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Chemical composition of circulating native and desialylated low density lipoprotein: what is the difference?

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ABSTRACT

Atherosclerosis and related cardiovascular disorders remain the leading global cause of morbidity and mortality. Modified low density lipoprotein (LDL) is considered to play a crucial role in atherosclerosis development. During the past decades, several types of atherogenic LDL modification have been discovered. Desialylation was one of the atherogenic modifications observed in circulating atherogenic LDL *in vivo*. Sialic acid level negatively correlates with triglyceride and cholesterol contents. Desialylated LDL is small, dense and highly susceptible to oxidation, as reported for hyperlipidemic conditions. This atherogenic modification leads to increased cholesterol intake by macrophages and smooth-muscle cells, and is also associated with other pathologies, such as diabetes mellitus. Moreover, these conditions provoke damage and desialylated LDL particles may trigger autoimmune reactions in macrophages and B-cells.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide. According to the American Heart Association, nearly 787,000 people in the US died from heart disease, stroke or other CVDs in 2011.[1] Atherosclerosis underlies most of the cardiovascular events in adults. Atherosclerotic plaque formation involves accumulation of cholesterol and its esters in the arterial intima, which results in migration and proliferation of various cell types (smooth muscle cells, macrophages, lymphocytes, neutrophils and dendritic cells) and inflammation, followed by necrosis and calcification.[2] Elevated blood pressure, diabetes mellitus, hyperlipidemia, family history and smoking are the major risk factors of atherosclerosis. These conditions provoke damage and lipid penetration into the arterial wall. According to current understanding, plasma low density lipoprotein (LDL) plays a crucial role in the pathogenesis of atherosclerosis because of its ability to deliver cholesterol from the liver to peripheral tissues, including the arterial wall. On the other hand, high density lipoprotein (HDL) negatively correlates with CVD and has protective effects.[3,4]

Currently, a high level of LDL cholesterol (LDL-C) is considered as a risk factor for CVD in clinical practice, and various treatment options (e.g. statins) are used to decrease it.[5,6] However, simple reduction of blood cholesterol level is not sufficient for effective atherosclerosis prevention. Moreover, this approach was demonstrated not to be efficient in several clinical studies.[7,8] The main drawback of statin therapy is the presence of several severe side-effects, such as statin-associated muscle symptoms, diabetes mellitus, and central nervous system disorders.[9] During the last decade, molecular mechanisms of atherosclerosis have become a subject of intensive research aimed at improving the clinical outcomes and developing novel therapies.

MODIFIED LDL AND ATHEROSCLEROSIS

Numerous studies have revealed that LDL subtypes form a heterogeneous group with different chemical and physical properties. Several types and subclasses of circulating LDL have different atherogenic effects. According to the widely accepted classification, the following LDL subtypes can be distinguished: small (dense), medium and large LDL. Dense LDL [with density (d) 1.044-1.060 g/mL] is considered to be the most atherogenic. Particles of this LDL subtype differ in size from 15 to 20 nm. For large LDL (d. 1.019-1.034 g/mL), mean particle size is 22 nm (up to 30 nm). Medium LDL (d. 1.034-1.044 g/mL) has a particle size in between small and large LDL.[10] An early study by Filipovic[11] and co-authors showed that LDL modification enhanced cholesterol intake by cultured cells. Subsequently, naturally modified LDL types were found in human blood.[11,12] During the past decades, numerous studies confirmed that LDL modifications, such as oxidation, desialylation and enzymatic processing, play a key role in increasing cholesterol intake, and the level of multiple modified LDL correlates with the risk of CVD.[13,14]

Other types of lipoproteins that are distinguished in some studies are electronegative LDL [LDL(-)] and lipoprotein (a) [Lp(a)]. The former subclass includes modified LDL with increased negative charge, which accounts for 3-5% of the total LDL in normolipidemic subjects. Several studies on LDL(-) showed that it represents a heterogeneous group of particles with various chemical modifications (oxidation, glycosylation, non-esterified fatty-acid enrichment, desialylation, and enzymatic modification) that share the common feature of increased electronegativity. Electronegative LDL is characterized by an enhanced ability to aggregate and is more susceptible to oxidation than native LDL (nLDL).[15,16] It was found that LDL(-) could accumulate in endothelial cells, monocytes, and lymphocytes through binding to scavenger receptors, such as platelet-activating factor receptor (PAF), lectin-like oxidized LDL receptors (LOX-1), and scavenger receptor A (SRA).[17,18] T-lymphocyte receptors (TCR) and CD14 are also involved in conveying LDL(-) biological effects.[19] It's worth mentioning that nLDL binding with oxidized forms of hemoglobin may cause changes in conformation and chemical composition of nLDL apolipoproteins.[20] High intracellular lipid level and activation of receptor pathways may result in cytotoxicity and the release of inflammatory cytokines.[21-24] On the other hand, recent studies showed that LDL(-) had an ability to induce anti-inflammatory cytokines [e.g. interleukin-10 (IL-10)] and counteract inflammatory effects promoted by lipopolysaccharides.[19,25] In that regard, the atherogenic role of LDL(-) needs further investigation. However, multiple studies confirmed that high LDL(-) level was a risk factor for CVD, which might be connected with other LDL(-) modifications, such as desialylation and oxidation.[16,26-28]

Lipoprotein (a) [Lp(a)] differs from LDL only by the presence of apoplipoprotein (a) bound to apolipoprotein B-100 (apoB-100) via a disulfide bridge. Lp(a) is normally present in the blood, and its plasma concentrations range from 1 to 1,000 mg/dL. High levels of Lp(a) are associated with some pathologies. For instance, Lp(a) level increased within 24 h after acute myocardial infarction, and its transient increase accompanied acute and chronic inflammatory processes.[29,30] Lp(a) gene polymorphism was associated with the incidence of cerebral vascular accident of large vessels, peripheral arterial disease, and abdominal aorta aneurysm.[31] Lp(a) level also correlated with IL-6, tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), and monocyte chemoattractant protein (MCP-1) levels.[32] In Korean population, patients with high Lp(a) level had higher CVD risk and worse disease course.[33] A Danish prospective study of 9,000 subjects revealed that extremely high plasma Lp(a) level (over 120 mg/dL) increased CVD risk 4-fold.[34] On the other hand, multiple prospective studies showed that a high Lp(a) level was not an independent risk factor for cardiovascular or cerebrovascular diseases.[29,35]

CHEMICAL COMPOSITION OF LDL

Non-modified, or nLDL, particle contains apolipoprotein B-100 (apoB-100) molecule, about 90 molecules of other regulatory proteins, a phospholipid monolayer, and a hydrophobic core, which accounts for 75% of LDL particle weight.[36] LDL contains proteins regulating apoB-100 metabolism and lipid transport [apolipoprotein C-II (apoC-II), apoC-III, apoE, apoA-I,

apoA-IV, and apoF], associated with inflammation (apoD, apoJ, apoM, serum amyloid A4, paraoxonase 1, prenylcysteine oxidase 1, migration inhibitory factor-related protein 8, and retinol binding protein), related with thrombosis (fibrinogen alpha chain) and components of the innate immunity system (lysozyme C, alpha-1 antitripsin, apoL-1, and transthyretin).[10] ApoB-100 is a large glycoprotein, which stabilizes and maintains LDL structure and composition. ApoB-100 has 24 potential N-glycosylation sites, with up to 16 asparagine residues actually glycosylated. Carbohydrates, including neutral and acidic carbohydrate chains, account for 5-9% of apoB-100 molecular weight. All chains contain N-acetylglucosamine and mannose residues. Acidic chains contain terminal sialic acid residues followed by galactose [Figure 1].[36-38] Loss of the terminal sialic acid residue results in exposure of galactose residues. It was suggested that almost all nLDL particles are partially monodesialylated because they have galactose ending chains.[39]

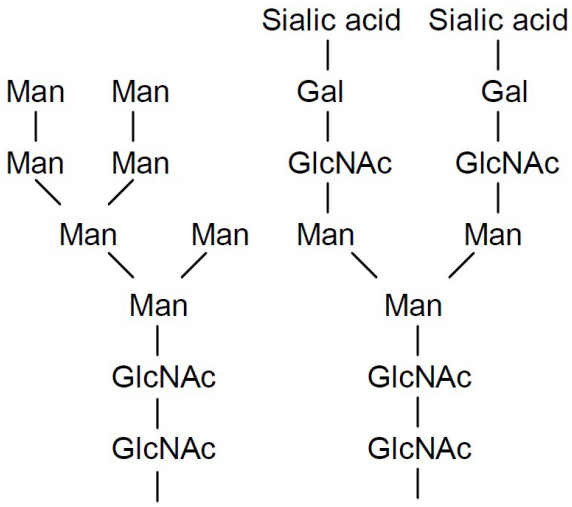

Figure 1: Carbohydrate chains in apoB-100. Both chains have mannose base (Man) connected to polypeptide chain by N-acetylglucosamine (GlcNAc). Acidic one has terminal sialic acid residues connected to galactose (Gal) molecules.[36-38]

The phospholipid monolayer contains phosphatidylcholine, sphingomyelin, lysophosphatidylcholine, phosphatidyl-ethanolamine, ceramide, and diacylglycerol. The hydrophobic core contains various lipid classes: non-esterified cholesterol, cholesterol esters, and triglycerides. Non-esterified cholesterol is also located on the surface of the LDL particle. nLDL transports 66% of serum gangliosides. Gangliosides are sialic-acid-rich glycosphingolipids and are thought to contain all the sialic acid residues associated with the LDL lipids.[36] The lipid part of nLDL also contains other monosaccharides: galactosamine and glucose.[40]

First studies of LDL carbohydrate composition revealed little or no variation in glucosamine, galactose and mannose values, but a marked variation in sialic acid levels [Table 1].[41] Further studies showed that in patients with coronary artery disease (CAD), LDL had a decreased sialic acid content. Isolated LDL from these patients, as well as *in vitro* desialylated LDL, caused atherogenic changes in cultured cells.[13,42,43] The most comprehensive study on chemical composition of LDL in patients with and without atherosclerosis was performed in 1993.[40] Carbohydrate content of LDL from patients with atherosclerosis was almost the same, except for sialic acid level, which was significantly (1.6 times) lower in patient LDL ($P < 0.05$) [Figure 2].[40] There was no significant difference in the levels of galactose, N-acetyl glucosamine and mannose between nLDL and modified LDL from healthy donors, as well as from patients with atherosclerosis. Sialic acid content in modified LDL was 30% lower than in nLDL from healthy subjects. Sialic acid level in modified LDL from patients was 2 to 3 fold lower than in nLDL. Comparison of nLDL obtained from healthy subjects and patients with atherosclerosis revealed no significant differences in carbohydrate contents. Modified LDL had a significantly lower level of sialic acid ($P < 0.05$). There was also a significant difference in the sialic acid content between modified LDL from healthy donors and from patients with atherosclerosis, $P < 0.05$.

Levels of all lipid-associated carbohydrates were 1.5 to 2 fold lower in LDL samples obtained from atherosclerosis patients in comparison to those from healthy subjects. Native and modified LDL obtained from patients and healthy subjects also differed by lipid composition. Modified LDL had decreased levels of cholesterol, cholesterol esters, triglycerides, phosphatidylcholine,

Table 1: Carbohydrate content of LDL according to early studies (percent dry weight)

	Sialic acid	Glucosamine	Galactose	Mannose
Schultze and Heide[87] (1960)	1.5	2.0	2.7	2.7
Ayrault-Jarrier[88] (1961)	1.3	1.2	-	-
Marshall and Kummerow[89] (1962)	0.35	1.2	3.23 (together)	
Kwiterovich *et al.*[90] (1974)	0.6	0.9	1.8	3.7
Swaminathan and Aladjem[41] (1976)	1.73	0.94	2.13	4.88

LDL: low density lipoprotein; "-": not measured

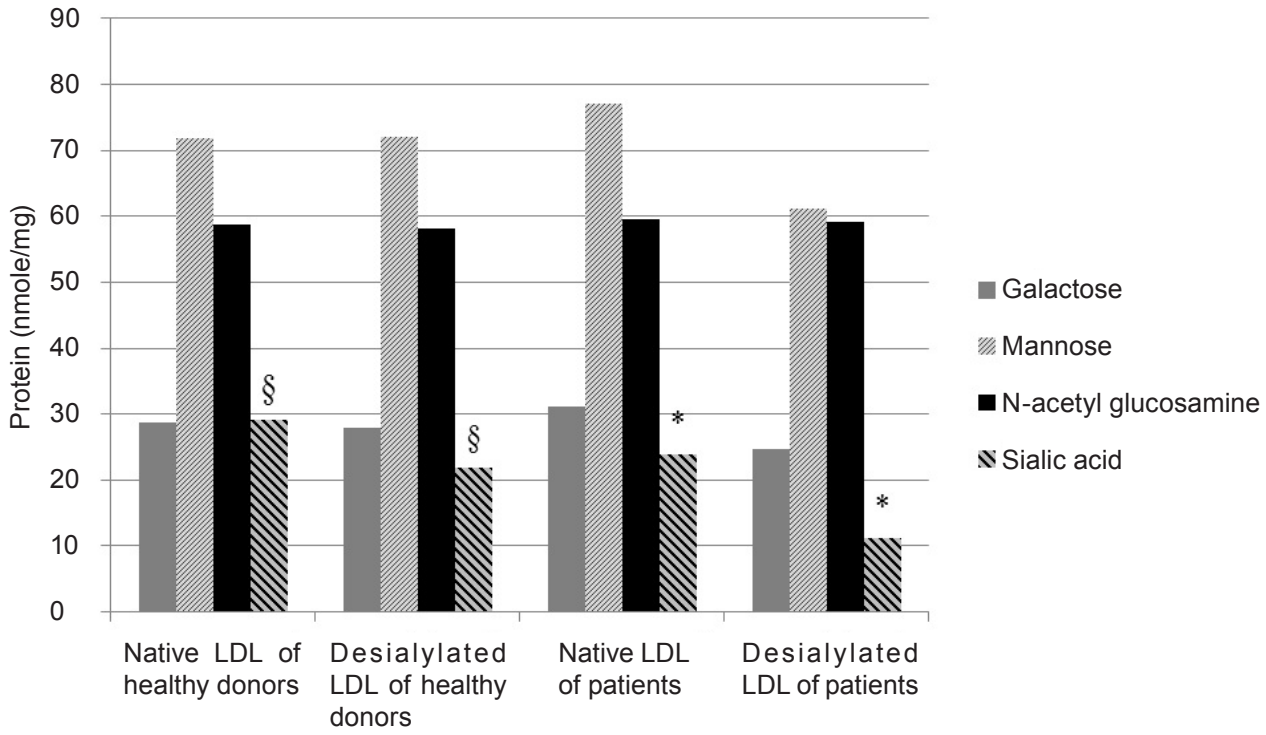

Figure 2: Mean content of carbohydrates in native and desialylated LDL (dLDL) of healthy donors and patients with atherosclerosis; §: significant difference in sialic acid content between native and desialylated LDL of healthy donors, $P<0.05$; *: significant difference in sialic acid content between native and dLDL in patients with atherosclerosis, $P<0.05$

and phosphatidylethanolamine, with higher levels of free fatty acids, mono- and diglycerides. At the same time, sphingomyelin content of modified LDL obtained from atherosclerosis patients was significantly decreased.[40] Further studies reported similar results.[42-44] Noteworthy, LDL desialylation positively correlated with particle density and negatively with particle size.[14,40,43-46] At the same time, several studies found no difference in LDL sialic acid content between subjects with and without atherosclerosis.[47-49]

To solve this dilemma, Lindbohn with co-authors suggested that the controversial results could be explained for a large part by the choice of study population, which was almost exclusively male. Another study was conducted on 22 middle-aged women with CAD and 11 control subjects. Patients' LDL had significantly lower sialic acid-to-apoB-100 ratio compared with the control group. A negative correlation was observed between sialic acid ratio and cholesterol, phospholipid and triglyceride concentration.[50] Recent studies confirmed previous results in CAD patients and revealed the crucial role of LDL desialylation in various other pathologies, such as aortic valve sclerosis, different types of hereditary hyperlipidemia and diabetes mellitus.[51-55]

OXIDIZED LDL

Studies conducted on cellular models demonstrated that *in vitro* oxidation caused increased uptake of LDL-C by cultured cells. Macrophages could not consume non-oxidized LDL because of receptor-dependent limitations. Oxidized LDL (oxLDL) can bind to various receptors for modified LDL [for example lectin-like oxidized LDL receptor (LOX-1)], which leads to increased cholesterol uptake and foam cell formation.[56] High levels of oxLDL were found in patients with atherosclerosis and, together with NO levels, were used as biomarkers of endothelial dysfunction.[56,57] To date, the precise LDL oxidation mechanism is not fully understood. Activated monocytes, macrophages and endothelial cells generate reactive oxygen species (ROS) and produce lipoxygenase, hypochlorous acid (HOCl) and myeloperoxidase. These substances, along with metal ions (Fe^{3+}, Cu^{2+}) are involved in LDL oxidation. It was shown that HOCl and hypothiocyanous acids can cause oxidation of the apoB-100 molecule.[58] Macrophages can recognize oxLDL with various receptors, including CD36, toll-like receptor 4 (TLR4), LOX-1, and receptor for advanced glycation end-products (RAGE).[59-61] Cholesterol and lipid accumulation in macrophages leads to the release of pro-inflammatory cytokines (e.g. TNF-α), which results in inflammation and recruitment of immune cells. OxLDL enter the endothelial cells through binding to LOX-1 receptors.[61] High lipid concentration results in IL-8 secretion, which stimulates inflammation and migration of smooth-muscle cells from the tunica media to the intima.

Chemical composition of oxLDL is characterized by 1.5 to 2 fold decreased levels of antioxidants, such as coenzyme Q10, tocopherols, β-carotene, and lycopene, and increased content of oxidation products. Intense oxidation of fatty acids, cholesterol and other lipids leads to accumulation of 13-hydroperoxylinoleic acid and other peroxides, hydroxides (e.g. 13-hydroxylinoleic acid), prostaglandin derivatives (isoprostanes), various aldehydes (malondialdehyde, oxovaleryl phosphatidylcholine, hexanal, *etc*.), lysophosphatidylcholine, 7-keto-cholesterol, various hydrocarbons, including pentane, and modified phosphatidyl ethanolamine/serine products. Products of protein oxidation include: protein carbonyls, non-enzymatic proteolyzed fragments, arginine, cysteine, modified cysteine, lysine, histidine, methionine, tyrosine, and tryptophan, protein cross-linking products due to tyrosine cross-links and bifunctional aldehydes, lipid-protein adducts which can be classified as ceroids (lipofuscins). Many of the above mentioned modifications, as well as conformational changes, might lead to increased antigenicity.[62] Lack of antioxidants makes oxLDL susceptible to further oxidation and apolipoprotein degradation. In the bloodstream, oxLDL is characterized by high density and increased negative charge. A controlled study of LDL structural changes due to *in vitro* oxidation with copper ions showed similar results. Small-angle X-ray scattering and dynamic light scattering techniques revealed high density, electrical charge, and increased degree of flexibility of the apoB-100.[63] However, oxidation should not be considered as the key modification leading to LDL electronegativity because the concentration of oxLDL in normolipidemic plasma is orders of magnitude lower than LDL(-) concentration.[17]

ELECTRONEGATIVE LDL

LDL(-) chemical composition is characterized by decreased sialic acid and antioxidant content, increased triglycerides, nonesterified fatty acids (NEFA), lysophosphatidylcholine, and ceramide levels compared to nLDL.[63,64] LDL(-) is also distinguished by phospholipolytic activities and abnormal apoB-100 conformation.[65] In nLDL, apoB-100 has a pentameric structure with alternating alpha helixes and beta pleated sheets. In LDL(-), apoB-100 has less alpha helixes and more beta sheets, as well as an altered pattern of exposed lysine residues that are involved in lipoprotein receptor binding interactions. Changes in apoB-100 structure may be caused by oxidation and nitration.[65] These chemical changes and presence of electronegative charge in desialylated LDL makes it possible to suggest that these two fractions are identical.[16]

GLYCATED LDL

Glycation of LDL occurs due to non-enzymatic reaction of glucose and its metabolites with free amino groups of apoB-100 lysine. This process is highly intensive in patients with diabetes mellitus and metabolic syndrome because of the high glucose blood level.[66] In non-diabetic patients, 4.8% of apoB-100 is glycated compared to 14.8% of total apoB glycated in patients with type II diabetes. It was demonstrated that small-dense LDL is more susceptible to glycation in patients with metabolic syndrome and type II diabetes than nLDL.[67] Glycation makes LDL more sensitive to oxidation. Formation of glycated LDL and other advanced glycation end products (AGEs) increases atherogenic properties of LDL and enhances lipid uptake by cultured aortic smooth-muscle cells. High concentration of AGEs leads to activation of the RAGE receptor pathway, which results in enhanced expression and NF-κB-dependent release of pro-inflammatory molecules. That, in turn, promotes vessel wall damage, endothelial dysfunction, monocyte and macrophage migration and recruitment to the vascular intima followed by oxidative stress, vascular wall remodeling and atherosclerotic lesion progression.[68] However, recent studies on diabetic patients showed that glycated LDL level was not an independent risk factor for CVD. At the same time, patients with type I and II diabetes had a high level of small dense desialylated LDL particles with oxidative modifications.[54] Therefore, glycation makes nLDL more susceptible to oxidation and enzymatic changes and may be the first step atherogenic modification of LDL in diabetic patients.

DESIALYLATION IMPACT ON ATHEROSCLEROSIS DEVELOPMENT

Under normal conditions, LDL lipid intake is controlled by lipoprotein receptors. Modification of LDL, such as oxidation and desialylation, allows LDL particles to escape this limitation and enter arterial cells via different pathways. Sialic acid provides LDL with negative charge, which protects the particle from binding to arterial proteoglycans. The increased ability of enzymatically desialylated LDL to interact with proteoglycans was confirmed by Millar *et al*.[45] However, small dense desialylated LDL are electronegative and can interact with macrophage lectin receptors, therefore mediating the lipid uptake.[68] Increased cholesterol accumulation may also result from macrophage scavenger receptor-mediated uptake followed by foam cell formation and macrophage cytokine release, which causes inflammation and monocyte migration in the intima.[70-72] Inhibition of Acyl-coenzyme A: cholesterol acyltransferase activity by desialylated LDL is also

considered as a possible mechanism of macrophage down-regulation.[14]

While some studies found no differences in lipid peroxide content between native and desialylated LDL,[73] Others reported that desialylation may cause both an increase and decrease in susceptibility to oxidation depending on LDL density and hyperlipidemia type.[74,75] Small dense LDL in type IIa hyperlipidemia was the most susceptible to oxidation.[75] Increased lipid peroxidation was found in desialylated LDL.[76] Another study showed that LDL sialic acid levels negatively correlates with thiobarbituric acid reactive substances and suggested that reactive oxygen substances may affect enzymatic desialylation *in vivo*.[77] It was suggested that a plasma enzyme called trans-sialidase is the possible cause of LDL desialylation in blood plasma.[78]

DESIALYLATION AND IMMUNE RESPONSE

Sialic acids belong to a group of *N*- or *O*- derivatives of neuraminic acid. N-acetylneuraminic acid (Neu5AC) is the most common type found in humans. Neu5AC is typically found at the terminal position of ganglioside glycan chains in the cellular glycocalyx. Sialic acids are involved in cell-cell interactions, including those between immune cells. Neu5AC refers to a self-associated molecular patterns (SAMPs) group because of their ability to suppress innate and adaptive autoimmune response.[79] Sialic-acid-binding immunoglobulin-like lectins (Siglecs) form a group of immune cell receptors that participate in the discrimination of “self” and “non-self” through recognition of cell glycan ligands. Macrophages have sialoadhesin (CD169), so-called Siglec-1, and B-cells have CD22, so-called Siglec-2. Studies on human immune cells discovered 14 members of the Siglec family. Siglec receptor binding with host-specific sialic acid provides negative regulation or even apoptosis in immune cells. For example, in B-cells activation of CD22 pathway leads to activation of Src homology region 2 domain-containing phosphatase-1 (SHP-1), which suppresses the activation of B-cell receptor (BCR).[80] Recent study showed that sialic acid binding domain mutations of Siglec-G resulted in decreased B-cell activation threshold.[81] Dysfunction of Siglec receptor interactions with sialic acid is associated with various autoimmune diseases.[79,80] Lack of sialoadhesin in macrophages causes activation of scavenger receptors and phagocytosis.[79-82] In atherosclerosis, decreased sialic acid content in desialylated LDL might result in increased cholesterol intake and inflammation through macrophage and B-cell activation.

Patients with various CVD have antibodies against modified LDL and lipoprotein-containing immune complexes (LDL-CIC) in the plasma. Immunoglobulin G (IgG) antibodies with high affinity for *in vitro* desialylated and malondialdehyde-modified LDL were detected in patients with angiographically assessed coronary atherosclerosis. On the other hand these antibodies have low affinity for native, glycated, acetylated, and LDL with other chemical modifications.[83] It was shown that IgG (subclasses G1, G3) against modified LDL have pro-atherogenic properties, while IgM antibodies are atheroprotective.[14,84] In 2013 Montano[85] and colleagues showed that monoclonal anti-oxLDL IgM (E06) inhibited oxLDL binding to macrophages in a dose dependent manner. Studies on LDL-CIC discovered that LDL in these complexes had atherogenic modifications, particularly LDL were small dense and had decreased sialic acid content. LDL-CIC stimulated lipid accumulation in cultured cells unlike nLDL.[86] Recent studies showed that IgG and LDL-CIC removal from patient sera reduced its atherogenic activity.[83] Level of LDL-CIC is used in diagnosis, prognosis and in several therapeutic approaches in CVD patients.[14,83,84]

CONCLUSION

Sialic acid level is decreased in atherogenic LDL and negatively correlates with triglyceride and cholesterol level in LDL. Desialylated LDL are small, dense and highly susceptible to peroxidation in several hyperlipidemia types. Desialylation results in atherogenic changes because of increased cholesterol intake in macrophages and smooth-muscle cells and is also associated with other pathologies, such as diabetes mellitus.

DECLARATIONS

Authors’ contributions

Analysis of literature, writing a draft: V.I. Alipov
Editing, writing a draft: V.N. Sukhorukov
Table and figures: V.P. Karagodin
Consultation: A.V. Grechko
English improvement: A.N. Orekhov

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Conflicts of interest

The authors declare that they have no competing interests.

Patient consent

Not applicable.

Ethics approval

Not applicable.

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