Biochemistry II third stage

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Lecture 1

Biosynthesis of Fatty Acids

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Biomedical Importance

In most mammals, glucose is the primary substrate for lipogenesis. The 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.

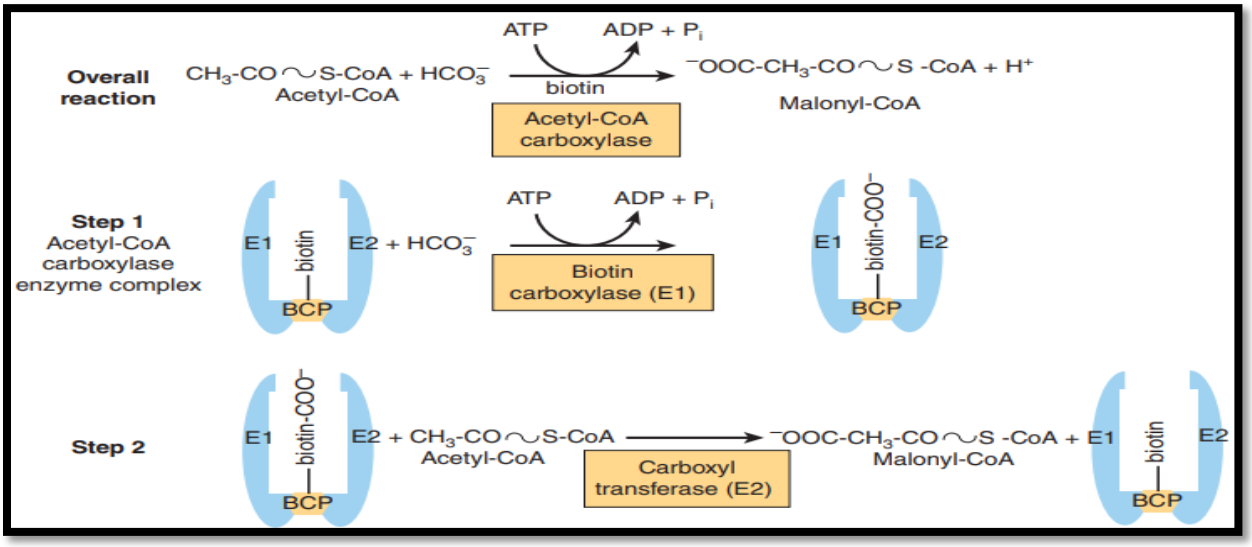
Animal tissues have limited capacity for desaturating fatty acids, and require certain dietary polyunsaturated fatty acids derived from plants. These essential fatty acids are used to form eicosanoic (C_{20}) fatty acids, which give rise to the eicosanoids prostaglandins, thromboxanes, leukotrienes, 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 de novo synthesis of fatty acids (lipogenesis)

Fatty acids are synthesized by an extramitochondrial system (cytosol), which is responsible for the complete synthesis of palmitate from acetyl-CoA. The lipogenesis 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.

A. Production of malonyl-CoA (initial & controlling step in lipogenesis)

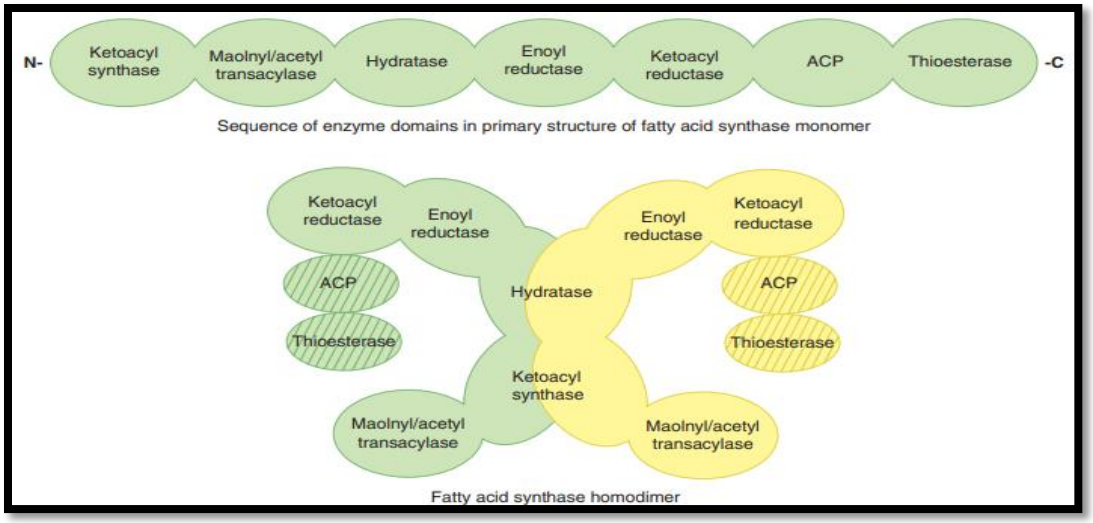
Bicarbonate as a source of CO_2 is required in the initial reaction for the carboxylation of acetyl-CoA to malonyl-CoA in the presence of ATP, B vitamin biotin and acetyl-CoA carboxylase. This enzyme has a **major role in the regulation of fatty acid synthesis**. Acetyl-CoA carboxylase is a multienzyme complex containing two enzymes, biotin carboxylase (E1) and a carboxyl transferase (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.



Biosynthesis of malonyl-CoA by acetyl carboxylase

B. The fatty acid synthase

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 enzyme is a homodimer, with two identical subunits, each containing 6 enzymes and an ACP, arranged in an X shape.



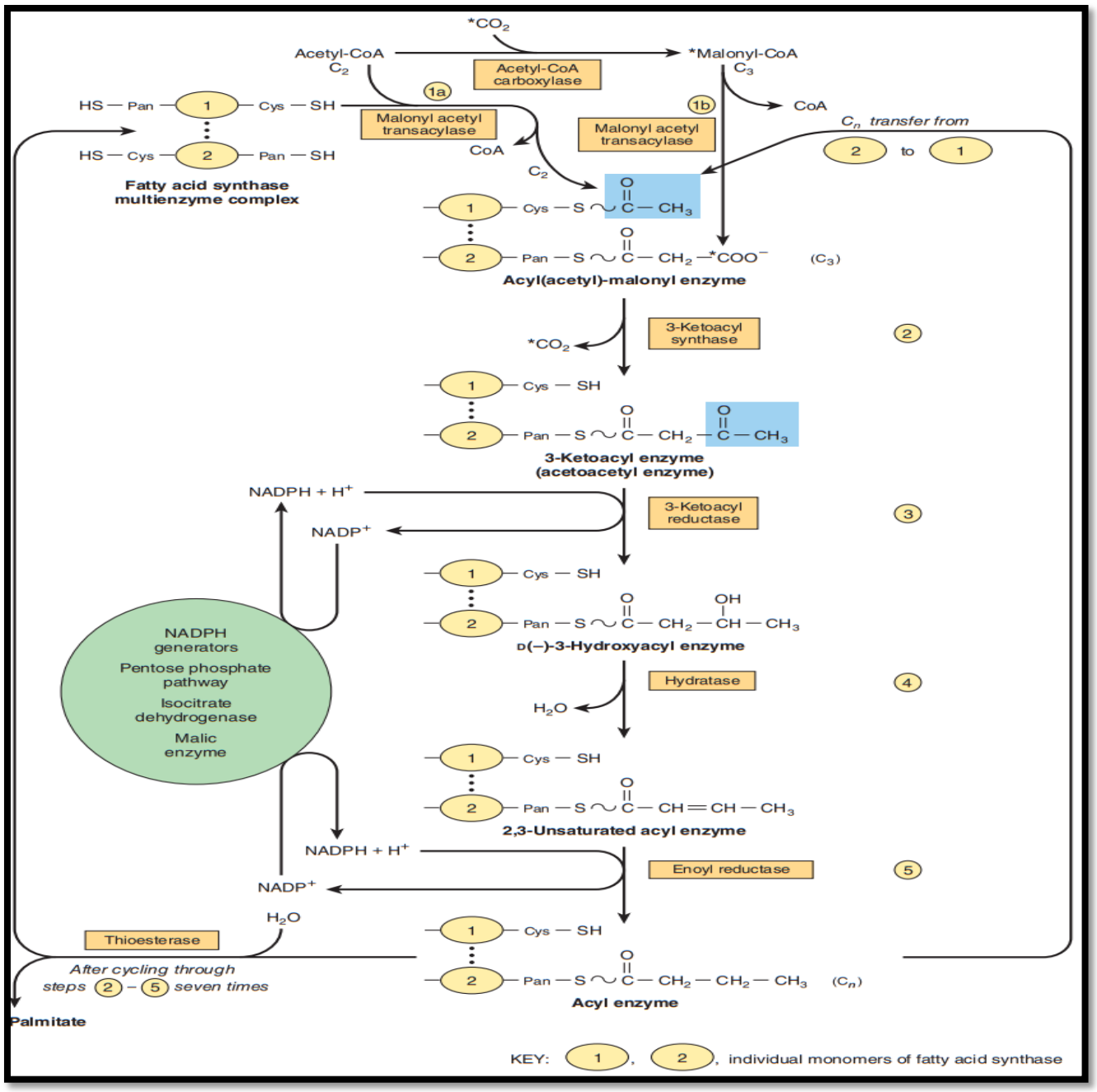
Initially, a priming molecule of acetyl-CoA combines with a cysteine -SH group, while malonyl-CoA combines with the adjacent -SH on the 4′-phosphopantetheine of ACP of the other monomer. These reactions are catalyzed by malonyl acetyl transacylase, to form acetyl (acyl)-malonyl enzyme. The **acetyl** group attacks the **methylene** group of the malonyl residue, catalyzed by 3-ketoacyl synthase, and liberates **CO_2**, forming 3-ketoacyl enzyme (acetoacetyl enzyme), freeing the cysteine -SH group.

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:

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

The acetyl-CoA used as a primer forms carbon atoms 15 and 16 of palmitate (even number of carbon atoms). 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.



Biosynthesis of long-chain fatty acids

The main source of NADPH for lipogenesis is the PPP

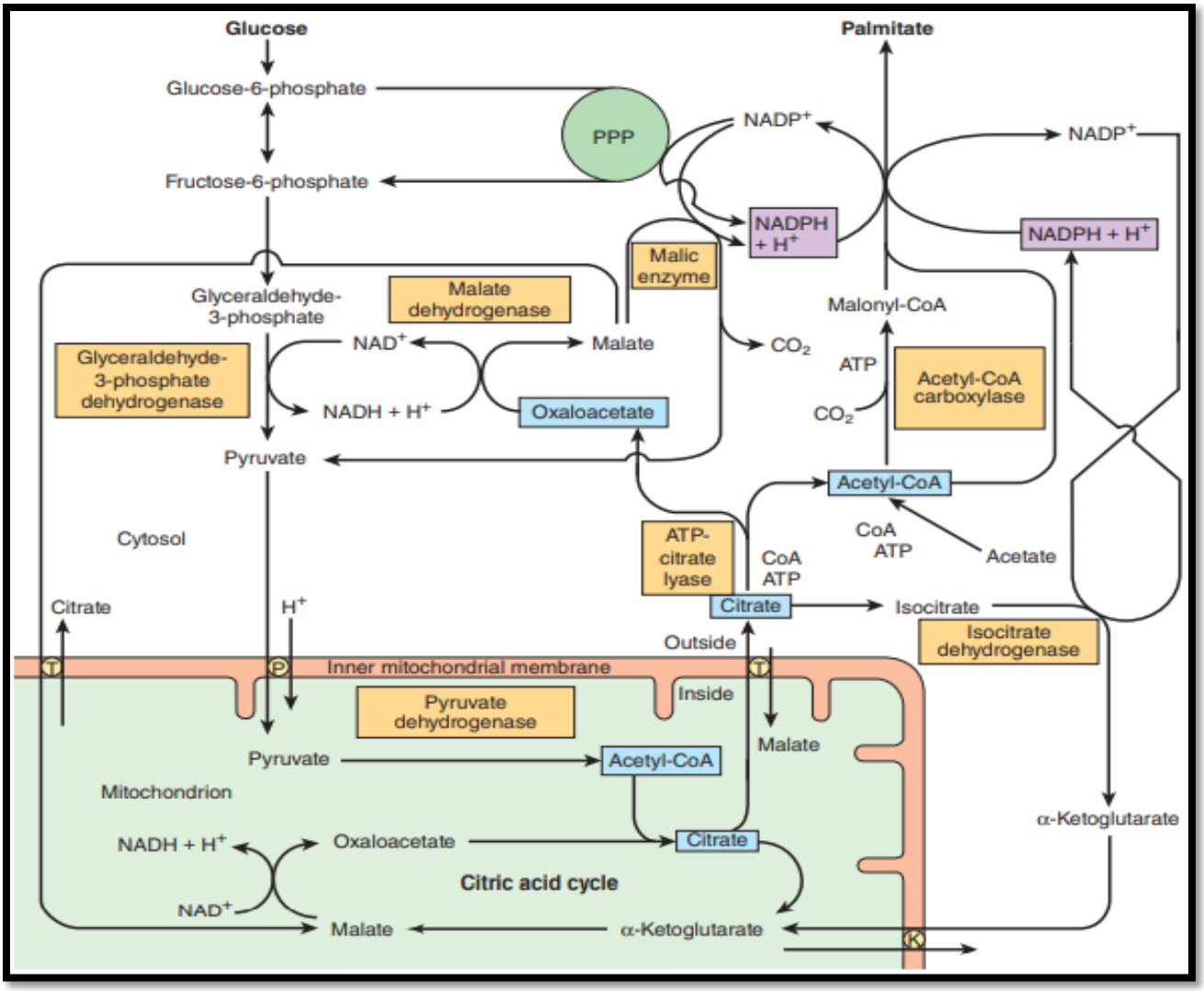
The oxidative reactions of the pentose phosphate pathway (PPP) are the chief source of the hydrogen (NADPH) required for the reductive synthesis of fatty acids (reactions 3 and 5). Significantly, tissues specializing in active lipogenesis ie, liver, adipose tissue, and the lactating mammary gland also possess an **active** pentose phosphate pathway. Moreover, both metabolic pathways are found in the cytosol of the cell. Other sources of NADPH include the "malic enzyme" (NADP malate dehydrogenase) and the extramitochondrial isocitrate dehydrogenase reaction.

Acetyl-CoA is the principal building block of fatty acids

Acetyl-CoA is formed from glucose via the oxidation of pyruvate in 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 (by **citrate synthase**) 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.

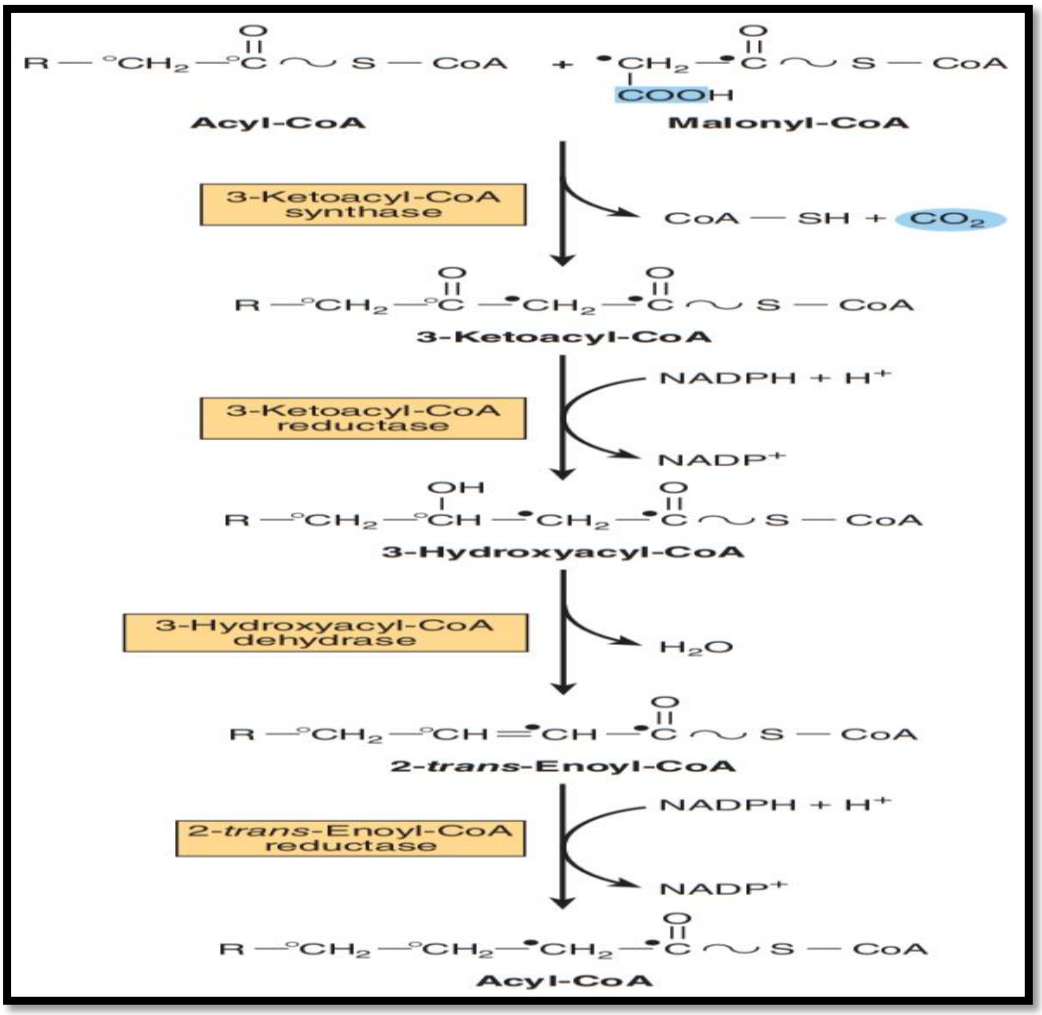
The resulting oxaloacetate can form **malate** via NADH-linked malate dehydrogenase, followed by the generation of NADPH via the malic enzyme. The NADPH becomes available for lipogenesis, and the pyruvate can be used to regenerate acetyl-CoA after transport into the mitochondrion. Alternatively, **malate** itself can be transported into the mitochondrion, where it is able to re-form oxaloacetate.



The provision of acetyl-CoA and NADPH for lipogenesis

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. Elongation of stearyl-CoA in brain increases rapidly during myelination in order to provide C_{22} and C_{24} fatty acids for sphingolipids.



Microsomal elongase system for fatty acid chain elongation

Regulation of lipogenesis (regulation by acetyl CoA carboxylase)

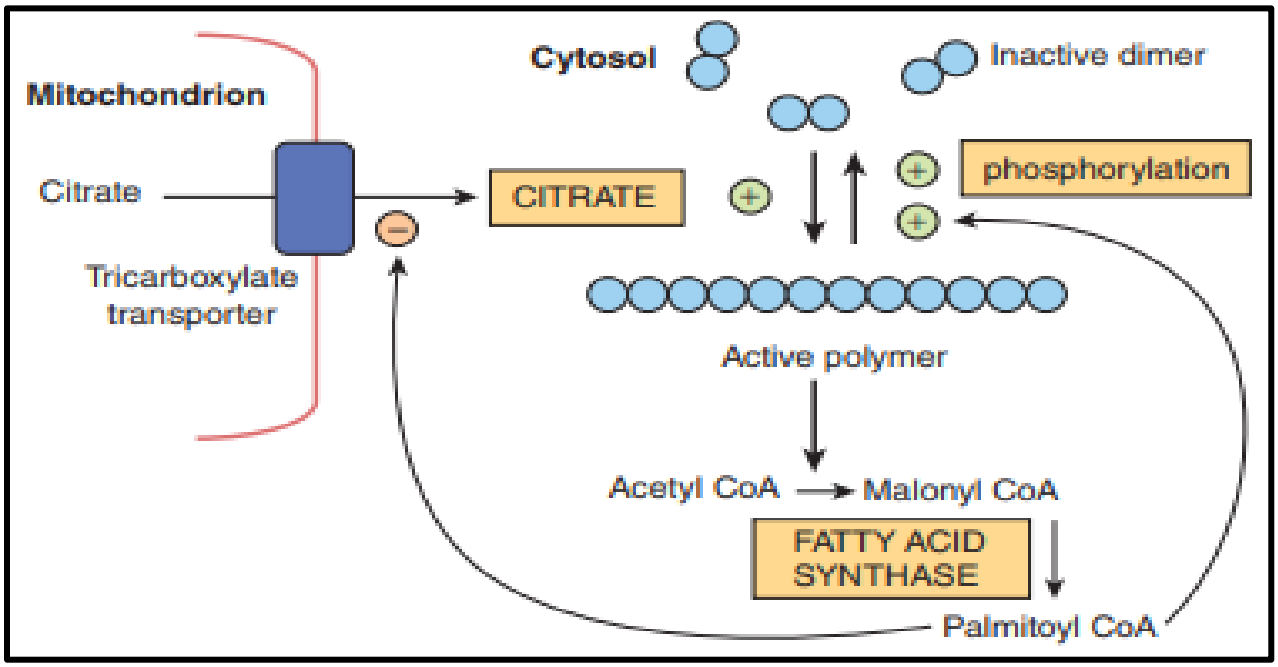
1. Short-term regulation of acetyl CoA carboxylase

A. Allosteric regulation: The carboxylation (acetyl CoA carboxylase) is the rate-limiting (regulation) step in fatty acid synthesis. The inactive form of acetyl CoA carboxylase is a (dimer). The enzyme undergoes allosteric activation by citrate, which causes dimers to polymerize, and allosteric inactivation by long-chain fatty acyl CoA (the end product of the pathway), which causes its depolymerization.

B. Covalent regulation of acetyl CoA carboxylase: The second mechanism of short term regulation is by phosphorylation. In the presence of counter regulatory hormones, such as epinephrine and glucagon, it stimulate protein kinase that phosphorylates and

inactivates acetyl CoA carboxylase. In the presence of insulin, it stimulate protein phosphatase that dephosphorylated and, thereby, activated acetyl CoA carboxylase.

Note: This is analogous to the regulation of glycogen synthase.



Regulation of acetyl CoA carboxylase

2. Long-term regulation of acetyl CoA carboxylase

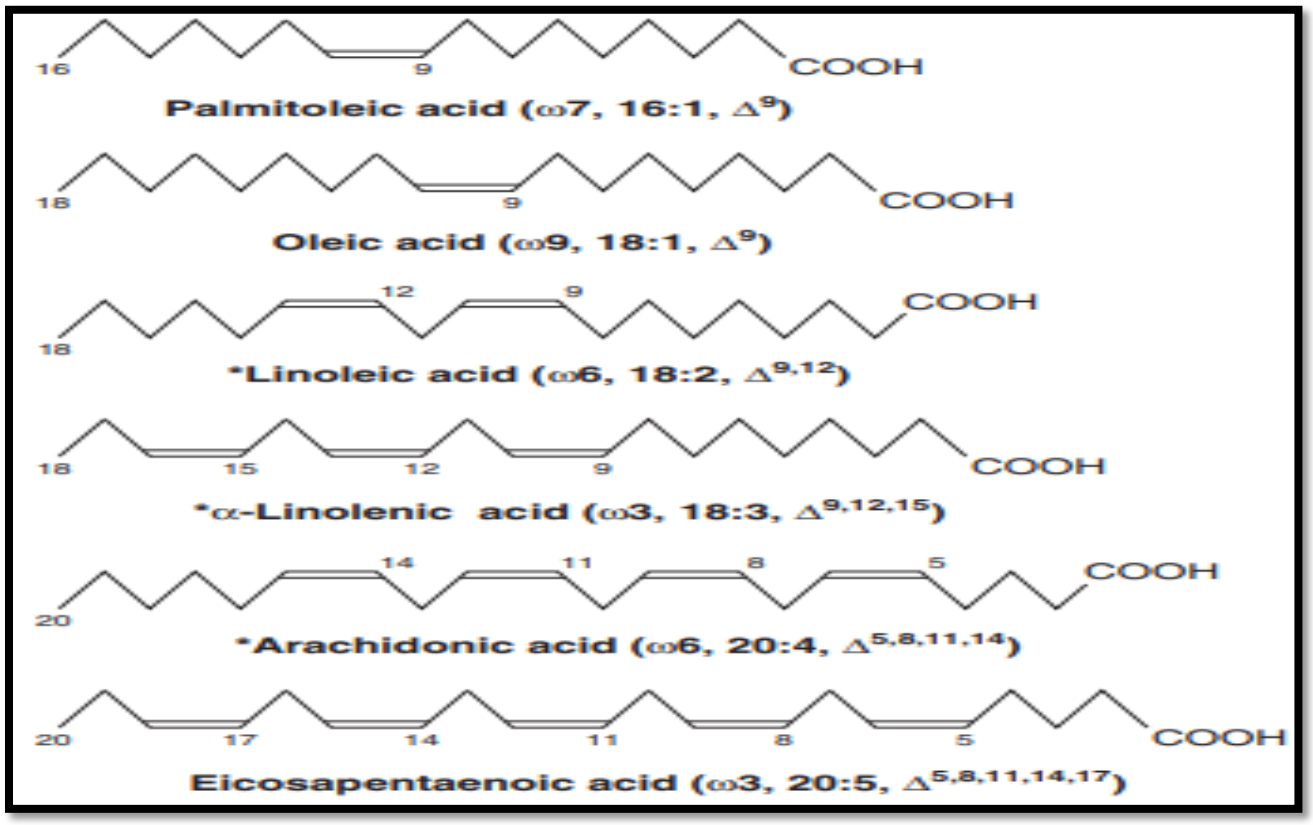
The long term by changes in gene expression governing rates of synthesis of enzymes. Prolonged consumption of a diet containing excess calories (particularly high calorie, high-carbohydrate diets) causes an increase in acetyl CoA carboxylase synthesis, thus increasing fatty acid synthesis. Conversely, a low calorie or a high-fat diet causes a reduction in fatty acid synthesis by decreasing the synthesis of acetyl CoA carboxylase.

Pyruvate dehydrogenase is also regulated by Acyl-CoA

Acyl-CoA causes an **inhibition** of pyruvate dehydrogenase by 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), (ATP)/(ADP) and (NADH)/(NAD^+) in mitochondria, **inhibiting** pyruvate dehydrogenase.

Some polyunsaturated fatty acids cannot be synthesized by mammals & are nutritionally essential

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.

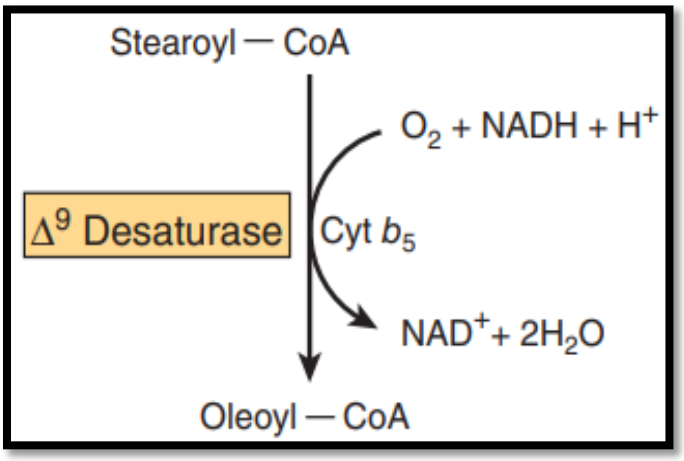


Structure of some unsaturated fatty acids

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 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.



Microsomal Δ^9 desaturase.

Synthesis of polyunsaturated fatty acids involves desaturase & elongase enzyme systems

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.

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 after the formation of saturated fatty acids by the pathways. However, linoleic (ω6) or α-linolenic (ω3) acids are required for the synthesis of the other members of the ω6 or ω3 families and must be supplied in the diet because the human cannot form double bond beyond Δ^9 position.

Linoleic acid (18:2 ω6) is converted and used for synthesis of arachidonic acid (AA) (20:4 ω6). The nutritional requirement for arachidonate may thus be dispensed with if there is adequate linoleate in the diet, while α-linolenic acids (18:3 ω3) is converted and used for synthesis of eicosapentaenoic acid (EPA) (20:5 ω3).