DISCUSSION PAPERS, LECTURES, NEW TRENDS IN MEDICAL SCIENCE

MODIFICATION OF LOW-DENSITY LIPOPROTEINS BY LOW MOLECULAR WEIGHT CARBONYL PRODUCTS OF FREE-RADICAL OXIDATION OF LIPIDS AND CARBOHYDRATES PLAYS A KEY ROLE IN ATHEROSCLEROTIC LESION OF THE VASCULAR WALL AND IN ENDOTHELIAL DYSFUNCTION

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ABSTRACT

The review presents evidence of the participation of low-density lipoproteins (LDL) modified by low molecular weight dicarbonyl compounds formed during free-radical oxidation of lipids (malondialdehyde) and carbohydrates in the development of endothelial dysfunction and atherosclerotic vascular lesions. The authors believe that it is they, and not oxidized (hydroperoxide-containing) LDL, that are the main factors of pathogenesis. The role of dicarbonyl-modified LDL in LOX-1 dependent induction of processes leading to the development of endothelial dysfunction is discussed. The results of studies proving that damage to the glycocalyx (a layer of macromolecules that prevent the development of endothelial dysfunction) covering the luminal surface of the endothelium is caused by hyperproduction of reactive oxygen species. Ways of pharmacological correction of free-radical oxidation processes are discussed, due to which inhibition of atherogenesis and diabetogenesis can be achieved.

Key words: *malondialdehyde, methylglyoxal, endothelial dysfunction, glycocalyx, low density lipoproteins, free radicals, atherosclerosis, diabetes mellitus*

For citation: Lankin V.Z., Tikhaze A.K., Kosach V.Ya., Konovalova G.G., Kudryashova A.V. Modification of low-density lipoproteins by low molecular weight carbonyl products of free-radical oxidation of lipids and carbohydrates plays a key role in atherosclerotic lesion of the vascular wall and in endothelial dysfunction. *Acta biomedica scientifica*. 2023; 8(3): 14-24. doi: 10.29413/ABS.2023-8.3.2

Received: 15.02.2023
Accepted: 26.06.2023
Published: 11.07.2023

МОДИФИКАЦИЯ ЛИПОПРОТЕИДОВ НИЗКОЙ ПЛОТНОСТИ НИЗКОМОЛЕКУЛЯРНЫМИ КАРБОНИЛЬНЫМИ ПРОДУКТАМИ СВОБОДНОРАДИКАЛЬНОГО ОКИСЛЕНИЯ ЛИПИДОВ И УГЛЕВОДОВ ИГРАЕТ КЛЮЧЕВУЮ РОЛЬ В АТЕРОСКЛЕРОТИЧЕСКОМ ПОВРЕЖДЕНИИ СТЕНКИ СОСУДОВ И ДИСФУНКЦИИ ЭНДОТЕЛИЯ

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РЕЗЮМЕ

В обзоре приводятся доказательства участия липопротеидов низкой плотности (ЛНП), модифицированных низкомолекулярными дикарбонильными соединениями, образующимися при свободнорадикальном окислении липидов (малоновый диальдегид) и углеводов, в развитии дисфункции эндотелия и атеросклеротического поражения сосудов. Авторы полагают, что именно они, а не окисленные (гидропероксид-содержащие) ЛНП являются основными факторами патогенеза. Обсуждается роль дикарбонил-модифицированных ЛНП в LOX-1-зависимой индукции процессов, приводящих к развитию дисфункции эндотелия. Рассматриваются результаты исследований, доказывающих, что к повреждению покрывающего люминальную поверхность эндотелия гликокаликса – слоя макромолекул, препятствующего развитию дисфункции эндотелия, – ведёт гиперпродукция активных форм кислорода. Обсуждаются пути фармакологической коррекции процессов свободнорадикального окисления, благодаря которой может достигаться торможение процессов атерогенеза и диабетогенеза.

Ключевые слова: *малоновый диальдегид, метилглиоксаль, дисфункция эндотелия, гликокаликс, липопротеиды низкой плотности, свободные радикалы, атеросклероз, сахарный диабет*

Для цитирования: Ланкин В.З., Тихазе А.К., Косач В.Я., Коновалова Г.Г., Кудряшова А.В. Модификация липопротеидов низкой плотности низкомолекулярными карбонильными продуктами свободнорадикального окисления липидов и углеводов играет ключевую роль в атеросклеротическом повреждении стенки сосудов и дисфункции эндотелия. *Acta biomedica scientifica*. 2023; 8(3): 14-24. doi: 10.29413/ABS.2023-8.3.2

Статья поступила: 15.02.2023
Статья принята: 26.06.2023
Статья опубликована: 11.07.2023

In the middle of the last century, Denhem Harman hypothesized that aging is associated with the accumulation of cell damage caused by products of spontaneous free-radical oxidation (FRO) [1, 2]. Since such pathologies as atherosclerosis and diabetes mellitus can be attributed to diseases of old age, D. Harman suggested that the emergence and development of these pathological conditions (he called them "free-radical diseases") are associated with the damaging effect of free-radical reactions [3]. J. Glavind et al. [4] in 1952 were the first to suggest that free-radical oxidation of lipids might be one of the triggering factors of atherosclerotic lesion of the vascular wall. Based on the analysis of autopsy materials, these authors concluded that the level of lipoperoxides in human aorta with atherosclerotic lesions is always higher than in the unaffected vascular wall. Unfortunately, a small number of samples were studied in this work, and an insufficient method of analysis was used to analyze the lipoperoxide content, which, as it was found later [5], is not specific enough. In spite of this, during the following decade the conclusions of the work of J. Glavind et al. did not raise doubts. Only in 1965, F.R. Woodford et al. [5] made an attempt to experimentally verify these results, using a highly specific method of iodometric titration with amperometric equivalence point determination developed earlier for the analysis of lipoperoxides [6]. The results obtained by F.R. Woodford et al. practically refuted the conclusion of J. Glavind et al. as statistically significant differences between the content of lipoperoxides in atherosclerotic lesion areas and intact areas of the aorta in autopsies could not be detected by these authors. The pessimistic conclusions of the publication by F.R. Woodford et al. cooled the interest in the free-radical theory of atherogenesis for a long time, despite its theoretical justification given in the articles by D. Harman [1–3]. At the same time, we detected an increase in the content of free radical oxidation products in the aorta of animals with experimental atherosclerosis [7]. Only two decades later our group using an adequate method of high-performance liquid chromatography (HPLC) proved a significant increase in the content of primary products of FRO – lipid hydroperoxides (LOOH) [8, 9] in the aorta damaged by atherosclerosis (compared to the unaffected area of the vascular wall), which increased with the progression of atherosclerotic lesion [8, 9]. It should be noted that these unique studies were performed using autopsy material during rapid autopsies of people who died in car accidents within 3 hours after the confirmation of death, i. e., when native samples were analyzed [8, 9]. (HPLC on a chiral phase column) with ratio of S- and R-stereoisomers proved that LOOHs detected in atherosclerotically damaged aorta are the result of spontaneous (non-enzymatic) free-radical oxidation of unsaturated lipids [8, 9]. Cholesterol esters is the major class of lipids accumulating in the areas of atherosclerotic lesions of the vascular wall [10, 11], and not only fatty acid residues [8, 9] but also the sterol part of the molecule are subjected to oxidation [10, 12]. A decrease in the activity of key antioxidant enzymes (Se-containing glutathione peroxidase (GSH-Px) and Cu,Zn-superoxide dismutase (Cu,Zn-SOD)) was also detected in the areas of human aortic atherosclerotic lesions, progressing with the increasing degree of damage [9, 13]. Then the hypothesis of imbalanced systems of FRO product formation and utilization in atherosclerosis was formulated [9, 14]. The same data provided convincing grounds for classifying atherosclerosis as a "free-radical pathology", i. e., a disease whose pathogenesis is strongly influenced by FRO processes [9, 14].

It should be noted that a significant increase in the level of primary and secondary products of lipid free-radical oxidation was detected in a representative epidemiological study in blood plasma of probands with diagnosed atherosclerosis [7, 9, 14]. In the same study, decreased activity of erythrocyte GSH-Px, LOOH utilizing enzyme, was found in patients with atherosclerosis [9, 14]. Based on these results, it could be assumed that nanoparticles of the lipid-transporting system – blood plasma lipoproteins – undergo oxidation during atherogenesis [7, 9, 14]. Indeed, it has been shown that "atherogenic" low-density lipoproteins (LDL) easily undergo oxidation both when incubated in the presence of vascular endotheliocytes and in the presence of free-radical oxidation initiators [15, 16]. It was found that chemical modification of blood plasma LDL particles with acetaldehyde makes them more «atherogenic» [17], i. e. capable of binding to the scavenger receptor and accumulating in macrophages of the vascular wall [17]. Later, numerous studies found that LDL particles subjected to free-radical oxidation also become «atherogenic» [18–25].

It is recognized that lipid FRO is a two-stage process: first, primary – unstable – LOOH oxidation products are formed. They further undergo oxidative degradation and form low molecular weight dicarbonyls, i. e. secondary products [26]. Consequently, with a sharp increase of LOOH in tissues, oxidative stress during atherogenesis must inevitably be accompanied by the accumulation of such active carbonyl products as hydroxynonenals and malondialdehyde (MDA), i. e., converted to carbonyl stress [14, 26]. In turn, aldehyde groups of dicarbonyls can easily react with the amino end groups of proteins by the Maillard reaction to form intra- and intermolecular cross-links in their molecules [26]. The possibility of MDA participating in modification of LDL apoprotein B-100 has been established [27], but nevertheless, the question of the mechanism of LDL oxidative modification, due to which LDL particles acquire "atherogenicity", has not been solved so far [14].

In strict terminology, "oxidized" LDLs contain hydroperoxy acyls in the phospholipids of the outer layer of the particles. Fundamentally, the accumulation of hydroperoxy acyls in the outer phospholipid monolayer of LDL can lead to changes in apoprotein B-100 conformation. Thus, during free-radical oxidation of unsaturated ("liquid") acyls of membrane phospholipids, an increase in membrane microviscosity is detected [9,

28] due to the "pushing" or "pulling" of more polar hydroperoxy acyls into the aqueous phase, as the relative content of saturated («solid») fatty acid residues increases in the membrane [9, 28]. It is highly likely that when fundamental properties of biomembranes such as microviscosity and polarity are significantly altered, the conformation of peripheral and integral proteins embedded in the phospholipid bilayer may be altered. In particular, we found a multidirectional change in the activity of membrane-bound enzymes in the same membrane during free-radical oxidation of liver microsomes biomembranes: the activity of some enzymes (sensitive to oxidation) decreased, while that of others (resistant to oxidation) increased [29], which can be explained by a physical change in the conformation of the molecules of these proteins when the physicochemical properties of membrane lipids change. Based on these results, it could be assumed that oxidation of phospholipids in LDL particles would lead to a change in the apoprotein B-100 conformation, as a consequence of which the efficiency of binding of such "oxidised" LDLs to the scavenger receptor of macrophages would also be changed.

The *in vitro* induction of LDL free radical oxidation using various initiators (such as azo initiators, hydrogen peroxide, superoxide anion radicals, metal ions of variable valency, etc.) leads to an increase in the concentration of both primary (LOOH) and secondary lipoperoxidation products (MDA) [30, 31]. Therefore, it is obvious that it is impossible to determine which lipid FRO products cause "atherogenic" modification of LDL particles using standard approaches. We were able to obtain truly oxidized LDLs without an admixture of MDA-modified LDLs [31] using a homogeneous preparation of rabbit reticulocyte 15-lipoxygenase capable of oxidizing polyene acyls of phospholipids [32]. At the same time, MDA-modified LDLs without an admixture of oxidized (LOOH-containing) LDL were obtained by incubation of LDLs with MDA [31]. When studying atherogenicity (efficiency of LDL particles capture by cultured human macrophages) of the two obtained LDL modifications, we experimentally proved that not oxidized (LOOH-containing LDLs) but exclusively MDA-modified LDLs bind to the scavenger receptors of macrophages [31]. Consequently, LDL particles modified by natural dicarbonyls rather than oxidized LDLs should be efficiently captured and accumulated in lipid vacuoles of vascular wall cells [31]. This leads to preaterosclerotic lesions of vascular walls and the transformation of macrophages and smooth muscle cells into "foam cells" forming lipoidosis zones [9, 14]. The obtained results do not just clarify the existing terminology, but are of principal character, since they substantiate the existence of a quite definite molecular mechanism of "atherogenic" modification of LDL particles with the participation of natural low-molecular carbonyl compounds. It was also found that the most cholesterol-rich LDL particles are also simultaneously MDA-modified [33]. Therefore, carbonyl modification of LDL particles may contribute to the efficient entry of cholesterol into the vascular wall [33]. In addition, there is evidence that increased accumulation of MDA-modified LDLs is characteristic of patients with certain mutations of apoprotein B-100, i. e. there is a possibility that carbonyl modification of LDLs may be genetically determined [34].

Protein molecules Cu,Zn-SOD and GSH-Px, similarly to LDL apoprotein B-100, also undergo modification during MDA accumulation during atherogenesis [35, 36], which is accompanied by suppression of their activity due to conformational changes in the structure of the active centre [35, 36]. It is obvious that dicarbonyl-dependent inhibition of antioxidant enzyme activity during atherogenesis must result in stimulation of oxidative stress. Thus, the development of oxidative (LOOH accumulation) and subsequent carbonyl stress (MDA accumulation) during atherogenesis leads to the formation of dicarbonyl-modified LDLs, which are the key factor causing preterogenic damage to the vascular wall and subsequent formation of atherosclerotic plaques [14].

Although the available literature attributes diabetes mellitus as a risk factor for atherosclerosis or a contributing factor to its development, a large number of diabetic patients die due to vascular incidents [37–39], no convincing pathophysiological explanation is provided. Nevertheless, an important role of FRO in the pathogenesis of diabetes mellitus has been hypothesized for quite some time [40]. The basis of this hypothesis is the assumption that in diabetes mellitus initially develops carbonyl rather than oxidative stress [41], in which active dicarbonyls such as glyoxal and methylglyoxal formed during oxidative transformations of glucose accumulate [41–43]. Glyoxylation during autoxidation of glucose and other hexatomic carbohydrates leads to the formation of glyoxal. Methylglyoxal is synthesized during enzymatic oxidation of glucose with the formation of triosophosphates [41, 44, 45]. Methylglyoxal, as we have shown, can also be formed when glucose derivatives are attacked by lipoperoxyl free radicals, i. e. non-enzymatically [46]. High blood glucose level in patients with type 2 diabetes mellitus contributes to LDL co-oxidation and a sharp increase in the rate of LDL lipid FRO accompanied by superoxide anion radical formation [47]. In the Maillard reaction, the interaction of methylglyoxal and amino end groups of apoprotein B-100 LDL can also generate superoxide radical [48]. Thus, diabetogenesis, unlike atherogenesis, is characterized by the primary development of carbonyl stress (accumulation of active carbonyl compounds), and at later stages, reactive oxygen species (ROS) generated by the reactions described above induce secondary oxidative stress.

On this basis, the stages of carbonyl stress development and subsequent oxidative stress characterized by the accumulation of various oxidation products should be distinguished during diabetogenesis. The accumulation of glyoxal and methylglyoxal in the blood plasma of diabetic patients has been repeatedly confirmed experimentally [41–43]. At the same time, the presence of oxidative stress in diabetes is evidenced by a de-

crease in telomere length in blood nuclear cells [49], as well as an increase in the level of 8-hydroxy-2'-deoxyguanosine, the end product of oxidative DNA destruction, in the blood and urine of type 2 diabetic patients [49]. It should be noted that 8-hydroxy-2-deoxyguanosine is a recognized biomarker of oxidative stress [50]. Its accumulation is not associated with the development of carbonyl stress. Increased levels of LOOH-containing LDLs [41] in the blood of type 2 diabetes patients also suggests that secondary induction of oxidative stress may indeed occur during atherogenesis. Similar to atherosclerosis, type 2 diabetes patients have increased carbonyl modification of LDLs [49] and a sharp drop in erythrocyte Cu,Zn-SOD and GSH-Px activity [49, 51], which is a characteristic reflection of carbonyl stress.

A significant increase in glyoxal and methylglyoxal levels in the blood of type 2 diabetes patients [41-43] can induce LDL modification, which is recognized by scavenger receptors of macrophages and thus can induce LDL accumulation in the vascular wall with subsequent development of lipoidosis lesions [41]. It has been shown that LDL modification by methylglyoxal significantly increases the "atherogenicity" of LDLs (increases their receptor capture by macrophages) [41, 52]. Based on the above data, we hypothesized a single molecular mechanism of vascular wall damage in atherosclerosis and diabetes mellitus, which includes increased chemical modification of LDL apoprotein B-100 by dicarbonyls accumulated during free radical oxidation of lipids in atherosclerosis or autoxidation of glucose molecules in diabetes mellitus [47]. This hypothesis satisfactorily explains the reasons for the stimulation of atherogenesis in diabetes and the fact that diabetes may increase the risk of atherosclerosis [47].

As it has been found in recent years, oxidized LDLs also play an important role in causing endothelial dysfunction [53–56]. The endotheliocyte scavenger receptor LOX-1 is thought to bind to oxidized LDLs, causing the expression of NADPH-oxidase, which generates superoxide anion radical, causing endothelial cell damage [57]. We found that strong expression of LOX-1 and NADPH-oxidase biosynthesis in human endotheliocytes is induced by culturing cells in the presence of dicarbonyl-modified (MDA-, glyoxal-, and methylglyoxal-modified) LDLs [58]. Consequently, the initial stages of vascular endothelial dysfunction, a process that plays a leading role in atherogenesis and diabetogenesis, are likely to be directly dependent on the formation of dicarbonyl-modified rather than "oxidized" LDLs. As a result, superoxide-dependent endotheliocyte damage provokes stimulation of apoptosis and endothelial cell death [53, 56, 57], which, in turn, obviously facilitates the penetration of modified LDLs into the vascular wall.

We have found that the enzyme antioxidant system of endotheliocytes is represented mainly by special classes of enzymes – peroxiredoxins [59], which, in accordance with our data, like Cu,Zn-SOD and GSH-Px [35, 51], are very sensitive to the inhibitory action of low-molecular-weight dicarbonyls accumulated under oxidative and carbonyl stress [60]. There is no doubt that suppression of peroxiredoxin activity attenuates the antiradical defence of endothelial cells, contributing to endothelial damage and dysfunction. Thus, the data obtained suggest that the formation of carbonyl-modified LDLs is a key factor in the development of endothelial dysfunction, a process that plays a leading role in atherogenesis and diabetogenesis.

Endothelial dysfunction must precede damage to the endothelial glycocalyx. The glycocalyx is a protective layer of macromolecules (such as proteoglycans and glycoproteins) covering the luminal surface of endotheliocytes [61, 62]. Damage to the glycocalyx is considered to be the earliest stage of vascular wall damage in various pathologies [63–66]. Glycocalyx controls the permeability of the vascular wall [67] and the adhesion of blood formed elements on endotheliocytes [68, 69]. In addition, the glycocalyx protects the endothelium from damaging factors such as viruses, pro-inflammatory cytokines and ROSs [70, 71]. It is likely that glycocalyx layer is the barrier preventing atherogenic LDLs (obviously, dicarbonyl-modified LDLs) from penetrating into the subendothelial space of the vascular wall [72]. A decrease in glycocalyx thickness due to its fragmentation has been observed in the process of ROS hyperproduction ("oxidative burst") during ischemia and/or ischemia/reperfusion [73–75], as well as an increase in the level of oxidized LDLs [76, 77]. These facts suggest that oxidatively modified LDLs (most probably dicarbonyl-modified LDLs), formed by oxidative and carbonyl stress, are the most important factors in atherogenesis. Consequently, preservation of the glycocalyx should prevent atherogenesis and diabetogenesis. Damage to the glycocalyx can be considered as the first step in atherosclerotic vascular damage.

The above proves that it is logical to use antioxidants to suppress lipoperoxidation in LDLs, and several clinical studies have used natural antioxidants such as vitamin E (α-tocopherol, α-TOH) for this purpose. In contrast to the very encouraging positive results obtained from studies involving animals with experimental atherosclerosis, trail data on antioxidant intervention (predominantly α-TOH, in some cases in combination with ascorbate and/or β-carotene) in cardiovascular diseases are quite ambiguous [78–82]. In randomized, double-blind, placebo-controlled trials, the use of antioxidant vitamins was found to statistically significantly reduce the risk of cardiovascular disease and cardiac mortality [83–85]. Moreover, angiography was performed as a control in one of the few studies [86], and the suppression of coronary stenosis in patients treated with antioxidants was documented [86]. Studies involving large cohorts of men [87] and women [88] have demonstrated that regular consumption of α-TOH for several years contributes to a statistically significant reduction in the risk of coronary artery disease (CAD) [87, 88]. More than 2000 patients involved in the Cambridge Heart Antioxidant Study (CHAOS) with angiographically confirmed atherosclerosis received high (400–800 IU/day) doses of α-TOH

for one year, and a statistically significant reduction in the risk of myocardial infarction was detected [89]. A statistically significant reduction in myocardial infarction incidents was noted during the SPACE study involving patients with CAD who were treated with hemodialysis and therapy including 800 IU/day of α-TOH for almost 1.5 years [90]. However, several other clinical trials have not demonstrated statistically significant reductions in cardiovascular complications and/or reductions in cardiac incident mortality with antioxidant administration [91–94]. For example, a study including a large number of male smokers who received α-TOH and/or β-carotene for 5–8 years did not show statistically significant increase in cardiovascular mortality [91]. In the GISSI-Prevenzione Trial, administration of 450 IU/day of α-TOH to patients with post-myocardial infarction (about 3 months) was not effective in reducing mortality, nor in reducing the incidence of new infarctions or strokes for 3.5 years [92]. In the Heart Outcomes Prevention Evaluation Study (HOPE), more than 1500 patients at high risk of cardiovascular disease who received 400 IU of α-TOH per day for 4.5 years showed no statistically significant reduction in cardiovascular mortality [93]. In the MRC/BHF Heart Protection Study, more than 20,000 patients with CAD who received an antioxidant vitamin complex (900 IU/day of α-TOH) for 5 years showed no increase in infarction- and stroke-related mortality [94]. The results of such studies, which, contrary to expectations, have not provided clear positive results regarding the use of antioxidants (including extremely high doses of α-TOH), gave a reason to believe that the antioxidants used had a negative effect [78, 79]. Obviously, it is not correct to interpret the lack of effect as a negative effect, but the ambiguity of results on the use of antioxidants in the clinic makes it necessary to critically analyze the reasons for this. It is important to note that no studies have found negative effects of antioxidants (e. g., increased mortality and/or cardiac complications), only a lack of expected positive effects. Based on the design of the conducted studies, the principles of selection of the used antioxidants and their doses, the criteria for the assessment of biochemical and clinical changes, it seems obvious that the results of such studies a priori cannot give an unambiguous answer to the questions posed in them. We might agree with the statements of ardent opponents of further research on the use of antioxidants in cardiology about the uselessness (or even pointlessness) of continuing such research [78, 79]. However, we should not radically change approaches to planning and conducting work.

It should be noted that the choice of α-TOH (vitamin E) as an antioxidant used in most of the above studies cannot be considered as sufficiently successful and justified. It is known that α-TOH, like other fat-soluble vitamins, is transported in the body as part of the hydrophobic lipid core of LDL particles [95]. Nevertheless, the protection of circulating LDL particles from free-radical oxidation in the bloodstream is performed not by α-TOH, but by the reduced (phenolic) form of coenzyme Q_{10} [9, 96–100]. Based on the fact that in 1 LDL particle only 1–2 molecules of coenzyme Q_{10} account for approximately 650 molecules of the substrate of free-radical oxidation – phospholipids [101, 102], effective inhibition of free-radical reactions in LDLs by this antioxidant is impossible without its bioregeneration, possibly involving radical intermediates α-TOH and ascorbate [102–107]. At the same time, it has been shown that administration of high doses of α-TOH does not affect LDLs oxidability in CAD patients [100]. Thus, it should be recognized that the use of α-TOH to inhibit LDL oxidability in clinical trials is not justified, and the use of coenzyme Q_{10} is more effective in protecting LDL from oxidation [9, 100] and other phenolic antioxidants, in particular, the non-toxic synthetic antioxidant probucol [9, 100, 108–110], whose efficacy in inhibiting LDL oxidation has been convincingly confirmed [9, 100]. It is obviously impermissible to apply generalizations about "negative" results obtained on the use of individual antioxidants such as α-TOH or β-carotene [91–93] to the whole rather heterogeneous group of antioxidants [79], which includes substances of different structure and mechanism of action. In addition, the data presented in this review suggest that in order to suppress atherogenesis and endothelial dysfunction it is necessary to inhibit not only the accumulation of primary products (LOOH) in LDLs, but also the accumulation of secondary products of free radical oxidation – low molecular weight dicarbonyls. There are already positive examples of effects on the intensity of free-radical oxidation using biguanides – dicarbonyl scavengers [100, 111–113] and imidazole-containing peptides [114, 115]. In particular, the use of biguanides significantly suppressed the manifestation of oxidative and carbonyl stress in diabetic patients without the administration of any antioxidants ("quasi-antioxidant effect") [100]. Obviously, preventive cardiology should aim to prevent the adverse effects of oxidative modification of LDL, because modified LDLs, as shown in this study, play an important role in the molecular mechanisms of atherogenesis and diabetogenesis. Currently, preventive cardiology is focused on the development of effective approaches to pharmacotherapy aimed at inhibiting the formation of primary and secondary products of free radical oxidation in order to control the level of potentially dangerous oxidized and modified LDLs.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

Financing

The study was financially supported by the Russian Science Foundation No. 22-15-00013.

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