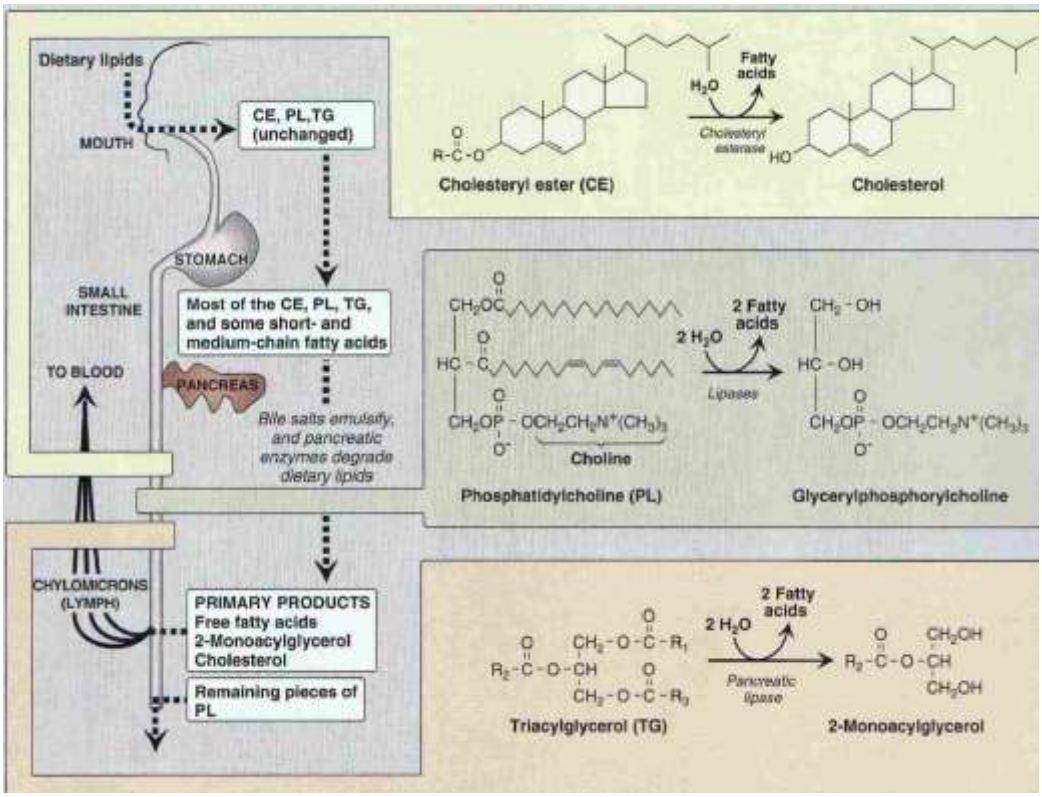
The digestion of fats and other lipids **poses a special problem because of *(a)* the insolubility of fats in water, and *(b)* because lipolytic enzymes, like other enzymes, are soluble in an aqueous medium.**

A. • ***Vegetable source:*** Various cooking oils from various seeds, viz. sunflower oil, groundnut oil, cotton seed oil, mustard oil, etc. and fats from other vegetable sources. However, unlike proteins, **vegetable fats are superior to animal fats because:**
They contain more of polyunsaturated fatty acids. Vegetable fats are less likely to go rancid due to presence of antioxidants.

1. **Digestion in mouth and stomach:** It was believed earlier that little or no fat digestion takes place in the mouth. Recently a *lipase* has been detected called ***lingual lipase*** which is secreted by the dorsal surface of the tongue (Ebner's gland). ***Lingual lipase:*** The pH of activity is 2.0 to 7.5 (optimal pH value is 4.0 to 4.5). *Lingual lipase* activity is continued in the stomach also where the pH value is low. Due to retention of food bolus for 2 to 3 hours, about 30 per cent of dietary triacyl glycerol (TG) may be digested. *Lingual lipase* is more active on TG having shorter FA chains and is found to be more specific for ester linkage at 3-position rather than position-1. Milk fats contain short and medium chain FA which tend to be esterified in the 3-position. Hence, ***milk fat appears to be the best substrate for this enzyme.*** The released short chain fatty acids are relatively more soluble and hydrophilic and can be absorbed directly from the stomach wall and enter the portal vein.

DIGESTION, ABSORPTION, SECRETION, AND UTILIZATION OF DIETARY LIPIDS

An adult ingests about 60 to 150 g of lipids per day, of which more than ninety percent is normally triacylglycerol (formerly called triglyceride). The remainder of the dietary lipids consists primarily of cholesterol, cholesteryl esters, phospholipids, and unesterified ("free") fatty acids. The digestion of dietary lipids is summarized.



These same triacylglycerols are also degraded by a separate gastric lipase, secreted by the gastric mucosa. Both enzymes are relatively acid-stable, with pH optimums of pH 4 to pH 6. These "acid lipases" play a particularly important role in lipid digestion in neonates, for whom milk fat is the primary source of calories. They are also important digestive enzymes in individuals with pancreatic insufficiency, such as those with cystic fibrosis. Lingual and gastric lipases aid these patients in degrading triacylglycerol molecules (especially those with short- to medium-chain length fatty acids) despite a near or complete absence of pancreatic lipase (see below). Emuisification of dietary lipid in the small intestine. The critical process of emuisification of dietary lipids occurs in the duodenum. Emuisification increases the surface area of the hydrophobic lipid droplets so that the digestive enzymes, which work at the interface of the droplet and the surrounding aqueous solution, can act effectively. Emulsification is accomplished by two complementary mechanisms, namely, use of the detergent properties of the bile salts, and mechanical mixing due to peristalsis. Bile salts, made in the liver and stored in the gallbladder, are derivatives of cholesterol. They consist of a sterol ring structure with a side chain to which a molecule of glycine or taurine is covalently attached by an amide linkage. These emulsifying agents interact with the dietary lipid particles and the aqueous duodenal contents, thereby stabilizing the particles as they become smaller, and preventing them from coalescing.

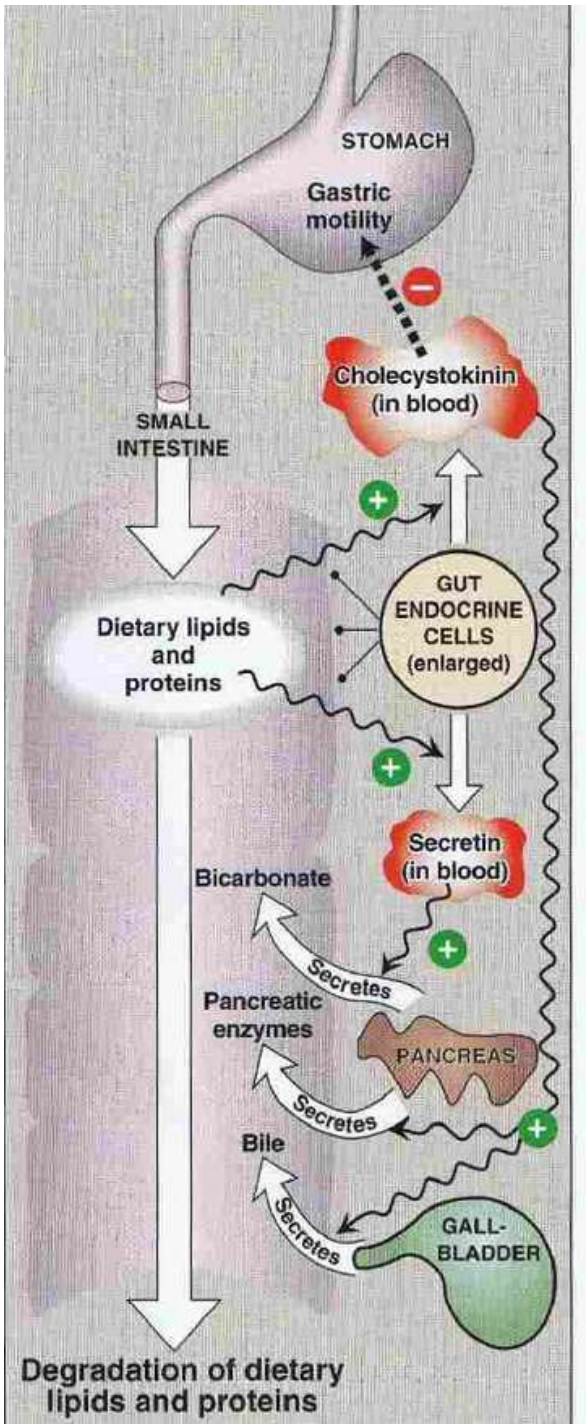
C. Degradation of dietary lipids by pancreatic enzymes

The dietary triacylglycerol, cholesteryl esters, and phospholipids are enzymically degraded ("digested") by pancreatic enzymes, whose secretion is hormonally controlled. „ r , Structure of glycocholic acid.

1. Triacylglycerol degradation: Triacylglycerol molecules are too large to be taken up efficiently by the mucosal cells of the intestinal villi. They are, therefore, acted upon by an esterase, pancreatic lipase, which preferentially removes the fatty acids at carbons 1 and 3. The primary products of hydrolysis are thus a mixture of 2 monoacylglycerol and free fatty acids. [Note: This enzyme is found in high concentrations in pancreatic secretions (two to three percent of the total protein present), and it is highly efficient catalytically, thus insuring that only severe pancreatic deficiency, such as that seen in cystic fibrosis, results in significant malabsorption of fat. A second protein, colipase, also secreted by the pancreas, binds the lipase at a ratio of one to one, and anchors it at the lipidaqueous interface. [Note: Colipase is secreted as the zymogen, procolipase, which is activated in the intestine by trypsin.] Orlistat, an antiobesity drug, inhibits gastric and pancreatic lipases, thereby decreasing fat absorption, resulting in loss of weight.

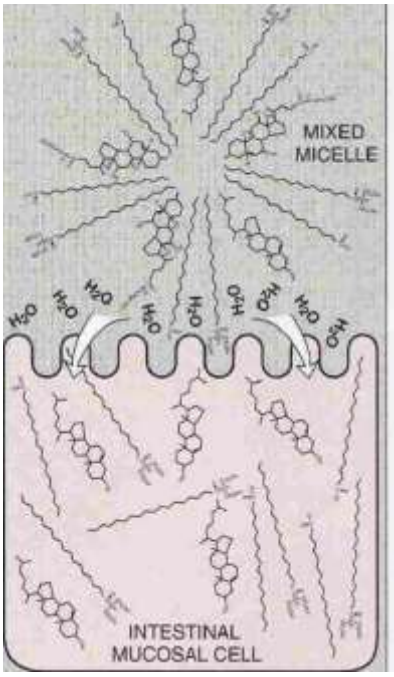
2. Cholesteryl ester degradation: Most dietary cholesterol is present in the free (nonesterified) form, with ten to fifteen percent present in the esterified form. Cholesteryl esters are hydrolyzed by pancreatic cholesterol ester hydrolase (cholesterol esterase), which produces cholesterol plus free fatty acids. Cholesteryl ester hydrolase activity is greatly increased in the presence of bile salts. Phospholipid degradation: Pancreatic juice is rich in the proenzyme of phospholipase A2 that, like procolipase, is activated by trypsin and, like cholesterol ester hydrolase, requires bile salts for optimum activity. Phospholipase A2 removes one fatty acid from carbon 2 of a phospholipid, leaving a lysophospholipid. For example, phosphatidylcholine (the predominant phospholipid during digestion) becomes lysophosphatidylcholine. The remaining fatty acid at carbon 1 can be removed by lysophospholipase, leaving a glycerylphosphoryl base that may be excreted in the feces, further degraded, or absorbed. Control of lipid digestion: Pancreatic secretion of the hydrolytic enzymes that degrade dietary lipids in the small intestine is hormonally controlled (Figure 15.4). Cells in the mucosa of the jejunum and lower duodenum produce a small peptide hormone, cholecystokinin (CCK, formerly called pancreozymin), in response to the presence of lipids and partially digested proteins entering these regions of the upper small intestine. CCK acts on the gallbladder (causing it to contract and release bile), and on the exocrine cells of the pancreas (causing them to release digestive enzymes). It also decreases gastric motility, resulting in a slower release of gastric contents into the small intestine. Other intestinal cells produce another small peptide hormone, secretin, in response to the low pH of the chyme entering the intestine. Secretin causes the pancreas and the liver to release a watery solution rich in bicarbonate that helps neutralize the pH of the intestinal contents, bringing them to the appropriate pH for enzymic digestive activity by pancreatic enzymes. Absorption of lipids by intestinal mucosal cells (enterocytes) Free fatty acids, free cholesterol, and 2-monoacylglycerol are the primary products of dietary lipid degradation in the jejunum. These, together with bile salts, form mixed micelles—disk-shaped clusters of amphipathic lipids that coalesce with their hydrophobic groups on the inside and their hydrophilic groups on the outside of the cluster.Mixed micelles are, therefore, soluble in the aqueous environment of the intestinal lumen. These particles approach the primary site of lipid absorption, the brush border membrane of the enterocytes (mucosal cell). This membrane is separated from the liquid contents of the intestinal lumen by an unstirred water layer that mixes poorly with the bulk fluid. The hydrophilic surface of the micelles facilitates the transport of the hydrophobic lipids through the unstirred water layer to the brush border membrane where they are absorbed. [Note: Short- and medium-chain length fatty acids do not require the

assistance of mixed micelles for absorption by the intestinal mucosa. This is an important consideration in the dietary therapy for individuals with malabsorption of other lipids.

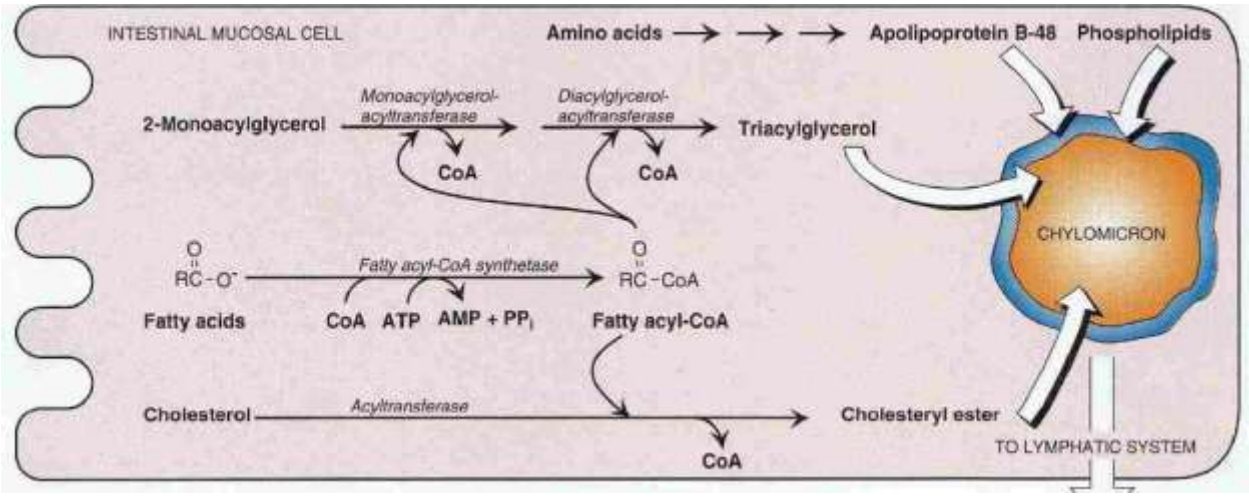


Resynthesis of triacylglycerol and cholesteryl esters

The mixture of lipids absorbed by the enterocytes migrates to the endoplasmic reticulum where biosynthesis of complex lipids takes place. Fatty acids are first converted into their activated form by fatty acyl CoA synthetase (thiokinase).

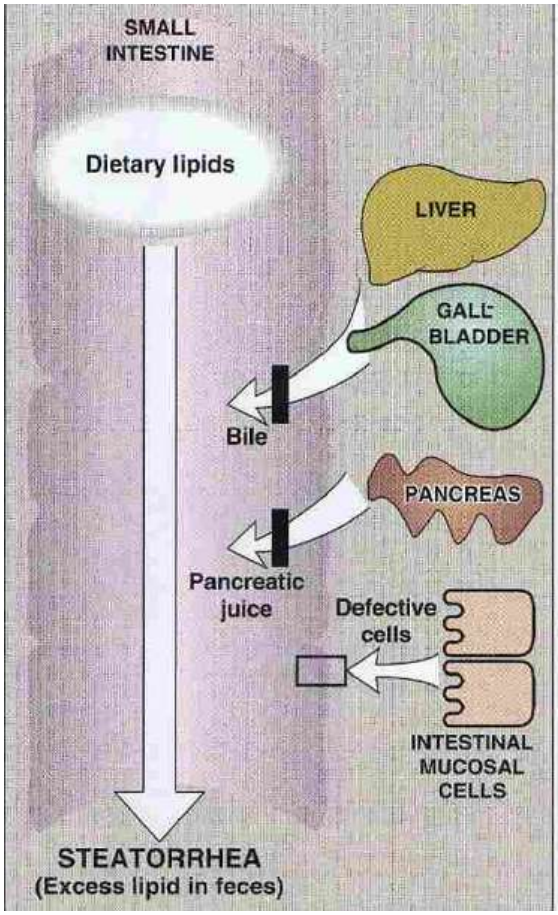


Using the fatty acyl CoA derivatives, the 2-monoacylglycerols absorbed by the enterocytes are converted to triacylglycerols by the enzyme complex, triacylglycerol synthase. This complex synthesizes triacylglycerol by the consecutive actions of two enzyme activities monoacylglycerol acyltransferase and diacylglycerol acyltransferase. Lysophospholipids are re-acylated to form phospholipids by a family of acyltransferases, and cholesterol is esterified to a fatty acid primarily by acyl CoA:cholesterolacyltransferase. [Note: Virtually all long-chain fatty acids entering the enterocytes are used in this fashion to form triacylglycerols, phospholipids, and cholesteryl esters. Short- and medium-chain length fatty acids are not converted to their CoA derivatives, and are not reesterified to 2-monoacylglycerol. Instead, they are released into the portal circulation, where they are carried by serum albumin to the liver.



Lipid malabsorption

Lipid malabsorption, resulting in increased lipid (including the fatsoluble vitamins A, D, E, and K, and essential fatty acids) in the feces (that is, steatorrhea), can be caused by disturbances in lipid digestion and/or absorption. Such disturbances can result from several conditions, including cystic fibrosis (causing poor digestion) and shortened bowel (causing decreased absorption).



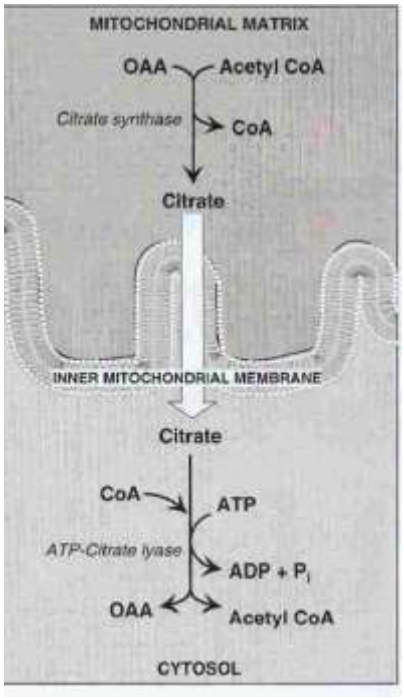
DE NOVO SYNTHESIS OF FATTY ACIDS

A large proportion of the fatty acids used by the body is supplied by the diet. Carbohydrates, protein, and other molecules obtained from the diet in excess of the body's needs for these compounds can be converted toatty acids, which are stored as triacylglycerols. In humans, fatty acid synthesis occurs primarily in the liver and lactating mammary glands and, to a lesser extent, in adipose tissue. The process incorporates carbons from acetyl CoA into the growing fatty acid chain, using ATP and reduced nicotinamide adenine dinucleotide phosphate (NADPH).

A. Production of cytosolic acetyl CoA

The first step in de novo fatty acid synthesis is the transfer of acetate units from mitochondrial acetyl CoA to the cytosol. Mitochondrial acetyl CoA is produced by the oxidation of pyruvate, and by the catabolism of fatty acids, ketone bodies, and certain amino acids. The coenzyme A portion

of acetyl CoA, however, cannot cross the mitochondrial membrane; only the acetyl portion is transported to the cytosol. It does so in the form of citrate produced by the condensation of oxaloacetate (OAA) and acetyl CoA. [Note: This process of translocation of citrate from the mitochondrion to the cytosol, where it is cleaved by ATP-citrate lyase to produce cytosolic acetyl CoA and OAA, occurs when the mitochondrial citrate concentration is high. This is observed when isocitrate dehydrogenase is inhibited by the presence of large amounts of ATP, causing citrate and isocitrate to accumulate.

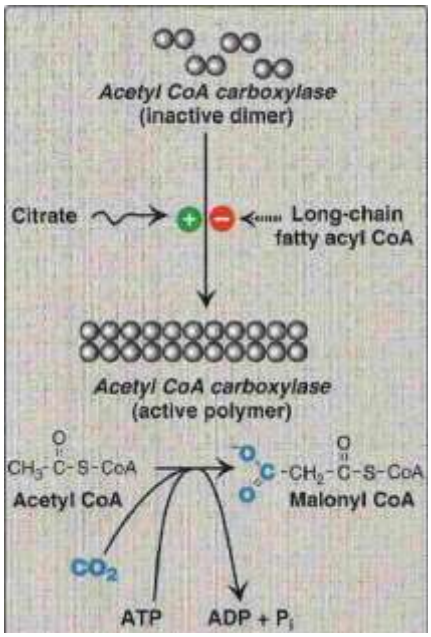


Therefore, cytosolic citrate may be viewed as a high-energy signal. Because a large amount of ATP is needed for fatty acid synthesis, the increase in both ATP and citrate enhances this pathway.

B. Carboxylation of acetyl CoA to form malony CoA

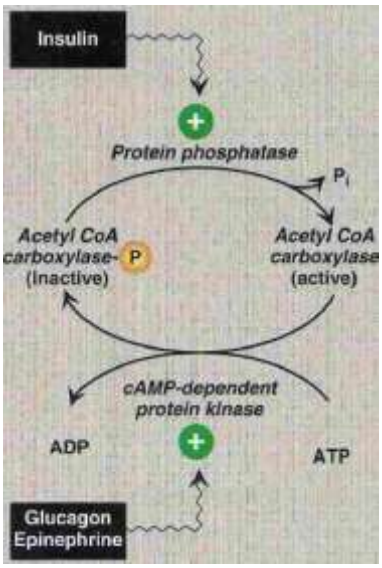
The energy for the carbon-to-carbon condensations in fatty acid synthesis is supplied by the process of carboxylation and then decarboxylation of acetyl groups in the cytosol. The carboxylation of acetyl CoA to form malonyl CoA is catalyzed by acetyl CoA carboxylase (Figure 16.7), and requires HC0 3" and ATP. The coenzyme is the vitamin, biotin, which is covalently bound to a lysyl residue of the carboxylase.

1. Short-term regulation of acetyl CoA carboxylase: This carboxylation is both the rate-limiting and the regulated step in fatty acid synthesis. The inactive form of acetyl CoA carboxylase is a protomer (dimer). The enzyme undergoes allosteric activation by citrate, which causes dimers to polymerize. The enzyme can be allosterically inactivated by long-chain fatty acyl CoA (the end product of the pathway), which causes its depolymerization.



A second mechanism of short-term regulation is by reversible phosphorylation. In the presence of counterregulatory hormones, such as epinephrine and glucagon, acetyl CoA carboxylase is phosphorylated and, thereby, inactivated. In the presence of insulin, acetyl CoA carboxylase is dephosphorylated and, thereby, activated.

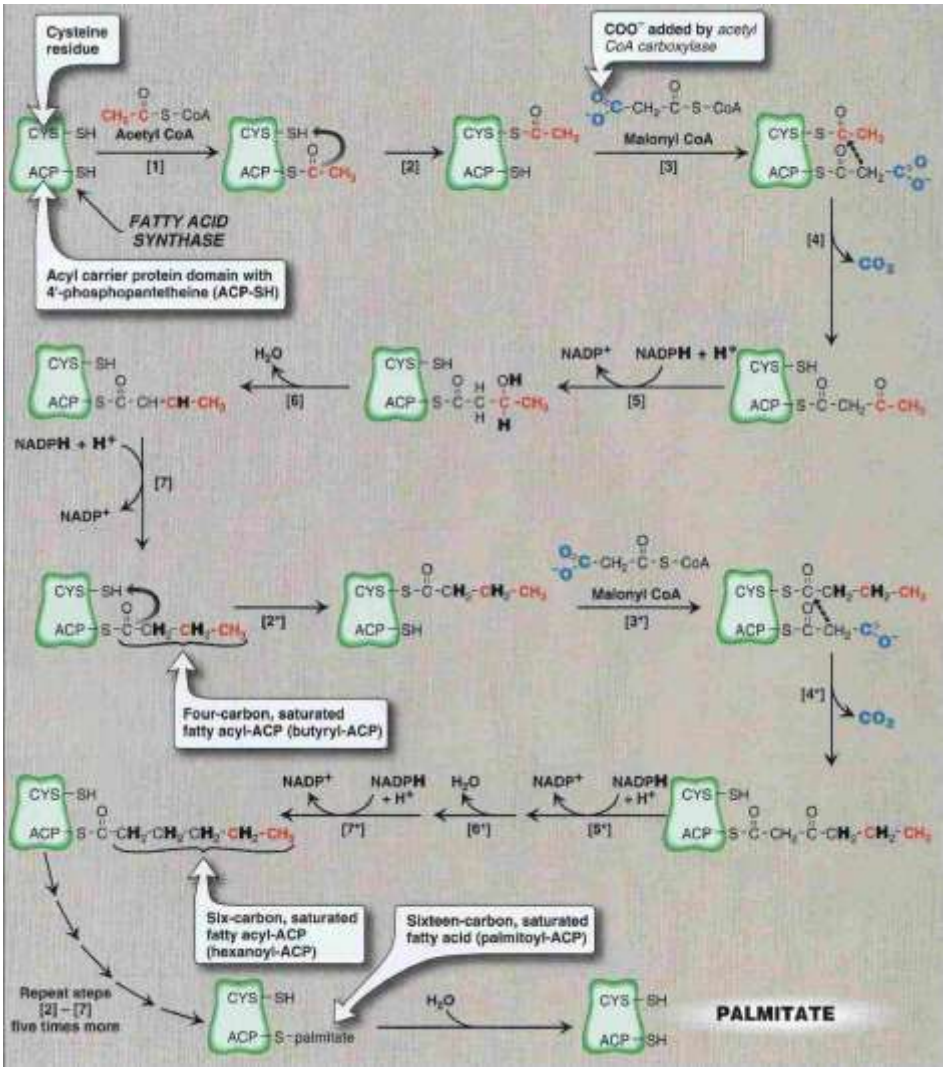
2. Long-term regulation of acetyl CoA carboxylase: Prolonged consumption of a diet containing excess calories (particularly, highcalorie, high-carbohydrate diets) causes an increase in acetyl CoA carboxylase synthesis, thus increasing fatty acid synthesis.



Conversely, a low-calorie diet or fasting causes a reduction in fatty acid synthesis by decreasing the synthesis of acetyl CoA carboxylase. [Note: Fatty acid synthase (see below) is similarly regulated by this type of dietary manipulation.

C. Fatty acid synthase: a multifunctional enzyme in eukaryotes

The remaining series of reactions of fatty acid synthesis in eukaryotes is catalyzed by the multifunctional, dimeric enzyme, fatty acid synthase. Each fatty acid synthase m o n o m e r is a multicatalytic polypeptide with seven different enzymic activities plus a domain that covalently binds a molecule of 4-phosphopantetheine. [Note: 4'-Phosphopantetheine, a derivative of the vitamin pantothenic acid, carries acetyl and acyl units on its terminal thiol (-SH) group during fatty acid synthesis. It also is a component of coenzyme A. In prokaryotes, fatty acid synthase is a multienzyme complex, and the 4'-phosphopantetheine domain is a separate protein, referred to as the acyl carrier protein (ACP). ACP is used below to refer to the phosphopantetheine-binding domain of the eukaryotic fatty acid synthase molecule. [Note: The enzyme activities listed are actually separate catalytic domains present in each multicatalytic fatty acid synthase monomer.



[1] A molecule of acetate is transferred from acetyl CoA to the –SH group of the ACP. Domain: Acetyl CoA-ACP acetyltransacylase.

[2] Next, this two-carbon fragment is transferred to a temporary holding site, the thiol group of a cysteine residue on the enzyme.

[3] The now-vacant ACP accepts a three-carbon malonate unit from malonyl CoA. Domain: Malonyl CoA-ACP-transacylase.

[4] The malonyl group loses the HCCV originally added by acetyl CoA carboxlyase, facilitating its nucleophilic attack on the thioester bond linking the acetyl group to the cysteine residue. The result is a four-carbon unit attached to the ACP domain. The loss of free energy from the decarboxylation drives the reaction. Domain: 3-Ketoacyl-ACP synthase. The next three reactions convert the 3-ketoacyl group to the corresponding saturated acyl group by a pair of reductions requiring NADPH and a dehydration step.

[5] The keto group is reduced to an alcohol. Domain: 3-Ketoacy ACP reductase.

[6] A molecule of water is removed to introduce a double bond. Domain: 3-Hydroxyacyl-ACP dehydratase.
[7] A second reduction step occurs. Domain: Enoyl-ACP reductase. The result of these seven steps is production of a four-carbon compound (butyryl) whose three terminal carbons are fully saturated, and which remains attached to the ACP. These seven steps are repeated, beginning with the transfer of the butyryl chain from the ACP to the cys residue [2*], the attachment of a molecule of malonate to the ACP [3*], and the condensation of the two molecules liberating HCCV [4*]. The carbonyl group at the (3-carbon (carbon 3—the third carbon from the sulfur) is then reduced (5*), dehydrated (6*), and reduced (7*), generating hexanoyl-ACP. This cycle of reactions is repeated five more times, each time incorporating a two-carbon unit (derived from malonyl CoA) into the growing fatty acid chain at the carboxyl end. When the fatty acid reaches a length of sixteen carbons, the synthetic process is terminated with palmitoyl-S-ACP. Palmitoyl thioesterase cleaves the thioester bond, producing a fully saturated molecule of palmitate (16:0). [Note: All the carbons in palmitic acid have passed through malonyl CoA except the two donated by the original acetyl CoA, which are found at the methyl-group end of the fatty acid.]
D. Major sources of the NADPH required for fatty acid synthesis The hexose monophosphate pathway is the major supplier of NADPH for fatty acid synthesis. Two NADPH are produced for each molecule of glucose that enters this pathway. (See p. 143 for a discussion of this sequence of reactions.) The cytosolic conversion of malate to pyruvate, in which malate is oxidized and decarboxylated by c y t o s o l i c malic enzyme (NADP*-dependent malate dehydrogenase), also produces cytosolic NADPH (and HC0 3- Figure 16.10). [Note: Malate can arise from the reduction of oxaloacetate (OAA) by c y t o s o l i c NAD+-dependent malate dehydrogenase.

One source of the cytosolic NADH required for this reaction is that produced during glycolysis (see 102). OAA, in turn, can arise from citrate. That citrate was shown to move from the mitochondria into the cytosol, where it is cleaved into acetyl CoA and OAA by ATP-citrate lyase.

Further elongation of fatty acid chains

Cytosolic conversion of oxaloacetate to pyruvate with the generation of NADPH. Although palmitate, a 16-carbon, fully saturated LCFA (16:0), is the primary end-product of fatty acid synthase activity, it can be further elongated by the addition of two-carbon units in the endoplasmic reticulum (ER) and the mitochondria. These organelles use separate enzymic

processes. The brain has additional elongation capabilities, allowing it to produce the very-long chain fatty acids (up to 24 carbons) that are required for synthesis of brain lipids.

Desaturation of fatty acid chains

Enzymes present in the ER are responsible for desaturating fatty acids (that is, adding cis double bonds). Termed mixed-function oxidases, the desaturation reactions require NADPH and 0 2. A variety of polyunsaturated fatty acids (PUFA) can be made through additional desaturation combined with elongation. [Note: Humans lack the ability to introduce double bonds between carbon 9 and the co-end of the chain and, therefore, must have the polyunsaturated linoleic and linolenic acids provided in the diet.

G. **Storage of fatty acids as components of triacylglycerols**

Mono-, di-, and triacylglycerols consist of one, two, or three molecules of fatty acid esterified to a molecule of glycerol. Fatty acids are esterified through their carboxyl groups, resulting in a loss of negative charge and formation of "neutral fat." [Note: If a species of acylglycero! is solid at room temperature, it is called a "fat"; if liquid, it is called an "oil."]

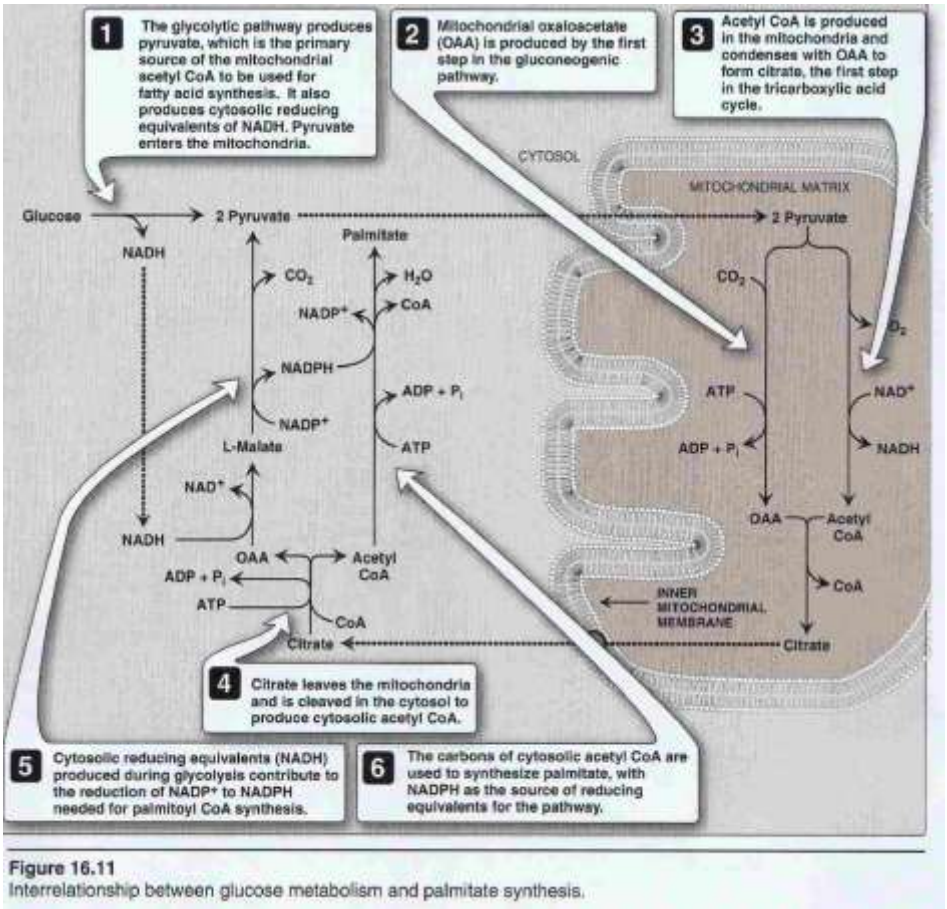


Figure 16.11
Interrelationship between glucose metabolism and palmitate synthesis.

MOBILIZATION OF STORED FATS AND OXIDATION OF FATTY ACIDS

Fatty acids stored in adipose tissue, in the form of neutral TAG, serve as the body's major fuel storage reserve. TAGs provide concentrated stores of metabolic energy because they are highly reduced and largely anhydrous. The yield from complete oxidation of fatty acids to $C0_2$ and H_20 is nine kcal/g of fat (as compared to four kcal/g of protein or carbohydrate.

A. Release of fatty acids from TAG

The mobilization of stored fat requires the hydrolytic release of fatty acids and glycerol from their TAG form. This process is initiated by hormone-sensitive lipase, which removes a fatty acid from carbon 1 and/or carbon 3 of the TAG. Additional lipases specific for diacylglycerol or monoacylglycerol remove the remaining fatty acids.

1. Activation of hormone-sensitive lipase (HSL): This enzyme is 'activated when phosphorylated by a 3 ',5'-cyclic AMP-dependent protein kinase. 3',5'-Cyclic AMP is produced in the adipocyte when one of several hormones (primarily epinephrine) binds to receptors on the cell membrane, and activates adenylate cyclase. The process is similar to that of the activation of glycogen phosphorylase. [Note: Because acetyl CoA carboxylase is inhibited by hormone directed phosphorylation when the cAMP-mediated cascade is activated, fatty acid synthesis is turned off when TAG degradation is turned on.] In the presence of high plasma levels of insulin and glucose, HSL is dephosphorylated, and becomes inactive

2. Fate of glycerol: The glycerol released during TAG degradation cannot be metabolized by adipocytes because they lack glycerol kinase. Rather, glycerol is transported through the blood to the liver, where it can be phosphorylated. The resulting glycerol phosphate can be used to form TAG in the liver, or can be converted to DHAP by reversal of the glycerol phosphate dehydrogenase reaction illustrated in Figure 16.13. DHAP can participate in glycolysis or gluconeogenesis.

3. Fate of fatty acids: The free (unesterified) fatty acids move through the cell membrane of the adipocyte, and immediately bind to albumin in the plasma. They are transported to the tissues, where the fatty acids enter cells, get activated to their CoA derivatives, and are oxidized for energy. [Note: Active transport of fatty acids across membranes is mediated by a membrane fatty acid binding protein.] Regardless of their blood levels, plasma free fatty acids cannot be used for fuel by erythrocytes, which have no mitochondria, or by the brain because of the impermeable blood-brain barrier.

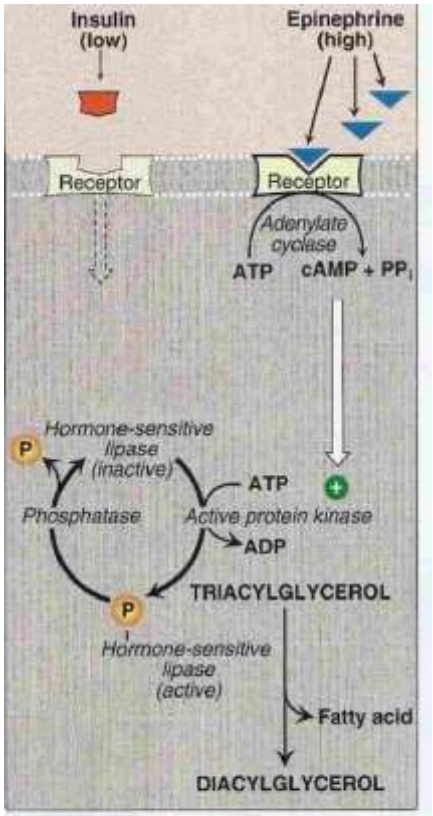


Figure 16.15
Hormonal regulation of triacylglycerol degradation in the adipocyte.

B. p-Oxidation of fatty acids

The major pathway for catabolism of saturated fatty acids is a mitochondrial pathway called [3-oxidation, in which two-carbon fragments are successively removed from the carboxyl end of the fatty acyl CoA, producing acetyl CoA, NADH, and FADH2.

1. Transport of long-chain fatty acids (LCFA) into the mitochondria: After a LCFA enters a cell, it is converted to the CoA derivative by long-chain fatty acyl CoA synthetase (thiokinase) in the cytosol (see p. 174). Because p-oxidation occurs in the mitochondrial matrix, the fatty acid must be transported across the mitochondrial inner membrane. Therefore, a specialized carrier transports the long-chain acyl group from the cytosol into the mitochondrial matrix. This carrier is carnitine, and the transport process is called the carnitine shuttle.

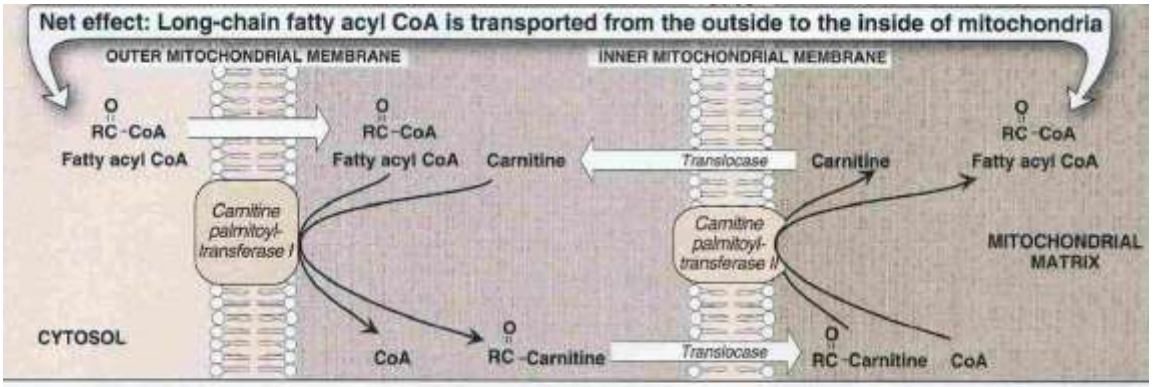


Figure 16.16
Carnitine shuttle.

Steps in LCFA translocation: First, an acyl group is transferred from the cytosolic coenzyme A to carnitine by carnitine palmitoyltransferase I (CPT-I)—an enzyme associated with the outer mitochondrial membrane. [Note: CPT-I is also known as CAT-I for carnitine acyltransferase. This reaction forms acylcarnitine, and regenerates free coenzyme A. Second, the acylcarnitine is transported into the mitochondrion in exchange for free carnitine by carnitine-acylcarnitine translocase. Carnitine palmitoyltransferase II (CPT-II, or CAT-II)—an enzyme of the inner mitochondrial membrane—catalyzes the transfer of the acyl group from carnitine to coenzyme A in the mitochondrial matrix, thus regenerating free carnitine.

b. Inhibitor of the carnitine shuttle: Malonyl CoA inhibits CPT-I, thus preventing the entry of long-chain acyl groups into the mitochondrial matrix. Therefore, when fatty acid synthesis is occurring in the cytosol (as indicated by the presence ofmalonyl CoA), the newly made palmitate cannot be transferred into the mitochondria and degraded.

c. Sources of carnitine: Carnitine can be obtained from the diet, where it is found primarily in meat products. Carnitine can also be synthesized from the amino acids lysine and methionine by an enzymatic pathway found in the liver and kidney but not in skeletal or heart muscle. Therefore, these tissues are totally dependent on carnitine provided by hepatocytes or the diet, and distributed by the blood. [Note: Skeletal muscle contains about 97 percent of all carnitine in the body.

d. Additional functions of carnitine: The carnitine system also allows the export from the mitochondria of branched-chain acyl groups (such as those produced during the catabolism of the branched-chain amino acids). In addition, the carnitine system is involved in the trapping and excretion via the kidney of acyl groups that cannot be metabolized by the body.
e. Carnitine deficiencies: Such deficiencies result in a decreased ability of tissues to use LCFA as a metabolic fuel, and can also cause the accumulation of toxic amounts of free fatty acids and branched-chain acyl groups in cells. Secondary carnitine deficiency occurs for many reasons, including 1) in patients with liver disease causing decreased synthesis of carnitine, 2) in individuals suffering from malnutrition or those on strictly vegetarian diets, 3) in those with an increased requirement for carnitine as a result of, for example, pregnancy, severe infections, burns, or trauma,

or 4) in those undergoing hemodialysis, which removes carnitine from the blood.

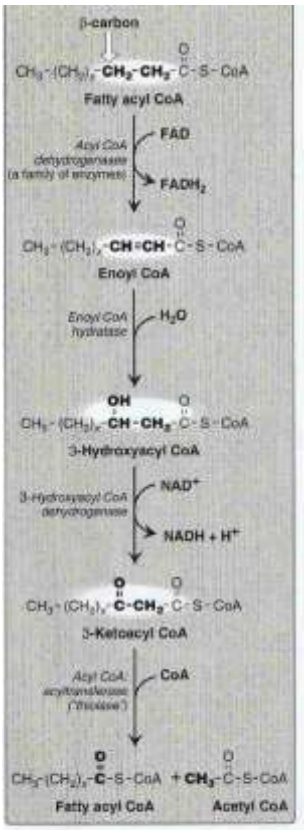


Figure 16.17
Enzymes involved in the β-oxidation of fatty acyl CoA.

Congenital deficiencies in one of the components of the carnitine palmatoyltransferase system, in tubular reabsorption of carnitine, or a deficiency in carnitine uptake by cells, can also cause carnitine deficiency. Genetic CPT-I deficiency affects the liver, where an inability to use LCFA for fuel greatly impairs that tissue's ability to synthesize glucose during a fast. This can lead to severe hypoglycemia, coma, and death. CPT-II deficiency occurs primarily in cardiac and skeletal muscle, where symptoms of carnitine deficiency range from cardiomyopathy, to muscle weakness with myoglobinemia following prolonged exercise. [Note: This is an example of how the impaired flow of a metabolite from one cell compartment to another results in pathology. Treatment includes avoidance of prolonged fasts, adopting a diet high in carbohydrate and low in LCFA, but supplemented with MCFA and, in cases of carnitine deficiency, carnitine.

2. Entry of short- and medium-chain fatty acids into the mitochondria: Fatty acids shorter than twelve carbons can cross the inner mitochondrial membrane without the aid of carnitine or the CPT system. Once inside the mitochondria, they are activated to their coenzyme

A derivatives by matrix enzymes, and are oxidized. [Note: MCFAs are plentiful in human milk. Because their oxidation is not dependent on CPT-I, it is not subject to inhibition by malonyl CoA.]

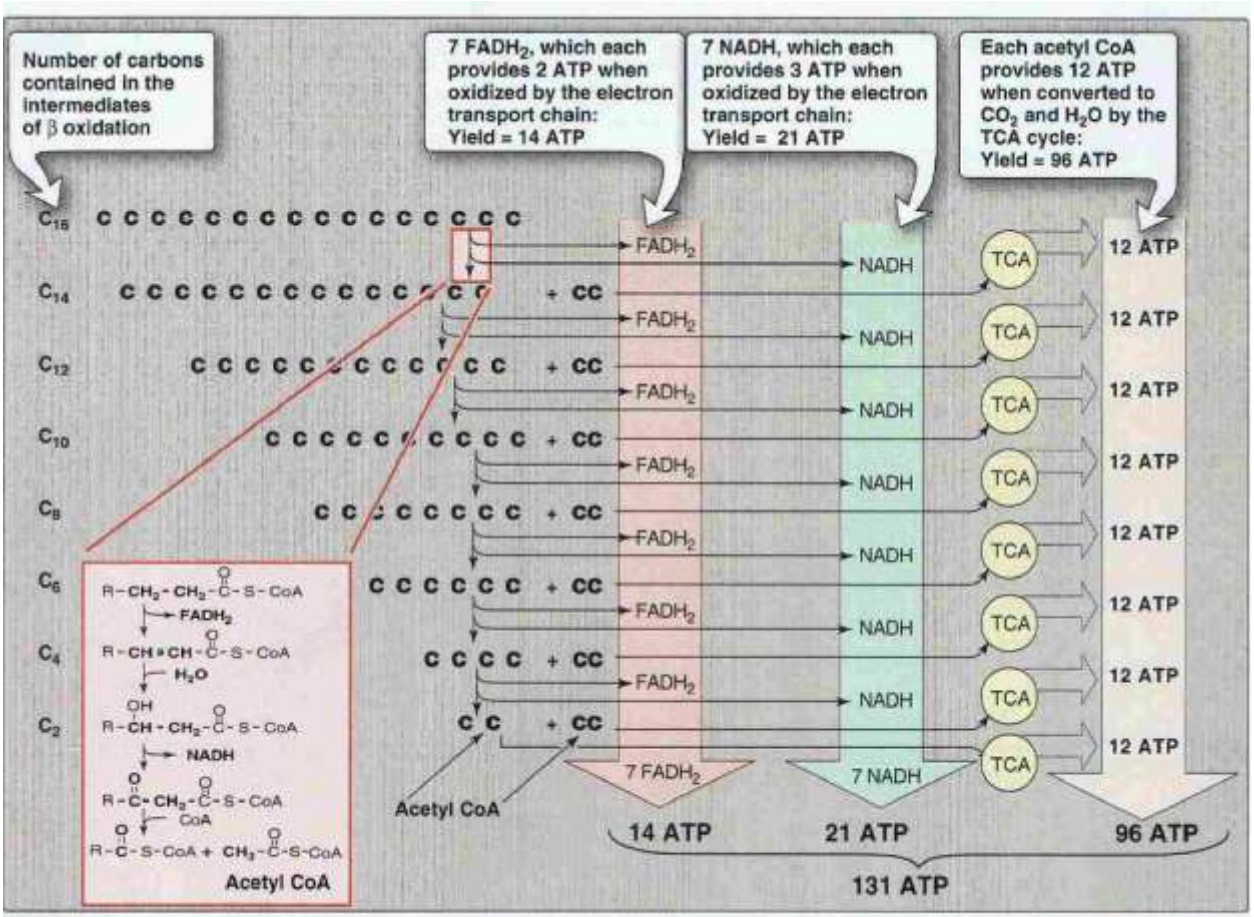


Figure 16.18
Summary of the energy yield from the oxidation of palmityl CoA (16 carbons). CC = acetyl CoA.

3. Reactions of β-oxidation: The first cycle of p-oxidation is shown in Figure 16.18. It consists of a sequence of four reactions that result in shortening the fatty acid chain by two carbons. The steps include an oxidation that produces FADH2, a hydration step, a second oxidation that produces NADH, and a thiolytic cleavage that releases a molecule of acetyl CoA. These four steps are repeated for saturated fatty acids of even-numbered carbon chains (n/2)-1 times (where n is the number of carbons), each cycle producing an acetyl group plus one NADH and one FADH2. The final thiolytic cleavage produces two acetyl groups. [Note: Acetyl CoA is a positive allosteric effector of pyruvate carboxylase (see p. 116), thus linking fatty acid oxidation and gluconeogenesis.

4. Energy yield from fatty acid oxidation: The energy yield from the p-oxidation pathway is high. For example, the oxidation of a molecule of palmitoyl CoA to $C0_2$ and H_20 yields 131 ATPs

(Figure 16.19). A comparison of the processes of synthesis and degradation of saturated fatty acids with an even number of carbon atoms is provided.

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

Figure 16.19
Comparison of the synthesis and degradation of even-numbered, saturated fatty acids.

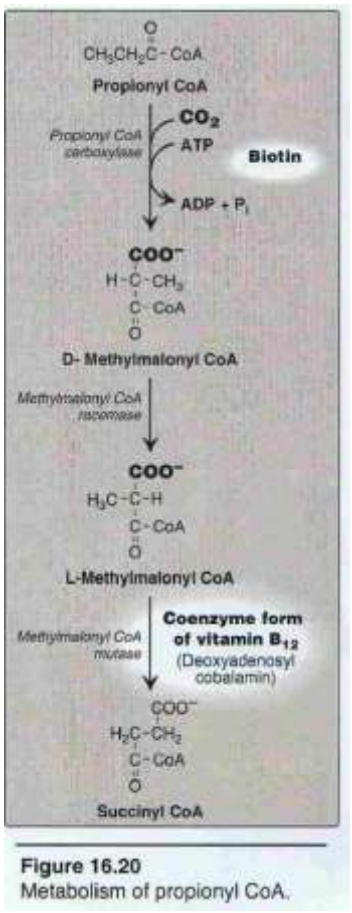


Figure 16.20
Metabolism of propionyl CoA.

5. Medium-chain fatty acyl CoA dehydrogenase (MCAD) deficiency: In mitochondria, there are four fatty acyl CoA dehydrogenase species, each of which has a specificity for either short-, medium-, long-, or very-long-chain fatty acids. MCAD deficiency, an autosomal, recessive disorder, is one of the most common inborn errors of metabolism, and the most common inborn error of fatty acid oxidation, being found in 1 in 12,000 births in the west, and 1 in 40,000 worldwide. It causes a decrease in fatty acid oxidation and severe hypoglycemia (because the tissues cannot obtain full energetic benefit from fatty acids and, therefore, must now rely on glucose). Treatment includes a carbohydrate-rich diet. [Note: Infants are particularly affected by MCAD deficiency, because they rely for their nourishment on milk, which contains primarily MCADs. MCAD dehydrogenase deficiency has been identified as the cause of some cases originally reported as sudden infant death syndrome (SIDS) or Reye's syndrome.

6. Oxidation of fatty acids with an odd number of carbons: The fi-oxidation of a saturated fatty acid with an odd number of carbon atoms proceeds by the same reaction steps as that of fatty acids with an even number, until the final three carbons are reached. This compound, propionyl CoA, is metabolized by a three-step pathway (Figure 16.21). [Note: Propionyl CoA is also produced during the metabolism of certain amino acids.

a. Synthesis of D-methylmalonyl CoA: First, propionyl CoA is carboxylated, forming D methylmalonyl CoA. The enzyme propionyl CoA carboxylase has an absolute requirement for the coenzyme biotin, as do other carboxylases.

b. Formation of L-methylmalonyl CoA: Next, the D-isomer is converted to the L-form by the enzyme, methylmalonyl CoA racemase. Synthesis of succinyl CoA: Finally, the carbons of L-methylmalonyl CoA are rearranged, forming succinyl CoA, which can enter the TCA cycle. The enzyme, methylmalonyl CoA mutase, requires a coenzyme form of vitamin Bi 2 (deoxyadenosylcobalamin) for its action. The mutase reaction is one of only two reactions in the body that require vitamin B12 (see p. 373). [Note: In patients with vitamin B12 deficiency, both propionate and methylmalonate are excreted in the urine. Two types of inheritable methylmalonic acidemia and aciduria have been described: one in which the mutase is missing or deficient (or has reduced affinity for the coenzyme), and one in which the patient is unable to convert vitamin B12 into its coenzyme form. Either type results in metabolic acidosis, with developmental retardation seen in some patients.

7. Oxidation of unsaturated fatty acids: The oxidation of unsaturated fatty acids provides less energy than that of saturated fatty acids because they are less highly reduced and, therefore, fewer reducing equivalents can be produced from these structures. Oxidation of monounsaturated fatty acids, such as 18:1(9) (oleic acid) requires one additional enzyme, 3,2-enoyl CoA isomerase, which converts the 3-cis derivative obtained after three rounds of p-oxidation to the 2-trans derivative that can serve as a substrate for the hydratase. Oxidation of polyunsaturated fatty acids, suchas 18:2(9,12) (linoleic acid), requires an NADPH-dependent reductase in addition to the isomerase.

8. β-Oxidation in the peroxisome: Very-long-chain fatty acids (VLCFA), twenty carbons long or longer, undergo a preliminary β-oxidation in peroxisomes. The shortened fatty acid is then transferred to a mitochondrion for further oxidation. In contrast to mitochondrial β-oxidation, the initial dehydrogenation in peroxisomes is catalyzed by an FAD-containing acyl CoA oxidase. The FADH2 produced is oxidized by molecular oxygen, which is reduced to H_20_2. The H_20_2 is reduced to H_20 by catalase.

V. KETONE BODIES: AN ALTERNATE FUEL FOR CELLS

Liver mitochondria have the capacity to convert acetyl CoA derived from fatty acid oxidation into ketone bodies. The compounds categorized as ketone bodies are acetoacetate, 3-hydroxybutyrate (formerly called p-hydroxybutyrate), and acetone (a nonmetabolizable side product, Figure 16.23). [Note: The two functional ketone bodies are actually organic acids.] Acetoacetate and 3-hydroxybutyrate are transported in the blood to the peripheral tissues. There they can be reconverted to acetyl CoA, which can be oxidized by the TCA cycle. Ketone bodies are important sources of energy for the peripheral tissues because 1) they are soluble in aqueous solution and, therefore, do not need to be incorporated into lipoproteins or carried by albumin as do the other lipids; 2) they are produced in the liver during periods when the amount of acetyl CoA present exceeds the oxidative capacity of the liver; and 3) they are used in proportion to their concentration in the blood by extrahepatic tissues, such as the skeletal and cardiac muscle and

renal cortex. Even the brain can use ketone bodies to help meet its energy needs if the blood levels rise sufficiently. [Note: This is important during prolonged periods of fasting, see p. 330.]

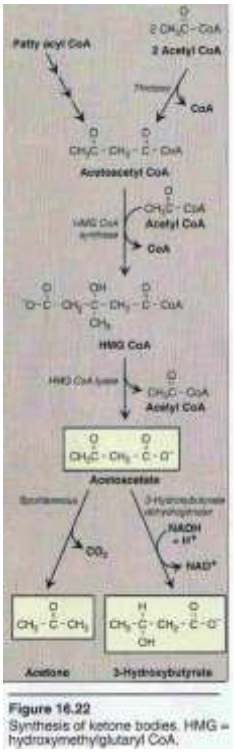


Figure 16.22
Synthesis of ketone bodies. HMG = hydroxymethylglutaryl CoA.

Synthesis of ketone bodies by the liver

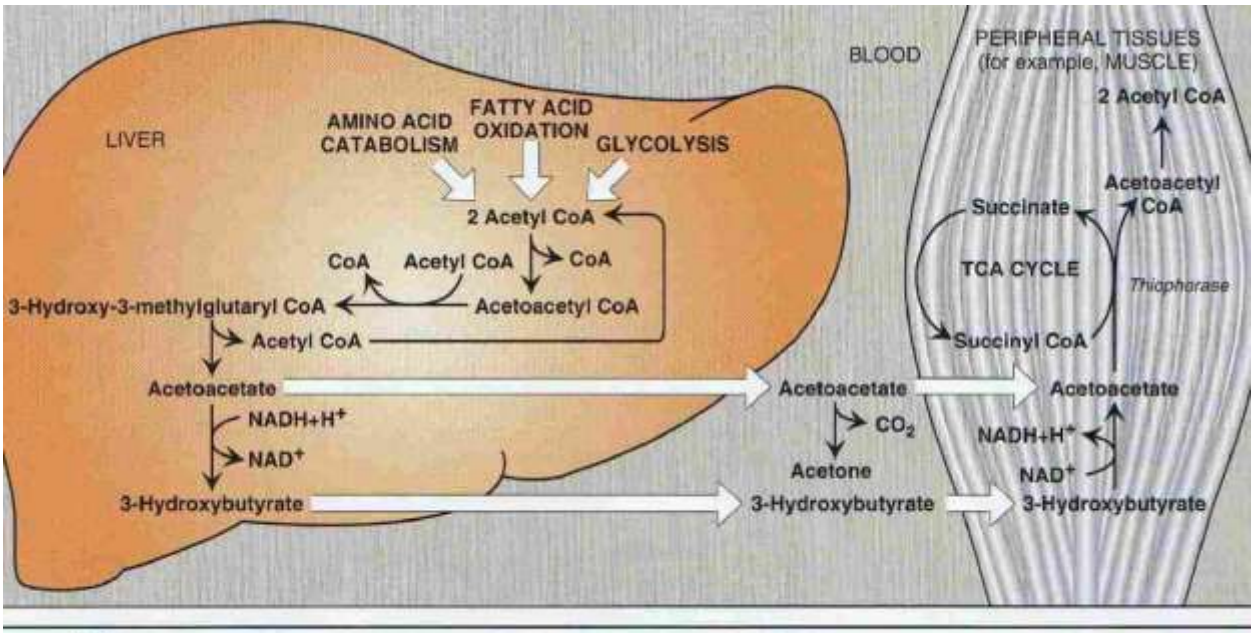
During a fast, the liver is flooded with fatty acids mobilized from adipose tissue. The resulting elevated hepatic acetyl CoA produced primarily by fatty acid degradation inhibits pyruvate dehydrogenase (see p. 108), and activates pyruvate carboxylase. The oxaloacetate thus produced is used by the liver for gluconeogenesis rather than for the TCA cycle. Therefore, acetyl Co A is channeled into ketone body synthesis.

- Synthesis of 3-hydroxy-3-methylglutaryl CoA (HMG CoA): The first synthetic step, formation of acetoacetyl CoA, occurs by reversal of the thioiase reaction of fatty acid oxidation (see Figure 16.18). Mitochondrial HMG CoA synthase combines a third molecule of acetyl CoA with acetoacetyl CoA to produce HMG CoA. [Note: HMG CoA is also a precursor of cholesterol (see p. 218). These pathways are separated by location in, and conditions of, the cell (see p. 218).] HMG CoA synthase is the ratelimiting step in the synthesis of ketone bodies, and is present in significant quantities only in the liver.
- Synthesis of the ketone bodies: HMG CoA is cleaved to produce acetoacetate and acetyl CoA. Acetoacetate can be reduced to form 3-hydroxybutyrate with NADH as the hydrogen donor. Acetoacetate can also spontaneously decarboxylate in the blood to form acetone—a volatile, biologically non-metabolized compound that can be released in the breath. [Note: The equilibrium between acetoacetate and 3-hydroxybutyrate is determined by the

NAD +/NADH ratio. Because this ratio is high during fatty acid oxidation, 3-hydroxybutyrate synthesis is favored.

B. Use of ketone bodies by the peripheral tissues

Although the liver constantly synthesizes low levels of ketone bodies, their production becomes much more significant during fasting when ketone bodies are needed to provide energy to the peripheral tissues. 3-Hydroxybutyrate is oxidized to acetoacetate by 3-hydroxybutyrate dehydrogenase, producing NADH (Figure 16.24). Acetoacetate is then provided with a coenzyme A molecule taken from succinyl CoA by succinyl CoA:acetoacetate CoA transferase (thiophorase). This reaction is reversible, but the product, acetoacetyl CoA, is actively removed by its conversion to two acetyl CoAs. Extrahepatic tissues, including the brain but excluding cells lacking mitochondria (for example, red blood cells), efficiently oxidize acetoacetate and 3-hydroxybutyrate in this manner. In contrast, although the liver actively produces ketone bodies, it lacks thiophorase and, therefore, is unable to use ketone bodies as fuel.



gure 16.23
tone body synthesis in the liver and use in peripheral tissues.

Excessive production of ketone bodies in diabetes mellitus

When the rate of formation of ketone bodies is greater than the rate of their use, their levels begin to rise in the blood (ketonemia) and eventually in the urine (ketonuria). These two conditions are seen most often in cases of uncontrolled, type 1 (insulin-dependent) diabetes mellitus. In such individuals, high fatty acid degradation produces excessive amounts of acetyl CoA. It also depletes the NAD+ pool and increases the NADH pool, which slows the TCA cycle (see p. 112). This forces the excess acetyl CoA into the ketone body pathway. In diabetic individuals with severe ketosis, urinary excretion of the ketone bodies may be as high as 5000 mg/24 hr, and the blood concentration may reach 90 mg/dl (versus less than 3 mg/dL in normal individuals). A frequent

symptom of diabetic ketoacidosis is a fruity odor on the breath which result from increased production of acetone. An elevation of the ketone body concentration in the blood results in acidemia. [Note: The carboxyl group of a ketone body has a pKa about 4. Therefore, each ketone body loses a proton (H^+) as it circulates in the blood, which lowers the pH of the body. Also, excretion of glucose and ketone bodies in the urine results in dehydration of the body. Therefore, the increased number of H^+, circulating in a decreased volume of plasma, can cause severe acidosis (ketoacidosis)]. Ketoacidosis may also be seen in cases of fasting.

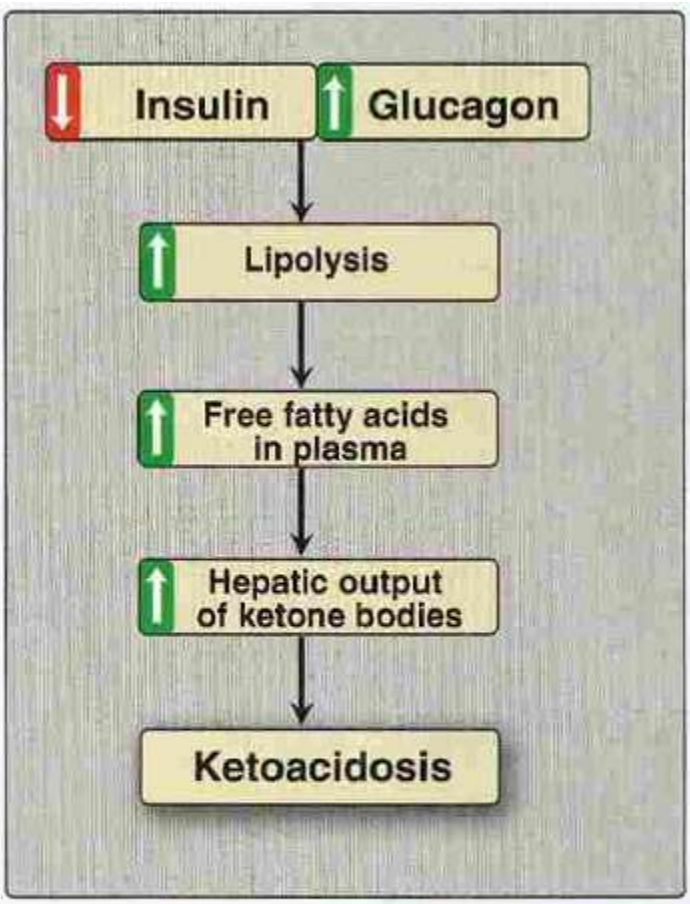


Figure 16.24
Mechanism of diabetic ketoacidosis seen in type 1 diabetes.