Biochemistry

Pavel Pestryakov

Novosibirsk State University
Institute of chemical biology and fundamental medicine, SB RAS

+7(913)892-3045
Pavel.pestryakov@niboch.nsc.ru

Synthesis

GLYCOGEN SYNTHESIS (GLYCOGENESIS)

The glycogen synthesis occurs by a pathway distinctly different from the reversal of glycogenbreakdown, which would prevent the operation of futile cycles. The steps are:

1. Activation of Glucose

UDP glucose is formed from glucose-1-phosphate and UTP (uridine triphosphate) by the enzyme UDP-glucose pyrophosphorylase.

Glucose-1-phosphate +UTP + PPi ————————→ UDP-glucose

2. Glycogen Synthase

The glucose moiety from UDP-glucose is transferred to a glycogen primer (glycogenin) molecule. The primer is essential to accept the glycosyl unit. The primer is made up of a protein-carbohydrate complex. It is a dimeric protein, having two identical monomers. An oligosaccharide chain of 7 glucose units is added to each monomer.

Glycogen primer (n) + UDP-glucose ————→ Glycogen (n+1) + UDP

In the next step, activated glucose units are sequentially added by the enzyme glycogen synthase. The glucose unit is added to the nonreducing (outer) end of the glycogen primer to form an alpha-1,4 glycosidic linkage and UDP is liberated.

Synthesis

GLYCOGEN SYNTHESIS (GLYCOGENESIS)

The glycogen synthesis occurs by a pathway distinctly different from the reversal of glycogenbreakdown, which would prevent the operation of futile cycles. The steps are:

i. The glycogen synthase can add glucose units only in alpha-1,4 linkage. A branching enzyme is needed to create the alpha-1,6 linkages.

ii. When the chain is lengthened to 11 – 12 glucose residues, the branching enzyme will transfer a block of 6 to 8 glucose residues from this chain to another site on the growing molecule. The enzyme amylo-[1,4]→[1,6]- transglucosidase (branching enzyme) formsthis alpha-1,6 linkage.

iii. To this newly created branch, further glucose units can be added in alpha-1,4 linkage by glycogen synthase.

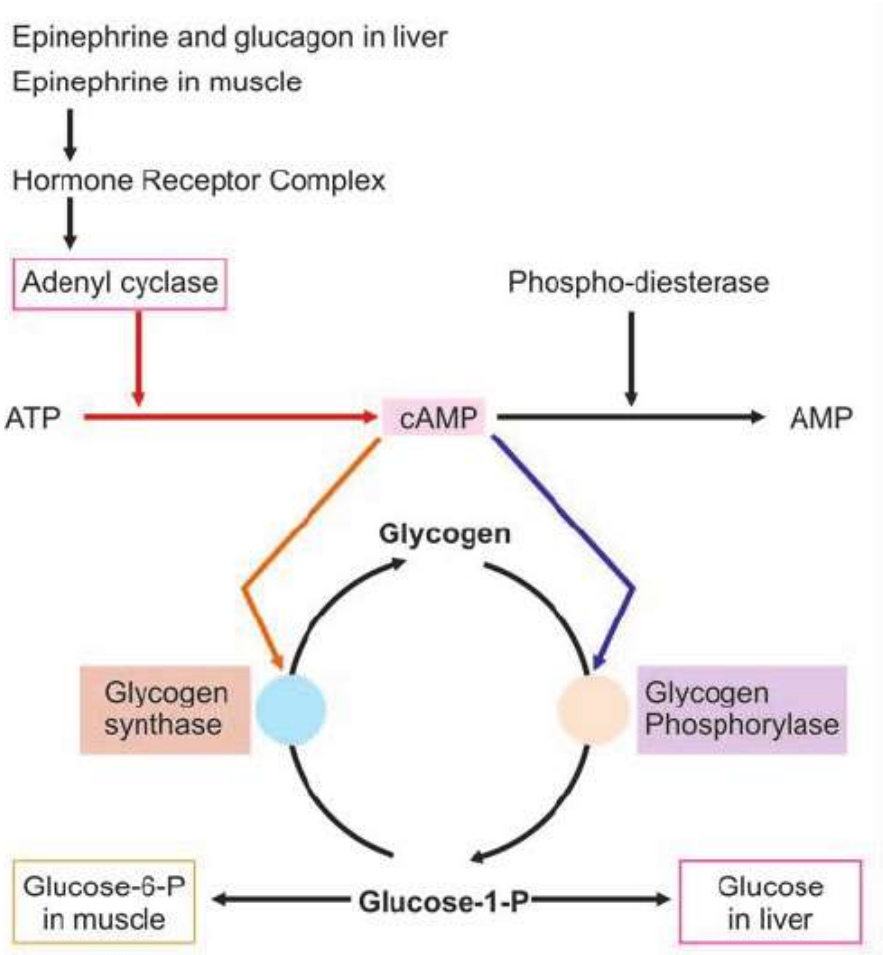
Regulation of Glycogen Metabolism

i. The synthetic and degradative pathways are reciprocally regulated to prevent futile cycles.

ii. The phosphorylated form of glycogen phosphorylase is active; but glycogen synthase becomes inactive on phosphorylation. The covalently modified phosphorylase is active even without AMP. Active (dephosphorylated) glycogen synthase is responsive to the action of glucose-6-phosphate. Covalent modification modulates the effect of allosteric regulators. The hormonal control by covalent modification and allosteric regulation are interrelated.

iii. These hormones act through a second messenger, cyclic AMP (cAMP).

iv. The covalent modification of glycogen phosphorylase and synthase is by a cyclic AMP mediated cascade. Specific protein kinases bring about phosphorylation and protein phosphatases cause dephosphorylation.



Synthesis

DE NOVO SYNTHESIS OF FATTY ACIDS

The process of fatty acid synthesis was studied by Feodor Lynen, who got Nobel prize in 1964. The pathway is referred to as Lynen's spiral.

It is not a reversal of oxidation. There are Important differences in synthesis and breakdown of fatty acids.

1 Fatty acids are synthesized mainly by a de novo synthetic pathway operating in the cytoplasm. So it is referred to as extramitochondrial or cytoplasmic fatty acid synthase system.

2 The major fatty acid synthesised de novo is palmitic acid, the 16C saturated fatty acid. The process occurs in liver, adipose tissue, kidney, brain, and mammary glands.

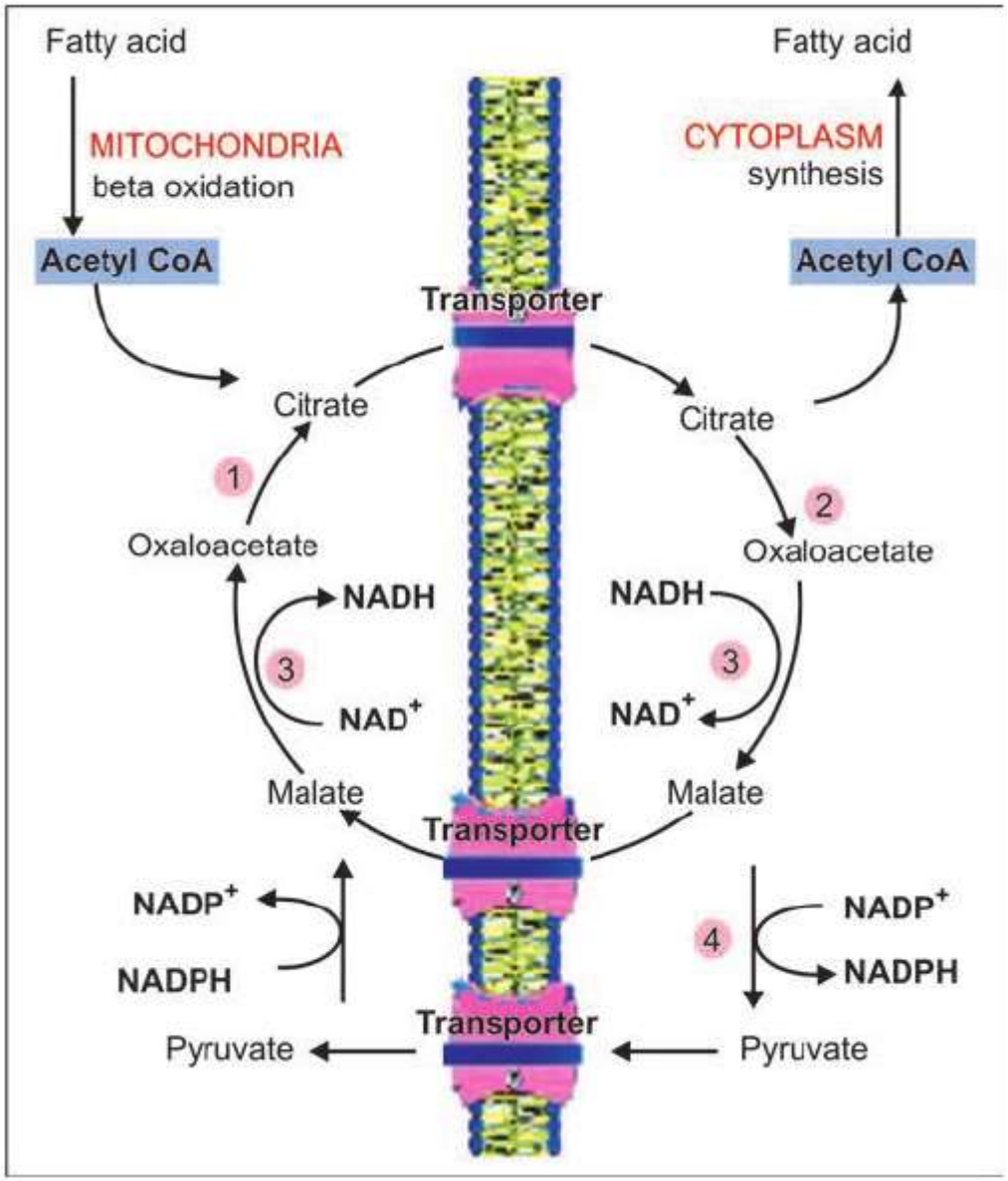
	Beta-oxidation	Fatty acid synthesis
Site	Mitochondria	Cytoplasm
Intermediates	Present as CoA derivatives	Covalently linked to SH group of ACP
Enzymes	Present as inde-pendent proteins	Multienzyme complex
Sequential units	2 carbon units split off as acetyl CoA	2 carbon units added, as 3 carbon malonyl CoA
Co-enzymes	NAD^+ and FAD are reduced	NADPH used as reducing power

Synthesis

DE NOVO SYNTHESIS OF FATTY ACIDS

Transport of Acetyl CoA to Cytoplasm

The starting material for de novo synthesis is acetyl CoA. It is formed inside the mitochondria from pyruvate. The inner membrane is not freely permeable to acetyl CoA. Hence the acetyl CoA units are delivered to the cytoplasm as citrate (yet another shuttle!). Citrate is transported from mitochondria by a tricarboxylic acid transporter. In the cytoplasm, citrate is cleaved to oxaloacetate and acetyl CoA. (The enzyme is ATP citrate lyase.) The xaloacetate can return to the mitochondria as malate or pyruvate.



Synthesis

DE NOVO SYNTHESIS OF FATTY ACIDS

Fatty Acid Synthase (FAS) Complex

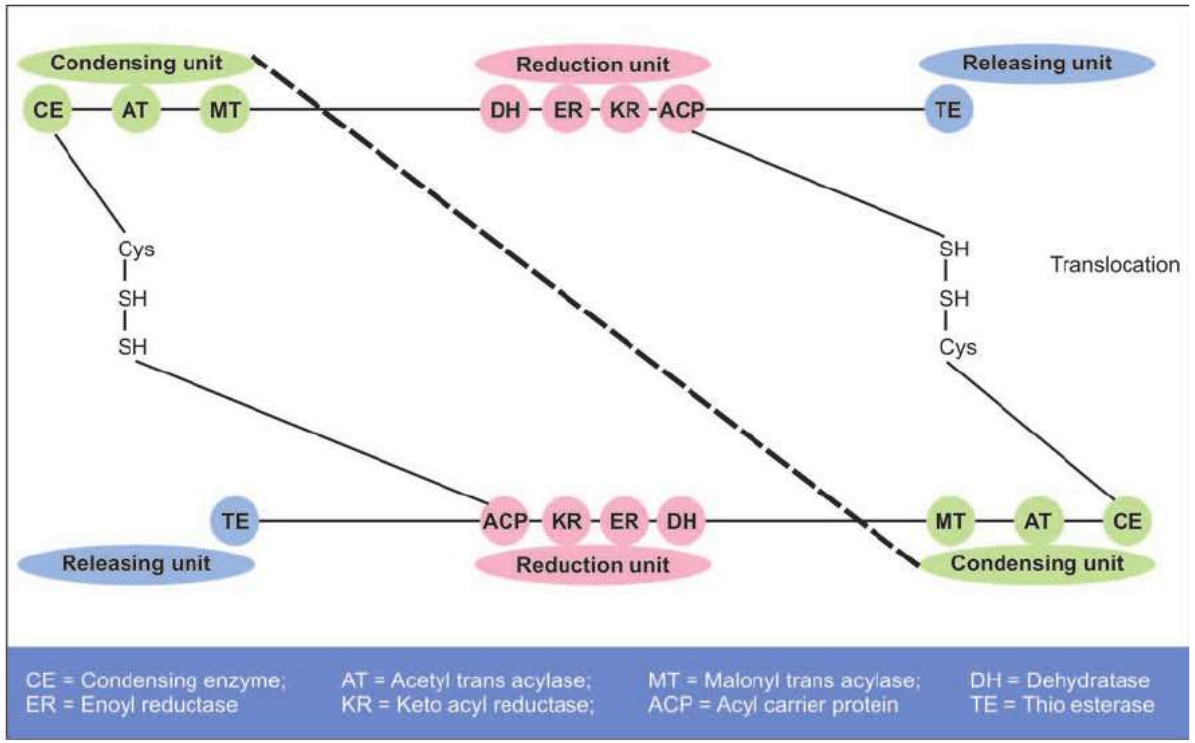
This system exists as a multi-enzyme complex. The enzymes form a dimer with identical subunits. Each subunit of the complex is organised into 3 domains with 7 enzymes.

Advantages of Multi-enzyme Complex

a. Intermediates of the reaction can easily interact with the active sites of the enzymes.

b. One gene codes all the enzymes; so all the enzymes are in equimolecular concentrations.

c. So the efficiency of the process is enhanced.



Synthesis

DE NOVO SYNTHESIS OF FATTY ACIDS

Fatty Acid Synthase (FAS) Complex

1st Domain or Condensing Unit

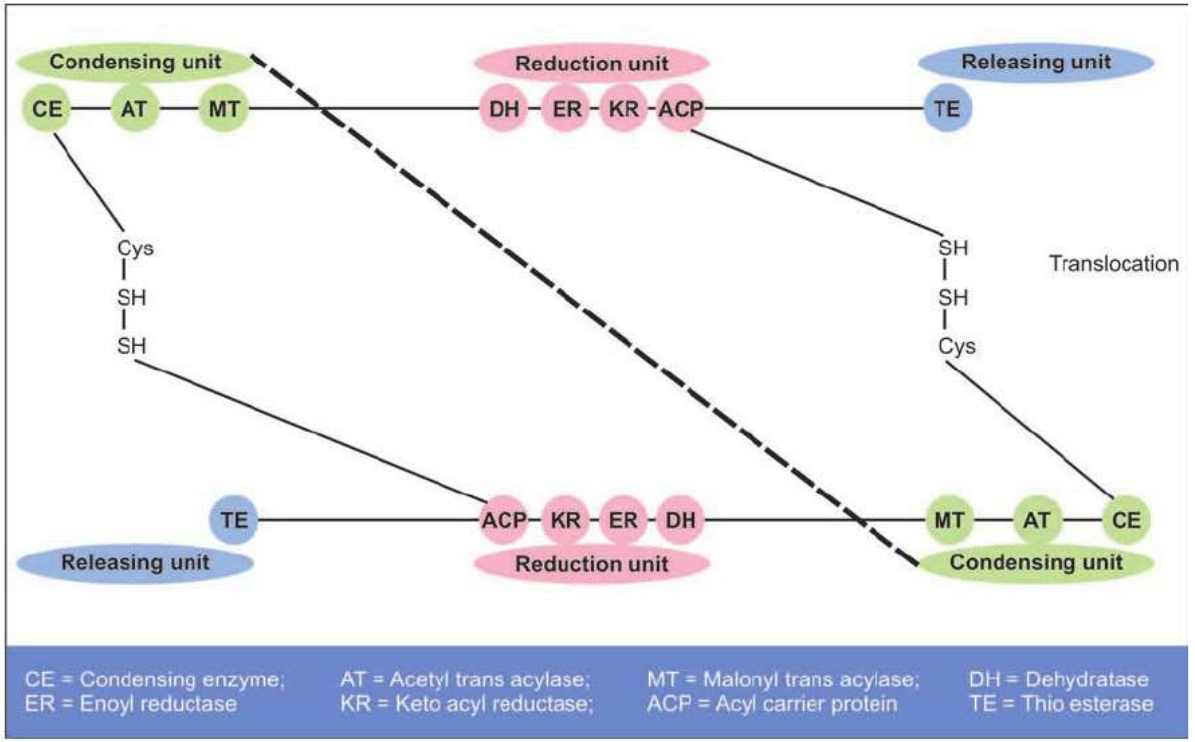
It is the initial substrate binding site. The enzymes involved are condensing enzyme (CE); acetyl transferase (AT) and malonyl trans acylase (MT).

2nd Domain or Reduction Unit

It contains the dehydratase (DH); enoyl reductase (ER); beta-keto acyl reductase (KR) and acyl carrier protein (ACP). The acyl carrier protein is a polypeptide chain having a phospho-panto-theine group, to which the acyl groups are attached in thioester linkage. So ACP acts like the CoA carrier for FA.

3rd Domain or Releasing Unit

It is involved in the release of the palmitate synthesised. It contains thio-esterase (TE) or deacylase.



Synthesis

DE NOVO SYNTHESIS OF FATTY ACIDS

Step 1: Carboxylation of Acetyl CoA

Step 2: Three C and Two C Units are Added

Step 3: Condensation

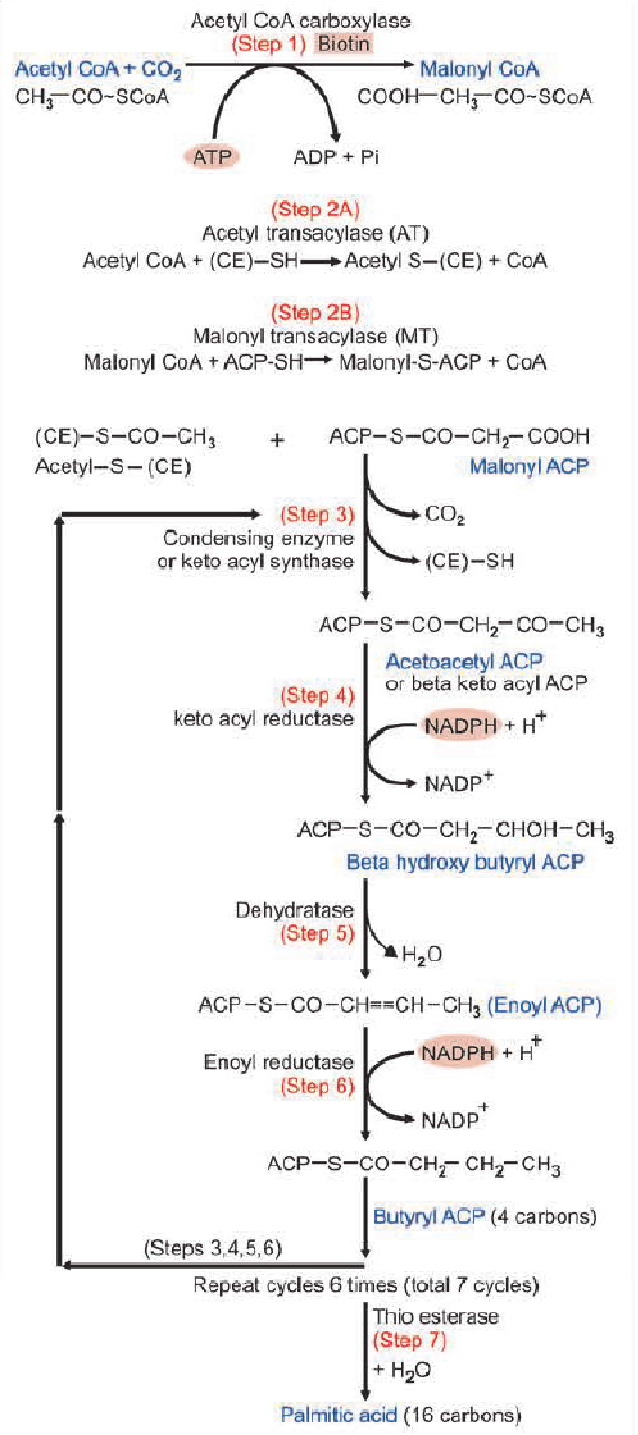
Step 4: Reduction

Step 5: Dehydration

Step 6: Second Reduction

Cycling of Reactions

Step 7: Palmitic Acid is Released



Synthesis

DE NOVO SYNTHESIS OF FATTY ACIDS

Step 1: Carboxylation of Acetyl CoA

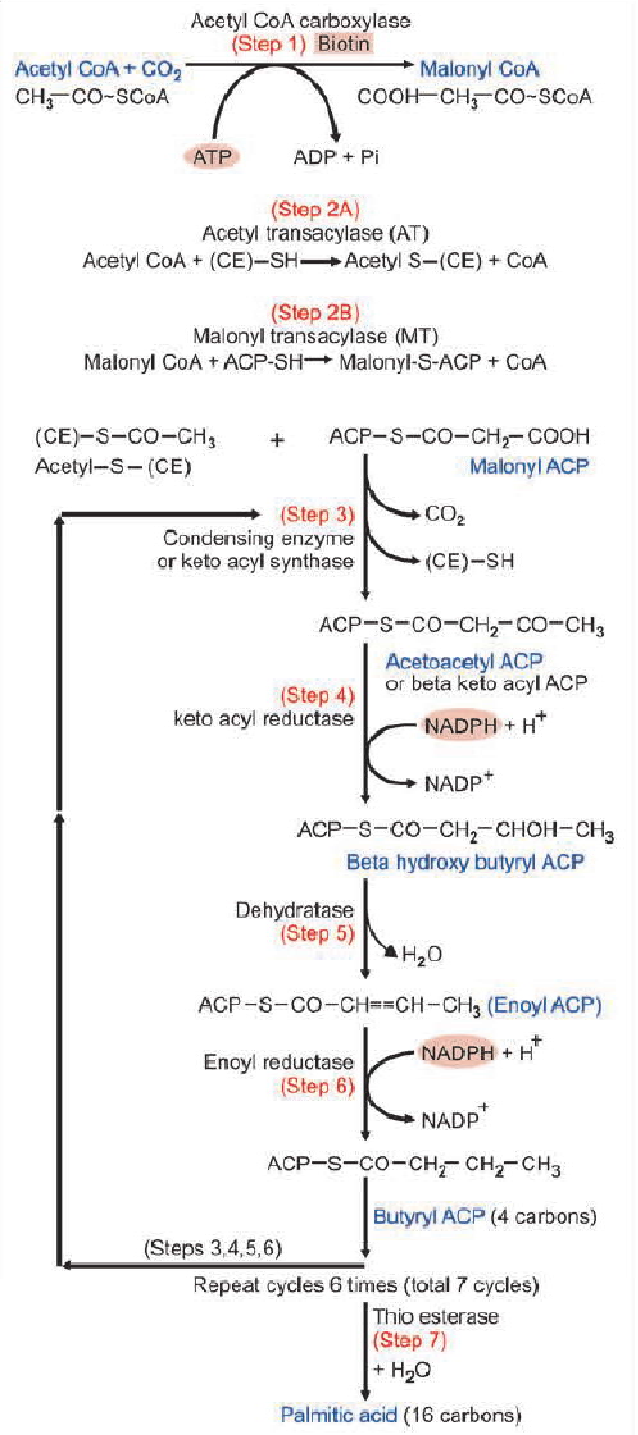
The first step in the fatty acid synthesis is the carboxylation of acetyl CoA to form malonyl CoA.

The 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.

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)**.



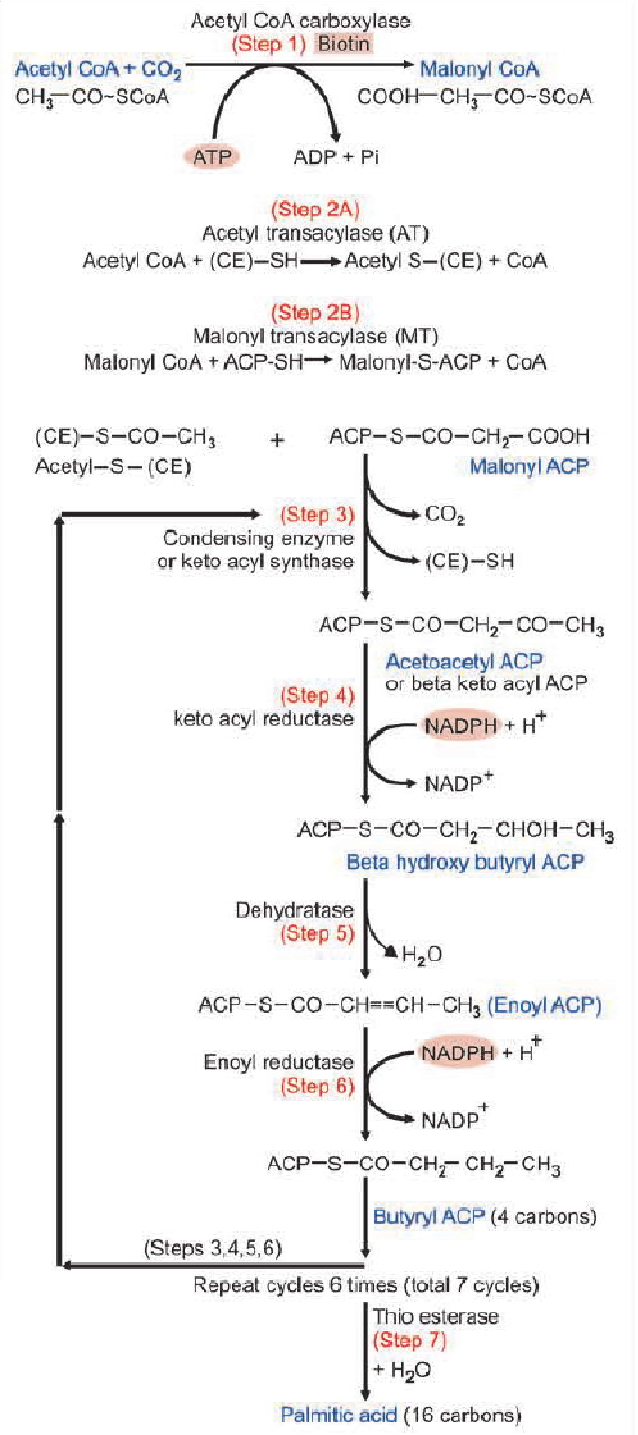
Synthesis

DE NOVO SYNTHESIS OF FATTY ACIDS

Step 2: Three C and Two C Units are Added

2-A: The acetyl transacylase (AT) catalyses 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).

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



Synthesis

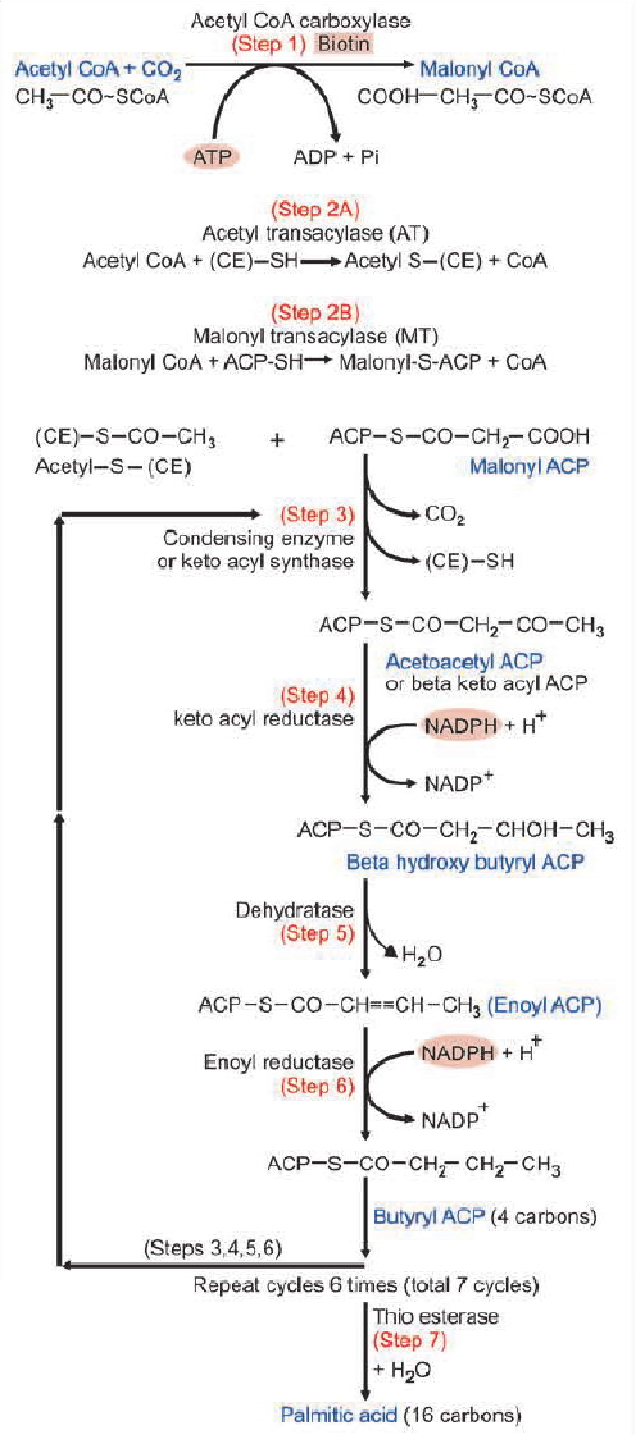
DE NOVO SYNTHESIS OF FATTY ACIDS

Step 3: Condensation

The acetyl (2C) and malonyl (3C) units are condensed to form beta-keto acyl ACP or aceto acetyl ACP (4C). During this process one carbon is lost as CO2 (step 3). The enzyme is called condensing enzyme or keto acyl synthase (CE).

Step 4: Reduction

The acetoacetyl ACP is reduced by NADPH dependent beta-keto acyl reductase (KR) to form beta-hydroxy fatty acyl ACP (step 4).



Synthesis

DE NOVO SYNTHESIS OF FATTY ACIDS

Step 5: Dehydration

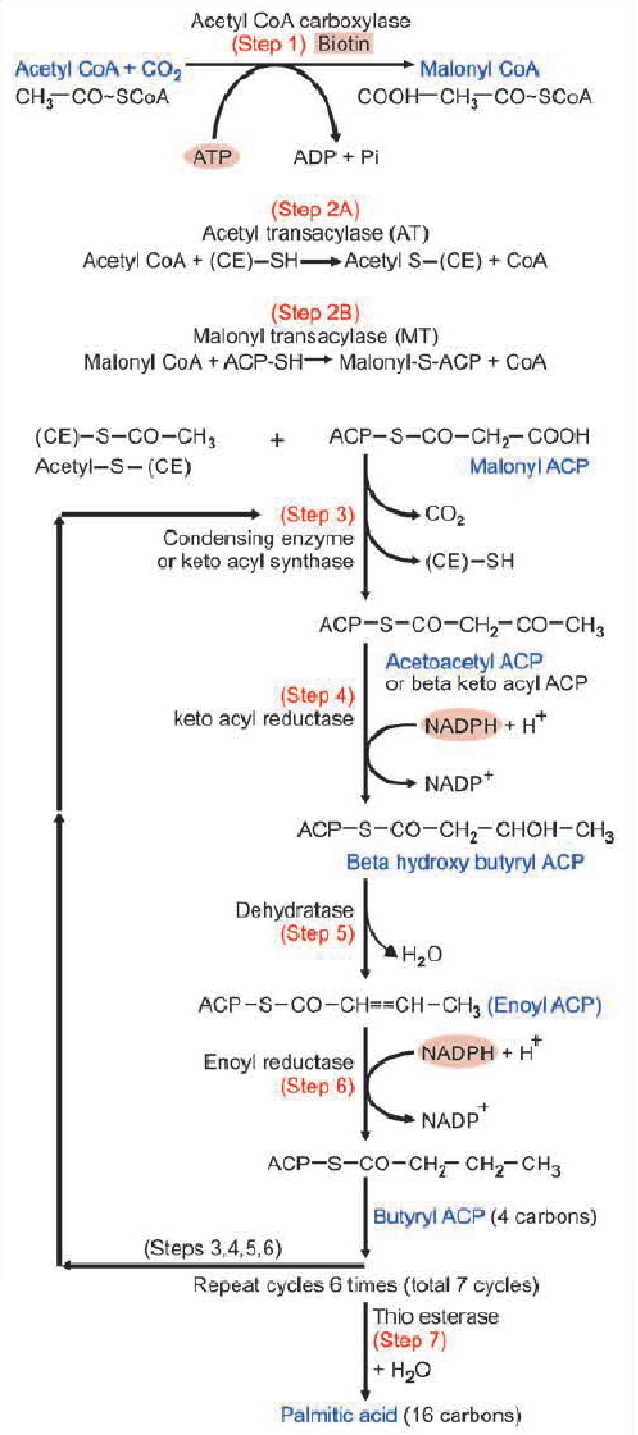
It is then dehydrated by a dehydratase (DH) to form enoyl ACP otherwise known as (alpha beta unsaturated acyl ACP) (step 5).

Step 6: Second Reduction

The enoyl ACP is again reduced by enoyl reductase (ER) utilizing a 2nd molecule of NADPH to form butyryl ACP (step 6).

Cycling of Reactions

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 phospho-pantothenyl SH group. The sequence of reactions, namely condensation, reduction, dehydration and reduction (steps 3,4,5,6) are repeated. The cycles are repeated a total of seven times, till the 16-carbon palmitic acid is formed.

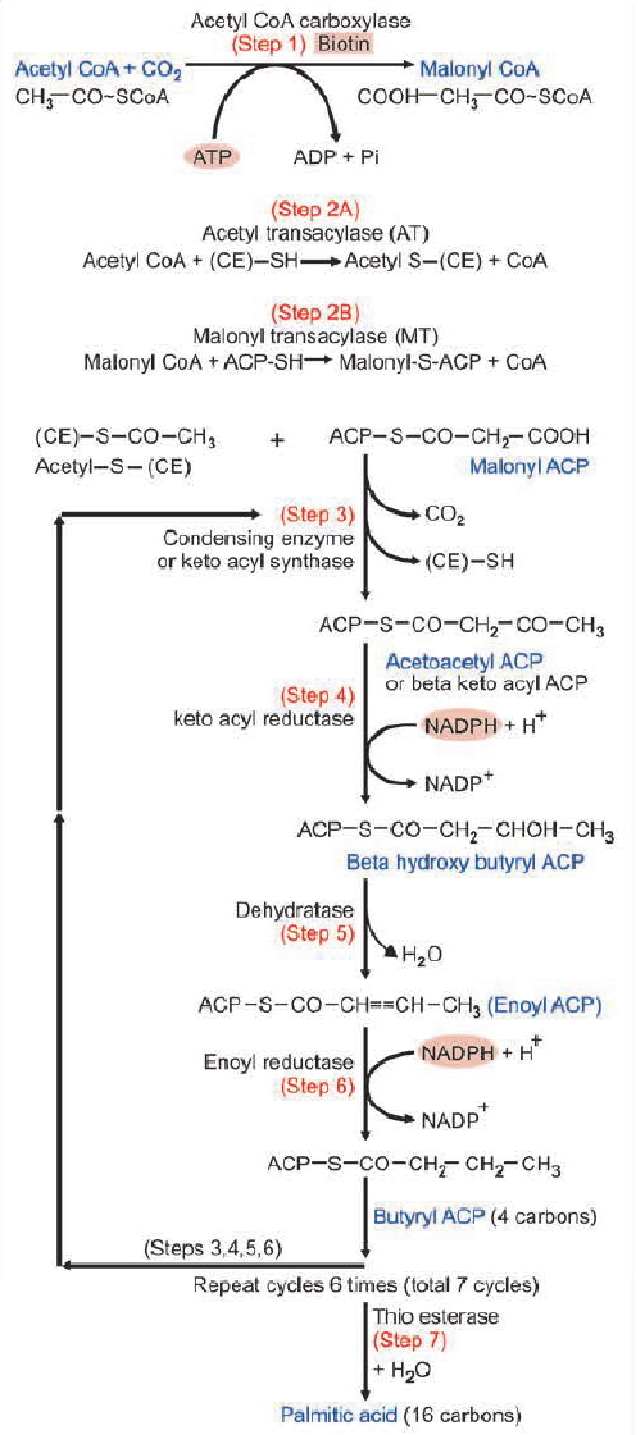


Synthesis

DE NOVO SYNTHESIS OF FATTY ACIDS

Step 7: Palmitic Acid is Released

The thio-esterase or de-acylase activity (TE) releases palmitate from the multienzyme complex (step 7). The end point is Palmitic acid (16 C) in liver and adipose tissue. But in lactating mammary gland, 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.



Summary of De Novo Synthesis

The net reaction of de novo synthesis of fatty acid may be summarized as:

1 Acetyl CoA + 7Malonyl CoA +14NADPH +14H+ →

1Palmitate + 7CO2 +14NADP+ + 8CoA+ 6H2O

Synthesis

DE NOVO SYNTHESIS OF FATTY ACIDS

Co-enzymes of Fatty Acid Synthesis

An important point to remember is that the co-enzyme utilised for de novo synthesis is **NADPH**. The sources of NADPH for fatty acid synthesis are:

1. **Pentose Phosphate Pathway**

This is the main source. Tissues having active lipogenesis (liver, adipose tissue, lactating mammary gland) have an active **HMP shunt pathway** also.

2. **Malic Enzyme**

Malate + NADP+ → Pyruvate + CO2 + NADPH + H+

The reaction also helps to transfer cytoplasmic oxaloacetate to the mitochondria. For every molecule of acetyl CoA delivered to the cytoplasm, one molecule of NADPH is formed and reducing equivalents are generated in cytoplasm.

Synthesis

DE NOVO SYNTHESIS OF FATTY ACIDS

Regulation of Fatty Acid Synthesis

1. Availability of Substrates

Fatty acid synthesis occurs when carbohydrate is abundant and the level of fatty acids is low. The availability of citrate in the cytoplasm is the most important regulatory factor producing a short-term effect.

2. Acetyl CoA Carboxylase

It is the key enzyme; citrate activates this enzyme. The citrate level is high only when both acetyl CoA and ATP are abundant. Covalent modification is another regulatory mechanism. Phosphorylation inactivates acetyl CoA carboxylase (similar to glycogen synthase under the effect of glucagon). Hence fatty acid synthesis decreases when glucose level is low. The enzyme is inhibited by palmitoyl CoA, the end product.

3. Insulin Favors Lipogenesis

Insulin enhances the uptake of glucose by adipocytes and increases the activity of pyruvate dehydrogenase, acetyl CoA carboxylase and glycerol phosphate acyl transferase. Insulin also depresses the hormone sensitive lipase.

4. Glucagon inhibits Lipogenesis

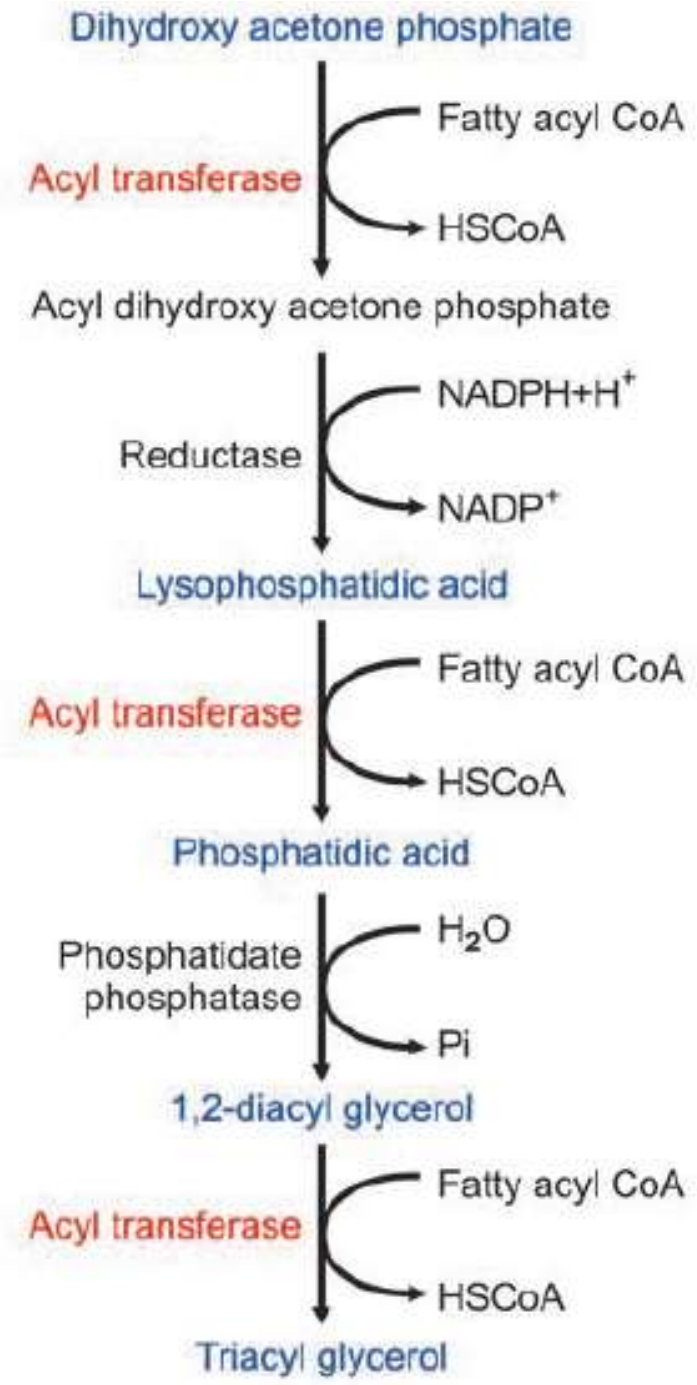
Glucagon and epinephrine inactivate the acetyl CoA carboxylase by phosphorylating the enzyme.

Synthesis

SYNTHESIS OF TRIGLYCERIDES (TAG)

Liver and adipose tissue are the major sites of triacylglycerol (TAG) synthesis. The TAG synthesis in adipose tissue is for storage of energy whereas in liver it is mainly secreted as VLDL and is transported to peripheral tissues. The TAG is synthesised by esterification **of fatty acyl CoA** with **either glycerol-3-phosphate or dihydroxy acetone** phosphate (DHAP). The glycerol part of the fat is derived from the metabolism of glucose. DHAP is a intermediate of glycolysis. Glycerol-3-phosphate may be formed by phos phorylation of glycerol or by reduction of dihydroxy acetone phosphate (DHAP). In adipose tissue, glycerol kinase is deficient and the major source is DHAP derived from glycolysis. However, in liver glycerol kinase is active.

The fatty acyl CoA molecules transfer the fatty acid to the hydroxyl groups of glycerol by specific acyl transferases.

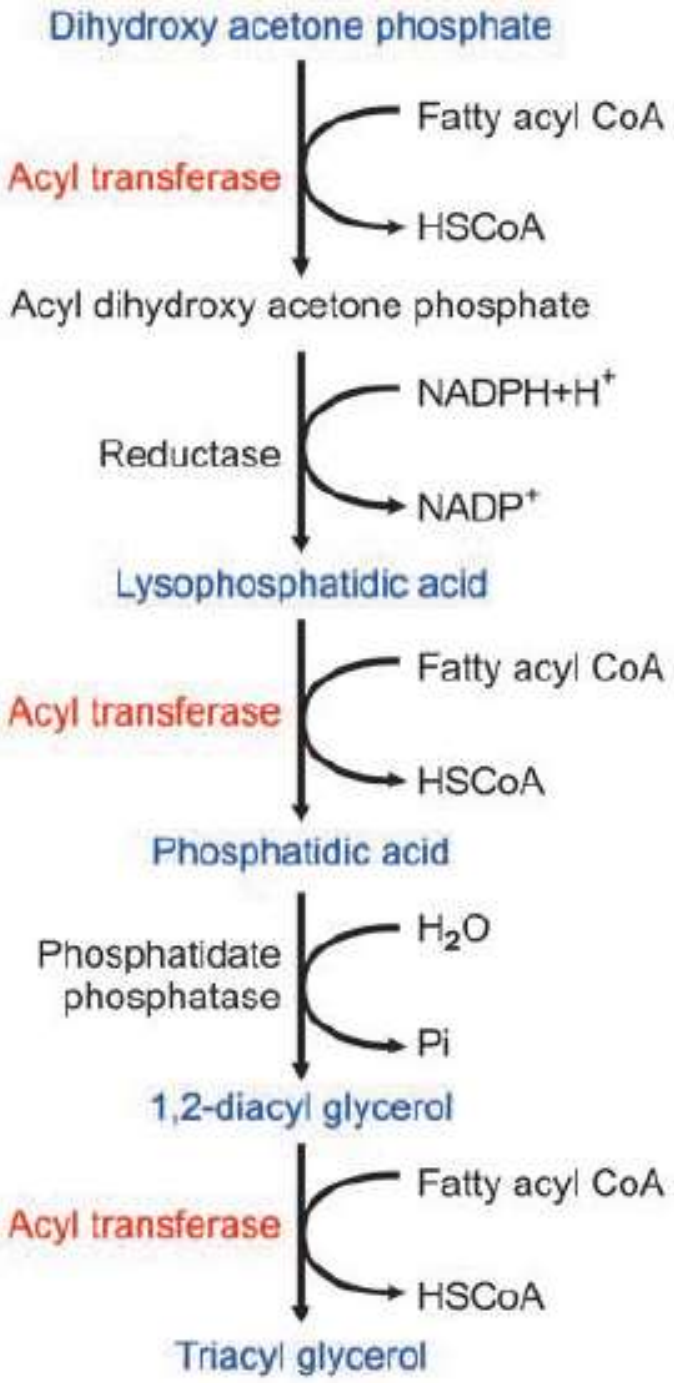


Synthesis

SYNTHESIS OF TRIGLYCERIDES (TAG)

In addition to these two pathways, in the **intestinal mucosal cells** the TAG synthesis occurs by the MAG pathway. The **2-MAG** absorbed is re-esterified with fatty acyl CoA to form TAG.

Esterification of fatty acyl CoA with glycerol phosphate to form triacyl glycerol occurs at a rapid rate during the fed state. Under conditions of fasting, it is seen that synthesis of triacyl glycerol occurs side by side with lipolysis, since the free fatty acid level is high in plasma. The ***glycerol phosphate is derived from the metabolism of glucose in the fed state by channeling dihydroxy acetone phosphate***, an intermediate of glycolysis. In the fasting state, the ***glycerol phosphate is derived from dihydroxy acetone phosphate formed during gluconeogenesis***. The activity of the enzyme PEPCK is enhanced in liver and adipose tissue during conditions of fasting, so that glycerol phosphate is available to esterify and store the excess fatty acid mobilized.



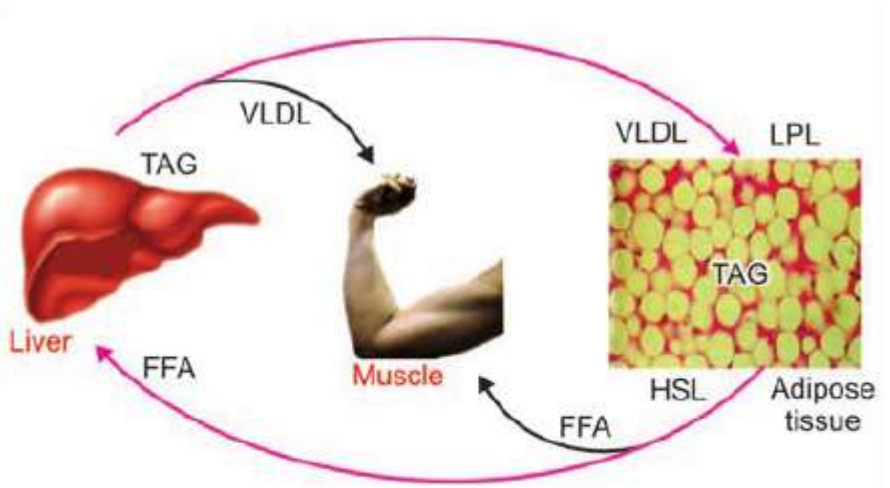
METABOLISM OF ADIPOSE TISSUE

The adipose tissue serves as a storage site for excess calories ingested. The triglycerides stored in the adipose tissue are not inert. They undergo a daily turnover with new triacyl glycerol molecules being synthesized and a definite fraction being broken down.

White Adipose Tissue

It is mainly concerned with energy storage. It is made up of spherical cells, with very few mitochondria. The triglycerides form the major component of white adipose tissue (about 80%) with oleic acid being the most abundant fatty acid (50%).

Brown adipose tissue is involved in thermogenesis. Brown adipose tissue cells are polygonal with more abundant cytoplasm. The brown color is due to the presence of numerous mitochondria. It is primarily important in newborn human beings and adult hibernating animals.

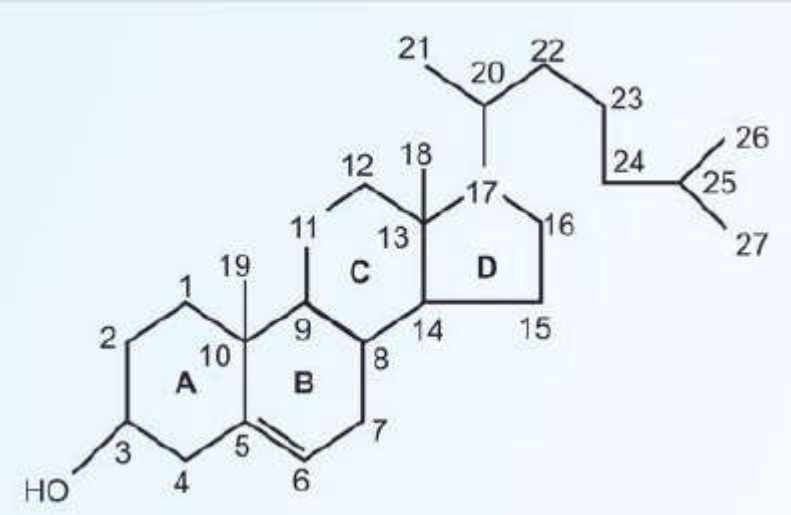


TAG = triacyl glycerol; VLDL = very low density lipoprotein;
LPL = lipoprotein lipase; HSL = hormone sensitive lipase;
FFA = free fatty acids

Liver-Adipose Tissue Axis

Liver produces fatty acid and TAG (triacyl glycerol), which is transported as VLDL (very low density lipoprotein) in the blood. The fatty acids from VLDL are taken up by adipose tissue with the help of lipoprotein lipase, and stored as TAG. This neutral fat is hydrolysed by hormone sensitive lipase into NEFA (NONESTERIFIED FA), which is carried by albumin in blood. The NEFA is utilised by the peripheral tissues, excess of which can be taken up by liver cells. Thus there is a constant flux of fat molecules from liver to adipose tissue and back.

Cholesterol/sterols/steroids



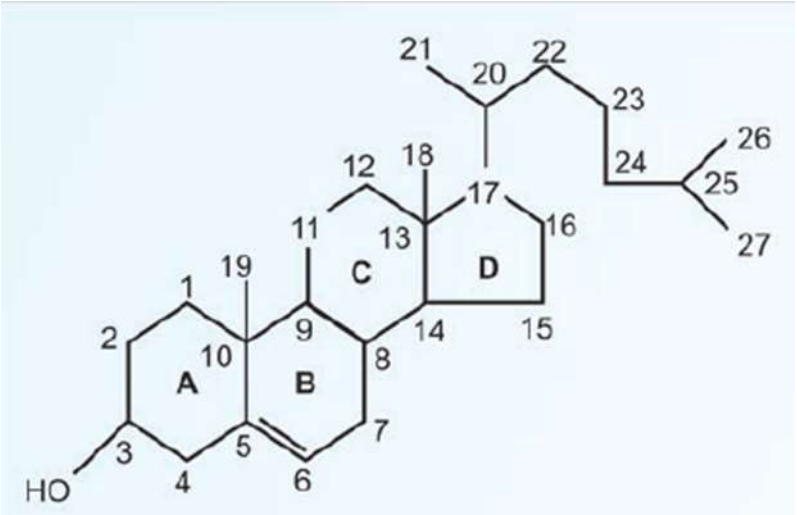
1. **Cell membranes:** Cholesterol is a component of membranes and has a modulating effect on the fluid state of the membrane.
2. **Nerve conduction:** Cholesterol has an insulating effect on nerve fibers.
3. **Bile acids and bile salts** are derived from cholesterol. Bile salts are important for fat absorption.
4. **Steroid hormones:** Glucocorticoids, androgens and estrogens are from cholesterol.
5. **Vitamin D_3** is from 7-dehydro-cholesterol.
6. **Esterification:** The OH group of cholesterol is esterified to fatty acids to form cholesterol esters. This esterification occurs in the body by transfer of a PUFA moiety by **lecithin cholesterol acyl transferase.**

Almost all nucleated cells (including arterial walls) can synthesise cholesterol. It is widely distributed in the body. In a 70 kg man, a total of about 140 g of cholesterol is available; which is roughly distributed as 30 g in brain and nerves, 30 g in muscles, 30 g in adipose tissue, 20 g in skin, 10 g in blood, 10 g in liver and spleen, 5 g in bone marrow, 3 g in alimentary tract, and 2 g in adrenal gland.

It is the most important animal steroid from which other steroid compounds are formed. Cholesterol is widely distributed in animal tissues. It is absent in prokaryotes.

Structure

All steroids have ***cyclopentano perhydro phenanthrene ring system***. It is a fused ring system made up of 3 cyclohexane rings designated as A, B and C and a cyclopentane ring D. The six-membered rings are in a phenanthrene arrangement.



Total 27 carbon atoms.

One hydroxyl group at third position which is characteristic of all sterols. The OH group is beta-oriented, projecting above the plane of ring.

Double bond between carbon atoms 5 and 6.

An ***eight carbon side chain***, beta-oriented, attached to 17th carbon.

BIOSYNTHESIS OF CHOLESTEROL

All carbon atoms of cholesterol are derived from acetyl CoA (Konrad Bloch, 1940, Nobel prize in 1964).

The biosynthetic pathway was described by
Sir John Cornforth and Vladimir Prelog; both of them got Nobel prizes in 1975.

The major sites of synthesis of cholesterol are liver, adrenal cortex, testes, ovaries and intestine. All nucleated cells can synthesise cholesterol, including arterial walls. The enzymes involved in the synthesis of holesterol are partly located in the endoplasmic reticulum and partly in the cytoplasm.

Step 1: Condensation

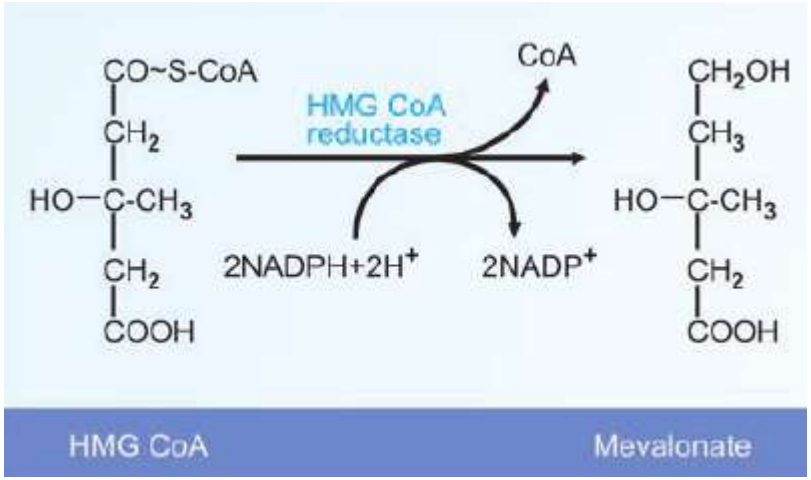
The acetyl CoA is provided by the ATP-citrate lyase reaction as in the case of fatty acid synthesis. Two molecules of acetyl CoA condense to form acetoacetyl CoA (CYTOPLASM)

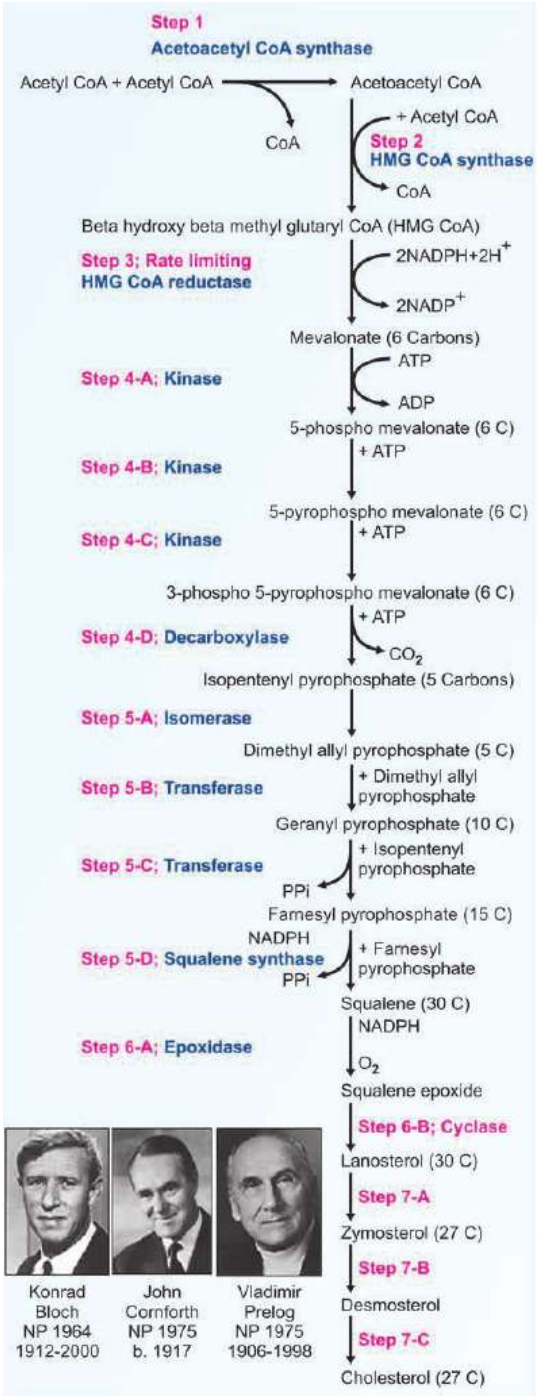
Step 2: Production of HMG CoA

A third molecule of acetyl CoA condenses with acetoacetyl CoA to form beta-hydroxy beta-methyl glutaryl CoA (HMG CoA) (CYTOPLASM)

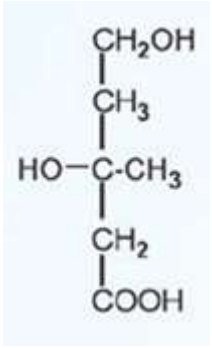
Step 3: The Committed Step

The reduction of HMG CoA to mevalonate is catalysed by HMG CoA reductase. It is a microsomal (endoplasmic reticulum) enzyme. It uses 2 molecules of NADPH. **RATE-LIMITING**





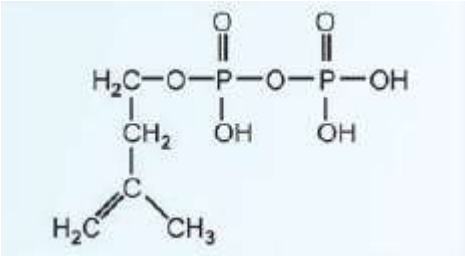
Step 4: Production of 5 Carbon Unit



EXTENSIVE PHOSPHORYLATION

5-PYRO + 3PHOSPHO

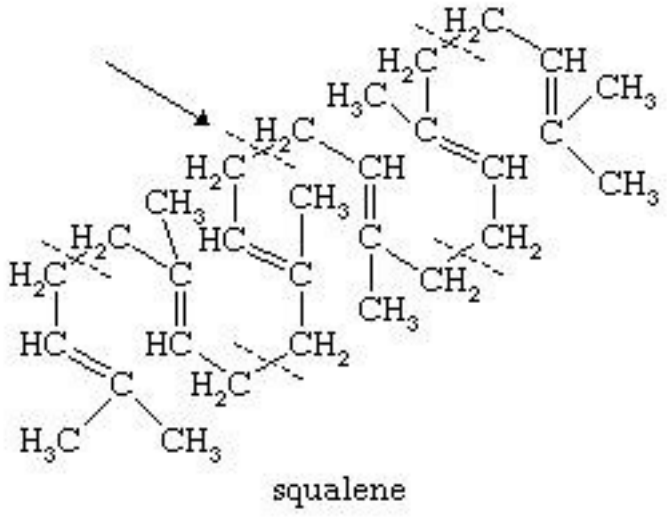
DECARBOXYLATION

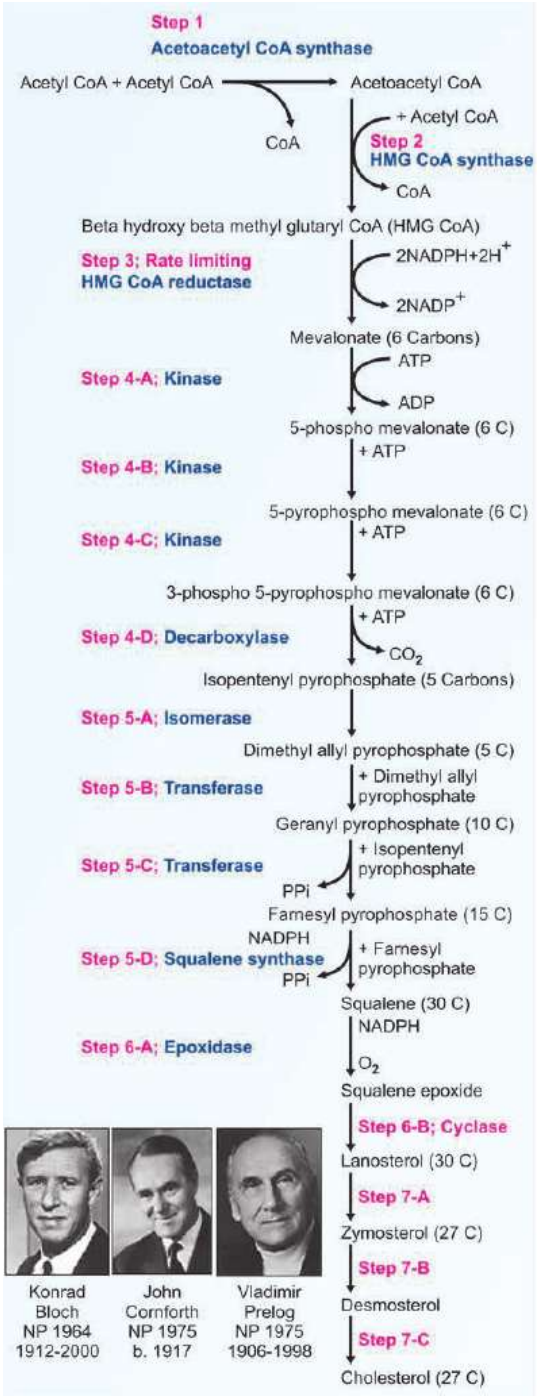


Step 5: Condensation of 5-Carbon Units

Thus, 6 numbers of 5-carbon units are condensed to form a 30 carbon compound, **Squalene**. In summary

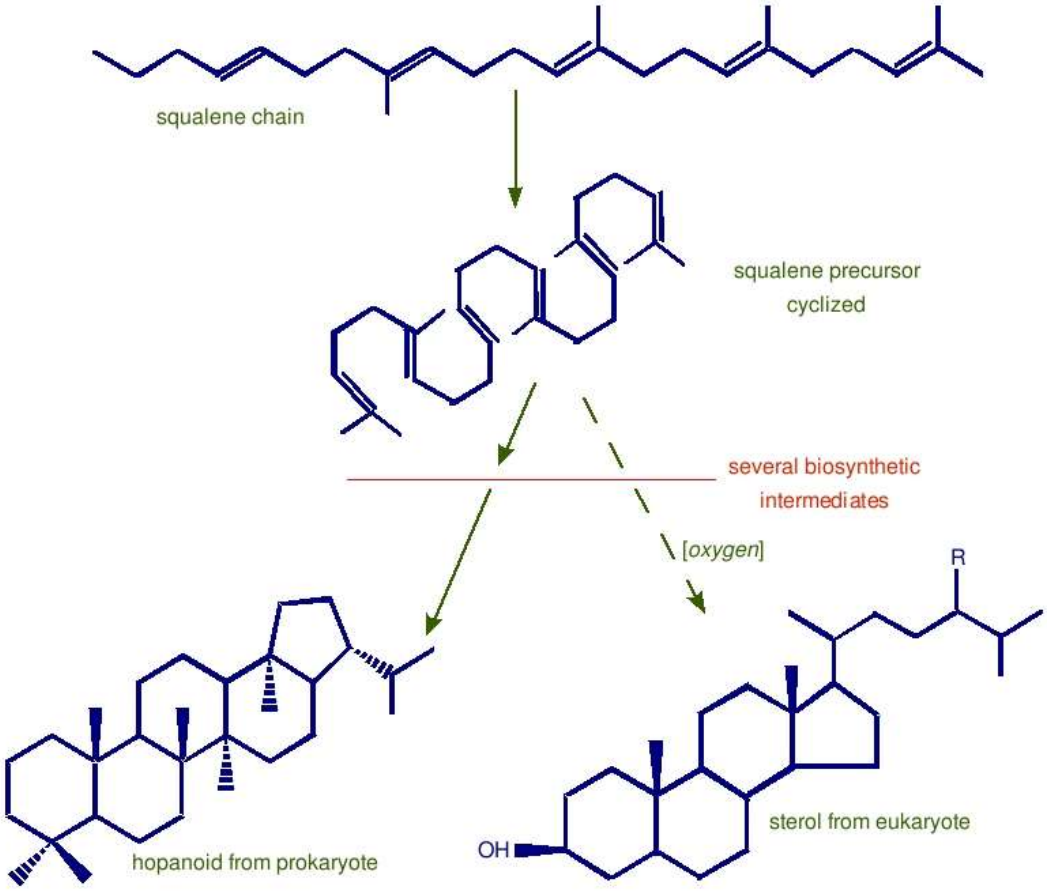
5C + 5C→10C; 10C+5C→15C; 15C+15C → 30C

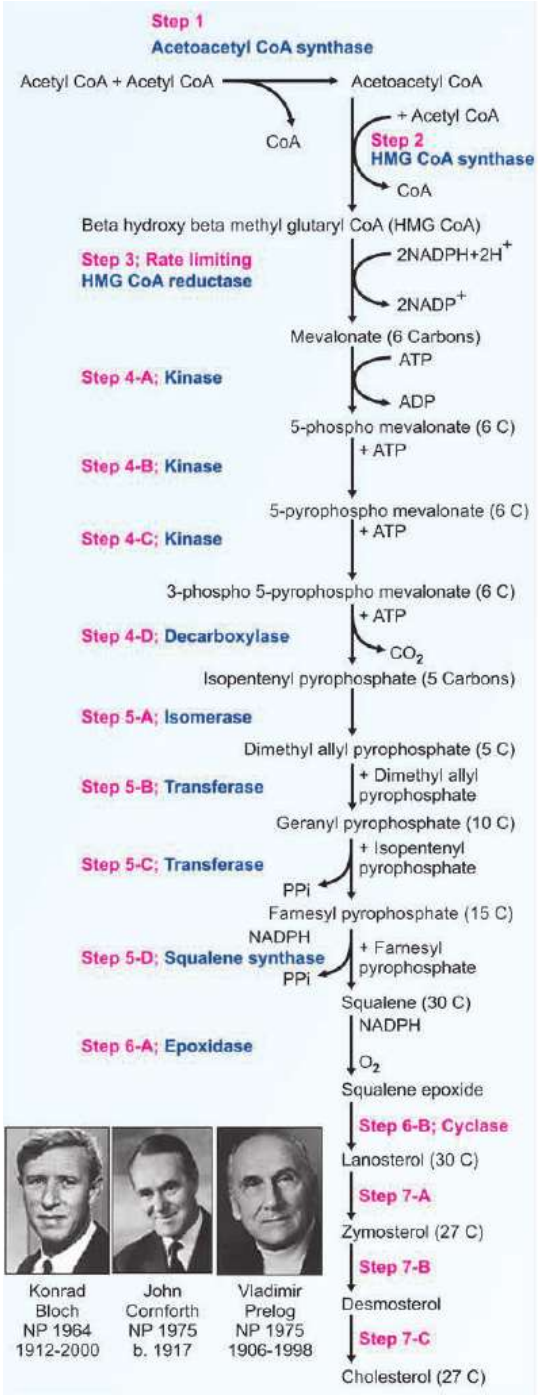




Step 6: Cyclization = EPOXIDE + CYCLIZATION

Step 7: Cutting to size (REMOVAL OF methyl GROUPS, MIGRATION/FORMATION OF DOUBLE BONDS)





Literature biochemistry

1. Lehninger Principles of Biochemistry (Nelson D.L., Cox M.M.)
2. Principles and Techiniques of Biochemistry and Molecular Biology (Wilson K., Walker J.)