Lipoproteins (clinic)

Dyslipidemia

Dyslipidemia (dyslipoproteinemia; formerly hyperlipoproteinemia – HLP) is a group of metabolic diseases that are characterized mainly by increased concentrations of lipids or lipoproteins in plasma due to their increased synthesis or decreased degradation. Often, however, an increase in some fraction of blood lipids is combined with a decrease in the concentration of HDL cholesterol.

Dyslipidemia represent one of the most significant risk factors for atherosclerosis. Complications of atherosclerosis (acute myocardial infarction, styeare, ischemic disease of the lower extremities) occupy a leading place in the causes of mortality and morbidity not only in our country, but practically in all developed countries of the world.

Classification of dyslipidemia

Dyslipidemia (DLP) can be divided according to various criteria:

1. Classification by cause

Primary dyslipidemia

There are genetically determined disorders of lipoprotein metabolism. It is believed that most HLPs are primary (e.g. familial hypercholesterolemia, familial hypertriacylglycerolemia).

Secondary dyslipidemia

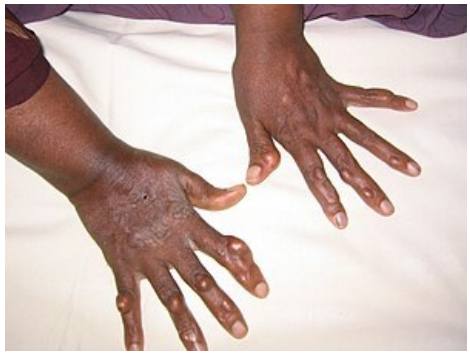
They are the result of another disease that disrupts lipid and lipoprotein metabolism. They can manifest themselves in an isolated increase in cholesterol or triacylglycerols or both. It often accompaniese.g. diabetes mellitus , hypothyroidism, liver disease, obesity, chronic alcoholism. Their danger lies in their long-term asymptomatic period, sudden manifestation then occurs as a complication of atherosclerosis in various places or as acute hemorrhagic pancreatitis.

2. Therapeutic classification

Classification of dyslipidemia according to the European Society for Atherosclerosis (1992)

Hypercholesterolemia

It represents a simple and practical division of DLP based on the determination of the concentration of cholesterol and triglycerides in serum into three groups. It is the basis for deciding on the therapeutic procedure.


Multiple hand xanthomas

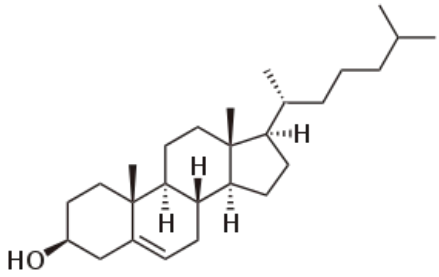
Isolated increase in total cholesterol, mainly in the LDL fraction. Usually we encounter familial hypercholesterolemia and polygenic hypercholesterolemia.

- **Familial hypercholesterolemia (FH)** is an autosomal dominant disease, the cause of which is a genetic disorder in the formation or function of LDL receptors. In homozygotes, LDL catabolism with LDL receptors is practically non-functional, in heterozygotes, the capacity of LDL receptors is halved. As a result, atherogenic LDL particles accumulate in the blood. Heterozygous form is more common and occurs about 1 case per 500 people. Homozygotes have been severely affected since childhood, have tendon and skin xanthomas and most of them die of myocardial infarction within 20 years. Affected heterozygous people are manifested by premature occurrence of cardiovascular diseases (CHD aged 30–50 years), *arcus senilis corneae*, *xanthelasma palpebrarum* or tendon xanthomatosis. Total cholesterol concentrations are around 7–10 mmol/l in heterozygotes and around 15–30 mmol/l in homozygotes. Changes in the lipoprotein spectrum correspond predominantly to phenotype IIa, less commonly IIb (according to Fredrickson).
- In **polygenic hypercholesterolemia**, genetic influences as well as environmental influences are applied. In industrialized countries, it is encountered very often. Total cholesterol values usually do not exceed 8 mmol/l, but already pose an increased risk of atherosclerosis. Changes in the lipoprotein spectrum correspond mainly to phenotype IIa, less often IIb.
- **Secondary hypercholesterolemia** can be found, for example, in hypothyroidism, nephrotic syndrome, in a diet rich in saturated fats.

Combined hyperlipidemia

Simultaneous increase in both cholesterol and triglycerides.

- **Familial combined hyperlipidemia** is one of the most common primary HLPs. It occurs at a frequency of 1:50 to 1:100. It has a basis in the genetically determined increased production of apolipoprotein B100. It is associated with an increased risk of vascular diseases. LDL and VLDL are usually increased, corresponding to phenotype IIb, but we also encounter phenotypes IIa, IV and V.
- **Secondary forms** are found, for example, in hypothyroidism, in the treatment with corticosteroids.



Cholesterol

Hypertriacylglycerolemia

Isolated increase in triacylglycerols:

- Genetically determined hypertriacylglycerolemia is **familial hypertriacylglycerolemia**, which affects about 0.2–0.3% of the population. It manifests itself in the multiplication of VLDL, probably on the basis of their increased formation. At the same time, we find a reduced level of HDL-cholesterol. In laboratory findings, we encounter slightly elevated triacylglycerols, usually up to 6 mmol/l at normal cholesterol concentrations. In patients, there is a danger of myocardial infarction.
- Rarely we can meet with**familial hyperlipoproteinemia type I**, characterized by hyperchylomicronemia. Patients are at risk of pancreatitis caused by high levels of triacylglycerols (often over 20 mmol/), which increases its risk.
- **The secondary form** of hypertriacylglycerolemia is often associated with diabetes mellitus, obesity, excessive alcohol intake or a high-carbohydrate diet.

3. Classification according to Fredrickson

The Fredrickson classification is the first classification of lipoprotein metabolism disorders. At present, it is being abandoned because it does not clarify the actual cause of the disease and is being replaced by a more modern approach, using new knowledge in the etiopathogenesis of HLP. We will briefly discuss it, because in older literature we still encounter it. Based on the concentration of cholesterol and triacylglycerols in serum and electrophoretic examination of lipoproteins, HLPs were divided into 5 lipoprotein types – phenotypes (according to who, type II was later divided into IIa and IIb). However, the lipoprotein type is only a current picture of the state of lipid and lipoprotein metabolism. Most lipoprotein types can be caused by several genetic disorders, and conversely, some genetically determined HLP can manifest themselves in one or more lipoprotein types depending on the diet or medication treatment.

Laboratory values

norm:

- **Total cholesterol:** < 5 mmol/l
- **LDL-cholesterol:** < 3 mmol/l
- **TAG:**< 1.7 mmol/l
- **HDL:** > 1 mmol/l for men; > 1.2 mmol/l for women

Biochemical examination in hyperlipoproteinemia

In recent years, the issue of lipid metabolism disorders has come to the forefront of interest mainly in connection with the prevention and treatment of cardiovascular diseases. Biochemical examinations play an essential role in the diagnosis of hyperlipoproteinemia and the monitoring of therapy.

The basic laboratory examination is the determination of total cholesterol and triacylglycerols in serum (plasma), as well as the determination of LDL- and HDL- cholesterol.

Other **more specialized examinations** include the determination of typical apoproteins apoA-I (HDL) and **apoB-100 (LDL), or lipoprotein(a)** and lipoproteinelectrophoresis, the importance of which is decreasing. Gradually, DNA diagnostics is also developing, affecting the causes of some HLP.

Laboratory diagnosis of lipid metabolism disorders must be based on blood lipid tests from at least two blood samples between 2 and 8 weeks, under normal lifestyle. Differences are found for each parameter and, if they are lower than the permissible values, the arithmetic mean for each analyte is calculated. If the critical difference between 2 consecutive analyzes is exceeded, a third examination is performed.

Critical differences for lipid metabolism analytes

Analyte Critical difference [%]:

- Total cholesterol > 20
- LDL-cholesterol > 25
- HDL-cholesterol > 25
- triacylglycerols > 65

It should also be taken into account that the examination of lipid metabolism is significantly influenced by **lifestyle** (dietary habits, physical activity, body weight), **drug therapy** (hormonal contraceptives, hormone replacement therapy and **ongoing acute or uncompensated disease**. Therefore, blood lipid testing should not be carried out when it can be assumed that the result will not tell about the situation under a normal lifestyle (shortly after vacation, when hospitalized for other reasons, acute diabetes mellitus, in pregnancy and half a year after it, etc.).

Determination of total cholesterol

Principle

Cholesterol is transported in the blood plasma as part of lipoproteins, mostly in the LDL fraction, less in HDL and VLDL. Of this cholesterol, approximately two-thirds are esterified with higher fatty acids, the rest is unesterified.

- It is common to determine total **cholesterol** in serum (plasma). Therefore, the actual determination of total cholesterol is preceded by hydrolysis of cholesterol esters to free cholesterol and fatty acids using the enzyme cholesterolesterase (CE).
- This is followed by the oxidation of unesterified cholesterol to 4-cholesten-3-one with the simultaneous formation of hydrogen peroxide in a reaction catalyzed by cholesterol oxidase (CHOD).
- The last reaction uses hydrogen peroxide to oxidatively copulate with 4-aminoantipyrine and phenol in the presence of another peroxidase enzyme (POD). A color product is formed, the absorbance of which is proportional to the amount of cholesterol.

Evaluation

- With an increasing concentration of total cholesterol, the **risk of atherosclerosis increases**. According to Czech and European recommendations, the concentration of total cholesterol should be < 5.0 mmol/l. Total cholesterol should be determined in all persons over 18 years of age as a precaution. Even in the case of a normal result, i.e. up to **5.0 mmol/l**, the examination should be repeated in 5 years.
- If the concentration is higher and further in patients with coronary heart disease and other people at risk, we proceed to a more detailed examination of the lipid spectrum by determining LDL- and HDL cholesterol and triacylglycerols.
- In patients at increased risk of cardiovascular disease (e.g. diabetics), total cholesterol should be lower < 4.5 mmol/l and in people with already manifest cardiovascular disease even < 4.0 mmol/l.
- In addition, elevated cholesterol concentrations are often found in diabetics or hypothyroidism. A decrease in the concentration of total cholesterol occurs, for example, in advanced liver cirrhosis or hyperthyroidism or malnutrition.
- It is a marker for overall mortality and a risk factor for CVD. The relationship of cholesterol to total mortality is nonlinear, has the shape of the letter J or U, which can be explained by the mechanism of reverse causality.

Evaluation limits

Serum total cholesterol concentration (S-total cholesterol): **2.9–5.0 mmol/l.**

Determination of LDL-cholesterol (LDL-C)

Principle

The concentration of LDL-cholesterol can be determined ***indirectly*** by calculation or ***direct*** analysis.

Indirect determination of LDL-C

- In the indirect determination of LDL-C, the so-called **Friedewald formula** is used.

LDL-cholesterol = total cholesterol – HDL-cholesterol – TG/2,2

- It is based on the assumption that serum cholesterol is contained in the HDL, LDL and VLDL fractions. The calculation requires the measurement of three different analytes – total cholesterol, HDL-cholesterol and triacylglycerols. This increases the possibility of analytical errors. Total cholesterol and HDL-cholesterol are determined directly. Detecting cholesterol in the VLDL is difficult. We determine it on the assumption that the molar cholesterol ratio in the VLDL is on average 45% of the total triacylglycerol content; then the fraction of TG/2.2 represents the cholesterol in the VLDL particles. The formula can only be used if the value of triacylglycerols does not exceed 4,5 mmol/l, chylomikra must not be present.

Direct determination of LDL-C

Direct determination of LDL-cholesterol by **homogeneous methods** without the need for separation steps and centrifugation, the advantage of which is the possibility of automation, is gradually being put into practice. Simplification of the analysis was achieved by using various detergents and other reagents that specifically block or dissolve individual lipoprotein fractions. In this way, LDL particles are separated, in which the cholesterol content is determined at the final stage of the analysis.

Several procedures have been developed for the direct determination of LDL-C. The analysis is divided into two steps.

- One of the procedures uses various detergents and other substances to **selectively block cholesterol in non-LDL particles** (HDL, VLDL and chylomicrons), whose cholesterol becomes inaccessible for the action of enzymes used to determine it. In unblocked LDL particles, after release, cholesterol is determined by the enzyme method described in total cholesterol using the reactions of cholesterolesterase, cholesterol oxidase and peroxidase.
- In another method, at the first stage, with the help of a detergent, cholesterol is released from chylomicrons, VLDL and HDL. The released cholesterol is converted with the help of enzymes up to 4-cholesten-3-one and hydrogen peroxide. The hydrogen peroxide formed at this stage is not used to form the colour product as it would interfere with the next step of determination. Therefore, it is decomposed by catalases into water and oxygen. With this reaction, discoloration does not develop. In the second phase, another detergent releases cholesterol from LDL and this is then determined similarly to total cholesterol.

There are other methods for determining direct LDL-cholesterol.

Evaluation

- An increased concentration of LDL-cholesterol is significantly involved **in the development of atherosclerosis**. The recommended concentration of LDL-cholesterol is < **3.0 mmol/l** according to current Czech and European recommendations.
-

Evaluation limits

Serum LDL-cholesterol: **1.2–3.0 mmol/l**

Determination of HDL-cholesterol (HDL-C)

Principle

Older methods for the determination of HDL-C

Older methods use procedures based on the determination of cholesterol in HDL particles after removal of non-HDL particles. Precipitated lipoprotein particles are separated by centrifugation. In the supernatant, the concentration of cholesterol in HDL particles is then determined using commonly used photometric methods.

Direct determination of HDL-C

Modern, easily automated methods use, similarly to LDL-C, direct determination of HDL-cholesterol using **homogeneous methods** without precipitation and centrifugation. Several procedures have been developed for the direct determination of HDL-C. The analysis is divided into two steps.

- In the first step, all non-HDL particles (VLDL, LDL, chylomicrons) are blocked. By blocking non-HDL particles, the reaction of the cholesterol contained in them with the enzymes used to determine it is impossible. Blocking can be achieved by immunoinhibition with antibodies directed against apoB, which is part of VLDL, LDL and chylomicrons. Antibodies produce non-HDL soluble immunocomplexes. In the next step, the detergent dissolves HDL particles and with the help of cholesterolesterase, cholesterol oxidase and peroxidase enzymes, cholesterol in HDL is measured in the same way as in the determination of total cholesterol, i.e. using enzymes.

- Another method is used to block polyanions and polymers that are selectively adsorbed to the surface of non-HDL lipoproteins. These are then protected against detergents that release cholesterol contained only in HDL particles. The second step of the analysis is analogous to the procedure described in the first method.

Evaluation

- HDL-cholesterol concentrations should be > **1.0 mmol/l in men and > 1.2 mmol/l in women**. Elevated HDL-C is considered a ***"negative risk"*** factor for the development of atherosclerosis, and levels below 1.0 mmol/L are associated with an increased risk of atherosclerosis.
- Low HDL is accompanied by metabolic syndrome, low physical activity, type 2 DM, cigarette smoking and consumption of large amounts of carbohydrates.

Evaluation limits

- Serum HDL-cholesterol (men): **1.0–2.1 mmol/l**
- Serum HDL-cholesterol (women): **1.2–2.7 mmol/l**

Determination of triacylglycerols

Principle

The recommended routine methods for the determination of triacylglycerols use several enzyme reactions.

- Lipoprotein lipase catalyzes the hydrolysis of triacylglycerols to glycerol and fatty acids.
- The released glycerol is converted by the action of glycerol kinase in the presence of ATP to glycerol-3-phosphate, which is oxidized to dihydroxyacetone phosphate by glycerol-3-phosphate oxidase.
- The simultaneously formed hydrogen peroxide is used in another reaction catalyzed by peroxidase to oxidative copulation of 4-aminoantipyrin with a phenol derivative. A quinonimine dye is formed, the absorbance of which is subtracted.

Evaluation

- An increased concentration of triacylglycerols is an independent risk factor for atherosclerosis. Czech and European recommendations consider the normal serum concentration of triacylglycerols to be **< 1.7 mmol/l**.
- A TG concentration of > 1.7 mmol/l (fasting) is considered an indicator of increased cardiovascular risk. Higher triglycerides tend to be associated with reduced HDL levels and predict high concentrations of cholesterol-rich remnants and small, dense LDL type B particles.
- The concentration of triacylglycerols increases within 2 hours after eating and reaches its maximum in 4–6 hours, therefore blood for the determination of triacylglycerols must be taken after a 12–14-hour lactation. Plasma samples with a TG concentration of more than 3.4 mmol/l opalesque, chylomicrons are present at TG levels above 11.3 mmol/l and the plasma is milky-hardened.
- In therapy, great emphasis is placed on a diet low in fats and sugars, increased physical activity, enough antioxidants in the diet and overall hypolipidemic treatment.

Evaluation limits

Serum triacylglycerol concentration (fS-triacylglycerols): **0.45–1.7 mmol/l**

Derived parameters of lipid metabolism

The aim of these calculations is to refine the risk determination resulting from the measured blood lipid values.

Atherogenic index

- From the measured values related to cholesterol, various atherogenic indices can be calculated. An index is delivered that takes into account the influence of the concentration of both total cholesterol and HDL-C on the risk of cardiovascular diseases. The index takes into account the fact that elevated HDL-C is a "negative risk factor" reducing the risk of coronary heart disease (CHD). Simultaneous increases in total and HDL cholesterol may not increase the risk of CHD.

Total cholesterol/HDL-cholesterol

Recommended value

Atherogenic index: < **5.0**

Non-HDL-cholesterol

- It is used to estimate the risk of atherosclerosis in cases where it is not possible to calculate the concentration of LDL-C. It represents cholesterol contained in potentially atherogenic lipoprotein particles (LDL, IDL, VLDL, residual chylomicrons).

Non-HDL-cholesterol (mmol/l) = total cholesterol (mmol/l) – HDL-cholesterol (mmol/l)

Recommended value

Non-HDL-cholesterol: **< 3.8 mmol/l**

Chylomicron test

Principle:

- Measurement of the concentration of chylomicrons is not commonly carried out. They should not occur in samples taken after a 12-hour emptying. Their presence is evidenced by a strong chylozity of the serum. For evidence, a chylomicron test can be performed, which consists of observing an opalescent to white ring after standing the serum for 12 hours at 4 °C. During this time, any chylomicrons present are flotating to the sample level. Any healthy serum should be completely clear after 12 hours of lactating.
- The appearance of the serum in the chylomicron test may also indicate other disorders in lipoprotein metabolism.

Evaluation

Appearance	Changes in lipoproteins
Clear	Normal serum or increased LDL particles
Milky cloudy top layer, the serum under this layer is clear	The milky turink of the upper layer corresponds to the chylomicrons that float to the surface during incubation
Diffuse clouding of the whole sample (chylous serum)	Increased VLDL particles, turbidity intensity depends on the amount of triacylglycerols in VLDL
Milky turbid top layer and diffuse turbidity of infranatant	Increased chylomicrons and VLDL
Narrow creamy top layer and diffuse turbidity of the infranatant	Increase in chylomicron residues and IDL

Electrophoresis of serum lipoproteins

Principle

- Lipoprotein particles are divided in an electric field according to the different size of the surface charge, in this case conditioned by the different amount of proteins in each fraction. It is mainly used for the detection **of some abnormal lipoproteins** and in the diagnosis **of less common types of hyperlipoproteinemia** and for the detection **of atherogenic lipoprotein Lp (a)**.
- The smallest particles with the highest protein content **α-lipoproteins (HDL)** move the fastest in the α area. At the level between α-2 and β as **pre-β-lipoproteins**, VLDL particles migrate. LDL particles usually represent the most pronounced fraction as **β-lipoproteins** in the β-globulin region. **Chylomicrons**, if present in the serum, remain at the start, sometimes they can form a stripe that is visible from the launch site to the α area. In the position between the α and β-lipoproteins (HDL and LDL), another fraction characteristic of lipoprotein (a) – Lp(a) may appear, which represents a risk factor for the development of atherosclerosis and cardiovascular diseases.
- The electrophoresis of serum lipoproteins is similar to that of serum protein electrophoresis, with the difference that lipophilic dyes such as Sudan black are used for staining. Electrophoresis of lipoproteins is usually performed on agarose gel, which allows good separation of pre-β-lipoproteins.

Evaluation

- The electrophoreogram can be evaluated **densitometrically** at a wavelength of 580 nm. The result is expressed as a percentage of the optical density for each fraction relative to the total colour area. The determined values, together with other indicators (total cholesterol, triacylglycerols), serve to characterize hyperlipoproteinemia (Fig. 7, 8, 9).
- In practice, only **visual** evaluation is also carried out. The biological finding shall be compared with the reference values and the result shall be assessed in terms of an increase (decrease) of the individual fractions.

Benchmarks

α-lipoproteins	23–46 %
pre-β-lipoproteins	3–18 %
β-lipoproteins	42–63 %

Links

- ws: Lipoproteiny (klinika)

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