

## LIPIDS AND ATHEROSCLEROSIS

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**Summary:** Lipids play a pivotal role in the atherogenesis, starting from initial changes. Their predictive value in coronary heart disease and development of novel therapeutic strategies is an increasingly addressed issue nowadays. LDL particles are fundamental, because reduction of LDL-cholesterol was proven to reduce morbidity and mortality associated with atherosclerosis. Besides the regulation of LDL-receptor expression, significant clinical importance is assigned to oxidized LDL (ox-LDL) and small dense LDL (sdLDL) that are generated through intravascular remodelling of triglyceride-rich lipoproteins, while the exact role of anti-oxLDL antibodies in atherosclerosis propagation and their clinical significance still remain unclear. In recent years, however, better understanding of the basic mechanisms involved in atherosclerosis development, as well as in the metabolic fate of lipids and lipoproteins, emphasizes the crucial role of other lipoprotein particles not only in the propagation, but also in the initiation of atherosclerosis and atherothrombosis.

**Key words:** lipids, lipoproteins, atherosclerosis

### Introduction

Lipids are important structural and bioregulatory components of human cells and plasma lipoproteins, representing their transporting circulating form. Plasma concentrations of major blood lipids are determined by the regulation mechanism of their cell synthesis and by complex metabolic reactions within their transportation pathway in the body. Predictive value of lipids on coronary heart disease (CHD) is an increasingly addressed issue nowadays, considering the pivotal role they play in atherogenesis, including initial changes, as well as development of complicated atherosclerotic lesions (1–5).

Atherosclerosis is a multifocal, smoldering, immunoinflammatory disease of the arteria undergoing gradual lipid deposition and intima thickening, resul-

ting in elasticity decrease, lumen occlusion and reduced blood flow (4, 5). Such a diffuse process may start early in childhood and progresses asymptotically through adult life as a relatively benign disease that is rarely fatal by itself (2). The most devastating complications of atherosclerosis, ie. life-threatening clinical events, such as acute coronary syndrome and stroke, are provoked by arterial occlusion precipitated by atherosclerotic plaque erosion or disruption with superimposed thrombosis. This process is defined as atherothrombosis (2, 3, 5).

Development of atherosclerosis involves multiple metabolic and cellular processes (1, 6). It is generally considered a chronic inflammatory disease developing as a response to endothelial activation and dysfunction that leads to progressive vasoconstrictive impact and proinflammatory, prothrombotic, procoagulative and proliferative processes in the vessel wall, ie. initiates the sequence of successive reactions prompting composition of atherosclerotic plaque (7).

Disruption and dysfunction of the endothelium may be the consequence of different factors, generally considered risk factors; however, central to this are dyslipoproteinemiae, particularly elevated LDL

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cholesterol levels (1,8,9). Over the past few years, better understanding of the basic mechanisms involved in atherosclerosis development and metabolic fate of lipids and lipoproteins has emphasized the vital role of other lipoprotein particles, not only in the progression, but also in the initiation of atherosclerosis and atherothrombosis. However, currently there is clear evidence only on the positive correlation between reduced level of LDL-cholesterol and decreased CHD morbidity and mortality (9, 10).

### Low-density lipoprotein (LDL)

LDL is the primary plasma lipid carrier with a single apolipoprotein (apo) molecule B-100 per one particle. Apo B-100 is a 500 kD peptide chain, one of the largest known monomeric proteins, highly insoluble in aqueous environment, which, contrary to other apo, is not substitutable by other lipoprotein particles. It is of hepatic origin, reaching the circulatory system via the very-low-density lipoproteins (VLDL), which constitute LDL through the process of gradual intravascular remodelling (4).

LDL are highly atherogenic particles that, dominantly over other lipoproteins, favour cholesterol accumulation in foam cells produced by macrophages. Abundant epidemiological and clinical studies have consistently demonstrated the causal relationship between LDL-cholesterol and atherosclerosis. Elevated LDL cholesterol is clearly identified as the primary target of lipid-lowering therapy for reducing CHD risk in the report of National Cholesterol Education Program (NCEP) (1, 8, 9). Of utmost clinical importance are modified forms of LDL, mainly oxidized LDL (ox-LDL), as well as LDL subfractions, primarily LDL-III or small dense LDL (sdLDL), which are products of intravascular remodelling of triglyceride-rich lipoproteins (TRL) (4, 11).

Plasma concentrations of LDL-cholesterol are primarily defined by the liver that is at the same time the site of formation and production of precursor lipoproteins, as well as the tissue abundant with LDL-receptors (LDL-R), which are a key component in maintaining the cholesterol homeostasis in the body through apoB-100 and apoE-lipoprotein particles clearance (10). Moreover, by the autoregulation mechanisms, LDL-R protect cells from excessive cholesterol efflux from plasma. Expression of LDL-R is predominantly regulated at the transcription level by the pool of intracellular cholesterol acting through the negative feed-back mechanism, including sterol responsive element binding proteins (SREBPs) as the transcription factors, and SREBP cleavage activating protein (SCAP) and insulin-induced gene (Insig) that play a role in the maturation process of SREBPs (10). The expression of LDL-R is regulated by genetic, environmental and hormonal factors (1). Atherogenic elevation of LDL-cholesterol level, thus, may be the

result of genetic defects related to the number or functionality of LDL-R or to the ligand-binding region of apo B-100 (familial hypercholesterolaemia). It is also associated with other genetic, hormonal or environmental factors inducing increased production and/or reduced clearance of these particles (1, 8, 10).

It has been hypothesized that LDL promotes atherosclerosis through complex inflammatory and immune mechanisms leading to lipid dysregulation and the formation of foam-cells, as well as to the maintenance of the vascular inflammation cycle and creation of a prothrombotic state of the endothelium that later contributes to complications in advanced stadiums of atherosclerosis and atherothrombosis. In that respect, oxidatively modified LDL is of highest clinical importance (4, 6, 12).

The subendothelial space, where LDL particles are less protected by antioxidants and more frequently exposed to various cell-derived oxidants, particularly in conditions of chronic oxidative stress, is the presumed site of LDL oxidation *in vivo*. The bidirectional transit of LDL across this space may result in a small amount of circulating oxidized LDL, which is significantly larger in cases of acute coronary syndrome and angiographically documented CHD (3, 4, 6). Because of the modification of lysine residues at apo B-100, oxLDL is not recognized by specific LDL-R, and they are avidly uptaken by macrophage scavenger receptors, including scavenger receptors A (SR-A) and B (SR-BI), CD36, CD68, lectin like oxLDL receptor (LOX-1) and scavenger receptor binding to phosphatidylserine (SR-PSOX) (4, 6). Lipid overladen macrophages transform to foam cells. At the same time, lipid oxidative products of ox-LDL through the ligand activation of peroxisome proliferator-activated receptors gamma (PPAR $\gamma$ ) regulate the gene expression in macrophages, such as SR-A and CD36, and some proinflammatory cytokines (13). Oxysterols may also promote the formation of foam cells in the atheroma by inhibiting the efflux of cholesterol induced by apo A-I (14). Discovery of the LOX-1 receptor in endothelial cells (EC) clearly indicated that oxidation of LDL, as an early, essential event in atherosclerosis, which induces endothelial dysfunction, may well contribute to the initiation of atherosclerosis (4, 6, 15). Proatherogenic and proinflammatory characteristics of ox-LDL are summarized in *Table 1*.

The immunogenicity of ox-LDL and modified low-density lipoprotein (mLDL) has been demonstrated both in humans and laboratory animals. Oxidation of LDL generates a wide spectrum of oxidatively modified lipids and proteins, which are highly immunogenic neoepitopes that provoke strong autoimmune response in the experimental model. Purified circulating human mLDL antibodies are predominantly an IgG isotype of the proinflammatory subclasses 1 and 3, which primarily react with malondialdehyde-lysine and carboxymethyl-lysine epitopes, but also

Table I Modifications of compositional and functional properties of HDL induced by oxidation (ox-HDL), glycation (Gly-HDL), homocysteinylation (Hcy-HDL) and tyrosylation (Tyr-HDL) (39–45)

Alteration	ox-HDL	Gly-HDL	Hcy-HDL	Tyr-HDL
Lipids: Levels of lipid peroxidation indexes	↑	↑		↑
Apolipoproteins: Denaturation/formation of dimers or heterodimers	↑	–	–	↑
Exposition of apoprotein Trp residues	↓	↓	–	–
Apoprotein-SH groups	–	–	↑	–
Physico-chemical properties: Molecular order	↓	↓	–	–
Fluidity	↑	↑	–	–
Cholesterol efflux	↓	↓	–	↑/↓
Cytotoxic effect	↑	↑	–	–
Protection against LDL peroxidation	↓	–	–	–
Enzymes activity: Paraoxonase	↓	↓	↓	–
LCAT	↓	↓	–	–
CETP	–	–	–	–
Antiinflammatory properties	↓	–	–	–

Legend: ↑, increase; ↓, decrease; LCAT, lecithin: cholesterol acyltransferase; PLTP, phospholipid transfer protein; CETP, cholesterol ester transfer protein.

recognizing minimally modified LDL (mmLDL) that do not contain significant amounts of those two epitopes. Contrary to that, some results of studies in animals suggest that ox-LDL antibodies are anti-atherogenic IgM (4, 12). Studies employing ox-LDL antibodies, advanced glycosylation end products-LDL (AGE-LDL), malondialdehyde-LDL (MDA-LDL) and carboxymethyl-LDL (CML-LDL) obtained by rabbit immunization revealed that principal modified forms of LDL exist in circulation as soluble immune complexes (IC). Data obtained by affinity chromatography of the isolated human circulating LDL-IC and antibodies to modified LDL primarily suggest their proinflammatory potential, which is supported by higher IgG concentration and enrichment in IgG1 determined in patients with diabetic nephropathy. Isolated, the antibodies exhibit even higher avidity (12). Several prospective studies in patients with type 1 diabetes revealed that LDL-IC levels significantly correlate with the development of CHD, even as predictors of disease progression (12).

Some recent investigation suggested that ox-LDL builds complexes with beta-2-glycoprotein I ( $\beta_2$ GPI) and/or C-reactive protein (CRP) in the intima of atherosclerotic lesions. Animal IgG autoantibodies to ox-LDL/ $\beta_2$ GPI significantly increase *in vitro* uptake of this complex by the macrophages, exhibiting the

proatherogenic effect, while natural IgM anti-oxLDL antibodies are proposed to be protective (4). CRP bound to ox-LDL enhances its binding to macrophages via the Fc $\gamma$  receptors. In diabetes patients with atherosclerotic cardiovascular disease, CRP/ox-LDL/ $\beta_2$ GPI complexes were detected (4).

High titer of anti-mLDL antibodies is detected in blood and atherosclerotic lesions of humans and animals. Epidemiology studies indicate strong correlation between their titer and standard risk factors, as well as some clinical markers of atherosclerotic disease severity. Nevertheless, their exact clinical significance still remains unclear (4, 6, 12).

### Lp(a) lipoprotein

Lp(a) lipoprotein is a genetically determined variety of LDL particles, generated by intravascular linking of the hepatic apo (a) at two different ends of the spherical LDL particle by a disulfide bridge between apo B-100 Cys 3734 and apo (a) kringle IV type 9 Cys 67. Structural and metabolic heterogeneity of Lp(a) relies mostly on quantitative polymorphism in the inner sequence of the apo (a) gene located on the chromosome 6 (6q26-27) in the region of plasminogen gene, towards which it exhibits pronounced structural similarity and immune cross-

reactivity. The paramount difference is the substitution of arginine by serine in the sequence Arg 560-Val 561 in kringle V of plasminogen, depriving apo (a) of most of its plasminogen's enzymic properties (16).

Nowadays, it seems likely that that Lp(a) offers an evolutionary advantage to the human species as an essential component of tissue reparation. It behaves as an acute-phase reactant, its plasma concentration significantly increasing after inducing different forms of acute phase response *in vivo* (17). Presence of apo (a) enhances its prompt binding to the broad variety of receptors at the surface of EC, monocytes, fibroblasts, thrombocytes and smooth muscle cells, particularly in the presence of defensin released from neutrophil granulocytes (17, 18). Furthermore, it binds to several components of the vascular wall and to sub-endothelial matrix, partly mediated by the lysine binding sites (LBS) on its apo (a) (17). Binding to fibronectin, fibrinogen, glycosaminoglycans and proteoglycans is determined by the presence of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions, and weaker interactions with laminin and beta-2 glycoprotein I were also observed (17, 19). Substantial amounts of cholesterol carried by Lp (a) can readily be extracted and used at the site of its accumulation.

However, these positive biological features may concurrently represent key mechanisms in promoting atherothrombosis, which is probably highly determined by the polymorphism of apo (a) (20). Lp (a) immunoreactive material was detected in the arterial vascular wall, and the amount of deposition is significantly related to the extent of atherosclerosis. Lp (a) could also be detected in growing atherosclerotic plaques and vein grafts, and accumulation of apo (a) was most likely preferential to that of other apolipoproteins (21).

The atherogenic potential of Lp(a) particles is undisputed. At the site of vascular damage, they can bind to a wide spectrum of cellular receptors and other unrecognized endosomal membrane proteins, and cellular uptake and degradation are enhanced by lipoprotein lipase and oxidative modification of the particles (16). The affinity towards TRL and LDL particles, particularly ox-LDL (6), and strong molecular interactions with some constituents of sub-endothelial matrix, further intensify the catabolism of Lp(a) via the alternative, but still unclear pathways, inducing an accelerated internalization and degradation of cholesterol-rich lipoproteins. Some recent biological research revealed that Lp(a) exhibits some growth factor-like characteristics for some vascular cells *in vitro* (16, 22, 23), initiates monocyte chemotaxis (24), alters the expression of intercellular adhesion molecule 1 (ICAM-1) and selectin in cell culture (23), leading to selective decrease in vasodilator capacity of receptor-mediated endothelial stimuli (25).

Moreover, thrombogenic potential of Lp(a) lipoprotein particles is quite likely. There is some clear

evidence that they attenuate plasminogen activation into plasmin induced by streptokinase as an exogenous activator, most likely by binding to streptokinase and inhibiting its binding to plasminogen, or by binding to the streptokinase-plasminogen complex, reducing its enzymatic activity (26). It is well established that Lp(a) interacts as a competitive inhibitor with the tissue-type plasminogen activator (t-PA) for binding with fibrinogen fragments in the presence of plasminogen. It binds directly to t-PA, inhibiting plasminogen activation (27), and inhibits secretion of t-PA from human EC (28). Furthermore, Lp(a) plays a role as a competitive inhibitor of plasminogen binding to plasminogen receptors in EC and blood cells. This competitive inhibition is established at the level of binding to tetranectin, the protein enhancing plasminogen activation (22, 29). It has been suggested that Lp(a) also binds to thrombospondin that, combined into the complex with plasminogen, accelerates its transformation into plasmin. Subsequently, Lp(a) possibly inhibits activation of the transforming growth factor-beta (TGF- $\beta$ ) by inhibiting formation of plasmin that is essential for its activation (22). Particularly in its oxidized form, the Lp(a) enhances endothelial synthesis and secretion of plasminogen activator inhibitors (PAI-1) *in vitro* by more than twofold (25). Finally, it is assumed that Lp(a) may remove the tissue factor pathway inhibitor (TFPI) from the surface of the endothelial cells, enhancing the activation of IX and X coagulation factor via the tissue factor-factor VII complex. Some preliminary research of these phenomena *in vivo* measuring the fibrinolytic parameters suggest that thrombolytic activity is less effective in individuals with elevated Lp(a) levels, which is primarily due to the attenuated fibrinogen and/or fibrin mediated increase of plasminogen activation by endogenous t-PA (30).

Lp(a) lipoprotein thus offers molecular explanation for the link between atherogenesis and thrombogenesis. It is likely that after endothelial injury occurs, the acute-phase response, concomitantly triggered by the cellular release of several mediators, involving primarily interleukin 6 (IL-6), stimulates the hepatic synthesis of newly-formed apo (a) molecules, probably followed by producing the Lp(a) complex in the blood stream. Subsequently, Lp(a) accumulates at the site of the vascular injury binding to cellular receptors, to the exposed sub-endothelial matrix and to immobilized fibrin, and most probably inhibits plasminogen binding and plasmin formation, thus inhibiting the lysis of the clot. Intensity of the binding of apo (a) to stabilized fibrin is increased in the presence of homocysteine and other sulfhydryl compounds, which might explain a reliable synergy between Lp(a) and homocysteine in the genesis of thrombotic disorders (31). Simultaneously, Lp(a) attenuates activation of TGF- $\beta$ , resulting in the migration and proliferation of smooth muscle cells into the vascular intima consequently to endothelium activation and expres-

sion of proinflammatory cytokines and adhesive molecules, which might be essential in the development of acute ischemic syndrome (23). At the same time, its growth factor-like properties promote vascular repair, and cell regeneration is ensured by the substantial amount of cholesterol carried by the lipoprotein. Through these mechanisms Lp(a) contributes to the stability of fibrin deposits, favouring cholesterol deposition at the sites of vascular damage and formation of atherosclerotic plaque.

Considering the well-established association between this atherogenic-thrombogenic particle and premature atherosclerosis, epidemiological and clinical surveys have characterized the Lp(a) as the most atherogenic of all lipoproteins; however, prospective studies conducted so far have clearly demonstrated the vital dependence on the LDL-cholesterol level (16, 20).

### High-density lipoproteins (HDL)

The HDL particles are composed of an outer amphipathic layer of free cholesterol, phospholipid, and several apolipoproteins, and of a triglyceride and cholesterol ester hydrophobic core. The particles also carry enzymes, such as paraoxonase (PON), platelet activating factor-acetylhydrolase (PAF-AH), lecithin: cholesterol acyltransferase (LCAT), and cholesteryl ester transfer protein (CETP) (32, 33).

It is well known that cholesterol concentration in HDL is an inverse predictor of future atherosclerotic cardiovascular disease. Abundant experimental evidence reveal that increasing the levels and/or function of HDL can have strong vascular protective effects, ranging from prevention to stabilization and regression, independent of total or non-high-density lipoprotein cholesterol (HDL-C) (1, 8, 9, 33, 34).

HDL accomplishes its basic antiatherogenic function by reducing cholesterol accumulation in the artery wall by uptaking it and transporting it back to the liver for removal from the body. Within the pathway of reverse cholesterol transport, the apo A-I, mainly synthesized in the liver, is released in the plasma, where it interacts with serum phospholipids and forms nascent discoidal HDL (ndHDL). The ndHDL initiates cholesterol release from the macrophages and fibroblasts in the subendothelial space, ie. its translocation into the extracellular space, the process known as cholesterol efflux. It encompasses 5 independent pathways, including ATP-binding cassette transporter-1 (ABCA1) playing a major role, SR-B1, caveolin, sterol 27-hydroxylase (CYP27A1), and passive diffusion. Major acceptors of the externalized cholesterol are apo A-I and ndHDL. Since maintaining of a concentration gradient is accomplished by cholesterol etherification by the LCAT and displacing of esters into the hydrophobic core, gradual particle enlargement and converting to the HDL3 and HDL2

occurs (32,35). Phospholipid transfer protein (PLTP) plays an important role in the conversion of HDL by accelerating *in vivo* efflux of phospholipids released from the TRL in the process of their lipolysis by lipoprotein lipase (LPL), which also triggers the process of particle fusion (36). Hepatic lipase (HL) is involved in HDL conversion by hydrolyzing triglycerides obtained from apo B-100 through the equimolar exchange with cholesteryl esters facilitated by CETP. The cholesterol uptaken from the peripheral tissues is then delivered back to the liver by the HDL, converted to bile salts, and eliminated through the gastrointestinal tract, either directly by SR-B1, or indirectly via the LDL-R (35).

Possible cardioprotective mechanisms of HDL are attributed to its antioxidative, antiinflammatory and antithrombotic activity (32, 37, 38). The antioxidative activity of HDL, ie. protecting the LDL particles from oxidation and reducing the activity of oxidized forms of LDL, is achieved through the apo A-I and enzymes, primarily PON-1, which prevent oxidation of LDL phospholipids, hydrolyze generated lipid peroxides, inhibit monocyte transmigration and transformation induced by ox-LDL in cell cultures, stimulate prostaglandin production in EC culture and enhance decreased endothelium-dependent relaxation by removing lysophosphatidylcholine from the ox-LDL (32, 37). Antiinflammatory function comprises inhibition of monocyte chemoattractant protein-1 (MCP-1) production in the EC, and limiting the expression of cytokines such as tumour necrosis factor- $\alpha$  (TNF- $\beta$ ) and IN-1 that are important regulators of expression of leukocyte adhesive molecules in EC (32).

Though primarily atheroprotective, a paradoxical change of its functional properties may occur by HDL modification in some individuals and/or situations, including small dense HDL (sdHDL) formed in the process of intravascular remodelling of TRL (32, 35, 39).

Several surveys demonstrated that HDL is rapidly modified to the oxidized HDL (ox-HDL) *in vitro* by a variety of oxidants. It is assumed that interstitial fluid of inflamed arterial intima are the major site of HDL oxidation *in vivo*, mediated by the phagocyte myeloperoxidase inside the growing atheroma (40–42). In conditions of hyperglycaemia, non-enzymatic glycation of HDL modification to the glycated HDL (Gly-HDL) that exhibits high susceptibility to oxidation (Gly-ox-HDL) occurs (43). Some recent research revealed the homocysteinylation of HDL to Hcy-HDL at incubation of HDL with homocysteine-thiolactone (44), as well as the tyrosylation of HDL to Tyr-HDL influenced by the effects of tyrosyl radicals generated by the activated human phagocytes (45). HDL is highly susceptible to structural modifications mediated by various mechanisms that, besides some structural and compositional alterations in lipids and

Table II The similarity of proatherogenic and proinflammatory properties of oxidized LDL (Ox-LDL) and remnant-like lipoprotein particles (RLP) in *in vitro* studies (6, 53)

Properties of ox-LDL and RLP
<ul style="list-style-type: none"> <li>a. Supported macrophage foam cell formation</li> <li>b. Were chemotactic for monocytes, T cells and tissue macrophages</li> <li>c. Enhance the expression of adhesion molecules on monocyte and endothelium</li> <li>d. Were mitogenic for smooth muscle cells and macrophages</li> <li>e. Altered inflammatory gene expression in vascular cells through a redox-sensitive mechanism</li> <li>f. Induced tissue factor, prothrombogenic molecules expression and platelet aggregation</li> <li>g. Impaired endothelium-dependent vasorelaxation (EDR) by altering NO activity</li> <li>h. Were cytotoxic and could induce apoptosis in endothelial and smooth muscle cells</li> <li>i. Activated lectin-like oxidized LDL receptor-1 (LOX-1) in endothelial cells</li> </ul>

proteins, may result in transformation of physico-chemical characteristics, which are of vital importance in regulating lipoprotein function, as presented in *Table II*.

Attenuated reverse cholesterol transport, which may induce premature development of atherosclerosis, partly results from the congenital impairment of affected genes. Numerous experiments on transgenic mice revealed that gene disruption, primarily apo A-I and ABCA-1, may induce atherosclerosis, whereas over-expression of pivotal proteins exhibits some cardioprotective effects (46). To that respect, the effects of CETP, PLTP, HL and LCAT still remain to be elucidated, since their function is regulated both by the proatherogenic and antiatherogenic factors (35).

Some *in vitro* studies demonstrated that various proinflammatory factors may decelerate the reverse cholesterol transport pathway, which is of paramount clinical importance. Thus, interferon-gamma (IFN- $\gamma$ ) affects the intracellular cholesterol efflux in the foam cell cultures (47). TNF- $\alpha$  and IL-1 $\beta$ , incubated with macrophages, decrease mRNA to ABCA-1 and ABCG-1 suggesting that host response to infection and inflammation may aggravate atherosclerosis (48). Contrary to that, antiinflammatory TGF- $\beta$  in macrophage culture increases cholesterol efflux through elevated ABCA-1 expression, and suppresses inhibition of cholesterol efflux by affecting the inhibitory effect of IFN- $\gamma$  to ABCA-1 expression. Moreover, it contributes to the stability of atherosclerotic plaque by inhibiting metalloproteinase activity and increasing matrix deposition (49).

Conversion of antiinflammatory HDL to the »amplifier« of vascular inflammation was demonstrated in HDL isolated in patients at high atherogenic risk or with coronary disease comprises an equivalent increase of LDL-induced monocytes adhering in the cell culture by increasing expression of MCP-1 (50). The potential of HDL particles to inhibit monocyte efflux and/or lipid peroxidation is likely to be modified within the acute phase response. It was demonstrated

that inflammation reduces the level of HDL-cholesterol by enhancing the activity of endothelial lipase and soluble phospholipase A2, as well as by substituting apo A-I with serum amyloid A (SAA) (51). Inflammation induces significant changes in the protein and lipid composition of HDL particles. Reduction of apo A-I, PON, PAF-AH, CETP, LCAT, PLTP, phospholipids and cholesterol esters was demonstrated in a mouse model, associated with simultaneous increase and incorporation of SAA, ceruloplasmin, apo J and secretory nonpancreatic phospholipase A2 (sPLA2) (52).

### Triglyceride-rich lipoproteins

Numerous clinical and epidemiological studies revealed that increased serum triglyceride levels are closely related to atherosclerosis, independently of serum levels of HDL and LDL (11, 53).

Hypertriglyceridaemia is characterized by increased secretion of triglyceride-overloaded VLDL-apo B particles by the liver. Mediated by CETP, HL and LCAT, a reciprocal transfer of lipids and lipoprotein remodelling is established, and, paradoxically, cholesterol-rich remnant-like lipoprotein particles (RLP) are produced. Cholesterol-poor sdLDL and sdHDL are generated with concomitant reduction of HDL-cholesterol and apo A-I (1, 53). Interrelationship between this atherogenic lipoprotein phenotype (ALP) and atherosclerosis has mainly been attributed to the loss of cardioprotective effects of sdHDL that are rapidly released from circulation and, to a great extent, to the modified LDL particles. Due to their lower affinity for their specific receptors, sdLDL promptly penetrates the arterial intima and intensively binds to subendothelial proteoglycans. They are more susceptible to oxidation, initiating abundant formation of foam cells and establishing of endothelial dysfunction (11). Predominance of sdLDL has been accepted as an emerging cardiovascular risk factor by the NCEP Adult Treatment Panel III (9).

It has recently been established that RLP produced by hydrolysis of TRL in the state of hypertriglyceridaemia and during the postprandial period can be considered atherogenic and independent coronary risk factors, rivalling LDL, particularly with respect to the metabolic syndrome. They involve series of particles that are heterogenous with respect to their size, density and lipid/apo composition (6, 53).

Isolation of apo B-100 lipoprotein in human atherosclerotic plaque revealed presence of significant quantities in the VLDL+IDL fraction. Moreover, VLDL apo B-100 was detected in the aorta of humans and experimental animals after its intravenous administration, thus it is likely that RLP have the ability of entering human atherosclerotic plaques in spite of its increasing size related to the LDL (54, 55).

Moreover, it was established that isolated RLP are oxidized or sensitive to oxidation in plasma. As summarized in *Table 1*, RLP exhibit similar proatherogenic and proinflammatory features as the ox-LDL, suggesting that they may contain the same oxidized phospholipids mediating their atherogenicity. There is abundant evidence that RLP, contrary to the LDL, can undergo the macrophage uptake by the remnant apo B-48 receptors and generate foam cells without previous oxidative modification (6, 56). Besides, they are able to bind to LOX-1 receptors in EC, which were primarily discovered as ox-LDL receptors. By activating these receptors, RLP could play a major role in the establishing of endothelial dysfunction and initiation of atherosclerosis, independently from plasma concentrations of LDL (6).

It is hypothesized that RLP promotes the atherogenesis by directly affecting vascular cells. In cell cul-

ture, RLP increase monocyte adhesion in vascular EC most probably by the mechanism of RhoA activation, which belong to the family of small GTP-binding proteins, via the protein kinase C (PKC), resulting in activation of focal adhesion kinase (FAK) and beta-1-integrin (53). Besides, RLP induce proliferation of smooth muscle cells via epidermal growth factor (EGF) receptor transactivation, and heparin-binding EGF-like growth factor (HB-EGF) shedding (57). Incubation of RLP with EC provokes expression of ICAM-1, vascular cell adhesion molecule-1 (VCAM-1) and MCP-1 most probably by the redox-sensitive mechanisms. RLP induce EC apoptosis by cytokine production through LOX-1 receptors (58).

It is also established that plasma levels of RLP significantly and independently correlate with decreased endothelium-dependent vasomotor function and that they decrease endothelium-dependent vasodilation in isolated rabbit aorta (53). It is suggested that endothelial dysfunction results from the elevated oxidative stress, and that RLP elevates expression of Rho-kinase in smooth muscle cells as well (6).

### Conclusion

Considering some new discoveries regarding the basic mechanism involved in the development of atherosclerosis and the possible role of lipoprotein particles in the initiation and propagation of atherosclerosis and atherothrombosis, development of novel pharmacological principles is expected in the near future. Appropriate prospective studies might further elucidate the predictive value of lipids and lipoproteins, and offer new strategies in the prevention and therapy of atherosclerotic cardiovascular diseases.

## LIPIDI I ATEROSKLEROZA

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*Kratak sadržaj:* Lipidi imaju značajnu ulogu u aterogenezi, počev od inicijalnih promena, te se danas najviše razmatra njihova prediktivna vrednost za koronarnu bolest srca, kao i mogućnosti novih terapijskih pristupa. Centralno mesto zauzimaju LDL čestice, jer je samo za redukciju LDL-holesterola dokazano da redukuje morbiditet i mortalitet udružen sa aterosklerozom. Osim regulacije ekspresije LDL-receptora, veliki klinički značaj imaju oksidizani LDL (ox-LDL) i male guste LDL (sdLDL) koje su produkt intravaskularnog remodelovanja trigliceridima bogatih lipoproteina, dok egzaktna uloga anti-oxLDL antitela u progresiji ateroskleroze i klinički značaj ostaju nejasni. Poslednjih godina, međutim, potpunije upoznavanje bazičnih mehanizama uključenih kako u aterosklerozu, tako i u metaboličku sudbinu lipida i lipoproteina, ističe i značajnu ulogu ostalih lipoproteinskih čestica, i to, ne samo u progresiji, već i u inicijaciji ateroskleroze i aterotromboze.

*Ključne reči:* lipidi, lipoproteini, ateroskleroza

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