UC 577,1; 61 ISSN 0354-3447

Jugoslov Med Biohem 22: 101–107, 2003

Pregledni članak
*Review article**

PATHOPHYSIOLOGY AND CLINICAL SIGNIFICANCE OF ATHEROGENIC LIPOPROTEIN PHENOTYPE AND SMALL DENSE LDL PARTICLES

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Summary: In spite of strong proofs supporting cholesterol hypothesis, serum cholesterol concentration is not a good discriminative factor in assessing the risk of coronary heart disease. The degree of reduction of coronary risk depends also on the level of serum triglycerides. Namely, within metabolic disturbance of triglyceride-rich lipoproteins, a reciprocal lipid transfer takes place in the course of delipidation cascade, yielding the remodelling of all the classes of lipoproteins and establishing the so-called atherogenic lipoprotein phenotype (increase in triglycerides, small dense LDL, and apolipoprotein B, and decrease in HDL cholesterol and apolipoprotein A-I). A major part of the atherogenic potential of this phenotype is related to the increase in the number of small dense LDL particles (phenotype B), and not because of the contribution to the serum cholesterol, but due to their lower affinity to LDL receptors, easier penetration to arterial intima, longer retention in subendothelium, accelerated oxidation, prompt takeover by macrophages and establishing of endothelial dysfunction.

Key words: triglyceride-rich lipoproteins, small dense LDL, postprandial metabolism, coronary artery disease

Introduction

The last decade was marked by numerous prospective and therapeutic studies, which finally put an end to discussions about the risk and benefits of lowering the level of total serum and LDL cholesterol (1 4). At the same time, results of these studies have also pointed to the significant role of mixed hyperlipidaemia as a coronary risk factor, and prompted more careful studies of the metabolism of triglycerides and triglyceride-rich lipoprotein particles. In this way also ended another discussion related to the independent role of hypertriglyceridemia in the development of premature atherosclerosis (5 8).

Now it is undoubtedly clear that hyperglyceridaemia is an integral part of metabolic characteristics in a prevalent lipoprotein phenotype, called atherogenic lipoprotein phenotype (ALP), involving at the same time an increase in VLDL-triglycerides and apolipoprotein B, and decrease in HDL-cholesterol and apolipoprotein A-I. Also, within such a disturbance in the metabolism of triglyceride-rich lipoproteins, the preponderance of small dense LDL particles has been established, which, despite a reduced cholesterol content, are highly atherogenic (3, 4, 9, 10).

Cholesterol hypothesis and paradox

Atherosclerosis is a degenerative disease characterized by the development of fibrolipid deposits on the interior walls of the large and medium arteries. The origin of cholesterol hypothesis can be probably related to the recognition of the yellowish fatty substance in atheromatous lesions in rats about 90 years ago (11), much before chemical identification of cholesterol. It was found that cholesterol on the arterial walls originated from the circulating lipoproteins, primarily LDL particles, and that it was deposited in the amounts that are directly proportional to its serum concentrations (12).

Convincing proofs supporting this hypothesis have been obtained later on the basis of extensive epidemiological studies that established the existence of a continual positive relationship between the serum cholesterol and mortality caused by coronary heart disease (13). These findings confirmed the previous observations of Keys (14) of the existence of a strong relationship among the increased dietary intake of saturated fatty acids, increased level of serum cholesterol and ischaemic heart disease. At that time, this hypoth-

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* Invited paper presented on 13th Congress of Medical Biochemistry and Laboratory Medicine, May 14 18, 2002, Niš, Yugoslavia

esis was strongly supported by two important discoveries. First, it was the Nobel-prize discovery of specific cell receptors of LDL particles, opening new therapeutic prospects (15), and second, the discovery of oxidative modification of LDL particles as a main prerequisite for its reception and deposition of its cholesterol on the arterial wall (16). Finally, this hypothesis has withstood a rigorous test of therapeutic studies confirming that the lowering of serum cholesterol level in individuals with ischaemic heart disease as well as those with hypercholesterolaemia disturbances, reduces the level of coronary risk (17, 18).

Hence, it was surprising that the interpretation of the results of the famous epidemiological prospective Framingham study showed that the concentration of serum cholesterol was not a good discrimination parameter to assess the risk of coronary heart disease (19). Besides, numerous therapeutic studies came to the conclusion that the degree of reduction of coronary risk is also dependent on the level of serum triglycerides (18, 20, 21). After being faced with this cholesterol paradox, researchers became reinterested in triglycerides.

Confusions about the atherogenicity of triglycerides

For years triglycerides have been pushed at the back of cholesterol, despite of the fact that numerous extensive epidemiological studies provided proofs of an unquestionable interrelation of the level of serum triglycerides, hypertriglyceridaemia prevalence and premature atherosclerosis, irrespective of the blood cholesterol level (6 9), and despite the fact that hypertriglyceridaemia was more frequent than isolated hypercholesterolaemia, not only in individuals with hyperlipoproteinaemia but also in those suffering from ischaemic heart diseases (1). The reason for neglecting triglycerides was a consequence of impossible of recognition of metabolic basis for explaining atherogenic potential of triglycerides. Therefore, the occurrence of premature atherosclerosis in individuals with increased levels of serum triglycerides has been mainly explained by an indirect mechanism a simultaneous existence and action of non-lipid atherogenic factors, effects on the haemostatic system, and most frequently, by low amounts of protective HDL cholesterol (5, 6).

Of decisive importance for the affirmation of serum triglycerides as an independent risk factor of premature atherosclerosis were strong proofs obtained in 1996 on the basis of a meta-analysis of 17 prospective population studies (22). This finding finally led to the acceptance of the existing knowledge and recognition of the fact that the atherogenic potential of triglycerides can be related primarily to the heterogeneity of LDL particles.

Structural heterogeneity of LDL lipoproteins

LDL particles, formed in circulation as an end catabolic product hepatic VLDL lipoproteins, are the main carriers of cholesterol to the peripheral tissues, transferring it to them by incorporation through the specific LDL receptors (15). These particles are spherical macromolecules that contain a hydrophobic core of cholesterol esters and triglycerides, surrounded by an amphipathic envelope of phospholipids, free cholesterol, and one molecule of apolipoprotein B-100 (23).

Absolutely there is no doubt that the risk of the development of premature atherosclerosis is directly related to the total concentration of LDL particles, and this concentration can best be assessed by determining their content of cholesterol or apolipoprotein B-100 (4, 23).

However, it has been recently established that apart from this simple quantitative concept, there is also a more subtle relationship involving the presence of LDL subpopulations, i.e. a spectrum of particles in the density range from 1.019 to 1.063 g/mL and dimensions of 20 30 nm, which are also heterogeneous in respect to the degree of their flotation, molecular mass, chemical composition, and qualitative characteristics, i.e. the atherogenic potential (23).

The methods of characterizing LDL particles distribution are: gradient gel electrophoresis (GGE), density gradient ultracentrifugation (DGUC), high-performance gel-filtration chromatography (HPGC), electron microscopy (EM), dynamic light scattering, secvential ultracentrifugation, nuclear magnetic resonance spectroskopy.

Using various methods based on the determination of the size, density and flotation degree (24 26), it was possible to distinguish seven subpopulations. However presently a common classification is accepted: light, large, floating LDL-I particles of an average diameter of 27 nm; intermediate LDL-II particles of a diameter of 26.6, and small, dense LDL-III particles of a diameter of 26.0 nm (1, 2, 23 28). Predominance of the large, floating LDL-I particles, designated as phenotype A, has been found in about 65 % of adult population, the phenotype of intermediate LDL-II particles in about 10 %, whereas the incidence of phenotype B, i.e., the predominance of small, dense LDL-III particles in the overall population of 25 44 % (2, 27, 29).

The qualitative differences, i.e. the diversity of their chemical composition, bring us close to the clinical significance of LDL subfractions. Thus, large LDL-I particles, which are predominant in healthy persons, carry more cholesterol, about 3000 molecules. Small, dense LDL-III particles, which predominate in individuals with premature atherosclerosis and different states characterized by hyperinsulinaemia and insuline resistance, contain less cholesterol (about 2000 mole-

cules) and phospholipids, but more triglycerides (2, 25, 28 30). In view of the fact that all subfractions, irrespective of the particle size, contain one molecule of apolipoprotein B-100, these small, dense LDL particles are characterized by a reduced ratio of cholesterol and apolipoprotein B (31).

Determination factors and clinical significance of small dense LDL

Numerous studies that have been carried out over the last two decades were directed to the determination of factors and clinical significance of LDL heterogeneity. Studies of healthy families, families with combined hyperlipidaemia and twins, have shown that there is still the effect of an unidentified single major gene, with dominant or additive mode of inheritance, whose prevalence in general population is about 30 % and with the simple allele frequency of phenotype B. The expression of this phenotype is more pronounced in males, increases with aging, and is influenced by non-genetic factors that are related to complex changes in the metabolism of triglycerides (25, 32).

A higher incidence of phenotype B LDL subfractions in the states of hyperinsulinaemia, insuline-resistance syndrome, glycose intolerance, insulin-independent diabetes, as well as in central obesity and low-fats-high-carbohydrates diet, could indicate the potential influence of hormonal and some environmental factors, exhibiting a significant regulatory effect of concurrent complex changes on the metabolism of triglyceride-rich lipoproteins (28 30).

Additional data related to the determination of factors of the phenotype of small, dense LDL were also obtained from the studies about the clinical importance of this subfraction with lower cholesterol content compared to the dominant LDL-I subfraction. A dozen of extensive epidemiological studies reported a higher frequency of phenotype of LDL-III particles in individuals with myocardial infarction and in angiographically-documented ischaemic heart disease (25). First prospective studies yielded that the presence of phenotype B LDL subfractions foreshadow the appearance of coronary heart disease, not as an independent risk factor but as one of the manifestations of complex changes in lipoproteins metabolism that can be due to certain genetic, hormonal, and environmental factors, whereby there is a strongest determining influence on the concentration of serum triglycerides. These findings explain the variations between 30 and 60 % of LDL value in different studies (33 35).

Lipoprotein cascade in normotriglyceridaemia

VLDL particles, serving as a vehicle for transportation of endogenous triglycerides from the liver to peripheral tissues, are relatively small under the conditions of normotriglyceridaemia, and have an appropriately small content of triglycerides. They exhibit a tendency of diminution, which takes place through the intravascular hydrolytic degradation of triglycerides under the action of lipoprotein lipase, accompanied with a simultaneous pushing on the cholesterol esters to the macromolecule centre, involving the enzyme lecithin: cholesterol acyl transferase (LCAT). At the same time, the detachment of fragments of the surface envelope, and of apolipoproteins of classes C and A, together with phospholipids and cholesterol esters takes place, which are then incorporated into the structure of HDL lipoproteins.

In the course of this delipidation cascade, a continuous conversion of VLDL through the three subfractions of different sizes and density, and through a stage of transitory lipoproteins of intermediate density, yields a decline in efficiency of lipoprotein lipase and increase in efficiency of hepatic lipase, which, by additional degradation of triglycerides, ensures final transformation of IDL to cholesterol-rich LDL particles that are being recognized by their specific receptors (2, 5, 6).

Lipoprotein cascade and reciprocal lipid transfer in hypertriglyceridaemia

Under the conditions of hypertriglyceridaemia, however, VLDL particles are relatively large and carry accordingly more triglycerides, so that they remain longer in circulation. By some very complex mechanisms involving cholesteryl ester transfer protein (CETP), LCAT enzyme, and hepatic lipase and, according to the most recent findings, with HDL particles playing the main role, a reciprocal transfer of different lipid components between the triglyceride-rich lipoproteins on the one hand and cholesterol-rich lipoproteins on the other, takes place. This is accompanied by significant modifications of not only chemical and physical but also of biological properties of lipoproteins.

From VLDL particles and chylomicron remnants, triglycerides are transfered first to HDL and then to LDL, whereas to the opposite direction cholesterol esters and phospholipids are transfered. By forming cholesterol esters and pushing them to the core of HDL particles, LCAT bound to these particles releases them from the surface lipids, making them more suitable for the acceptance of new amounts of lipids of the surface envelope of LDL particles within their further remodelling.

As a consequence of these reactions LDLs are converted to a subpopulation that is rich in triglycerides and poor in phospholipids and free and esterified cholesterol, to be finally modified to small, dense LDL subfractions with the participation of hepatic lipase, which performs hydrolysis of transfered triglycerides (1, 23, 36).

Atherogenic potential of triglycerides and the mechanisms of LDL-III atherogenicity

It is now accepted that this reciprocal lipid transport, i.e. the characteristic bridging between the lipoprotein classes whose role is to transfer triglycerides to the periphery and the particles enabling transfer of cholesterol from the liver and peripheral tissues, is responsible for the majority of the effects of triglicerzdes. Thus, the formation of VLDL takes place and chylomicron remnants carry huge amounts of cholesterol esters, so that when these particles are being taken over by non-specific receptors on the macrophages, cholesterol reaches the arterial walls, where it is deposited in the form of atheromatous plaques (1).

Thereafter, subpopulations are formed of small, dense HDL particles with a higher content of triglycerides and lower contents of cholesterol esters. As they are quickly removed from circulation, their availability for participation in the reversible transport of cholesterol from the periphery to the liver is diminished, i.e. they lose a major part of their cardioprotective effect (1).

Finally, small, dense LDL subpopulations to which an extremely high atherogenic potential is ascribed are formed. In comparison to LDL particles isolated from the subjects with familial hypercholesterolaemia, whose affinity for binding to LDL receptors is similar to that of LDL particles from the subjects with normolipidaemia, the LDL-III subfractions present in subjects with hypertriglyceridaemia exhibit a lower affinity of binding to their specific receptors; this might probably be a consequence of structural changes yielding also to changes in the arrangement of apolipoprotein B-100 epitope. The degree of reduction of the affinity of LDL receptors is proportional to the degree of hypertriglyceridaemia (23, 25, 37).

Because of evident removal from blood, the conditions are created for potentiating the interaction of these LDL subpopulations with the arterial walls. Thus, they can much easier penetrate to arterial intima and remain longer there, probably because of the lowered content of neutral carbohydrates. Afterwards, they are increasingly bound to subendothelial proteoglycans, and hence they exhibit an increased proneness to oxidation compared with the larger LDL subpopulations, which, however, might also be a consequence of a lower content of antioxidants, or could be related to the reduced mass of free cholesterol. This phenomenon is also related to plasmatic levels of triglycerides (23, 25, 27).

Apart from contributing significantly to the formation of foam cells, LDL-III subpopulations contribute to the appearance of endothelial dysfunction through the inhibitory effects on endothelium-dependent vasodilatation and synthesis of nitrogen monoxide, as well as selective induction of the expression of adhesive molecules (10).

Atherogenic lipoprotein phenotype

However, despite the fact that a number of atherogenic mechanisms have been proposed, small, dense LDL particles are not an independent risk factor responsible for the development of premature atherosclerosis. However their presence is a primary indication for the existence of a wider metabolic disturbance that encompasses also hypertiglyceridaemia, increase in VLDL triglycerides and apolipoprotein B, and decrease in HDL cholesterol and apolipoproteins A-I. Such a constellation of abnormalities of lipids, lipoproteins and apolipoproteins might be a marker of a subordinate physiological process which yields to an increased risk of premature atherosclerotic development through some multiple mechanisms, which are not mutually exclusive but contrary, they act synergistically. Hence, we can rightly talk about the atherogenic lipoprotein phenotype (3, 9, 10, 25, 28, 29).

In the expression of phenotype B LDL subfractions and determination complex changes in the metabolism of lipids and lipoproteins that lead to a classical presentation of the atherogenic lipoprotein phenotype, a central place belongs to the serum concentration of triglycerides, which controls the rate of the reaction of reciprocal lipid transfer that results in remodelling of lipoproteins (1, 3).

According to a number of researchers, the serum triglyceride concentration that would be safe in respect of establishing the atherogenic lipoprotein phenotype, is below the desirable level of 1.7 mmol/L. It has even been suggested that it is possible that there is no a concentration threshold, and that is desirable to have the lowest possible concentrations (1).

Clinical significance of the atherogenic lipoprotein phenotype and LDL-III

In view of the fact that in individuals with coronary heart disease the frequency of hypertriglzceridaemic state is higher compared to that of isolated hypercholesterolaemia (5 7), it is probable that the clinical significance of atherogenic lipoprotein phenotype exceeds that of LDL cholesterol. The elevated risk of the development of premature atherosclerosis in this lipoprotein phenotype is a consequence of a characteristic distribution of LDL subfractions. As the triglyceride concentration increases and HDL decreases, the distribution of LDL shifts towards the atherogenic subfraction of small, dense particles. The combined hyperlipidaemia is additionally characterized by an increase in LDL cholesterol, which almost entirely circulates in the most atherogenic of the three LDL subfractions; this is in contrast with LDL cholesterol observed in familial hypercholesterolaemia that is mainly bound to particles of intermediate density and dimensions (1).

Furthermore, what contributes most to the extreme clinical significance of this atherogenic phenotype,

is the fact that this phenotype is an integral part of metabolic characteristics of abdominal obesity, insulin-independent diabetes and other insulin-resistant states (28 30). Similar atherogenic changes in physicochemical properties have also been observed in postprandial period. This phenomenon is independently predictive of coronary heart disease (38). Finally, the first completed therapeutic studies have shown that in individuals with predominant small, dense LDL, there is a greater benefit of applying an intensive programme of reducing coronary risk, because the conversion to higher floating LDL particles is accompanied by favorable angiographic changes, i.e. regression of the coronary disease (39, 40).

Repercussions on the routine laboratory diagnostics

Would it be justified to have, in the near future, routine determination of LDL subfractions? If we would make a comparison with the relationship between the total and LDL cholesterol amounts, we could conclude that there would be a need for determination of LDL subclasses because their different distribution in the persons with the identical concentrations of LDL cholesterol may lead to significant differences in respect of the risk of coronary heart disease. The presently available methods are, however, very involving, technically complex, and time-consuming (24 26). Alternatively, there is a possibility of calculating the ratio of cholesterol and apolipoprotein B, as an essential characteristic of small, dense LDL subfractions, using relatively simple formulas (31).

It is possible that the answer to the above question will be obtained when simple and fast methods, suitable for serial work, become available to routine practise, like the recently described NMR spectroscopic method enabling quantification of 15 different subclasses, 4 for LDL, and obtaining for each subject the so-called »lipoprofile«, which, in addition to quantitative data, indicates the concentrations of individual subclasses in the form of graphs (26).

PATOFIZIOLOGIJA I KLINIČKI ZNAČAJ ATEROGENOG LIPOPROTEINSKOG FENOTIPA I LDL ČESTICA MALE GUSTINE

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Kratak sadržaj: I pored snažnih dokaza u prilog holesterolske hipoteze, koncentracija serumskog holesterola nije dobar diskriminacioni parametar za procenu rizika koronarne srčane bolesti. Stepen redukcije koronarnog rizika zavisi i od nivoa serumskih triglicerida. U okviru poremećaja u metabolizmu lipoproteina bogatih u trigliceridima, naime, tokom delipidacione kaskade ostvaruje se recipročni prenos lipida koji dovodi do karakterističnog remodeliranja svih klasa lipoproteina i uspostavljanja tzv. aterogenog lipoproteinskog fenotipa (povišenje triglicerida, LDL male gustine i apolipoproteina B, a sniženje HDL holesterola i apolipoproteina AI). Glavnina aterogenog potencijala ovog fenotipa vezana je za povećanje broja LDL male gustine (fenotip B), ali ne zbog doprinosa serumskom holesterolu, već usled njihovog nižeg afiniteta za LDL receptore, lakšeg penetriranja u intimu arterija, dužeg zadržavanja u subendotelu, ubrzane oksidacije, promptnog preuzimanja od strane makrofaga i uspostavljanja endotelne disfunkcije.

Ključne reči: lipoproteini bogati u trigliceridima, LDL male gustine, postprandijalni metabolizam, koronarna arterijska bolest

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Received: December 10, 2002

Accepted: February 15, 2003