Biosynthesis of Fatty Acids

BIOMEDICAL IMPORTANCE

Fatty acids are synthesized by an **extramitochondrial** system, which is responsible for the complete synthesis of palmitate from **acetyl-CoA** in the **cytosol**. In most mammals, **glucose** is the primary substrate for lipogenesis, but in ruminants it is **acetate**, the main fuel molecule they obtain from the diet. Critical diseases of the pathway have not been reported in humans. However, inhibition of lipogenesis occurs in type 1 (insulin-dependent) **diabetes mellitus**, and variations in the activity of the process affect the nature and extent of obesity.

Unsaturated fatty acids in phospholipids of the cell membrane are important in maintaining membrane fluidity. A high ratio of polyunsaturated fatty acids to saturated fatty acids (P:S ratio) in the diet is considered to be beneficial in preventing coronary heart disease. Animal tissues have limited capacity for desaturating fatty acids, and require certain dietary polyunsaturated fatty acids derived from plants. These essential fatty acids are used to form eicosanoic (C_{20}) fatty acids, which give rise to the eicosanoids prostaglandins, thromboxanes, leukotrienes, and lipoxins. Prostaglandins mediate inflammation, pain, and induce sleep and also regulate blood coagulation and reproduction. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen act by inhibiting prostaglandin synthesis. Leukotrienes have muscle contractant and chemotactic properties and are important in allergic reactions and inflammation.

THE MAIN PATHWAY FOR DE NOVO SYNTHESIS OF FATTY ACIDS (LIPOGENESIS) OCCURS IN THE CYTOSOL

This system is present in **many tissues**, including liver, kidney, brain, lung, mammary gland, and adipose tissue. Its cofactor requirements include NADPH, ATP, Mn^{2+}, biotin, and HCO_3^- (as a source of CO_2). **Acetyl-CoA is the immediate substrate**, and free palmitate is the end product.

Production of Malonyl-CoA Is the Initial & Controlling Step in Fatty Acid Synthesis

Bicarbonate as a source of CO_2 is required in the initial reaction for the carboxylation of acetyl-CoA to malonyl-CoA in the presence of ATP and **acetyl-CoA carboxylase**. This enzyme has a **major role** in the regulation of fatty acid synthesis. Acetyl-CoA carboxylase has a requirement for the **B vitamin biotin** and is a **multienzyme protein** containing

biotin, biotin carboxylase, biotin carboxyl carrier protein, and a carboxyl transferase, as well as a regulatory allosteric site. One subunit of the complex contains all the components, and variable number of subunits form polymers in the active enzyme (Figure 6). The reaction takes place in two steps: (1) carboxylation of biotin involving ATP and (2) transfer of the carboxyl group to acetylCoA to form malonyl-CoA (Figure 1).

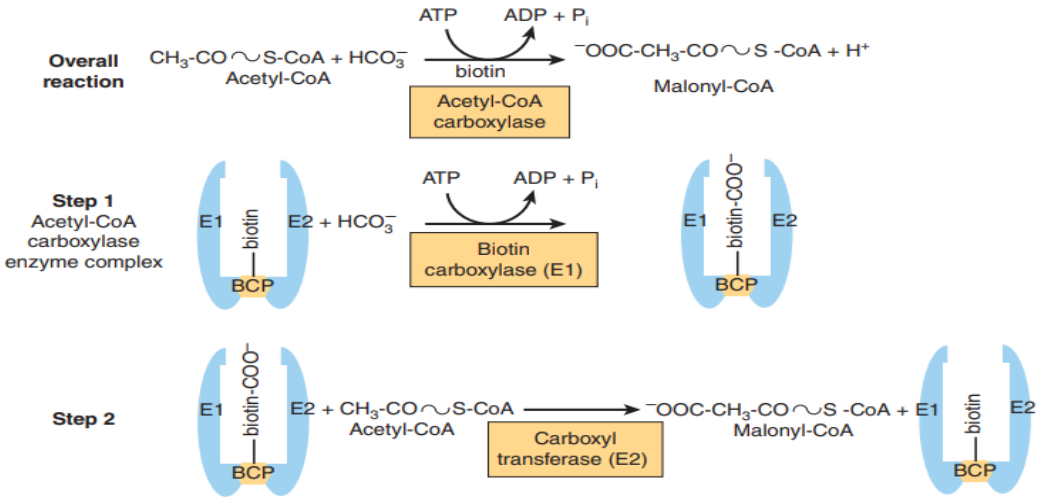

Figure 1 Biosynthesis of malonyl-CoA by acetyl carboxylase. Acetyl carboxylase is a multienzyme complex containing two enzymes, biotin carboxylase (E1) and a carboxyltransferase (E2) and the biotin carrier protein (BCP). Biotin is covalently linked to the BCP. The reaction proceeds in 2 steps. In step 1, catalysed by E1, biotin is carboxylated as it accepts a COO^- group from HCO_3^- and ATP is used. In step 2, catalyzed by E2, the COO^- is transferred to acetyl-CoA forming malonyl-CoA.

The Fatty Acid Synthase Complex Is a Homodimer of Two Polypeptide Chains Containing Six Enzyme Activities

After the formation of malonyl-CoA, fatty acids are formed by the **fatty acid synthase enzyme complex**. The individual enzymes required for fatty acid synthesis are linked in this **multienzyme polypeptide complex** that incorporates the acyl carrier protein (ACP), which has a similar function to CoA in the β-oxidation pathway. It contains the **vitamin pantothenic acid in the form of 4'-phosphopantetheine**. In the primary structure of the protein, the enzyme domains are linked in the sequence as shown in Figure 2. X-ray crystallography of the three-dimensional structure, however, has shown that the complex is a **homodimer**, with two identical subunits, each containing **6 enzymes** and an ACP, arranged in an X shape (Figure 2). The position of the ACP and thioesterase domains cannot be resolved as yet by x-ray crystallography, possibly because they are too flexible, but they are thought to lie close to the 3-ketoacylreductase enzyme. The

use of one multienzyme functional unit has the advantages of achieving the effect of compartmentalization of the process within the cell without the erection of permeability barriers, and synthesis of all enzymes in the complex is coordinated since it is encoded by a single gene.

Initially, a **priming molecule of acetyl-CoA** combines with a cysteine —SH group (Figure 3, reaction 1a), while malonyl-CoA combines with the adjacent —SH on the 4'-phosphopantetheine of ACP of the other monomer (reaction 1b). These reactions are catalyzed by malonyl acetyl transacylase, to form acetyl (acyl)-malonyl enzyme. The acetyl group attacks the methylene group of the malonyl residue, catalyzed by 3-ketoacyl synthase, and liberates CO_2 , forming 3-ketoacyl enzyme (acetoacetyl enzyme) (reaction 2), freeing the cysteine —SH group. **Decarboxylation allows the reaction to go to completion**, pulling the whole sequence of reactions in the forward direction. The 3-ketoacyl group is reduced, dehydrated, and reduced again (reactions 3-5) to form the corresponding saturated acyl-S-enzyme. A new malonyl-CoA molecule combines with the —SH of 4'-phosphopantetheine, displacing the saturated acyl residue onto the free cysteine —SH group. The sequence of reactions is repeated six more times until a saturated 16-carbon acyl radical (palmitoyl) has been assembled. It is liberated from the enzyme complex by the activity of the sixth enzyme in the complex, **thioesterase (deacylase)**. The free palmitate must be activated to acyl-CoA before it can proceed via any other metabolic pathway. Its possible fates are esterification into acylglycerols, chain elongation or desaturation, or esterification into cholesteryl ester. In mammary gland, there is a separate thioesterase specific for acyl residues of C_8 , C_{10}, or C_{12}, which are subsequently found in milk lipids.

The equation for the overall synthesis of palmitate from acetyl-CoA and malonyl-CoA is

$$\begin{aligned} &CH_3CO—S—CoA + 7HOOCCHCO—S—CoA + 14NADPH + 14H^+ \\ &\rightarrow\ CH_3(CH_2)_{14}COOH + 7CO_2 + 6H_2O + 8CoA—SH + 14NADP^+ \end{aligned}$$

The acetyl-CoA used as a primer forms carbon atoms 15 and 16 of palmitate. The addition of all the subsequent C_2 units is via malonyl-CoA. **Propionyl CoA acts as primer** for the synthesis of long-chain fatty acids having an odd number of carbon atoms, found particularly in ruminant fat and milk.

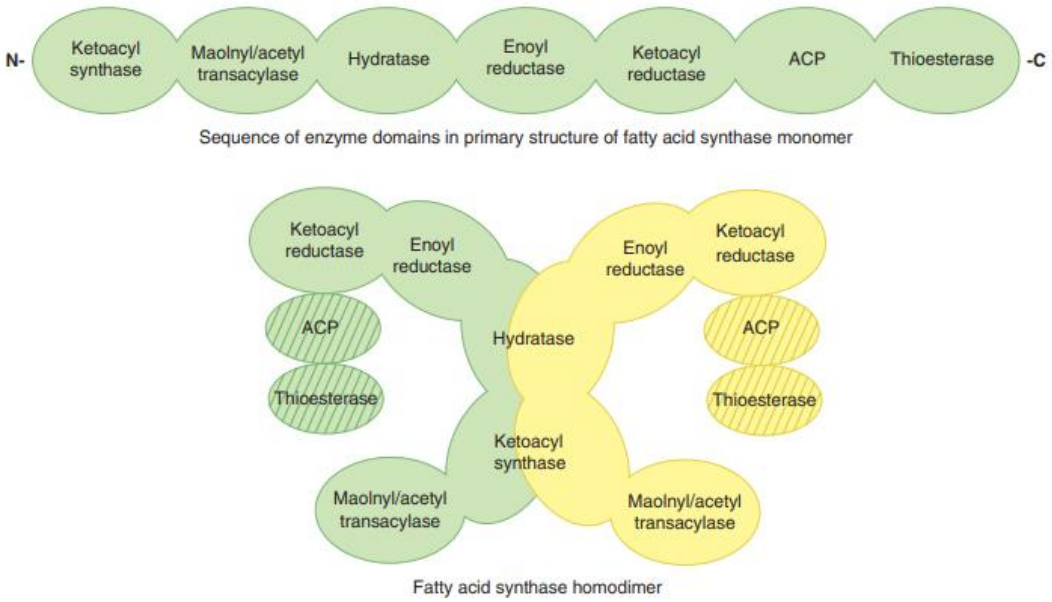

Figure 2 Fatty acid synthase multienzyme complex. The complex is a dimer of two identical polypeptide monomers in which six enzymes and the acyl carrier protein (ACP) are linked in the primary structure in the sequence shown. X-ray crystallography of the three-dimensional structure has demonstrated that the two monomers in the complex are arranged in an X-shape. The position of the ACP and thioesterase is not yet resolved, but they are thought to be close to the 3 ketoacyl reductase enzyme domain.

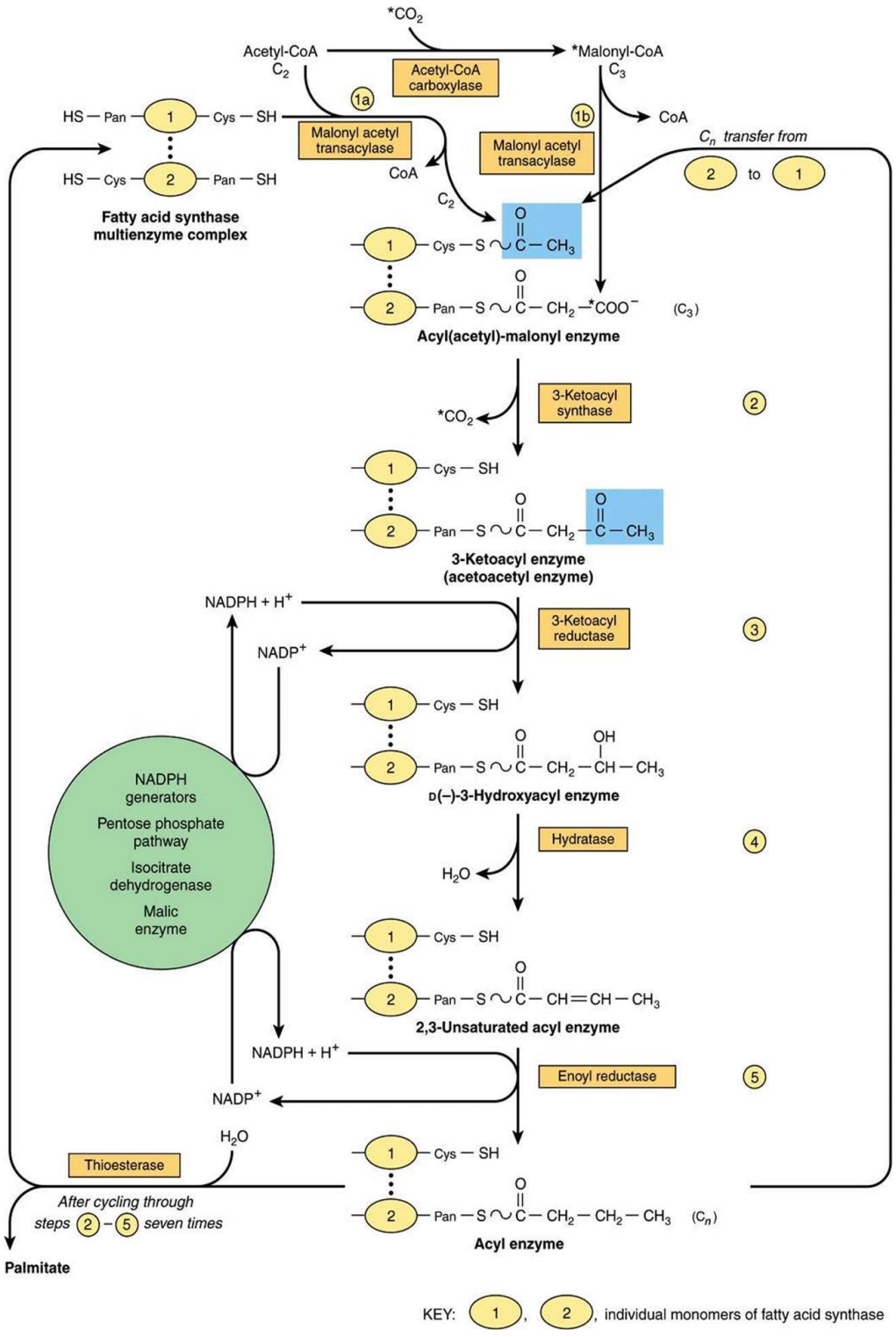

Figure 3 Biosynthesis of long-chain fatty acids. Details of how addition of a malonyl residue causes the acyl chain to grow by two carbon atoms. (Cys, cysteine residue; Pan, 4′-phosphopantetheine.) The blocks highlighted in blue contain initially a C2 unit derived from acetyl-CoA (as illustrated) and subsequently the Cn unit formed in reaction 5.

The Main Source of NADPH for Lipogenesis Is the Pentose Phosphate Pathway

NADPH is involved as a donor of reducing equivalents in both the reduction of the 3-ketoacyl and of the 2,3-unsaturated acyl derivatives (Figure 3, reactions 3 and 5). The oxidative reactions of the **pentose phosphate pathway** are the chief source of the hydrogen required for the reductive synthesis of fatty acids. Significantly, tissues specializing in active lipogenesis—ie, liver, adipose tissue, and the lactating mammary gland— also possess an active pentose phosphate pathway. Moreover, both metabolic pathways are found in the cytosol of the cell; so, there are no membranes or permeability barriers against the transfer of NADPH. Other sources of NADPH include the reaction that converts malate to pyruvate catalyzed by the **"malic enzyme" (NADP malate dehydrogenase)** (Figure 4) and the **extramitochondrial isocitrate dehydrogenase** reaction (probably not a substantial source, except in ruminants).

Acetyl-CoA Is the Principal Building Block of Fatty Acids

Acetyl-CoA is formed from glucose via the oxidation of pyruvate in the matrix of the mitochondria. However, as it does not diffuse readily across the mitochondrial membranes, its transport into the cytosol, the principal site of fatty acid synthesis, requires a special mechanism involving **citrate**. After condensation of acetyl-CoA with oxaloacetate in the citric acid cycle within mitochondria, the citrate produced can be translocated into the extramitochondrial compartment via the tricarboxylate transporter, where in the presence of CoA and ATP, it undergoes cleavage to acetyl-CoA and oxaloacetate catalyzed by **ATP-citrate lyase**, which increases in activity in the **well-fed state**. The acetyl-CoA is then available for malonyl-CoA formation and synthesis of fatty acids (Figure 4). The resulting oxaloacetate can form malate via NADH-linked malate dehydrogenase, followed by the generation of NADPH via the malic enzyme. The NADPH becomes available for lipogenesis, and the pyruvate can be used to regenerate acetyl-CoA after transport into the mitochondrion. This pathway is a means of transferring reducing equivalents from extramitochondrial NADH to NADP. Alternatively, malate itself can be transported into the mitochondrion, where it is able to re-form oxaloacetate. Note that the **citrate (tricarboxylate)** transporter in the mitochondrial membrane requires malate to exchange with citrate. There is little ATP-citrate lyase or malic enzyme **in ruminants**, probably because in these species **acetate (derived from carbohydrate digestion in the rumen and activated to acetyl-CoA extramitochondrially)** is the main source of acetyl-CoA.

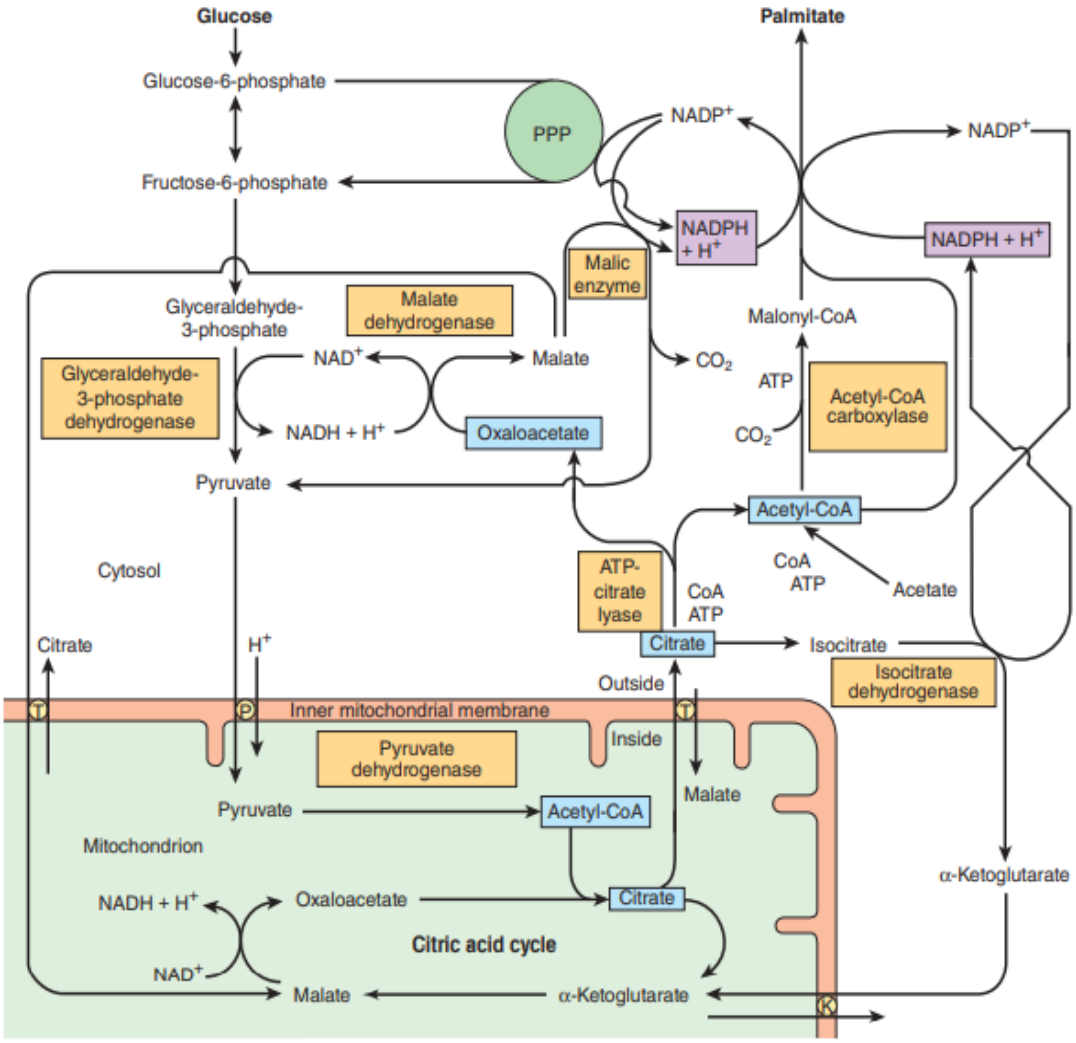

Figure 4 The provision of acetyl-CoA and NADPH for lipogenesis. (K, α-ketoglutarate transporter; P, pyruvate transporter; PPP, pentose phosphate pathway; T, tricarboxylate transporter.)

Elongation of Fatty Acid Chains Occurs in the Endoplasmic Reticulum

This pathway (the "microsomal system") elongates saturated and unsaturated fatty acyl-CoAs (from C_{10} upward) by two carbons, using **malonyl-CoA as the acetyl donor** and **NADPH as the reductant**, and is catalyzed by the **microsomal fatty acid elongase system** of enzymes (Figure 5). Elongation of stearyl-CoA in brain increases rapidly during myelination in order to provide C_{22} and C_{24} fatty acids for sphingolipids.

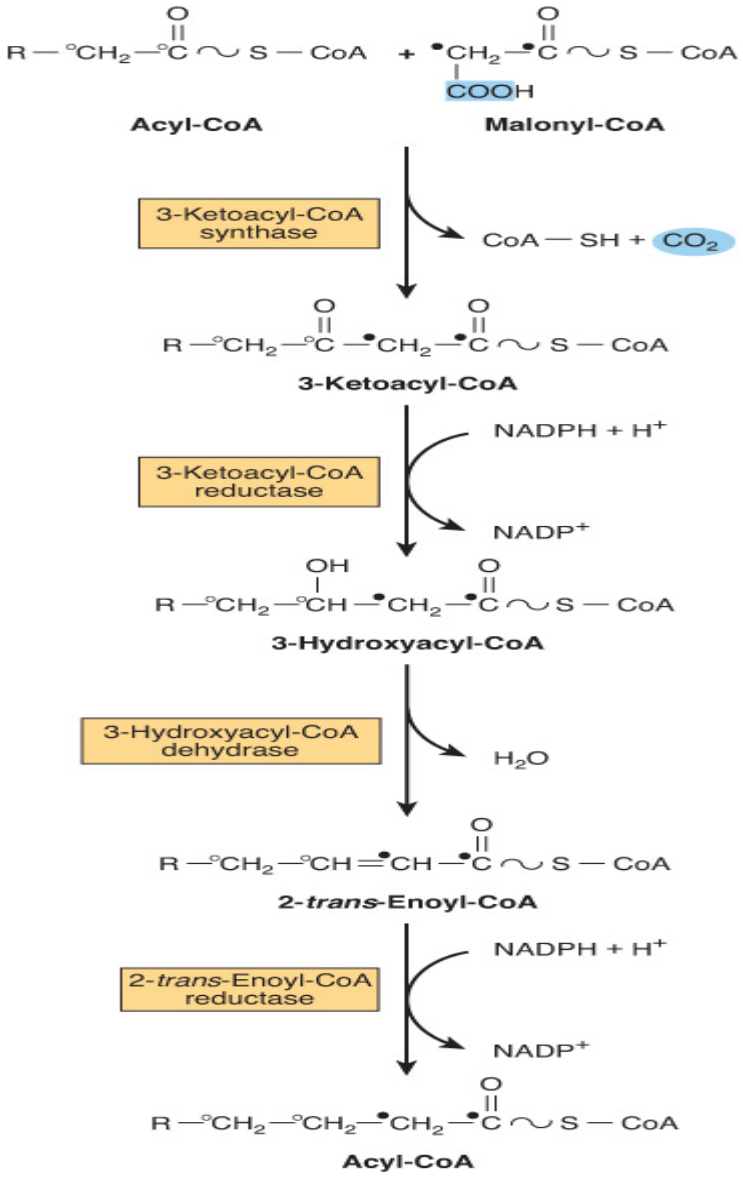

Figure 5 Microsomal elongase system for fatty acid chain elongation. NADH is also used by the reductases, but NADPH is preferred.

THE NUTRITIONAL STATE REGULATES LIPOGENESIS

Excess carbohydrate is stored as fat in many animals in anticipation of periods of caloric deficiency such as starvation, hibernation, etc, and to provide energy for use between meals in animals, including humans, that take their food at spaced intervals. Lipogenesis converts surplus glucose and intermediates such as pyruvate, lactate, and acetyl-CoA to fat, assisting the anabolic phase of this feeding cycle. The **nutritional state** of the organism is the **main factor** regulating the rate of lipogenesis. Thus, the rate is high in the well-fed animal whose diet contains a high proportion of carbohydrate. It is

depressed by restricted caloric intake, high-fat diet, or a deficiency of insulin, as in diabetes mellitus. These latter conditions are associated with increased concentrations of plasma-free fatty acids, and an inverse relationship has been demonstrated between hepatic lipogenesis and the concentration of serum-free fatty acids. Lipogenesis is increased when **sucrose** is fed instead of glucose because fructose bypasses the phosphofructokinase control point in glycolysis and floods the lipogenic pathway.

SHORT- & LONG-TERM MECHANISMS REGULATE LIPOGENESIS

Long-chain fatty acid synthesis is controlled in the **short term** by allosteric and covalent modification of enzymes and in the **long term** by changes in gene expression governing rates of synthesis of enzymes.

Acetyl-CoA Carboxylase Is the Most Important Enzyme in the Regulation of Lipogenesis

Acetyl-CoA carboxylase is an allosteric enzyme and is **activated by citrate**, which increases in concentration in the well-fed state and is an indicator of a plentiful supply of acetylCoA. Citrate promotes the conversion of the enzyme from an inactive dimer (two subunits of the enzyme complex) to an active polymeric form, with a molecular mass of several million. **Inactivation is promoted by phosphorylation of the enzyme and by long-chain acyl-CoA molecules**, an example of negative feedback inhibition by a product of a reaction (Figure 6). Thus, if acyl-CoA accumulates because it is not esterified quickly enough or because of increased lipolysis or an influx of free fatty acids into the tissue, it will automatically reduce the synthesis of new fatty acid. Acyl-CoA also inhibits the mitochondrial tricarboxylate transporter, thus preventing activation of the enzyme by egress of citrate from the mitochondria into the cytosol (Figure 6).

Acetyl-CoA carboxylase is also regulated by hormones such as glucagon, epinephrine, and insulin via changes in its phosphorylation state (Figure 7).

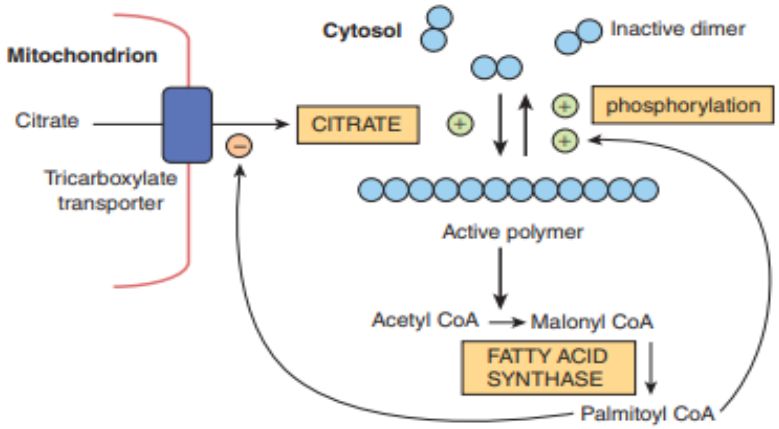

Figure 6 Regulation of acetyl CoA carboxylase. AcetylCoA carboxylase is activated by citrate, which promotes the conversion of the enzyme from an inactive dimer to an active polymeric form. Inactivation is promoted by phosphorylation of the enzyme and by long-chain acyl-CoA molecules such as palmitoyl CoA. In addition, acyl-CoA inhibits the tricarboxylate transporter, which transports citrate out of mitochondria into the cytosol, thus decreasing the citrate concentration in the cytosol and favoring inactivation of the enzyme.

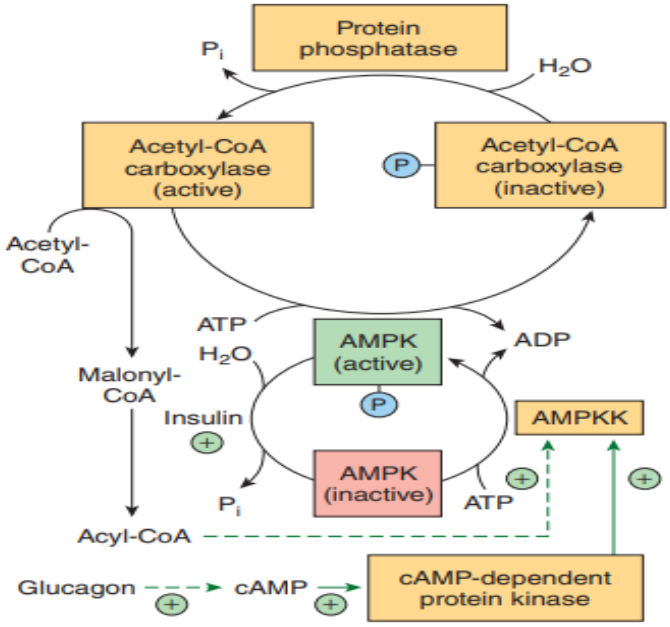

Figure 7 Regulation of acetyl-CoA carboxylase by phosphorylation / dephosphorylation. The enzyme is inactivated by phosphorylation by AMP-activated protein kinase (AMPK), which in turn is phosphorylated and activated by AMP-activated protein kinase kinase (AMPKK). Glucagon (and epinephrine) increase cAMP, and thus activate this latter enzyme via cAMP-dependent protein kinase. The kinase kinase

enzyme is also believed to be activated by acyl-CoA. Insulin activates acetyl-CoA carboxylase via dephosphorylation of AMPK.

Pyruvate Dehydrogenase Is Also Regulated by Acyl-CoA

Acyl-CoA causes an inhibition of pyruvate dehydrogenase by inhibiting the ATP-ADP exchange transporter of the inner mitochondrial membrane, which leads to increased **intramitochondrial (ATP)/(ADP)** ratios and therefore to conversion of active to inactive pyruvate dehydrogenase, thus regulating the availability of acetyl-CoA for lipogenesis. Furthermore, oxidation of acyl-CoA due to increased levels of free fatty acids may increase the ratios of **(acetyl-CoA)/(CoA)** and **(NADH)/(NAD$^+$)** in mitochondria, inhibiting pyruvate dehydrogenase.

Insulin Also Regulates Lipogenesis by Other Mechanisms

Insulin stimulates lipogenesis by several other mechanisms as well as by increasing acetyl-CoA carboxylase activity. It increases the transport of glucose into the cell (eg, in adipose tissue), increasing the availability of both pyruvate for fatty acid synthesis and glycerol-3-phosphate for triacylglycerol synthesis via esterification of the newly formed fatty acids, and also converts the inactive form of pyruvate dehydrogenase to the active form in adipose tissue, but not in liver. Insulin also—by its ability to depress the level of intracellular cAMP—inhibits lipolysis in adipose tissue and reducing the concentration of plasma-free fatty acids and, therefore, long-chain acyl-CoA, which are inhibitors of lipogenesis.

The Fatty Acid Synthase Complex & AcetylCoA Carboxylase Are Adaptive Enzymes

These enzymes adapt to the body's physiologic needs via changes in gene expression which lead to increases in total amount present in the fed state and decreases during intake of a high-fat diet and in conditions such as starvation, and diabetes mellitus. Insulin plays an important role, causing gene expression and induction of enzyme biosynthesis, and glucagon (via cAMP) antagonizes this effect. Feeding fats containing polyunsaturated fatty acids coordinately regulates the inhibition of expression of key enzymes of glycolysis and lipogenesis. These mechanisms for longer term regulation of lipogenesis take several days to become fully manifested and augment the direct and immediate effect of free fatty acids and hormones such as insulin and glucagon.

SOME POLYUNSATURATED FATTY ACIDS CANNOT BE SYNTHESIZED BY MAMMALS & ARE NUTRITIONALLY ESSENTIAL

Certain long-chain unsaturated fatty acids of metabolic significance in mammals are shown in Figure 8. Other C_{20}, C_{22}, and C_{24} polyenoic fatty acids may be derived from oleic, linoleic, and α-linolenic acids by chain elongation. **Palmitoleic and oleic acids are not essential** in the diet because the tissues can introduce a double bond at the Δ^9 position of a saturated fatty acid. **Linoleic and α-linolenic acids are the only fatty acids known to be essential** for the complete nutrition of many species of animals, including humans, and are termed the **nutritionally essential fatty acids**. In most mammals, arachidonic acid can be formed from linoleic acid. Double bonds can be introduced at the Δ^4 , Δ^5 , Δ^6 , and Δ^9 positions in most animals, but never beyond the Δ^9 position. In contrast, plants are able to synthesize the nutritionally essential fatty acids by introducing double bonds at the Δ^{12} and Δ^{15} positions.

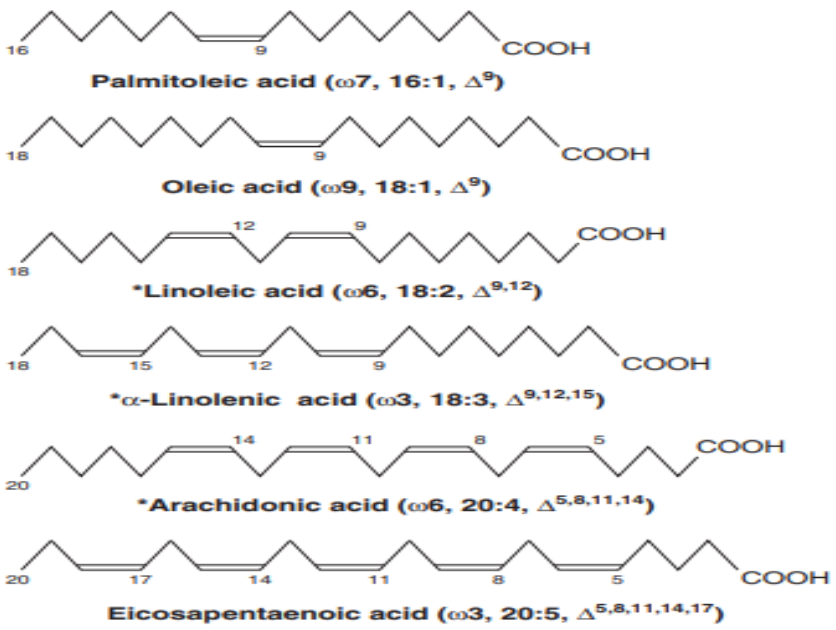

Figure 8 Structure of some unsaturated fatty acids. Although the carbon atoms in the molecules are conventionally numbered—ie, numbered from the carboxyl terminal—the ω numbers (eg, ω7 in palmitoleic acid) are calculated from the reverse end (the methyl terminal) of the molecules. The information in parentheses shows, for instance, that α-linolenic acid contains double bonds starting at the third carbon from the methyl terminal, has 18 carbons and 3 double bonds, and has these double bonds at the 9th, 12th, and 15th carbons from the carboxyl terminal. (∗Classified as "essential fatty acids.")

MONOUNSATURATED FATTY ACIDS ARE SYNTHESIZED BY A Δ^9 DESATURASE SYSTEM

Several tissues including the liver are considered to be responsible for the formation of nonessential monounsaturated fatty acids from saturated fatty acids. The first double bond introduced into a saturated fatty acid is nearly always in the Δ^9 position. An enzyme system—**Δ^9 desaturase** (Figure 9)—in the endoplasmic reticulum catalyzes the conversion of palmitoyl-CoA or stearoyl-CoA to palmitoleoyl-CoA or oleoyl-CoA, respectively. **Oxygen and either NADH or NADPH** are necessary for the reaction. The enzymes appear to be similar to a monooxygenase system involving cytochrome b5.

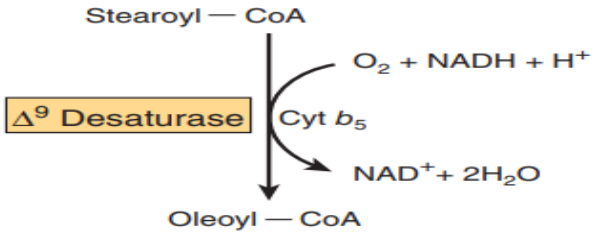

Figure 9 Microsomal Δ^9 desaturase.

SYNTHESIS OF POLYUNSATURATED FATTY ACIDS INVOLVES DESATURASE & ELONGASE ENZYME SYSTEMS

Additional double bonds introduced into existing monounsaturated fatty acids are always separated from each other by a methylene group (methylene interrupted) except in bacteria. Since animals have a Δ^9 desaturase, they are able to synthesize the ω9 (oleic acid) family of unsaturated fatty acids completely **by a combination of chain elongation and desaturation** (Figures 9 and 10) after the formation of saturated fatty acids by the pathways. However, as indicated above, **linoleic (ω6) or α-linolenic (ω3) acids** are required for the synthesis of the other members of the ω6 or ω3 families (pathways shown in Figure 10) and **must be supplied in the diet**. Linoleic acid is converted to arachidonic acid (20:4 ω6) via γ-linolenic acid (18:3 ω6). The nutritional requirement for arachidonate may thus be dispensed with if there is adequate linoleate in the diet. Cats, however, cannot carry out this conversion owing to the absence of Δ^6 desaturase and must obtain arachidonate in their diet. The desaturation and chain elongation system is greatly diminished in the starving state, in response to glucagon and epinephrine administration, and in the absence of insulin as in type 1 diabetes mellitus.

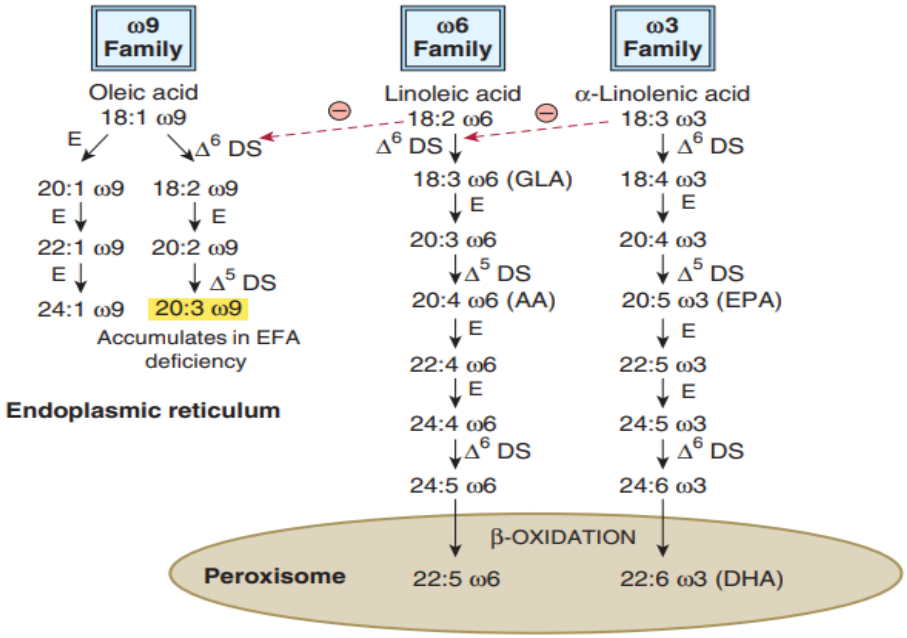

Figure 10 Biosynthesis of the ω9, ω6, and ω3 families of polyunsaturated fatty acids. In animals, the ω9, ω6, and ω3 families of polyunsaturated fatty acids are synthesized in the endoplasmic reticulum from oleic, linoleic and β-linolenic acids, respectively, by a series of elongation and desaturation reactions. The production of 22:5 ω6 (osbond acid) or 22:6 ω3 (docosahexanoic acid (DHA)), however, requires one cycle of β-oxidation which takes place inside peroxisomes after the formation of 24:5 ω6 or 24:6 ω3. AA, arachidonic acid; E, elongase; EFA, essential fatty acids; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; DS, desaturase. ⊖, Inhibition.

SUMMARY

■ The synthesis of long-chain fatty acids (lipogenesis) is carried out by two enzyme systems: acetyl-CoA carboxylase and fatty acid synthase.

■ The pathway converts acetyl-CoA to palmitate and requires NADPH, ATP, Mn^{2+} , biotin, and pantothenic acid as cofactors.

■ Acetyl-CoA carboxylase converts acetyl-CoA to malonyl-CoA, and then fatty acid synthase, a multienzyme complex consisting of two identical polypeptide chains, each containing six separate enzymatic activities and ACP, catalyzes the formation of palmitate from one acetyl-CoA and seven malonyl-CoA molecules.

■ Lipogenesis is regulated at the acetyl-CoA carboxylase step by allosteric modifiers, phosphorylation/dephosphorylation, and induction and repression of enzyme synthesis. The enzyme is allosterically activated by citrate and deactivated by long-chain acyl-CoA.

Dephosphorylation (eg, by insulin) promotes its activity, while phosphorylation (eg, by glucagon or epinephrine) is inhibitory.

■ Biosynthesis of unsaturated long-chain fatty acids is achieved by desaturase and elongase enzymes, which introduce double bonds and lengthen existing acyl chains, respectively.

■ Higher animals have Δ4 , Δ5 , Δ6 , and Δ9 desaturases but cannot insert new double bonds beyond the position 9 of fatty acids. Thus, the essential fatty acids linoleic (ω6) and α-linolenic (ω3) must be obtained from the diet.