ÚSTAV LÉKAŘSKÉ BIOCHEMIE A LABORATORNÍ DIAGNOSTIKY 1. LF UK

Lipids
(fatty acids, lipoperoxidation, digestion)

General Medicine

Lenka Fialová

translated and edited by Jan Plášeník

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Theory

Lipids are structurally heterogenous group of organic substances of hydrophobic or amphipathic nature. They share ***poor solubility in water***, but dissolve well in organic solvents (chloroform, benzene, ether). Regarding their structures, lipids are ***derivatives of fatty acids***.

1. Fatty acids

Fatty acids (FA) are ***carboxylic acids with alifatic hydrocarbon chain whose length is usually 4-26 carbon atoms***. They represent the richest source of energy. Typically they are found as components of lipids; only rarely they occur separately. The fatty acids in natural fats usually have an ***even number of carbons and unbranched chain***. Their classification is based on the length of hydrocarbon chain and degree of its saturation:

According to the length of hydrocarbon chain we classify the fatty acids to these groups:

- Short chain fatty acids (SCFA) < 6 carbons
- Medium chain fatty acids (MCFA) 6 – 12 carbons
- Long chain fatty acids (LCFA) 14 – 20 carbons
- Very long chain fatty acids (VLCFA) > 20 carbons

According to the degree of saturation we recognize:

- Saturated fatty acids (SFA);
- Unsaturated fatty acids.

Fatty acids possessing one double bond are called ***monounsaturated or monoenoic fatty acids*** (MUFA); if there are two or more double bonds in the FA molecule we talk about ***polyunsaturated or polyenoic fatty acid***s (PUFA).

The double bonds in polyunsaturated fatty acids are usually non-conjugated, i.e. separated with methylene bridges. For further PUFA classification positions of the double bonds are important, especially where the first double bond is placed with respect to the terminal methyl group (denoted as n or ω):

- ***n-3 (ω- 3)*** first double bond is on the third carbon counted from the terminal methyl;
- ***n-6 (ω- 6)*** first double bond is on the sixth carbon counted from the terminal methyl;
- ***n-9 (ω- 9)*** first double bond is on the nineth carbon counted from the terminal methyl.

The spatial configuration in the naturally occurring unsaturated fatty acids is mostly *cis*.

Important fatty acids from the n-6 group are ***linoleic acid***, a nutritionally essential one, and also ***arachidonic acid***, serving as a precursor for group of biologically active substances known as eicosanoids. The n-3 group is represented with ***α-linolenic acid***, which is also essential and found mainly in plant oils, and eicosapentaenoic and docosahexaenoic acids occurring in the fish oil. The relative abundance of some fatty acids in selected fats is shown in table 1.

In the storage fat of humans and other warm-blooded animals the saturated fatty acids prevail. In the phospholipids of biological membranes that must keep fluidity, both saturated and unsaturated fatty acids are found.

Polyunsaturated fatty acids readily undergo an oxidative damage (lipid peroxidation – see below).

Tab. 1 Relative abundance in % of some fatty acids in % selected fats (Velíšek, 1999)

Fatty acid	Olive oil	Sunflower oil	Soya oil	Sardine oil	Pork lard	Milk fat (cow)
Palmitic a.	8 – 20	5 – 8	10 – 13	9 – 22	20 – 32	20 – 32
Stearic a.	1 – 5	3 – 7	3.0 – 5	2 – 7	5 – 24	8 – 14
Oleic a.	55 – 83	13 – 40	18 – 25	7 – 17	35 – 62	17 – 26
Linoleic a.	4 – 21	40 – 74	50 – 57	1 – 3	3 – 16	0 – 2
Linolenic a.	0 – 2	< 0.5	6 – 10	0 – 1	< 1.5	0 – 1
Arachidonic a.	–	–	–	–	–	0 – 1
Eicosapentaenoic a.	–	–	–	9 – 35	–	–
Docosahexaenoic a.	–	–	–	4 – 13	–	–

2. Lipid peroxidation

2.1. The terms lipoperoxidation and free radicals

The ***lipid peroxidation – lipoperoxidation*** means an oxidative damage to higher fatty acids through an uncontrolled autocatalytic process resulting in formation of lipid hydroperoxides and further secondary products including aldehydes.

The lipoperoxidation can be started by any substance that has sufficiently high affinity to electrons and can abstract a hydrogen atom (proton and electron) from a methylene group of a fatty acid hydrocarbon chain. Substances that can do this involve ***free radicals, which are substances (elements, compounds, ions) with one or more unpaired electrons in the outer shell***. They are very reactive and display a ***tendency to chain reactions***, in which a radical can donate its unpaired electron, accept one, or combine with another molecule. As examples, we can introduce some of the oxygen free radicals that most often participate in lipoperoxidation:

- hydroxyl radical $HO^{\bullet}$ – the most reactive oxygen radical, with the shortest half-life;
- alkoxyl radical $RO^{\bullet}$ – a highly reactive organic radical;
- peroxyl radical $ROO^{\bullet}$ – formally derived from abstraction of hydrogen atom from hydroperoxides, e.g. lipid ones.

The rate of lipoperoxidation is significantly accelerated by ions of some metals, such as iron and copper.

The substrate for lipoperoxidation is especially ***unsaturated higher fatty acids*** present as components of phospholipids found in biological membranes and plasma lipoproteins. The most susceptible ones are the carboxylic acids with two and more double bonds, such as linoleic acid (18:2), linolenic acid (18:3) and arachidonic acid (20:4). It is because presence of at least two double bonds separated with methylene group ($-CH_2-$), which displays a high reactivity, is required for sensitivity to lipoperoxidation. Saturated higher fatty acids rarely get oxidized and also higher carboxylic acids with one double bond, such as oleic acid, undergo lipoperoxidation only in extreme conditions.

2.2. Course of lipoperoxidation

The process of lipoperoxidation proceeds in several steps (Fig. 1):

- ***Initiation***

In the initiation phase a hydrogen atom (proton and electron) is removed from a fatty acid hydrocarbon chain by action of free radicals producing free radical from the fatty acid and water:

$$L{-}H + HO^{\bullet} \rightarrow H_2O + L^{\bullet}$$ free radical of fatty acid

Following the hydrogen abstraction electrons in the fatty acid hydrocarbon chain rearrange so that there is only one single bond between two double bonds while in the original fatty acid two single bonds separated the double ones. A ***conjugated diene*** originates, which in aerobic environment very rapidly reacts with oxygen giving a peroxyl radical.

$$L^{\bullet} + O_2 \rightarrow L{-}O{-}O^{\bullet} \text{ peroxyl radical}$$

- ***Propagation***

As ***propagation*** we mean the phase of lipoperoxidation in which oxidation of further chain of polyunsaturated fatty acid is initiated.

The peroxyl radical is highly reactive and able to abstract hydrogen atom from a fatty acid of an adjacent lipid, converting it to a new radical. The original peroxyl radical is changed in this reaction to a hydroperoxide – lipoperoxide (LOOH) with a conjugated arrangement of double bonds. This reaction sequence can be many times repeated, that is why we talk about chain reaction.

$$L{-}O{-}O^{\bullet} + L{-}H \rightarrow L^{\bullet} + L{-}O{-}O{-}H \text{ hydroperoxide (lipoperoxide)}$$

Lipoperoxides with the same number of double bonds as the original fatty acids, but position of the double bond usually shifted one carbon either to the carboxyl group, or to the terminal methyl, are the ***primary reaction products of lipoperoxidation***.

The primary fatty acid oxidation products can further change in several ways:

- Reactions giving rise to cyclic products, in which number of carbons in molecule does not change
- Reactions, in which the molecule is cleaved and forms products with less carbon atoms, having character of aldehydes, hydrocarbons or oxoacids. Substances like ethane or penthane are exhaled; while aldehydes 4-hydroxynonenal and malondialdehyde are toxic and firmly bind to proteins, altering their functions
- Polymerisation reactions

These reactions lead to formation of ***secondary lipoperoxidation products***.

- ***Termination*** of lipoperoxidation occurs when:
  - Substrate is exhausted
  - Two radicals react together. If their concentration in the reaction system is high enough, two radicals are quite likely to react together yielding a non-radical, fairly stable product, which terminates the chain reaction.

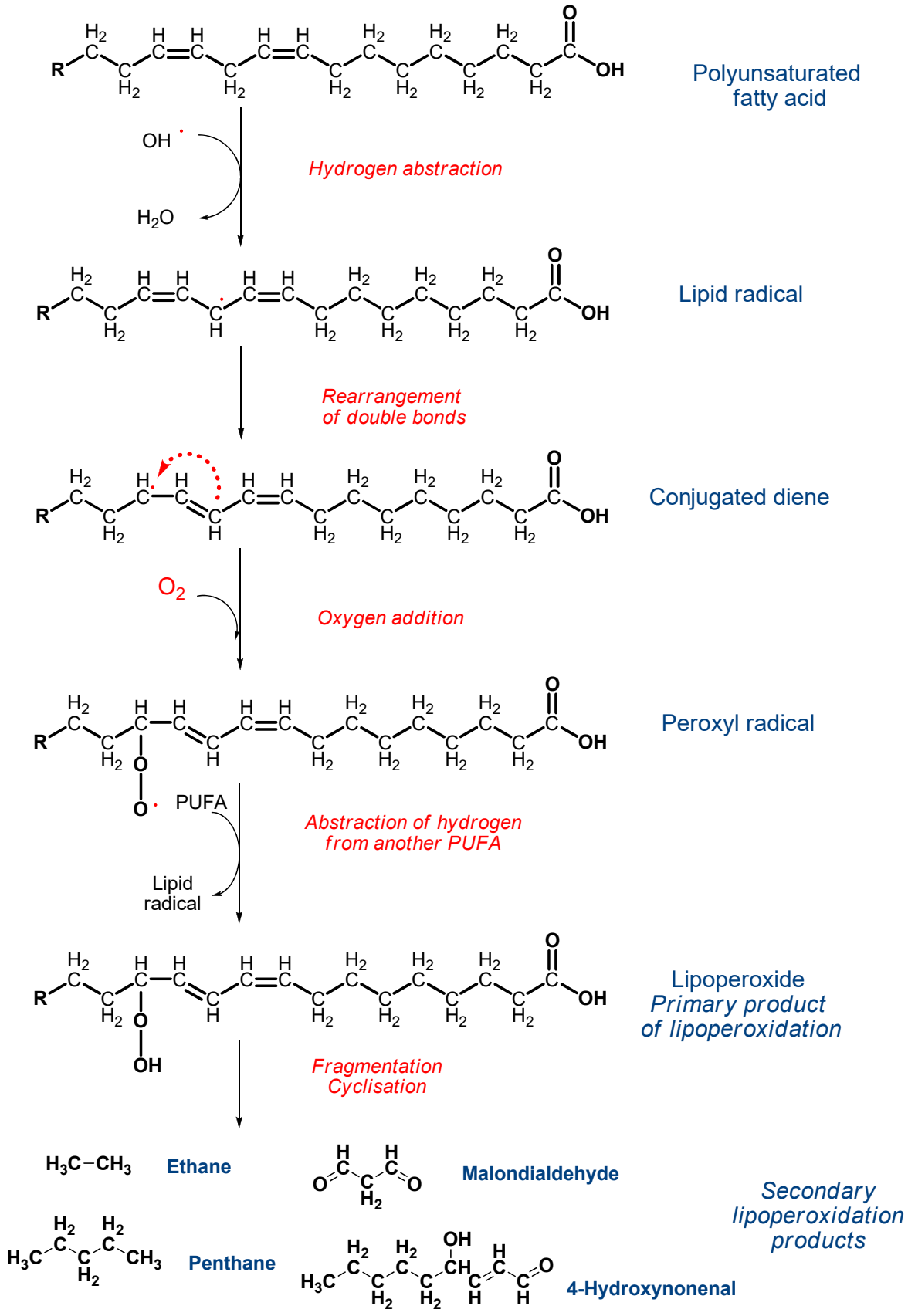
$$L^{\bullet} + L^{\bullet} \rightarrow L{-}L$$
$$L^{\bullet} + L{-}O{-}O^{\bullet} \rightarrow L{-}O{-}O{-}L$$
$$L{-}O{-}O^{\bullet} + L{-}O{-}O^{\bullet} \rightarrow L{-}O{-}O{-}L + O_2$$

- Action of antioxidants, such as tocopherol.

$$L{-}O{-}O^{\bullet} + T{-}OH \rightarrow L{-}O{-}O{-}H + T{-}O^{\bullet}$$

T–OH tocopherol
T–O$^{\bullet}$ tocopheryl (radical) – not sufficiently reactive to cleave further lipid molecule.

Fig. 1 Lipoperoxidation of unsaturated fatty acids



2.3. Significance of lipoperoxidation

1. Lipoperoxidation, if running out of control, is a destructive process *in vivo*. Free radicals react rapidly not only with the lipids present in cell membranes, but also with membrane proteins, embedded enzymes and nucleic acids. Action of free radicals alters the structure and physiologic function of the affected compounds. Likewise, structure of lipoprotein particles circulating in blood can be impaired by lipoperoxidation, which contributes to acceleration of atherosclerosis.
2. Peroxidation of lipids *in vitro* is subject of study in food chemistry. The edible fats can undergo lipoperoxidation during storage or processing. At ambient temperatures only unsaturated fatty acids are oxidized by atmospheric oxygen. At high temperatures likely to occur during baking, frying, and roasting even saturated fatty acids can autoxidize. The oxidation reactions of lipids are associated with alterations in sensoric quality of food, and are known as ***rancidity of fat***.

3. Digestion and absorption of lipids

In a healthy adult human the dietary fat should provide for about 25-30 % of energy intake. Digestion and absorption of triacylglycerols (TG), which in adults takes place mostly in the small intestine, represents a process encompassing several different steps – emulgation, hydrolysis of triacylglycerols to fatty acids and monoacylglycerols, their dispersion in the aqueous medium, and uptake by enterocytes.

3.1. Emulgation of fats

Cleavage of fat takes place on the oil-water phase boundary. Triacylglycerols as substances insoluble in water are aggregated in a form of large fat droplets. Under this condition only the triacylglycerols on the very surface of the droplet could be accessed by the digestive enzymes present in the aqueous phase, and digestion would be rather ineffective.

Emulgation of fats breaks large droplets to many small ones, expanding their total surface and thus facilitating the hydrolysis by pancreatic lipase. Two mechanisms contribute to the emulgation – ***peristaltic movements together with the action of bile acids and phospholipids***, which are present in bile. The peristaltic movements start in stomach and continue in the duodenum.

Bile acids

Bile acids are amphipathic molecules. Their structure is based on the sterane ring that represents the non-polar part of molecule and orientates itself to non-polar surfaces. In contrast, the polar groups in molecule (1 – 3 OH groups, glycine or taurine) direct to the aqueous phase (Fig. 2). This structure together with the molecular shape enables the bile acids to act ***as detergents and effectively emulsify fats***, and hence prepare them for cleavage by pancreatic lipase.

The bile acids are synthesized in hepatocytes from cholesterol. The ***primary bile acids*** are ***cholic acid and chenodeoxycholic acid*** (Fig. 3), which are further through their carboxyl groups conjugated with amino acids glycine or taurine. In this way four primary conjugated bile acids originate: glycocholic, taurocholic, glycochenodeoxycholic and taurochenodeoxycholic. Conjugation increases polarity of the original bile acids. The conjugated primary bile acids are secreted in bile to the intestine. Majority of the bile acids is subjected to the ***enterohepatic circulation***, which means they are absorbed from the small intestine and return to the liver. The endogenous bacterial flora in the intestine can bring about deconjugation and removal of OH groups on the carbon 7 of the primary bile acids. These modifications lead to conversion of some primary bile acids to ***secondary bile acids – deoxycholic acid*** (from cholic acid) ***and lithocholic acid*** (from chenodeoxycholic acid) (Fig. 3). The secondary bile acids (lithocholic acid to very limited extent) are also absorbed from the intestine and in the liver conjugated with glycine or taurine.

Fig. 2 Structure of bile acid and its salt with glycine
(structural formula and 3D arrangement; oxygen atoms are red, nitrogen atom is blue)

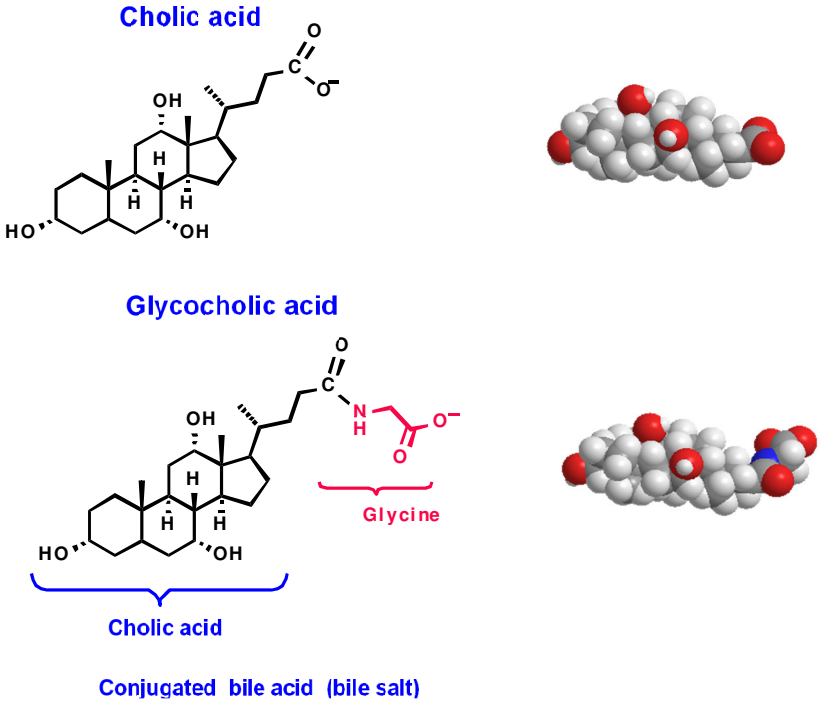
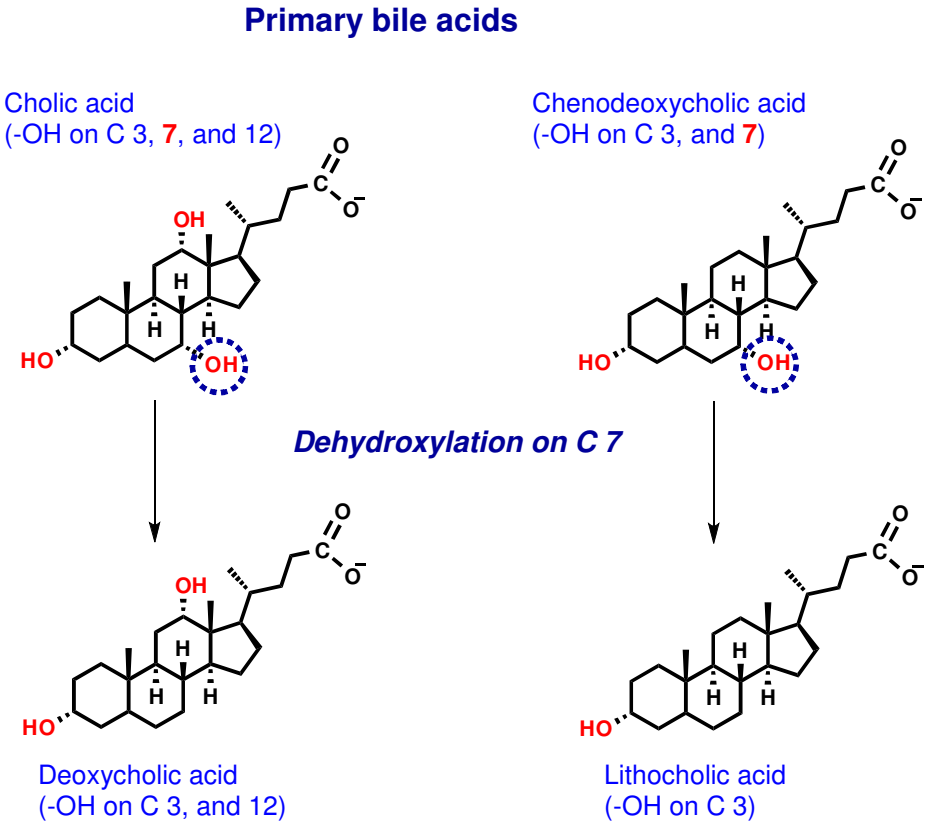


Fig. 3 Primary and secondary bile acids



The emulgation of fats results in ***emulsion of lipid droplets*** of 200 – 500 nm in size, whose surface is coated with salts of bile acids. Because of their negative charges the lipid droplets repel each other and their aggregation is prevented.

3.2. Hydrolysis of fats by pancreatic lipase

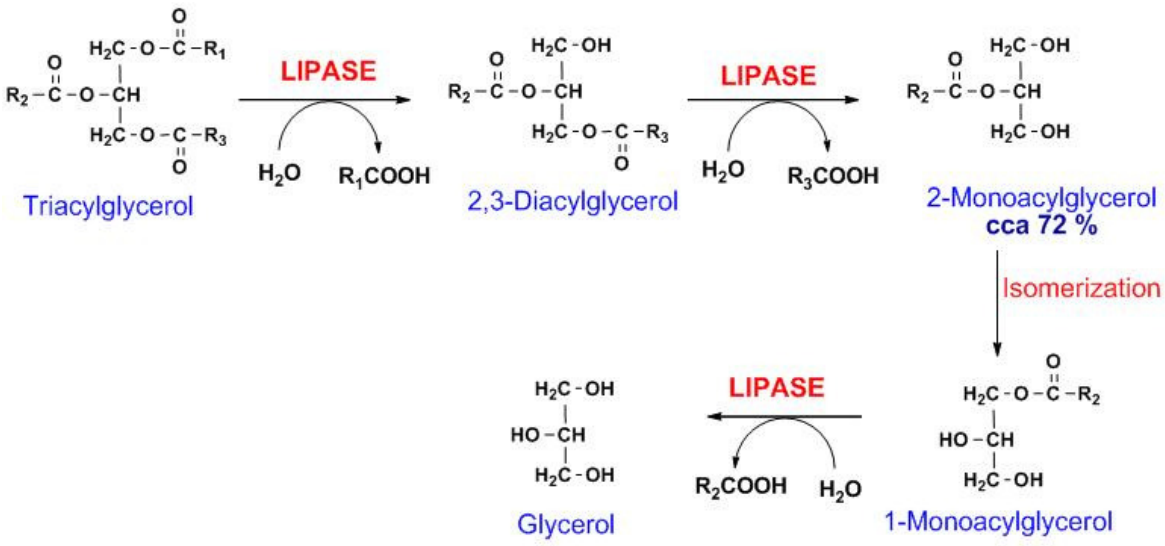
The digestion of fats takes place on the surface of the droplets of emulsified fat by the action of enzyme ***pancreatic lipase*** (triacylglycerol lipase, EC 3.1.1.3), which is secreted by exocrine part of the pancreatic gland.

Pancreatic lipase acts only on the oil-water boundary of the emulsion of triacylglycerols and the rate of triacylglycerol hydrolysis depends on the area of the emulgated substrate. Its catalytic site is localized in the N-terminal globular hydrophobic domain. The C-terminal domain contains a place for attachment of ***colipase***. It is a small protein cofactor, produced also by the pancreas roughly in the amount equimolar to the lipase. The colipase releases bile acids from the surface of micelles and helps to anchor the lipase to the oil-water boundary.

The pancreatic lipase occurs in two different conformations – ***active (open)*** and ***inactive (closed)***. The enzyme active site is hidden in the inactive conformation. Colipase forms a complex with the lipase that results in conformation changes unmasking the active site. In addition, the pancreatic lipase together with the colipase forms together a large hydrophobic area that interacts with the triacylglycerols.

Pancreatic triacylglycerol lipase catalyzes predominantly hydrolysis of primary ester bond in a triacylglycerol (on the first and third carbon) yielding a molecule of 2-monoacylglycerol and two molecules of fatty acids. These are the main cleavage products of pancreatic lipase. The secondary ester bond is resistant to hydrolysis by pancreatic lipase. It can isomerize to the first or third carbon of monoacylglycerol, and then it can be removed by the lipase. However, this process is slow and so less than ¼ of triacyglycerols undergoes complete cleavage to glycerol and fatty acids (Fig. 4)

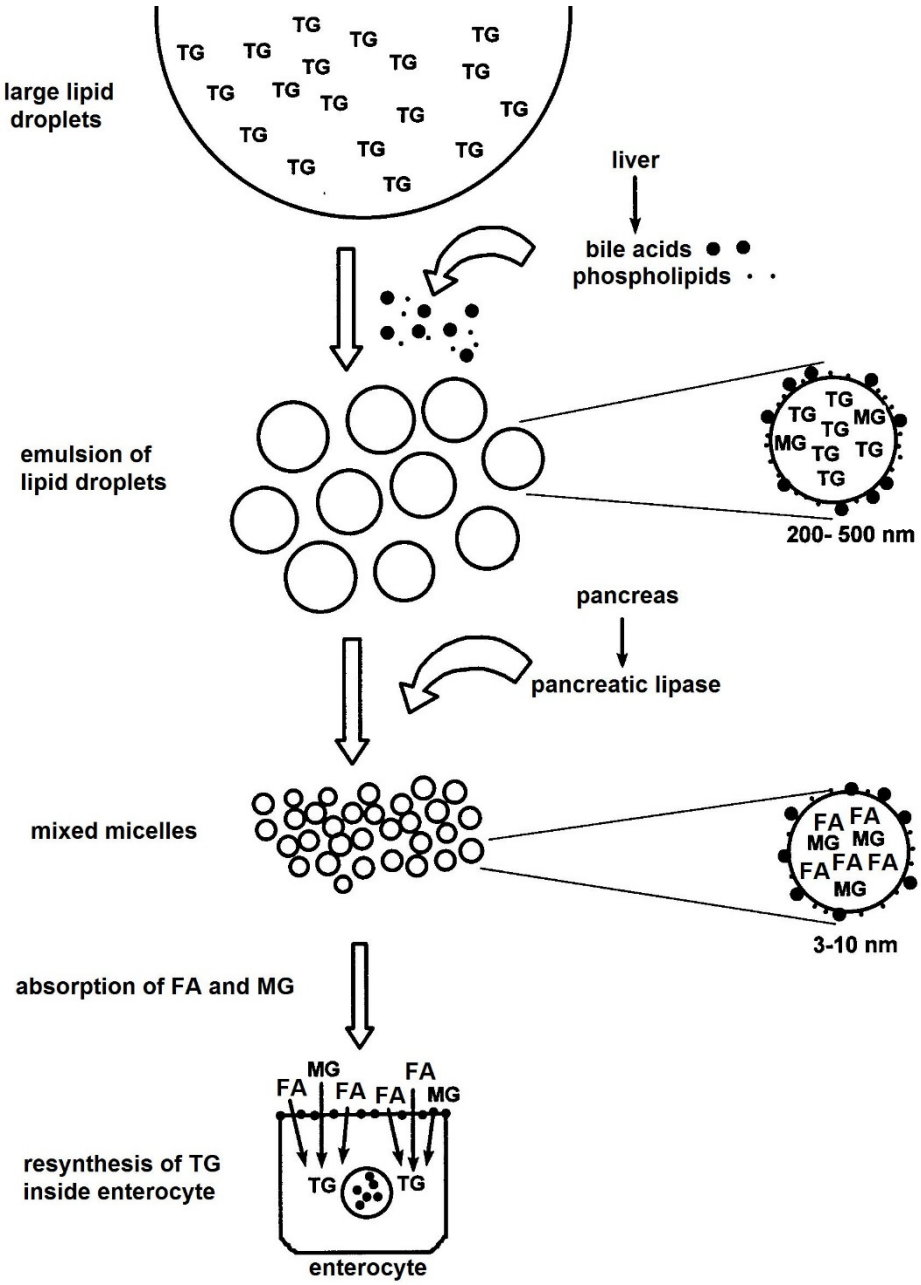
Fig. 4 Hydrolysis of triacylglycerols by pancreatic lipase



Clinical chemistry note

Examination of pancreatic lipase in serum or plasma is used in diagnostics of pancreatic diseases. Increased activity in serum is found especially in patients with *acute pancreatitis*. The estimation of pancreatic lipase in acute pancreatitis is more specific in comparison to α-amylase. Unlike the α-amylase, pancreatic lipase activity does not rise in disorders of salivary glands as it is absent from parotic gland. The elevation of pancreatic lipase peaks 24 hours after an attack of acute pancreatitis; it is usually parallel with α-amylase, but more pronounced and persistent.

Fig. 5 Scheme of digestion and absorption of fats
(MG – monoacylglycerol, TG – triacylglycerol, FA – fatty acid)



3.3. Dispersion of hydrolytic products in aqueous medium

The action of pancreatic lipase diminishes the volume of the oily phase and the lipolytic products are stabilized to ***mixed micelles*** that have an arrangement similar to the lipid emulsions, but much smaller particle size (3 – 10 nm). The micelles are high-molecular-weight aggregates of round or cylindric shape consisting of the hydrolytic lipase products (fatty acids and 2-monoacylglycerols), bile acids and phospholipids arranged so that the polar groups of particular molecules direct to the aqueous medium while the non-polar groups or structures are dipped to the non-polar core. The hydrophobic core has an ability to attract other non-polar molecules such as cholesterol, carotenoids, tocopherol, or uncleaved triacylglycerol.

3.4. Absorption of fats

Monoacylglycerols and long chain fatty acids are transported through the brush border to the enterocytes, where their re-esterification back to triacylglycerols takes place and in the form of chylomicrons they are released to the lymph. Fatty acids with lower number of carbons (less then 10) pass directly to the portal blood. The digestion and absorption of fats is summarized in Fig. 5.

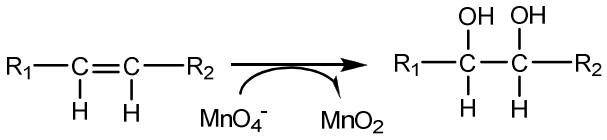
Practical tasks

1. Demonstration of unsaturated bonds in fatty acids

Principle:

Compounds containing multiple bonds are very sensitive to oxidation. One posibility how to demonstrate the presence of double bonds is an addition of hydroxyl groups to the double bond during its oxidation by potassium permanganate. A bifunctional alkohol results. The permanganate is reduced during the reaction; its typical violet color is lost and replaced with brown color.

Oxidation of double bond with potassium permanganate



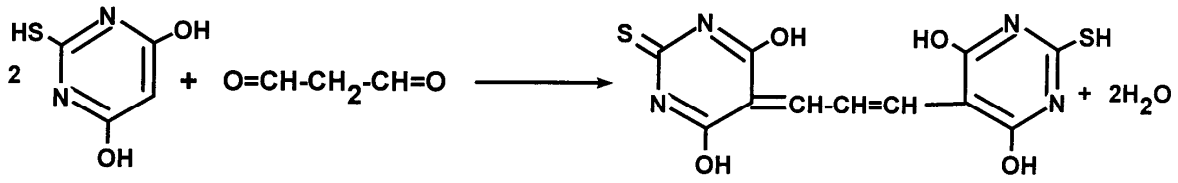
2. Estimation of malondialdehyde

Principle:

Malondialdehyde is considered to be the most abundant end-product of lipoperoxidation. It originates from oxidative damage to fatty acids having at least three double bonds.

Measurement of malondialdehyde is used for assessment of the lipoperoxidation levels both in the living body and in the food industry. Commonly, the determination of malondialdehyde utilizes its ***reaction with thiobarbituric acid in acidic medium***. A violet product originates, consisting of two molecules of thiobarbituric acid and one molecule of malondialdehyde (Fig. 6). The reaction product is quantitated by means of spectrophotometry or fluorimetry.

Fig. 6 Reaction of malondialdehyde with thiobarbituric acid



2-thiobarbituric acid **malondialdehyde** **colored condensation product**

The test with thiobarbituric acid will be performed with two samples of plant oil: one fresh and the other one expired. The development of color will be assessed only qualitatively.

3. Effect of bile on the hydrolysis of fat by pancreatic lipase

Principle:

Pancreatic lipase hydrolyzes predominantly the terminal ester bonds of triacylglycerols. The amount of released fatty acids is determined by means of neutralization titration with sodium hydroxide. A similar principle can be used for estimation of catalytic concentration of pancreatic lipase in biological material (serum, plasma, pancreatic juice).

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