

Ministry of Health of Ukraine
Zaporizhzhya State Medical University
Biochemistry & Laboratory Diagnostics Department

Metabolism of lipids and its regulation

*A manual for independent work at home and in class
for students of International Faculty (the second year of study)
speciality: 7.120 10001 «General Medicine»*

Zaporizhzhya

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The manual is created to help students of international faculty (the second year of study) speciality: 7.120 10001 «General Medicine» in the study of Biochemistry. It was confirmed on the Central Methodological Council of ZSMU on «_____» _____2015, the protocol №_____

This manual is recommended to use for students for independent work at home and in class.

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INTRODUCTION

The educational process for students of medical department requires the use not only the basic literature but also that one which is discussed as additional literature sources. This is because each day we have new scientific researches in biochemistry, later which can improve our understanding of theoretical questions of biochemistry. Sometimes it is difficult for students to find out the main important notions for study of biochemistry in basic literature that is recommended. This textbook is proposed by authors as additional manual for study of biochemistry to be as the helper in study of lipids metabolism and its regulation in human organism.

Authors

LIPOPROTEINS OF BLOOD PLASMA. METABOLISM OF TRIACYLGLYCEROLS AND OF GLYCEROPHOSPHOLIPIDS

Lipids are some organic compounds non-soluble in the water but soluble in organic solvents represented in human tissues.

Among them are naturally-occurring molecule, such as fats, oils, waxes, cholesterol, steroids, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, triacylglycerols phospholipids, and others.

Although the term “lipids” is sometimes used as a synonym for fats, fats are a subgroup of lipids called triglycerides and should not be confused with the term fatty acid. Lipids also encompass molecules such as fatty acids and their derivatives (including tri-, di-, and monoglycerides and phospholipids), as well as other sterol-containing metabolites such as cholesterol.

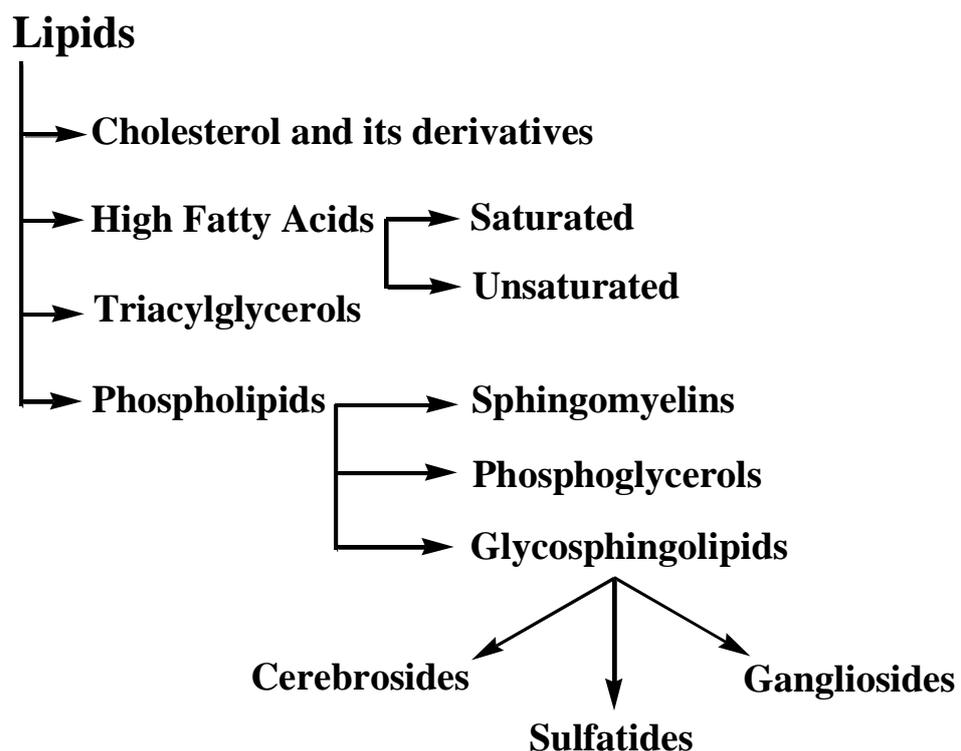


Figure 1. The classification of lipids.

The main biological functions of lipids include energy storage, acting as structural components of cell membranes, and participating as important signaling molecules.

hydration that is associated with stored polysaccharides (2 g per gram of polysaccharide).

Phospholipids

A 1,2-diacylglycerol that has a phosphate group esterified at carbon atom 3 of the glycerol backbone is a **glycerophospholipid**, also known as a *phosphoglyceride* or a *phosphatidic acid* (Fig. 3). These lipids form one of the largest class of natural lipids and one of the most important. They are components of cell membranes and are found in small concentrations in other parts of cells, too. It should be noted that all glycerophospholipids are members of the broader class of lipids known as **phospholipids**.

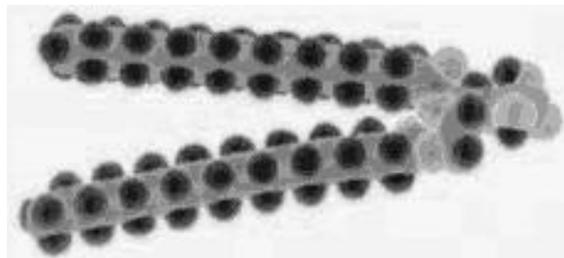
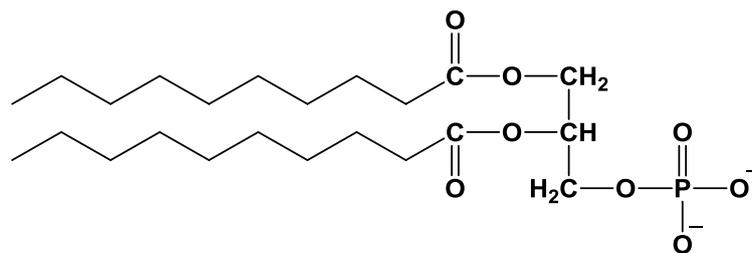


Figure 3. Phosphatidic acid, the parent compound for glycerophospholipids.

Phosphatidic acid, the parent compound for the glycerol-based phospholipids, consists of glycerol-3-phosphate, with fatty acids esterified at the 1- and 2-positions. Phosphatidic acid is found in small amounts in most natural systems and is an important intermediate in biosynthesis of more common glycerophospholipids (Fig. 4). In these compounds, a variety of polar groups are esterified to the phosphoric acid moiety of the molecule. The phosphate, together with such esterified entities, is referred to as a “head” group. Phosphatides with

choline or ethanolamine are referred to as **phosphatidylcholine** (known commonly as **lecithin**) or **phosphatidylethanolamine**, respectively. These phosphatides are two of the most common constituents of biological membranes. Other common *head groups* found in phosphatides include glycerol, serine, and inositol. Another kind of glycerol phosphatide found in many tissues is **diphosphatidylglycerol**. First observed in heart tissue, it is also named **cardiolipin**. In cardiolipin, a phosphatidylglycerol is esterified through the C-1 hydroxyl group of the glycerol moiety of the head group to the phosphoryl group of another phosphatidic acid molecule.

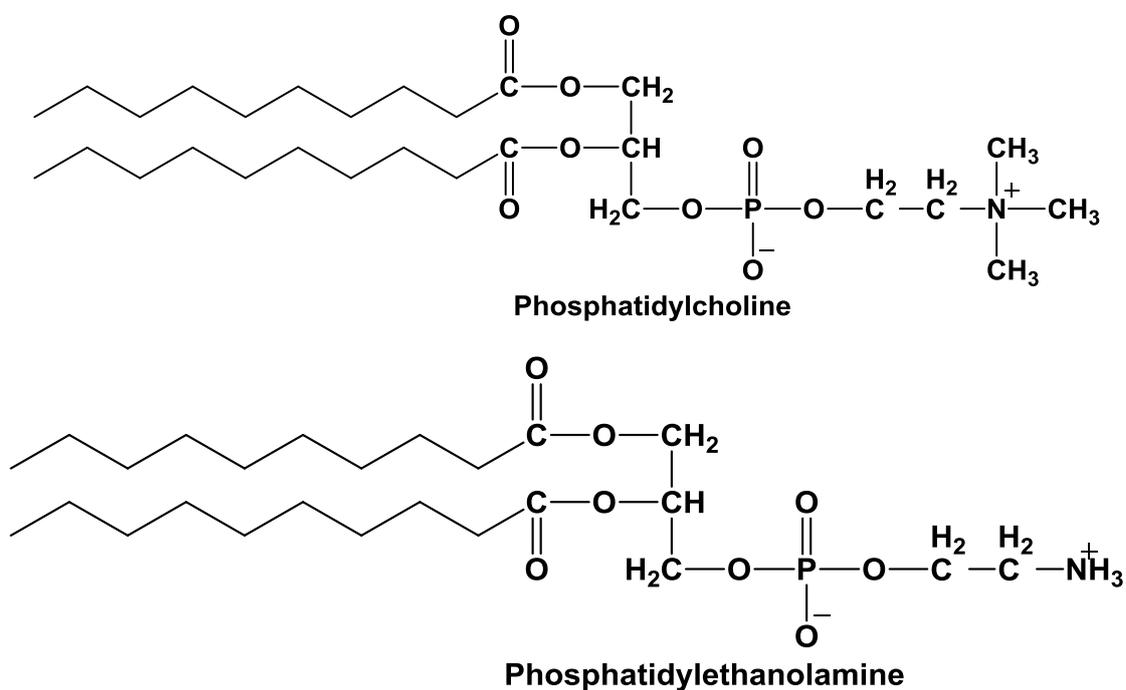


Figure 4. Structures of several glycerophospholipids.

Phosphatides exist in many different varieties, depending on the fatty acids esterified to the glycerol group. As we shall see, the nature of fatty acids can greatly affect the chemical and physical properties of phosphatides and the membranes that contain them. In most cases, glycerol phosphatides have a saturated fatty acid at position 1 and an unsaturated fatty acid at position 2 of glycerol. Thus, **1-stearoyl-2-oleoyl-phosphatidylcholine** is a common constituent in natural membranes, but **1-linoleoyl-2-palmitoylphosphatidylcholine** is not.

Sphingolipids

These lipids are found in the cellular membranes of all eukaryotic cells, although the concentration is highest in cells of central nervous system. Sphingolipids do not have a glycerol backbone; they are formed from aminoalcohol sphingosine. (Fig. 5). Sphingosine is derived from serine and a specific fatty acid, palmitate. Ceramides are amides formed from sphingosine by attaching fatty acid to its amino group. Various sphingolipids are then formed by attaching different groups to hydroxyl group on ceramide. As reflected in the names for cerebrosides and gangliosides, these sphingolipids contain sugars attached to hydroxyl group of ceramide through glycosidic bonds. They are glycolipids (more specifically, glycosphingolipids). Sphingomyelin, which contains a phosphorylcholine group attached to ceramide, is a component of cell membranes and the myelin sheath around neurons.

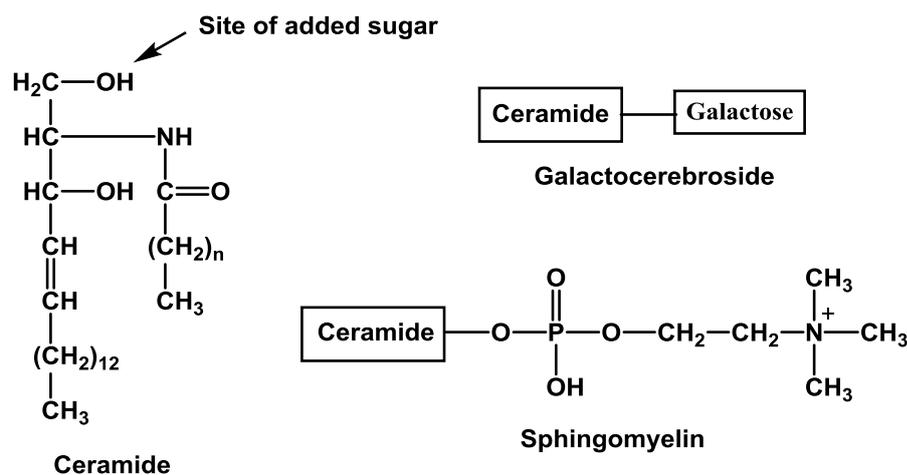


Figure 5. Sphingolipids, derivatives of ceramide.

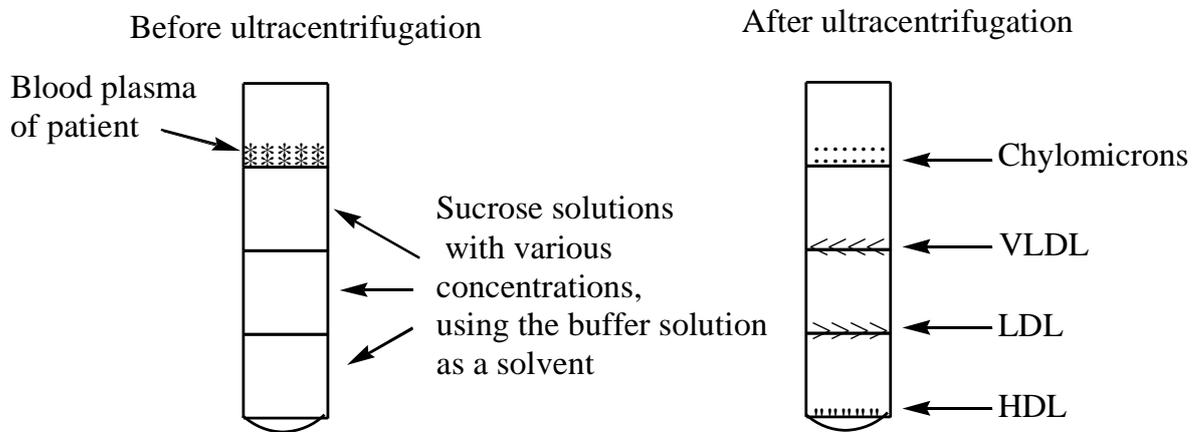
The investigation methods for lipids and lipoproteins

Two main methods for separation of lipids and lipoproteins are used: ultracentrifugation and electrophoresis.

Ultracentrifugation

Sucrose solutions with various concentrations are added step by step to the test centrifugal tube and the blood plasma of patient is plotted on the surface of this

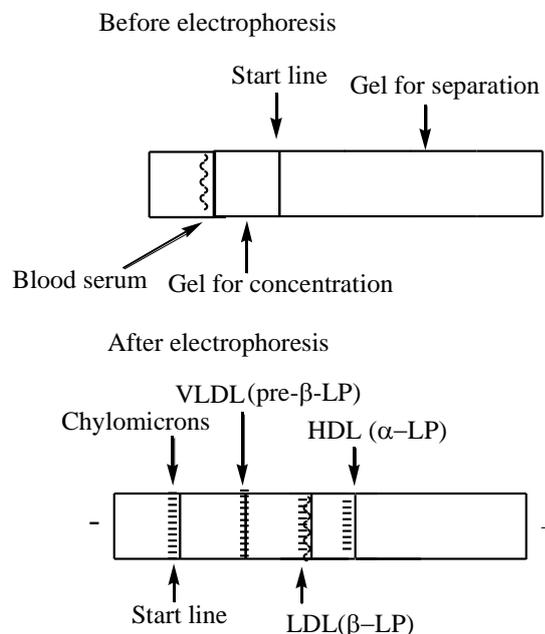
mixture. Then there is the centrifugation using special centrifuge (~ 24 hours) that gives 50000g.



Then there is the ability to take any fraction of lipoproteins for further study.

Electrophoresis

Poly Acryl Amide Gel (PAAG) may be used as a carrier for plotting of blood serum (0,2 mL). pH of buffer solution must be about 7,4-7,6 and after electrophoresis and painting of fractions we can see the result:



Plasma lipoproteins: classification, structure, composition and function

The term lipoprotein can describe any protein that is covalently linked to lipid groups (e.g., fatty acids or prenyl groups), it is most often used for a group of

molecular complexes found in the blood plasma of mammals (especially humans). Plasma lipoproteins transport lipid molecules (triacylglycerols, phospholipids, and cholesterol) through the bloodstream from one organ to another. Lipoproteins also contain several types of lipid-soluble molecules (fat-soluble vitamins A, E, D, K and several carotenoids) (Fig. 6). The protein components of lipoproteins are named **apolipoproteins** or **apoproteins**.

The most hydrophobic TG and cholesterol esters are placed in site of those lipoprotein particles named **micella**. Phospholipids create monolayer turned to around medium. Proteins are placed mainly on the surface of those micella to be involved in receptor-dependent endocytosis.

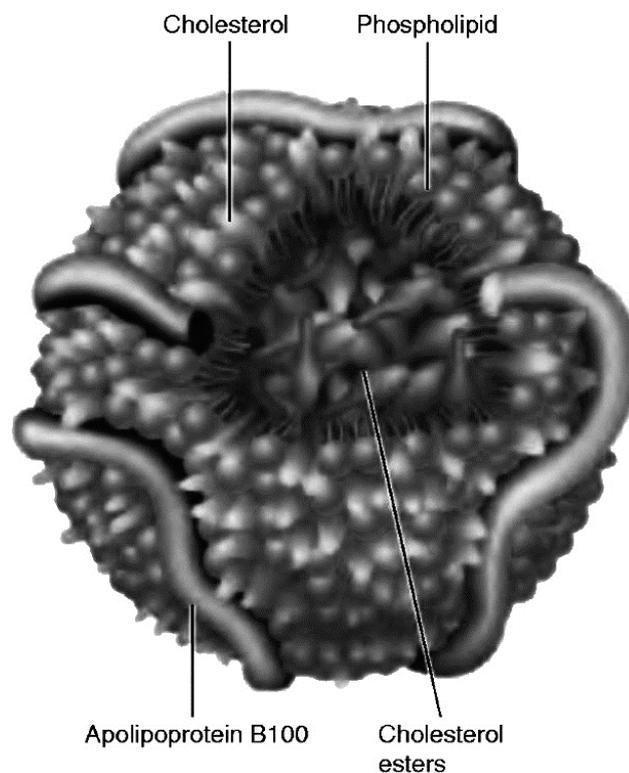


Figure 6. Plasma Lipoprotein micella structure.

The small particles of plasma lipoprotein, which carry triacylglycerols, may be separated according to their densities by centrifugation. They have been classified into five groups of increasing density: **chylomicrons (Chm)**, very low density lipoproteins (**VLDL**), intermediate density lipoproteins (**IDL**), low density (**LDL**), and high density lipoproteins (**HDL**) (Figure 7). Each lipoprotein particle contains one or more apolipoproteins, whose sizes vary from the enormous 4536-

residue apoB-100 to apoC-II and apoC-III, each of which contains just 79 residues, and the 57-residue apoC-I (Figure 8).

Figure 7. Classes of Lipoprotein Particles

Class	Diameter (nm)	Density (g/ml)	Composition (weight %)*				
			Surface components			Core lipids	
			Protein	Phospho-lipid	Cholesterol	Cholesteryl esters	Triacyl-glycerol
Chylo-microns	75-1200	0.930	2	7	2	3	86
VLDL	30-80	0.930-1.006	8	18	7	12	55
IDL	25-35	1.006-1.019	19	19	9	29	23
LDL	18-25	1.019-1.063	22	22	8	42	6
HDL2	9-12	1.063-1.125	40	33	5	17	5
HDL3	5-9	1.125-1.210	45	35	4	13	3
Lp(a)	25-30	1.040-1.090					

*Data from Havel, R. J., and Kane, J. P. (1995) in *The Metabolic and Molecular Bases of Inherited Disease*, 7th ed., Vol. II (Scriver C. R., Beaudet A.L., Sly W. S., and Valle D., eds), pp. 1841 – 1852, McGraw-Hill, New York.

Chylomicrons (Chm), which are assembled by the intestinal mucosa, function to keep exogenous triacylglycerols and cholesterol absorbed from the gastrointestinal tract. Precursors (**nascent** forms – newly released, synthesized, immature) involved into different interactions during their circulation in the blood stream to be converted to **remnant** (parts left over after the loss of components) forms which are ready for the degradation. These lipoproteins are released into the intestinal lymph (known as chyle), which are transported through the lymphatic vessels before draining into the large body veins via the thoracic duct. After a fatty meal, the otherwise clear chyle takes on a milky appearance in the blood.

Chm reach the liver across vena porta and adhere to binding sites on the inner surface (endothelium) of the capillaries in skeletal muscle and adipose tissue. There, within minutes after entering the bloodstream, the chylomicron

triacylglycerols are hydrolyzed through the action of lipoprotein lipase (LPL), an extracellular enzyme that is activated by apoC-II. Then tissues take up the liberated monoacylglycerol and fatty acid hydrolysis products. The chylomicrons shrink as their triacylglycerols are progressively hydrolyzed until they are reduced to cholesterol-enriched chylomicron remnants. The chylomicron remnants reenter the circulation by dissociating from the capillary endothelium and are subsequently taken up by the liver. Utilization of Chm is due to apoE-receptor depended endocytosis. It should be noted that any lipoprotein class is degraded due to the same mechanism. Chm therefore function to deliver dietary triacylglycerols and cholesterol to the liver, muscle and adipose tissue (Fig. 9).

Figure 8. Properties of Major Plasma Apolipoproteins

Designation	No residues	Mass (kDa)	Source	Function
A-I	243	29		Major HDL protein, cofactor of LCAT
A-II	–	17.4	Liver and intestine	Unknown
A-IV	376	44.5	Intestine	Unknown
B-100	4536	513	Liver	VLDL formation; ligand for LDL receptor
B-48	2152	241	Intestine	Chylomicron formation, ligand for liver Chm receptor
C-I	57	6.6	Liver	Inhibition of Cholesteryl ester transfer protein
C-II	79	8.9	Liver	Cofactor for lipoprotein lipase
C-III	79	8.8	Liver	Inhibits lipoprotein lipase and hepatic lipase
D	–	31	Many tissues	Structural component of lipocalins (are a family of proteins which transport small hydrophobic molecules such as steroids, bilins, retinoids and lipids)
E	299	34	Liver, VLDL	Ligand for Chm receptor
(a)	Variable			Ligand for liver chylomicron receptor

VLDL, which are synthesized in the liver as lipid transport vehicles, are also degraded by lipoprotein lipase. The VLDL remnants appear in the circulation, first as IDL and then as LDL. In the transformation of VLDL to LDL, all their proteins but apoB-100 are removed and much of their cholesterol is esterified by the HDL-

associated enzyme lecithin–cholesterol acyltransferase (LCAT), which is activated by apoA-I and catalyzes those reaction: esterification of cholesterol by LCAT is made at the moment of a contact of HDL with these lipoprotein or with outer surface of cellular membrane of any peripheral cell where excess cholesterol occurs.

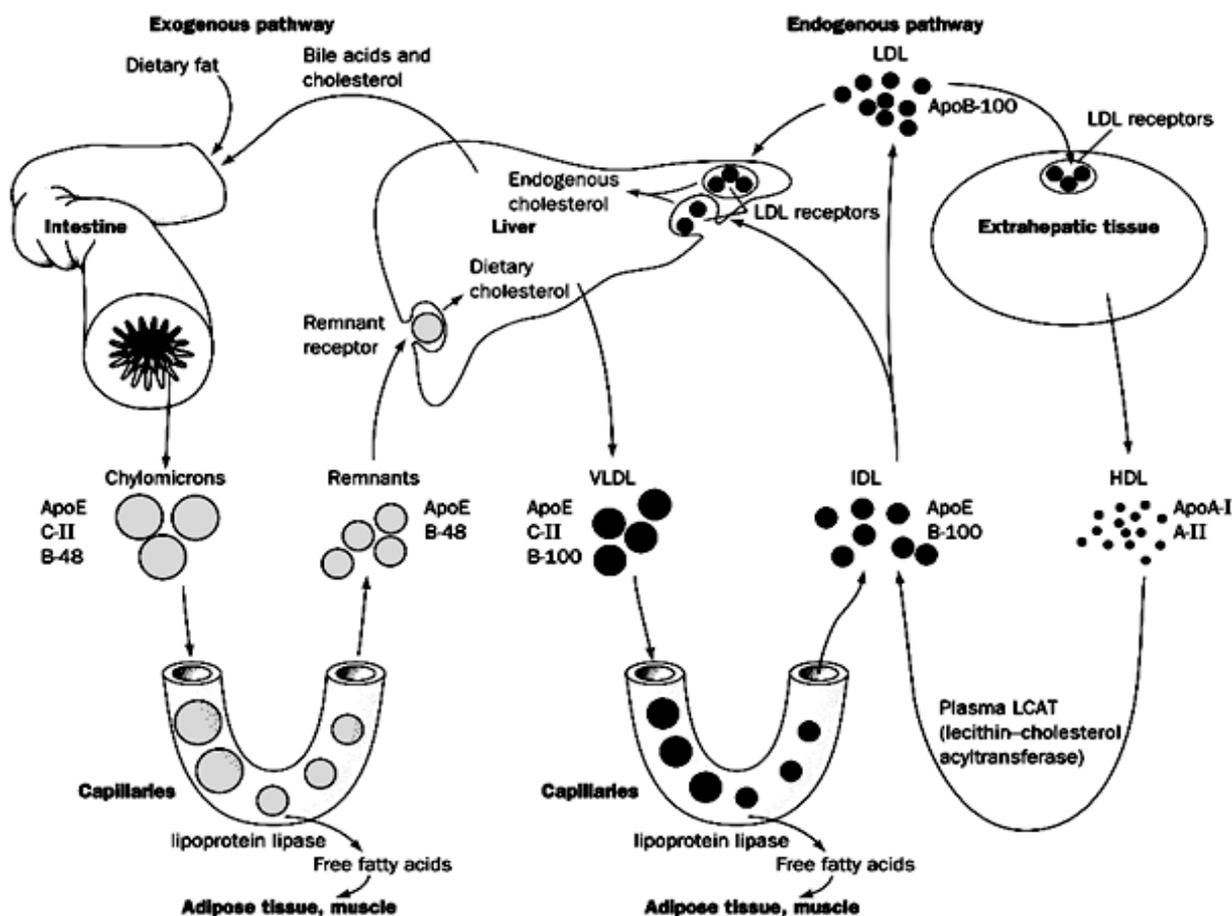


Figure 9. Model for plasma triacylglycerol and cholesterol transport in humans.

ApoB-100, a 4536-residue monomeric glycoprotein (and thus one of the largest monomeric proteins known), has a hydrophobicity approaching that of integral proteins and contains relatively few amphipathic helices. Hence, in contrast to the other, less hydrophobic plasma apolipoproteins, apoB-100 is neither water-soluble nor transferred between lipoprotein particles. Each LDL particle contains but one molecule of apoB-100, which immunoelectron microscopy

indicates has an extended form that covers at least half of the particle surface. Chylomicrons, however, contain apoB-48, a 2152-residue protein that is identical in sequence to the N-terminal 48% of apoB-100. Indeed, both proteins are encoded by the same gene.

Cholesterol is an essential component of animal cell membranes. The cholesterol may be externally supplied or, if this source is insufficient, internally synthesized. Michael Brown and Joseph Goldstein have demonstrated that cells obtain exogenous cholesterol mainly through the endocytosis (engulfment) of LDL in complex with LDL receptor (LDLR), a cell-surface transmembrane glycoprotein that specifically binds apoB-100. LDLR also binds chylomicron remnants via their apoE components. Such receptor-mediated endocytosis (Fig. 10) is a general mechanism whereby cells take up large molecules, each through a corresponding specific receptor. LDL specifically binds to LDL receptors (LDLRs) on clathrin-coated pits (1). These bud into the cell (2) to form coated vesicles (3), whose clathrin coats depolymerize as triskelions, resulting in the formation of uncoated vesicles (4). These vesicles then fuse with vesicles called endosomes (5), which have an internal pH of ~ 5.0. The acidity induces LDL to dissociate from LDLR. LDL accumulates in the vesicular portion of the endosome, whereas LDLR concentrates in the membrane of an attached tubular structure, which then separates from the endosome (6) and subsequently recycles LDLR to the plasma membrane (7). The vesicular portion of the endosome (8) fuses with a lysosome (9), yielding a secondary lysosome (10), wherein the apoB-100 component of LDL is degraded to its component amino acids and the cholesteryl esters are hydrolyzed by a lysosomal lipase to yield cholesterol and fatty acids. An LDLR molecule cycles in and out of the cell every 10 to 20 minutes during its ~ 20-hour lifetime.

Any excess intracellular cholesterol is reesterified for storage within the cell through the action of acyl-CoA:cholesterol acyltransferase (ACAT). The overaccumulation of cellular cholesteryl esters is prevented by two feedback mechanisms:

1. High intracellular levels of cholesterol suppress the synthesis of LDLR, thus decreasing the rate of LDL accumulation by endocytosis.

2. Excess intracellular cholesterol inhibits the biosynthesis of cholesterol.

HDL has essentially the opposite function of LDL: It removes cholesterol from the tissues. Nascent forms of HDL are produced in the liver, but remnant HDL are assembled in the plasma from components obtained largely through the degradation of other lipoproteins. Circulating HDL acquires its cholesterol by extracting it from cell-surface membranes and converts it to cholesteryl esters through the action of LCAT, an enzyme that is activated by apoA-I. HDL therefore functions as a cholesterol scavenger.

The liver is the only organ capable of disposing of significant quantities of cholesterol (by its conversion to bile acids). This occurs through the mediation of both LDLR and a specific HDL receptor named SR-BI (for scavenger receptor class B type I). About half of the VLDL, after its degradation to IDL and LDL, is taken up by the liver via LDLR-mediated endocytosis (Fig. 10). However, hepatocytes (liver cells) take up cholesteryl esters from HDL by an entirely different mechanism: rather than being engulfed and degraded, the SR-BI-bound HDL selectively transfers its component cholesteryl esters to the cell. The lipid-depleted HDL then dissociates from the cell and reenters the blood circulation.

Reasons of Hyperlipoproteinemias development in human

Hyperlipoproteinemia is a metabolic disorder characterized by abnormally elevated concentrations of specific lipoprotein particles in the plasma.

According to the classification (Fridrickson E., et al) hyperlipoproteinemias are divided into five types:

Type I (Hyperchylomicronemia). Defect in human organism: decreased lipoprotein lipase, altered ApoC-II. Diagnostic results: very high level of chylomicrons in the blood serum on empty stomach. Slightly higher levels for VLDL and triacylglycerols. Xanthomatosis in patients is associated with this type of hyperlipoproteinemia.

Type II (Hyper- β -lipoproteinemia). Subtype IIa. Defect in human organism: LDL receptor deficiency. Diagnostic results: high levels of LDL and total cholesterol in patients. Subtype IIb. Defect in human organism: decreased LDL receptor and increased ApoB. Diagnostic results: high levels of LDL, VLDL, cholesterol and triacylglycerols. Ischemic heart disease and hypertension is observed at patients.

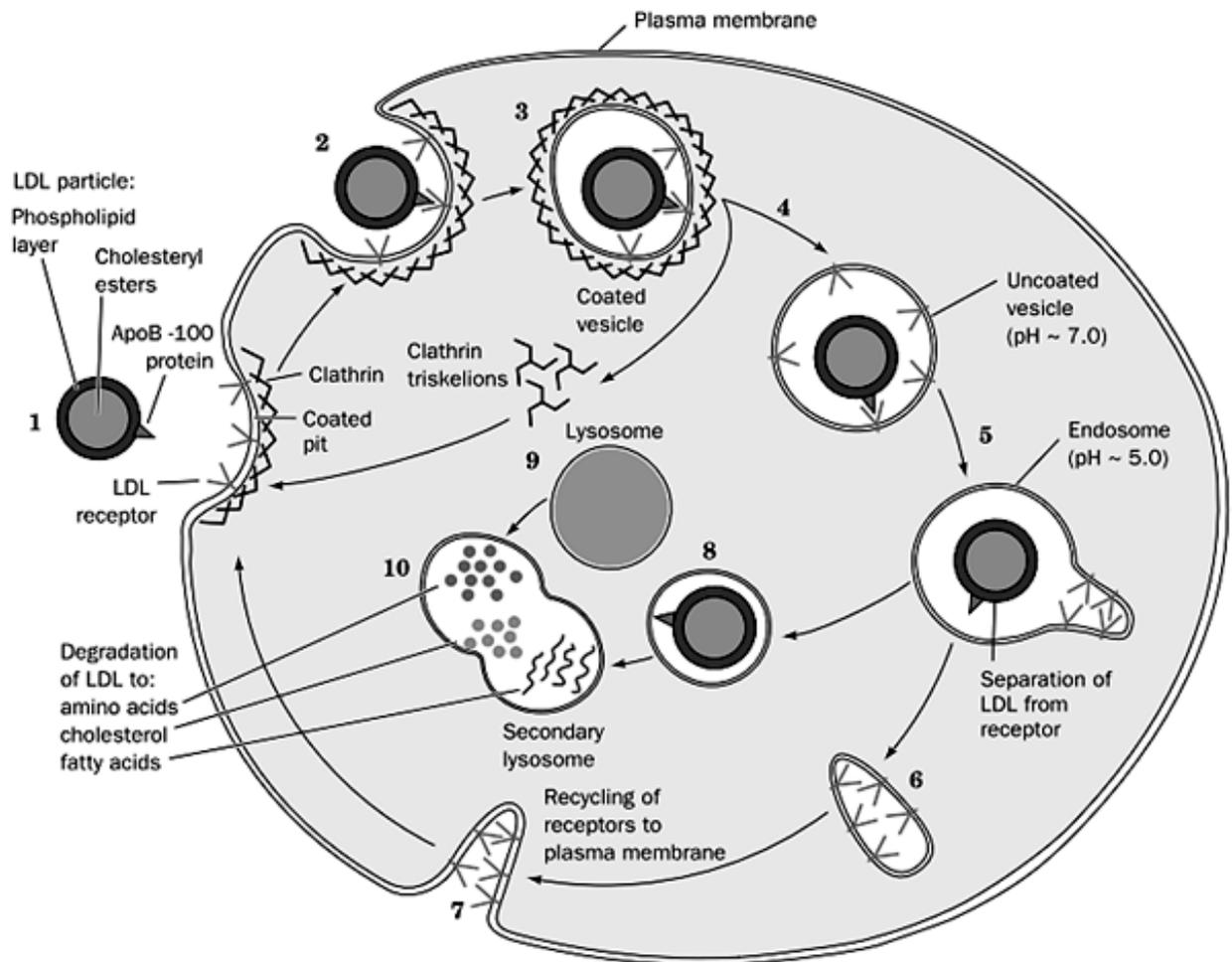


Figure 10. Sequence of events in the receptor-mediated endocytosis of LDL.

Type III (Dis- β -lipoproteinemia). Defect in human organism: ApoE-II synthesis. Diagnostic results: high levels of IDL in the blood plasma that are absent at healthy adults, high levels of cholesterol. These changes associated with problems in heart system: atherosclerosis of blood vessels, thrombosis may be at patient too.

Type IV (Hyper-pre- β -lipoproteinemia). Diagnostic results: high levels of VLDL, but LDL are slight higher or normal. Chylomicrons are absent. Diabetes mellitus with obesity and ischemic heart disease are associated with this type of hyperlipoproteinemia.

Type V (Hyper-pre- β -lipoproteinemia accompanied with Hyperchylomicronemia). Diagnostic results: high levels of chylomicrons and VLDL. Xanthomatosis is represented, also. This state may be in patients with latent form of insulin-independent diabetes mellitus, but ischemic heart disease is not observed at patient, in this case.

Triacylglycerols degradation in adipose tissue

The mobilization of fatty acids from triacylglycerol stores depends upon hormone-sensitive lipase (Fig. 11). This enzyme is activated by cAMP-dependent phosphorylation (catalyzed by protein kinase A) and moves from the cytoplasm to surfaces of lipid droplets in response to catecholamines and other lipolytic hormones (glucagon, STH, ACTH) (Fig. 12). The increased level of cyclic AMP then stimulates protein kinase A, which phosphorylates two key proteins: perilipin A, a fat-droplet-associated protein, and hormone-sensitive lipase. Fatty acids are a major fuel for aerobic cells (except neurons).

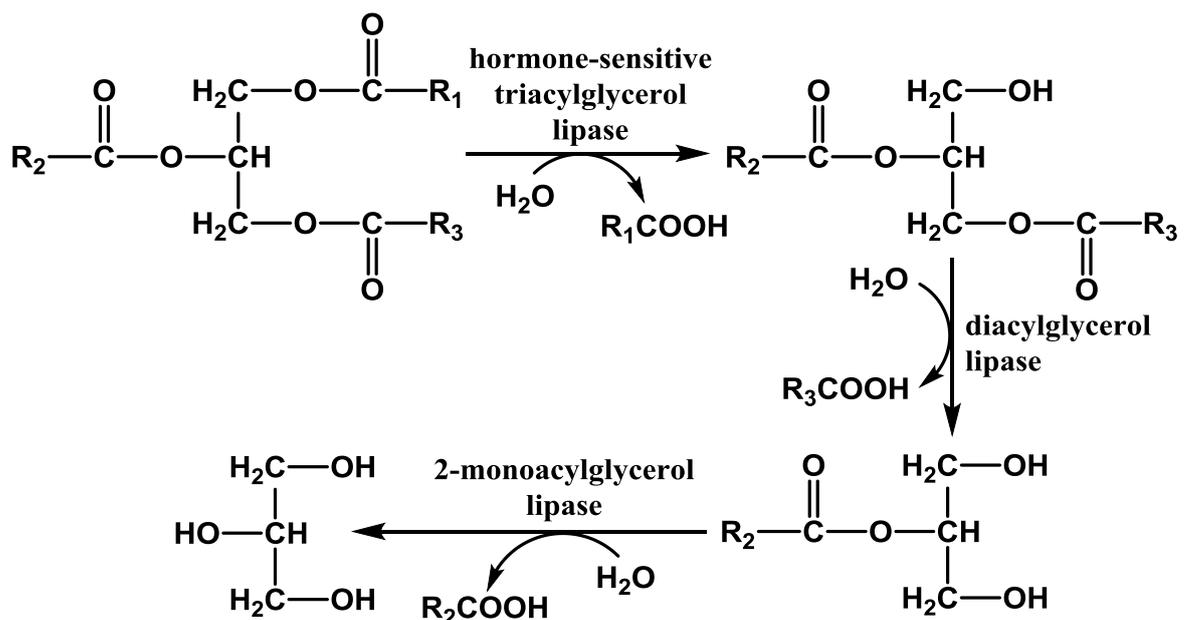


Figure 11. Hydrolysis of triacylglycerols.

Both products of lipolysis (i.e., fatty acids and glycerol) are released into the blood. Fatty acids are carried to tissues for use in synthesis of triacylglycerols, phospholipids, and other membrane lipids. Glycerol is transported to the liver where it can be used in lipid or glucose synthesis (Fig. 13). After their transport across the adipocyte plasma membrane, fatty acids become bound to serum albumin. The albumin-bound fatty acids are carried to tissues throughout the body, where they are oxidized to generate energy. Fatty acids are transported into cells by a protein in the plasma membrane. This process is linked to the active transport of sodium. The amount of fatty acid that is transported depends on its concentration in blood and the relative activity of the fatty acid transport mechanism.

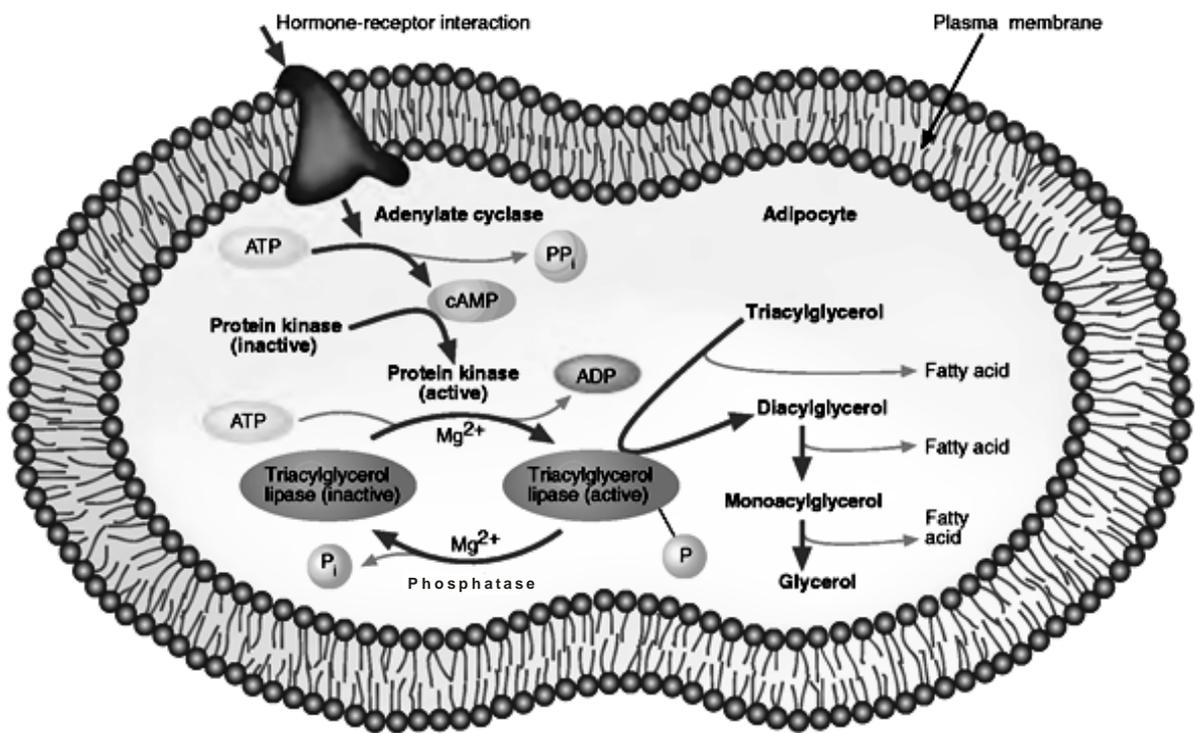


Figure 12. Diagrammatic view of lipolysis.

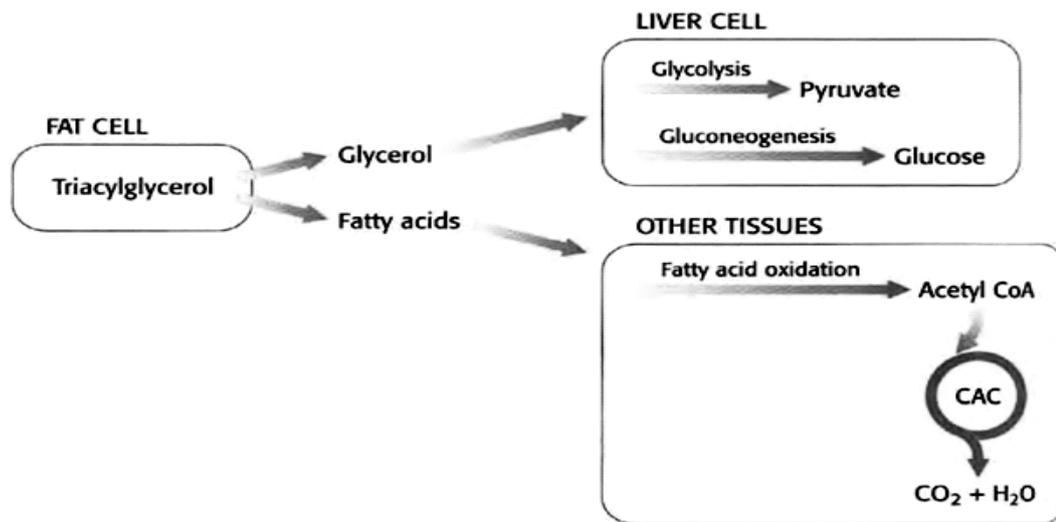


Figure 13. Lipolysis products and their metabolism in tissues:

Starvation → Glucagon↑ → Triacylglycerol lipase activity ↑;

Physical loading → Epinephrine, STH↑ → Triacylglycerol lipase activity ↑;

Emotional stress → ACTH, Epinephrine → Triacylglycerol lipase activity ↑;

Extensive growing → STH → Triacylglycerol lipase activity ↑.

Synthesis of Triacylglycerols

Triacylglycerols are synthesized from fatty acyl-CoA esters and glycerol-3-phosphate or dihydroxyacetone phosphate (Fig. 14). The initial step in this process is catalyzed either by glycerol-3-phosphate acyltransferase in mitochondria and the ER, or by dihydroxyacetone phosphate acyltransferase in the ER or peroxisomes. In the liver glycerol-3-phosphate is formed from glycerol under glycerolkinase action. In the latter case, the product acyl-dihydroxyacetone phosphate is reduced to the corresponding lysophosphatidic acid by an NADPH-dependent reductase. The lysophosphatidic acid is converted to a triacylglycerol by the successive actions of 1-acylglycerol-3-phosphate acyltransferase, phosphatidic acid phosphatase, and diacylglycerol acyltransferase. The intermediate phosphatidic acid and 1,2-diacylglycerol (DAG) can also be converted to phospholipids. The acyltransferases are not completely specific for particular fatty acyl-CoAs, either in chain length or in degree of unsaturation, but in triacylglycerols of human adipose

tissue, palmitate tends to be concentrated at position 1 and oleate at position 2 of glycerol fragment.

The dihydroxyacetone phosphate used to make glycerol-3-phosphate for triacylglycerol synthesis comes either from glucose via the glycolytic pathway or from oxaloacetate via an abbreviated version of gluconeogenesis termed glyceroneogenesis. Glyceroneogenesis is necessary in times of starvation, since approximately 30% of the fatty acids that enter the liver during a fast are reesterified to triacylglycerol and exported as VLDL. Adipocytes also carry out glyceroneogenesis in times of starvation. They do not carry out gluconeogenesis but contain the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK), which is upregulated when glucose concentration is low, and participates in the glyceroneogenesis required for triacylglycerol biosynthesis.

Lipid biosynthesis is controlled by long-term regulation, with insulin stimulating and starvation inhibiting the synthesis of acetyl-CoA carboxylase and fatty acid synthase. The presence in the diet of polyunsaturated fatty acids also decreases the concentrations of these enzymes. The amount of adipose tissue lipoprotein lipase, the enzyme that initiates the entry of lipoprotein-packaged fatty acids into adipose tissue for storage, is also increased by insulin and decreased by starvation. In contrast, the concentration of heart lipoprotein lipase, which controls the entry of fatty acids from lipoproteins into heart tissue for oxidation rather than storage, is decreased by insulin and increased by starvation. Starvation and/or regular exercise, by decreasing the glucose concentration in the blood, change the body's hormone balance. This situation results in long-term changes in gene expression that increase the levels of fatty acid oxidation enzymes and decrease those of lipid biosynthesis.

If balanced diet is created by human person ability for TG synthesis is considered mainly in the liver after portion of carbohydrates intake with food products or due to appearance of 2-monoacylglycerols after their absorption or resynthesis in the small intestine wall and transport with Chm across vena portae to the liver.

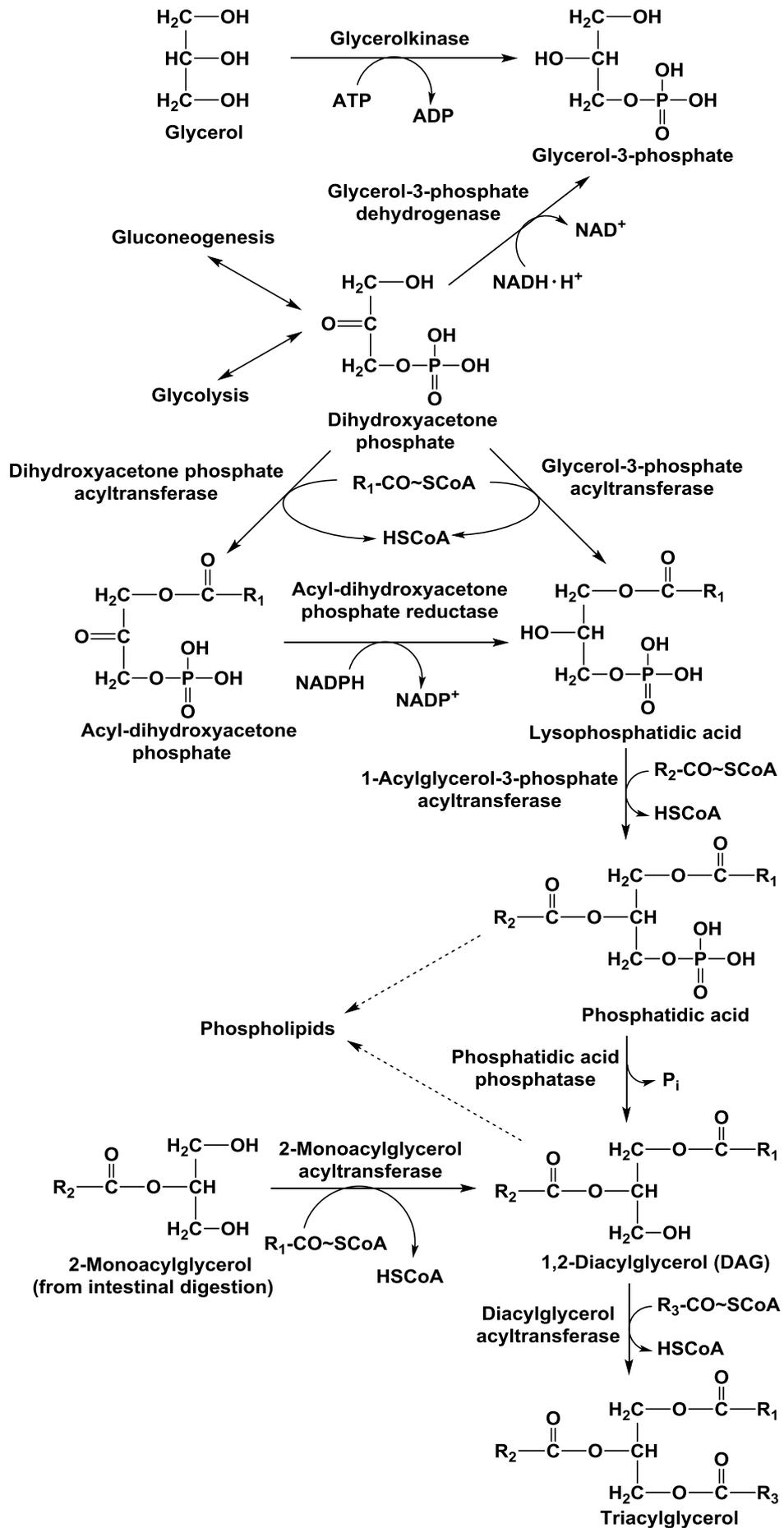


Figure 14. The reactions of triacylglycerol biosynthesis.

Main signal to stimulate enzymes of TG synthesis is accumulation of HFA acyl-CoAs inside the hepatocyte.

Beginning of TG synthesis in adipose tissue is observed in humans under excess intake of exogenous carbohydrates and TG. To like eating cakes with butter cream – direct way to obesity whose development is promoted by described metabolic pathway duration.

Phospholipid metabolism

The “complex lipids” are dual-tailed amphipathic molecules composed of either 1,2-diacylglycerol or N-acylsphingosine (ceramide) linked to a polar head group that is either a phosphate ester (Fig. 4). Note also that these substances are synthesized in membranes, mostly on the cytosolic face of the endoplasmic reticulum, and from there are transported to their final cellular destinations.

The triacylglycerol precursors 1,2-diacyl glycerol and phosphatidic acid are also the precursors of certain glycerophospholipids (Figs. 15). Activated phosphate esters of the polar head groups react with the C3 OH group of 1,2-diacylglycerol to form the phospholipid’s phosphodiester bond. In some cases the phosphoryl group of phosphatidic acid is activated and reacts with the unactivated polar head group.

The mechanism of activated phosphate ester formation is the same for both the polar head groups ethanolamine and choline (Fig. 16):

1. ATP first phosphorylates the OH group of choline or ethanolamine.
2. The phosphoryl group of the resulting phosphoethanolamine or phosphocholine then attacks CTP, displacing PP_i , to form the corresponding CDP derivatives, which are activated phosphate esters of the polar head group.
3. The C3 OH group of 1,2-diacylglycerol attacks the phosphoryl group of the activated CDP-ethanolamine or CDP–choline, displacing CMP to yield the corresponding glycerophospholipid.

The liver also converts phosphatidylethanolamine to phosphatidylcholine by trimethylating its amino group, using S-adenosylmethionine as the methyl donor.

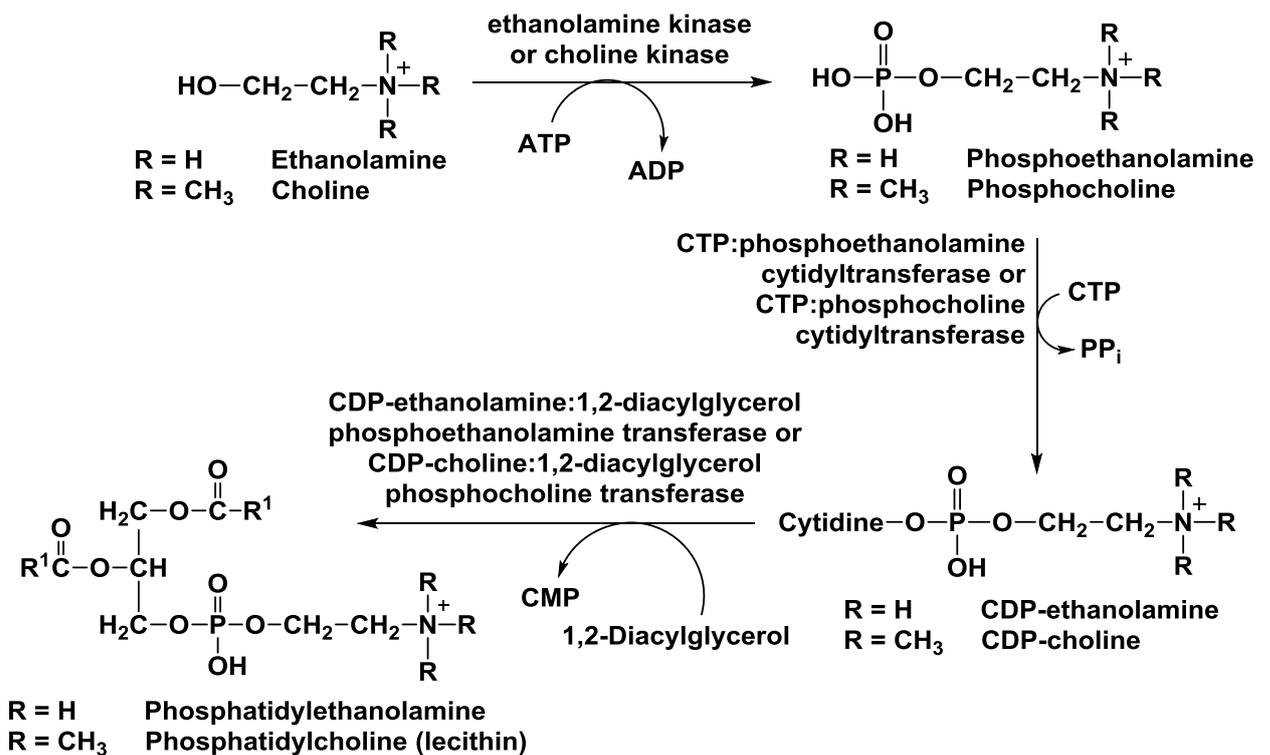


Figure 15. The biosynthesis of phosphatidylethanolamine and phosphatidylcholine.

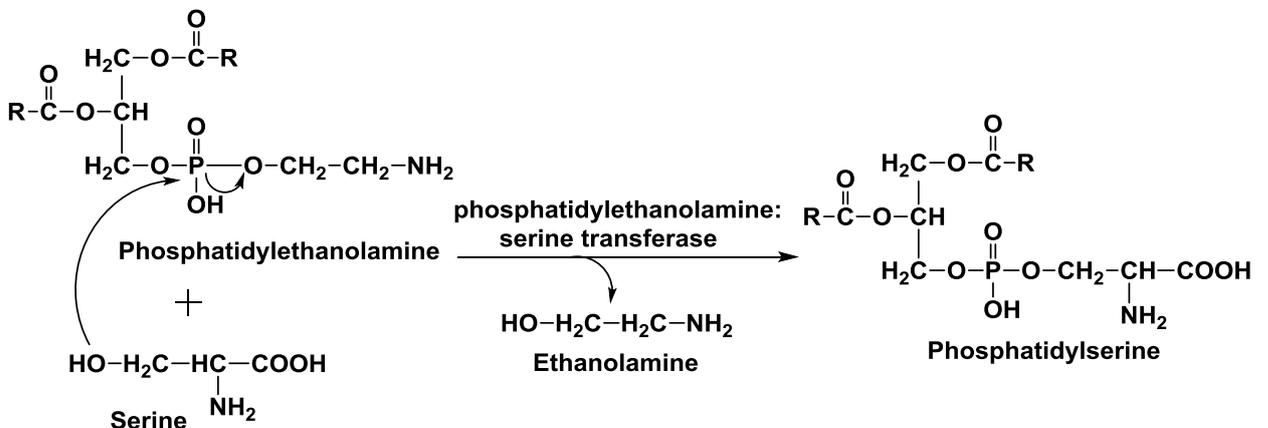


Figure 16. Phosphatidylserine synthesis.

Phosphatidylserine is synthesized from phosphatidylethanolamine by a head group exchange reaction catalyzed by phosphatidylethanolamine:serine transferase in which serine's OH group attacks the donor's phosphoryl group (Fig. 16). The original head group is then eliminated, forming phosphatidylserine.

In the synthesis of phosphatidylinositol and phosphatidylglycerol, the hydrophobic tail is activated rather than the polar head group. Phosphatidic acid,

the precursor of 1,2-diacylglycerol, attacks the α -phosphoryl group of CTP to form the activated CDP-diacylglycerol and PP_i . Phosphatidylinositol results from the attack of inositol on CDP-diacylglycerol. Phosphatidylglycerol is formed in two reactions: attack of the C1-OH group of glycerol-3-phosphate on CDP-diacylglycerol, yielding phosphatidylglycerol phosphate; and hydrolysis of the phosphoryl group to form phosphatidylglycerol.

It should be noted that phospholipids and TG synthesis in the liver have common steps in the beginning of their duration. After phosphatidic acid formation their transportations are differ. What pathway will be predominated? The answer for this question is depended situation in hepatocytes which mostly realized due to reaction Glycerol:HFA:Lipotropic factors. Lipotropic factors are Choline, Methionine, Vitamins B₆, B₁₂, CTP. The lower content of lipotropic factors – the higher rate for TG synthesis.

Laboratory works

The determination of β -lipoproteins (LDL) content in the blood serum

The principle of the method:

β -Lipoproteins precipitate in the presence of calcium chloride and heparin: the turbidity is appeared. It is explained that heparin can form with β -lipoproteins a complex, which is precipitated in the presence of calcium chloride. The concentration of β -lipoproteins in the blood serum correlates with the rate of turbidity.

The course of laboratory work:

Pour 2 mL of 0.27 % calcium chloride solution and 0.2 mL of blood serum in a test tube, mix. Determine the optical density of this solution (E_1) apposite 0.27 % of calcium chloride solution at red-colour filter in cuvettes (5 mm thick layer). A solution from experimental cuvette transfer again into the test tube, add 0.04 mL of 1 % heparin solution, mix and exactly (in 4 minutes later) determine the optical density of this one again (E_2) with the same condition. Calculate LDL content according to the formula:

$$X = (E_2 - E_1) \cdot 1000, \text{ where}$$

X - concentration of LDL in the blood serum, mg %;

E₁ - optical density of experimental sample before heparin adding;

E₂ - optical density of experimental sample after heparin adding;

1000 – empiric recalculation coefficient.

Clinical significance of β -lipoproteins (LDL) content determination in the blood serum

The content of β -lipoproteins in the blood serum is normal when it equals 300-450 mg% or 3.0-4.5 g/L.

The increased β -lipoproteins content is observed at states: hyperlipoproteinemias such types as IIa, IIb, III (Fredrikson classification), which correlates with the increase of the total cholesterol content in plasma. The specified conditions promote the development of atherosclerotic damages of the vessels at patients with a hypertension, myocardial ischemia (MI) or at diseases, which are accompanied with development of secondary hyperlipoproteinemias at diabetes mellitus (obvious and the latent form), hypothyroid edema, nephritis syndrome, chronic kidney insufficiency.

The determination of total phospholipids content in the blood serum

The principle of the method:

Phospholipids are precipitated by trichloroacetic acid together with blood proteins. The sediment is digested during the boiling with perchloric acid up to the simple substances: some of them are free phosphates. These ones form with ammonium molybdate at the presence of an ascorbic acid the coloured complex.

The course of laboratory work:

Pour 3 mL of distilled water into a test tube and add 0.2 mL of blood serum. Add 3 mL of 10% trichloroacetic acid solution (the first 1.5 mL in drops, shaking up permanently), the rest 1.5 mL - more quickly. Let it stay for 2 minutes, then centrifuge at 3000 g for 5 minutes. Pour off supernatant and turn over the test-tube on the Petri dish until all liquid flows down. Add 1 mL of 50-80% perchloric acid to the deposit and boil for 20-30 min on a sand bath (180 °C) until the mixture will become colourless. It is recommended during the first 5 minutes to observe the

process until the scum will be over. After the sample's cooling add distilled water up to the 7 mL volume.

Prepare the control test and two standards. Pour 0.8 mL of 50-80% perchloric acid solution into the control test-tube and make the volume of 7 mL with distilled water. Put 2 mL of working standard solution into each standard test tube, add 0.8 mL of 50-80% perchloric acid solution and make the volume of solutions with distilled water to be equal 7 mL.

Add into every test tube 1 mL of ammonium molybdate solution, mix and pour to each of them 1 mL of ascorbic acid solution, make the volumes of solutions with distilled water to be equal 10 mL, stir again. Leave all test tubes on the table for 20 minutes, then measure the optical density at red colour filter (630-690 nm) in ditches (10 mm thick layer) against control test.

Calculation by formula:

$$X = 10 (E_{exp} / E_{st}) \cdot C, \text{ where}$$

X - concentration of phosphorus in serum, mg%;

E_{exp} - extinction of experimental test;

$E_{st} = (E_{st1} + E_{st2})/2$ - average extinction of standard tests;

C - concentration of working standard phosphate solution; [P]=1.0 mg %.

The concentration of lipid phosphorus in plasma of adults is normal when equals 6.1-14.5 mg%. For putting the results in mmol/L the received data is multiplied by index 0.3229. The lipids phosphorus content normally equals 1.97-4.68 mmol/L.

Total phospholipids concentration is calculated by multiplying of the estimated phosphorus concentration (mg%) on 25 (lipid phosphorus makes up 4% of the phospholipids molecular mass).

Clinical significance of phospholipids content determination in the blood serum

The increase of total phospholipids content in blood serum is observed at heavy form of diabetes, acute nephritis, chronic nephritis, essential hyperlipoproteinemia, at obstructive jaundice, liver coma, an anemia. The decrease

For example, *cis*- Δ^9 means that there is a *cis* double bond between carbon atoms 9 and 10; *trans*- Δ^2 means that there is a *trans* double bond between carbon atoms 2 and 3. Alternatively, the position of a double bond can be denoted by counting from the distal end, with the ω -carbon atom (the methyl carbon) as number 1. An ω -3 fatty acid, for example, has the structure shown in Fig. 17.

Figure 18. Common biological fatty acids.

Number of carbon	Common name	Symbol	Structure
<i>Saturated fatty acids</i>			
12	Lauric acid	12:0	CH ₃ (CH ₂) ₁₀ COOH
14	Myristic acid	14:0	CH ₃ (CH ₂) ₁₂ COOH
16	Palmitic acid	16:0	CH ₃ (CH ₂) ₁₄ COOH
18	Stearic acid	18:0	CH ₃ (CH ₂) ₁₆ COOH
20	Arachidic acid	20:0	CH ₃ (CH ₂) ₁₈ COOH
22	Behenic acid	22:0	CH ₃ (CH ₂) ₂₀ COOH
24	Lignoceric acid	24:0	CH ₃ (CH ₂) ₂₂ COOH
<i>Unsaturated fatty acids (all double bonds are cis)</i>			
16	Palmitoleic acid	16:1	CH ₃ (CH ₂) ₅ CH=CH(CH ₂) ₇ COOH
18	Oleic acid	18:1	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₇ COOH
18	Linoleic acid	18:2	CH ₃ (CH ₂) ₄ (CH=CHCH ₂) ₂ (CH ₂) ₆ COOH
18	α -Linolenic acid	18:3	CH ₃ CH ₂ (CH=CHCH ₂) ₃ (CH ₂) ₆ COOH
18	γ -Linolenic acid	18:3	CH ₃ (CH ₂) ₄ (CH=CHCH ₂) ₃ (CH ₂) ₃ COOH
20	Arachidonic acid	20:4	CH ₃ (CH ₂) ₄ (CH=CHCH ₂) ₄ (CH ₂) ₂ COOH
24	Nervonic acid	24:1	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₁₃ COOH

Saturated fatty acid chains can pack closely together to form ordered, rigid arrays under certain conditions, but unsaturated fatty acids prevent such close packing and produce flexible, fluid aggregates. Some fatty acids are not synthesized by mammals and yet are necessary for normal growth and life. These *essential fatty acids* include *linoleic* and *γ -linolenic acids*. These must be obtained by mammals in their diet (specifically from plant sources). *Arachidonic acid* may be synthesized in mammals from linoleic acid. At least one function of the essential fatty acids is to serve as a precursor for the synthesis of *eicosanoids*, such as *prostaglandins*, a class of compounds that exert hormone-like effects in many physiological processes.

β -Oxidation of high fatty acids

After FA transport across the adipocyte plasma membrane, fatty acids become bound to serum albumin. Appearance of free FA in the blood plasma mainly associated with previous tissue lipolysis duration in a ratio 1 Alb: 4 FA. The albumin-bound fatty acids are carried to tissues throughout the body, where they may be oxidized to generate energy. Fatty acids are a major fuel for aerobic cells (except neurons). Fatty acids are transported into cells by a protein in the plasma membrane. This process is linked to the active transport of sodium. The amount of fatty acid that is transported depends on its concentration in blood and the relative activity of the fatty acid transport mechanism.

Most fatty acids are degraded by the sequential removal of two-carbon fragments from the carboxyl end of fatty acids. During this process, referred to as β -oxidation, acetyl-CoA is formed as the bond between the α - and β -carbon atoms is broken.

β -Oxidation occurs primarily within mitochondria. Before β -oxidation begins, each fatty acid is activated in a reaction with ATP and CoASH (derivative of pantothenic acid, vitamin B₅). The enzyme that catalyzes this reaction, acyl-CoA synthetase, is found in the outer mitochondrial membrane. Because the mitochondrial inner membrane is impermeable to most acyl-CoA molecules, a special carrier named carnitine is used to transport acyl groups into the mitochondria (Fig. 19).

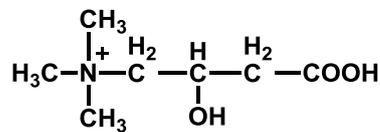


Figure 19. Structure of Carnitine.

Carnitine-mediated transport of acyl groups into the mitochondrial matrix is accomplished through the following mechanism (Fig. 20):

1. Each acyl-CoA molecule is converted to an acylcarnitine derivative (Fig. 21):

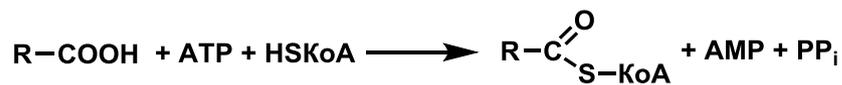


Figure 21. Activation of fatty acids.

This reaction is catalyzed by carnitine acyltransferase I.

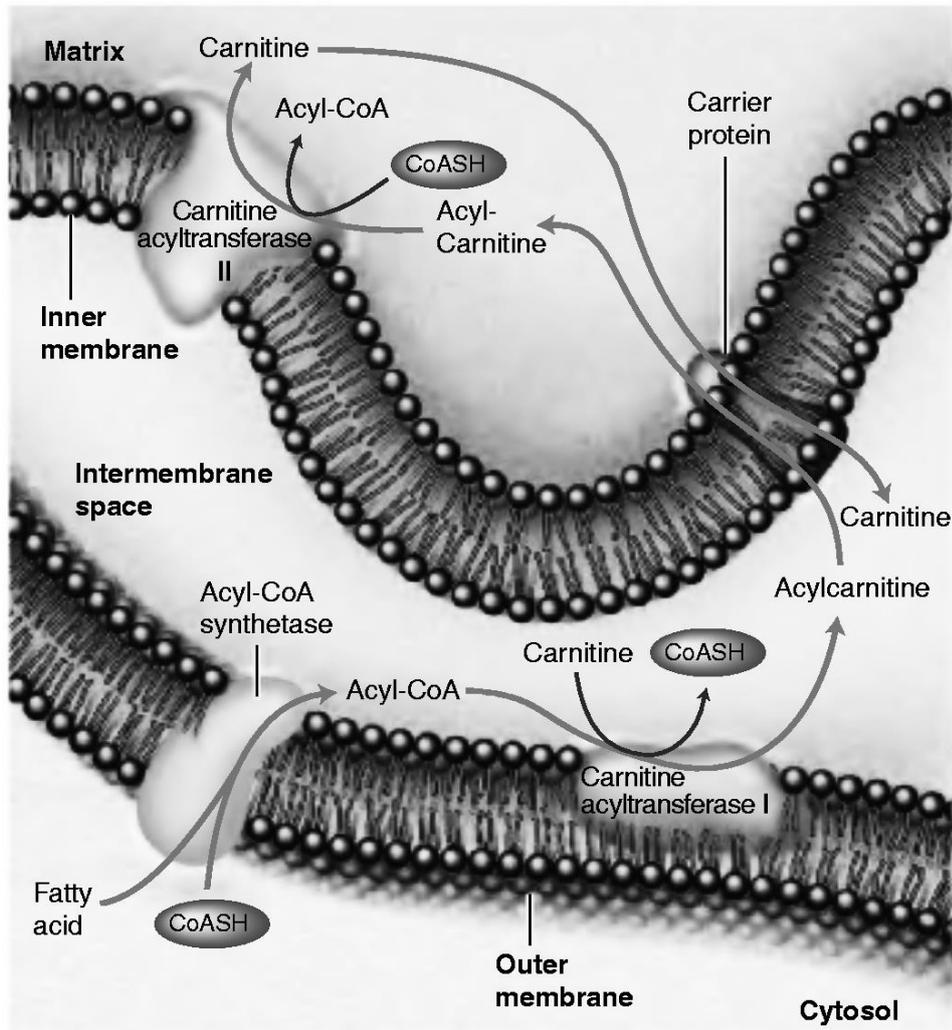


Figure 20. Fatty Acid Transport into the Mitochondria.

2. A carrier protein (carnitine-acylcarnitine translocase) within the mitochondrial inner membrane transfers acylcarnitine into the mitochondrial matrix.

3. Acyl-CoA is regenerated by carnitine acyltransferase II.

4. Carnitine is transported back into the intermembrane space by the carrier protein. It then reacts with another acyl-CoA.

A summary of the reactions of the β -oxidation of saturated fatty acids is shown in Figure 22. The pathway begins with an oxidation-reduction reaction, catalyzed by acyl-CoA dehydrogenase (an inner mitochondrial membrane

flavoprotein), in which one hydrogen atom each is removed from the α - and β -carbons and transferred to the enzyme-bound FAD. The FADH_2 produced in this reaction then donates 2 electrons to the mitochondrial electron transport chain. There are several isozymes of acyl-CoA dehydrogenase, each specific to a different fatty acid chain length. The product of this reaction is trans- α,β -enoyl-CoA.

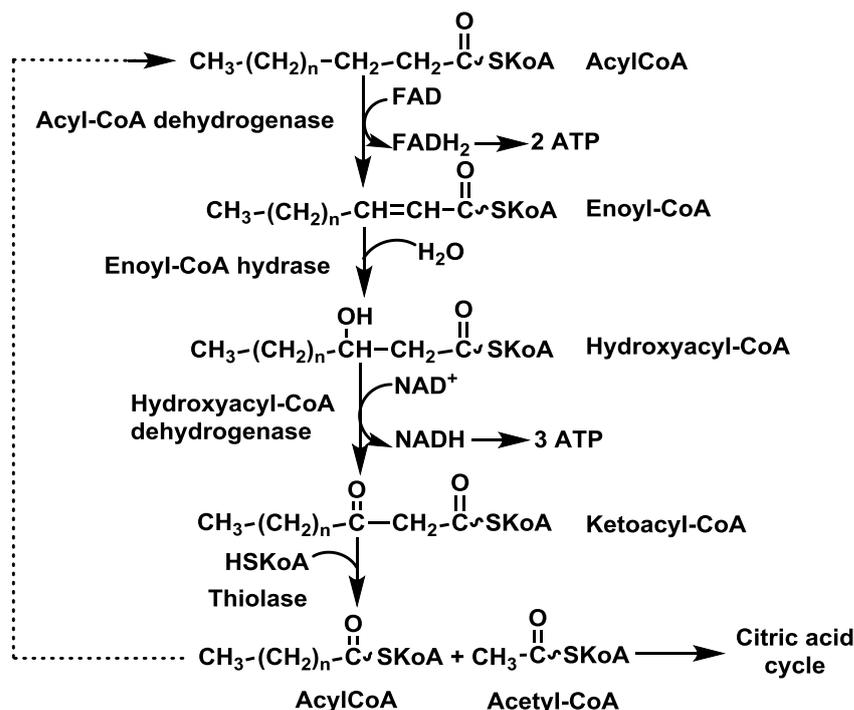


Figure 22. β -Oxidation of saturated Acyl-CoA.

The second reaction, catalyzed by enoyl-CoA hydratase, involves a hydration of the double bond between the α - and β -carbons. The β -carbon is now hydroxylated. In the third reaction this hydroxyl group is oxidized. The production of a β -ketoacyl-CoA is catalyzed by β -hydroxyacyl-CoA dehydrogenase. The electrons transferred to NAD^+ are later donated to Complex I of the ETC. Finally, thiolase (sometimes referred to as β -ketoacyl-CoA thiolase) catalyzes a $\text{C}_\alpha\text{-C}_\beta$ cleavage. In this reaction, sometimes named a *thiolytic cleavage*, an acetyl-CoA molecule is released. The other product, an acyl-CoA, now contains two fewer C atoms. The four steps constitute one cycle of β -oxidation. During each later cycle, a two-carbon fragment is removed. This process, sometimes called the β -oxidation spiral, continues until, in the last cycle, a four-carbon acyl-CoA is cleaved to form two molecules of acetyl-CoA.

β -Oxidation of unsaturated and odd-chain fatty acids

The oxidation of unsaturated fatty acids presents some difficulties, yet many such FA are available in the diet. Most of these reactions are the same as those for saturated fatty acids. In fact, only two additional enzymes – an isomerase and a reductase – are needed to degrade a wide range of unsaturated FA.

Consider the oxidation of palmitoleate. This C_{16} unsaturated fatty acid, which has one double bond between C-9 and C-10, is activated and transported across the inner mitochondrial membrane in the same way as saturated fatty acids. Palmitoleoyl-CoA then undergoes three cycles of degradation, which are carried out by the same enzymes as those in the oxidation of saturated fatty acids. However, the cis- Δ^3 -enoyl-CoA formed in the third round is not a substrate for acyl-CoA dehydrogenase. The presence of a double bond between C-3 and C-4 prevents the formation of another double bond between C-2 and C-3. This impasse is resolved by a new reaction that shifts the position and configuration of the cis- Δ^3 double bond. Cis- Δ^3 enoyl-CoA isomerase converts this double bond into a trans- Δ^2 double bond. The subsequent reactions are those of the saturated fatty acid oxidation pathway, in which the trans- Δ^2 -enoyl-CoA is a regular substrate.

Another problem arises with the oxidation of polyunsaturated fatty acids. Consider linoleate, a C_{18} polyunsaturated fatty acid with cis- Δ^9 and cis- Δ^{12} double bonds. The cis- Δ^3 double bond formed after three rounds of β -oxidation is converted into a trans- Δ^2 double bond by the aforementioned isomerase. The acyl-CoA produced by another round of oxidation contains a cis- Δ^4 double bond. Dehydrogenation of this species by acyl-CoA dehydrogenase yields a 2,4-dienoyl intermediate, which is not a substrate for the next enzyme in the β -oxidation pathway. This impasse is circumvented by 2,4-dienoyl-CoA reductase, an enzyme that uses NADPH to reduce the 2,4-dienoyl intermediate to trans- Δ^3 -enoyl-CoA. Cis- Δ^3 -enoyl-CoA isomerase then converts trans- Δ^3 -enoyl-CoA into the trans- Δ^2 form, a customary intermediate in the β -oxidation pathway. Only two extra enzymes are needed for the oxidation of any polyunsaturated fatty acid. Odd-

numbered double bonds are handled by the isomerase, and even-numbered ones by the reductase and the isomerase.

Most fatty acids have even numbers of carbon atoms and are therefore completely converted to acetyl-CoA. Some plants and marine organisms, however, synthesize fatty acids with an odd number of carbon atoms. The final round of β -oxidation of these fatty acids forms propionyl-CoA, which is converted to succinyl-CoA for entry into the citric acid cycle.

The conversion of propionyl-CoA to succinyl-CoA involves three enzymes (Fig. 23). The first reaction is that of propionyl-CoA carboxylase, a biotin-dependent enzyme with subunit composition $\alpha_6\beta_6$.

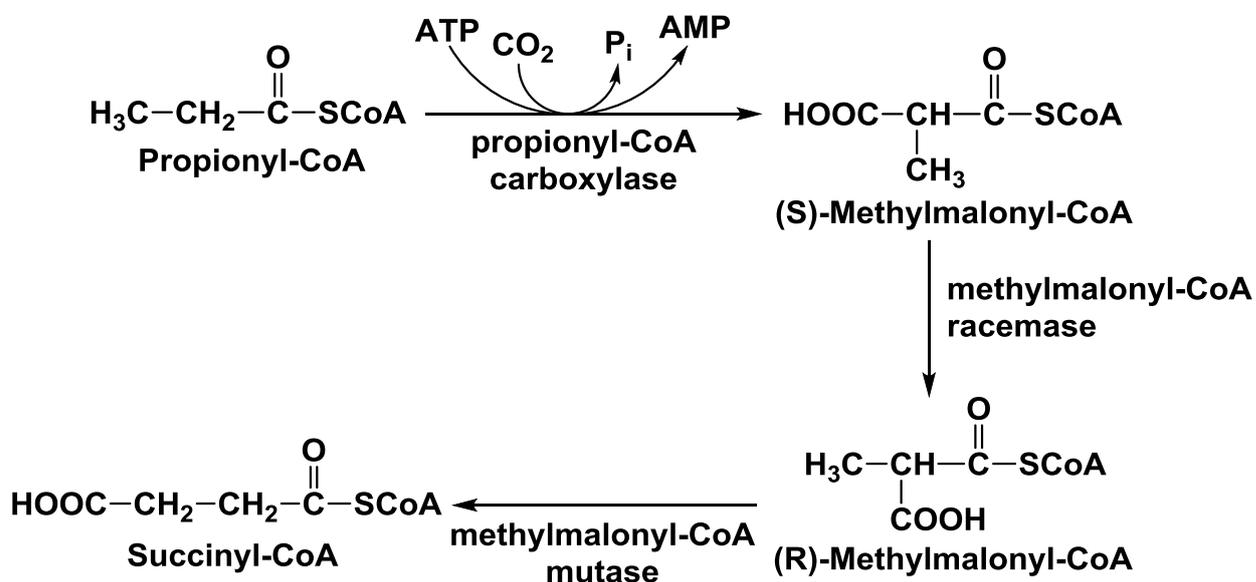


Figure 23. Conversion of propionyl-CoA to succinyl-CoA.

Methylmalonyl-CoA mutase, which catalyzes the third reaction of the propionyl-CoA to succinyl-CoA conversion (Fig. 23), is specific for (R)-methylmalonyl-CoA even though propionyl-CoA carboxylase stereospecifically synthesizes (S)-methylmalonyl-CoA. This diversion is rectified by methylmalonyl-CoA racemase, which interconverts the (R) and (S) configurations of methylmalonyl-CoA. Methylmalonyl-CoA mutase catalyzes the conversion of a metabolite to a succinyl-CoA (C₄) citric acid cycle intermediate.

Fatty acids biosynthesis

FA biosynthesis occurs through condensation of C_2 units, the reverse of the β -oxidation process. The pathway of fatty acid synthesis differs from that of fatty acid oxidation. Figure 24 outlines fatty acid oxidation and synthesis with emphasis on the differences between these pathways. Whereas fatty acid oxidation occurs in the mitochondrion and utilizes fatty acyl-CoA esters, fatty acid biosynthesis occurs in the cytosol with the growing fatty acids esterified to acyl-carrier protein (ACP). ACP, like CoA, contains a phosphopantetheine group that forms thioesters with acyl groups. The phosphopantetheine phosphoryl group is esterified to a Ser OH group of ACP, whereas in CoA it is esterified to AMP. In animals, ACP is part of a large multifunctional protein (Type I ACP), whereas in *E. coli* it is a 125-residue polypeptide (Type II ACP). The phosphopantetheine group is transferred from CoA to apo-ACP to form the active holo-ACP by phosphopantetheine transferase (alternatively, ACP synthase).

The redox coenzymes of the animal fatty acid oxidative and biosynthetic pathways differ (NAD^+ and FAD for oxidation; NADPH for biosynthesis) as does the stereochemistry of their intermediate steps, but their main difference is the manner in which C_2 units are removed from or added to the fatty acyl thioester chain. In the oxidative pathway, β -ketothiolase catalyzes the cleavage of the C_α - C_β bond of β -ketoacyl-CoA so as to produce acetyl-CoA and a new fatty acyl-CoA, which is shorter by a C_2 unit. In the biosynthetic pathway, the condensation reaction is coupled to the hydrolysis of ATP, thereby driving the reaction to completion. This process involves two steps: (1) the ATP-dependent carboxylation of acetyl-CoA by acetyl-CoA carboxylase (ACC) to form malonyl-CoA, and (2) the exergonic decarboxylation of the malonyl group in the condensation reaction catalyzed by fatty acid synthase.

The synthesis of FA from acetyl-CoA and malonyl-CoA involves seven enzymatic reactions that yield mainly palmitic acid, which takes place in the cytoplasm. Acetyl-CoA must be transferred from mitochondria to the cytoplasm (Fig. 25). Mitochondria, however, are not readily permeable to acetyl-CoA. Recall

that carnitine carries only long-chain fatty acids. The barrier to acetyl-CoA is bypassed by citrate, which carries acetyl groups across the inner mitochondrial membrane. Citrate is formed in the mitochondrial matrix by the condensation of acetyl CoA with oxaloacetate. When present at high levels, citrate is transported to the cytoplasm, where it is cleaved by citrate lyase.

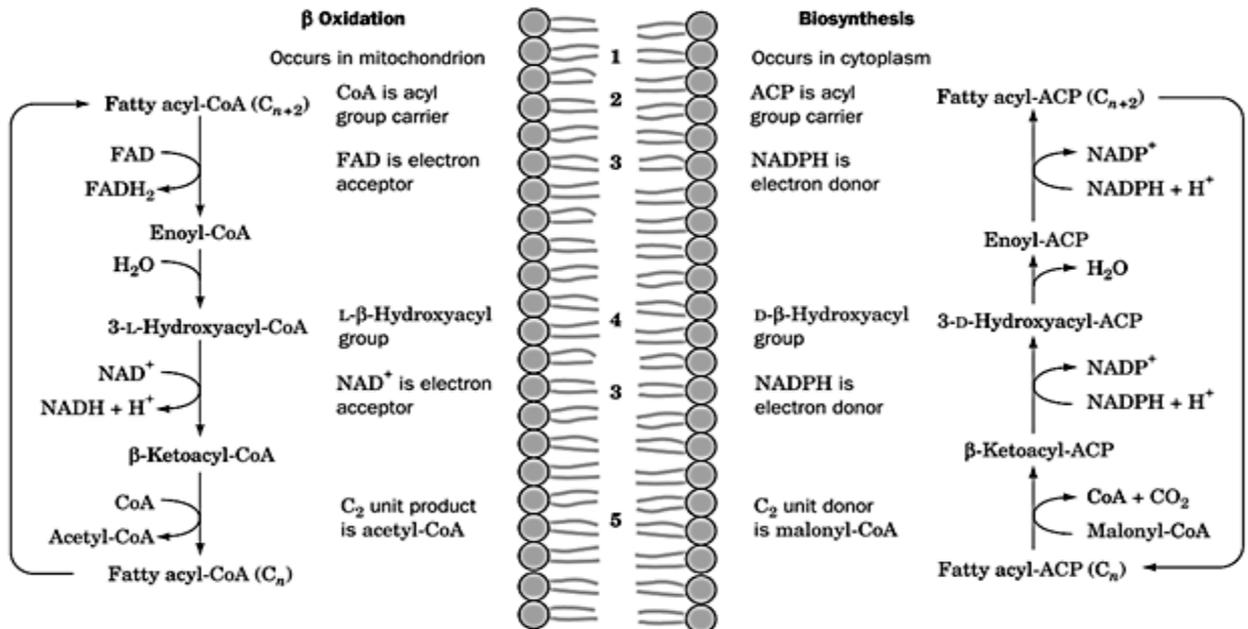


Figure 24. Comparison of fatty acid β -oxidation and fatty acid biosynthesis.

Differences occur in (1) cellular location, (2) acyl group carrier, (3) electron acceptor/donor, (4) stereochemistry of the hydration/dehydration reaction, and (5) the form in which C_2 units are produced/donated.

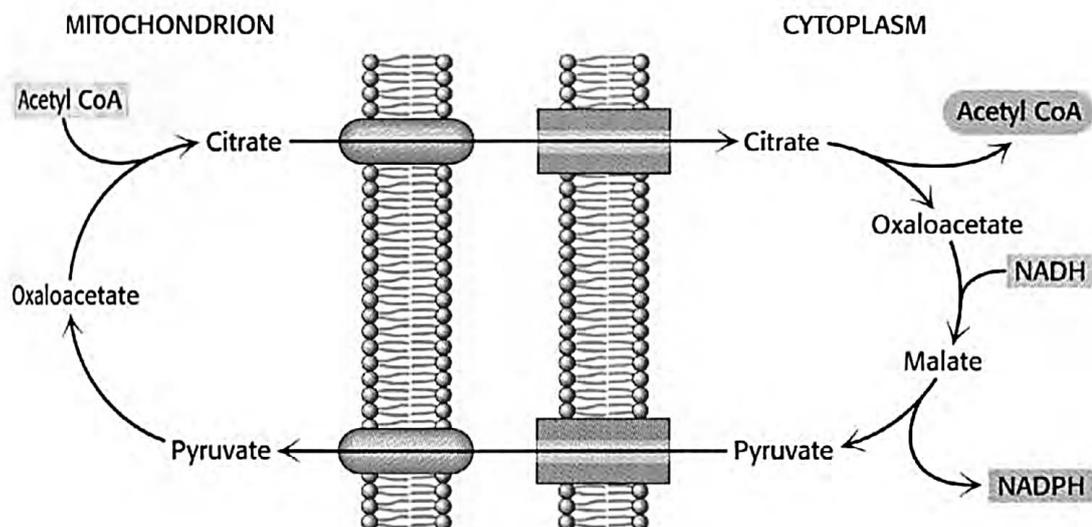
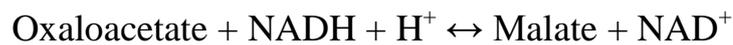
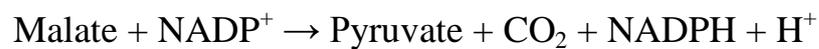


Figure 25. Transfer of acetyl-CoA to the cytoplasm.

Oxaloacetate formed in the transfer of acetyl groups to the cytoplasm must be returned to the mitochondria. The inner mitochondrial membrane is impermeable to oxaloacetate. Hence, a series of bypass reactions are needed. Most important, these reactions generate much of the NADPH needed for fatty acid synthesis. First, oxaloacetate is reduced to malate by NADH. This reaction is catalyzed by a malate dehydrogenase in the cytoplasm.



Second, malate is oxidatively decarboxylated by an NADP⁺-linked malate enzyme (also named malic enzyme).

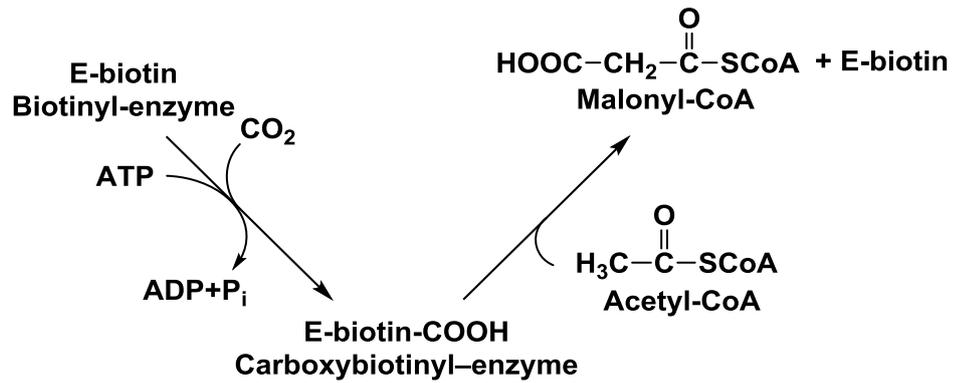


The pyruvate formed in this reaction readily enters mitochondria, where it is carboxylated to oxaloacetate by pyruvate carboxylase.



Thus, one molecule of NADPH is generated for each molecule of acetyl-CoA that is transferred from mitochondria to the cytoplasm. Hence, eight molecules of NADPH are formed when eight molecules of acetyl-CoA are transferred to the cytoplasm for palmitate synthesis. The additional six molecules of NADPH required for this process come from the pentose phosphate pathway.

ACC (acetyl-CoA carboxylase) is a biotin-dependent enzyme that catalyzes the first committed step of fatty acid biosynthesis and one of its rate-controlling steps. It is a member of a family of biotin-dependent carboxylases that, in humans, has only three members besides ACC: propionyl-CoA carboxylase, pyruvate carboxylase, and β -methylcrotonyl-CoA carboxylase. The ACC reaction, like those of other biotin-dependent carboxylases, occurs in two steps, a CO₂ activation and a carboxylation:



Acetyl-CoA carboxylase plays an essential role in regulating fatty acid synthesis and degradation. Recall that this enzyme catalyzes the committed step in fatty acid synthesis: the production of malonyl-CoA (the activated two-carbon donor). This important enzyme is subject to both local and hormonal regulation.

Acetyl-CoA carboxylase is switched off by phosphorylation and activated by dephosphorylation (Fig. 26). cAMP-dependent protein kinase (cAMPK) converts the carboxylase into an inactive form by modifying a single serine residue. cAMPK is essentially a fuel gauge; it is activated by cAMP. Thus, the carboxylase is inactivated when the energy charge is low. Fats are not synthesized when energy is required.

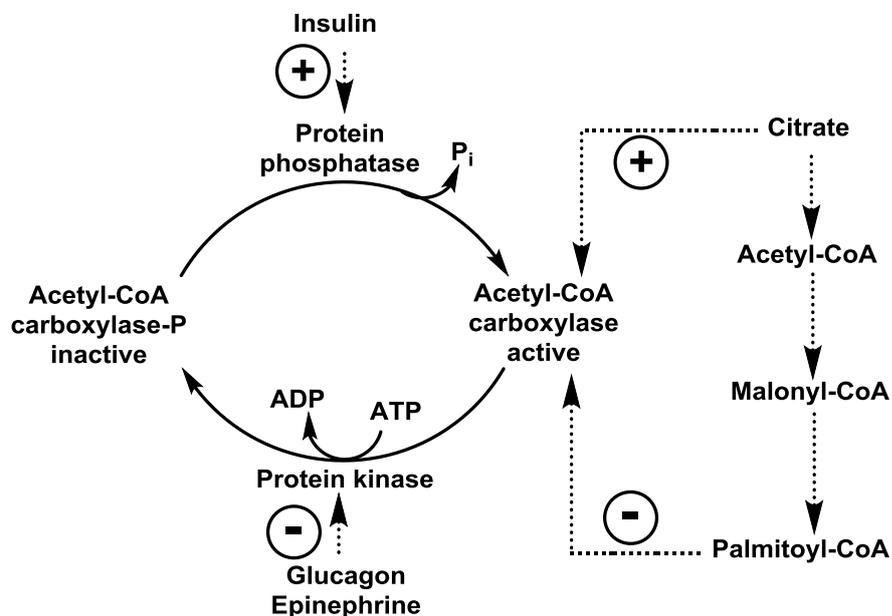


Figure 26. Control of acetyl-CoA carboxylase.

The carboxylase is also allosterically stimulated by citrate. Citrate acts in an unusual manner on inactive acetyl-CoA carboxylase, which exists as isolated

dimers. Citrate facilitates the polymerization of the inactive dimers into active filaments (Fig. 27). Citrate-induced polymerization can partly reverse the inhibition produced by phosphorylation. The level of citrate is high when both acetyl-CoA and ATP are abundant, signifying that raw materials and energy are available for fatty acid synthesis. The stimulatory effect of citrate on the carboxylase is counteracted by palmitoyl-CoA, which is abundant when there is an excess of fatty acids. Palmitoyl-CoA causes the filaments to disassemble into the inactive subunits. Palmitoyl-CoA also inhibits the translocase that transports citrate from mitochondria to the cytoplasm, as well as glucose 6-phosphate dehydrogenase, which generates NADPH in the pentose phosphate pathway.

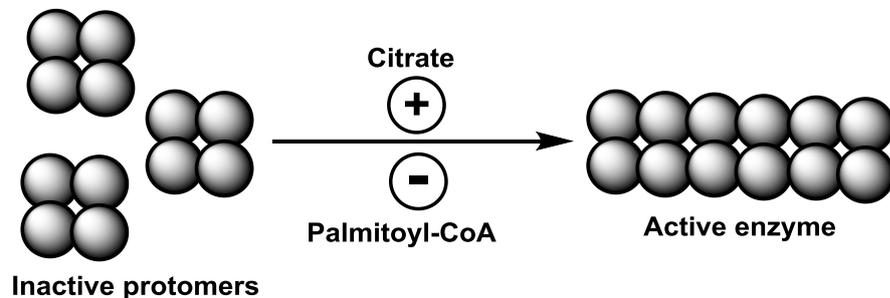


Figure 27. Activation of acetyl-CoA carboxylase by polymerization.

Acetyl-CoA carboxylase also plays a role in the regulation of fatty acid degradation. Malonyl-CoA, the product of this reaction, is present at a high level when fuel molecules are abundant. Malonyl-CoA inhibits carnitine acyltransferase I, preventing the entry of fatty acyl-CoAs into mitochondrial matrix in times of plenty. Malonyl-CoA is an especially effective inhibitor of carnitine acyltransferase I in heart and muscle, tissues that have little fatty acid synthesis capacity of their own. In these tissues, acetyl-CoA carboxylase may be a purely regulatory enzyme.

Carboxylase is controlled by hormones glucagon, epinephrine, and insulin, which reflect the overall energy status of the organism. *Insulin stimulates fatty acid synthesis by activating the carboxylase, whereas glucagon and epinephrine have the reverse effect.*

Regulation by Glucagon and Epinephrine. Consider, again, a person who has just awakened from a night's sleep and begins a bout of exercise. As mentioned, glycogen stores will be low, but lipids are readily available for mobilization.

As stated earlier, the hormones glucagon and epinephrine, present under conditions of fasting and exercise, will stimulate the release of fatty acids from triacylglycerols in fat cells, which will be released into the blood, and probably from muscle cells, where they will be used immediately as fuel. These same hormones will inhibit fatty acid synthesis by inhibiting acetyl-CoA carboxylase. Although the exact mechanism by which these hormones exert their effects is not known, the net result is to augment the inhibition by the cAMP-dependent kinase. This result makes sound physiological sense: when the energy level of the cell is low, as signified by high concentration of AMP, and the energy level of the organism is low, as signaled by glucagon, fats should not be synthesized. Epinephrine, which signals the need for immediate energy, enhances this effect. Hence, these hormones switch off fatty acid synthesis by keeping the carboxylase in inactive phosphorylated state.

Regulation by Insulin. Now consider the situation after the exercise has ended and the runner has had a meal. In this case, the hormone insulin inhibits the mobilization of FA from adipocytes and stimulates their accumulation in a form of triacylglycerols in adipose tissue. Insulin also stimulates fatty acid synthesis by activating acetyl-CoA carboxylase. Insulin stimulates the carboxylase by stimulating the activity of a protein phosphatase that dephosphorylates and activates acetyl-CoA carboxylase. Thus, the signal molecules glucagon, epinephrine, and insulin act in concert on triacylglycerol metabolism and acetyl-CoA carboxylase to carefully regulate the utilization and storage of fatty acids.

Reactions of fatty acid synthesis are catalysed by a multifunctional enzyme known as fatty acid synthase (FAS) complex. In eukaryotic cells, including man, FAS exists as a dimer with two identical units (Fig. 28). Each monomer possesses the activities of seven different enzymes and an acyl carrier protein (ACP) bound

to 4'-phosphopantetheine. FAS functions as a single unit catalysing all the seven reactions to produce at the same time two palmityl-CoA. Dissociation of the synthase complex in two monomers results in loss of the enzyme activities.

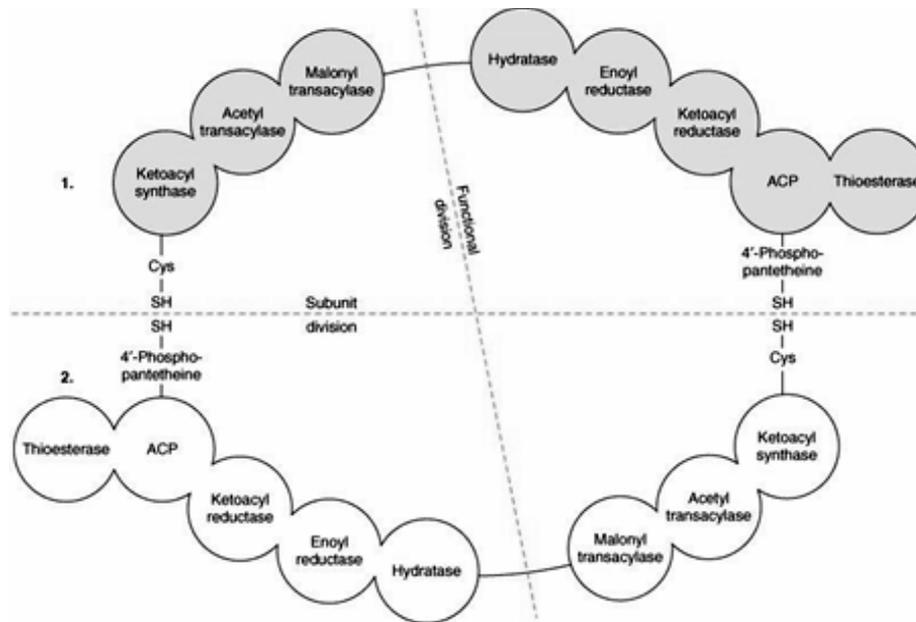


Figure 28. Fatty acid synthase complex.

The sequence of reactions of the extramitochondrial synthesis of fatty acids (palmitate) is depicted in Fig. 29-31.

1. The two carbon fragment of acetyl CoA is transferred to ACP of fatty acid synthase, catalysed by the enzyme, acetyl CoA-ACP transacylase. The acetyl unit is then transferred from ACP to cysteine residue of the enzyme. Thus ACP site falls vacant.

2. The enzyme malonyl-CoA-ACP transacylase transfers malonate from malonyl-CoA to bind to ACP.

3. The acetyl unit attached to cysteine is transferred to malonyl group (bound to ACP). The malonyl moiety loses CO_2 which was added by acetyl-CoA carboxylase. Thus, CO_2 is never incorporated into fatty acid carbon chain. The decarboxylation is accompanied by loss of free energy which allows the reaction to proceed forward. This reaction is catalyzed by β -ketoacyl ACP synthase.

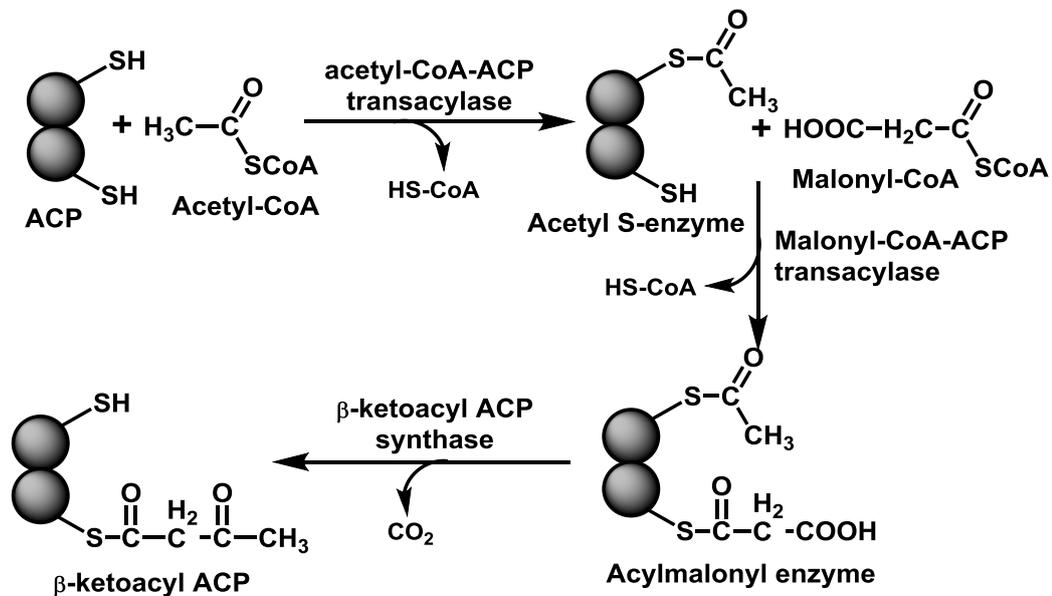


Figure 29. β -Ketoacyl ACP formation.

4. β -Ketoacyl ACP reductase reduces ketoacyl group to hydroxyacyl group. The reducing equivalents are supplied by NADPH.

5. β -Hydroxyacyl ACP undergoes dehydration. A molecule of water is eliminated and a double bond is introduced between α and β carbons.

6. A second NADPH-dependent reduction, catalyzed by enoyl-ACP reductase occurs to produce acyl-ACP. The four-carbon unit attached to ACP is butyryl group. The carbon chain attached to ACP is transferred to cysteine residue and the reactions 2-6 are repeated 6 more times. Each time, the fatty acid chain is lengthened by a two-carbon unit (obtained from malonyl-CoA). At the end of 7 cycles, the fatty acid synthesis is complete and a 16-carbon fully saturated fatty acid-namely palmitate-bound to ACP is produced.

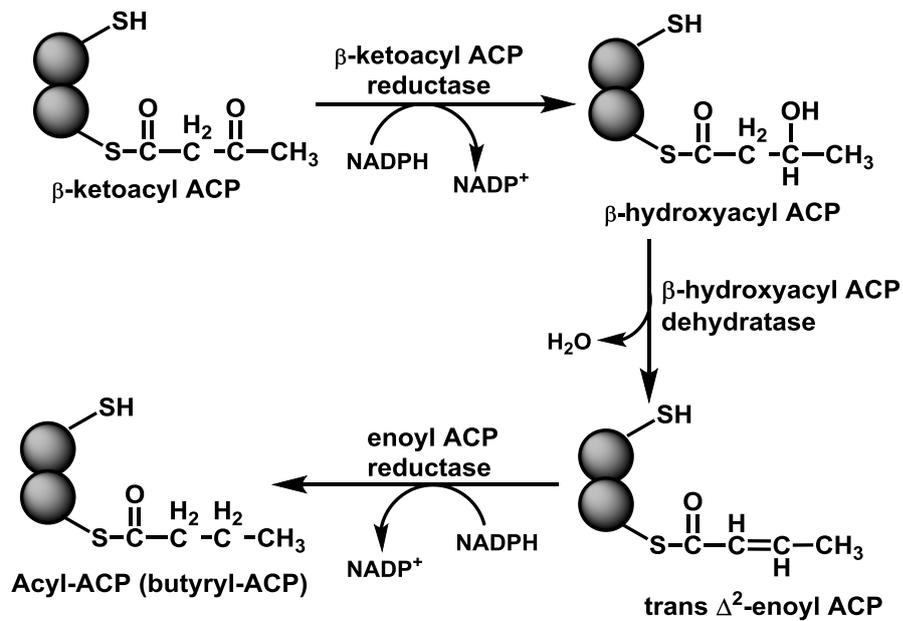


Figure 30. Butyryl ACP synthesis.

7. The enzyme palmitoyl thioesterase separates palmitate from fatty acid synthase. This completes the synthesis of palmitate.

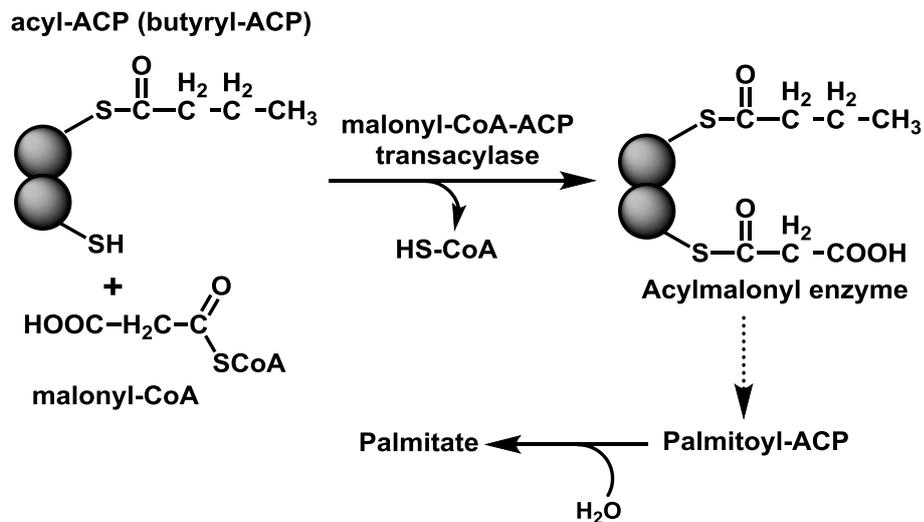


Figure 31. Palmitate formation.

Palmitate (16:0), the normal product of the animal fatty acid synthase pathway, is the precursor of longer chain saturated and unsaturated fatty acids through the actions of elongases and desaturases. Elongases are present in both the mitochondrion and the ER (endoplasmic reticulum) but the mechanisms of elongation at the two sites differ. Mitochondrial elongation occurs by successive addition and reduction of acetyl units; the only chemical difference between these two pathways occurs in the final reduction step in which NADPH takes the place

of FADH₂ as the terminal redox coenzyme (Fig. 32). Elongation in the ER involves the successive condensations of malonyl-CoA with acyl-CoA. These reactions are each followed by NADPH-associated reductions similar to those catalyzed by FAS, the only difference being that the fatty acid is elongated as its CoA derivative rather than as its ACP derivative.

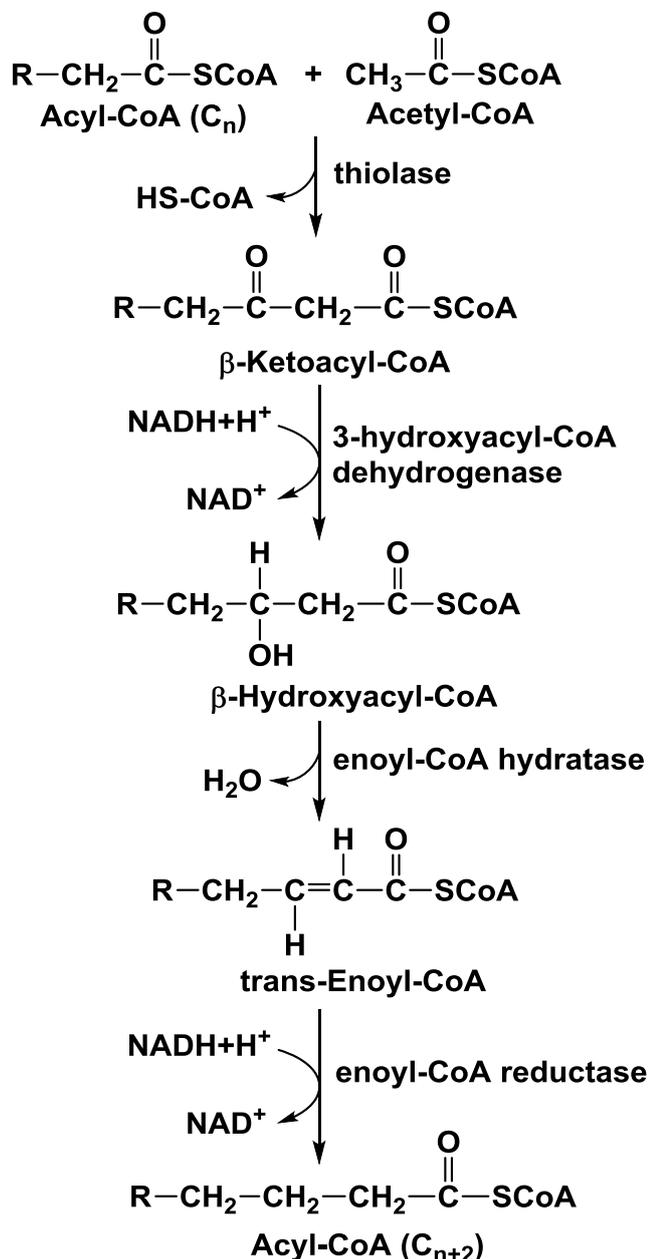
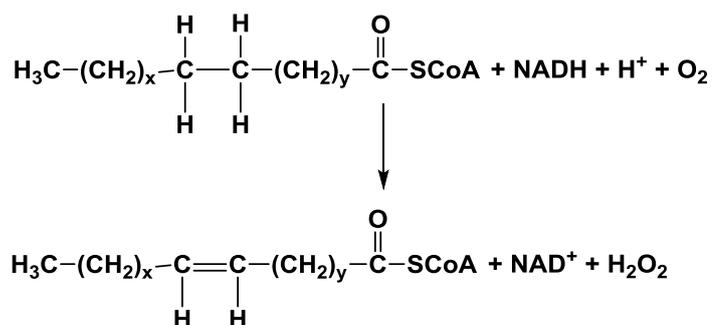


Figure 32. Mitochondrial fatty acid elongation.

Unsaturated fatty acids are produced by terminal desaturases. Mammalian systems contain four terminal desaturases of broad chain-length specificities designated Δ^9 -, Δ^6 -, Δ^5 -, and Δ^4 -fatty acyl-CoA desaturases. These membrane-bound, nonheme iron-containing enzymes catalyze the general reaction



where x is at least 5 and where $(\text{CH}_2)_x$ can contain one or more double bonds. The $(\text{CH}_2)_y$ portion of the substrate is always saturated. Double bonds are inserted between existing double bonds in the $(\text{CH}_2)_x$ portion of the substrate and the CoA group such that the new double bond is three carbon atoms closer to the CoA group than the next double bond (not conjugated to an existing double bond) and, in animals, never at positions beyond C9. Mammalian terminal desaturases are components of mini-electron-transport systems that contain two other proteins: cytochrome b_5 and NADH-cytochrome b_5 reductase. The electron-transfer reactions mediated by these complexes occur at the inner surface of the ER membrane (Fig. 33) and are therefore not associated with oxidative phosphorylation.

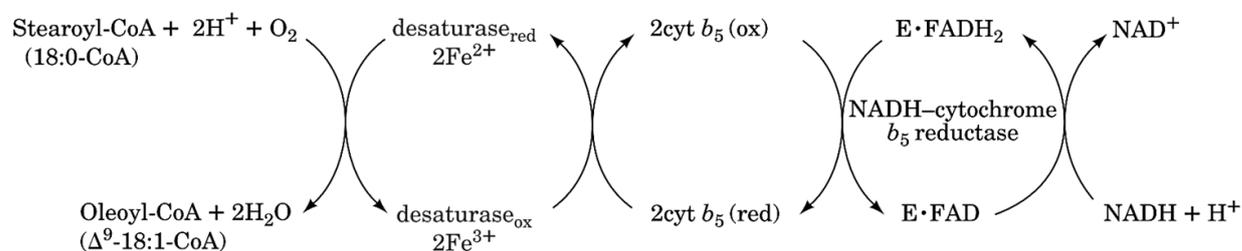
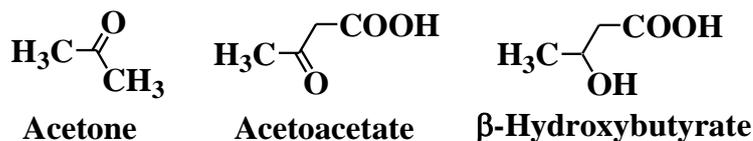


Figure 33. The electron-transfer reactions mediated by the Δ^9 -fatty acyl-CoA desaturase complex.

Ketone bodies

Acetate, Acetone and β -Hydroxybutyrate are named as Ketone bodies. They are preferred as energy source during their conversions in the heart, skeletal muscle and kidney. The brain, under normal circumstances, uses only glucose as its energy source (fatty acids are unable to pass the blood-brain barrier), but during starvation, ketone bodies become the brain's major fuel source. Ketone bodies are water-soluble equivalents of fatty acids except acetone. The end product for their

catabolic pathway is acetyl-CoA that is involved in Krebs Cycle to be utilized there. Acetone cannot be utilized in humans, it is very inert molecule.



Ketone bodies synthesis is activated in the case of acetyl-CoA accumulation in the cytoplasm of hepatocytes. It may be during extended β -oxidation of HFA and tissue lipolysis.

Acetoacetate formation occurs in three reactions (Fig. 34):

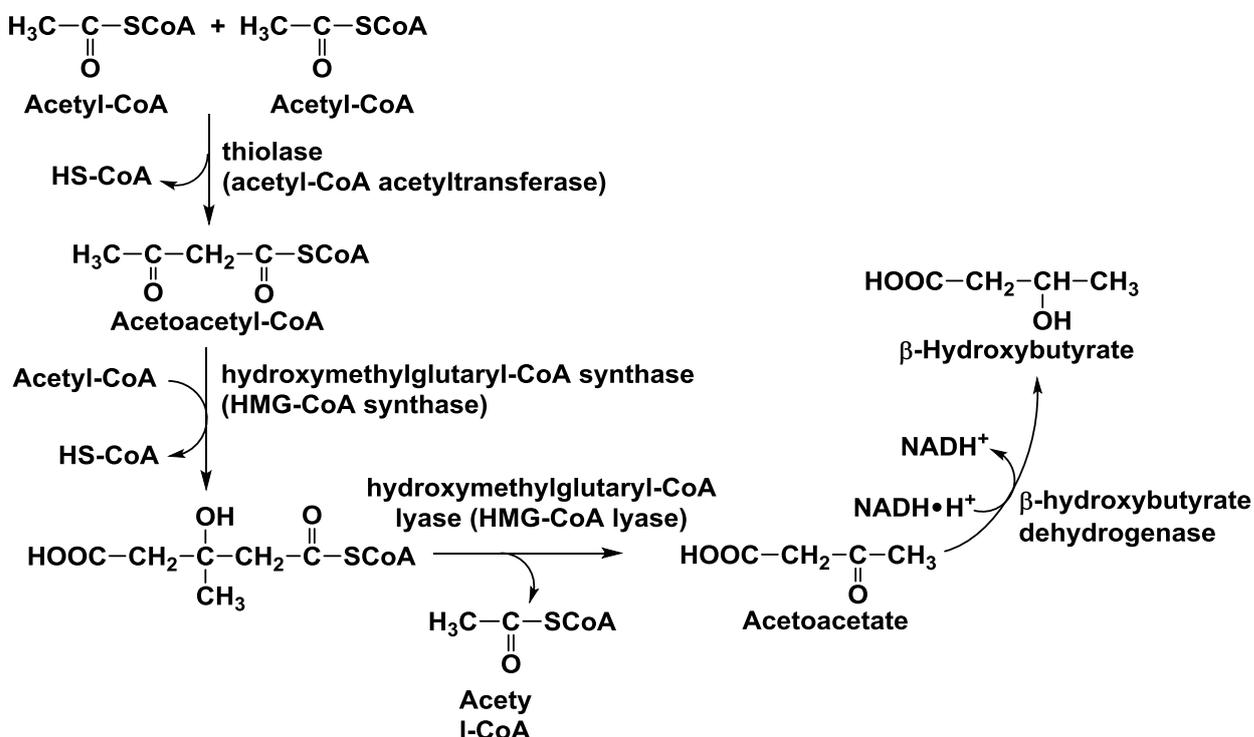


Figure 34. Ketogenesis.

1. Two molecules of acetyl-CoA are condensed to acetoacetyl-CoA by thiolase (also called acetyl-CoA acetyltransferase) working in the reverse direction from the way it does in the final step of β -oxidation.

2. Condensation of the acetoacetyl-CoA with a third acetyl-CoA by HMG-CoA synthase forms β -hydroxy- β -methylglutaryl-CoA (HMG-CoA).

3. Degradation of HMG-CoA to acetoacetate and acetyl-CoA is catalyzed by HMG-CoA lyase.

Acetoacetate may be reduced to β -hydroxybutyrate by β -hydroxybutyrate dehydrogenase.

The liver releases acetoacetate and β -hydroxybutyrate, which are carried by the bloodstream to the peripheral tissues for use as alternative fuels. There, these products are converted to acetyl-CoA as is diagrammed in Fig. 35. Succinyl-CoA, which acts as the CoA donor, can also be converted to succinate with the coupled synthesis of GTP in the succinyl-CoA synthetase reaction of the citric acid cycle. The “activation” of acetoacetate bypasses this step and therefore “costs” the free energy of GTP hydrolysis. The liver lacks 3-ketoacyl-CoA transferase, which permits it to supply ketone bodies to other tissues.

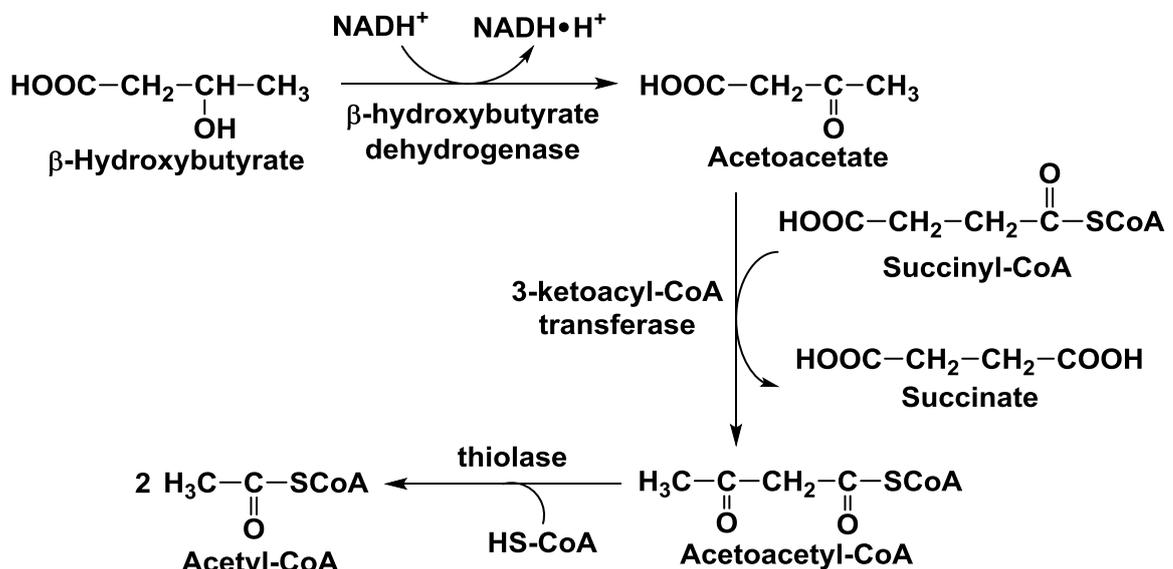


Figure 35. The metabolic conversion of ketone bodies to acetyl-CoA.

The ketone bodies level of the blood serum of healthy humans must be in the region 0.034-0.43 mmol/L. In the blood ketone bodies levels increase sufficiently, as they do after about 20 days of starvation, they are a valuable energy substrates for the brain and may account for up to 75% of brain oxidation. Ketone bodies concentration is elevated in patients with diabetes mellitus, too.

The most common of these conditions is diabetic ketosis in patients with insulin-dependent diabetes mellitus. It curtails fatty acid mobilization by adipose tissue. The absence of insulin has two major biochemical consequences (Fig. 36).

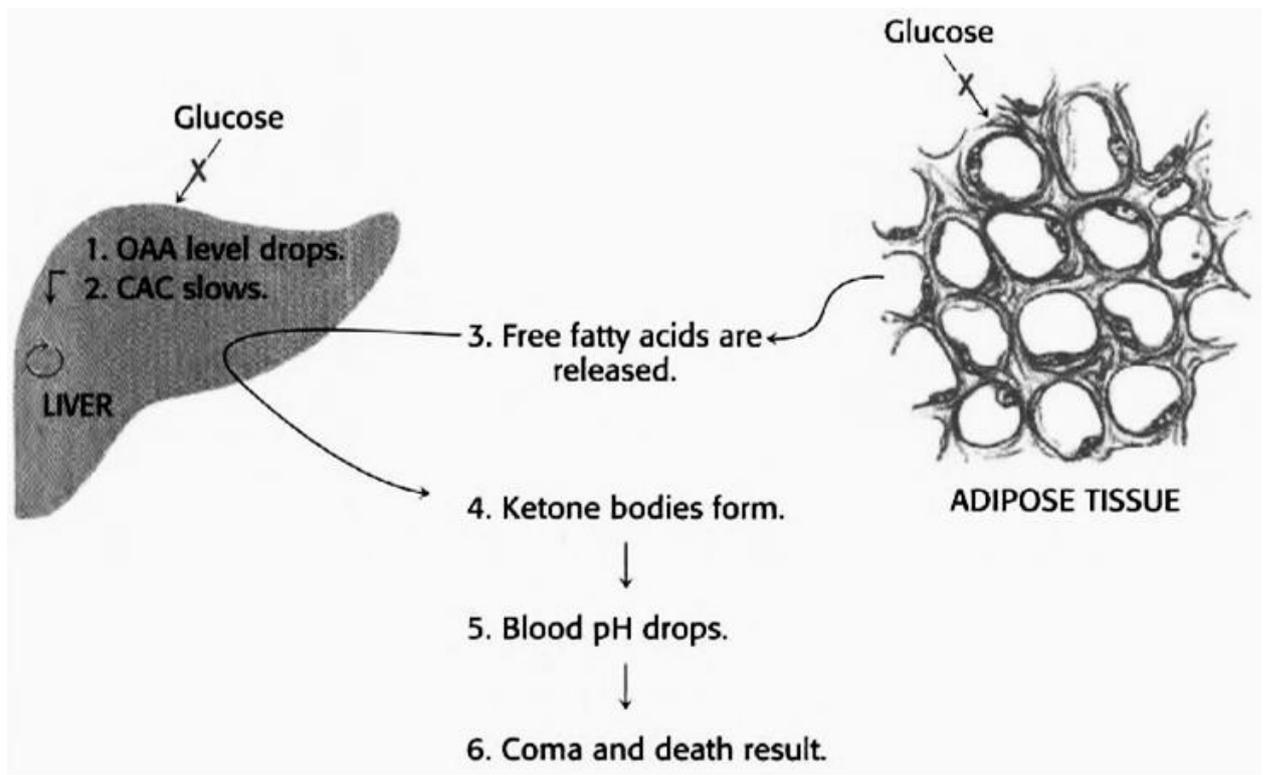


Figure 36. Diabetic ketosis results when insulin is absent.

OAA – glucose-derived oxaloacetate; CAC – citric acid cycle.

First, the liver cannot use glucose and consequently cannot provide oxaloacetate to process fatty acid-derived acetyl- CoA. Second, adipose cells continue to release fatty acids into the bloodstream, which are taken up by the liver to be involved in β -oxidation with the production of excess Acetyl-CoA which is converted into ketone bodies. The liver thus produces large amounts of ketone bodies, which are moderately strong acids, except acetone. As the result for patient is severe metabolic acidosis. The decrease in pH impairs tissue function, most importantly in the central nervous system.

Laboratory works

Qualitative tests for ketone bodies

Lieber's test

The principle of the method:

Acetone reacts with iodine turning into iodophorm in the presence of alkali. The formation of it is recognized by a specific odour.



The course of laboratory work:

Add 5-6 drops of 10% NaOH solution and 3-4 drops of Lugol's reagent to 1 mL of acetone solution. Iodophorm will be formed. In case of large quantity of acetone in the urine the crystalline precipitate of iodophorm may be formed.

Legal's reaction

The principle of the method:

In alkaline environment acetone and acetoacetic acid form an orange-red colour complex with sodium nitroprusside. After adding of glacial acetic acid (100% solution) a cherry-coloured compound will be formed.

The course of laboratory work:

Pour 1 mL of acetone into a test-tube, add some drops of 10% NaOH solution and then pour some drops of fresh sodium nitroprusside solution. The red colouring will appear. The intensity of colouring grows due to the addition of acetic acid.

Clinical significance of qualitative tests for ketone bodies

They are used usually for the determination of ketone bodies in the urine of patients at long time starvation, in severe form of diabetes mellitus, in patients with high rate of tissue lipolysis.

CHOLESTEROL METABOLISM. THE REGULATION AND DISORDERS OF LIPIDS METABOLISM: OBESITY, ATHEROSCLEROSIS

Steroids

Steroids contain a four-ring structure named the steroid nucleus (Fig. 36). Cholesterol is the steroid precursor in human cells from which all of the steroid hormones are synthesized by modifications to the ring or C₂₀ side chain. Although cholesterol is not water soluble, it is converted to amphipathic water-soluble bile salts such as cholic acid. Bile salts line the surfaces of lipid droplets called micelles in the lumen of the intestine, where they keep the droplets emulsified in the aqueous environment.

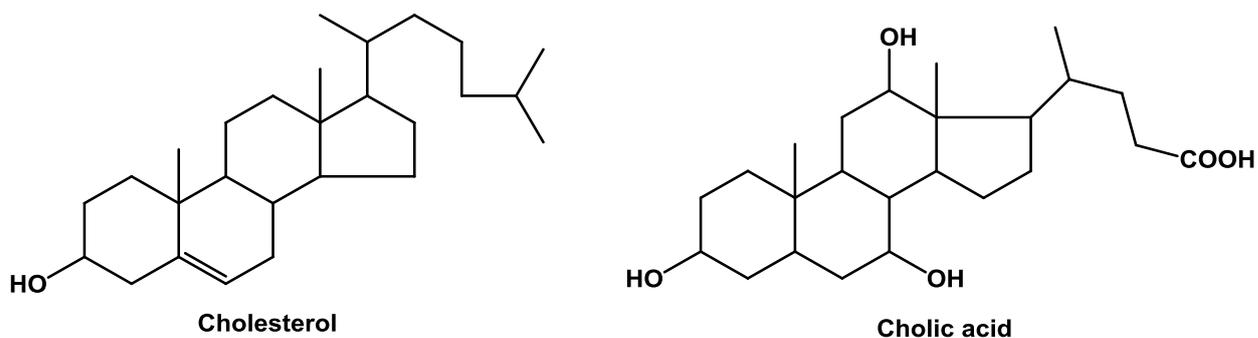


Figure 36. Cholesterol and cholic acid.

Cholesterol metabolism in humans

All ways of the cholesterol metabolism and its utilization are represented in the figure 37.

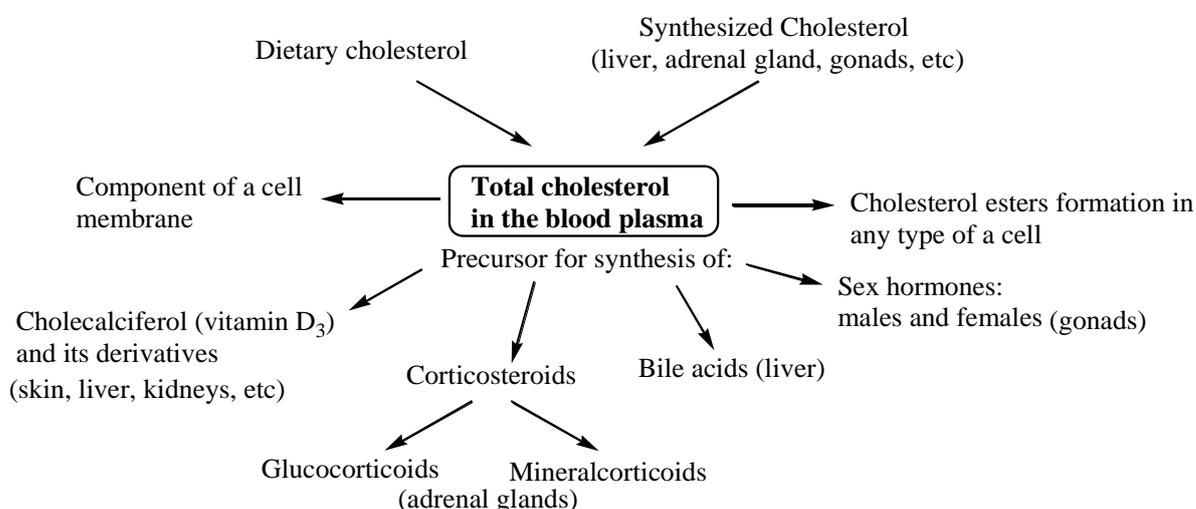


Figure 37. All the ways for the formation of cholesterol pool in the blood plasma.

Synthesis of cholesterol

Almost all the tissues of the body participate in cholesterol biosynthesis. The largest contribution is made by liver (50 %), intestine (15 %), skin, adrenal cortex, reproductive tissue etc. The enzymes involved in cholesterol synthesis are found in the cytosol and microsomal fractions of the cell.

For the production of one mole of cholesterol, 18 moles of acetyl-CoA, 36 moles of ATP and 16 moles of NADPH are required.

All 27 carbon atoms of cholesterol are derived from acetyl-CoA in a five-stage synthetic process:

1. Synthesis of β -hydroxy- β -methylglutaryl-CoA (HMG-CoA)

2. Synthesis of mevalonate
3. Synthesis of isoprene units
4. Synthesis of squalene
5. Squalene cyclization and cholesterol formation.

1. Synthesis of β -hydroxy- β -methylglutaryl-CoA (HMG-CoA).

The first stage in the synthesis of cholesterol is the formation of β -hydroxy- β -methylglutaryl-CoA from acetyl-CoA (Fig. 38). Two moles of acetyl-CoA condense to form acetoacetyl-CoA. Another molecule of acetyl-CoA is then added to produce HMG-CoA. HMG-CoA synthesis requires the participation of two enzymes: thiolase and HMG-CoA synthase. The enzymes forming the HMG-CoA leading to ketone bodies occur in the mitochondria, whereas those responsible for the synthesis of the HMG-CoA that is destined for cholesterol biosynthesis are located in the cytosol. Their catalytic mechanisms, however, are identical.

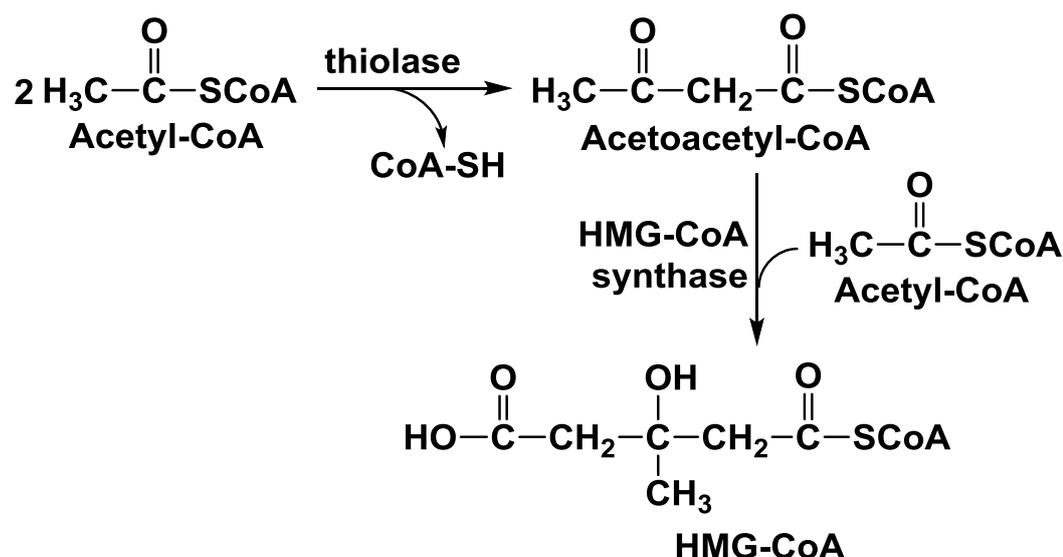


Figure 38. Synthesis of β -hydroxy- β -methylglutaryl-CoA (HMG-CoA)

2. Synthesis of mevalonate.

The CoA thioester group of HMG-CoA is reduced to an alcohol in an NADPH-dependent four-electron reduction catalyzed by HMG-CoA reductase, yielding mevalonate (Fig. 39).

4. Synthesis of squalene.

Four isopentenyl pyrophosphates and two dimethylallyl pyrophosphates condense to form the C₃₀ cholesterol precursor squalene in three reactions catalyzed by two enzymes (Fig. 41):

1. Prenyltransferase (farnesyl pyrophosphate synthase) catalyzes the head-to-tail (1'-4) condensation of dimethylallyl pyrophosphate and isopentenyl pyrophosphate to yield geranyl pyrophosphate.

2. Prenyltransferase catalyzes a second head-to-tail condensation of geranyl pyrophosphate and isopentenyl pyrophosphate to yield farnesyl pyrophosphate.

3. The endoplasmic reticulum enzyme squalene synthase then catalyzes the head-to-head (1-1') condensation of two farnesyl pyrophosphate molecules to form squalene. Farnesyl pyrophosphate is also a precursor of dolichol, farnesylated and geranylgeranylated proteins, and ubiquinone.

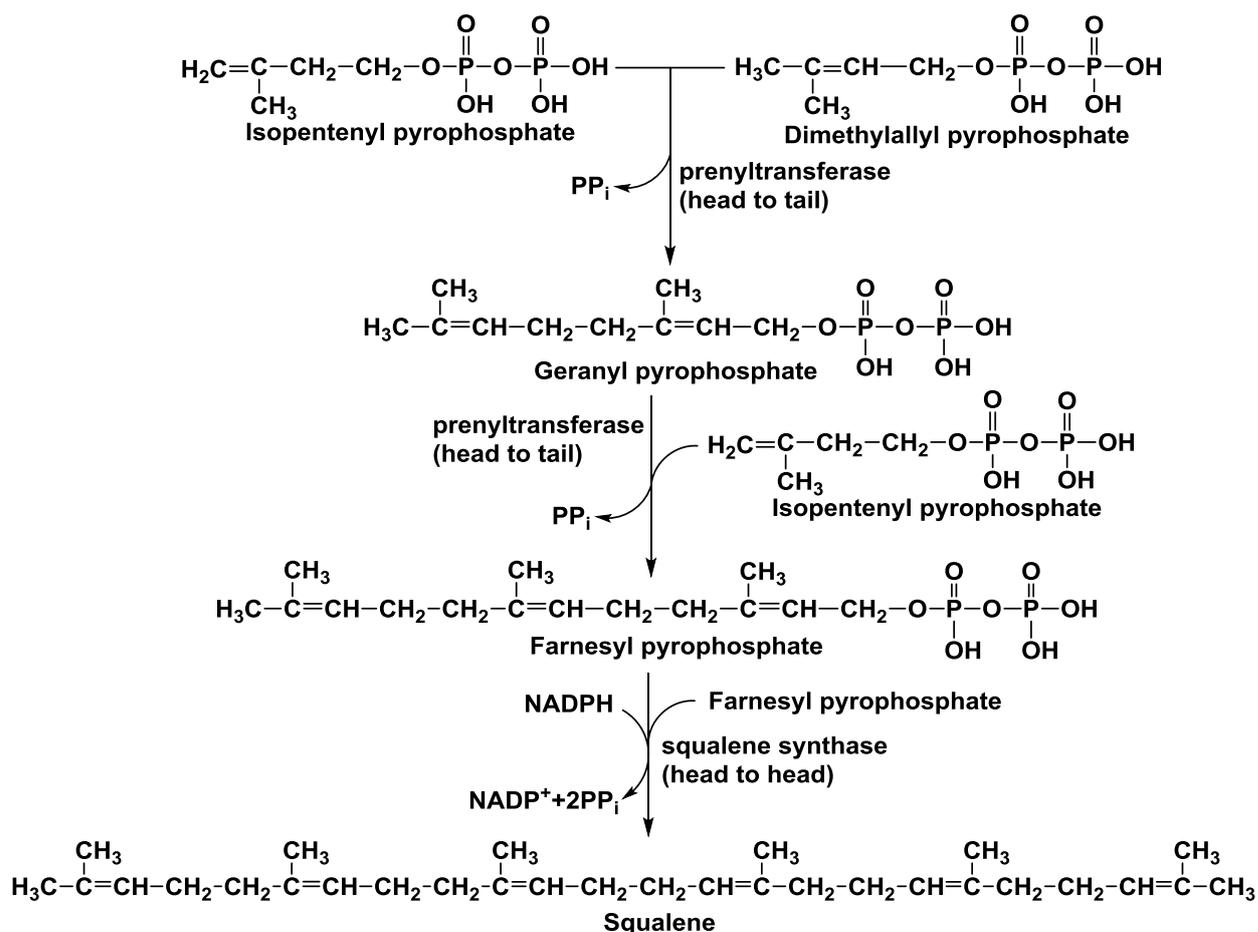


Figure 41. Synthesis of squalene

5. Squalene cyclization and cholesterol formation.

Squalene undergoes hydroxylation and cyclization utilizing O_2 and NADPH to form lanosterol. The formation of cholesterol from lanosterol is a multistep process with a series of about 19 enzymatic reactions (Fig. 42).

The following are the most important reactions:

- Elimination the carbon atoms from 30 to 27.
- Removal of two methyl groups from C_4 and one methyl group from C_{14} .
- Shift of double bond from C_8 to C_5 .
- Reduction in the double bond present between C_{24} and C_{25} .

Control of Cholesterol Biosynthesis

Cholesterol may be obtained from the diet or it may be synthesized de novo. An adult person on a low-cholesterol diet typically synthesizes about 800 mg of cholesterol per day. The rate of cholesterol formation by these organs is highly responsive to the cellular level of cholesterol. This feedback regulation is mediated primarily by changes in the amount and activity of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase).

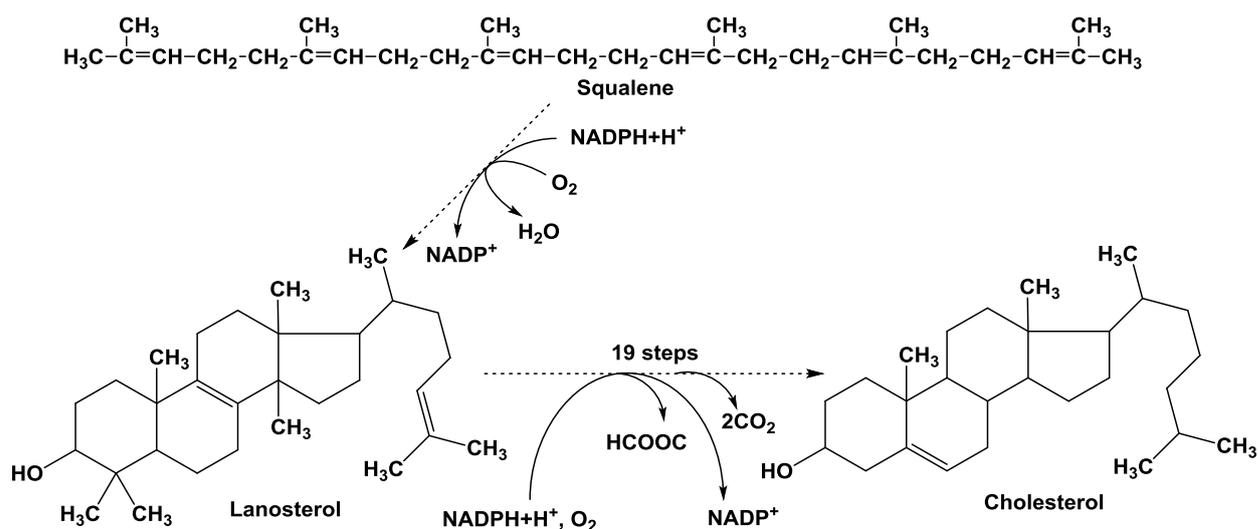


Figure 42. Squalene cyclization and cholesterol formation

The main way in which HMG-CoA reductase is controlled is by long-term feedback control of the amount of enzyme present in a cell. When either LDL-cholesterol or mevalonate levels fall, the amount of HMG-CoA reductase present in the cell can rise as much as 200-fold, due to an increase in enzyme synthesis combined with a decrease in its degradation. When LDL-cholesterol or mevalonolactone (an internal ester of mevalonate that is hydrolyzed to mevalonate and metabolized in the cell) are added back to a cell, these effects are reversed.

The mechanism by which cholesterol serves to control the expression of the >20 genes involved in its biosynthesis and uptake, such as those encoding HMG-CoA reductase and the LDL receptor, has been elucidated by Michael Brown and Joseph Goldstein.

The rate of mRNA synthesis of HMG-CoA reductase is controlled by the sterol regulatory element binding protein (SREBP). This transcription factor binds to a short DNA sequence named the sterol regulatory element (SRE) on the 5' side of the reductase gene. It binds to the SRE when cholesterol levels are low and enhances transcription. In its inactive state, the SREBP resides in the endoplasmic reticulum membrane, where it is associated with the SREBP cleavage activating protein (SCAP), an integral membrane protein. SCAP is the cholesterol sensor. When cholesterol levels fall, SCAP escorts SREBP in small membrane vesicles to the Golgi complex, where it is released from the membrane by two specific proteolytic cleavages (Fig. 43). The released protein migrates to the nucleus and binds the SRE of the HMG-CoA reductase gene, as well as several other genes in the cholesterol biosynthetic pathway, to enhance transcription. When cholesterol levels rise, the proteolytic release of the SREBP is blocked, and the SREBP in the nucleus is rapidly degraded. These two events halt the transcription of genes of the cholesterol biosynthetic pathways.

The degradation of the reductase is stringently controlled. The enzyme is bipartite: its cytoplasmic domain carries out catalysis and its membrane domain senses signals that lead to its degradation. The membrane domain may undergo structural changes in response to increasing concentrations of sterols, and it makes

the enzyme more susceptible to proteolysis. The reductase may be further degraded by ubiquitination and targeting to the 26S proteasome under some conditions.

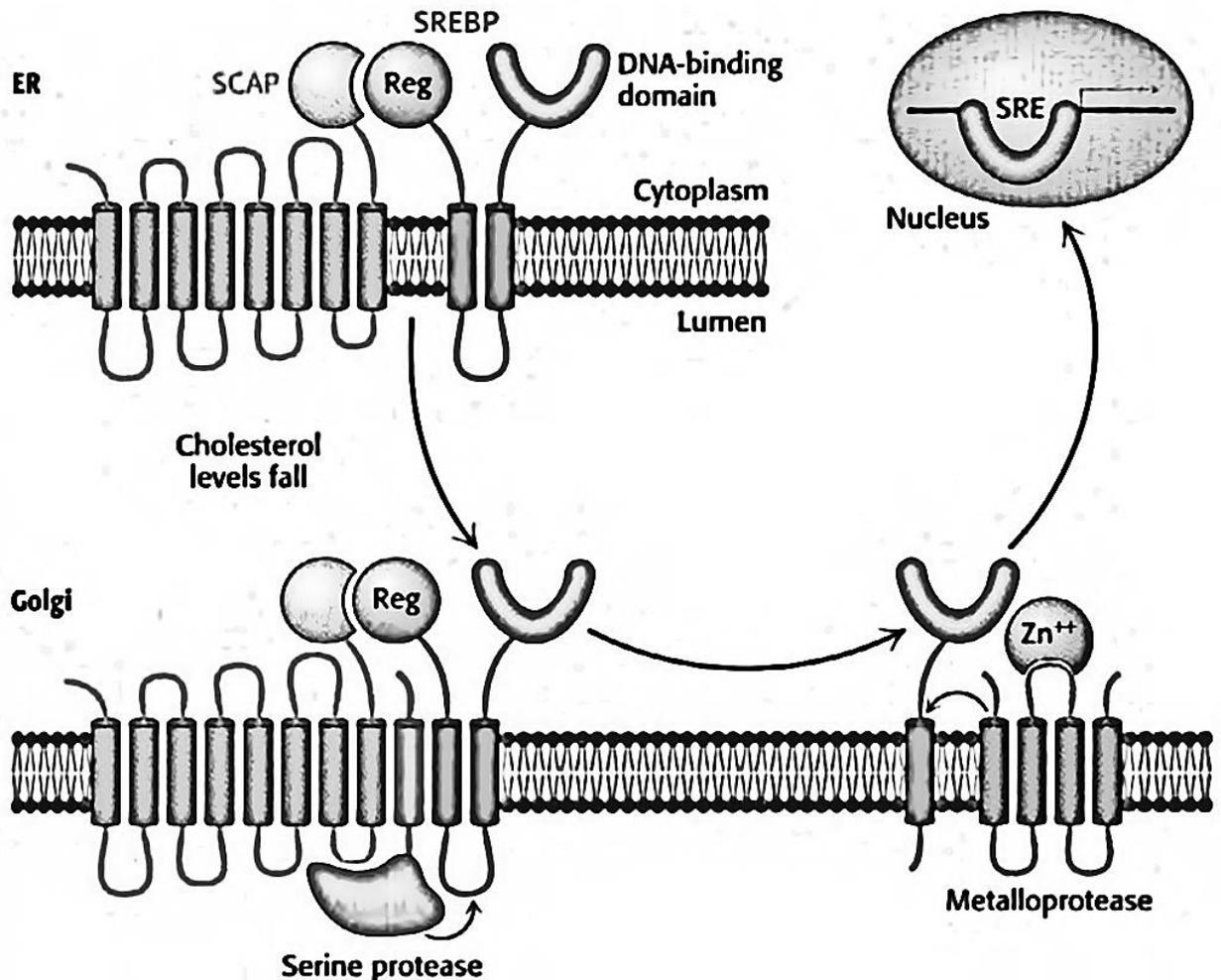


Figure 43. The cholesterol-mediated proteolytic activation of SREBP (Goldstein, J., Rawson, R.B., and Brown, M., *Arch. Biochem. Biophys.* 397, 139 (2002)).

HMG-CoA reductase exists in interconvertible more active and less active forms, as do glycogen phosphorylase, glycogen synthase, pyruvate dehydrogenase, and acetyl-CoA carboxylase, among others. The unmodified form of HMG-CoA reductase is more active and the phosphorylated form is less active. HMG-CoA reductase is phosphorylated (inactivated) at its Ser 871 in a bicyclic cascade system by the covalently modifiable enzyme AMP-dependent protein kinase (AMPK), which also acts on acetyl-CoA carboxylase. It appears that this control is exerted to conserve energy when ATP levels fall and AMP levels rise, by

inhibiting biosynthetic pathways. This hypothesis was tested by Brown and Goldstein, who used genetic engineering techniques to produce hamster cells containing a mutant HMG-CoA reductase with Ala replacing Ser 871 and therefore incapable of phosphorylation control. These cells respond normally to feedback regulation of cholesterol biosynthesis by LDL-cholesterol and mevalonate but, unlike normal cells, do not decrease their synthesis of cholesterol on ATP depletion, supporting the idea that control of HMG-CoA reductase by phosphorylation is involved in energy conservation.

In this way glucagon and glucocorticoids favour the formation of inactive HMG-CoA reductase (phosphorylated form) and, thus, decrease cholesterol synthesis. On the other hand, insulin and thyroxine increase cholesterol production by enhancing the formation of active HMG-CoA reductase (dephosphorylated form).

The drugs lovastatin (also called mevinolin and sold as Mevacor), pravastatin (Pravachol), and simvastatin (Zocor) are fungal products, which are used to decrease the serum cholesterol level in patients with hypercholesterolemia. The synthetic inhibitor atorvastatin (Lipitor) is presently one of the most widely prescribed drugs in USA. These drugs, collectively known as statins, are competitive inhibitors of HMG-CoA reductase and, therefore, reduce cholesterol synthesis. About 50 to 60 % decrease in serum cholesterol level has been reported by a combined use of these two drugs.

Cholesterol Degradation Pathways

The steroid nucleus (ring structure) of the cholesterol cannot be degraded to CO_2 and H_2O . Cholesterol (50 %) is converted to bile acids (excreted in feces), serves as a precursor for the synthesis of steroid hormones, vitamin D_3 , coprostanol and cholestanol. The latter two are the fecal sterols, besides cholesterol.

Bile Salts Formation. Bile salts are polar derivatives of cholesterol. These compounds are highly effective detergents because they contain both polar and nonpolar regions. Bile salts are synthesized in the liver, stored and concentrated in

the gall bladder, and then released into the small intestine. Bile salts, the major constituent of bile, solubilize dietary lipids. Solubilization increases the effective surface area of lipids with two consequences: (1) more surface area is exposed to the digestive action of lipases and (2) lipids are more readily absorbed by the intestine. Bile salts are also the major breakdown products of cholesterol.

Bile acid synthesis, which occurs in the liver, is outlined in Figure 44.

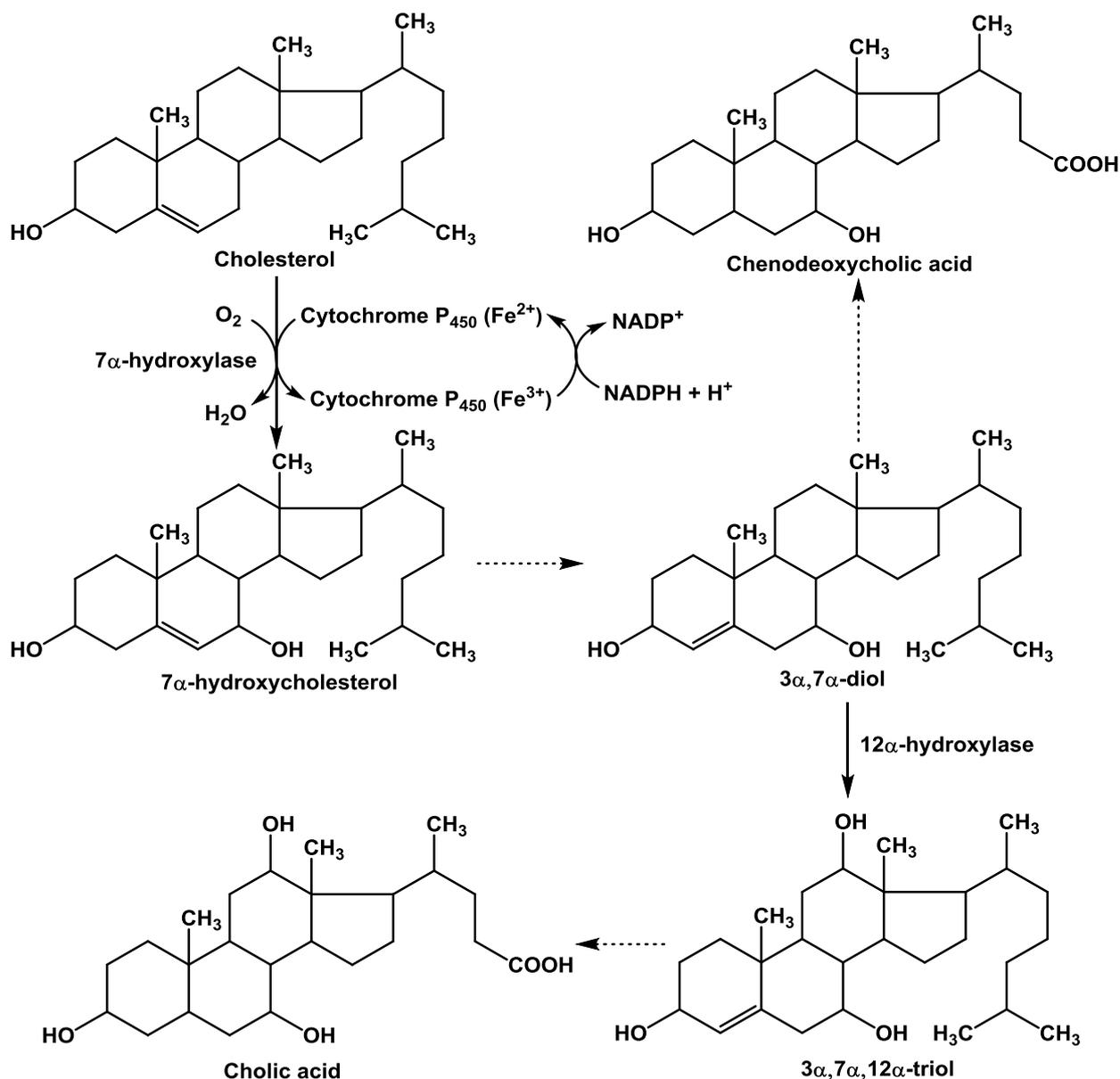


Figure 44. Synthesis of bile acids.

The conversion of cholesterol to 7 α -hydrocholesterol, catalyzed by cholesterol-7 α -hydroxylase (a microsomal enzyme), is the rate-limiting reaction in bile acid synthesis. In later reactions, the double bond at C-5 is rearranged and reduced, and an additional hydroxyl group is introduced. Two different sets of

compounds are produced. One set has α -hydroxyl groups at positions 3, 7, and 12, and produces the cholic acid series of bile salts. The other set has α -hydroxyl groups only at positions 3 and 7 and produces the chenodeoxycholic acid series. Three carbons are removed from the side chain by an oxidation reaction. The remaining 5-carbon fragment attached to the ring structure contains a carboxyl group. The products of this process, cholic acid and chenodeoxycholic acid, are converted to bile salts by microsomal enzymes that catalyze conjugation reactions. These conversion steps help to increase solubility of intermediate metabolites. Most bile acids are conjugated with glycine or taurine (Fig. 45).

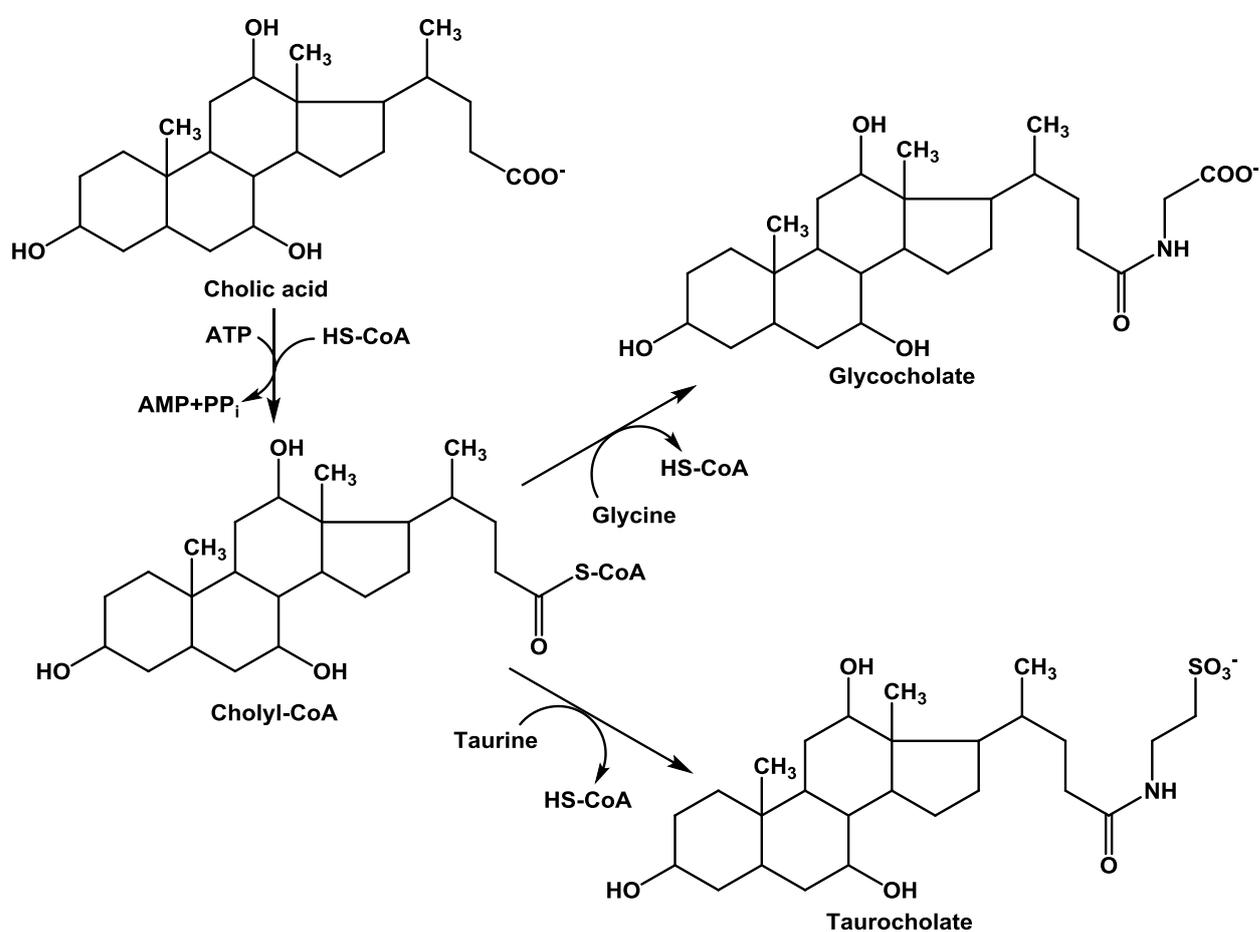


Figure 45. Synthesis of glycine and taurine conjugates.

In the bile, the conjugated bile acids exist as sodium and potassium salts which are known as bile salts. In the intestine, a portion of primary bile acids undergoes deconjugation and dehydroxylation to form secondary bile acids (deoxycholic acid and lithocholic acid). These reactions are catalyzed by bacterial enzymes in the intestine.

Enterohepatic circulation. The conjugated bile salts synthesized in the liver accumulate in gall bladder. From there they are secreted into the small intestine where they serve as emulsifying agents for the digestion and absorption of fats and fat soluble vitamins. A large portion of bile salts (primary and secondary) are reabsorbed and returned to the liver through portal vein. Thus the bile salts are recycled and reused several times in a day. This is known as enterohepatic circulation. About 12-32 g of bile salts are secreted into the intestine each day and reabsorbed. However, a small portion of about 0.2-0.6 g/day is lost in the feces. An equal amount (0.2-0.6 g/day) is synthesized in liver to replace the lost bile salts. The fecal excretion of bile salts is the only route for the removal of cholesterol from the body (Fig. 46).

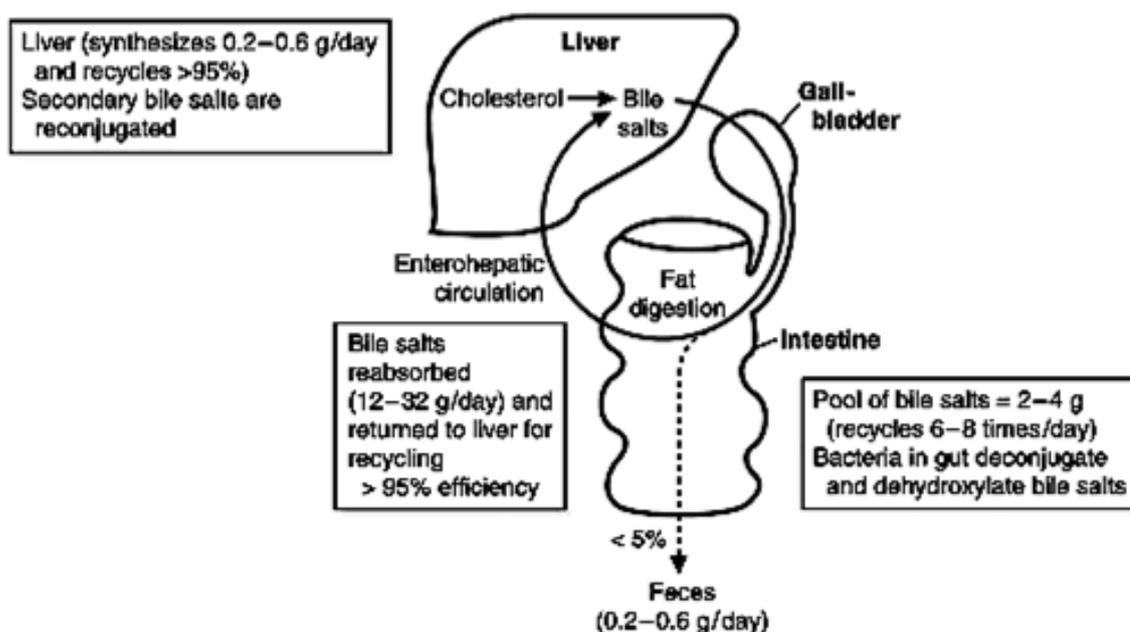


Figure 46. Overview of bile salt metabolism.

Steroid Hormones Formation. Cholesterol is the precursor of the five major classes of steroid hormones: progestagens, glucocorticoids, mineralocorticoids, androgens, and estrogens. These hormones are powerful signal molecules that regulate a host of organismal functions.

Progesterone, a progestagen, prepares the lining of the uterus for the implantation of an ovum. Progesterone is also essential for the maintenance of

pregnancy. The major site of synthesis of this class of hormones is the corpus luteum.

Androgens (such as testosterone) are responsible for the development of male secondary sex characteristics, whereas estrogens (such as estrone) are required for the development of female secondary sex characteristics. The major sites of synthesis of this class of hormones are the testes.

Estrogens, along with progesterone, also participate in the ovarian cycle. Glucocorticoids (such as cortisol) promote gluconeogenesis and the formation of glycogen, enhance the degradation of fat and protein, and inhibit the inflammatory response. They enable animals to respond to stress; indeed, the absence of glucocorticoids can be fatal. The major sites of synthesis of this class of hormones are the ovaries.

Mineralocorticoids (primarily aldosterone) act on the distal tubules of the kidney to increase the reabsorption of Na^+ and the secretion of K^+ and H^+ , which leads to an increase in blood volume and blood pressure. The major site of synthesis of this class of hormones is the adrenal cortex.

Steroid hormones bind to and activate receptor molecules that serve as transcription factors to regulate gene expression. These small similar molecules are able to have greatly differing effects because the slight structural differences among them allow interactions with specific receptor molecules.

Cholesterol is converted to progesterone in the first two steps of synthesis of all steroid hormones. Cytochrome P450_{scc} (side-chain cleavage enzyme system or cholesterol desmolase) is located in the mitochondrial inner membrane and removes six carbons from the side chain of cholesterol, forming pregnenolone, which has 21 carbons (Fig. 47). The next step, the conversion of pregnenolone to progesterone, is catalyzed by 3 β -hydroxysteroid dehydrogenase, an enzyme that is not a member of the cytochrome P450 family. Other steroid hormones are produced from progesterone by reactions that involve members of the P450 family. As the synthesis of the steroid hormones is discussed, notice how certain enzymes are used in more than one pathway. Defects in such enzymes will lead to multiple

abnormalities in steroid synthesis, which, in turn, results in a variety of abnormal phenotypes.

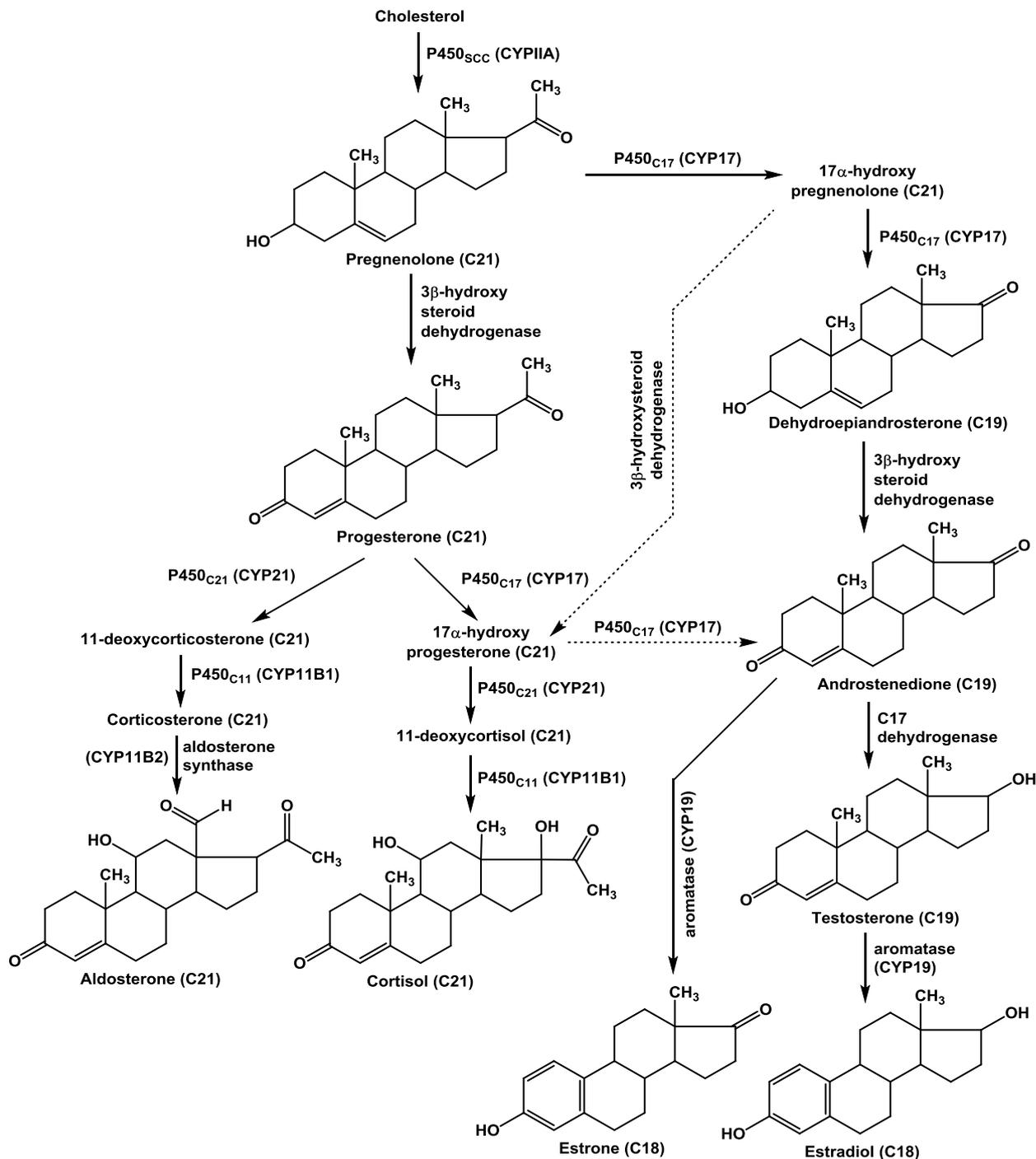


Figure 47. Synthesis of steroid hormones.

Synthesis of cortisol. The adrenocortical biosynthetic pathway that leads to cortisol synthesis occurs in the middle layer of the adrenal cortex known as the zona fasciculata. Free cholesterol is transported by an intracellular carrier protein to the inner mitochondrial membrane of cells (Fig. 48), where the side chain is

cleaved to form pregnenolone. Pregnenolone returns to the cytosol, where it forms progesterone.

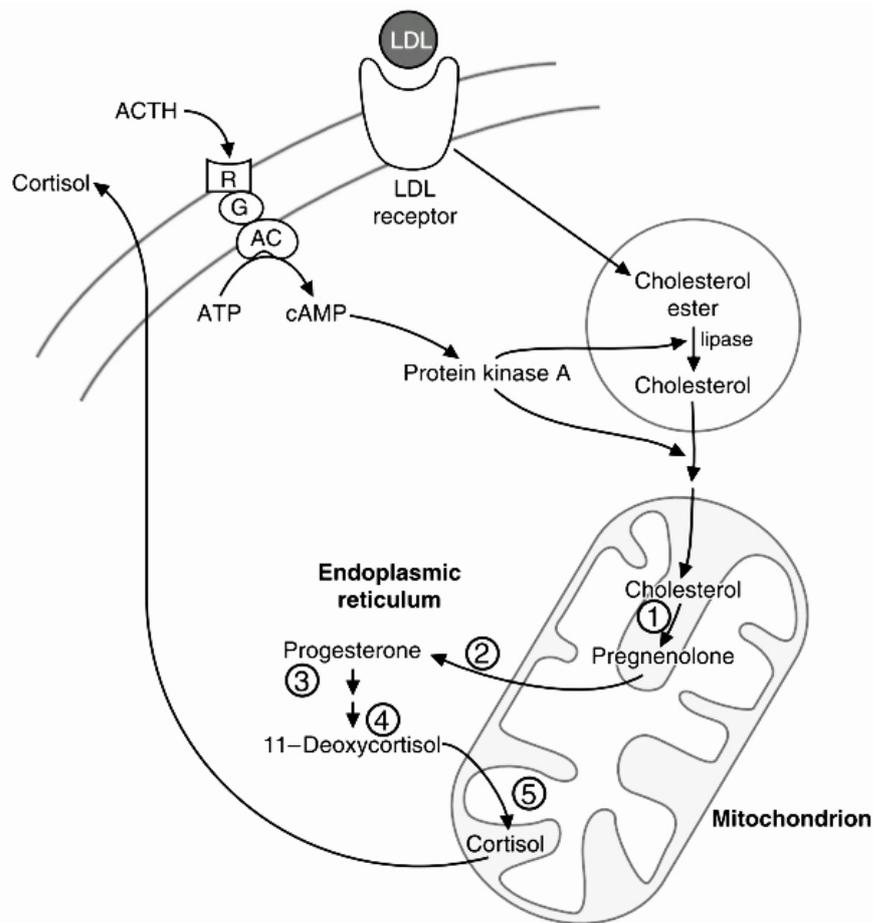


Figure 48. Cellular route for cortisol synthesis.

1 – Cholesterol desmolase (involved in side chain cleavage); 2 – 3β -hydroxysteroid dehydrogenase; 3 – 17α -hydroxylase; 4 – 21-hydroxylase; 5 – 11β -hydroxylase.

In the membranes of the endoplasmic reticulum, the enzyme $P450_{C17}$ catalyzes the hydroxylation of C17 of progesterone or pregnenolone and can also catalyze the cleavage of the 2-carbon side chain of these compounds at C17 (a C17-C20 lyase activity). These two separate functions of the same enzyme allow further steroid synthesis to proceed along two separate pathways: the 17-hydroxylated steroids that retain their side chains are precursors of cortisol (C21), whereas those from which the side chain was cleaved (C19 steroids) are precursors of androgens (male sex hormones) and estrogens (female sex hormones).

In the pathway of cortisol synthesis, the 17-hydroxylation of progesterone yields 17- α -hydroxyprogesterone, which, along with progesterone, is transported to the smooth endoplasmic reticulum. The membrane-bound P450_{C21} (21- α -hydroxylase) enzyme catalyzes the hydroxylation of C21 of 17- α -hydroxyprogesterone there to form 11-deoxycortisol (and of progesterone to form deoxycorticosterone, a precursor of the mineralocorticoid, aldosterone) (Fig. 47).

The final step in cortisol synthesis requires transport of 11-deoxycortisol back to the inner membrane of the mitochondria, where P450_{C11} (11- β -hydroxylase) receives electrons from electron transport protein intermediates (adrenodoxin, which when oxidized is reduced by adrenodoxin reductase). This enzyme transfers these reducing equivalents by way of oxygen to 11-deoxycortisol for hydroxylation at C11 to form cortisol. The rate of biosynthesis of cortisol and other adrenal steroids is dependent on stimulation of the adrenal cortical cells by adrenocorticotrophic hormone (ACTH).

Synthesis of aldosterone. The synthesis of the potent mineralocorticoid aldosterone in the zona glomerulosa of the adrenal cortex also begins with the conversion of cholesterol to progesterone (Figs. 47, 48). Progesterone is then hydroxylated at C21, a reaction catalyzed by P450_{C21}, to yield deoxycorticosterone. The P450_{C11} enzyme system then catalyzes the reactions that convert deoxycorticosterone to corticosterone. The terminal steps in aldosterone synthesis, catalyzed by the P450 aldosterone system, involve the oxidation of corticosterone to 18-hydroxycorticosterone, which is oxidized to aldosterone.

The primary stimulus for aldosterone production is the octapeptide angiotensin II, although hypernatremia (greater than normal levels of sodium in the blood) may directly stimulate aldosterone synthesis as well. ACTH has a permissive action in aldosterone production. It allows cells to respond optimally to their primary stimulus, angiotensin II.

Synthesis of the adrenal androgens. Adrenal androgen biosynthesis proceeds from cleavage of the 2-carbon side chain of 17-hydroxypregnenolone at C17 to form the 19-carbon adrenal androgen dehydroepiandrosterone (DHEA) and

its sulfate derivative (DHEAS) in the zona reticulosa of the adrenal cortex (Fig. 47). These compounds, which are weak androgens, represent a significant percentage of the total steroid production by the normal adrenal cortex, and are the major androgens synthesized in the adrenal gland.

Androstenedione, another weak adrenal androgen, is produced when the 2-carbon side chain is cleaved from 17α -hydroxyprogesterone by the C17-C20 lyase activity of P450_{C17}. This androgen is converted to testosterone primarily in extraadrenal tissues. Although the adrenal cortex makes very little estrogen, the weak adrenal androgens may be converted to estrogens in the peripheral tissues, particularly in adipose tissue.

Synthesis of testosterone. Luteinizing hormone (LH) from the anterior pituitary stimulates the synthesis of testosterone and other androgens in Leydig cells of human testicle. In many ways, the pathways leading to androgen synthesis in the testicle are similar to those described for the adrenal cortex. In the human testicle, the predominant pathway leading to testosterone synthesis is through pregnenolone to 17α -hydroxypregnenolone to DHEA (the Δ^5 pathway), and then from DHEA to androstenedione, and from androstenedione to testosterone (Fig. 47). As for all steroids, the rate-limiting step in testosterone production is the conversion of cholesterol to pregnenolone. LH controls the rate of side-chain cleavage from cholesterol at carbon 21 to form pregnenolone, and thus regulates the rate of testosterone synthesis. In its target cells, the double bond in ring A of testosterone is reduced through the action of 5α -reductase, forming the active hormone dihydrotestosterone (DHT).

Synthesis of estrogens and progesterone. Ovarian production of estrogens, progestins (compounds related to progesterone), and androgens requires the activity of the cytochrome P450 family of oxidative enzymes used for the synthesis of other steroid hormones. Ovarian estrogens are C18 steroids with a phenolic hydroxyl group at C3 and either a hydroxyl group (estradiol) or a ketone group (estrone) at C17. Although the major steroid-producing compartments of the ovary (the granulosa cell, the theca cell, the stromal cell, and the cells of the corpus

luteum) have all of the enzyme systems required for the synthesis of multiple steroids, the granulosa cells secrete primarily estrogens, the thecal and stromal cells secrete primarily androgens, and the cells of the corpus luteum secrete primarily progesterone.

The ovarian granulosa cell, in response to stimulation by follicle-stimulating hormone (FSH) from the anterior pituitary gland and through the catalytic activity of P450 aromatase, converts testosterone to estradiol, the predominant and most potent of the ovarian estrogens (Fig. 47). Similarly, androstenedione is converted to estrone in the ovary, although the major site of estrone production from androstenedione occurs in extraovarian tissues, principally skeletal muscle and adipose tissue.

Vitamin D₃. The D₃ vitamin is sterol derivative in which the steroid B ring is disrupted at its 9,10 position. The natural form of the vitamin, vitamin D₃ (cholecalciferol), is nonenzymatically formed in the skin of animals through the photolytic action of UV light on 7-dehydrocholesterol (is an intermediate in cholesterol synthesis) (Fig. 49). On the basis of its mechanism of action in the body, cholecalciferol should be called a prohormone, a hormone precursor. Dietary forms of vitamin D are absorbed through the aid of bile salts in the small intestine. Whether absorbed in the intestine or photosynthesized in the skin, cholecalciferol is then transported to the liver by a specific vitamin D-binding protein (DBP), also known as transcalferrin. Vitamin D₃ gains biological activity through further metabolic processing, first in the liver and then in the kidney:

1. In human liver, vitamin D₃ is hydroxylated to form 25-hydroxycholecalciferol in an O₂-requiring reaction catalyzed by either of two cytochrome P450's: CYP27A1 and CYP2R1 (or 25-hydroxylase).

2. The 25-hydroxycholecalciferol is transported to the kidney, where it is further hydroxylated by CYP27B1 (or 1 α -hydroxylase) to yield the active hormone 1,25-dihydroxycholecalciferol [1,25(OH)₂D₃]. CYP27B1 is activated by PTH, so this reaction is an important control point in Ca²⁺ homeostasis.

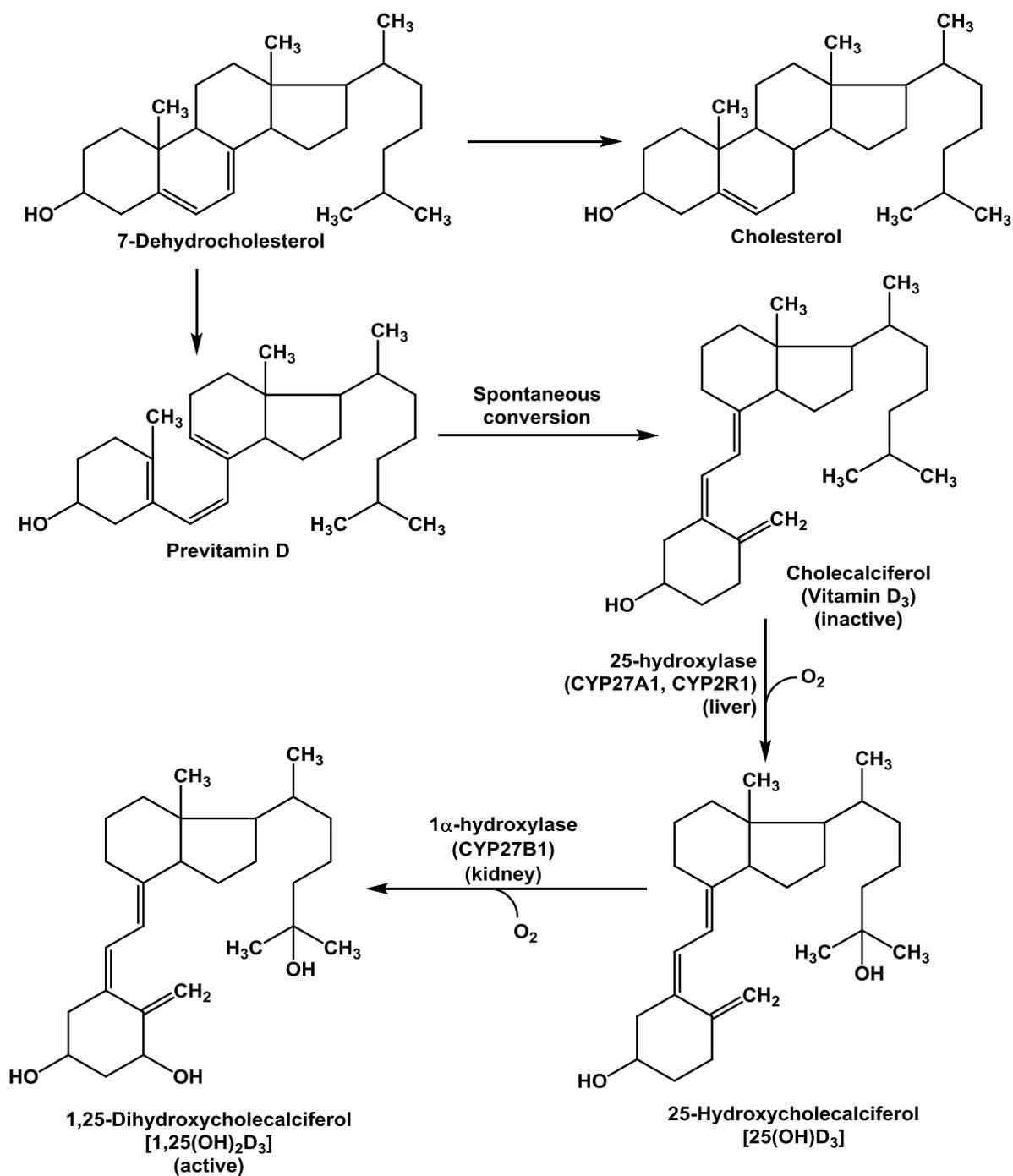


Figure 49. Synthesis and activation of vitamin D₃.

1,25(OH)₂D₃ acts to increase serum [Ca²⁺] by promoting the intestinal absorption of dietary Ca²⁺ and by stimulating Ca²⁺ and PO₄³⁻ reabsorption in kidneys. Intestinal Ca²⁺ absorption is stimulated through increased synthesis of a Ca²⁺-binding protein, which functions to transport Ca²⁺ across the intestinal mucosa. 1,25(OH)₂D₃ binds to cytoplasmic receptors in intestinal epithelial cells that, on transport to the nucleus, function as transcription factors for the Ca²⁺-

binding protein. The maintenance of electroneutrality requires that Ca^{2+} transport be accompanied by that of counterions, mostly P_i (H_3PO_4), so that $1,25(\text{OH})_2\text{D}_3$ also stimulates the intestinal absorption of P_i . The observation that $1,25(\text{OH})_2\text{D}_3$, like PTH, stimulates the release of Ca^{2+} and P_i from bone seems paradoxical in view of the fact that low levels of $1,25(\text{OH})_2\text{D}_3$ result in subnormal bone mineralization. Presumably the increased serum $[\text{Ca}^{2+}]$ resulting from $1,25(\text{OH})_2\text{D}_3$ -stimulated intestinal uptake of Ca^{2+} causes bone to take up more Ca^{2+} than it loses through direct hormonal stimulation. In addition, vitamin D_3 has been shown to modulate the immune response, provide protection against certain types of cancers, and has been implicated in preventing/reversing heart disease.

Vitamin D_3 , unlike the water-soluble vitamins, is retained by the body, so that excessive intake of vitamin D_3 over long periods causes vitamin D_3 intoxication (although note that most individuals, particularly the elderly and those with limited sun exposure, have less than the recommended levels of vitamin D_3 in their blood). The consequent high serum $[\text{Ca}^{2+}]$ results in aberrant calcification of a wide variety of soft tissues. The kidneys are particularly prone to calcification, a process that can lead to the formation of kidney stones and ultimately kidney failure. In addition, vitamin D_3 intoxication promotes bone demineralization to the extent that bones are easily fractured. The observation that the level of skin pigmentation in indigenous human populations tends to increase with their proximity to the equator is explained by the hypothesis that skin pigmentation functions to prevent vitamin D intoxication by filtering out excessive solar radiation.

Lipid metabolism disorders

Obesity

Obesity is an abnormal increase in the body weight due to excessive fat deposition. Men and women are considered as obese if their weight due to fat (in adipose tissue), respectively, exceeds more than 20 % and 25 % of body weight.

There is strong evidence to suggest that obesity has genetic basis. Thus, a child born to two obese people has about 25% chances of being obese. One gene namely *ob gene*, expressed in adipocytes (of white adipose tissue) producing a protein named *leptin*, is closely associated with obesity.

Leptin is regarded as a body weight regulatory hormone. It binds to a specific receptor in the brain and functions as a lipostat. When the fat stores in the adipose tissue are adequate, leptin levels are high. This signals to restrict the feeding behaviour and limit fat deposition. Further, leptin stimulates lipolysis and inhibits lipogenesis. Any genetic defect in leptin or its receptor will lead to extreme overeating and massive obesity. Treatment of such obese individuals with leptin has been shown to reverse obesity.

During starvation, leptin levels fall which promote feeding, and fat production and its deposition.

Obesity is a major problem in developed and increasingly in underdeveloped countries. There is particular concern about the marked increase in obesity in children, which can lead to major health problems in later life and will result in a massive increase in financial expenditure on health provision in the future: some obese children are now developing type 2 diabetes as young as 12.

There are at least three concerns about the diet of children in developed countries since they may lead to disease in adulthood or even earlier. The concerns are related to:

- the fat content;
- the trans-fatty acid content;
- the sugar (sucrose) content.

Fat content. The large amount of fat (as a high percentage of energy intake) is one factor that can lead to obesity, which increases the risk of developing type 2 diabetes. The increased availability of “fast food” and snacks that contain a high percentage of fat are temptations to children, in whom appetite is large, to accommodate sufficient intake of food to support growth. If the energy intake is higher than expenditure, obesity can result.

Trans-fatty acids. The phospholipids in the plasma and in membranes of all cells contain long-chain polyunsaturated fatty acids (PUFA). During periods of growth and development of organs, PUFAs are required for phospholipid synthesis. The PUFAs are, of course, obtained from dietary triacylglycerol and phospholipids. The double bonds in most natural fatty acids are cis not trans. Nonetheless trans-fatty acids do occur in dietary fats. If the diet contains trans-fatty acids, they might be incorporated into the phospholipids along with the cis-fatty acids and hence into membranes. The presence of these abnormal fatty acids will modify the structure of the phospholipids which could impair the function of the membrane. There are two main sources of trans-fatty acids in the diet: foods produced from ruminants contain trans-fatty acids due to the activity of bacteria in the rumen; commercial hydrogenation of oils results in conversion of some cis into trans bonds. These artificially hydrogenated fats are used in the preparation of children's favourite foods such as potato crisps (chips), biscuits and pastries, and fast food such as beefburgers. Cells particularly exposed to such fatty acids are the endothelial cells. Damage to membranes of endothelial cells can lead to local inflammation and predispose to atherosclerosis.

Sugar. The hydrolysis of sucrose in the intestine produces both glucose and fructose, which are transported across the epithelial cells by specific carrier proteins. The fructose is taken up solely by the liver. Fructose is metabolised in the liver to the triose phosphates, dihydroxyacetone and glyceraldehyde phosphates. These can be converted either to glucose or to acetyl-CoA for lipid synthesis. In addition, they can be converted to glycerol 3-phosphate which is required for, and stimulates, esterification of fatty acids. The resulting triacylglycerol is incorporated into the VLDL which is then secreted. In this way, fructose increases the blood level of VLDL.

The metabolism of VLDL by lipoprotein lipase in the capillaries in many tissues results in the formation of low density lipoprotein (LDL), which is atherogenic, so that diets high in sucrose are a risk factor for development of atherosclerosis. Many children in developed countries now consume large

quantities of soft drinks containing sucrose or fructose. According to the above discussion, this could lead to atherosclerosis in later life.

Atherosclerosis of blood vessels in humans

Atherosclerosis of blood vessels in humans usually associated with the state of Hypercholesterolemia. It is determined in patients with cholesterol level more than 5.72 mmole/L (normal is 3,9-5,72 mmol/L) in the blood plasma. This state is correlated with one of the following abnormalities:

- 1) elevated concentrations of VLDL with normal concentrations of LDL;
- 2) elevated LDL with normal VLDL;
- 3) elevation of both lipoprotein fractions;
- 4) the inverse relationship between HDL and LDL may be also. The most predictive relationship is the LDL:HDL cholesterol ratio, and it must be not higher than 3.5 for healthy people.

The accumulation of LDL in the blood may be in patients with genetic defects associated with:

- a) a defect of synthesis of receptors to LDL – apoB-100 receptors. It results in high levels of cholesterol (about 18 mmol/L) and LDL;
- b) a decrease of synthesis of triacylglycerol lipase linked with VLDL. This defect results the increase of VLDL, then LDL (cholesterol and TG concentrations are increased);
- c) the low activity of Endothelial triacylglycerol lipase (the same results);
- d) a decrease of synthesis of Lecithin Cholesterol Acyltransferase (LChATase) linked with HDL. In this case the surplus of cholesterol can't be transformed into cholesterol ester, and transported from peripheral cell to the liver by HDL.

These changes can cause the atherosclerosis and are considered as primary reasons of this pathology. The surplus of cholesterol accumulates in the arterial walls. LDL and VLDL (in high levels) can penetrate the vascular wall from the blood plasma to act subsequently as a primary substrate causing the atherosclerotic lesion of arteries.

The secondary reasons of atherosclerosis may be the diseases such as diabetes mellitus, lipid nephrosis, hypothyroidism, and other conditions of hyperlipidemia.

Coronary heart disease is caused by atherosclerosis of arteries and can be finished by myocardium infarction in patient.

The treatment of hypercholesterolemia state depends upon its reason. It may include:

- 1) ***Cholesterol free diet*** (for all reasons of this state);
- 2) ***Unsaturated High fatty acids*** must be in high concentration in food products (for all reasons of this state)

Fish oils. These contain high levels of omega-3 fatty acids, which have a number of properties that could explain why fish oils or a diet high in oily fish have a protective effect:

- They lower the blood level of VLDL and therefore they lower plasma level of LDL.
- They decrease the formation of blood clots.
- They decrease the formation of thromboxane A₂ and prostacyclin I₂ in favour of thromboxane A₃ and prostacyclin I₃, changes which protect against thrombosis.
- They have a hypotensive effect.

The major omega-3 fatty acid in fish oil is eicosapentaenoic acid, which contains five double bonds compared with only four present in the omega-6 fatty acid, arachidonic acid. When eicosapentaenoic acid is substrate for eicosanoid production, it gives rise to prostacyclins and thromboxanes of the three series whereas when arachidonic acid is substrate, it gives rise to the two series, thromboxane A₂ and prostacyclin I₂. Thromboxane A₃ has much less of a thrombolytic effect than thromboxane A₂ whereas prostacyclin I₃ has more of an antithrombotic activity than prostacyclin I₂. Hence, the risk of formation of a thrombus is decreased when omega-3 fatty acids are the substrate for the cyclooxygenase. There is considerable epidemiological evidence that fish oils are protective against atherosclerosis;

3) **Antioxidants.** These are naturally occurring compounds that have the ability to lower the levels of free radicals: they include vitamins C and E, the carotenoids and the flavonoids. Vitamin E and the carotenoids are particularly important in preventing oxidation of the unsaturated fatty acids within the LDL particle and within membranes of cells;

4) **Physical activity.** Evidence for the beneficial effects of physical activity on the development of atherosclerosis first arose from a series of epidemiological studies. This activity is now known to cause several changes, all of which are beneficial in decreasing the risk of development of atherosclerosis. These are:

- a fall in the total serum level of cholesterol;
- an increase in the serum HDL-cholesterol level;
- a fall in the serum LDL-cholesterol level;
- a fall in the plasma triacylglycerol level;
- loss of weight;
- reduction of blood pressure.

It also increases the sensitivity of tissues to insulin, which may provide better control of the blood glucose level to minimise the risk of damage to LDL by glycosylation;

5) **The use of drugs – inhibitors for β -hydroxy- β -methylglutaryl-CoA reductase:** Lovastatin, Mevastatin;

6) **The use of drug – Cholestyramine resin** to block the reabsorption of bile acids in the small intestine. In this case the cholesterol is utilized in higher quantity up to bile acids.

7) **The use of drugs Clofibrate and gemfibrozil** that divert the hepatic inflow of free fatty acids into oxidation, thus decreasing the secretion of VLDL by the liver. These drugs stimulate the hydrolysis of VLDL triacylglycerols by lipoprotein lipase;

8) **The use of drug Probucol.** It increases the rate of LDL catabolism via receptor-independent pathways, but its antioxidant properties may be more important in preventing accumulation of oxidized LDL in arterial walls;

9) ***The use of Nicotinic acid*** to reduce the flux of fatty acids by inhibiting of adipose tissue lipolysis thereby inhibiting VLDL production by the liver.

Genetic disorders of phospholipid metabolism

Hexosaminidase A deficiency results in a group of neurodegenerative disorders caused by intralysosomal storage of the specific glycosphingolipid, GM2 ganglioside. The prototype hexosaminidase A deficiency is ***Tay-Sachs disease***, also known as the acute infantile variant. Tay-Sachs disease is characterized by progressive weakness, loss of motor skills, decreased attentiveness, and increased startle response beginning between ages three and six months with progressive evidence of neurodegeneration including: seizures, blindness, spasticity, eventual total incapacitation, and death, usually before age four years. The juvenile (subacute), chronic, and adult-onset variants of hexosaminidase A deficiency have later onsets, slower progression, and more variable neurologic findings, including: progressive dystonia, spinocerebellar degeneration, motor neuron disease, and, in some individuals with adult-onset disease, a bipolar form of psychosis.

Acute infantile hexosaminidase A deficiency (Tay-Sachs disease, TSD). Affected infants generally appear to be completely normal at birth. Mild motor weakness begins between age three and six months, along with myoclonic jerks and an exaggerated startle reaction to sharp noise.

By age six to ten months, the infant fails to achieve new motor skills or even loses previously demonstrated skills. Decreasing visual attentiveness and unusual eye movements are associated with pallor of the perifoveal macula of the retina with prominence of the fovea centralis, the so-called cherry-red spot, which is seen in virtually all affected children.

After age eight to ten months, progression of the disease is rapid. Spontaneous or purposeful voluntary movements diminish, and the infant becomes progressively less responsive. Vision deteriorates rapidly. Seizures are common by age 12 months. Subtle partial complex seizures or absence attacks typically become more frequent and more severe.

Progressive enlargement of the head typically begins by age 18 months; it results from reactive cerebral gliosis, not hydrocephalus.

Further deterioration in the second year of life results in: decerebrate posturing, difficulties in swallowing, worsening seizures, and finally an unresponsive, vegetative state. Death from bronchopneumonia usually occurs between age two and four years.

Juvenile (subacute) hexosaminidase A deficiency. Juvenile hexosaminidase A deficiency often begins with ataxia and incoordination between age two and ten years. Speech, life skills, and cognition decline. Spasticity and seizures are present by the end of the first decade of life. Loss of vision occurs much later than in the acute infantile form of the disease, and a cherry-red spot is not consistently observed. Instead, optic atrophy and retinitis pigmentosa may be seen late in the course. A vegetative state with decerebrate rigidity develops by age ten to 15 years, followed within a few years by death, usually from infection. In some cases, the disease pursues a particularly aggressive course, culminating in death in two to four years.

Chronic and adult-onset hexosaminidase A deficiency. These conditions represent a spectrum of later-onset, more slowly progressive neurodegenerative disorders, associated with low levels of residual HEX A enzyme activity. Early symptoms may range from muscle weakness to extrapyramidal findings to altered cerebellar manifestations.

In the chronic form, central nervous system involvement is widespread, although certain neurologic findings may predominate over others. Psychomotor regression may be less prominent. The age of onset ranges from early childhood to the end of the first decade. In some individuals, extrapyramidal signs of dystonia, choreoathetosis, and ataxia may be evident. In others, cerebellar signs of dysarthria, ataxia, incoordination, and abnormalities of posture develop between age two and ten years; mentation and verbal skills tend to be involved later in the course. The clinical presentation of the chronic form of hexosaminidase A

deficiency may suggest possible diagnosis of spinocerebellar degeneration, Friedreich ataxia, or amyotrophic lateral sclerosis (ALS).

Individuals with adult-onset disease tend to show progressive muscle wasting, weakness, fasciculations, and dysarthria, indistinguishable from progressive adolescent-onset spinal muscular atrophy (Kugelberg-Welander disease) or early-onset ALS. Upper motor neuron signs, nonspecific cerebellar atrophy, and abnormalities of saccades may be present.

Cognitive dysfunction and dementia can be observed. As many as 40% of individuals have psychiatric manifestations (without dementia) including: recurrent psychotic depression, bipolar symptoms, and acute hebephrenic schizophrenia with disorganization of thought, agitation, delusions, hallucinations, and paranoia. Impairment of executive functioning and memory has also been observed.

Laboratory works

The determination of total cholesterol content in the blood serum (Ilk's method)

The principle of the method:

Cholesterol at the presence of reagent N1 (a mixture of acetic anhydride, acetic and sulfuric acids) will have a green colouring. The intensity of colouring is proportional to the cholesterol concentration.

The course of laboratory work:

All the operations are made in ventilation system only!

Prepare reactive solution according to scheme:

<i>Add (in mL)</i>	<i>N1 (test sample)</i>	<i>N2 (control sample)</i>
<i>Blood serum</i>	<i>0.05 mL</i>	—
<i>0.9 % NaCl solbtion</i>	—	<i>0.05 mL</i>
<i>Reagent N1</i>	<i>1.05 mL</i>	<i>1.05 mL</i>

Pour 0.05 mL of blood serum on the bottom of a dry test tube, then add reagent N1. Shake up the content of the test tube quickly and vigorously for 10-12 times (in ventilation system), close and leave them for 20 minutes in the thermostat (37 °C). The green colouring will appear. If there is the sediment in the test tube

you have to centrifuge it. Measure the optical density at red colour filter (630-690 nm) in cuvettes (3 mm) opposite control solution (reagent N1). Use the obtained value to find out the cholesterol content by graph curve. Multiply the cholesterol index using the factor of conversion into SI units (mmol/L) – 0.0258. The total cholesterol content (free and esters) in the blood serum of healthy adults varies within 2.6-6.46 mmol/L.

Clinical significance of cholesterol content determination in the blood serum

At newborns the total CH concentration is very low (< 2.6 mmol/L), and till 10 years does not increase (usually 4.1 mmol/L). Then CH concentration will grow in the early period of puberty. Risk of the development myocardial ischemia (MI) considerably grows at the adult person at CH>5.2 mmol/L, therefore it is more preferable to estimate the concentration ratio concerning the ideal for given one individually. In the domestic literature a range of norm is in wider limits (2.6-8.79 mmol/L), than in the foreign literature (2.6-6.46 mmol/L).

Hypercholesterinemia is observed at patients with a hypertension, MI, diabetes mellitus, obesity, hypothyroid edema, nephritis syndrome, kidney insufficiency, cholestasis, and also at some genetic disease of lipid metabolism. Hypercholesterinemia accompanies hyperlipoproteinemia: such types as IIa, II b, III, IV and V.

Hypocholesterinemia is observed at parenchyma damage of the liver, starvation, tuberculosis, hyperthyroidism, cancer of some organs.

EXERCISES FOR INDEPENDENT WORK

1. Which statement is a true for the comparison of phospholipids (PL) and triacylglycerols (TAG)?
 - A. Both molecules contain a phosphate group.
 - B. TAG may be saturated or unsaturated, but all fatty acids in PL structure are saturated
 - C. Both TAG and PL are the primary storage form for fats in our bodies.
 - D. PL contain a cyclic steroid ring, whilst triglycerides maintain a straight-chain form.
 - E. PL molecules have a distinctly polar 'head' and a distinctly non-polar 'tail,' whilst triglycerides are predominantly non-polar.

2. Point out the type of lipoproteins transporting great bulk of triacylglycerols by blood from intestine to tissues:
 - A. VLDL
 - B. LDL
 - C. HDL
 - D. Chylomicrons
 - E. Apoproteins

3. Which statement is correct about high density lipoproteins (HDL)?
 - A. Lecithin cholesterol acyltransferase is activated by apoC-II in HDL
 - B. Nascent HDL pick up triacylglycerols from peripheral cells
 - C. Lecithin cholesterol acyltransferase produces bile acids from cholesterol in HDL
 - D. HDL are synthesized in the adrenal gland
 - E. HDL transfer cholesteryl esters to liver

4. Which one of the following properties is not characteristic of LDL?
 - A. LDL is smaller than both VLDL and chylomicrons

- B. LDL contains more cholesteryl esters than triacylglycerols
- C. The major protein component of LDL is apoB-48
- D. LDL are more dense than chylomicrons
- E. LDL have specific high affinity receptors in most cells

5. Which of the following statements is correct for situation when mobilization of fat from adipose tissue occurs:

- A. The increase of fatty acids content in blood is observed
- B. The decrease of fatty acids content in blood is observed
- C. The activity of triacylglycerol lipase is decreased
- D. Hormone-sensitive triacylglycerol lipase is dephosphorylated
- E. cAMP level is decreased in adipocytes

6. Which of the following descriptions is not correct for hormone-sensitive triacylglycerol lipase:

- A. It is synthesized in adipocytes
- B. It is activated by phosphorylation
- C. It is activated under effect of glucagon
- D. It is activated under effect of epinephrine
- E. It is activated under effect of insulin

7. There are two sources of glycerol 3-phosphate for triacylglycerol synthesis. Adipose tissue is strictly dependent on glucose uptake to produce dihydroxyacetone phosphate for glycerol 3-phosphate formation. But liver can use diverse (glucose independent) way for glycerol 3-phosphate synthesis. Point out the liver specific enzyme for this transformation:

- A. Glycerol 3-phosphate dehydrogenase
- B. Glycerol kinase
- C. Enolase
- D. Aldolase

E. Acetyl CoA carboxylase

8. This enzyme is present in lysosomes and specifically cleaves the bond between phosphate and glycerol of phospholipids. Point out the enzyme described above:

- A. Phospholipase A₁
- B. Phospholipase A₂
- C. Phospholipase B
- D. Phospholipase C
- E. Phospholipase D

9. What are the constituents of cardiolipin are obtained as the result of its degradation?

- A. 3 glycerols, 4 fatty acids, 2 phosphates
- B. 2 glycerols, 4 fatty acids, 1 phosphate
- C. 2 glycerols, 2 fatty acids, 1 phosphate
- D. 1 sphingosine, 1 fatty acid, 1 sugar
- E. 2 sphingosines, 2 fatty acids, 1 oligosaccharide

10. Which statement is incorrect about phosphatidylcholine (PC)?

- A. PC may be synthesized from phosphatidylserine or from diacylglycerol and CDP-choline.
- B. PC together with phosphatidylethanolamine and phosphatidylserine are major phospholipid components of cell membranes.
- C. PC participates in signal transmission via activated phospholipase C.
- D. PC is an important component of the surface shell of plasma lipoproteins.
- E. PC can be hydrolyzed by phospholipase A₂ into lysolecithin and a fatty acid.

11. The removal of two-carbon units from a fatty acyl CoA involves four sequential reactions. Which of the following reaction sequences is correct for the pathway of β -oxidation:

- A. Oxidation, dehydration, oxidation, cleavage
- B. Hydrogenation, dehydration, hydrogenation, cleavage
- C. Dehydrogenation, hydration, dehydrogenation, cleavage
- D. Reduction, hydration, dehydrogenation, cleavage
- E. Reduction, dehydration, reduction, cleavage

12. The formation of the “active form” of a fatty acid is endergonic process in which the ATP energy is consumed. But there is another necessary participant of the fatty acid activation. Choose it:

- A. Acetyl CoA
- B. CoASH
- C. GTP
- D. UTP
- E. Succinyl CoA

13. Choose the products for one round of stearic acid β -oxidation:

- A. 129 ATP
- B. 1 Oleyl CoA, 12 ATP
- C. 2 acetyl CoA, 2 FADH₂, 1 ATP
- D. 1 palmitoyl CoA, 1 acetyl CoA, 1 FADH₂, 1 NADH
- E. 1 stearyl CoA, 1 acetyl CoA, 1 FADH₂, 1 NADH

14. This vitamin is precursor for the formation of non-protein parts of both enzymes: acyl CoA synthase and fatty synthase complex. Name it:

- A. Thiamine
- B. Riboflavin
- C. Biotin
- D. Nicotinic acid
- E. Pantothenic acid

15. What sentence is reasonable in regard to acetyl CoA:
- A. It serves as a donor of acetyl groups in fatty acid synthesis.
 - B. It cannot be formed from protein
 - C. It cannot be used to make cholesterol and ketone bodies
 - D. It allosterically activates pyruvate dehydrogenase
 - E. It is carboxylated by pyruvate carboxylase to form malonyl CoA

16. Point out the intracellular location of high fatty acid synthesis:
- A. Inner mitochondrial membrane
 - B. Cytosol
 - C. Outer mitochondrial membrane
 - D. Nucleus
 - E. Mitochondrial matrix

17. Acetyl CoA is the source of carbon atoms while NADPH provides the reducing equivalents for fatty acid synthesis. Which of the following enzymes take part in the formation of mentioned substrates:

- A. Citrate synthase, pyruvate dehydrogenase (PDH), Glucose 6 phosphate dehydrogenase (G 6-P DH)
- B. Citrate lyase, malate dehydrogenase (MDH), malic enzyme
- C. Citrate lyase, PDH, MDH
- D. Glucose 6 phosphate dehydrogenase (G 6-P DH), gluconolactone hydrolase, phosphogluconate dehydrogenase
- E. Pyruvate decarboxylase, PDH, MDH

18. A microsomal enzyme system called fatty acyl CoA desaturase is responsible for the formation of monounsaturated fatty acids: oleic and palmitoleic acids.

Name a coenzyme of the enzyme system:

- A. NADH
- B. FADH₂

- C. Biotin
- D. Phosphopantetheine
- E. CoASH

19. Which of the following substances is immediate precursor of acetoacetate in pathway ketogenesis?

- A. β -Hydroxybutyrate
- B. Acetoacetyl CoA
- C. β -Hydroxybutyryl CoA
- D. Acetyl CoA
- E. β -Hydroxy- β -methylglutaryl CoA

20. Which of the following enzyme controls a committed step in fatty acid synthesis:

- A. Ketoacyl synthase
- B. Acetyl transacylase
- C. Acetyl CoA carboxylase
- D. Malonyl transacylase
- E. Thioesterase

21. Which of the following statements is believed to be the most important step in the regulation of the rate of cholesterol biosynthesis from acetyl-CoA?

- A. The condensation of malonyl-CoA with acetyl-CoA
- B. The cleavage of HMG-CoA to form acetoacetate and acetyl-CoA
- C. The reactions involved in the cyclization of the polyisoprenoid precursor of the sterol ring system
- D. The enzymatic reduction of HMG-CoA.
- E. The introduction of the 3-beta-hydroxyl group into the steroid ring system.

22. The main chemical compounds for cholesterol synthesis are:

- A. Acetyl CoA, NADPH, ATP
- B. Malonyl CoA, NADPH, ATP
- C. Acetyl CoA, GTP, pantothenate
- D. Acyl CoA, carnitine, ATP
- E. Glycerol, phosphate, choline

23. Point out the key regulatory enzyme of cholesterol biosynthesis in the liver:

- A. Mevalonate kinase
- B. Mitochondrial β -hydroxy β -methylglutaryl CoA (HMG CoA) synthase
- C. Cytosomal HMG CoA synthase
- D. HMG CoA reductase
- E. Thiolase

24. The conversion of cholesterol catalyzed by 7α -hydroxylase is inhibited by:

- A. Cortisol
- B. Bile acids
- C. Testosterone
- D. Calcitriol
- E. Glycine and taurine

25. All hereinafter stated compounds are products of cholesterol catabolic conversion except one. Choose it:

- A. Glucuronic acid
- B. Cholic acid
- C. Chenodeoxycholic acid
- D. Glucocorticoids
- E. Mineralocorticoids

26. Which of the following statements is not true for fat mobilization description?

- A. Free fatty acid concentration is higher than normal one

- B. Blood epinephrine levels are elevated
- C. Hormone-sensitive lipase is phosphorylated
- D. Hormone-sensitive lipase is dephosphorylated
- E. Blood glucagon levels are elevated

27. Which statement is not associated with situation of fat deposition?

- A. Insulin secretion is increased
- B. Blood free fatty acid levels are elevated
- C. Hormone sensitive triacylglycerol lipase activity is increased
- D. Blood VLDL and chylomicrone levels are elevated
- E. Lipoprotein lipase activity is increased

28. Point out the atherogenic lipoproteins:

- A. Nascent chylomicrones
- B. Fatty acid-albumins complex
- C. Low density lipoproteins
- D. High density lipoproteins
- E. Remnant chylomicrones

29. Which of the following statements explains correctly metabolic alterations that are specific for persons disposed to obesity beside people having standart weight?

- A. Coupling of respiration with oxidative phosphorylation is much more
- B. There is any genetic defect in leptin
- C. Rate of Krebs cycle reactions is higher
- D. Calorie intake is much less
- E. Rate of fatty acid β -oxidation is much less

30. All of the following statements regarding hypercholesterolemia (type IIa hyperlipidemia) are correct except:

- A. There is an increased risk of coronary artery disease

- B. The blood serum cholesterol levels are increased
- C. The blood serum triacylglycerol levels are elevated
- D. The blood serum low density lipoprotein (LDL) levels are high
- E. It is due to a deficiency of LDL receptors

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