Endocrinol Nutr. 2013;60(9):518–528

ENDOCRINOLOGÍA Y NUTRICIÓN

www.elsevier.es/endo

REVIEW ARTICLE

Modified lipoproteins as biomarkers of cardiovascular risk in diabetes mellitus☆

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Received 17 September 2012; accepted 19 December 2012

KEYWORDS
Diabetes mellitus;
Atherosclerosis;
Modified lipoproteins;
Cardiovascular risk;
Biomarkers

Abstract Prevention of high incidence of cardiovascular disease in diabetes is one of the challenges of endocrinology. Validation of new biomarkers that may contribute to a better assessment of cardiovascular risk and help implement treatment strategies is one of the promising approaches in research on prevention and reduction of cardiovascular risk. Modification of low density lipoprotein (LDL) is a key element in development of atherosclerotic lesions. Several pathophysiological characteristics of diabetes are crucial for the LDL of these patients to have higher modification rates as compared to the healthy population. Diabetic dyslipidemia, hyperglycemia, and oxidative stress synergistically promote the occurrence of lipoperoxidation, glycosylation and glycoxidation processes, which will generate modified lipoproteins that stimulate development of atherosclerosis. This article reviews the role of different types of modified LDL in development of atherosclerosis in diabetes, as well as the possibility of using its quantification in cardiovascular risk prediction.

PALABRAS CLAVE
Diabetes mellitus;
Arteriosclerosis;
Lipoproteínas modificadas;
Riesgo cardiovascular;
Biomarcadores

Lipoproteínas modificadas como marcadores de riesgo cardiovascular en la diabetes mellitus

Resumen La prevención de la alta incidencia de enfermedad cardiovascular en la diabetes es uno de los retos de la endocrinología. La validación de nuevos biomarcadores que puedan contribuir a una mejor evaluación del riesgo cardiovascular y ayuden a implementar estrategias terapéuticas es una de las aproximaciones prometedoras en la investigación dirigida a la prevención y a la reducción del riesgo cardiovascular. La modificación de las lipoproteínas de baja densidad (LDL) es un elemento clave en el desarrollo de la lesión arteriosclerótica. Varias características fisiopatológicas de la diabetes contribuyen decisivamente a que la LDL de estos pacientes tenga unos índices de modificación más elevados que la de la población sana. La

☆ Please cite this article as: Sánchez-Quesada JL, Pérez A. Lipoproteínas modificadas como marcadores de riesgo cardiovascular en la diabetes mellitus. Endocrinol Nutr. 2013;60:518–528.

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2173-5093/$ – see front matter

dislipidemia diabética, la hiperglicemia y el estrés oxidativo favorecen de manera sinérgica la aparición de procesos de lipoperoxidación, glicosilación y glicoxidación que van a generar lipoproteínas modificadas que estimulan el desarrollo de la arteriosclerosis. Este artículo revisa el papel de los diferentes tipos de LDL modificada en el desarrollo de la arteriosclerosis en la diabetes, así como en la posibilidad de utilizar su cuantificación en la predicción del riesgo cardiovascular.

Introduction

Cardiovascular disease derived from atherosclerotic conditions is the leading cause of death in patients with diabetes mellitus. This disease has a number of characteristics contributing to an increased cardiovascular risk (CVR) through independent mechanisms. Overall, three events with a relevant role in the development of atherosclerosis may be distinguished in patients with diabetes: (1) diabetic dyslipidemia; (2) non-enzymatic glycosylation of proteins; and (3) oxidative stress.[1,2] These processes are in principle independent but, as we will attempt to explain in this review, are closely interconnected events (Fig. 1). The consequence of the high incidence of these conditions in diabetes is that lipoproteins, mainly low density lipoproteins (LDL), of these patients are modified, so that they lose their natural characteristics and function.[3] These modified LDLs are a determinant for the accelerated development of atherosclerosis experienced by subjects with diabetes. The direct implication of modified LDL in the evolution of atheromatous lesions suggests that quantification of LDL in plasma could be a very helpful tool for both the prediction of CVR and the monitoring of treatment aimed at decreasing this risk.[4]

Diabetic dyslipidemia

Metabolic syndrome (MS), defined as abdominal obesity, insulin resistance, hypertension, and an abnormal lipid profile,[5] is frequently associated with type 2 diabetes. Although all four factors are associated with the early development of atherosclerosis, an abnormal lipid profile is probably the factor most directly related to atherogenesis. This lipid profile, known as diabetic or atherogenic dyslipidemia, is characterized by hypertriglyceridemia, decreased high density lipoprotein (HDL) cholesterol levels, increased apolipoprotein B (apoB) levels,[6] and increased postprandial lipidemia.[7,8]

ApoB is the main protein component of atherogenic lipoproteins, LDL and very low density lipoproteins (VLDL). However, LDL cholesterol levels are usually normal. Since 80–90% of apoB is associated with LDL, this observation may seem paradoxical. This is explained by the abundance of small, dense LDL (sdLDL) particles with lower relative cholesterol content and greater apoB content, resulting from the deficient metabolization of VLDL.[9] Thus, although patients with diabetes often have normal plasma total cholesterol levels, their lipid profile is far from what may be considered as an atheroprotective factor. This suggests that, despite the fact that drug treatment of dyslipidemia is quantitatively efficient,[10] the incidence of cardiovascular events in the diabetic population continues to be high.

Significant qualitative changes in HDL and LDL occur in diabetic dyslipidemia. The effect on the antiatherogenic role of HDL, which is determinant for reverse cholesterol transport and has anti-inflammatory and antioxidant properties protecting LDL from oxidation, should first be considered.[11] HDL levels are not only decreased in patients with diabetes, but are also partially dysfunctional, with a decreased capacity to stimulate reverse cholesterol transport and a decreased antioxidant and anti-inflammatory capacity.[12] HDL dysfunctionality therefore promotes the formation of oxidized LDL (oxLDL).

sdLDL is more atherogenic than LDL of normal size and density because of a number of distinctive characteristics. It has less affinity for the LDL receptor, which implies a lower plasma clearance rate and a longer residence time in circulation. In addition, sdLDL crosses the endothelial barrier more easily than native LDL, because this process mainly depends on lipoprotein particle size. It also binds with greater affinity to proteoglycans forming the arterial wall, promoting subendothelial lipoprotein retention. Moreover, sdLDL has greater susceptibility to modification by oxidative and non-enzymatic glycosylation mechanisms.[13] The latter relates diabetic dyslipidemia to the other two processes involved in the abovementioned increase in CVR, namely non-enzymatic glycosylation and oxidative stress.

Non-enzymatic glycosylation

A continued hyperglycemia state enhances the non-enzymatic glycosylation processes of the different macromolecules. This has a highly significant impact on protein function. This process occurs when glucose reacts with amino acids containing amino groups (mainly lysine and arginine) to form a Schiff base and, subsequently, a stable product called the Amadori product.[14] This modification may affect the function of a very high number of proteins. Although the proteins most affected by non-enzymatic glycosylation are of course structural proteins with a long half-life, lipoproteins may also be glycosylated during their residence time in circulation.[15] However, the most widely accepted concept is that this process is enhanced in LDL retained in the arterial wall for a longer time. This modification affects LDL function, as exemplified by the fact that glycosylated LDL loses affinity for the LDL receptor.[16] SdLDL, common in patients with diabetes, is more susceptible to non-enzymatic glycosylation processes than LDL of normal size,[17] which confers on this modification a greater relevance in patients with diabetic dyslipidemia. Non-enzymatic

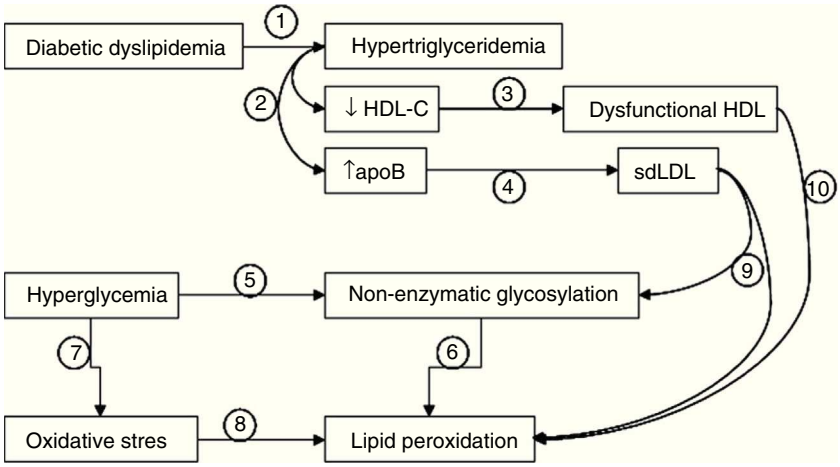

Figure 1 Interactions between diabetic dyslipidemia, hyperglycemia, and oxidative stress. Diabetic dyslipidemia is characterized by hypertriglyceridemia (1), which results in decreased HDL cholesterol levels and increased apoB levels (2). In these patients, HDL is not only quantitatively decreased, but is partly dysfunctional (3), and elevated apoB levels are associated with the presence of small, dense LDL particles (sdLDL). On the other hand, hyperglycemia promotes the non-enzymatic glycosylation of proteins (5), a process that stimulates the occurrence of lipid peroxidation events (6). Hyperglycemia also increases cellular oxidative stress (7), enhancing lipid peroxidation (8). The fact that LDL particles are small and dense (9) (more oxidizable and glycosylable) and the fact that HDL is partly dysfunctional (10) (less antioxidant potential) also contribute decisively to increases in non-enzymatic glycosylation and lipid peroxidation.

glycosylation in turn induces the formation of oxygen free radicals with the resultant stimulation of the oxidative processes, a phenomenon known as glycoxidation.[18] This process results in a rearrangement of the molecular bonds and leads to the formation of advanced glycation end-products (AGE).[18] This is a heterogeneous group of compounds which irreversibly alter protein function. All these processes occur in vivo, which has allowed for the detection of glycosylated LDL (glLDL) and modified LDL with AGE (AGE-LDL) in plasma circulation.[19] AGE-LDL, both generated in vitro and isolated from plasma circulation, has inflammatory properties and induces apoptosis in vascular wall cells,[20–22] processes which are both involved in the development of atherosclerosis.

The relatively short half-life of LDL in circulation (2.5–3.5 days) has always been an argument against a significant in vivo effect of non-enzymatic glycosylation on LDL during plasma circulation because, in the absence of reducing agents, 6–7 days are usually required for glucose to significantly change proteins.[23] Thus, it has implicitly been assumed that the formation of glLDL, and particularly of AGE-LDL, mainly occurs in LDL which has been retained in the arterial wall for a longer time than its plasma life.[23] It is therefore assumed that the glLDL and AGE-LDL detected in plasma circulation were formed in damaged arterial wall areas and that their presence in blood reflects the development of atherosclerotic areas.

An additional type of change related to hyperglycemia but not directly affecting glucose is modification by methylglyoxal (MG) or other similar compounds.[24] Different researchers have studied the effect of dicarbonyl glucose metabolites with a high reducing power, the most important of which is MG. This metabolite is able to rapidly react with arginine residues. Studies by Thornalley et al. showing the presence in blood of LDL modified by MG (MG-LDL) and that its levels were increased in patients with diabetes and decreased after treatment with metformina are particularly important.[25] LDL minimally modified by MG has a number of atherogenic characteristics including smaller size, increased susceptibility to aggregation, and greater binding affinity for proteoglycans in the arterial wall.[26] Arginine modification with MG results in a heterocyclic compound (hydroimidazolone) which is part of the heterogeneous family of AGE compounds, so that MG-LDL is a specific form of the AGE-LDL group. However, the relevant point to make about MG-LDL, within the whole group of AGE-LDL, is that because of the high reactivity of MG, it could be formed during the plasma circulation of LDL.

Oxidative stress

The modification of proteins by glycosylation and subsequent AGE formation is not the only mechanism by which oxidative stress is involved in the development of atherosclerosis in patients with diabetes. In fact, increased systemic oxidative stress is a characteristic of diabetes.[1] The main cause is probably that, as the result of hyperglycemia, an increase occurs in mitochondrial activity which favors the production of radical oxygen species (ROS).[27] Because of this, changes in the parameters quantifying oxidative stress are very frequently detected in plasma from these subjects. Oxidative stress plays a particularly significant role in the subendothelial space of the arterial wall, a microenvironment surrounded by metabolically active cells (endothelial cells, smooth muscle cells, macrophages) which generate ROS and lacking the abundant antioxidant defenses of blood plasma. Oxidative modification may affect all macromolecules, but lipoproteins, and specifically LDL, are highly sensitive to oxidative attack by ROS.[28] These radicals mainly oxidize the unsaturated fatty acids of phospholipids located on the lipoprotein surface, a process called lipid peroxidation. Three decades of research, starting with pioneering

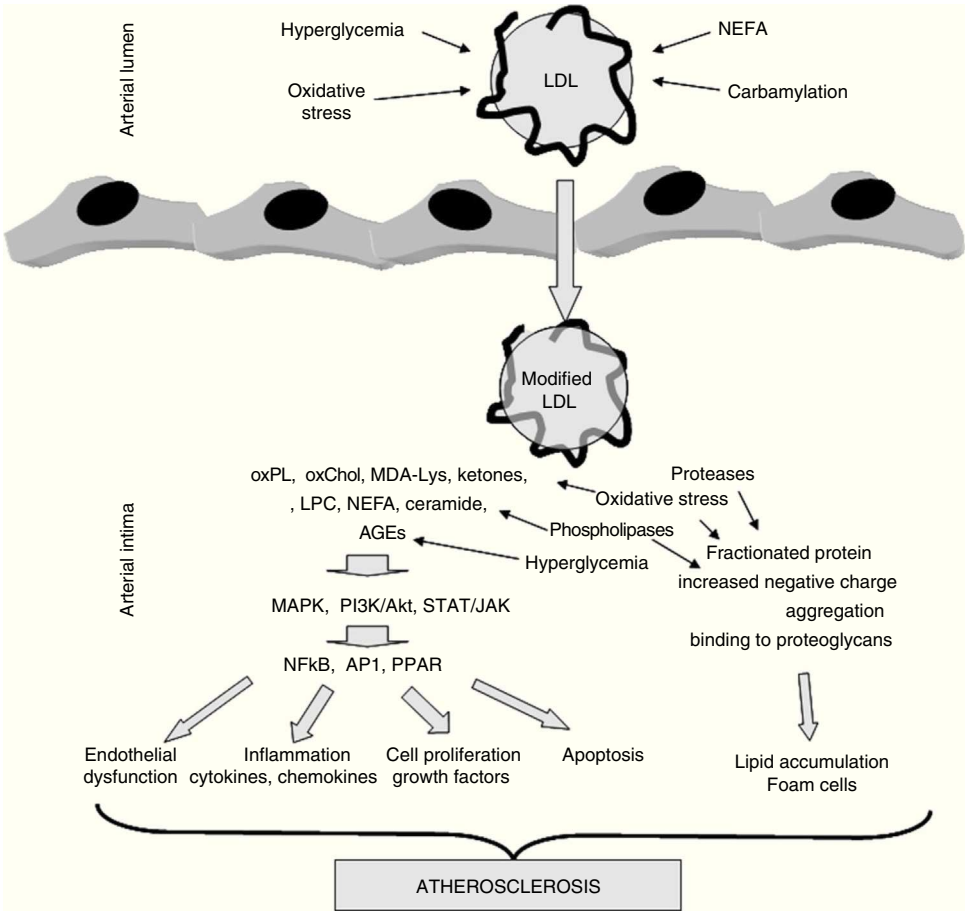

Figure 2 The role of LDL modification in the development of atherosclerosis. LDL may be modified by different mechanisms during its residence time in circulation (hyperglycemia, oxidative stress, carbamylation, NEFA overload) or once it has been trapped into the arterial wall (hyperglycemia, oxidative stress, lipolysis, proteolysis, aggregation). These modifications result in compounds, mainly of a lipid nature (oxidized phospholipids, oxidized cholesterol, ketones, lysophosphatidylcholine, NEFA, ceramide), but also derived from apoB (MDA-Lys adducts, AGE), with inflammatory, apoptotic, and proliferative potential. These compounds activate kinase-mediated signaling pathways (MAPK, PI3K/Akt, TAT/JAK) which stimulate in turn the translocation and/or activation of transcription factors (NFkB, AP1, PPARγ). These factors cause endothelial dysfunction and stimulate the secretion of cytokines, chemokines, growth factors, and mediators of apoptosis. On the other hand, other modifications break down apoB, increase negative charge, promote aggregation, and promote binding to proteoglycans forming the arterial wall. This results in the stimulation of subendothelial LDL accumulation and the formation of foam cells characteristic of atheromatous lesions.

research by Steinberg et al. in the early 1980s, have shown that oxidative LDL modification is a key event in the development of atherosclerosis.[28–30] Unlike native, unmodified LDL, which has no potentially atherogenic properties, oxLDL is able to promote and/or be involved in virtually all events occurring during the evolution of the atherosclerotic lesion. Thus, oxLDL is able to induce a massive intracellular accumulation of cholesterol esters by macrophages, inducing foam cell formation, which is probably the most characteristic pathological feature of the atherosclerotic lesion.[31] This is due to a loss of affinity for the LDL receptor, which is associated with an increased affinity for scavenger receptors (SR), whose expression is not regulated by intracellular cholesterol content. In addition, oxLDL may induce different arterial wall cells to express cytokines, chemokines, and growth factors. OxLDL thus promotes the chronic inflammatory process and cell proliferation characteristic of atherosclerosis.[30,32,33] It is also cytotoxic and apoptotic, promoting the formation of the necrotic core found in advanced atheromatous lesions.[34] Fig. 2 shows the impact of modification, either by oxidative or other different mechanisms, on LDL and its implication in the development of atherosclerosis.

Other modifications affecting low density lipoprotein

Multiple evidences suggest that LDL may be modified by other mechanisms in addition to oxidative changes and non-enzymatic glycosylation processes. Fig. 3 summarizes the different processes that may generate the different types of modified LDL.[35] In atherosclerotic lesions there is an overexpression of lipolytic enzymes such as phospholipase A3 (PLA2), sphingomyelinase (SMase), or cholesterol esterase (CEase), and also of various proteases (metalloproteinases, cathepsins).[36–41] LDL retained in the arterial wall is

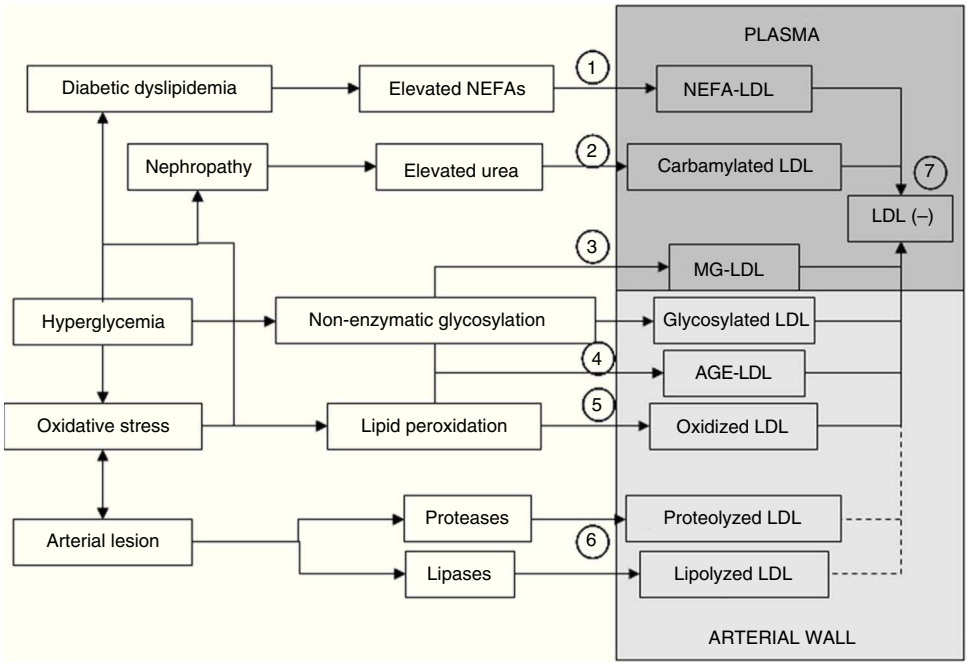

Figure 3 Mechanisms through which modified LDL is formed. LDL may be modified in plasma or the arterial wall by different mechanisms. Elevated plasma NEFA levels characteristic of diabetic dyslipidemia promote LDL overload with NEFAs (1). If nephropathy and hyperuremia exist, LDL carbamylation is stimulated (2). Another process that may occur in plasma circulation is modification by methylglyoxal (MG) (3). Significant modification of LDL in plasma by non-enzymatic glycosylation is more difficult. The formation of glycosylated LDL and AGE-LDL is therefore more likely to occur in the arterial wall (4). Similarly, although oxidative modification may occur in plasma, it is also more likely to occur more extensively in the arterial wall (5). Modification mediated by lipases and proteases necessarily also preferentially occurs in the arterial wall (6). A common characteristic to all these modifications is an increase in the negative electric charge of the LDL particle, which is reflected in the formation of electronegative LDL (LDL(−)), a modified form of LDL that may be isolated from plasma circulation (7). LDL(−) consists at least of NEFA-LDL, carbamylated LDL, MG-LDL, glycosylated LDL, AGE-LDL, and oxidized, and maybe also lipolyzed and/or proteolyzed LDL from the arterial wall.

therefore assumed to be affected not only by lipid peroxidation and glycoxidation processes, but also by proteases and lipases. Indirect evidence of this is that lipoproteins isolated from the arterial wall show fragmentation of apoB (representing 98–99% of the protein in LDL) and high contents of various products of the enzymatic breakdown of lipids such as lysophosphatidylcholine, ceramide, and non-esterified cholesterol.[31,42,43] Some of these products may also be generated from oxidative processes (apoB fragmentation, lysophosphatidylcholine), but others cannot be explained by these events. In addition, the fact that oxidation products are not excessively elevated in atherosclerotic lesions has resulted in the contribution of oxidative modification to the development of atherosclerotic lesions being played down.[44] The lack of positive results in large clinical trials using different antioxidant molecules has also contributed to this.[45,46] The current general perception is that, while it is still accepted that lipid peroxidation plays a significant role in atherogenesis, other mechanisms of LDL modification, such as enzymatic modification through lipases or proteases, may play a role even more preponderant than oxidation in the generation of modified LDL in the arterial wall.[44,47]

In addition to the processes described so far, preferentially occurring in the arterial wall, LDL may also be modified by other mechanisms in the bloodstream. The presence of carbamylated LDL in plasma has recently been reported.[48,49] Carbamylation is a chemical change generated by a reaction with the cyanate molecule derived from thiocyanate formed from urea.[48] This type of modification has been shown to be particularly important in smokers because tobacco smoke promotes the formation of thiocyanate, and in patients with chronic uremia due to severe renal failure. This suggests the possibility that carbamylated LDL is increased in patients with diabetes and kidney disease. However, this has yet to be experimentally confirmed.

The presence in the circulation of desialized LDL, i.e. LDL with a decreased content in sialic acid, one of the carbohydrates forming the enzymatic glycosylation chains of apoB, has also been reported. Desialized LDL is increased in patients with diabetes and is able to induce foam cell formation. It is therefore potentially atherogenic.[50] Desialization has been attributed to oxidation processes, because these promote the loss of sialic acid bound to apoB. Desialized LDL may therefore reflect the presence of oxLDL.[51]

Another LDL modification, which may quantitatively be highly relevant, is overload with non-esterified fatty acids (NEFAs). These compounds are usually transported in blood associated with albumin. When albumin transport capacity is exceeded due to increased plasma NEFA levels, NEFAs bind to other macromolecules, mainly lipoproteins.[52] LDL with increased NEFA contents has a greater inflammatory potential and an altered structure which promotes its aggregation.[53–55] This phenomenon is important in diabetes, where plasma NEFA levels are frequently increased. In this

context, LDL in diabetic patients has been reported to have high NEFA contents.[56] This may explain observations in other studies showing that LDL from these subjects is more inflammatory than LDL from subjects with no diabetes, despite the fact that lipid peroxidation indices are not increased.[57,58]

A property common to the different forms of modified LDL previously described is an increase in the electric charge of the particle.[59] Using this characteristic, Avogaro et al. were the first to isolate from plasma a fraction of modified LDL with an increased negative charge they called electronegative LDL(LDL(−)).[60] LDL(−) may be considered as a pool that contains the different modified forms of LDL (Fig. 2) present in blood and may account for approximately 5% of total LDL in healthy subjects. LDL(−) is increased 2- to 4-fold in different groups of subjects with high CVR or advanced atherosclerosis, including patients with type 1 and type 2 diabetes.[59,61,62] The proportion of LDL(−) is much higher than the values usually reported for oxLDL or AGE-LDL (0.1–1%).[63,64] Both oxLDL and AGE-LDL can therefore be considered minority forms within LDL(−) and most LDL(−) is probably LDL with increased NEFA contents, other associated minority apolipoproteins (other than apoB) and/or greater density (sdLDL). LDL(−) therefore reflects the metabolic abnormalities occurring in the different diseases, while oxLDL or AGE-LDL are related to the presence of underlying atherosclerotic lesions.

The value of modified low density lipoprotein as a biomarker

Regardless of the relative significance of each type of modification for the generation of modified LDL and the atherogenic properties conferred to LDL by each of these mechanisms, LDL modification is widely accepted as playing a key role in atherogenesis. Many researchers have therefore suggested that the quantitation of modified LDL may be used as a CVR marker, and may even serve to estimate the extent and evolution of atherosclerotic lesions found in patients with atherosclerosis.[4,64] Although the possibility that part of oxLDL and AGE-LDL has formed during its residence time in plasma circulation cannot be ruled out completely, the general perception is that oxLDL and AGE-LDL have been formed in the arterial wall. The occurrence in plasma of these modified LDL forms may therefore reflect the silent presence of active atherosclerotic lesions, because LDL is oxidized and/or glycoxidized in injured areas of the arterial wall but is not found in healthy areas. OxLDL may thus be considered to be not only a biomarker of atherosclerosis, but also a potential indicator of unstable and/or ruptured atherosclerotic plaques which release part of their contents into circulation.[64–69]

To show this involvement of modified LDL and to assess its value as a biomarker, rapid, reproducible, and relatively simple methods allowing for quantitation in large groups of patients have been developed. From the early 1990s, advances in the development of immunoassays have made it possible to detect the presence of different modified LDL forms in plasma circulation. Holvoet et al. developed an immunoassay able to detect MDA-LDL, a form of modified LDL resulting from oxidation, and were the first to report an increased concentration in patients with atherosclerosis.[70] Since then, at least three methods based on different antibodies (4E6, E06, and DLH3) recognizing different oxidative epitopes generated in oxLDL (adduct MDA-Lys, phosphorylcholine, and oxidized phosphatidylcholine respectively) have been marketed.[4,70–72] Cohen et al. developed in 1993 an immunoassay method to quantitate glycosylated LDL[73] which was marketed soon afterwards. Methods for detecting AGE-LDL have also been developed[74] and, more recently, immunoassays able to detect carbamylated LDL[49] and LDL(−) have been implemented. However, these latter methods have not been marketed, which has limited to date the development of multicenter studies that may validate these types of modified LDL as CVR markers. The objective of many researchers has been to use these tools to determine whether, in addition to being a causative agent, modified LDL could be quantitated and serve as a biomarker of atherosclerotic disease, providing additional information to the more traditional risk factors. A vast majority of studies have quantitated oxLDL, but studies assessing AGE-LDL and LDL(−) have become increasingly relevant in recent years.

Association of oxidized low density lipoprotein with cardiovascular risk

Many population studies have reported increased oxLDL levels in groups of patients with high CVR, including hypercholesterolemia, hypertriglyceridemia, metabolic syndrome, obesity, diabetes, hypertension, and severe kidney disease.[4,64,70,75–77] Increased oxLDL levels have also been reported in plasma from patients with angiographically documented atherosclerosis and have been associated with severity of coronary artery disease.[65,78–80] However, although the involvement of oxLDL in the development of atherosclerosis is widely accepted, its value as an independent biomarker of CVR is moderate.[81–83] This may be due to different reasons. On the one hand, a strong correlation exists with lipid parameters, particularly total cholesterol and LDL, especially in subjects with dyslipidemia.[81] This masks the role of oxLDL as a biomarker and plays down the value of its quantitation. Another factor explaining the lack of conclusive studies is that the different immunoassays using antibodies that recognize different epitopes generated during the oxidative process in LDL are not standardized.[4,64] As this process is tremendously complex and results, in a more or less sequential form, in products which occur in some oxidation phases and are degraded in other phases, the different immunoassays may detect different oxidative states of LDL. The complete standardization of these methods is clearly needed in order that the results achieved in the studies conducted can be compared.

The observation that oxLDL levels, regardless of the measurement method used, transiently increase during the acute phase of acute myocardial infarction or stroke, and also after percutaneous transluminal angiography, is more consistent and probably more applicable.[84–86] These observations support the concept that circulating oxLDL comes from the injured arterial wall and suggest that the quantitation of oxLDL in blood may be a very helpful tool for increasing our understanding of the vulnerability of

atheromatous lesions and for the secondary prevention of cardiovascular events in patients with atherosclerosis.

Modified low density lipoprotein as a cardiovascular risk biomarker in diabetes

Most studies conducted in patients with both type 1 and type 2 diabetes have shown increased levels of the different types of modified LDL, including oxLDL, glycosylated LDL, AGE-LDL, and LDL(−).[62,73,87–92] Overall, it has been noted that poor glucose control is associated with higher levels of modified LDL and that the optimization of glucose control results in decreases in these levels.[62,93,94] Similarly to the findings made in studies conducted in subjects without diabetes, treatment with lipid lowering agents also decreases levels of modified LDL in patients with diabetes, with a clear relationship being found with the effects on lipid profile.[90,95,96] Thus, its predictive value as an independent factor of clinical cardiovascular events is not completely clear. In type 2 diabetes, where lipid profile is usually altered, studies have found particularly significant discrepancies.[97,98] Some studies conducted in populations with diabetes have reported that oxLDL is a predictor of the occurrence of cardiovascular events, although some authors did not find this independent association when lipid profile abnormalities were considered.[99–101] However, in contrast to the weak association of modified LDL with clinical events, other studies found independent associations with other markers of the course of atherosclerosis such as carotid intima-media thickness or diabetic nephropathy.[102–106]

A significant advance in the use of LDL as a biomarker is the quantitation of the immune complexes formed by antibodies and modified LDL (oxLDL-IC o AGE-LDL-IC), mainly in studies conducted in subjects with type 1 diabetes.[107] One of the properties of the different types of modified LDL is their immunogenic capacity.[108–113] This property has allowed for the detection in plasma of specific autoantibodies against the different modified LDLs, including oxLDL, AGE-LDL, and LDL(−). Various studies have shown that levels of these autoantibodies are associated with the presence of atherosclerotic disease, although many discrepancies exist in this regard. The current evidence would appear to suggest that IgG antibodies are positively associated with the development of atherosclerosis, while IgM antibodies play an atheroprotective role.[114]

The most relevant studies in this regard were conducted by the group of Virella and Lopes-Virella, mainly in subjects with type 1 diabetes. Using in vitro studies, these authors showed that LDL-ICs have greater atherogenic potential than modified LDL not bound to antibodies.[97,115] This could be due to the fact that, in addition to activating the pathway mediated by scavenger receptors recognizing modified LDL, LDL-ICs also activate in monocytes the pathway of the antibody-specific Fc receptor, enhancing the inflammatory process.[20,115,116] It should also be noted that most of the oxLDL and AGE-LDL present in plasma circulate as ICs.[20,74,117] This supports the concept that immune complexes with oxLDL or AGE-LDL play in vivo a more relevant role in the development of atherosclerosis than free oxLDL and AGE-LDL.

In agreement with this concept, studies conducted by this group have shown that levels of oxLDL-ICs and AGE-LDL-ICs are strongly associated with carotid intima-media thickness and its progression in type 1 diabetes independently of other risk factors.[88] These ICs are also associated with the extent of coronary calcification,[118] the risk of developing nephropathy,[119] and the progression of retinopathy.[120] The significance of these studies lies in the fact that they have been conducted on a large population enrolled in the cohort of the DCCTR/EDIC study, and the consistency of the results is therefore high. However, no studies analyzing the association of LDL-ICs with the incidence of coronary events in patients with type 1 diabetes have been conducted yet. Such an association was however analyzed in type 2 diabetics (the VADT cohort).[102] Interestingly, the incidence of clinical events in these patients was not found to be associated with oxLDL-ICs or AGE-LDL-ICs, but with the levels of immune complexes of MDA-LDL, a specific form of oxLDL which was not associated with the progression of atherosclerosis in type 1 diabetes.[88] The reasons for these differences are not clear, but they may be attributed to the different evolution of lesions between both types of patients with diabetes. The main disadvantage of this test method is the need for a precipitation step of immune complexes before quantitation using an immunoassay for modified LDL. This increases the technical complexity of the measurement, which is therefore difficult to perform in most clinical laboratories.

Conclusions

Plasma levels of different modified LDLs are increased in patients with diabetes, although this observation has often been strongly associated with the presence of dyslipidemia. However, recent studies assessing levels of modified LDL associated with immune complexes have shown an association with the presence and progression of atherosclerosis in both type 1 and type 2 diabetes. This is a promising approach which may help to better predict CVR not only in patients with diabetes, but also in other diseases with an accelerated development of atherosclerosis. However, additional studies conducted by different research groups on patients with different diseases or conditions associated with early atherosclerosis are needed to better define the implication of each type of modified LDL in the development of atherosclerosis.

Conflict of interest

The authors state that they have no conflict of interest.

Acknowledgements

The authors of this study have been funded by Instituto de Salud Carlos III (CIBERDEM, FIS PI05-2099, FIS CP06-0220 and FIS PI10-00265) and the Regional Government of Catalonia (2009-SGR-1205).

References

1. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107:1058–70.
2. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. Nat Clin Pract Endocrinol Metab. 2009;5:150–9.
3. Levitan I, Volkov S, Subbaiah PV. Oxidized LDL: diversity, patterns of recognition, and pathophysiology. Antioxid Redox Signal. 2010;13:39–75.
4. Fraley AE, Tsimikas S. Clinical applications of circulating oxidized low-density lipoprotein biomarkers in cardiovascular disease. Curr Opin Lipidol. 2006;17:502–9.
5. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome—a new world-wide definition. A Consensus Statement from the International Diabetes Federation. Diabet Med. 2006;23:469–80.
6. Farmer JA. Diabetic dyslipidemia and atherosclerosis: evidence from clinical trials. Curr Diab Rep. 2008;8:71–7.
7. Sahade V, França S, Badaró R, Fernando Adán L. Obesity and postprandial lipemia in adolescents: risk factors for cardiovascular disease. Endocrinol Nutr. 2012;59:131–9.
8. Wagner Fahlin AM, Sánchez Quesada JL, Pérez Pérez A. Diabetes mellitus y lipemia posprandial. Endocrinol Nutr. 2000;47:311–21.
9. Vergès B. Abnormal hepatic apolipoprotein B metabolism in type 2 diabetes. Atherosclerosis. 2010;211:353–60.
10. Ascaso JF. Advances in cholesterol-lowering interventions. Endocrinol Nutr. 2010;57:210–9.
11. Escolà-Gil JC, Rotllan N, Julve J, Blanco-Vaca F. In vivo macrophage-specific RCT and antioxidant and antiinflammatory HDL activity measurements: new tools for predicting HDL atheroprotection. Atherosclerosis. 2009;206:321–7.
12. Kontush A, Chapman MJ. Why is HDL functionally deficient in type 2 diabetes? Curr Diab Rep. 2008;8:51–9.
13. Chapman MJ, Guérin M, Bruckert E. Atherogenic, dense low-density lipoproteins. Pathophysiology and new therapeutic approaches. Eur Heart J. 1998;19 Suppl. A:A24–30.
14. Cerami A, Vlassara H, Brownlee M. Role of advanced glycosylation products in complications of diabetes. Diabetes Care. 1988;11 Suppl. 1:73–9.
15. Curtiss LK, Witztum JL. Plasma apolipoproteins AI, AII, B, CI, and E are glucosylated in hyperglycemic diabetic subjects. Diabetes. 1985;34:452–61.
16. Wang X, Bucala R, Milne R. Epitopes close to the apolipoprotein B low density lipoprotein receptor-binding site are modified by advanced glycation end products. Proc Natl Acad Sci U S A. 1998;95:7643–7.
17. Younis N, Charlton-Menys V, Sharma R, Soran H, Durrington PN. Glycation of LDL in non-diabetic people: small dense LDL is preferentially glycated both in vivo and in vitro. Atherosclerosis. 2009;202:162–8.
18. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med. 1988;318:1315–21.
19. Lopes-Virella MF, Klein RL, Virella G. Modification of lipoproteins in diabetes. Diabetes Metab Rev. 1996;12:69–90.
20. Virella G, Atchley D, Koskinen S, Zheng D, Lopes-Virella MF. Proatherogenic and proinflammatory properties of immune complexes prepared with purified human oxLDL antibodies and human oxLDL. Clin Immunol. 2002;105:81–92.
21. Isoda K, Folco E, Marwali MR, Ohsuzu F, Libby P. Glycated LDL increases monocyte CC chemokine receptor 2 expression and monocyte chemoattractant protein-1-mediated chemotaxis. Atherosclerosis. 2008;198:307–12.
22. Hodgkinson CP, Laxton RC, Patel K, Ye S. Advanced glycation end-product of low density lipoprotein activates the toll-like 4 receptor pathway implications for diabetic atherosclerosis. Arterioscler Thromb Vasc Biol. 2008;28:2275–81.
23. Younis N, Sharma R, Soran H, Charlton-Menys V, Elseweidy M, Durrington PN. Glycation as an atherogenic modification of LDL. Curr Opin Lipidol. 2008;19:378–84.
24. Rabbani N, Thornalley PJ. Glyoxalase in diabetes, obesity and related disorders. Semin Cell Dev Biol. 2011;22:309–17.
25. Rabbani N, Chittari MV, Bodmer CW, Zehnder D, Ceriello A, Thornalley PJ. Increased glycation and oxidative damage to apolipoprotein B100 of LDL cholesterol in patients with type 2 diabetes and effect of metformin. Diabetes. 2010;59:1038–45.
26. Rabbani N, Godfrey L, Xue M, Shaheen F, Geoffrion M, Milne R, et al. Glycation of LDL by methylglyoxal increases arterial atherogenicity: a possible contributor to increased risk of cardiovascular disease in diabetes. Diabetes. 2011;60:1973–80.
27. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005;54:1615–25.
28. Steinberg D. The LDL modification hypothesis of atherogenesis: an update. J Lipid Res. 2009;50:S376–81.
29. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med. 1989;320:915–24.
30. Navab M, Berliner JA, Watson AD, Hama SY, Territo MC, Lusis AJ, et al. The Yin and Yang of oxidation in the development of the fatty streak. A review based on the 1994 George Lyman Duff Memorial Lecture. Arterioscler Thromb Vasc Biol. 1996;16:831–42.
31. Stary HC. Natural history and histological classification of atherosclerotic lesions: an update. Arterioscler Thromb Vasc Biol. 2000;20:1177–8.
32. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med. 1999;340:115–26.
33. Navab M, Ananthramaiah GM, Reddy ST, van Lenten BJ, Ansell BJ, Fonarow GC, et al. The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. J Lipid Res. 2004;45:993–1007.
34. Ross R. Cell biology of atherosclerosis. Annu Rev Physiol. 1995;57:791–804.
35. Sánchez-Quesada JL, Villegas S. Modified forms of LDL in plasma. In: Parthasarathy S, editor. Atherogenesis. Rijeka, Croatia: InTech; 2011. p. 447–72.
36. Oörni K, Kovanen PT. Lipoprotein modification by secretory phospholipase A(2) enzymes contributes to the initiation and progression of atherosclerosis. Curr Opin Lipidol. 2009;20:421–7.
37. Katsuda S, Coltrera MD, Ross R, Gown AM. Human atherosclerosis. IV. Immunocytochemical analysis of cell activation and proliferation in lesions of young adults. Am J Pathol. 1993;142:1787–93.
38. Pentikäinen MO, Oörni K, Ala-Korpela M, Kovanen PT. Modified LDL – trigger of atherosclerosis and inflammation in the arterial intima. J Intern Med. 2000;247:359–70.
39. Williams KJ, Tabas I. Lipoprotein retention—and clues for atheroma regression. Arterioscler Thromb Vasc Biol. 2005;25:1536–40.
40. Fenske D, Dersch K, Lux C, Zipse L, Suriyaphol P, Dragneva Y, et al. Enzymatically hydrolyzed low-density lipoprotein modulates inflammatory responses in endothelial cells. Thromb Haemost. 2008;100:1146–54.
41. Klouche M, Rose-John S, Schmiedt W, Bhakdi S. Enzymatically degraded, nonoxidized LDL induces human vascular smooth muscle cell activation, foam cell transformation, and proliferation. Circulation. 2000;101:1799–805.
42. Stary HC. Composition and classification of human atherosclerotic lesions. Virchows Arch A Pathol Anat Histopathol. 1992;421:277–90.

43. Williams KJ, Tabas I. The response-to-retention hypothesis of atherogenesis reinforced. Curr Opin Lipidol. 1998;9:471–4.
44. Tabas I. Nonoxidative modifications of lipoproteins in atherogenesis. Annu Rev Nutr. 1999;19:123–39.
45. Steinberg D, Witztum JL. Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis? Circulation. 2002;105:2107–11.
46. Witztum JL, Steinberg D. The oxidative modification hypothesis of atherosclerosis: does it hold for humans? Trends Cardiovasc Med. 2001;11:93–102.
47. Bhakdi S, Lackner KJ, Han SR, Torzewski M, Husmann M. Beyond cholesterol: the enigma of atherosclerosis revisited. Thromb Haemost. 2004;91:639–45.
48. Apostolov EO, Ray D, Savenka AV, Shah SV, Basnakian AG. Chronic uremia stimulates LDL carbamylation and atherosclerosis. J Am Soc Nephrol. 2010;21:1852–7.
49. Apostolov EO, Shah SV, Ok E, Basnakian AG. Quantification of carbamylated LDL in human sera by a new sandwich ELISA. Clin Chem. 2005;51:719–28.
50. Tertov VV, Sobenin IA, Gabbasov ZA, Popov EG, Jaakkola O, Solakivi T, et al. Multiple-modified desialylated low density lipoproteins that cause intracellular lipid accumulation. Isolation, fractionation and characterization. Lab Invest. 1992;67:665–75.
51. Tertov VV, Bittolo-Bon G, Sobenin IA, Cazzolato G, Orekhov AN, Avogaro P. Naturally occurring modified low density lipoproteins are similar if not identical: more electronegative and desialylated lipoprotein subfractions. Exp Mol Pathol. 1995;62:166–72.
52. Lagrost L, Florentin E, Guyard-Dangremont V, Athias A, Gandjini H, Lallemant C, et al. Evidence for nonesterified fatty acids as modulators of neutral lipid transfers in normolipidemic human plasma. Arterioscler Thromb Vasc Biol. 1995;15:1388–96.
53. Benítez S, Camacho M, Arcelus R, Vila L, Bancells C, Ordóñez-Llanos J, et al. Increased lysophosphatidylcholine and non-esterified fatty acid content in LDL induces chemokine release in endothelial cells. Relationship with electronegative LDL. Atherosclerosis. 2004;177:299–305.
54. Gaubatz JW, Gillard BK, Massey JB, Hoogeveen RC, Huang M, Lloyd EE, et al. Dynamics of dense electronegative low density lipoproteins and their preferential association with lipoprotein phospholipase A(2). J Lipid Res. 2007;48:348–57.
55. Lu M, Gantz DL, Herscovitz H, Gursky O. Kinetic analysis of thermal stability of human low density lipoproteins: a model for LDL fusion in atherogenesis. J Lipid Res. 2012;53:2175–85.
56. Phillips C, Owens D, Collins P, Tomkin GH. Low density lipoprotein non-esterified fatty acids and lipoprotein lipase in diabetes. Atherosclerosis. 2005;181:109–14.
57. Sánchez-Quesada JL, Benítez S, Pérez A, Wagner AM, Rigla M, Carreras G, et al. The inflammatory properties of electronegative low-density lipoprotein from type 1 diabetic patients are related to increased platelet-activating factor acetylhydrolase activity. Diabetologia. 2005;48:2162–9.
58. Benítez S, Pérez A, Sánchez-Quesada JL, Wagner AM, Rigla M, Arcelus R, et al. Electronegative low-density lipoprotein subfraction from type 2 diabetic subjects is proatherogenic and unrelated to glycemic control. Diabetes Metab Res Rev. 2007;23:26–34.
59. Sánchez-Quesada JL, Benítez S, Ordóñez-Llanos J. Electronegative low-density lipoprotein. Curr Opin Lipidol. 2004;15:329–35.
60. Avogaro P, Bon GB, Cazzolato G. Presence of a modified low density lipoprotein in humans. Arteriosclerosis. 1988;8:79–87.
61. Mello AP, da Silva IT, Abdalla DS, Damasceno NR. Electronegative low-density lipoprotein: origin and impact on health and disease. Atherosclerosis. 2011;215:257–65.
62. Sánchez-Quesada JL, Vinagre I, de Juan-Franco E, Sánchez-Hernández J, Blanco-Vaca F, Ordónez-Llanos J, et al. Effect of improving glycemic control in patients with type 2 diabetes mellitus on low-density lipoprotein size, electronegative low-density lipoprotein and lipoprotein-associated phospholipase A2 distribution. Am J Cardiol. 2012;110:67–71.
63. Sánchez-Quesada JL, Estruch M, Benítez S, Ordóñez-Llanos J. Electronegative LDL: a useful biomarker of cardiovascular risk. Clin Lipidol. 2012;7:345–59.
64. Ishigaki Y, Oka Y, Katagiri H. Circulating oxidized LDL: a biomarker and a pathogenic factor. Curr Opin Lipidol. 2009;20:363–9.
65. Holvoet P, Mertens A, Verhamme P, Bogaerts K, Beyens G, Verhaeghe R, et al. Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscler Thromb Vasc Biol. 2001;21:844–8.
66. Holvoet P, Collen D, van de Werf F. Malondialdehyde-modified LDL as a marker of acute coronary syndromes. JAMA. 1999;281:1718–21.
67. Nishi K, Itabe H, Uno M, Kitazato KT, Horiguchi H, Shinno K, et al. Oxidized LDL in carotid plaques and plasma associates with plaque instability. Arterioscler Thromb Vasc Biol. 2002;22:1649–54.
68. Tsimikas S. Oxidized low-density lipoprotein biomarkers in atherosclerosis. Curr Atheroscler Rep. 2006;8:55–61.
69. Uno M, Kitazato KT, Nishi K, Itabe H, Nagahiro S. Raised plasma oxidised LDL in acute cerebral infarction. J Neurol Neurosurg Psychiatry. 2003;74:312–6.
70. Holvoet P, Perez G, Zhao Z, Brouwers E, Bernar H, Collen D. Malondialdehyde-modified low density lipoproteins in patients with atherosclerotic disease. J Clin Invest. 1995;95:2611–9.
71. Itabe H, Yamamoto H, Imanaka T, Shimamura K, Uchiyama H, Kimura J, et al. Sensitive detection of oxidatively modified low density lipoprotein using a monoclonal antibody. J Lipid Res. 1996;37:45–53.
72. Tsimikas S, Witztum JL. Measuring circulating oxidized low-density lipoprotein to evaluate coronary risk. Circulation. 2001;103:1930–2.
73. Cohen MP, Lautenslager G, Shea E. Glycated LDL concentrations in non-diabetic and diabetic subjects measured with monoclonal antibodies reactive with glycated apolipoprotein B epitopes. Eur J Clin Chem Clin Biochem. 1993;31:707–13.
74. Virella G, Derrick MB, Pate V, Chassereau C, Thorpe SR, Lopes-Virella MF. Development of capture assays for different modifications of human low-density lipoprotein. Clin Diagn Lab Immunol. 2005;12:68–75.
75. Holvoet P, Theilmeier G, Shivalkar B, Flameng W, Collen D. LDL hypercholesterolemia is associated with accumulation of oxidized LDL, atherosclerotic plaque growth, and compensatory vessel enlargement in coronary arteries of miniature pigs. Arterioscler Thromb Vasc Biol. 1998;18:415–22.
76. Tsimikas S, Clopton P, Brilakis ES, Marcovina SM, Khera A, Miller ER, et al. Relationship of oxidized phospholipids on apolipoprotein B-100 particles to race/ethnicity, apolipoprotein(a) isoform size, and cardiovascular risk factors: results from the Dallas Heart Study. Circulation. 2009;119:1711–9.
77. Frostegård J, Wu R, Lemne C, Thulin T, Witztum JL, de Faire U. Circulating oxidized low-density lipoprotein is increased in hypertension. Clin Sci (Lond). 2003;105:615–20.
78. Holvoet P, Vanhaecke J, Janssens S, van de Werf F, Collen D. Oxidized LDL and malondialdehyde-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. Circulation. 1998;98:1487–94.
79. Tsimikas S, Aikawa M, Miller Jr FJ, Miller ER, Torzewski M, Lentz SR, et al. Increased plasma oxidized phospholipid:apolipoprotein B-100 ratio with concomitant depletion of oxidized phospholipids from atherosclerotic lesions after dietary lipid-lowering: a potential biomarker of early

atherosclerosis regression. Arterioscler Thromb Vasc Biol. 2007;27:175–81.
80. Tsimikas S, Kiechl S, Willeit J, Mayr M, Miller ER, Kronenberg F, et al. Oxidized phospholipids predict the presence and progression of carotid and femoral atherosclerosis and symptomatic cardiovascular disease: five-year prospective results from the Bruneck study. J Am Coll Cardiol. 2006;47:2219–28.
81. Herder C, Baumert J, Zierer A, Roden M, Meisinger C, Karakas M, et al. Immunological and cardiometabolic risk factors in the prediction of type 2 diabetes and coronary events: MONICA/KORA Augsburg case-cohort study. PLoS One. 2011;6:e19852.
82. Wu T, Willett WC, Rifai N, Shai I, Manson JE, Rimm EB. Is plasma oxidized low-density lipoprotein, measured with the widely used antibody 4E6, an independent predictor of coronary heart disease among U.S. men and women? J Am Coll Cardiol. 2006;48:973–9.
83. Meisinger C, Baumert J, Khuseyinova N, Loewel H, Koenig W. Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. Circulation. 2005;112:651–7.
84. Tsimikas S, Bergmark C, Beyer RW, Patel R, Pattison J, Miller E, et al. Temporal increases in plasma markers of oxidized low-density lipoprotein strongly reflect the presence of acute coronary syndromes. J Am Coll Cardiol. 2003;41:360–70.
85. Tsimikas S, Lau HK, Han KR, Shortal B, Miller ER, Segev A, et al. Percutaneous coronary intervention results in acute increases in oxidized phospholipids and lipoprotein(a): short-term and long-term immunologic responses to oxidized low-density lipoprotein. Circulation. 2004;109:3164–70.
86. Ehara S, Ueda M, Naruko T, Haze K, Itoh A, Otsuka M, et al. Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes. Circulation. 2001;103:1955–60.
87. Hoogeveen RC, Ballantyne CM, Bang H, Heiss G, Duncan BB, Folsom AR, et al. Circulating oxidised low-density lipoprotein and intercellular adhesion molecule-1 and risk of type 2 diabetes mellitus: the Atherosclerosis Risk in Communities Study. Diabetologia. 2007;50:36–42.
88. Lopes-Virella MF, Hunt KJ, Baker NL, Lachin J, Nathan DM, Virella G. Levels of oxidized LDL and advanced glycation end products-modified LDL in circulating immune complexes are strongly associated with increased levels of carotid intima-media thickness and its progression in type 1 diabetes. Diabetes. 2011;60:582–9.
89. Sánchez-Quesada JL, Pérez A, Caixàs A, Rigla M, Payés A, Benítez S, et al. Effect of glycemic optimization on electronegative low-density lipoprotein in diabetes: relation to nonenzymatic glycosylation and oxidative modification. J Clin Endocrinol Metab. 2001;86:3243–9.
90. Zhang B, Kaneshi T, Ohta T, Saku K. Relation between insulin resistance and fast-migrating LDL subfraction as characterized by capillary isotachophoresis. J Lipid Res. 2005;46:2265–77.
91. Nakhjavani M, Asgharani F, Khalilzadeh O, Esteghamati A, Ghaneei A, Morteza A, et al. Oxidized low-density lipoprotein is negatively correlated with lecithin-cholesterol acyltransferase activity in type 2 diabetes mellitus. Am J Med Sci. 2011;341:92–5.
92. Bastos AS, Graves DT, Loureiro AP, Rossa Júnior C, Abdalla DS, Faulin Tdo E, et al. Lipid peroxidation is associated with the severity of periodontal disease and local inflammatory markers in patients with type 2 diabetes. J Clin Endocrinol Metab. 2012;97:E1353–62.
93. Akanji AO, Abdella N, Mojiminiyi OA. Determinants of glycated LDL levels in nondiabetic and diabetic hyperlipidaemic patients in Kuwait. Clin Chim Acta. 2002;317:171–6.
94. Abe M, Maruyama N, Okada K, Matsumoto S, Matsumoto K, Soma M. Effects of lipid-lowering therapy with rosuvastatin on kidney function and oxidative stress in patients with diabetic nephropathy. J Atheroscler Thromb. 2011;18:1018–28.
95. Sánchez-Quesada JL, Otal-Entraigas C, Franco M, Jorba O, González-Sastre F, Blanco-Vaca F, et al. Effect of simvastatin treatment on the electronegative low-density lipoprotein present in patients with heterozygous familial hypercholesterolemia. Am J Cardiol. 1999;84:655–9.
96. Zhang B, Matsunaga A, Rainwater DL, Miura S, Noda K, Nishikawa H, et al. Effects of rosuvastatin on electronegative LDL as characterized by capillary isotachophoresis: the ROSARY Study. J Lipid Res. 2009;50:1832–41.
97. Virella G, Lopes-Virella MF. The pathogenic role of the adaptive immune response to modified LDL in diabetes. Front Endocrinol (Lausanne). 2012;3:76.
98. Rizzo M, Berneis K, Koulouris S, Pastromas S, Rini GB, Sakellariou D, et al. Should we measure routinely oxidised and atherogenic dense low-density lipoproteins in subjects with type 2 diabetes? Int J Clin Pract. 2010;64:1632–42.
99. Shimada K, Mokuno H, Matsunaga E, Miyazaki T, Sumiyoshi K, Kume A, et al. Predictive value of circulating oxidized LDL for cardiac events in type 2 diabetic patients with coronary artery disease. Diabetes Care. 2004;27:843–4.
100. Holvoet P, Harris TB, Tracy RP, Verhamme P, Newman AB, Rubin SM, et al. Association of high coronary heart disease risk status with circulating oxidized LDL in the well-functioning elderly: findings from the Health, Aging, and Body Composition study. Arterioscler Thromb Vasc Biol. 2003;23:1444–8.
101. Hsu RM, Devaraj S, Jialal I. Autoantibodies to oxidized low-density lipoprotein in patients with type 2 diabetes mellitus. Clin Chim Acta. 2002;317:145–50.
102. Lopes-Virella MF, Hunt KJ, Baker NL, Virella G, Moritz T. The levels of MDA-LDL in circulating immune complexes predict myocardial infarction in the VADT study. Atherosclerosis. 2012;224:526–31.
103. Ujihara N, Sakka Y, Takeda M, Hirayama M, Ishii A, Tomonaga O, et al. Association between plasma oxidized low-density lipoprotein and diabetic nephropathy. Diabetes Res Clin Pract. 2002;58:109–14.
104. Gokulakrishnan K, Deepa R, Velmurugan K, Ravikumar R, Karkuzhali K, Mohan V. Oxidized low-density lipoprotein and intimal medial thickness in subjects with glucose intolerance: the Chennai Urban Rural Epidemiology Study-25. Metabolism. 2007;56:245–50.
105. Piarulli F, Lapolla A, Sartore G, Rossetti C, Bax G, Noale M, et al. Autoantibodies against oxidized LDLs and atherosclerosis in type 2 diabetes. Diabetes Care. 2005;28:653–7.
106. Brown WV, Goldberg RB, Lopes-Virella M, Reaven P. Reducing vascular disease risk in the type 2 diabetic patient. J Clin Lipidol. 2011;5:3–11.
107. Lopes-Virella MF, Thorpe SR, Derrick MB, Chassereau C, Virella G. The immunogenicity of modified lipoproteins. Ann N Y Acad Sci. 2005;1043:367–78.
108. Lopes-Virella MF, Virella G. Immune mechanisms in the pathogenesis of atherosclerosis. Adv Exp Med Biol. 1991;285:383–92.
109. Witztum JL, Koschinsky T. Metabolic and immunological consequences of glycation of low density lipoproteins. Prog Clin Biol Res. 1989;304:219–34.
110. Tsimikas S, Palinski W, Witztum JL. Circulating autoantibodies to oxidized LDL correlate with arterial accumulation and depletion of oxidized LDL in LDL receptor-deficient mice. Arterioscler Thromb Vasc Biol. 2001;21:95–100.
111. Korpinen E, Groop PH, Akerblom HK, Vaarala O. Immune response to glycated and oxidized LDL in IDDM patients with and without renal disease. Diabetes Care. 1997;20:1168–71.
112. Oliveira JA, Sevanian A, Rodrigues RJ, Apolinário E, Abdalla DS. Minimally modified electronegative LDL and its autoantibodies

in acute and chronic coronary syndromes. Clin Biochem. 2006;39:708–14.
113. Virella G, Thorpe SR, Alderson NL, Stephan EM, Atchley D, Wagner F, et al. Autoimmune response to advanced glycosylation end-products of human LDL. J Lipid Res. 2003;44:487–93.
114. Lopes-Virella MF, Virella G. Clinical significance of the humoral immune response to modified LDL. Clin Immunol. 2010;134:55–65.
115. Saad AF, Virella G, Chassereau C, Boackle RJ, Lopes-Virella MF. OxLDL immune complexes activate complement and induce cytokine production by MonoMac 6 cells and human macrophages. J Lipid Res. 2006;47:1975–83.
116. Lopes-Virella MF, Binzafar N, Rackley S, Takei A, la Via M, Virella G. The uptake of LDL-IC by human macrophages: predominant involvement of the Fc gamma RI receptor. Atherosclerosis. 1997;135:161–70.
117. Virella G, Carter RE, Saad A, Crosswell EG, Game BA, Lopes-Virella MF. Distribution of IgM and IgG antibodies to oxidized LDL in immune complexes isolated from patients with type 1 diabetes and its relationship with nephropathy. Clin Immunol. 2008;127:394–400.
118. Lopes-Virella MF, Baker NL, Hunt KJ, Lachin J, Nathan D, Virella G. Oxidized LDL immune complexes and coronary artery calcification in type 1 diabetes. Atherosclerosis. 2011;214:462–7.
119. Lopes-Virella MF, Carter RE, Baker NL, Lachin J, Virella G. High levels of oxidized LDL in circulating immune complexes are associated with increased odds of developing abnormal albuminuria in type 1 diabetes. Nephrol Dial Transplant. 2012;27:1416–23.
120. Lopes-Virella MF, Baker NL, Hunt KJ, Lyons TJ, Jenkins AJ, Virella G. High concentrations of AGE-LDL and oxidized LDL in circulating immune complexes are associated with progression of retinopathy in type 1 diabetes. Diabetes Care. 2012;35:1333–40.