Biochemical examination in hyperlipoproteinemia

In recent years, the issue of lipid metabolism disorders has come to the forefront of interest, especially in connection with the prevention and treatment of cardiovascular diseases. Biochemical examinations play an important role in the diagnosis of hyperlipoproteinemias 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 lipoproteins electrophoresis (but the importance decrease). DNA diagnostics is also gradually developing, affecting the causes of some HLP (hyperlipoproteinemia).

Laboratory diagnostics of lipid metabolism disorders must be based on examination of blood lipids from at least two blood samples in the range of 2-8 weeks, under normal lifestyle. Differences are found for each parameter and, if lower than the allowable values, an arithmetic mean is calculated for each analyte. If the critical difference between 2 consecutive analyses is exceeded, a third examination is perform

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

Determination of total cholesterol

Principle

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

- Serum (plasma) is routinely determined for **total cholesterol**. Therefore, the actual determination of total cholesterol is preceded by the hydrolysis of cholesterol esters to free cholesterol and fatty acids by the enzyme cholesterol esterase (CE).
- This is followed by oxidation of non-esterified cholesterol to 4-cholesten-3-one to form hydrogen peroxide in a cholesterol oxidase (CHOD) catalyzed reaction.
- The later reaction uses hydrogen peroxide to oxidatively couple 4-aminoantipyrine and phenol in the presence of another enzyme peroxidase (POD). A colored product is formed. Its absorbance is proportional to the amount of cholesterol.

Evaluation

- ***The risk of atherosclerosis increases*** with increasing total cholesterol levels. According to Czech and European recommendations, the concentration of total cholesterol should be <5.0 mmol/l. Total cholesterol should be determined in all people over the age of 18 as part of prevention. The test should be repeated in 5 years even in the case of a normal result, i.e. up to 5.0 mmol/l.

- If the concentration is higher and also in patients with ischemic heart disease and other high-risk individuals, 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 <4.5 mmol/l and in people with already manifested cardiovascular disease even <4.0 mmol/l.
- Elevated cholesterol levels are also often found in diabetics or hypothyroidism. Total cholesterol levels are reduced, for example, in advanced liver cirrhosis or hyperthyroidism, or malnutrition.
- It is a marker for overall mortality and a risk factor for CVD (cardiovascular disease). The relationship of cholesterol to overall mortality is non-linear, J-shaped, or U-shaped, which can be explained by the mechanism of reverse causality.

Evaluation limits

Serum total cholesterol (total S-cholesterol): **2,9–5,0 mmol/l**.

Determination of LDL-cholesterol (LDL-C)

Principle

LDL-cholesterol levels can be determined ***indirectly*** by calculation or ***directly*** by analysis.

Indirect determination of LDL-C

- The so-called ***Friedewald's formula*** is used for the indirect determination of LDL-C.

  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. We determine total cholesterol and HDL-cholesterol directly. Cholesterol detection in VLDL is difficult. We determine it on the assumption that the molar ratio of cholesterol in VLDL is on average 45% of the total triacylglycerol content; then the TG/2.2 fraction represents cholesterol in the VLDL particles. The formula can only be used if the value of triacylglycerols does not exceed 4.5 mmol/l, chylomicrons must not be present.

Direct determination of LDL-C

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

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

- One method uses various detergents and other substances to ***selectively block cholesterol in non-LDL particles*** (HDL, VLDL, and chylomicrons), whose cholesterol becomes inaccessible to the enzymes used to determine it. Cholesterol is determined in unblocked LDL particles after release by the enzyme method described for total cholesterol using the cholesterol esterase, cholesterol oxidase, and peroxidase reactions.
- In another method, cholesterol is released from chylomicrons, VLDL, and HDL in the first phase using a detergent. The released cholesterol is converted by enzymes to 4-cholesten-3-one and hydrogen peroxide. The hydrogen peroxide formed at this stage is not used to form a colored product, as it would interfere in the next step of the determination. Therefore, it is decomposed by catalase into water and oxygen. In this reaction, no color develops. In the second phase, another detergent releases cholesterol from the LDL, which is then determined similarly to total cholesterol.

There are also other methods for determining direct LDL-cholesterol.

Evaluation

- Elevated LDL-cholesterol levels significantly contribute ***to the development of atherosclerosis***. According to current Czech and European recommendations, the recommended concentration of LDL-cholesterol is **< 3,0 mmol/l**.
- LDL concentrations <2.6 mmol/l are recommended for patients at increased risk of cardiovascular disease and <1.8 mmol/l for patients with already developed cardiovascular disease.

Evaluation limits

Serum LDL-cholesterol: **1,2–3,0 mmol/l**

Determination of HDL-cholesterol (HDL-C)

Principle

Older methods for HDL-C determination

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

Direct determination of HDL-C

Modern, easily automated methods use, as with LDL-C, the 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 usually divided into two steps.

- In the first step, all non-HDL particles (VLDL, LDL, chylomicrons) are blocked. By blocking the non-HDL particles, the reaction of the cholesterol contained in them with the enzymes used for its determination is prevented. Blockade can be achieved by immunoinhibition with antibodies directed against apoB, which is part of VLDL, LDL, and chylomicrons. Antibodies form soluble immunocomplexes with non-HDL. In the next step, the detergent dissolves the HDL particles, and the cholesterol in the HDL is measured by the cholesterol esterase, cholesterol oxidase, and peroxidase in the same way as in the determination of total cholesterol, i.e. by the enzymes.
- Another method is used to block polyanions and polymers that selectively adsorb 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 levels 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 conversely, 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 (males): **1,0–2,1 mmol/l**
- Serum HDL-cholesterol (females): **1,2–2,7 mmol/l**

Determination of triacylglycerols

Principle

Recommended routine methods for the determination of triacylglycerols use several enzymatic reactions.

- Lipoprotein lipase catalyzes the hydrolysis of triacylglycerols to glycerol and fatty acids.
- The released glycerol is converted by glycerol kinase in the presence of ATP to glycerol-3-phosphate, which is oxidized to dihydroxyacetone phosphate by glycerol-3-phosphate oxidase.
- The co-formed hydrogen peroxide is used in another peroxidase-catalyzed reaction to oxidatively couple 4-aminoantipyrine with a phenol derivative. A quinone imine dye is formed, the absorbance of which is deducted.

Evaluation

- Elevated triacylglycerol levels are an independent risk factor for atherosclerosis. Czech and European recommendations consider a normal serum triacylglycerol concentration to be ***<1,7 mmol/l***.
- TG concentration >1.7 mmol/l (fasting) is considered an indicator of increased cardiovascular risk. Higher triacylglycerols are associated with decreased HDL levels and predict high concentrations of cholesterol-rich remnants and small dense B-type LDL particles.
- The concentration of triacylglycerols increases within 2 hours after a meal and reaches a maximum in 4-6 hours, therefore blood must be collected for the determination of triacylglycerols after 12-14 hours of fasting. Plasma samples with a TG concentration higher than 3.4 mmol/l opalesce, chylomicrons are present at TG levels above 11.3 mmol/l and the plasma is milky turbid.
- Therapy places great emphasis on a diet low in fat and sugar, increased physical activity, plenty of 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 determination of the risk that results from the measured values of blood lipids.

Atherogenic index

- Various atherogenic indices can be calculated from the measured values concerning cholesterol. The recommended index is the one that takes into account the effect of total cholesterol and HDL-C on the risk of cardiovascular disease. The index takes into account the fact that elevated HDL-C is a "negative risk factor" that reduces the risk of ischemic heart disease. Simultaneous increases in both total and HDL cholesterol may not increase the risk of ischemic heart disease.

Recommended value

Atherogenic index: **< 5,0**

Non-HDL-cholesterol

- It is used to estimate the risk of atherosclerosis in cases where LDL-C levels cannot be calculated. 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 chylomicron concentration is not routinely performed. They should not be present in samples taken after 12 hours of fasting. Their presence is evidenced by the strong chylosity of the serum. For a proof, a chylomicron test can be performed. It consists of observing an opalescent to white ring in the serum, that stood for 12 hours at 4°C. During this time, any chylomicrons present float to the sample surface. Each healthy serum should be completely clear after 12 hours of fasting.
- The appearance of the serum by the chylomicron test may also indicate other disorders in lipoprotein metabolism.

Evaluation

Appearance	Changes in lipoproteins
pure	normal serum or elevated LDL particles
Milky turbid upper layer, serum under this layer is clear	the milky turbid top layer corresponds to chylomicrons that float to the surface during incubation
Diffuse turbidity of the whole sample (chlorosis serum)	increased VLDL particles, the intensity of turbidity 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

Serum lipoprotein electrophoresis

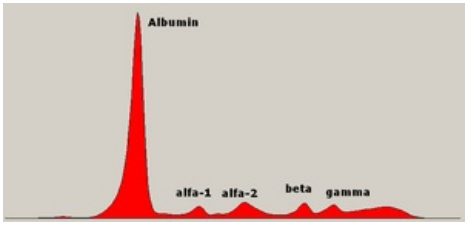
Principle

- Lipoprotein particles are divided in an electric field according to the different size of the surface charge, in this case, conditioned by the different amounts of proteins in the individual fractions. It is mainly used for ***the detection of some abnormal lipoproteins*** and in the diagnosis of ***less common types of hyperlipoproteinemias*** 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 region. At the level between α-2 and β VLDL particles migrate as ***pre-β-lipoproteins***. LDL particles usually represent the most prominent fraction as ***β-lipoproteins*** in the β-globulin region. ***Chylomicrons***, when present in the serum, remain at the start, sometimes forming a band that is visible from the start point to the α region. In the position between α and β-lipoproteins (HDL and LDL), another fraction characteristic of lipoprotein (a) – Lp (a) may appear, which is a risk factor for the development of atherosclerosis and cardiovascular diseases.
- The performance of serum lipoprotein electrophoresis is similar to serum protein electrophoresis, except that lipophilic dyes such as Sudan Black are used for staining. Lipoprotein electrophoresis is usually performed on

an agarose gel, which allows good separation of pre-β-lipoproteins.

Evaluation

- The electropherogram 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 color area. The determined values together with other indicators (total cholesterol, triacylglycerols) serve to characterize hyperlipoproteinemias.
- In practice, only ***visual*** evaluation is performed. The biological finding is compared with the reference values and the result is assessed in terms of an increase (decrease) in the individual fractions.



Normal protein electrophoresis

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

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Related Articles

- Dyslipidemy
- Lipoproteins (clinics)
- Lipoproteins
- Hypolipidemics
- Hypolipidemic treatment

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