BIOCHEMISTRY **1st Year, 2nd Semester**

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Lecture 6

III. LIPID METABOLISM

III.1. Digestion and absorption

The daily lipids intake is of 100-150 grams, of which 90 - 95% triglycerides, the rest being cholesterol, phospholipids and liposoluble vitamins. Within the mouth cavity and within the stomach, lipases active in the pH domain of 3-6 are secreted. During meals, the food proteins partly buffer the acid pH from the stomach, allowing for the action of these lipases. They hydrolize the triacylglycerols into diacylglycerols and short and medium-chain fatty acids. These short-chain, relatively hydrophilic acids, are absorbed through the intestinal wall reaching the portal vein. The gastric and oral lipases hydrolize about 30% of the lipids from the breast milk in newborns. Diacylglycerols and the long chain fatty acids reach the duodenum, where the lipases from the intestine will act. Within the duodenum, digestion is the result of the conjugated action of lipases from the pancreatic juice, of the biliary salts and of the alkaline pH.

Thus, the bile salts, strongly tensioactive compounds, play the role of detergents, emulsifying the large lipid droplets into small particles, facilitating the access of lipases.

The weak alkaline pH will alter to the right the balance of the acylgliceroles hydrolysis reaction, due to turning the fatty acids (reaction products) into soaps by the reaction with the alkaline environment. Additionally, the soaps resulted, which are tensioactive compounds, will also participate to the emulsification of the lipids within the intestine lumen.

In order for the pancreatic lipase to be able to act on the acylgliceroles, these will form a mycelium with the biliary salts, where the biliary salts will have the –OH groups oriented towards the outside and will bind the lipase. The action of the lipase will be favored by the presence of the pancreatic colipase, a protein which makes the bond between the lipase and the lipid mycelium. This way, both the dispersion of the lipid droplets, as well as the lipid – lipase contact is accomplished. The action of dispersion of the lipid droplets is accelerated by the fatty acids salts, by lysolecithin and the degraded proteins.

Throughout digestion, the pancreatic lipase specifically hydrolizes the ester bonds from posotions 1 and 3 within triglycerides, a process which leads to 2 molecules of fatty acids and 2-monoacylglycerol. About 75% of the digested triglycerides are transformed and absorbed under the form of 2-monoacylglycerols, while the rest of 25%, although initially turned into 2-monoacylglycerol, will then isomerize to 1-monoacylglycerol which, under the action of lipase, will hydrolyze into glycerol and fatty acid.

This way, the triglycerides digestion produces 2-monoacylglycerol, fatty acids and glycerol.

Phospholipids are hydrolyzed by the phospholipase from the pancreatic juice, first at position 2, the outcome being lysolecithin, and then for the rest of the positions producing fatty acids, glycerol and amino alcohols.

Cholesterol is found as esterified cholesterol (food, bile, desquamated cells) which under the action of cholesterol esterase is turned into free cholesterol and fatty acids.

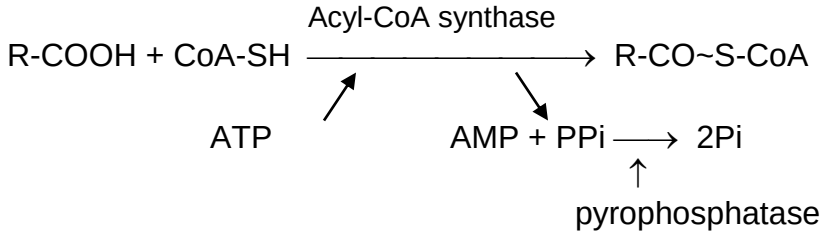
Absorption

The micelles containing fatty acids and cholesterol make contact with the microvilli of the epithelial cells at the level of the proximal region of the jejunum, allowing the 2-monoacylglycerols and fatty acids to cross the intestinal wall, using specific transporters.

Glycerol, which is a hydrophilic molecule, will cross the intestinal wall, using the transmembrane channels called aquaglyceroporins. As the free fatty acids are toxic for the cell, in the cytoplasm they are attached to specific proteins called FABP (fatty acid binding proteins).

Chylomicrons formation

Within the intestinal cell, from the absorbed lipids, the triglycerides and phospholipids are resynthesized and the cholesterol esterification takes place. The fatty acids are first activated to acyl–CoA within the endoplasmic reticulum:



The glycerol is activated by phosphorylation to glycerol–3–phosphate. Triglycerides will be resynthesized using as precursors 2-monoacilglycerol, glycerol-3-phosphate and acyl CoA. The cholesterol is esterified with various acids under the action of enzyme acyl-CoA-cholesterol-acyltransferase (ACAT). The resynthesized triglycerides, along with other types of lipids (phospholipids, cholesterol, cholesterol esters, and liposoluble vitamins) and proteins (apo B-48 and apo C-2) will be incapsulated within specific lipoprotein particles, called chylomicrons. These are as fatty droplets with a diameter of 1 micron and the density of 0,95 grams/cm^3, which contain 2% proteins and 98% lipids, of which 88% triglycerides, 8% phospholipids, 3% esterified cholesterol and 1% free cholesterol.

The short chain fatty acid and the glycerol will go straight into the portal vein.

The chylomicrons are secreted outside the cells, collected by the local lymphatic vessels and transported in the left subclavian vein from where they go into the blood circulation. Early postprandial, the plasma has an increased chylomicrons level, which

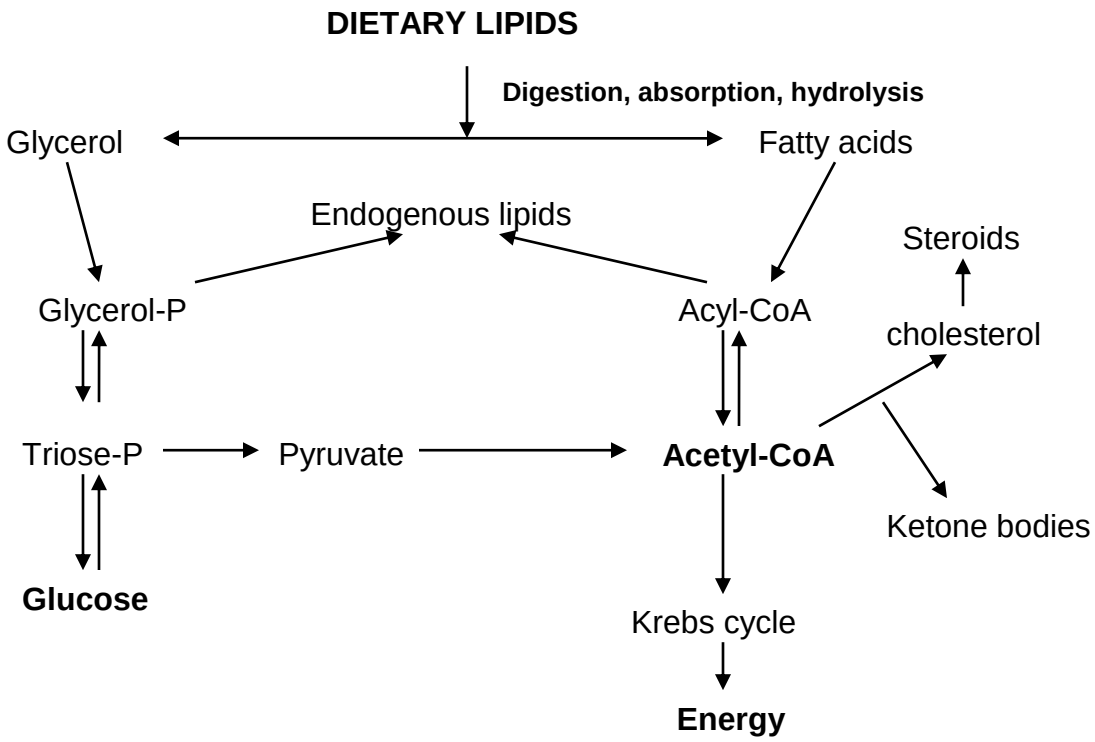
gives it a white, opalescent aspect. At the level of the tissue capillaries, but **not the brains and the liver,** there is the lipoprotein lipase enzyme which is attached by a heparan-sulfate proteoglycan arm to the surface of the capillary endothelium. Upon the passing of the chylomicrons, the triglycerides within them will be hydrolyzed by lipoprotein lipase to fatty acids and glycerol, most of which will migrate to the tissue. The lipoprotein lipase can be made soluble by heparine injection, so that the assessment of the lipoprotein lipase activity in the laboratory is made by analyzing the activity before and after injecting heparine.

As a result of lipase action, most of the components from chylomicrons pass into tissues, the chylomicrons reducing their volume by 90%, and the plasma becomes clear again (plasma clearing).

After the hydrolysis of triglycerides at tissue level, the residual chylomicrons are captured by the liver where the remaining components, the phospholipids and esterified cholesterol, are hydrolyzed to basic components. The life span of chylomicrons, from enterocitary secretion up to hepatic endocytosis, is of about an hour.

Further on, the metabolism of the lipids components takes place at the intracellular level.

The lipids metabolization pathways can be summarized as follows:



III.2. Catabolism of fatty acids

The fatty acids are compulsory constituents of all lipids categories. They are also the organic molecules from the body with the highest energetic potential, which also explains the using of triglycerides as an energy storage form within the adipose tissue. The total oxidation of 1 mol of palmitic acid releases 2338 kcal, while the oxidation of 1 mol of glucose releases 686 kcal.

The catabolism of fatty acids takes place within all tissues, except for the brain and the erythrocytes (glucose dependent tissues). The catabolism takes place inside the mitochondrion, within an area adjacent to the respiratory chain, which facilitates the transfer of hydrogen resulted from the dehydrogenation of fatty acids, straight to the respiratory chain. In order to be catabolized at mitochondrial level, the fatty acids from the cytoplasm must undergo the following stages:

III.2.1. Activation of fatty acids

It is made at the level of the external mitochondrial membrane and consists in the reaction:

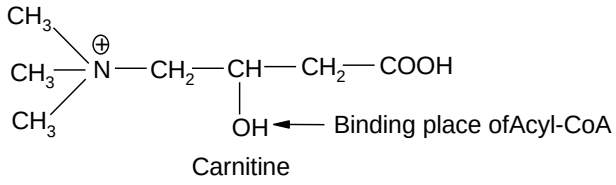
$$\textbf{Fatty acid + CoA-SH + ATP} \xrightarrow{thiokinase} \textbf{acyl~S-CoA + AMP + } PP_i$$

$$PP_i \xrightarrow{pyrophosphorylase} 2P_i$$

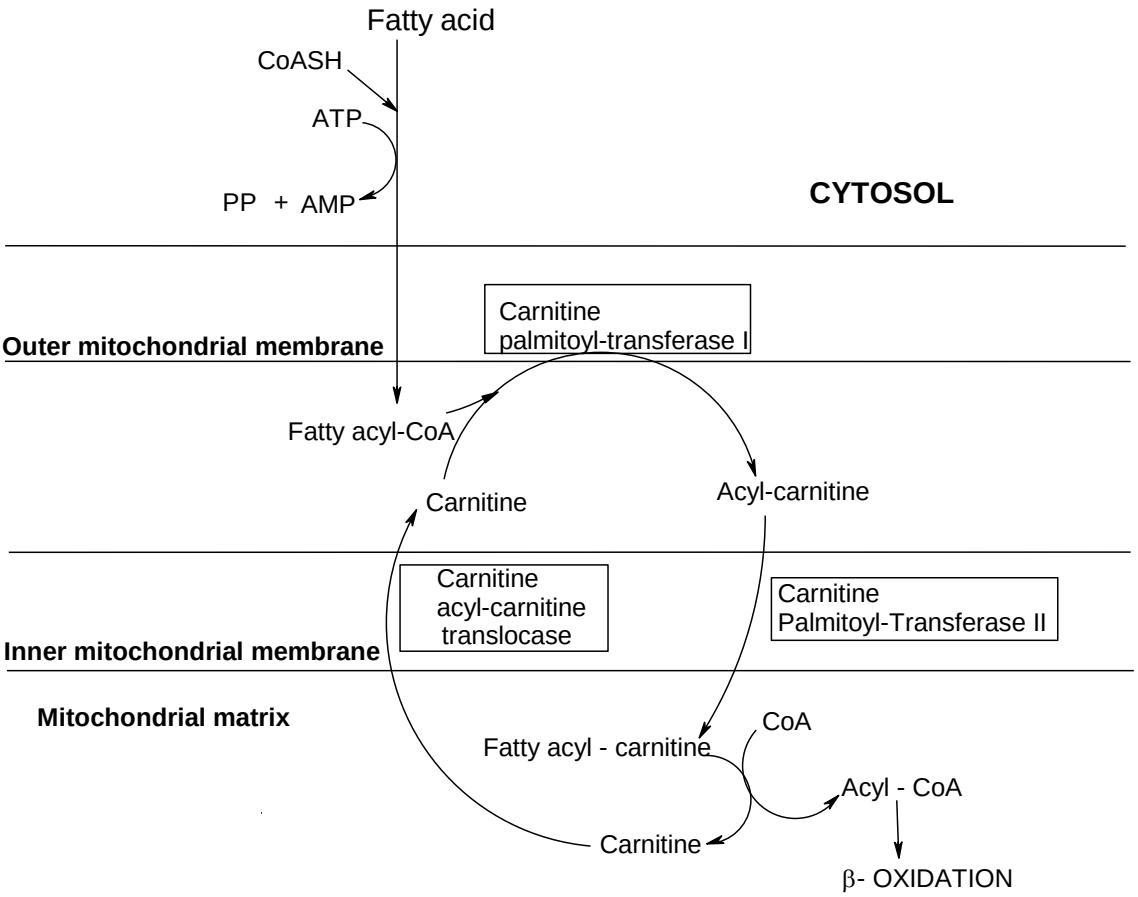
Pyrophosphate hydrolysis ensures the irreversibility of the process, as well as forming the macroergic bond acyl ~ S-CoA.

III.2.2. Transport into the mitochondria

Short chain acyl~S-CoA may cross directly the mitochondrial membrane, while medium and long acyl~S-CoA use a specific transporter, called carnitine:



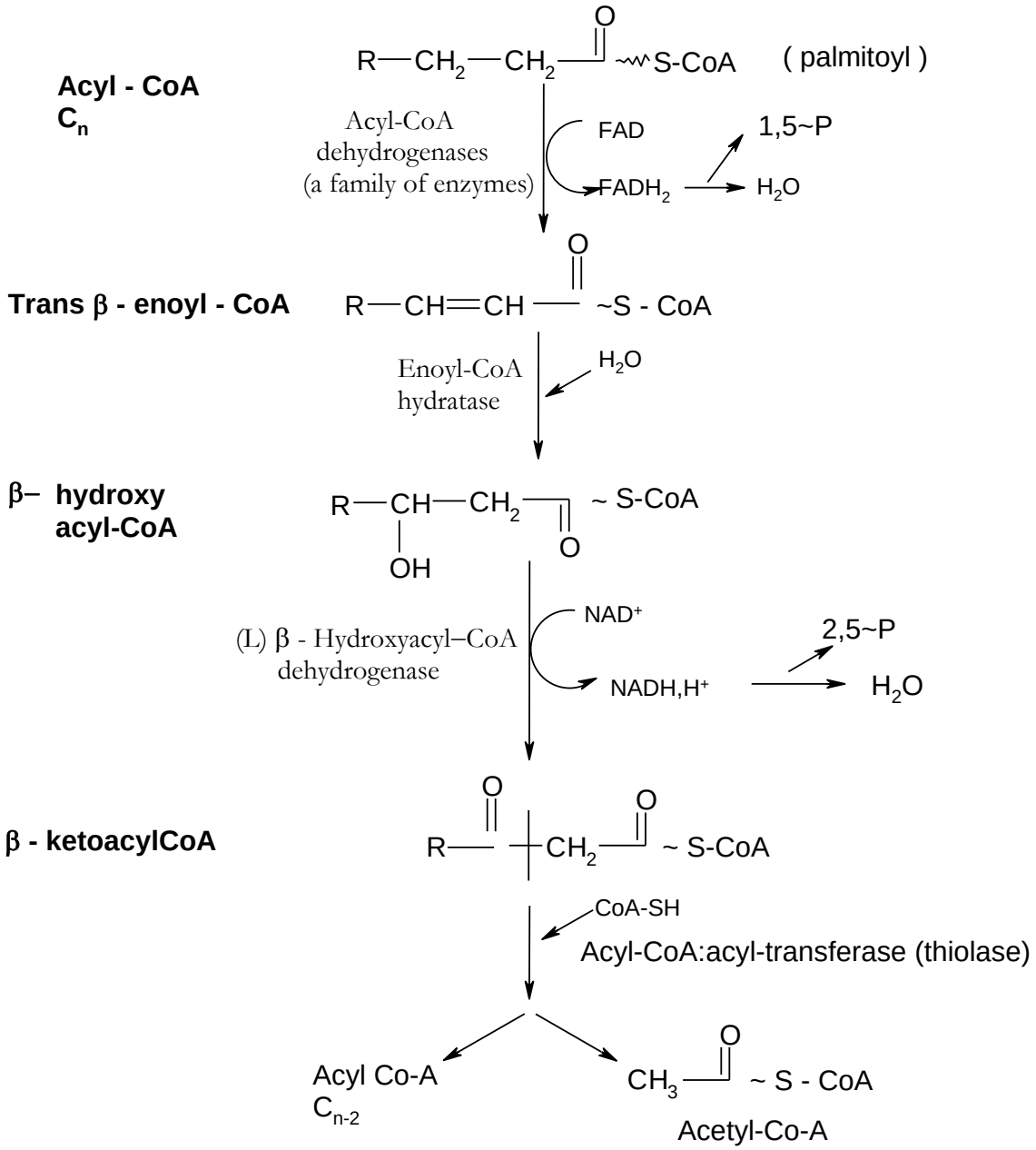
Carnitine



Carnitine deficit, caused by a faulty process within biosynthesis, transportation, or urinary elimination, occurs in general in the case of the prematurely-born and causes hypoglycemia, hypoketonemia, muscle weakness, liver fatty degeneration. Although carnitine absorption from food is limited, the carnitine is used by sportsmen for accelerating beta – oxidation and thus obtaining additional energy.

III.2.3. β - Oxidation of fatty acids

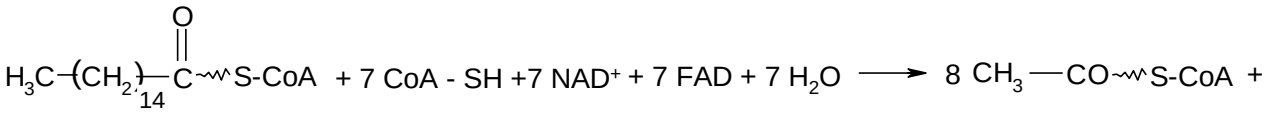
β - Oxidation is the main way of fatty acids catabolism, transforming the acyl–CoA into acetyl–CoA, and the resulting reducing equivalents transported by $NADH+H^+$ and $FADH_2$ are directly transferred to the respiratory chain located in the immediate neighborhood of the β-oxidation enzymes.



The reactions succession, through which a molecule of acetyl–CoA is detached from acyl–CoA with n carbon atoms, is repeated n/2 – 1 times, the final product being n/2 molecules of acetyl–CoA. The metabolic pathway described above is valid for the saturated fatty acids with even number of carbon atoms.

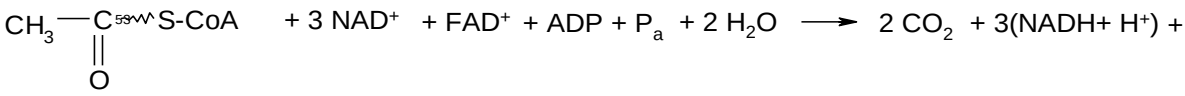
III.2.4. The balance and energetic yield of β - oxidation

The general equation of β-oxidation of the palmitic acid is:



+ 7 $FADH_2$ + 7($NADH + H^+$)

The molecules of acetyl – CoA will be oxidized within the TCA cycle, as per the equation:



+$FADH_2$ + ATP + CoA - SH

By the oxidation of 8 molecules of acetyl–CoA within the TCA cycle, followed by the oxidative phosphorylation in the respiratory chain, about 108 ATP molecules will be produced:

TOTAL 24 $NADH,H^+$ + 7 $NADH,H^+$ → 31 $NADH,H^+$ x 2.5 ATP → 77.5 ATP

7 $FADH_2$ + 8 $FADH_2$ → 15 $FADH_2$ x 1.5 ATP → 22.5 ATP

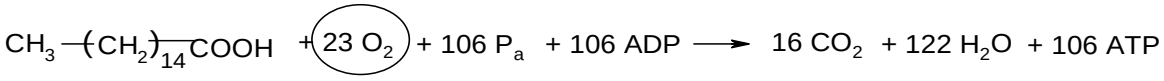
Substrate oxidative phosphorylation → 8 ATP

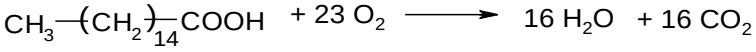
108 ATP

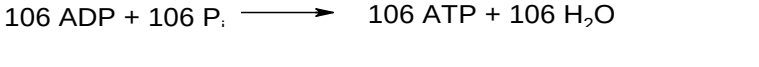
ATP consume for Acyl-CoA activation - 2 ATP

106 ATP

The global equation:







η=(941.7/2338)*100=33%

III.2.5. β- oxidation related pathology

The main pathological condition is due to the congenial deficit of acyl–CoA–dehydrogenase which oxidizes the medium chain fatty acids of 5–10 carbon atoms (there are specific enzymes for the short and long chain fatty acids as well). The main symptom of the disease, in up to 2 year old children, is hypoglycemia throughout the times between meals. In case of infections, when the infant eats little, the disease may be fatal, as the brain depends on the hepatic gluconeogenesis. Upon authopsy, the disease is also confirmed by the fatty infiltration of the liver. For the other patients, it is required to avoid long fasting periods and carnitine is administered.

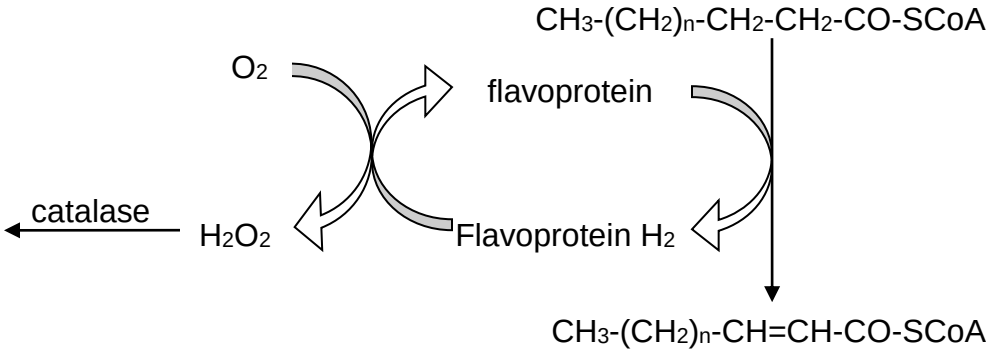
III.2.6. α - oxidation of fatty acids

It is a secondary pathway of catabolism for the fatty acids, consisting in the oxidation at the carbon in α position from the acyl group. It is significant for the metabolization of phytanic acid (acid 3,7,11,15 – tetra methyl hexadecanoic) from milk, animal fat (herbivores) and chlorofyll (contains an alcohol called phytol).

The existence of the methyl radical in position β does not allow for the β-oxidation, so that the acid first goes through an α-oxidation, eliminating a molecule of CO_2, then followed by normal β oxidation. A congenial defect by which α oxidation is blocked is the origin of a rare disease, called **Refsum disease**, in which the patient accumulates phytanic acid within the cells. The disease manifests itself through neurological disorders (cerebellar ataxia, nerve deafness, etc). The theraphy consists in diet without green vegetables, milk and herbivores meat, food which contains great quantities of phytanic acid.

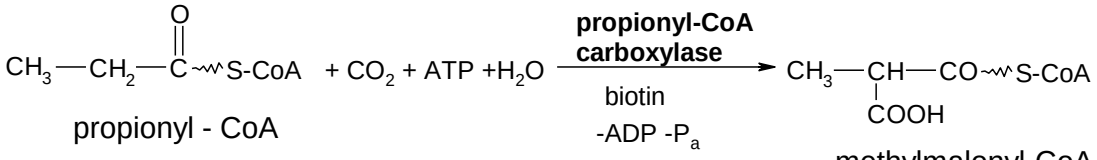
III.2.7. Oxidation of fatty acids within peroxisomes

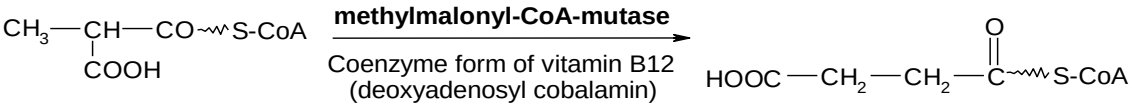
It is considered that the β-oxidation of long chain fatty acids with more than 20 carbon atoms, acids which are not oxidized within mitochondria, begins within peroxisoms. These are oxidized until the stage of octanoyl-CoA, and then the oxidation continues within mitochondria. The involvement of peroxisomes into the lipid metabolism is suggested by the fact that the medication which reduces the level of triglycerides from the blood, increases in parallel the level of peroxisomes. A particular feature of the process is the first stage of oxidation, catalized by an oxidized system, generating hydrogen peroxide. The system is not sensitive to cyanide.

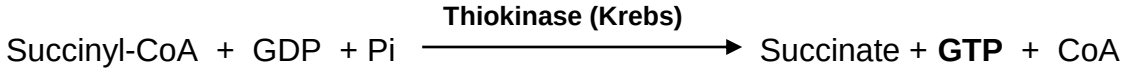


The congenital absence of peroxisomes will produce the Zellweger syndrome, a fatal malady during the first months of life.

In the case of fatty acids with **an odd number of carbon atoms,** β-oxidation takes place in a similar manner, except for the last stage when the last acyl–CoA resulting is propyonyl–CoA instead of butyryl–CoA. The propionyl-CoA catabolism will continue on a different pathway, comprising the following reactions:

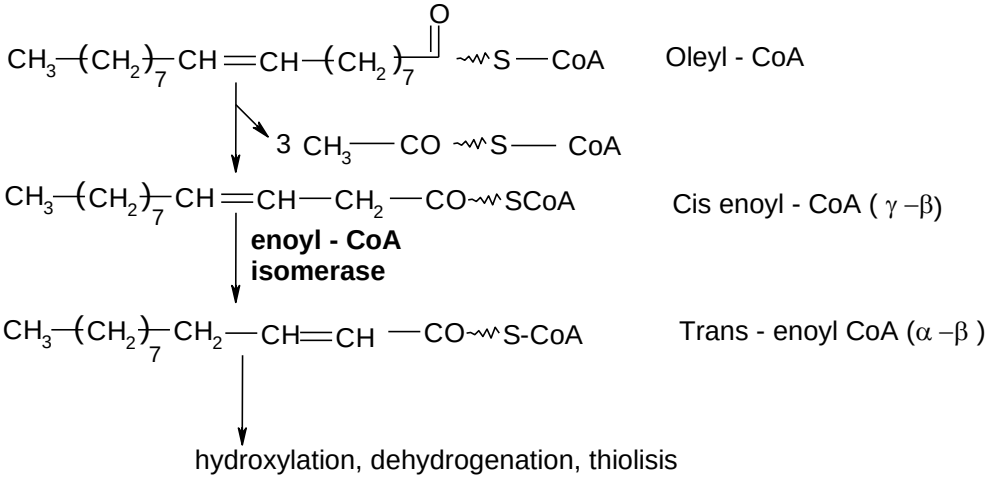






III.2.8. Oxidation of unsaturated fatty acids

The unsaturated fatty acids (oleic, linoleic, linolenic, arachidonic, etc.) are catabolized normally through β-oxidation, until the shortening of the chain by two carbon atoms reaches the forming of an intermediary containing a double bond in position cis Cβ - Cγ and not in the normal position trans Cα - Cβ.



This way, enoyl-CoA isomerase alters both the position as well as the orientation of the double bond, thus allowing the normal resuming of the β-oxidation process. The energetic balance of the unsaturated fatty acids catabolism is similar to that of the

saturated fatty acids, less the forming of a molecule of $FADH_2$ for each already existing double bond.

III.3. Biosynthesis of fatty acids

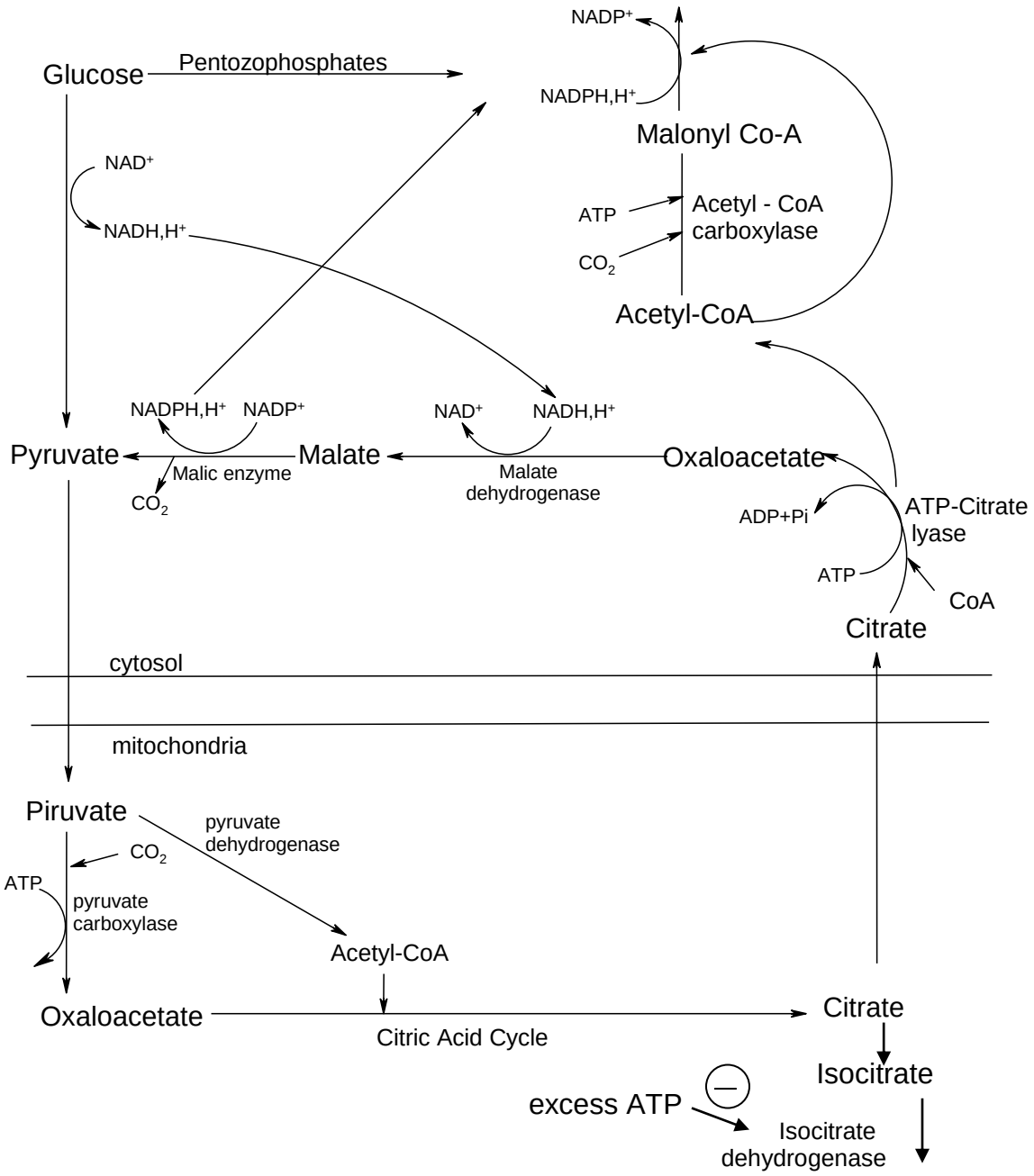
The excess of calories, usually glucidic in nature, is stored by conversion into fatty acids and is stored as triglycerides within all tissues, but mostly within the adipose tissue.

The precursor of synthesis is acetyl–CoA, a compound mainly coming from the glucidic catabolism and secondarily from the protein catabolism.

The conditions needed for the biosynthesis are:

- saturation of the TCA cycle (supplying the energy needs)
- supplying the acetyl CoA needs at the place of synthesis
- supplying the hydrogen needs, transported by $NADPH,H^+$
- the normal functioning of the biosynthesis enzymes.

The location of the process depends on the biosynthesis type. Thus, the process of de novo synthesis takes place within cytoplasm, while the extention of the chain of existing fatty acids is made within mitochondria and the endoplasmic reticulum.



Processes taking place ahead of synthesis. Acetyl–CoA is consumed primarily within the TCA cycle, where in the first stage (irreversible) it condenses with the oxaloacetic acid, forming citric acid. Supplying all energy needs leads to the saturation of the TCA cycle by the inhibiting action of ATP upon the isocitrate dehydrogenase, which generates citric acid accumulation.

Acetyl–CoA, formed within mitochondria, cannot cross the mitochondrial membrane, so that the acetyl CoA excess will enter the cytoplasm (the place for fatty acids synthesis) using citrate as a shuttling system. Thus, the citrate which may cross

the mitochondrial membrane, within cytoplasm will split into acetyl CoA and oxaloacetate. The acetyl CoA will be used for fatty acids synthesis, while the oxaloacetate will be turned back into pyruvate and sent back into mitochondria, where it will form a new citric acid molecule.

The transportation by means of citric acid is the bond between the citric acid and the biosynthesis of the fatty acids, both for providing the acetyl–CoA needs, as well as of the reducing equivalents. Actually, the cycle of reactions which transports a molecule of acetyl – CoA from mitochondria into cytoplasm, will have the following balance:

$$NADH+H^+ + NADP^+ + ATP + H_2O \longleftrightarrow NAD^+ + NADPH+H^+ + ADP + P_I$$

Therefore, in the case of the synthesis of a palmitic acid molecule, 8 molecules of acetyl-CoA and 14 of $NADPH,H^+$ will be necessary, of which 8 $NADPH,H^+$ formed upon transportation into the cytoplasm of the 8 molecules of acetyl CoA, and the remaining 6 $NADPH,H^+$ being provided by the pentose phosphates pathway.

After providing the reactants acetyl–CoA and $NADPH,H^+$, the fatty acids synthesis will take place within the cytoplasm, and comprises two stages:

- malonyl–CoA synthesis
- acyl–CoA synthesis (the biosynthesis itself).

Malonyl CoA synthesis

Although acetyl CoA supplies all carbon atoms within the structure of the newly synthesized fatty acids, the synthesis process requires the involvement of CO_2.

$$CH_3\text{-}CO\sim SCoA + ATP + {}^*CO_2 \longrightarrow HOOC^*\text{-}CH_2\text{-}CO\sim SCoA + ADP + P_I$$

Acetyl CoA carboxylase has biotin as prosthetic group, which it bonds by means of a ε-amino group of a lysine residue.
The reaction will comprise the stages:

$$HCO_3^- + ATP + biotin – enzyme \leftrightarrow carboxybiotin – enzyme + ADP + P_i$$
$$Carboxybiotin – enzyme + acetyl\ CoA \leftrightarrow malonyl – CoA + biotin – enzyme$$

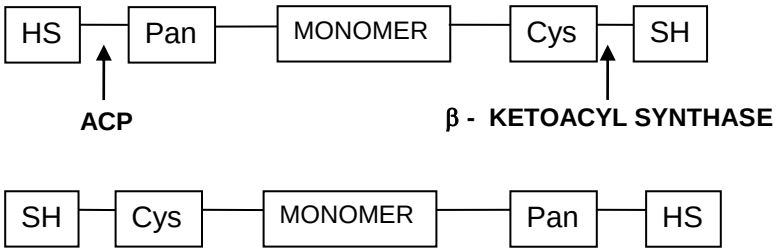
Carboxilation of acetyl CoA is the regulatory stage of fatty acids biosynthesis, acetyl-CoA-carboxylase being the rhythm enzyme of the metabolic pathway.

The active enzyme has a polymeric structure, activated by citrate and inhibited by palmytoil-CoA. The enzyme is also regulated by the process of phosphorylation–dephosphorylation, the active form being the dephosphorylated one. This type of regulation is under hormonal control, the insulin stimulates the enzyme dephosphorylation, therefore having an activating effect, while glucagon stimulates phosphorylation, inhibiting the enzyme activity.

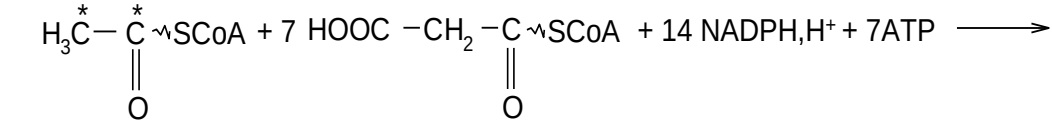
Acyl-CoA synthesis (biosynthesis itself)

The process takes place under the action of a multienzymatic complex called fatty acid synthetase. This has a dimer structure, being made up of two identical monomers, and layed out complementarily in a head to tail system. Each monomer

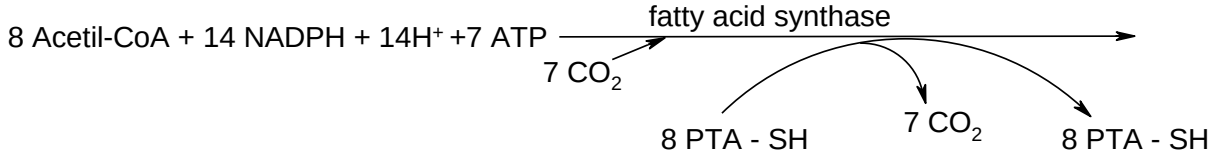
contains 7 different catalytic domains, acting successively. The transfer of the reaction intermediates from one catalytic domain to another is made by the acyl carrier protein (ACP), binding the acyl intermediaries through a thioester bond at the level of a prosthetic group of 4–phosphopantotheine (Pan–SH). One of the catalytic domains, β - ketoacyl sinthase, also has a bonding site - SH for the acyl intermediaries at the level of a cysteine residue (Cis–SH). This way, each monomer of fatty acid synthase may be represented as having 2 binding centers for the reaction intermediaries:



The reaction cycle 1- 4 is repeated 7 times, each time involving a new malonyl ~S-CoA residue, until palmitoyl ~S-CoA is formed. This is then detached from the multienzymatic complex by a specific enzyme, deacetylase (thioesterase). It appears that the two monomeric subunits of the fatty acid synthase act complementarily: the product of a cycle of reactions from a monomer, for instance butyryl~S-CoA, is taken over by the second monomer, which subjects it to another cycle of reactions transforming it into caproyl~S-CoA, and then it is transferred again to the first monomer and so on. One can say that the two monomer subunits of the enzymatic complex synthesize concomitantly 2 fatty acid molecules.

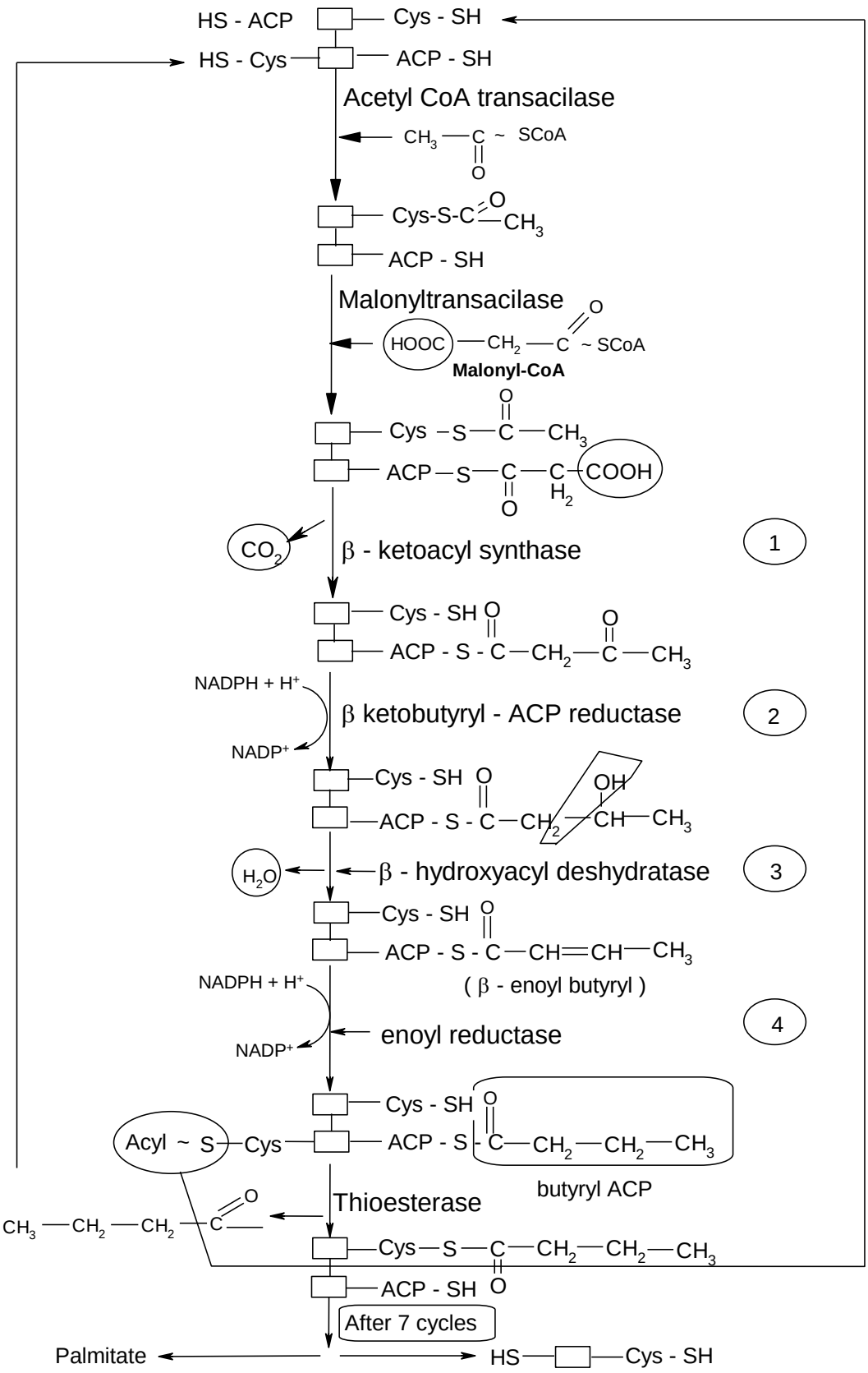


The acetyl~CoA is the primer molecule of the future fatty acid molecule, the rest of the atoms coming from the molecules of malonyl~CoA. The global equation of the process can be considered as follows:



$CH_3(CH_2)_{14}COOH + 8\ CoASH + 14\ NADP^+ + 7\ ADP + 7\ Pa + 6\ H_2O$

Within the mammary gland and within the liver, instead of acety~CoA, it is also possible to start from butyryl ~CoA, while the propionyl ~CoA induces the synthesis reaction of the fatty acids with long chain and odd number of carbon atoms in the molecule. The mammary gland also contains specific thioesterases for fatty acids with 8, 10, 12 carbon atoms, acids which can be found within the milk lipids.



The fatty acids synthesis

Regulation of fatty acids biosynthesis

From a metabolic point of view, the fatty acids biosynthesis is a process characteristic to the anabolic state and depends on the available ATP and NADPH, H^+. The enzyme which controls the metabolic pathway is acetyl–CoA–carboxylase. The enzyme is regulated by a phosphorylation–dephosphorylation process (the active for is dephosphorylated), having as positive allosteric effectors citrate and glycerophosphate, and as negative allosteric effector acyl-CoA (feed back regulation by the end product).

Insulin stimulates the process of fatty acids synthesis both directly, by stimulating the activity of the enzymes acyl-CoA-carboxylase and ATP-citrate-lyase, as well as indirectly, by stimulating the metabolic pathways which provide precursor molecules for the synthesis: the pentose phosphate pathway $\rightarrow$NADPH, H^+ and the complete oxidative catabolism of glucose $\rightarrow$acetyl-CoA .

The hyperglycemic hormones adrenaline and glucagon inhibit the process by inhibiting (stimulating the phosphorylation) the activity of the enzyme acyl-CoA-carboxylase.