Al-Mustaqbal University

جامعة المستقبل
AL MUSTAQBAL UNIVERSITY

College of Health and Medical Techniques

Medical Laboratories Techniques Department

Biochemistry Lectures for 2nd Year Students

(2 Credit Hrs. Theory + 2 Credit Hrs. Practice / Week = 3 Credit Unit

Academic Year: 2024 - 2025

Course Organizers:

1. **Prof. Dr. Fadhil Jawad Al-Tu'ma, Ph.D., Professor of Clinical Biochemistry.**
2. **Dr. Dalya Shakir Obaida, Ph.D. Lecturer of Clinical Biochemistry.**

Second Semester

Lecture No. 5

Date: March, 2nd, 2025

Lipid Metabolism
Extra-mitochondrial Fatty Acids Biosynthesis

Objectives: The student should understand the following:

1. Describe the reaction catalyzed by acetyl-CoA carboxylase and understand the mechanisms by which its activity is regulated to control the rate of fatty acid synthesis.
2. Outline the structure of the fatty acid synthase multienzyme complex, indicating the sequence of two peptide chains of homodimer enzyme.
3. Explain how long-chain fatty acids are synthesized by the repeated condensation of two carbon units, with formation of the 16-carbon palmitate and identify the cofactors required.
4. Indicate the sources of reducing equivalents (NADPH + H^+) for fatty acid synthesis.
5. How fatty acid synthesis is regulated by nutritional status and identifies other control mechanisms that operate in addition to modulation of the activity of acetyl-CoA carboxylase.
6. Explain how polyunsaturated fatty acids are synthesized by desaturase and elongation enzymes.

Biomedical Importance:

Fatty acids are synthesized by an **extra-mitochondrial system**, which is responsible for the complete biosynthesis 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 produced by the diet. Inhibition of lipogenesis occurs in **type 1** (insulin-dependent) **diabetes mellitus,** and variations in its activity may affect the nature and extent of **obesity**.

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.

De Novo Bioynthesis of Fatty Acids:

The process of fatty acid biosynthesis (lipogenesis) is referred to as *de novo* biosynthetic pathway operating in the **cytoplasm**, so, it is referred to as **extra-mitochondrial** fatty acid synthase system. The major product is **palmitic acid**, the (16 C) saturated fatty acid. The process occurs in liver, adipose tissue, kidney, brain, lung and mammary glands. Its cofactor requirements include NADPH + H^+, 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.

Fatty acid degradation and synthesis are relatively simple processes that are essentially the reverse of each other. The process of degradation converts an aliphatic compound into a set of activated acetyl units (acetyl-CoA) that can be processed by the citric acid cycle.

Fatty acid biosynthesis is essentially the reverse of this process. Because the result is a polymer, the process starts with monomers in this case with activated acyl group (most simply, an acetyl unit) and malonyl units. The malonyl unit is condensed with the acetyl unit to form a four-carbon fragment. To produce the required hydrocarbon chain, the carbonyl must be reduced forming of butyryl-CoA. Another activated malonyl group condenses with the butyryl unit and the process is repeated until a C16 fatty acid is synthesized.

Fatty acid biosynthesis is not simply a reversal of the degradative pathway. Rather, it consists of a new set of reactions, again exemplifying the principle that biosynthetic and degradative pathways are almost always distinct. Important differences between the biosynthesis and degradation of fatty acids are given in **Table 1.**

Table 1: Differences between fatty acid oxidation and biosynthesis

	β-Oxidation of Fatty Acids	Fatty Acid Biosynthesis
Site	Mitochondrial matrix	Cytoplasm
Intermediates	Present as CoA derivatives	Covalently linked to SH group of ACP
Enzymes	Present as independent proteins	Multi-enzyme complex
Sequential units	Two carbon units split off as acetyl CoA	Two carbon units added, as 3 carbon malonyl CoA
Co-enzymes required	NAD^+ and FAD	NADPH used as reducing power
Carrier	Carnitine	Citrate
Sequence of chemical reaction per each cycle	Oxidation , Hydration, Oxidation, Cleavage	Condensation, Reduction, Dehydration, Reduction
Stereoisomerism of Intermediate	L - Stereoisomerism	D-Stereoisomerism
Product	Acetyl-CoA	Palmitic Acid
Regulation	Insulin ↓ , Glucagon ↑	Insulin ↑ , Glucagon ↓

Biosynthesis of Fatty Acids Occurs in the Cytosol:

A large proportion of the fatty acids used by the body are 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 to fatty acids, which are stored as triacylglycerols. This system is present in many

tissues, including liver, kidney, brain, lung, mammary gland, and adipose tissue. Its cofactor requirements include NADPH + H^+, ATP, Mn^{2+}, biotin, and HCO_3^- (as a source of CO_2).

Source of NADPH for Fatty Acid Biosynthesis:

The reduced NADPH + H^+ is involved as donor of reducing equivalents are derived from 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 i.e, liver, adipose tissue, and the lactating mammary gland. 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 + H^+.

Other sources of NADPH + H^+ include the reaction that converts malate to pyruvate catalyzed by the **"malic enzyme"** ($NADP^+$ malate dehydrogenase) (see below) and the extramitochondrial **isocitrate dehydrogenase** reaction.

Production of Cytosolic Acetyl-CoA :

Transfer of acetyl groups from mitochondria to the cytosol was indicated in **Fig. 1.** The mitochondrial outer membrane is freely permeable to all these compounds.

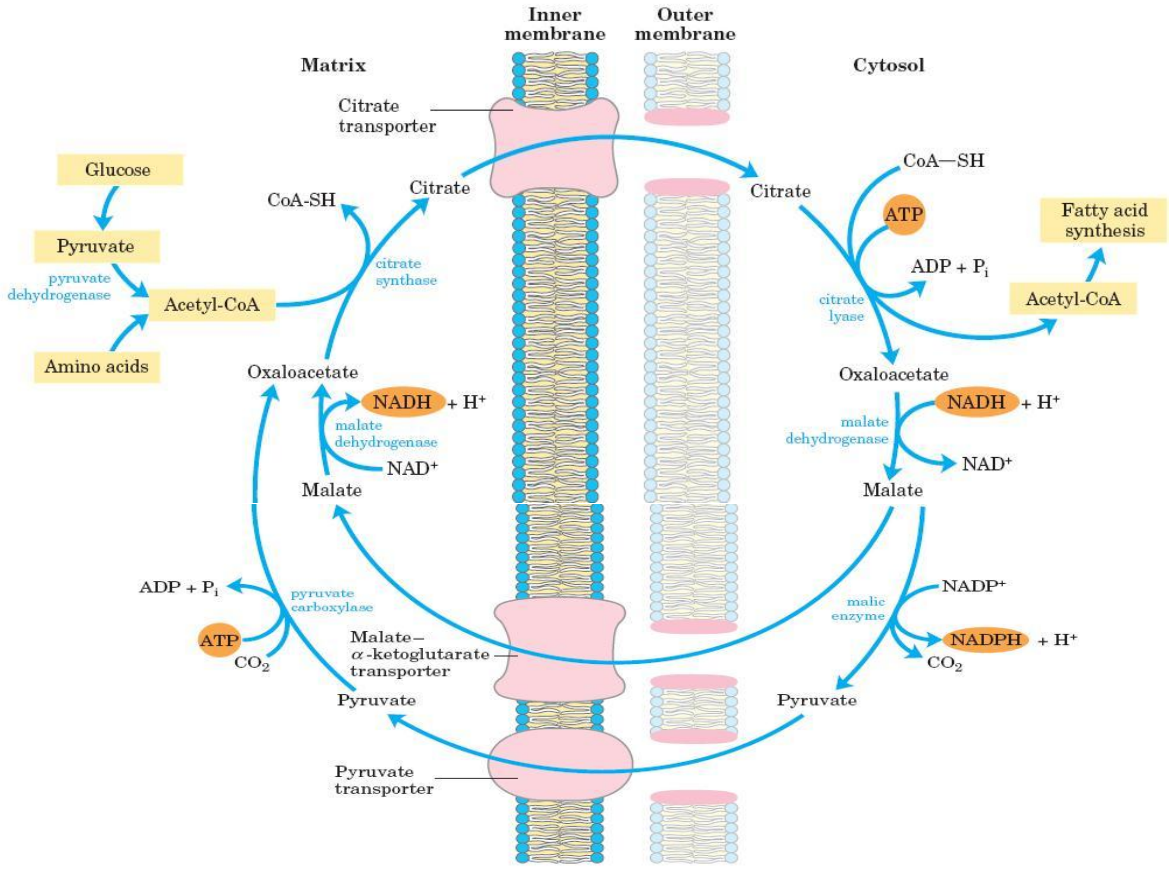


Fig. 1: Citrate-malate shuttle

Pyruvate derived from amino acid catabolism in the mitochondrial matrix, or from glucose by glycolysis in the cytosol, is converted to acetyl-CoA in the matrix. Acetyl groups pass out of the mitochondrion as citrate; in

the cytosol they are delivered as acetyl-CoA for fatty acid synthesis. Oxaloacetate is reduced to malate, which returns to the mitochondrial matrix and is converted to oxaloacetate. An alternative fate for cytosolic malate is oxidation by malic enzyme to generate cytosolic NADPH; the pyruvate produced returns to the mitochondrial matrix.

Malonyl-Co A Biosynthesis is the Committed Step:

Fatty acid biosynthesis starts with the carboxylation of acetyl-CoA to *malonyl-CoA*. This irreversible reaction is the committed step in fatty acid biosynthesis, **Fig. 2.**

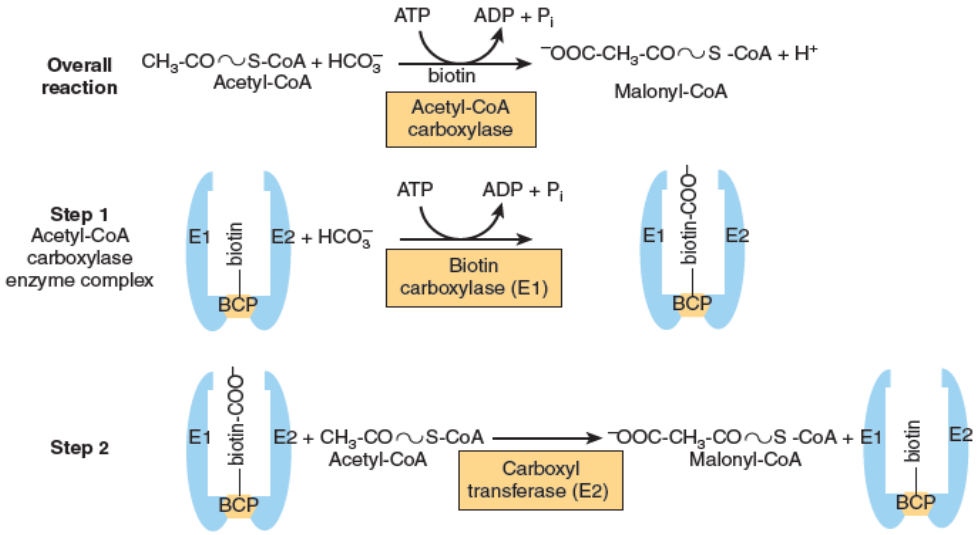
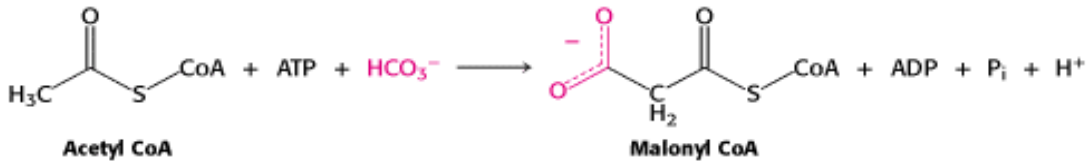


Fig. 2: Biosynthesis of malonyl-CoA by multienzyme complex acetyl carboxylase which containing two enzymes, biotin carboxylase (E1) and a carboxyltransferase (E2) and the biotin carrier protein (BCP). Biotin is covalently linked to the BCP. The reaction proceeds in 2 steps. In step 1, catalyzed by E1, 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 synthesis of malonyl-CoA is catalyzed by biotin-dependent ***acetyl-CoA carboxylase***. The enzyme is a **multienzyme protein** containing a variable number of identical subunits, each containing biotin, biotin carboxylase, biotin carboxyl carrier protein, and transcarboxylase, as well as a regulatory allosteric site.



The reaction takes place in two steps as shown below:

1. Carboxylation of biotin as a prosthetic group involving ATP.

2. Transfer of the carboxyl to acetyl-CoA to form malonyl-CoA.

$$\text{Biotin-enzyme} + \text{ATP} + HCO_3^- \rightleftharpoons CO_2\text{-biotin-enzyme} + \text{ADP} + P_i$$

$$CO_2\text{-biotin-enzyme} + \text{acetyl CoA} \longrightarrow \text{malonyl CoA} + \text{biotin-enzyme}$$

The Fatty Acid Synthase Complex:

In mammals, the fatty acid synthase complex is a dimer comprising two identical monomers, each containing all seven enzyme activities of fatty acid synthase on one polypeptide chain, **Fig. 3**. Initially, a priming molecule of acetyl-CoA combines with a cysteine-SH group catalyzed by **acetyl transacylase**. The advantages of multi-enzyme complex:

- **a.** Intermediates of the reaction can easily interact with the active sites of the enzymes.
- **b.** One gene code all the enzymes; so, all the enzymes are in equimolecular concentrations.
- **c.** So, the efficiency of the process is enhanced.

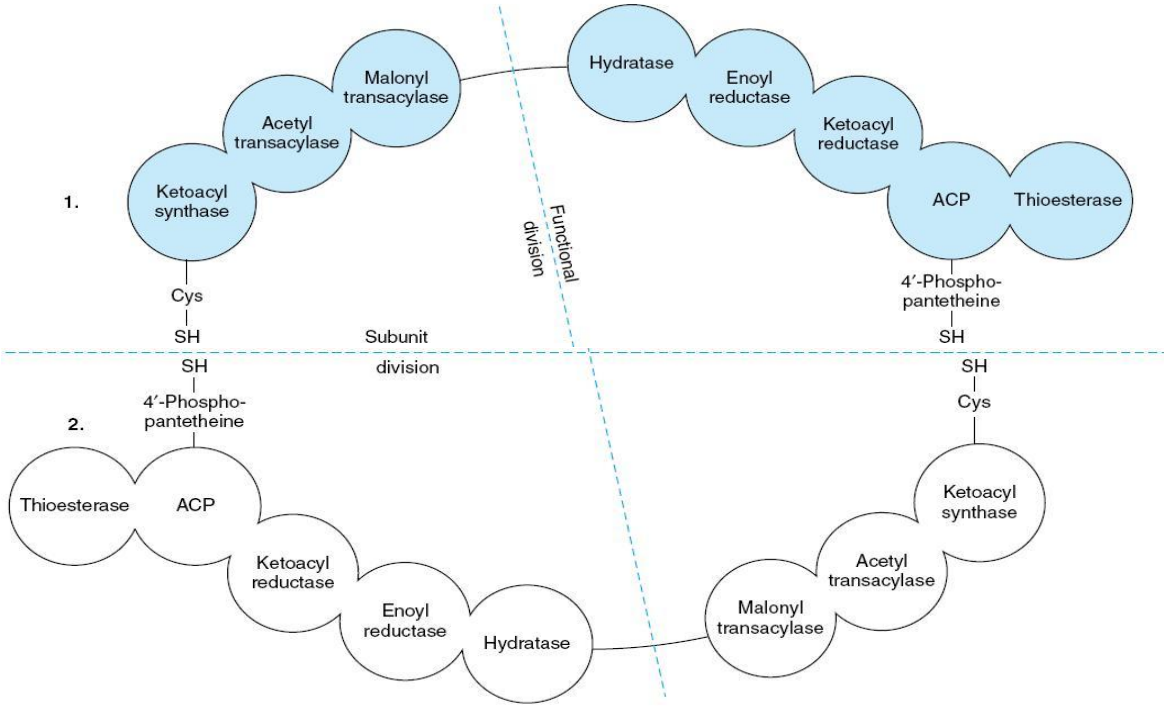


Fig. 3. Fatty acid synthase multienzyme complex. The complex is a dimer of two identical polypeptide monomers, 1 and 2, each consisting of seven enzyme activities and the acyl carrier protein (ACP). (Cys-SH, cysteine thiol). The -SH of the 4′-phosphopantetheine of one monomer is in close proximity to the -SH of the cysteine residue of the ketoacyl synthase of the other monomer, suggesting a "head-to-tail" arrangement of the two monomers.

Step 1: Carboxylation of Acetyl-CoA

The first step in the fatty acid synthesis is the carboxylation of acetyl-CoA to form malonyl-CoA. Acetyl-CoA carboxylase is not a part of the multi-enzyme complex. But it is the **rate-limiting enzyme. Biotin**, a member of B complex vitamins, is necessary for this reaction (Step 1 in **Fig. 4**). The enzyme is allosterically regulated, the major effectors being citrate (positive)

and palmitoyl-CoA (negative). The reaction is similar to carboxylation of pyruvate to form oxaloacetate. The elongation of the fatty acid occurs by addition of 2 carbon atoms at a time. But the 2-carbon units are added as 3-carbon, **malonyl units**. The whole reaction sequence occurs while the intermediates are bound to ACP (acyl carrier protein).

Step 2: Three C and Two C Units are Added

A. Acetyl transacylase catalyzes the transfer of the acetyl group (2 carbons) to the cysteinyl SH group of the **condensing enzyme** (CE) of the other monomer of the fatty acid synthase complex (step 2A in **Fig. 4**).

B. One molecule of acetyl-CoA (2 carbon) and one molecule of malonyl-CoA (3 carbon) bind to the multienzyme complex. **Malonyl transacylase** transfers the malonyl group to the SH group of the ACP of one monomer of the enzyme (step 2B in **Fig. 3**).

Step 3: Condensation

Malonyl-CoA combines with the adjacent -SH on the 4′-phosphopantetheine of ACP of the other monomer, catalyzed by **malonyl transacylase**, to form **acetyl (acyl)-malonylenzyme.** Acetyl (2C) and malonyl (3C) units are condensed by condensing enzyme or keto acyl synthase to form beta-keto acyl ACP or acetoacetyl ACP (4C). During this process one carbon is lost as CO_2 (step 3 in **Fig. 4**).

The acetyl group attacks the methylene group of the malonyl residue, catalyzed by **3-ketoacylsynthase,** and liberates CO_2, forming 3-ketoacyl enzyme (acetoacetyl enzyme), 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 to form the corresponding saturated acyl-S-enzyme.

Step 4: Reduction

Acetoacetyl-ACP is reduced by **NADPH** dependent beta-keto acyl **reductase** to form beta-hydroxy fatty acyl-ACP (step 4 in **Fig. 4**).

Step 5: Dehydration

It is then dehydrated by a **dehydratase** (DH) to form enoyl-ACP otherwise known as (alpha beta unsaturated acyl-ACP) (step 5 **Fig. 4**).

Step 6: Second Reduction

The enoyl-ACP is again reduced by enoyl reductase (ER) utilizing a 2^{nd} molecule of **NADPH** to form butyryl-ACP (step 6 in **Fig. 4**).

Cycling of Reactions

To start the next cycle of four reactions that lengthens the chain by two more carbons, another malonyl group is linked to the now unoccupied phospho-pantetheine-SH group of ACP.

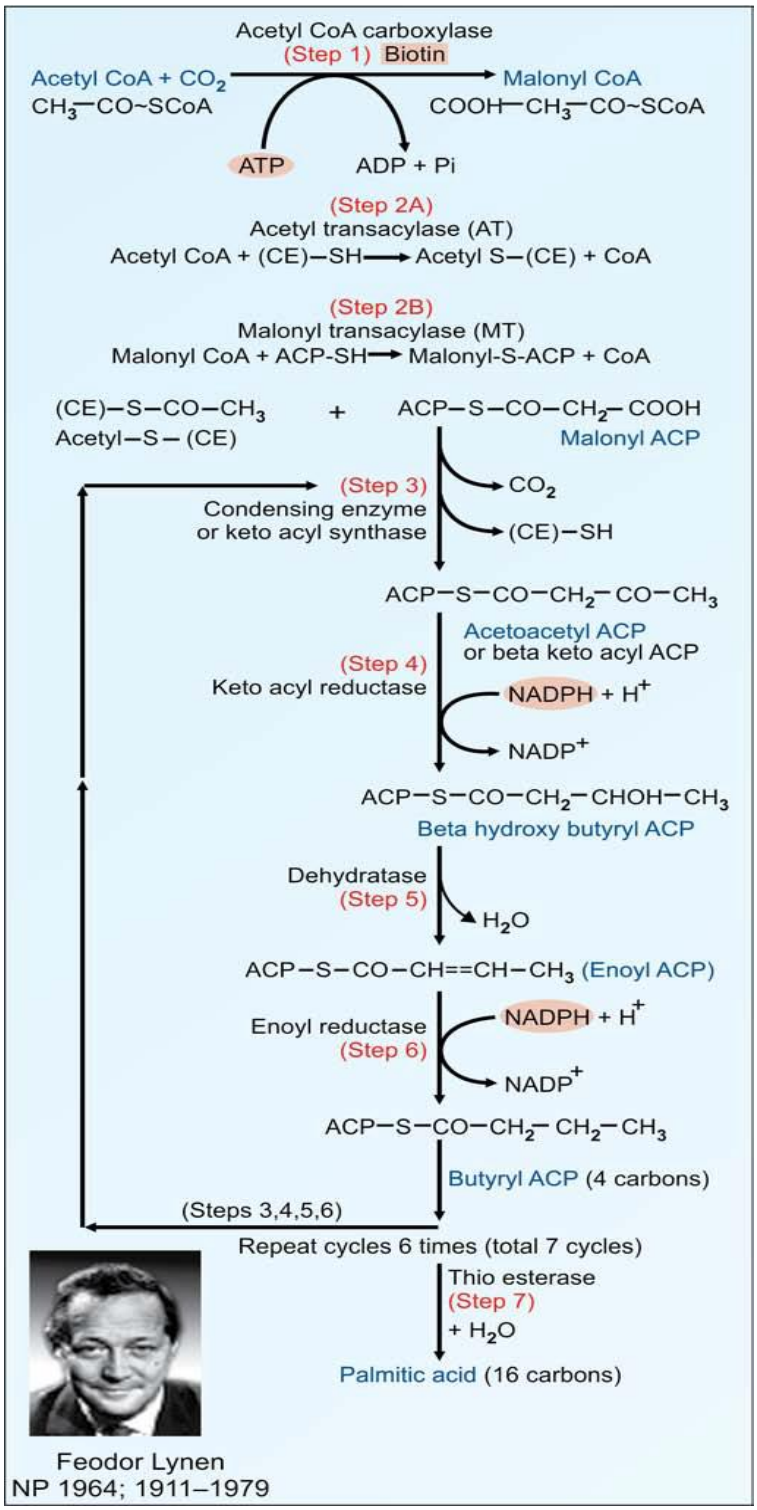


Fig. 4: De novo synthesis of fatty acid (Lynen cycle). Steps 4 and 6 utilize NADPH

Condensation occurs as the butyryl group (4C) is now transferred to the SH group of the condensing enzyme on the other monomer and a 2nd malonyl-CoA molecule binds to the phosphopantothenyl-SH group displacing the saturated acyl residue onto the free cysteine-SH group. The sequence of reactions in the cycle, namely condensation, reduction, dehydration and reduction (steps 3,4,5,6) are repeated six more times until a saturated 16-carbon acyl radical (palmitoyl) has been assembled or produced. The product of this condensation is a six-carbon acyl group,

covalently bound to the phosphopantetheine-SH group. Its β-keto group is reduced in the next three steps of the synthase cycle to yield the saturated acyl group, exactly as in the first round of reactions.

Step 7: Palmitic acid is Released

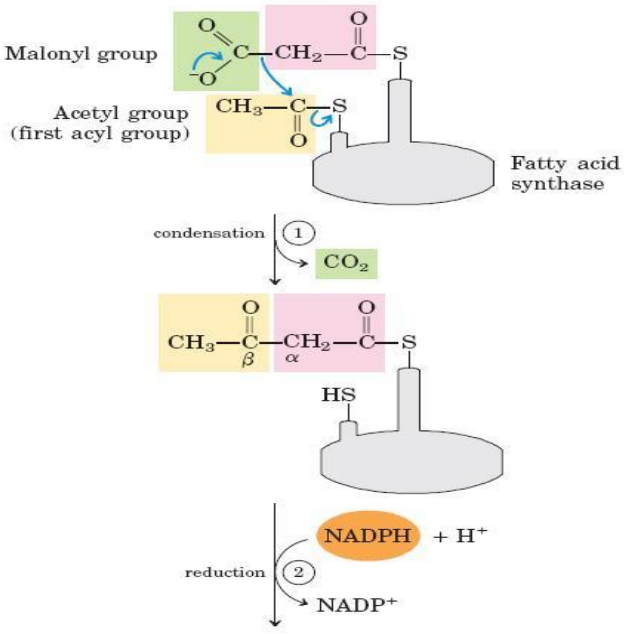
Thio-esterase or de-acylase activity (TE) releases palmitate from the multi-enzyme complex (step 7, **Fig. 4**). The end point is palmitic acid (16 C) in liver and adipose tissue. But in lactating mammary gland, there is a separate thioesterase specific for acyl residues of C8, C10, or C12, which are subsequently found in milk lipids, the end products are capric (10 C) and lauric (12 C) acids. Mother's milk contains these medium-chain fatty acids. Cow's milk contains odd numbered fatty acids. The free palmitate must be activated to acyl-CoA before it can proceed via any other metabolic pathway. Its usual fate is esterification into acylglycerols, chain elongation or desaturation, or esterification to cholesteryl ester.

Fatty Acid Synthesis Proceeds in a Repeating Reaction Sequence:

The long carbon chains of fatty acids are assembled in a repeating four-step sequence, **Fig. 5.** The equation for the overall synthesis of palmitate from acetyl-CoA and malonyl-CoA is:

$$CH_3\,CO\text{-}SCoA + 7HOOC\text{-}CH_2CO\text{-}SCoA + 14\,NADPH + 14H^+ \longrightarrow CH_3(CH_2)_{14}COOH + 7CO_2 + 6H_2O + 8CoA\text{-}SH + 14NADP^+$$

The acetyl-CoA used as a primer forms carbon atoms 15 and 16 of palmitate. The addition of all the subsequent C2 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.



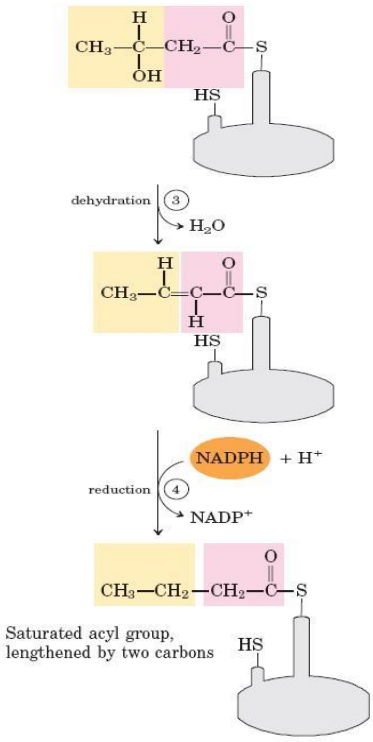


Fig. 5: Addition of two carbons to a growing fatty acyl chain: a four-step sequence. Each malonyl group and acetyl (or longer acyl) group is activated by a thioester that links it to fatty acid synthase, a multienzyme.

The overall process of palmitate biosynthesis (16:0.) was shown in **Fig. 6.** The fatty acyl chain grows by two-carbon units donated by activated malonate, with loss of CO_2 at each step. The initial acetyl group is shaded yellow, C-1 and C-2 of malonate are shaded pink, and the carbon released as CO_2 is shaded green. After each two-carbon addition, reductions convert the growing chain to a saturated fatty acid of four, then six, then eight carbons, and so on.

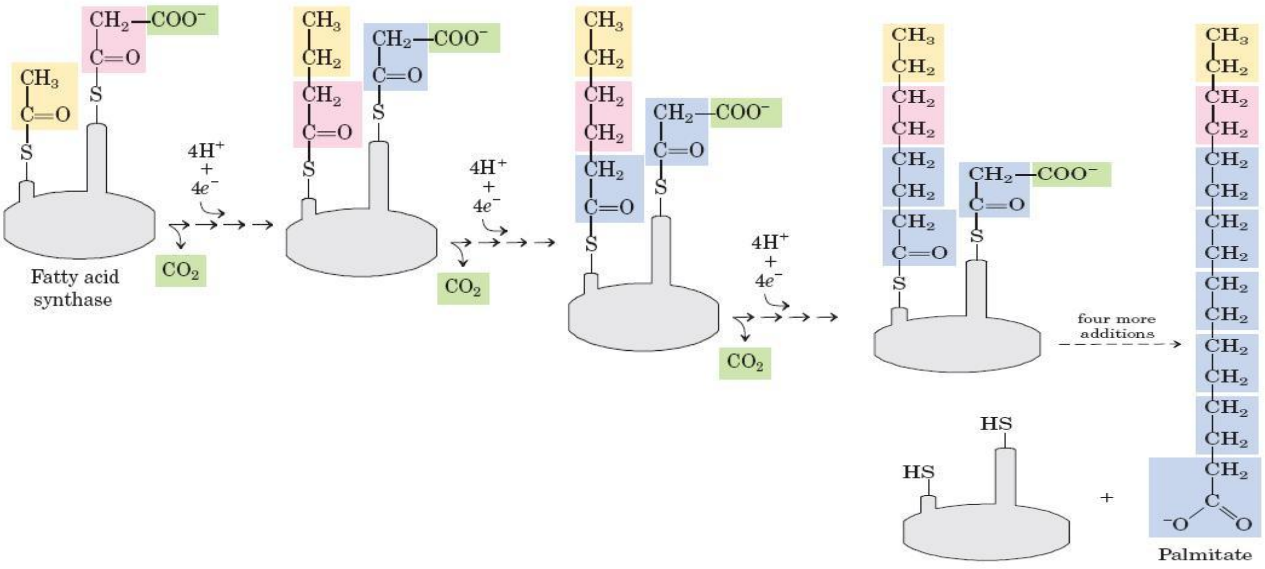


Fig. 6: Palmitate biosynthesis

Acyl carrier protein (ACP), Fig. 7 is a small protein (*M*r 8,860) containing the prosthetic group **4'-phosphopantetheine** (see below). Hydrolysis of thioesters is highly exergonic, and the energy released helps to make two different steps (1 and 5) in fatty acid synthesis (condensation) thermodynamically favorable.

Before the condensation reactions that build up the fatty acid chain can begin, the two thiol groups on the enzyme complex must be charged with the correct acyl groups. First, the acetyl group of acetyl-CoA is transferred to the Cys-SH group of the β-ketoacyl-ACP synthase. This reaction is catalyzed by **acetyl-CoA–ACP transacetylase** (AT).

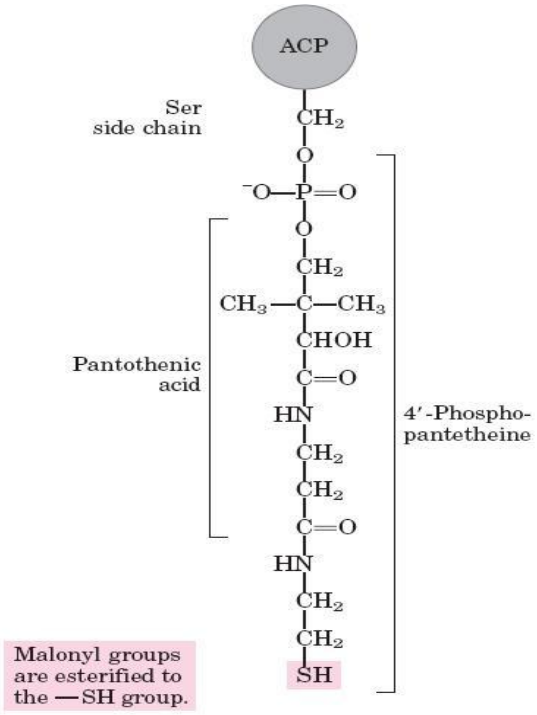


Fig. 7. Acyl carrier protein

Long-Chain Saturated Fatty Acids Are Synthesized from Palmitate:

This pathway (the “microsomal system”) elongates saturated and unsaturated fatty acyl-CoAs (from C10 upward) by two carbons, using malonyl-CoA as acetyl donor and NADPH as 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 C22 and C24 fatty acids for sphingolipids. Palmitate is the precursor of other long-chain fatty acids (see below). It may be lengthened to form stearate (18:0) or even longer saturated fatty acids by further additions of acetyl groups, through the action of **fatty acid elongation systems** present in the smooth endoplasmic reticulum and in mitochondria. Although different enzyme systems are involved, and coenzyme A rather than ACP is the acyl carrier in the reaction, the mechanism of elongation in the ER is otherwise

identical to that in palmitate synthesis: donation of two carbons by malonyl-CoA, followed by reduction, dehydration, and reduction to the saturated 18-carbon product, stearoyl-CoA, **Fig. 8**.

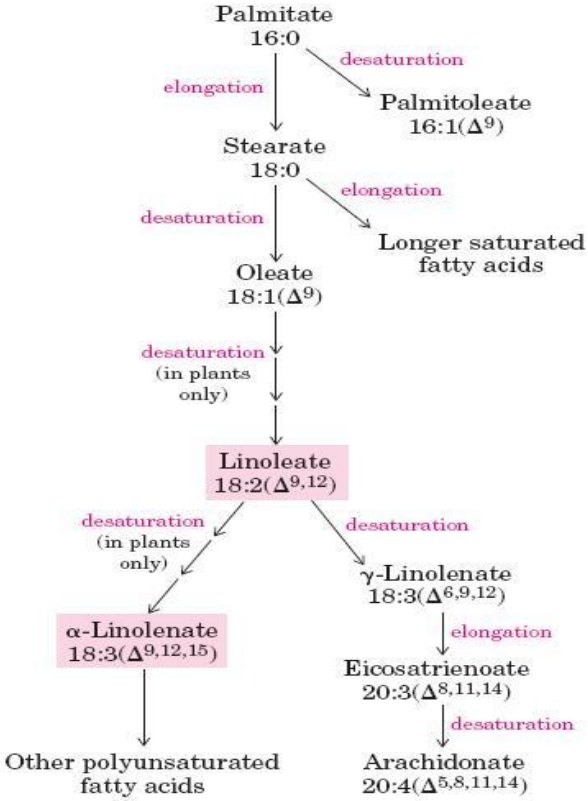


Fig. 8. Desaturation of Fatty Acids Requires a Mixed-Function Oxidase

Palmitate and stearate serve as precursors of the two most common monounsaturated fatty acids of animal tissues: palmitoleate, 16:1(Δ^9), and oleate, 18:1(Δ^9); both of these fatty acids have a single cis double bond between C-9 and C-10. The double bond is introduced into the fatty acid chain by an oxidative reaction catalyzed by **fatty acyl-CoA desaturase** (a **mixed-function oxidase**) **as shown in Fig. 9**.

Mammalian hepatocytes can readily introduce double bonds at the Δ^9 position of fatty acids but cannot introduce additional double bonds between C-10 and the methyl-terminal end. Thus mammals cannot synthesize linoleate, 18:2($\Delta^{9,12}$), or *α*-linolenate, 18:3($\Delta^{9,12,15}$) therefore, these two fatty acids must be provided by the dietary sources as essential fatty acids.

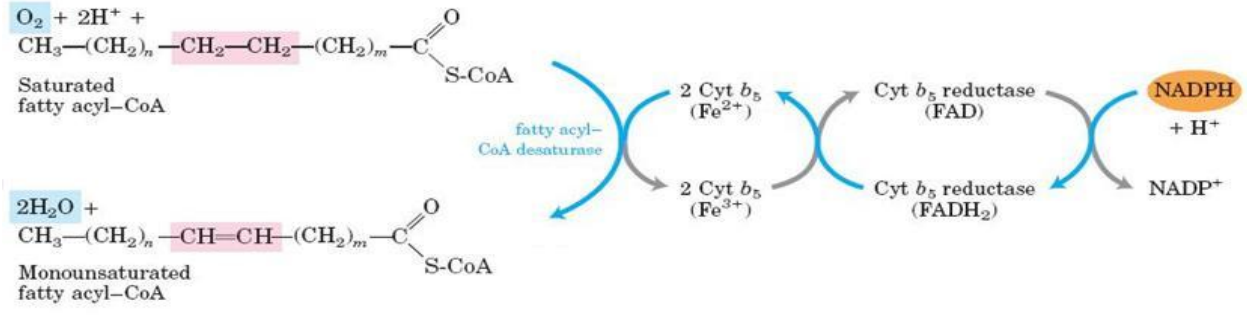


Fig. 9: Desaturation mechanism of fatty acids

Plants, however, can synthesize both; the desaturases that introduce double bonds at the Δ^{12} and Δ^{15} positions are located in the ER and the chloroplast. The ER enzymes act not on free fatty acids but on a phospholipid, phosphatidylcholine, that contains at least one oleate linked to the glycerol. Both plants and bacteria must synthesize polyunsaturated fatty acids to ensure membrane fluidity at reduced temperatures.

Example of Clinical Symptoms Due to Essential Fatty Acids Deficiency:

These fatty acids are found in high concentrations in vegetable oils and in small amounts in animal products. Essential fatty acids are required for prostaglandin, thromboxane, leukotriene, and lipoxin formation, and they also have various other functions. They are found in the structural lipids of the cell, often in the position 2 of phospholipids, and are concerned with the structural integrity of the mitochondrial membrane. Arachidonic acid is present in membranes and accounts for 5-15% of the fatty acids in phospholipids. Docosahexaenoic acid (DHA; ω3, 22:6), which is synthesized to a limited extent from α-linolenic acid or obtained directly from fish oils, is present in high concentrations in retina, cerebral cortex, testis, and sperm. DHA is particularly needed for development of the brain and retina and is supplied via the placenta and milk. Patients with **retinitis pigmentosa** are reported to have low blood levels of DHA.

Acetyl-CoA Carboxylase and Regulation of Lipogenesis:

Example of acetyl-CoA regulation is short-term regulation. This carboxylation is both the rate-limiting and the regulated step in fatty acid biosynthesis. 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, which causes its depolymerization. A second mechanism of short-term regulation is by reversible phosphorylation. In the presence of counter-regulatory hormones, such as epinephrine and glucagon, *acetyl-CoA carboxylase* is phosphorylated and, thereby, inactivated, **Fig. 10**. **In the presence of insulin, *acetyl-CoA carboxylase* is dephosphorylated and, thereby, activated.**

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 acetyl-CoA. 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.

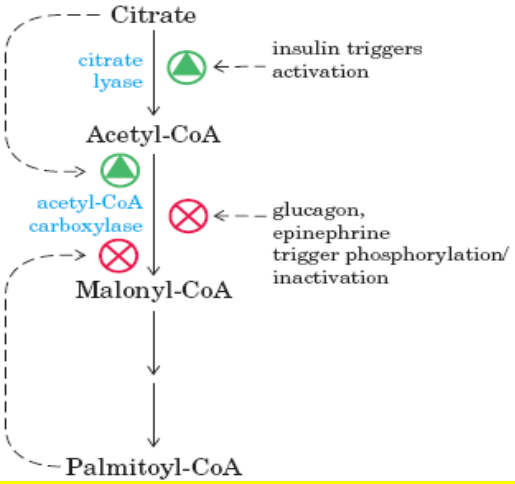


Fig. 10. Regulation of acetyl-CoA carboxylase

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, **Fig. 11**. 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.

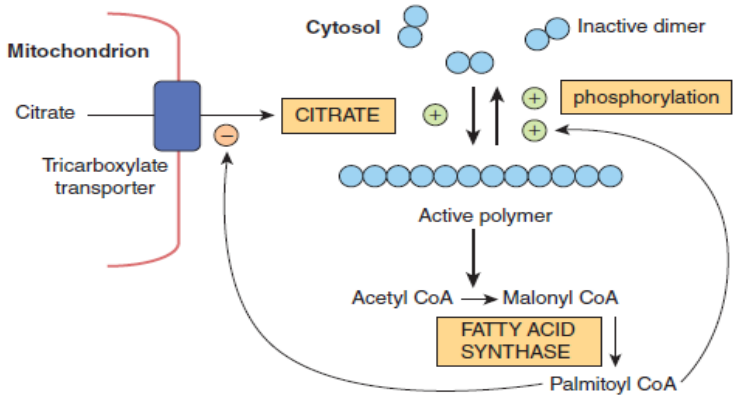


Fig. 11: Regulation of acetyl-CoA carboxylase. It activated by citrate, which promotes the conversion of the enzyme from an inactive dimer to an active polymeric form. Inactivation is promoted by phosphorylation of the enzyme and by long-chain acyl-CoA molecules such as palmitoyl-CoA. In addition, acyl-CoA 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.

Insulin Also Regulates Lipogenesis by Other Mechanisms

Insulin stimulates lipogenesis by several other mechanisms as well as by increasing acetyl-CoA carboxylase activity.

1. It increases the transport of glucose into the cell (e.g. in adipose tissue).

2. 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.
3. Converts the inactive form of pyruvate dehydrogenase to the active form in adipose tissue, but not in liver.
4. 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.

Long-term regulation of acetyl-CoA carboxylase:

Prolonged consumption of a diet containing excess calories such as high-carbohydrate diets cause 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 biosynthesis by decreasing the synthesis of *acetyl-CoA carboxylase.*

Fatty acid synthase complex and acetyl-CoA carboxylase are adapted 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.