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Review

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A novel insight into the nature of modified low-density lipoproteins and their role in atherosclerosis

Dmitry A. Kashirskikh[1,2], Shuzhen Guo[3], Suraphan Panyod[4], Nelya R. Chicherina[2,5], Mariam Bagheri Ekta[6], Anastasia I. Bogatyreva[6], Andrey V. Grechko[7]

[1]Laboratory of Angiopathology, The Institute of General Pathology and Pathophysiology, Moscow 125315, Russia.
[2]Institute for Atherosclerosis Research, Moscow 121096, Russia.
[3]School of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing 100029, China.
[4]Institute of Food Science and Technology, National Taiwan University, Taipei 10617, Taiwan.
[5]Sirius University of Science and Technology, Sochi 354340, Russia.
[6]Laboratory of Cellular and Molecular Pathology of Cardiovascular System, Avtsyn Research Institute of Human Morphology of FSBI "Petrovsky National Research Center of Surgery", Moscow 117418, Russia.
[7]Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, Moscow 109240, Russia.

Correspondence to: Dmitry A. Kashirskikh, Institute for Atherosclerosis Research, Osennyaya Street 4-1-207, Moscow 121609, Russia. E-mail: dim.kashirsckih@gmail.com; Shuzhen Guo, School of Traditional Chinese Medicine, Beijing University of Chinese Medicine,11 Bei San Huan Dong Lu, Chaoyang District, Beijing 100029, China. E-mail: guoshz@bucm.edu.cn

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Abstract

Atherosclerosis plays a significant role in the development of cardiovascular diseases, the leading cause of death worldwide. Modification of low-density lipoproteins (LDLs) is a critical event in atherogenesis. Native LDL undergoes several modifications that can lead to the formation of atherogenic modified LDLs. LDL modifications change their physicochemical and biological properties. Possible modifications include changes in the lipoprotein particle's structure, size, charge, and composition. Uptake and utilization of modified LDLs are impaired in cells. Macrophages take up modified LDLs that promote forming of foam cells, one of the critical cellular components of atherosclerotic lesions. Nevertheless, the direct role of each atherogenic LDL modification in atherogenesis remains uncertain. This review highlights LDL's most critical atherogenic modifications, including oxidized, enzyme-modified, non-oxidative, desialylated, glycated and carbamylated LDLs. Studying the role of each type of LDL modification will clarify the unknown elements of atherosclerosis progression and facilitate the development of effective methods for its diagnosis, treatment, and prevention.

Keywords: Atherosclerosis, modified LDL, oxidized LDL, enzyme-modified non-oxidized LDL, desialylated LDL, glycated LDL, carbamylated LDL

INTRODUCTION

According to the World Health Organization, cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide, representing 32% of all deaths. Atherosclerosis is associated with the occurrence and development of CVDs, including coronary heart disease (CHD), myocardial infarction, stroke, and peripheral arterial disease. The study of atherosclerosis initiation and early development would make it possible to develop practical diagnostic tools for the asymptomatic stages and promising therapeutic approaches[1].

Atherosclerosis is a chronic progressive disease of the elastic arteries characterized by the accumulation and retention of cholesterol ApoB-containing lipoproteins, primarily low-density lipoproteins (LDLs), in the arterial wall[2]. The pathogenic mechanism of atherosclerosis is a cascade of events leading to the formation of atherosclerotic lesions. Currently, there are disputes about the processes that initiate atherosclerosis. The critical events in the formation of atherosclerotic lesions are changes in endothelial permeability, migration of smooth muscle cells, synthesis of extracellular matrix components, retention of lipoproteins in the vessel wall, modification of lipoproteins, turbulent blood flow, and inflammation[3-6]. This review is focused on the modification of LDL. One of the critical events of atherogenesis is LDL modification[4,7,8]. It has been repeatedly demonstrated that modified LDL causes excessive intracellular accumulation of lipids in cell cultures, which determines lipoprotein atherogenicity[9-12]. It has been shown that incubating culture of human aortic intima cells with native LDL does not lead to intracellular lipid accumulation[12,13]. Modified LDLs can be found in atherosclerotic lesions and in the blood of patients with atherosclerosis[14-17]. Circulating LDLs in patients with atherosclerosis CHD are modified several times, and modified LDLs may differ in terms of the modification from one patient to another[18-20]. Such features of LDL are likely to be associated with various combinations of modifications[13,20]. Modified LDLs can be classified as oxidized LDL (OxLDL), enzyme-modified non-oxidized LDL (eLDL), desialylated LDL (desLDL), glycated LDL (gLDL), and carbamylated LDL (cLDL) [Figure 1].

There is continuing interest in studying LDL modifications and their role in atherogenesis. Over the past five years, 13,758 full-text articles with the keywords "modified LDL and atherosclerosis" have been registered in the PubMed Central system. These articles account for about half of all publications that mention a combination of these terms from 1979 to 2022. However, several LDL modifications in atherogenesis remain incompletely elucidated. Therefore, this review highlights the role of various mechanisms of LDL modifications in the early stages of atherosclerosis development.

OXIDIZED LDL

The oxidative modification hypothesis

The oxidative modification hypothesis arose from the study of atherosclerosis in patients with homozygous hypercholesterolemia. Although this disease is characterized by the absence of expression of the LDL receptor (LDLR), foam cell formation is observed in patients with homozygous hypercholesterolemia[21]. It is essential to note that LDLR has usually involved in LDL receptor-mediated endocytosis[22]. In 1979, Goldstein and Brown suggested that LDL modification occurs before the LDL uptake by macrophages with subsequent transformation into foam cells[21]. In addition, it was assumed that an alternative receptor is required to uptake modified LDLs. Goldstein and Brown showed that adding chemically acetylated LDL (acLDL) to cell culture led to their uptake and more significant intracellular cholesterol levels than native

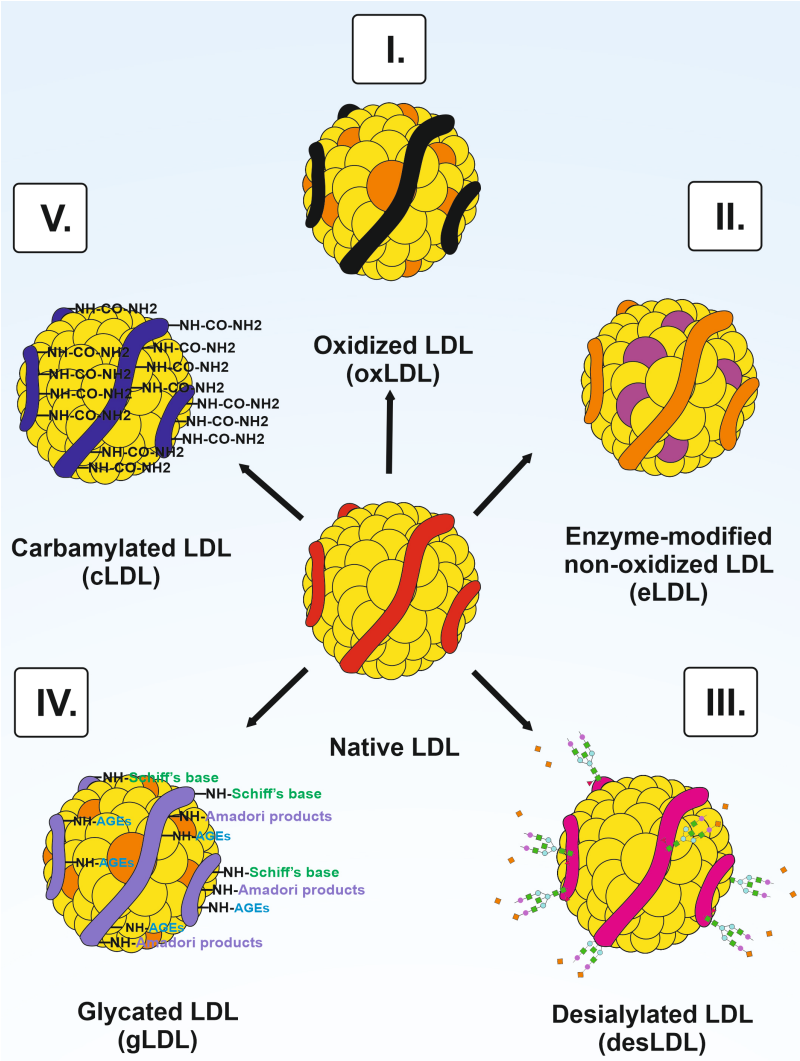

Figure 1. The scheme of LDL modifications. (I) Oxidation of native LDL with reactive oxygen species, reactive nitrogen species, cyclooxygenase 2, lipoxygenases, cytochrome P450 monooxygenases, myeloperoxidase, and lipoxygenase 12/15. (II) Enzymatic non-oxidative modification of native LDL by matrix metalloproteases 2 and 9, plasmin, trypsin, cholesterol esterase, chymases, sphingomyelinase, phospholipase A2, and cathepsins (cathepsin H, G, and F). (III) Desialylation of native LDL by endogenous sialidases, trans-sialidase, viral neuraminidases, and reactive oxygen species. (IV) Glycation of native LDL by excess glucose and nornicotine. (V) Carbamylation of native LDL with urea, cyanate, and thiocyanate with myeloperoxidase and H_2O_2.

LDL[23]. The acLDL receptor was characterized and named scavenger receptor A (SR-A)[24]. Goldstein and Brown demonstrated the participation of modified LDL in atherogenesis; however, acLDL has never been found *in vivo*; therefore, a search is required to establish new modified LDLs involved in atherogenesis[21].

Developing the idea of the participation of modified LDL in atherogenesis, Steinbrecher and Hessler suggested that free radical lipids are involved in LDL modification[25,26]. Incubation of native LDL with endothelial cells and human dermal fibroblasts results in LDL oxidation by lipid peroxidation (LPO)[25,26]. The evidence supports the oxidative modification hypothesis of LDL: (1) there is OxLDL *in vivo*; (2) OxLDL leads to the formation of foam cells compared to native LDL; and (3) high levels of ROS and their products present in atherosclerotic lesions[18,27-29]. In addition, the predictive value of serum OxLDL levels in determining the progression of subclinical atherosclerosis has recently been demonstrated[30].

There are several current assumptions regarding the moment when LDL oxidation occurs: before or after macrophage uptake. According to the response-to-retention hypothesis, native LDL accumulates in the vessel wall[3]. One explanation for the excess accumulation of lipids is a change in the length of chondroitin sulfate chains in the tunica intima[31,32]. In addition, changes in the length of macrophage chondroitin sulfate proteoglycans have been shown to affect OxLDL binding on the surface of peritoneal macrophages[33,34]. According to the oxidative modification hypothesis, atherosclerosis is initiated by LDL oxidation in the vascular wall[35]. Moreover, it is essential to note that OxLDL in the blood was excluded due to several facts: (1) LDL contains an antioxidant component (coenzyme-Q10, α-tocopherol, and carotenoids); and (2) high activity of the antioxidant system in the blood[4,36]. LDL oxidation can be divided into two successive stages[4]. In the first stage, the lipid part of LDL is oxidized, while the protein part is almost not modified. In the second stage, the formed mildly oxidized LDL undergoes strong oxidation of the LDL protein part, leading to the formation of highly oxidized LDL[37]. Modifying ApoB-100 promotes increased uptake of OxLDL by macrophages and subsequent formation of foam cells[23,38]. Thus, forming foam cells is a pathological characteristic of atherosclerosis in the early stages[39].

The lysosomal pathway is considered the second possible pathway of LDL oxidation[40]. Aggregated LDL can be oxidized in macrophage lysosomes in the presence of iron[41]. Acidic pH is required for cholesterol esterase (CEase) activity and proteases for LDL degradation in normal conditions. Incubation of THP-1 macrophages with LDL aggregated by sphingomyelinase (SMase-LDL) leads to an increase in pH and a decrease in the degradation of endocytosed LDL[42]. The lysosome-targeted antioxidant cysteamine can prevent intracellular LDL accumulation and oxidation. Cysteamine prevents an increase in pH in lysosomes, maintaining the proteolytic activity of lysosomes and reducing LDL oxidation[42]. The mechanism of lysosomal LDL oxidation likely explains the failure of common antioxidants in clinical trials to reduce free radical processes[42,43].

It remains unclear whether oxidative stress is a cause or a consequence of atherosclerosis. However, lipoprotein oxidation is thought to be one of the processes present in the early stages of atherosclerosis[4,7]. In addition, according to recent data, OxLDL is a risk factor for CVD and dyslipidemia[44].

Mechanism of LDL oxidation

The oxidation process involves transferring electrons from an electron donor to an electron acceptor. LDL oxidation can be carried out both with the participation of specialized enzymes (cyclooxygenases, lipoxygenases, cytochrome P450 monooxygenases, and myeloperoxidase) and without them (metals with variable valences)[45-48]. Moreover, LDL's protein and lipid parts are prone to oxidation[26] [Figure 2]. LPO products can form Schiff bases, and Michael adducts with ApoB-100 residues. Oxidation of the LDL protein part leads to impaired recognition of OxLDL by LDLR, increasing the circulating lifetime of LDL. Moreover, OxLDL undergoes subsequent alternative binding to scavenger receptors (SR)[15]. The primary components of the LDL lipid part, such as cholesterol esters (CE), phospholipids (PL), and triglycerides, undergo LPO, followed by the formation of hydroperoxides and aldehyde derivatives (malonic dialdehyde and 4-hydroxynonenal[10,49,50]. Furthermore, there is a clear relationship between the degree of LPO and polyunsaturated fatty acids of LDL[51]. Well-known polyunsaturated fatty acids such as arachidonic acid (AA), eicosapentaenoic acid, and linoleic acid also undergo oxidative modification. The lipoxygenase 12/15 (12/15-LOX) carries out oxidative modification of LDL direct oxygenation and LRP-mediated 12/15-LOX membrane translocation. In the former case, 12/15-LOX oxygenates esterified fatty acids in lipoproteins and phospholipids with the formation of biologically active compounds[52,53]; as a result, 12-hydroperoxyeicosatetraenoic acid (12-HPETE) and 15-HPETE from AA and 13-hydroperoxyoctadecadienoic acid (13-HPETE) from linoleic acid are formed[54,55]. In the latter case, it was shown that 12/15-LOX moves from the cytoplasm to the plasma membrane and oxidizes LDL during pre-

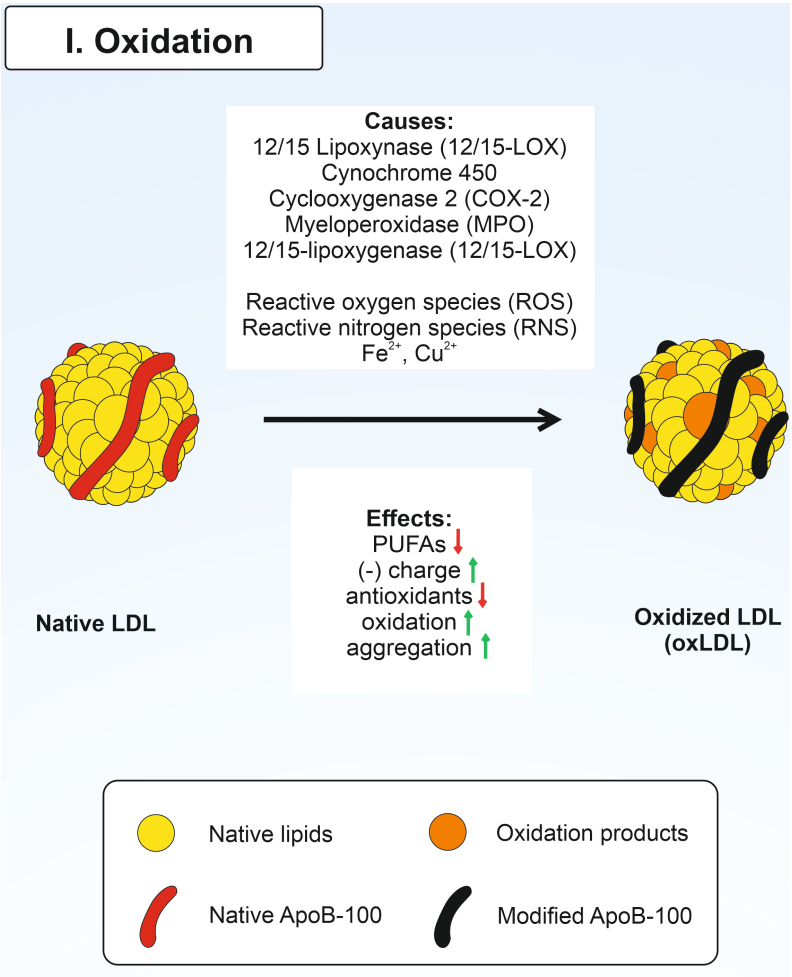

Figure 2. Oxidation of native LDL.

incubation of macrophages with LDL[56]. This process is closely related to the receptor-related protein (LRP)[57]. However, the exact mechanism of LRP-mediated 12/15-LOX membrane translocation remains unclear[58]. 12/15-LOX has been reported to be directly involved in OxLDL formation[59,60]. 12/15-LOX has been shown to have a pro-atherogenic role by affecting the area of atherosclerotic lesions and the sensitivity of LDL to oxidation in transgenic mice[46,61]. Importantly, lipoxygenase-15 has been found in atherosclerotic lesions where it can colocalize with OxLDL[62,63].

Cyclooxygenases catalyze the oxygenation of AA to prostaglandins (PGs)[64]. PGs, as a consequence, are rapidly converted to PGE2, PGD2, PGF2, and PGJ2, which initiate many of the responses associated with inflammation and vascular reactivity[65]. In addition, there is evidence of pro-atherogenic and anti-atherogenic effects of cyclooxygenase 2 (COX-2)[45,66]. The various biological effects of COX-2 products are determined by the type of tissue in which they are produced[66]. Cytochrome P450 monooxygenases catalyze the conversion of AAs to epoxyeicosatrienoic acids[64,67], which regulate vascular tone and are associated with anti-inflammatory effects[68-70].

Another enzyme responsible for LDL oxidation is MPO[71]. It has been reported that there is an association between serum MPO levels and acute coronary syndrome[72,73]. MPO forms a range of reactive compounds

such as hypochlorous acid (HOCl), chloramines, tyrosyl radicals, and nitrogen dioxide (NO_2) that oxidize the protein, lipid, and antioxidant components of LDL[47]. In addition, myeloperoxidase-oxidized LDL (Mox-LDL) is found in atherosclerotic lesions and in the blood of patients with atherosclerosis[14,15].

Metals with variable valences, such as iron and copper ions, are involved in LDL oxidation by interacting with lipid hydroperoxides[48]. Moreover, there is evidence of the participation of ROS in LDL oxidation by forming hydroxyl radicals from hydrogen peroxide and interacting with superoxide radicals[74,75]. It is essential to note that the primary enzymatic sources of ROS are NADPH oxidase, endothelial nitric oxide synthase (eNOS), and xanthine oxidase (XO)[76]. Likely, the generation of reactive nitrogen species such as peroxynitrite may contribute to LDL oxidative modification[47].

Effects of oxidized LDL

The oxidation process of native LDL leads to the appearance of pro-atherogenic properties in modified LDLs. Oxidative modification of the protein part impairs LDL recognition by LDLR. Macrophages actively take up OxLDL by scavenger receptors (CD36, SR-A, and SR-B1) and lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) to remove excess OxLDL from the arterial wall[77-79]. OxLDL may be involved in ROS production, leading to endothelial cell damage[80]. OxLDL stimulates monocyte adhesion and triggers the differentiation of intimal monocytes into resident macrophages[81]. Furthermore, OxLDL induces the production of pro-inflammatory cytokines and chemokines by monocytes, endothelial cells, and smooth muscle cells (SMCs)[82]. In addition, OxLDL inhibits autophagy, a cellular pathway for the degradation of organelles and macromolecules. Autophagy leads to excessive accumulation of intracellular lipids and endothelial cell apoptosis[11]. Thus, excessive uptake of OxLDL by macrophages leads to lipid accumulation followed by foam cell formation, which is the hallmark of the early stages of atherosclerosis[83]. Mox-LDL causes endothelial dysfunction through the uptake by LOX-1, contributing to the initiation or development of atherosclerosis[14]. The use of substances lowering the uptake of OxLDL, such as dioscin and nifedipine, can reduce the development of atherosclerosis[84,85].

As mentioned earlier, these lines of evidence support the involvement of OxLDL in atherosclerosis in the early stages. However, most of the experiments were carried out using cell cultures, so further studies in animal models are required.

ENZYME-MODIFIED NON-OXIDIZED LDL

Mechanism of enzymatic non-oxidative modification of native LDL

According to the hypothesis of the enzymatic non-oxidative LDL modification, native LDLs infiltrate the intima and are enzymatically modified[86]. Some enzymes can modify LDL *in vitro* without oxidation processes. Such modified LDL particles are called enzyme-modified non-oxidized LDL (eLDL) [Figure 3]. Enzymatic non-oxidative modification of LDL occurs due to the activity of ubiquitous hydrolytic enzymes such as matrix metalloproteases 2 and 9, plasmin, trypsin, CEase, chymases, sphingomyelinase (SMase), phospholipase A2 (PLA_2) and cathepsins H, G and F[16,42,87-92]. eLDL is prone to aggregation and fusion, increasing lipoprotein retention by human aortic proteoglycans due to active ApoB-100 lysine residues[92]; eLDL phagocytosed by macrophages is transported to lysosomes, where it can be oxidized[42].

Hydrolytic enzymes affect the protein and lipid parts of LDL. Plasmin can induce fragmentation of LDL ApoB-100, while trypsin and cathepsins proteolyze the protein part of LDL, followed by the formation of aggregates from its fragments[90,93]. Lipolytic modifications of LDL are carried out by lipases such as SMase and PLA_2. SMase catalyzes the hydrolysis of sphingomyelins by forming ceramides and phosphorylcholines[91]. Moreover, eLDL has a lower density and exhibits less electrophoretic mobility than

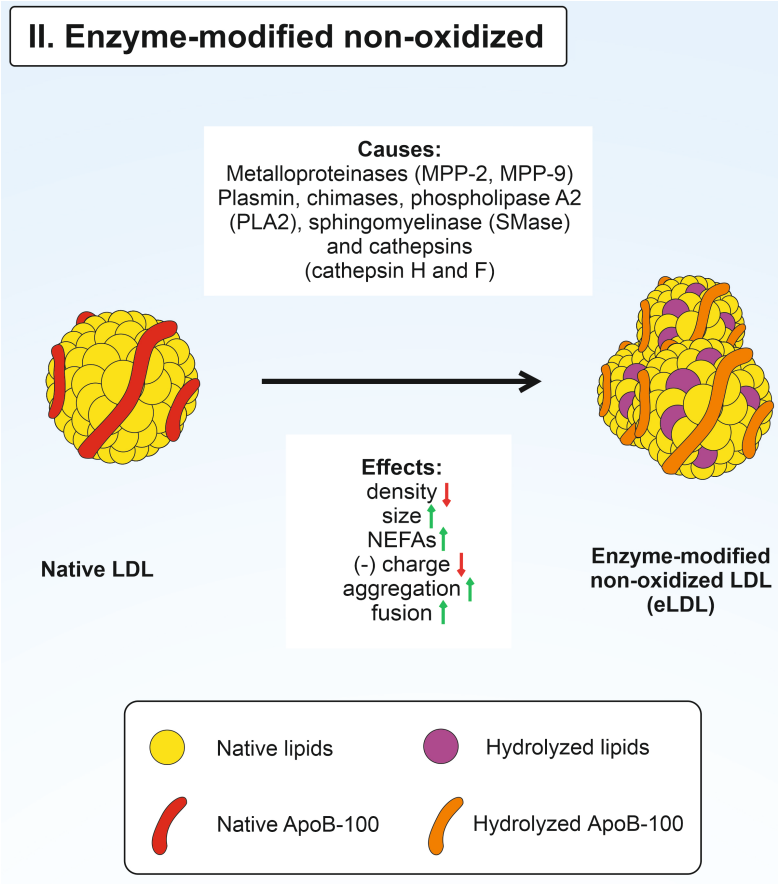

Figure 3. Enzymatic non-oxidative modification of native LDL.

native LDL, indicating its lower negative charge[93,94]. PLA_2 degrades LDL phospholipids to form non-esterified fatty acids and lysophospholipids[95]. Treatment of native LDL with SMase leads to aggregation and fusion of particles, while incubation of LDL with PLA_2 leads only to aggregation[92]. Thus, this event leads to their increased uptake by macrophages[96]. There is also a lipoprotein-associated PLA_2 that preferentially hydrolyzes oxidized phospholipids of OxLDL [97]. Human aortic proteoglycans actively retain such an eLDL[86,92].

The LDL core lipids, such as cholesteryl esters and triglycerides, may undergo hydrolysis. CEase catalyzes the conversion of cholesterol ester to non-esterified cholesterol and fatty acid. CE hydrolysis leads to the formation of cholesterol crystals found in early and advanced atherosclerotic lesions[98-100]. Furthermore, eLDL induces intracellular lipid accumulation by cells and promotes the formation of lipid droplets[10].

It is most likely that native LDL undergoes lipolytic and proteolytic modifications. It has been shown that the combined treatment of native LDL with trypsin, Cease, and neuraminidase leads to the formation of eLDL with characteristics similar to the eLDL from atherosclerotic lesions[86,101].

Effects of enzyme-modified non-oxidative LDL

It is essential to note that eLDL has pro-atherogenic properties. It is actively taken up by SMCs of coronary arteries and macrophages derived from monocytes[101]. Uptake of eLDL has been shown to occur via micropinocytosis and is independent of scavenger receptors[94]. Nevertheless, eLDL activates the major

OxLDL scavenger receptor LOX-1 in SMCs[94]. Thus, eLDL aggregation and fusion have been shown to promote eLDL uptake by macrophages and foam cell formation[42]. There is evidence of an association between the aggregation-susceptibility of LDL particles and future cardiovascular events in patients with atherosclerosis[102,103].

There is evidence of the association of eLDL with inflammatory responses in atherosclerosis. Thus, eLDL activates the complement system in a C-reactive protein-dependent fashion[104,105]. Other potential triggers for the complement system activation in the arterial wall include modified lipoproteins and cholesterol crystals[106,107]. Modified and aggregated eLDL activate macrophages and induce the secretion of pro-inflammatory cytokines and chemokines. Incubation of THP-1-derived macrophages with SMase-LDL induces secretion of TNF, IL-1β, IL-6, and MCP-1[42,98]. Treatment of native LDL with PLA_2 or SMase leads to the generation of pro-inflammatory lipid mediators from AA[108].

eLDL triggers migration and osteogenic differentiation of SMCs and OxLDL[16,109,110]. The development of the advanced atherosclerotic lesion is characterized by plaque calcification and SMC migration from the media to the intima of the arterial wall. SMCs play an essential role in forming the protective fibrous cap; therefore, their dysfunction usually leads to plaque rupture[111]. eLDL has been found in atherosclerotic lesions and aortic valve sclerosis[16,112,113].

In general, eLDL is involved in the pathological processes that characterize early and advanced atherosclerotic lesions. Pharmacological reduction of SMase and PLA_2 activity would decrease eLDL formation[114,115]. Moreover, using substances inhibiting macropinocytosis and eLDL aggregation is a promising approach for atherosclerosis treatment[16,116]. However, further research is needed on the many mechanisms of eLDL formation and their effects due to the ubiquitous presence of various hydrolytic enzymes in atherosclerotic lesions.

DESIALYLATED LDL

The desialylation modification hypothesis

Chazov *et al.* first reported serum atherogenicity in patients with CHD in 1986[117]. The addition of serum from patients with CHD to a primary culture of human aortic intima subendothelial cells increased intracellular lipid accumulation, in contrast to serum from healthy patients. The separation of lipoproteins into classes made it possible to identify LDL as an atherogenic serum component of CHD patients[118,119]. Further study of lipoproteins showed that atherogenic LDL is desialylated. This atherogenic LDL had decreased sialic acid level (by 2-5 times) compared to LDL isolated from non-atherogenic plasma. Furthermore, native LDL from healthy donors became atherogenic after incubation with neuraminidase from *Clostridium perfringens*[9]. Subsequently, a subfraction of desialylated LDL was isolated from the atherogenic serum of CHD patients using lectin blotting with *Ricinus communis*[120,121]. Moreover, multi-modified LDL is significantly different from native LDL; the densest subfractions of desialylated LDL caused intracellular lipid accumulation in cell culture[122]. Finally, the trans-sialidase, which transfers sialic acids from protein donors to other acceptors, has been isolated from human atherogenic serum[123]. The discovery of this enzyme and the results of subsequent studies led to conclusions about the possible effect of trans-sialidase on the initiation and development of atherosclerosis[124].

Despite these facts, there is a limited understanding of the nature of the desialylation process and its role in atherosclerosis. Further research on enzymatic and non-enzymatic desialylation mechanisms is required.

Sialylation

Sialylation is the glycosylation process of biological molecules such as glycolipids and glycoproteins. Sialylation plays a significant role in many biological processes, including embryonic development, reprogramming of somatic cells, immune responses, and oncogenesis[125-127]. It is essential to note that sialylation is carried out by sialyltransferase, which attaches activated cytidine-5'-monophosphate-sialic acid to the end of the O-glycan of serine or threonine or the terminal N-glycan of asparagine. Sialic acids are usually attached either to galactose or N-acetylgalactosamine residues of glycans at the α-2,3- or α-2,6-bond position or to other sialic acid moieties via α-2,8- or α-2,9 bonds.

Mechanism of LDL desialylation

Sialic acids can be cleaved from gangliosides by specific enzymes such as sialidases and without them. This process is called desialylation [Figure 4]. Desialylation and sialylation are essential parts of sialic acid metabolism[128]. However, desialylation is often associated with pathological processes such as sporadic prion disease, infectious diseases, and atherosclerosis[123,129,130]. Desialylation affects endothelial permeability in atherosclerosis. A recent study reported visualization of changes in the vascular endothelium after exposure to neuraminidase[131]. The enzyme cleaved the sialic acid residues, which reduced the depth of the endothelial glycocalyx and increased vascular permeability.

Desialylation may occur non-enzymatically. The formation of ROS can likely promote the cleavage of terminal sialic acids of glycans[132]. Previously, it was assumed that free radical processes are involved in LDL desialylation. Oxidation of LDL with copper ions led to a dose-dependent decrease in sialic acid content *in vitro*[133]. In another study, the sialic acid content on the cell surface decreased during the addition of hypoxanthine and xanthine oxidase (HX/XO) to HL60 cell culture[132]. This finding was explained by HX/XO causing the generation of a superoxide anion in the presence of metal ions of variable valence and hydrogen peroxide leading to the elimination of terminal sialic acid from glycosides. The cleavage of sialic acid from 4MU-Neu5Ac led to induced fluorescence. It is also essential to note that Neu5Ac exhibits antioxidant activity by neutralizing H_2O_2 with the formation of its oxidation product, 4-(acetylamino)-2, 4-dideoxy-D-glycero-D-galacto-octonic acid[134].

Another example of the involvement of ROS in desialylation is a study on Long-Evans Cinnamon (LEC) rats, which are used to model hepatitis[135]. The inbred mutant strain serum samples were characterized by an increased level of hydrogen peroxide, copper, and LPO. In addition, serum glycolipids from LEC rats were desialylated compared to wild-type rats. During the exposure of healthy rats to copper ions and hydrogen peroxide, a decrease in trisialic acid chains and an increase in bi- and asialic acid chains of transferrin glycans were noted, indicating desialylation[135]. Another study using rabbits demonstrated increased levels of thiobarbituric acid reactive substances as atherosclerosis progressed, suggesting an increase in LPO[136]. Increased serum sialidase activity of serum and decreased sialic acid levels in the LDL fraction were also revealed in rabbits. Despite the evidence mentioned above about the mechanism of ROS-induced desialylation, further study is required.

Enzymatic desialylation is the most studied process of the cleavage of sialic acids from glycolipids and glycoproteins[137]. Sialidases (also known as neuraminidases) are a large group of enzymes. Hydrolytic sialidases and trans-sialidases belong to the same class of exo-alpha-sialidases (EC 3.2.1.18). Mammals have four neuraminidases: lysosomal (NEU1), cytosolic (NEU2), plasmatic (NEU3), and mitochondrial (NEU4)[138-141]. Hydrolytic sialidases can cleave α2-3-, α2-6-, and α2-8 terminal sialic acids from a wide range of biomolecules. Several studies demonstrated the role of endogenous neuraminidase in atherogenesis. Hypomorphic gene expression of *Neu1* was shown to reduce inflammatory cell infiltration into vascular intima and decrease serum LDL cholesterol levels and atherosclerotic lesions size[142]. In another study,

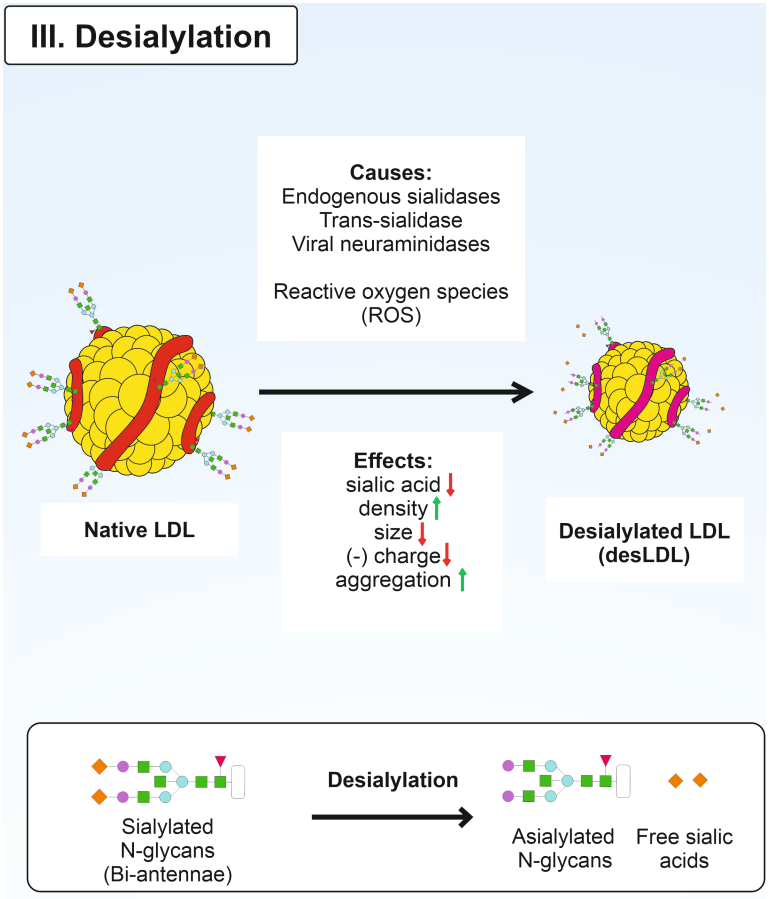

Figure 4. Desialylation of native LDL.

Neu1-deficient and Neu4-knockout *Apoe*$^{-/-}$ mice markedly slowed the development of atherosclerotic lesions compared to control *Apoe*$^{-/-}$ mice[143]. The use of endogenous sialidase inhibitors contributed to a decrease in the size of the atherosclerotic lesions in *Apoe*$^{-/-}$ mice[143]. These findings suggest that sialidase inhibition is a promising approach for atherosclerosis treatment[144].

The trans-sialidases may be responsible for LDL desialylation. Trans-sialidase, including protozoan trans-sialidase, is found in the blood of CHD patients, where it interacts with α2-3-terminal sialic acids, cleaving and transferring them to other glycolipids and glycoproteins[145,146]. As a sialic acid donor, this enzyme can use plasma lipoproteins, gangliosides, plasma protein glycoconjugates, and erythrocyte glycoconjugates. Erythrocyte glycoconjugates, plasma lipoproteins, and plasma proteins can be used as sialic acid acceptors. Sialic acid can be attached to terminal galactose, acetylgalactosamine, or other sialic acids at the α-2.3 or α-2.6 position and less commonly at α-2.8 position[145]. Native LDL incubated with trans-sialidase acquires atherogenic properties, subsequently causing intracellular cholesterol accumulation in human aortic intima SMCs[123].

The possible causes of atherogenic LDL modification could be viral and bacterial sialidases. A significant association has been found between influenza virus infection and an increased risk of acute myocardial infarction[147]. Furthermore, the influenza virus has been shown to aggravate OxLDL-induced endothelial cell apoptosis[148]. A seasonal pattern of the viral sialidase activity in the blood was also found, which might

be an additional atherogenic factor[149]. Modeling of high sialidase activity in the blood by injection of *Vibrio cholerae* neuraminidase contributed to a reduced LDL sialic acid content in wild-type mice[8].

There are other enzymes with sialidase activity. The soluble form of the Klotho protein acts as a sialidase, removing terminal α-2,6-sialic acids from the N-glycans of the TRPV5 and ROMK1 ion channel protein chain[150,151]. This enzyme regulates cell surface glycoproteins'activity, affecting the balance of Ca^{2+} and K^{+} ions. It is essential to note that the Klotho protein has anti-atherogenic effects. This enzyme attenuates OxLDL-induced oxidative stress by activating the PI3K/Akt/eNOS pathway and downregulating LOX-1 expression[152]. There is also evidence of catalytic sialidase activity of abzymes. Abzymes desialylate molecules such as glycoproteins, gangliosides, and erythrocyte membranes, likely facilitating the clearance of apoptotic cells[153].

Effects of desialylated LDL

Desialylation is a critical atherogenic modification of LDL in the early stages of atherosclerosis[8]. An inverse correlation was found between LDL sialic acid content and lipoprotein atherogenicity[20]. Moreover, there is evidence that CHD patients have increased levels of asialylated LDLs characterized by the absence of terminal sialic acids on the glycans of protein chains[154]. LDL changes its density, particle size, lipid composition, and charge after desialylation[20]. DesLDL is subjected to another atherogenic modification of LDL, such as oxidation[20]. Consequently, LDL is considered multi-modified LDL, characterized by reduced sialic acid content, changes in lipid composition, reduced particle size, and acquisition of a negative charge[13,20,155]. There is an assumption that the desLDL subfraction may be electronegative LDL[121].

Furthermore, desLDL tends to aggregate, increasing its atherogenicity[12,156]. Active uptake of multi-modified LDL by arterial cells has been demonstrated by the addition of desLDL to aortic intima SMCs[157]. This phenomenon has been confirmed in experiments on mouse models. Injection of fluorescence-labeled desLDL to *Apoe*$^{-/-}$ mice resulted in active lipoprotein uptake by arterial cells[143]. In addition, desLDL can cause intracellular lipid accumulation[158]. This finding was confirmed in a study using cultured human aortic intima cells due to the increased uptake and low rate of intracellular degradation of desLDL. It has also been shown that uptake of desLDL by macrophages can be mediated by a lectin receptor such as asialoglycoprotein receptor 1[143,159]. Increased proliferative activity and the synthesis of fibrous extracellular matrix components were found during intracellular lipid accumulation induced by desLDL[160]. These events are the first evidence of cellular aspects of atherosclerosis.

The role of LDL desialylation in atherogenesis is undeniable; nevertheless, further study of the nature of sialidase activity in humans is required. Further studies will allow the modeling of LDL desialylation in animals.

GLYCATED LDL

Mechanism of glycation and effects of gLDL

Glycation is an atherogenic LDL modification. The cause of gLDL production is glycation, i.e., non-enzymatic glycosylation in hyperglycemia[161]. Non-enzymatic glycosylation increases the total negative charge of modified LDL[162]. The carbonyl groups of glucose interact with the free amino groups of ApoB-100, resulting in the formation of a Schiff base, which is converted to Amadori products via rearrangement, which in turn are converted to advanced glycation end products (AGEs) [Figure 5]. The glycation process is revealed in patients with diabetes mellitus and metabolic syndrome[163]. Incubation of native LDL with glucose resulted in dose-dependent glycation of LDL and increased LPO *in vitro*[164]. Glycation of the free amino groups of ApoB-100 lysine occurs in the LDLR binding domain. This event leads to the decreased

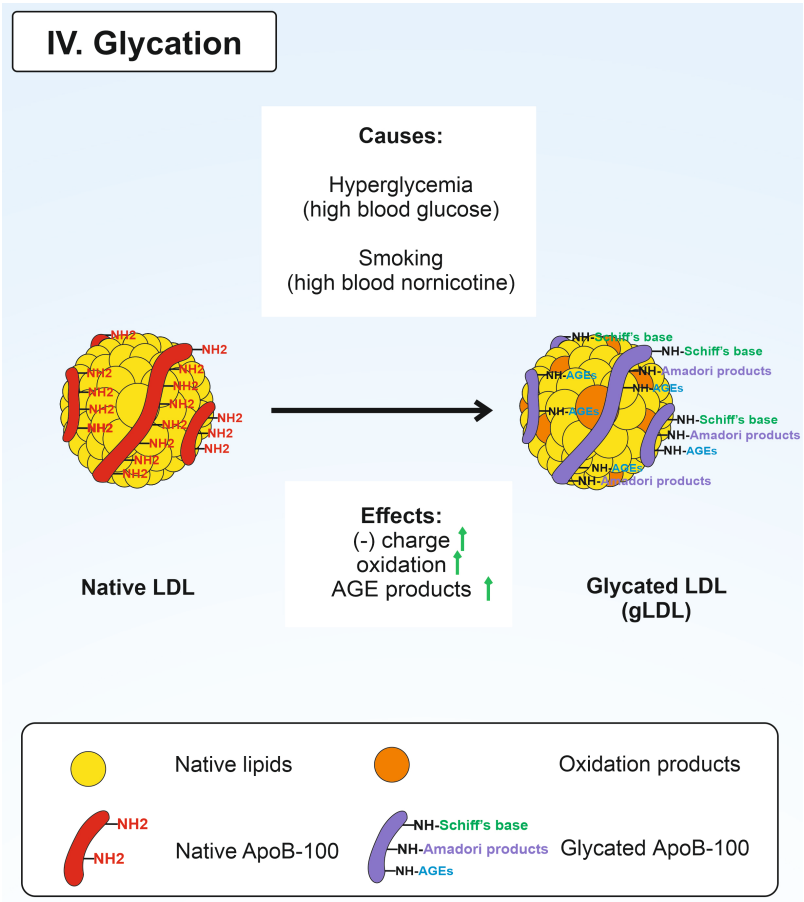

Figure 5. Glycation of native LDL.

affinity of LDL to their receptor and an increased average plasma lifetime[165,166]; gLDL binds to human macrophage scavenger receptors, which are hypothesized to promote the intracellular accumulation of CE and foam cell formation in atherosclerotic lesions[17].

The high degree of LDL glycation contributes to LDL oxidation, leading to the formation of highly oxidized LDL and increasing LDL atherogenicity[19,167]. Particles such as small dense LDL are most susceptible to glycation, even in non-diabetic patients[164,168,169]. A subfraction of *in vivo* modified atherogenic LDL was found in the blood of patients with diabetes mellitus[17]; this was a subfraction of small dense electronegative desialylated and glycated LDL that induced intracellular lipid accumulation in cell culture. It is critical to note that the synergistic effect of several LDL modifications (particularly glycation and desialylation) on the enhancement of intracellular lipid accumulation has been demonstrated in an experiment with cultured aortic SMCs obtained from healthy donors[170,171].

Non-enzymatic glycation also produces AGEs from the interaction of aldehyde groups of the reducing sugars that interact with proteins, lipids, and nucleic acids[172]. In addition to diabetes, another risk factor for CVD and myocardial infarction is smoking[173-175]. Elevated levels of ApoB-100 and albumin AGEs have been found in the blood of smokers[176]. The nicotine metabolite nornicotine explains this in the blood, which causes aberrant protein glycation[177]. The process of formation of AGE-protein adducts is irreversible. AGE-protein adducts are characterized by high stability[178]. In addition, the interaction of AGE with its receptor

leads to increased expression, oxidative stress, and the release of pro-inflammatory agents[179]. AGE formation induces atherosclerosis through the proliferation of vascular SMCs, increased expression of pro-atherogenic mediators, and vascular remodeling[180].

These findings suggest the critical role of classical risk factors for atherosclerosis, such as smoking and diabetes mellitus[181-183].

CARBAMYLATED LDL

Mechanism of LDL carbamylation

The final post-translational modification of LDL is carbamylation. The carbamoyl moiety of cyanate or thiocyanate non-enzymatically binds to the free functional amino groups of ApoB-100 LDL to form carbamylated LDL (cLDL) [Figure 6][184]. This modification changes proteins' structural and functional properties, causing partial or complete loss of functionality, disruption of protein-protein interactions, and binding to receptors[185]. Carbamylation of LDL increases the particle's electrophoretic mobility because the modified lysine's positive charge is neutralized[186].

Patients with chronic kidney disease (CKD) and smokers were found to have an increased risk of developing atherosclerosis[187,188]. The enhanced process of carbamylation explains these patterns in patients due to increased concentrations of urea and thiocyanate. Active urea breakdown results in elevated blood cyanate levels, followed by carbamylation of proteins in CKD patients[189]. This finding was confirmed in a study using oral administration of urea to *Apoe*$^{-/-}$ mice leading to an eight-fold increase in blood cLDL levels and more severe progression of atherosclerosis than in control mice[190]. Due to myeloperoxidase, there is an alternative mechanism in which thiocyanate is oxidized in the presence of H_2O_2 to cyanate in smokers[191,192].

Effects of carbamylated LDL

The atherogenic properties of cLDL are well-known; cLDL is prone to oxidation and has high cytotoxicity for endothelial cells *in vitro*[18]. Elevated levels of the soluble form of the lectin-like oxidized low-density lipoprotein receptor-1 and cLDL significantly increase the risk of CHD in patients with metabolic syndrome[193]. Moreover, cLDL activates the LOX-1 receptor, promoting a prothrombotic effect in vascular cells and platelets in mice[194]. Endothelial cell injury occurs due to the induction of autophagy proteins such as LC3-I, beclin-1, and Atg5 in response to cLDL[195]. In another study, cLDL promoted monocyte recruitment, adhesion to endothelial cells, and the proliferation of SMCs in coronary arteries[196,197]. The atherogenic effect of cLDL has been demonstrated in *Apoe*$^{-/-}$ mice with surgically-induced CKD. Uremic mice with high cLDL levels had more atherosclerotic lesions than control animals[190]. In addition, cLDL has reduced clearance from the blood, which may also lead to atherosclerosis progression[198].

There is robust evidence linking elevated cLDL levels to progressive atherosclerosis in humans and animal models. Nevertheless, the direct role of carbamylated LDL in the mechanism of atherogenesis remains to be established.

CONCLUSION

Native LDL is subjected to many modifications in pathological processes that increase lipoprotein atherogenicity. It is most likely that several types of LDL modifications are involved in atherogenesis. Further studies of atherogenesis in animal models will allow the use of modified LDL as a biomarker for diagnosing the subclinical form of atherosclerosis in humans. Likely, promising therapeutic strategies affecting atherogenesis will effectively treat atherosclerosis. Inhibitors of sialidases, sphingomyelinase, phospholipase A2, specific antioxidants, and substances that prevent the aggregation and uptake of

modified LDL by cells can be used as probable therapeutic approaches.

It is also critical to focus on the study of risk factors for atherosclerosis, including hypertension, diabetes mellitus, smoking, and obesity, which contribute to atherogenic modifications of native LDL. Studies of the association of risk factors with atherogenesis will reveal reliable biomarkers and improve the prevention of CVDs.

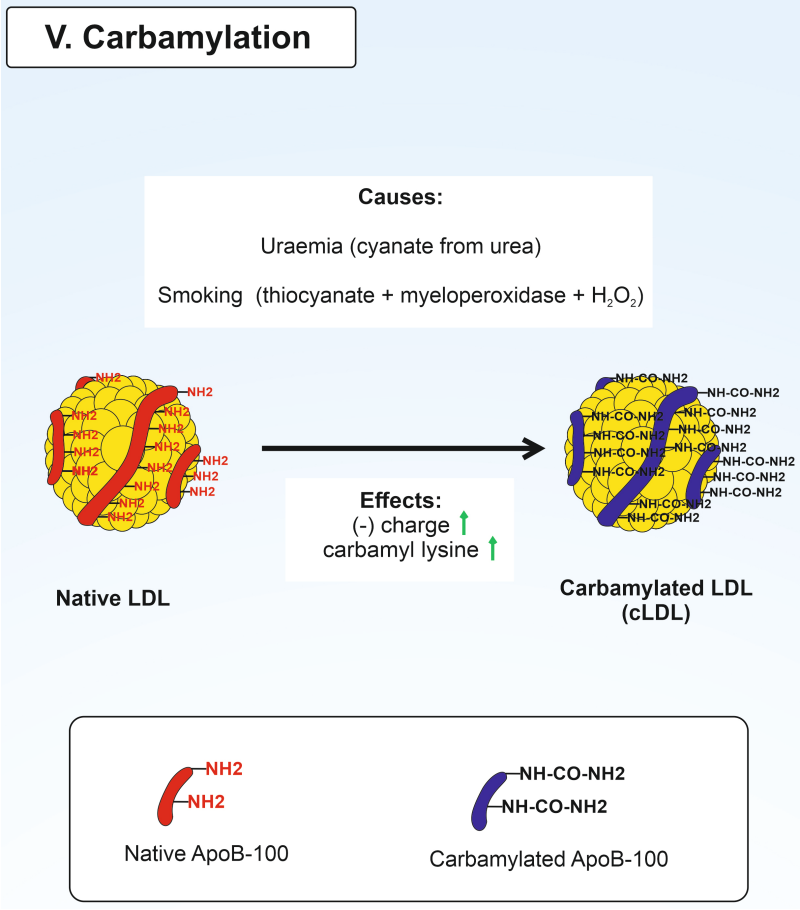

Figure 6. Carbamylation of native LDL.

DECLARATIONS

Authors' contributions

Conceptualized the manuscript: Kashirskikh DA, Guo S
Wrote the manuscript text and made a visualization: Kashirskikh DA
Reviewed the text: Bagheri Ekta M, Guo S, Grechko AV, Bogatyreva AI
Developed the methodology: Bagheri Ekta M, Chicherina NR
Completed the formal analysis: Panyod S, Guo S

Availability of data and materials

Not applicable.

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Conflicts of interest
All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate
Not applicable.

Consent for publication
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