 The reciprocal relationship between LDL metabolism and type 2 diabetes mellitus

Isabella Bonilha1, Eric Hajduch2, Beatriz Luchiari1, Wilson Nadruz3, Wilfried Le Goff4**\***, Andrei C. Sposito1**\***

1 Atherosclerosis and Vascular Biology Laboratory (AtheroLab), Cardiology Division, State University of Campinas (Unicamp), Campinas, 13083-887, Brazil

2 Centre de Recherche des Cordeliers, INSERM, Sorbonne Université, F-75006 Paris, France

3 Cardiology Division, State University of Campinas (Unicamp), Campinas, 13083-887, Brazil

4 Sorbonne Université, Inserm, Unité de recherche sur les maladies cardiovasculaires, le métabolisme et la nutrition, ICAN, F-75013 Paris, France

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Address for correspondence

Professor Andrei C. Sposito

Cardiology Division, Faculty of Medical Sciences,

State University of Campinas (Unicamp),

13084-971, Campinas, Sao Paulo, Brazil.

Tel 55 19 3521 7098

Fax 55 19 3289 4107

Email: andreisposito@gmail.com

\* The two last authors contributed equally to this work.

**Abstract**

Type 2 diabetes mellitus and insulin resistance feature substantial modifications of the lipoprotein profile, including a higher proportion of smaller and denser low-density lipoprotein (LDL) particles. In addition, qualitative changes occur in the composition and structure of LDL, including changes in electrophoretic mobility, enrichment of LDL with triglycerides and ceramides, prolonged retention of modified LDL in plasma, and increased uptake by macrophages and the formation of foam cells. These modifications affect LDL functions and favor an increased risk of cardiovascular disease in diabetic individuals. In this review, we discuss the main findings regarding the structural and functional changes in LDL particles in diabetes pathophysiology and therapeutic strategies targeting LDL in patients with diabetes.

**Keywords**

Type 2 diabetes mellitus; low-density lipoprotein; oxidation; glycation; modified LDL; small and dense LDL; endothelial dysfunction; deleterious effects

1. **Background**

The prevalence of type 2 diabetes mellitus (T2DM) has consistently increased worldwide for several decades, shifting the overall picture of cardiovascular disease and its pathophysiological background. The combination of disarrangements in both glucose and lipid metabolism frameworks comprises the complex pathophysiology of T2DM, favoring accelerated and severe atherosclerotic disease. Dyslipidemia is observed in 72–85% of T2DM patients and predicts the presence of cardiovascular disease (CVD) (1). Typically, diabetic dyslipidemia is characterized by low levels of high-density lipoproteins (HDL), hypertriglyceridemia, and an increase in the proportion of small, dense, low-density lipoproteins (LDL) (2, 3). Nevertheless, several other changes in the lipid profile have been reported, notably on LDL particles, which may influence cardiovascular risk.

An imperative factor for the change in the LDL phenotype during T2DM is the decline in insulin sensitivity (4). Insulin resistance increases the triglyceride content of LDL and decreases LDL particle size (5). Additionally, in patients with T2DM, LDL particles exhibit a wide range of oxidation levels, ranging from minimally oxidized LDL (MM-LDL) to fully oxidized LDL (Ox-LDL) (6). The degree of oxidation of LDL is directly correlated with the magnitude of insulin resistance, accumulation of visceral fat, presence of metabolic syndrome, and T2DM duration (7, 8). On the other hand, elevated Ox-LDL concentration is associated with an increased risk of incident diabetes due to its effects on β-cells (9) and with a higher risk of developing obesity and cardiovascular disease (7). This indicates a bidirectional association between Ox-LDL and T2DM. Lastly, glycation and the change in lipid composition of LDL have also been reported to increase the atherogenic potential (10). In this review, we discuss recent advances in the evidence of biological mechanisms that underlie LDL phenotype changes during T2DM pathology and its clinical and therapeutic implications.

1. **Low-density lipoproteins**

LDL are particles that differ in size, density, chemical composition, and atherogenicity (11). ApoB-containing lipoprotein biogenesis begins with intrahepatic cholesterol, either from intestinal absorption or from *de novo* synthesis incorporated into very-low-density lipoprotein (VLDL) particles (12). In the bloodstream, VLDL is converted by lipoprotein lipase, cholesteryl ester transfer protein (CETP) (13), and hepatic lipase (14) into cholesterol-enriched lipoproteins such as the LDL (13). The LDL particle has an average diameter of 22 nm and comprises a 700 phospholipid-molecule surface layer and 170 triglycerides (TG) and 1600 cholesteryl ester (CE) molecule core. Additionally, this lipoprotein contains approximately 600 molecules of non-esterified cholesterol (15, 16), with one-third located in the core and two-thirds on the surface (17). Each LDL particle contains a single apoB-100 molecule composed of an amphoteric α-helical domain related to the amphiphilicity of LDL particles and a β-sheet domain that corresponds to 40% of the structure of apoB100 and is related to the stability of LDL particles (18).

The density of lipoproteins is directly proportional to their protein content (19). With a density of 1.019 to 1.063 g/ml, LDL has a composition of 20% protein and 50% cholesterol, including both CE and free cholesterol (FC) (19). Traditionally, LDL can be fractionated into apparently discrete components by density gradient centrifugation or affinity chromatography, according to its decreasing size and increased density (19). The largely used classification groups LDL in 5 subfractions (20, 21), ranging from the largest and less dense to the smallest and most dense.

Insulin resistance-induced dyslipidemia is characterized by the presence of small, dense LDL (sdLDL) particles, (4) which have a more substantial atherogenic potential compared to other LDL subfractions (22). As insulin resistance becomes more severe the size of LDL particles has decreased (23). Moreover, sdLDL circulation times are longer due to their relatively reduced affinity to LDL receptors (LDLR) (24), secondary to apoB100 glycation (25) (see the section below). In addition, sdLDL displays increased affinity to the arterial wall (4), contributing to atherogenesis (26).

1. **Influences on LDL subfractions heterogeneity**

In 1990, two LDL phenotypes were discovered (27). Individuals with a higher proportion of sdLDL subfraction also termed the 'B' phenotype (particle diameter <25.5 nm) (18), had three times greater risk of having a myocardial infarction when compared with a higher prevalence of larger LDL particles (27), termed type 'A', regardless of total LDL concentration (28). In 1994, a study postulated that, in LDL particles, TG content *per se* does not influence the overall apoB100 structure, whereas LDL size plays a significant role in determining apoB100 conformation near its receptor recognition site and, thus, its affinity for the LDLR (29). Overall, this evidence supports that LDL particle size is more crucial than its core composition.

The LDL metabolism is also modified in T2DM. It has been suggested that there is a metabolic channeling within the lipoproteins VLDL-intermediate density lipoprotein (IDL)-LDL delipidation cascade so that parallel processing pathways generate different LDL products. Compared to individuals with type 'A' phenotype, type 'B' is associated with an approximately 2-fold increase in plasma TG, higher plasma apoB100 and IDL levels, and reduced HDL cholesterol (HDL-C) and Apo-AI concentrations (22). This evidence indicates that variations in the availability of hepatic TG may determine the quantities of sequential lipoproteins (30). Therefore, plasma levels of VLDL correlate with increased density and decreased size of LDL (5), resulting from the exchange of TG from VLDL to CE from LDL mediated by CETP. This TG-rich LDL is a substrate for hepatic lipase (4). In addition, CETP-mediated transfer of CE from HDL to sdLDL and to VLDL-1, the precursors of sdLDL, contributes to the formation of sdLDL in T2DM and is dependent of the degree of triglyceridemia (40). The sdLDL levels were associated with a metabolic condition characterized by increased hepatic lipase concentration (31) and decreased lipoprotein lipase activity (32) (Figure 1). Conversely, the increase in lipoprotein lipase activity induced by a high-fat diet contributed to a significant increase of large LDL I subtype concentration (32).

The sdLDL differs from other subtypes due to its binding affinity to proteoglycans, caused by its apoB100 conformation (33). More specifically, sdLDL displays a lower binding affinity for LDLR and, thus, longer circulation time (34). Plasma levels of sdLDL (35) as the proportion of small LDL/large LDL are significantly elevated in diabetic individuals (36). An increase in sdLDL is a risk factor for developing atherosclerosis and coronary heart disease in patients with or without diabetes mellitus (DM). The formation of sdLDL appears to be increased in individuals with overt insulin resistance and hypertriglyceridemia (37). The intima-media thickness layer (IMT) of the carotid artery of diabetic patients was strongly associated with sdLDL, followed by apoB100 and LDL cholesterol (LDL-C) (36). These results show that sdLDL was a good lipid marker in the assessment of carotid atherosclerosis among the tested lipid parameters (36).

**Figure 1:** **Schematic representation of the mechanism behind the generation of sdLDL in T2DM**. Mechanisms leading to sdLDL in T2DM. Insulin resistance promotes triglyceride lipolysis in adipocytes and the release of free fatty acids (FFA) in the circulation. Uptake and accumulation of FFA in the liver results in hepatic gluconeogenesis and affects lipid metabolism. FFAs taken up by hepatocytes are used to form new TG-rich VLDL particles. Remodeling of TG-rich VLDL through the action of CETP, HL and LPL enzymes promotes the formation of small and dense LDL, more atherogenic particles. Consequently, these particles are electronegative and stay longer in the plasma, being more susceptible to oxidation and glycation, are more prone to the subendothelial space binding to proteoglycans of the arterial vessel wall and interact with beta2-glycoprotein I forming autoimmune complexes. On the other hand, there is a decrease in affinity with LDLR.

*FFA: free fatty acid, TG, triglyceride, HL: hepatic lipase, LPL: lipoprotein lipase, CETP: Cholesteryl Ester Transfer Protein, VLDL: very low-density lipoprotein, IDL: intermediate density lipoprotein, LDL: low-density lipoprotein, sdLDL: small dense lipoprotein, LDLR: LDL receptor.*

1. **LDL modification due to T2DM**

Proatherogenic factors may also mediate other non-atherosclerotic cardiovascular changes related to diabetes, such as diabetic cardiomyopathy. Insulin resistance is the primary precursor of the pathophysiology of diabetic cardiomyopathy (38). Regarding the lipid profile, insulin resistance may be related to increased fat accumulation in muscle and liver tissue and mitochondrial dysfunction in skeletal muscle in diabetic patients even without obesity (39). As a result of insulin resistance, intracellular lipase increases the release of non-esterified fatty acids (NEFA) from TG stored in adipose tissue. High levels of NEFA and glycogen reserves promote hepatic TG production, which is associated with increased apoB100 secretion (40, 41). Evidence shows that insulin activates the phosphatidylinositol-3 kinase (PI-3K) pathway that inhibits apoB100 secretion while activating a mitogen-activated protein kinase (MAPK) that down-regulates the microsomal TG-transfer protein (MTP) expression. This mechanism contributes to the increased in VLDL secretion in T2DM (16). Therefore, in T2DM patients, TG-rich VLDLs are part of the central mechanism to generate sdLDL and reduce plasma HDL levels through CETP-mediated transfer of CE (42), as commented earlier. Interestingly, LDL binding of heparin increases in T2DM, triggering pro-atherogenic LDL modifications. Therefore, the effects of heparin binding are negatively associated with atherogenesis for VLDL, but positively for LDL (43). LDL enriched with TG is then a substrate for lipases linked to the endothelium, and its action leads to the formation of smaller particles depleted of lipids (34). Also, LDL with high NEFA contents has a more significant inflammatory potential and an altered structure which promotes its aggregation (44).

Moreover, particles of TG-rich LDL are a preferential substrate for hepatic lipase or lipoprotein lipase action, resulting in lipid-depleted sdLDL (41). *In vitro*, sdLDL showed a reduced affinity with its receptor, greater propensity for transport to the subendothelial space, increased arterial binding to wall proteoglycans, and susceptibility to oxidative modifications, with atherogenic potential (5). In addition to sdLDL, a fraction of LDL with an increased negative charge, called electronegative LDL (-) (45), is increased in patients with T2DM and high cardiovascular risk (46). In anion exchange chromatography techniques, LDL (-) exhibited a high TG content, suggesting a relationship between insulin resistance and LDL (-) since glycemic control did not reduce LDL (-) in T2DM (47). As we will discuss later, all these changes indicate modifications of the recognized functions of these lipoproteins.

* 1. *LDL oxidation*

The chemical composition of LDL makes these particles susceptible to oxidation, and atherogenic modifications exacerbate this vulnerability. The polyunsaturated acyl chains of CE, phospholipids and TG are vulnerable to oxidative stress (48). Most evidence suggests that oxidative modification in LDL plays a vital role in the onset of diabetes and the pathogenesis of atherosclerosis (49). However, previous studies hit the limitation of evaluating oxidation of LDL particles only indirectly through the analysis of serum autoantibodies against oxidized LDL (50, 51), which does not reflect the overall extent of oxidative damage. The LDL oxidation process will be described in the paragraphs below.

LDL oxidation occurs in two main pathways. Initially, oxidative changes in LDL lipids can result in the absence or slight change of apoB100, known as MM-LDL, preserving its function of LDLR ligand. This modified lipoprotein does not bind to scavenger receptors (6), has a slight negative charge, activates anti-apoptotic signaling, and induces endothelial cells to express tissue factors and chemokines (52). Subsequently, LDL lipids are oxidized and become cytotoxic and pro-apoptotic (52). Inflammatory signaling pathways are activated, leading to inflammatory cell recruitment and further LDL modification. Importantly, continuous oxidation of LDL causes modification of apo-B100. After the oxidation process is complete, Ox-LDL loses its affinity for LDLR and binds to scavenger receptors (SRs) (18), such as SR-A1, CD36, LOX-1, SREC, SR-PSOX and CD68 (53). This phenomenon results in the formation of cholesterol-loaded foam cells via PI-3K/protein kinase B (Akt)-dependent mechanisms (52), but can be upregulated via JNK, Wnt and NF-κB signaling (54). As lesions advance, a necrotic nucleus is formed in atheromatous lesions (44).

Particles of Ox-LDL also interact with beta2-glycoprotein I (β2GPI), promoting the formation of the Ox-LDL/β2GPI complex as a putative autoantigen in autoimmune-mediated atherosclerotic vascular disease (55). C-reactive protein (CRP), an acute phase protein and predictor of cardiovascular risk (56), is associated with the complex. CRP/Ox-LDL/β2GPI complexes were found in the serum of patients with T2DM and atherosclerosis, suggesting a crosstalk between arterial inflammation, hyperglycemia, and hypercholesterolemia (55).

Several studies have revealed that Ox-LDL represents a potent regulator of macrophage gene expression (57) involved in the inflammatory response, including those encoding tumor necrosis factor (TNF)-α, the interleukins -1α, -1β, -6, and Platelet Derived Growth Factor (PDGF) (49). Moreover, the oxidized lipoprotein activates peroxisome proliferator-activated receptor γ (PPAR-γ)-dependent transcription through a signaling mechanism involving scanning receptor-mediated cell uptake (58). Two oxidized metabolites of linoleic acid (9-HODE and 13-HODE) can bind and activate PPAR-γ, functioning as pathological endogenous ligands (59). Consequently, these oxidized metabolites act by regulating the expression of the gene on macrophages during atherogenesis. Both free and esterified HODE metabolites are increased by 49% in the LDL of diabetic patients, reflecting free radical peroxidation of 18: 2 n-6, and were in line with the decrease of 18: 2 n-6 in lipid-rich classes in polyunsaturated fatty acids (PUFA), CE and phosphatidylcholines. Furthermore, lipid peroxidation in LDL in diabetic patients is twice higher than in healthy patients (60). In that regard, LDL susceptibility to oxidation is shown to be greater in hyperglycemia, as a positive correlation between Ox-LDL and glycated hemoglobin (HbA1c) concentrations in diabetics has been demonstrated (48).

More specifically, lectin-like oxidized LDL receptor-1 (LOX-1), a type II membrane glycoprotein belonging to the C-type lectin family (61), has been identified as the primary Ox-LDL receptor. LOX-1 is mainly found in endothelial cells and plays an essential role in the pathogenesis of diabetic vasculopathy (62). Evidence shows that LOX-1 expression is increased in the vascular endothelium of diabetic rats, which reinforces its role in diabetes-associated endothelial dysfunction. Moreover, elevated glucose levels increase LOX-1 expression at the gene and protein level (63). In this scenario, LDL oxidation can occur by either enzymatic and non-enzymatic processes, as described in the following sections.

* + 1. *LDL oxidation by an enzymatic process*

The enzymatic process involves many systems, such as lipoxygenases, myeloperoxidase (MPO), reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, and nitric oxide synthase (48). Lipoxygenases catalyze the stereospecific incorporation of one oxygen molecule into unsaturated fatty acids (64, 65). Its activity is suggested to play a significant role in LDL oxidation by endothelial cells and activated monocytes (66) and macrophages (67). The dual-specificity 12/15-lipoxygenases is implicated in the oxidative modification of LDL and foam cell formation (68). *In vivo* data obtained with 12/15-lipoxygenase-deficient mice crossbred to apolipoprotein E deficiency have established a pro-atherogenic role for this pathway (69).

*In vitro* reaction of hypochlorous acid (HOCl), the primary oxidant generated by the MPO system, with LDL results primarily in modifications of apo-B100, with slight lipid oxidation (70). HOCl reacts readily with the ε-amino groups of apoB100 lysine residues, resulting in the formation of N-chloramines (70). As a result, LDL charge is modified, leading to macrophages' uncontrolled uptake of HOCl-modified LDL. A small proportion of the LDL-associated chloramines break down to form aldehydes. This last may be associated with the cross-linking and aggregation of HOCl-exposed LDL particles via Schiff base formation and the formation of advanced glycation end products (AGEs) (70). Apo-B100 oxidation affects fractional LDL catabolism, the *in vivo* consequence of this has been shown to be a 2-fold longer residence time of buoyant LDL and a > 4-fold longer residence time of sdLDL in familial defective apo-B100 when compared to controls (71).

The NADPH oxidase, which produces superoxide anion (∙O2-) radicals, was also involved in the macrophage-mediated oxidation of LDL (72). This multi-component enzyme consists of cytosolic accessory proteins (Rac, p47phox, p67phox) that, when stimulated, associate with membrane catalytic subunits (Nox, p22phox) to facilitate ∙O2- generation (73). The level of NADPH can be significantly increased when cells are exposed to mitogenic and transforming growth factors, high glucose levels, or hyperlipidemia. These signals increase reactive oxygen species (ROS) production and contribute to oxidative stress development (74).

By reducing nitric oxide (NO) synthesis and availability, Ox-LDL disrupts the vessel wall balance and results in impaired endothelium-dependent vasodilation (75), which, in turn, explains the pro-inflammatory, pro-oxidant, pro-thrombotic, and vasoconstrictor actions of Ox-LDL in the endothelium (75). Tests performed on porcine subepicardial arterioles suggest that Ox-LDL up-regulates arginase I, which contributes to endothelial dysfunction by reducing L-arginine availability to endothelial NO synthase (eNOS) for NO production and thus vasodilation (76).

Attention has been paid to lipoprotein-associated phospholipase A2 (Lp-PLA2), which is believed to play an atherogenic role in T2DM when linked to LDL (77). The amount of Lp-PLA2 in sdLDL or LDL (-) is 5 to 10 times greater than normal-sized or electropositive LDL particles (78). Previous studies suggested that Lp-PLA2 binds with greater affinity to LDL through the conformational change that occurs in apoB100 (78), due to glycation, for example.

* + 1. *LDL oxidation by non-enzymatic process*

The non-enzymatic process of LDL oxidation involves free transition metal ions in the catalyzation of lipid peroxidation. The most common are those initiated by the ∙O2-/hydrogen peroxide/hydroxyl radical system of NO and by non-radical ROS such as singlet oxygen (1ΔgO2) and ozone (O3) (79). LDL exposure to ROS may result in chemical damage by generating lipid peroxides and ultimately apo-B100 protein adducts. The oxidative modification of apo-B100 changes its ligand properties and marks its removal by scavenger receptors (51). The degree of LDL modification is shown to be directly proportional to the rate of ∙O2- production by cells (80). Arterial smooth muscle cells in culture that generated O2- modified LDL by a ∙O2--dependent free radical process catalyzed by Fe or Cu. This process resulted in an increased uptake of modified LDL by macrophages, thus, in foam cell formation and atherogenesis (80).

While LDL is oxidized, an increase in∙O2- production occurs, accompanying a time- and concentration-dependent decrease in phosphorylation of eNOS in Thr495 (81). Furthermore, protein kinase C (PKC), which phosphorylates the Thr495 residue, showed diminished activity in cells incubated with Ox-LDL, which induced dissociation of eNOS and Golgi membranes (81).

* 1. *Glycated LDL*

Advanced Glycation End-products (AGEs) synthesis initiates with condensation reaction between the carbonyl group of glucose and the primary amino groups of lipoproteins. Subsequently, the Schiff bases formed are transformed into Amadori products and other irreversible adducts (82). Another complex class of compounds is the advanced lipoxidation end-products (ALEs), which result from successive oxidation cascades. ALEs are formed by reactive carbonyl species derived from a lipid peroxidation process, such as malondialdehyde, 4-hydroxynonenal, and acrolein, detected in inflammatory and oxidative-based diseases (83) such as T2DM. The half-life of LDL in the bloodstream ranges from 2.5 to 3.5 days. However, it takes 6 to 7 days for non-enzymatic glycosylation to occur. Altogether, these data suggest that glycation occurs in the LDL particles retained in the arterial wall for longer than their lifespan in circulation (44).

The primary amino acid of apo-B100 that undergoes glycation is lysine. About 2 to 17% of its residues are glycated (84), which continues with the formation of sugar-amino acid adducts (82). The lysine residue is essential for specific recognition by the LDLR. Thus, glycation of this amino acid promotes an increase in the average half-life of glycated LDL. In this scenario, circulating glycated LDL levels are increased (85) approximately two-fold (86) in diabetic patients with concomitant hyperglycemia compared to non-diabetics (85). *In vitro*, glycated LDL had impaired binding with fibroblasts and greater glycation of lysine, which residues resulted in lower recognition by the LDLR (87). Regarding LDL subfraction phenotype, it was observed that oxidized, glycated, and electronegative LDL was increased in the T2DM group with poor glycemic control. In these patients, the prevalence of phenotype 'B' was associated with increased oxidized LDL and glycated LDL activity, compared to phenotype 'A' (88). Furthermore, metformin therapy in T2DM patients decreased the oxidative damage mediated by AGEs derived from arginine and methionine sulfoxide (89). Metformin therapy also showed lower dicarbonyl-derived AGE residues than patients who did not receive metformin therapy (89).

* 1. *Alteration of LDL lipidome in T2DM: Ceramides*

Saturated fatty acids and specific sphingolipids such as sphingomyelin can serve as precursors to ceramides (CER). CER, central lipids of sphingolipid metabolism, consist of a sphingoid base linked to a fatty acid *via* an amide bond. While these lipids play an important structural role in cell membranes, they are also involved in many processes such as the regulation of apoptosis, cell differentiation and insulin signaling (90). CER are mainly synthesized through three distinct pathways: sphingomyelinase pathway, recycling pathway and *de novo* pathway. Nevertheless, the predominant synthesis pathway observed in conditions of obesity and lipid excess is the *de novo* synthesis pathway (90). CER produced de novo (especially C16 and C18 CER species) have been well described to play a crucial role in the development of tissue insulin resistance through targeting several actors of the insulin signalling pathway, including insulin receptor substrates (IRS) and Akt (90, 91).

Studies have also reported an increase in circulating CER concentrations in obese T2D patients compared to plasma CER concentrations in healthy individuals (92). There is clear clinical evidence that circulating CER are mainly secreted by the liver and correlates with systemic insulin resistance (93). For 7 years, a study followed more than 1,500 non-diabetic American subjects from the multiethnic “Dallas Heart Study” cohort, and a positive correlation between plasma concentrations of CER and insulin resistance was demonstrated (94). In addition, circulating concentrations of dihydroCER, CER lipid precursors, are significantly increased in individuals who develop diabetes (95), up to 9 years before the onset of the first symptoms of the disease (96).

Circulating CER are mainly associated with lipoproteins in the circulation (90% of circulating CER), and 60% of CER are found in LDL in healthy individuals (93). Interestingly, evidence shows that an increase in plasma LDL-CER in T2DM and the prevalence of obesity correlates with the severity of insulin resistance and elevated plasma TNF-α levels (92), but not with the degree of obesity (97). Interestingly, the main CER species present in LDL are C24 CER (93), and myotubes treated with LDL artificially enriched with C24 CER exhibited reduced insulin-stimulated glucose transport and an inhibition of the insulin signaling pathway (97). In view of these data, and in addition of both C16 and C18 CER produced *de novo*, circulating C24 CER could also exert a major negative action on peripheral tissues.

Unlike LDL, no study has suggested a role for transported CER by VLDL and by HDL in insulin resistance. However, and as with LDL, C24 CER are the species of ceramides found predominantly in VLDL and HDL from healthy individuals (93). Nevertheless, a study showed that mice whose gene encoding the VLDL receptor was invalidated and subjected to a fatty diet exhibited better tolerance to glucose and better sensitivity to insulin than wild mice subjected to the same diet (98), suggesting the possibility that VLDL-CER may play a role in modulating the insulin sensitivity of these animals.

* 1. *Deleterious effects of LDL from T2DM patients*

As mentioned above, DM is a chronic condition with inflammatory factors, such as pro-inflammatory cytokines, that play an atherogenic role. The investigation of the influence on the expression of matrix metalloproteinases (MMP) or ADAM (a disintegrin and metalloproteinase) genes by LDL from individuals with T2DM showed in monocytic cell line (THP-1) that there was an expression of MMP-1, MMP-9, ADAM28, ADAM17, and ADAM15 genes compared to incubation with healthy LDL (99). This result suggests a prolonged inflammatory state in these patients.

LDL modified by glycation and oxidation also acts on platelet arachidonic acid metabolism (100). The LDL isolated from the plasma of T2DM individuals promoted phosphorylation of platelet p38-MAPK and the production of thromboxane B and was rich in malondialdehyde (100), which contributed to platelet aggregation hyperactivation.

* 1. *Deleterious effects of modified LDL from T2DM patients*

As previously cited, Ox-LDL contributes to DM progression. cAMP-responsive element modulator (CREM) expression is inducible by activation of the cAMP signaling pathway. The induced transcript encodes a novel repressor, called inducible cAMP early repressor (ICER) (101). β cells in the presence of Ox-LDL increased the abundance of ICER, which compromised the expression of insulin and anti-apoptotic islet 1 brain gene (102), and hindered insulin production and secretion (103).

The effects of Ox-LDL also extend to the loss of pericytes, an initial characteristic of diabetic retinopathy. Human retinal capillary pericytes exposed to Ox-LDL had endoplasmic reticulum (ER) stress resulting in mitochondrial dysfunction, apoptosis, and autophagy (104). ER stress increased in the diabetic human retina and was correlated with the severity of diabetic retinopathy (104). It caused dysfunction and was present in the retinas of diabetic patients, again correlating with disease severity (105). The formation of oxidized LDL immune complexes exerted more significant toxicity concerning retinal capillary pericytes, decreasing their viability and increased secretion of inflammatory cytokines, and reduced secretion of a critical anti-angiogenic factor, PEDF (105). These findings allow the search for new therapies to reduce retinal damage in diabetic patients caused by Ox-LDL.

* 1. *Endothelial dysfunction in diabetes by modified LDL*

Endothelial dysfunction is one of the first manifestations of T2DM and cardiovascular disease. As mentioned in the previous paragraphs, the exposure of endothelial cells to hyperglycemia is accompanied by its change in secretory action, demonstrated by the overdevelopment of the rough endoplasmic reticulum and Golgi complexes, enrichment of the intermediate filaments, enlargement of inter-endothelial junctions, and increase in the number of plasmalemmal vesicles (82). Together, these modifications increase in endothelial cells permeability and favor the subendothelial accumulation of modified LDL (82). Markers of endothelial dysfunction are often elevated years before any evidence of microangiopathy becomes evident (106). Because of this, endothelial dysfunction has received significant attention in DM. Under physiological conditions, there is a balance between contraction and relaxation factors derived from the endothelium, but this can be altered in diabetes, contributing to the progression of vascular damage (106).

High glucose concentration also promotes more LDL glycation in diabetics, as shown above. In endothelial dysfunction, glycated LDL promotes increased expression of adhesion molecules and modulates the fibrinolytic potential of vascular endothelial cells (107).

In addition, glycated LDL increases ROS production, which reduces the interaction between eNOS and caveolin-1, and thus, impairs NO production (108). This finding was confirmed in a 24-h incubation of human endothelial cell culture with the addition of 100 µg/ml of glycated LDL that induced inhibition of eNOS expression and increased inducible NO synthase (iNOS) expression and, consequently, ROS production (109). *In vitro*, the pro-apoptotic activity of glycated LDL gradually increased as the concentration increased, contributing to endothelial dysfunction (107). Measuring the reactivity of the mesenteric arteries incubated with glycated LDL showed a significant decrease in the vasodilator response to acetylcholine and sodium nitroprusside (110). This evidence shows that glycated LDL inhibits endothelium-dependent relaxation.

The decrease in capillary density of the endothelium influences the development of atherogenic changes in lipoprotein concentrations through the aggravating activity of lipoprotein lipase (LPL) bound to the endothelium. A reduced capillary endothelial surface area may impair TG-rich lipoproteins' access to LPL (108). These lipoproteins indirectly affect endothelial function through the production of sdLDL (111). An increase in ∙O2- release in endothelial cells exposed to LDL from individuals with T2MD was observed, suggesting that glycation or difference in composition of LDL initiates this process (112). Also, NO bioactivity was reduced with the addition of LDL from subjects with diabetes. This imbalance between NO and ∙O2- contributes to endothelial dysfunction (112). In Goto Kakizaki rats, a model with insulin resistance, an increase in ∙O2- was observed in the vasculature of the abdominal aorta and nitrotyrosine, indicative of the formation of peroxynitrite (113). These are mechanisms that reduce NO bioavailability and impair endothelial function.

Transfer of Ox-LDL across the endothelium of the endothelial wall occurs at sites of endothelial disruption and also through inflammation. In this process, Ox-LDL induces the expression of adhesion molecules such as monocyte chemoattractant protein-1 and macrophage colony-stimulating factor. Nevertheless, Ox-LDL increases the expression of matrix metalloproteinase-9, which causes vascular remodeling and fibrous cap rupture. Clearly, Ox-LDL promotes endothelial dysfunction (114).

1. **Potential therapeutic targets**

Dyslipidemia in diabetic patients is a significant risk factor for the development of atherosclerotic cardiovascular disease. Cholesterol-lowering therapy has become the most robust strategy to reduce MACEs and cardiovascular mortality in a large spectrum of clinical conditions. There are several guidelines for the treatment of dyslipidemia in diabetic patients. In this review, we will address them to provide the best decision about the cardiovascular risk involved.

* 1. *Statins*

Reducing the risk of cardiovascular disease in primary and secondary prevention with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) is well established. By blocking hepatic cholesterol synthesis and increasing the availability of LDLR in hepatocytes, statins reduce serum atherogenic lipoprotein concentrations (115). In 2010, estimates showed that only 58.2% of individuals with cardiovascular disease and 52% with T2DM over 40 years old were on statin therapy (116). More diabetics currently use statins, with about 65% on low-intensity statin therapy (116). In this setting, a meta-analysis suggested that with every 1mmol/L reduction cholesterol LDL (LDL-C) in plasma, there was a reduction in the incidence of heart attack (13%), myocardial revascularization (19%), and stroke (16%) (117), by about a fifth in a wide range of high-risk participants, largely irrespective of baseline lipid profile or other presenting characteristics, including DM (118). Thus, standard doses of statins reduce LDL cholesterol by about 40%, suggesting that such an absolute reduction would prevent patients from major vascular events (118).

Most recommendations in clinical studies involve the use of moderate-intensity statins for diabetic patients over 40 years of age, proposing adjusting doses to reach LDL-C levels below previously accepted targets, based on the fact that the correlation between the risk of cardiovascular disease and LDL-C in diabetic patients was more robust at this level (119). Among individuals with T2DM without known vascular disease, the average risk of a major vascular event was about 2.9% per year. The absolute benefit in this group over an average of 4.3 years of statin therapy was significant (118).

In type 2 diabetic patients, rosuvastatin significantly increased LDL-apoB100 catabolism, with positive effects on TG-rich lipoprotein catabolism and VLDL1-apoB catabolism (120). Therefore, therapy with statins is beneficial for individuals with diabetes, even without overt coronary disease or high concentrations of cholesterol (121). In addition to these effects, there is also a reduction in high-sensitivity C-reactive protein and other markers of inflammation that improve endothelial function and decrease vascular inflammation and oxidative stress (122).

* 1. *Ezetimibe*

Cholesterol absorption inhibitors (ezetimibe) appear to increase insulin sensitivity in patients with insulin resistance. They promote a moderate reduction in sdLDL and a more significant reduction in intermediate and large-sized LDL subfractions (123). Their mechanism of action involves the inhibition of intestinal cholesterol absorption by selectively blocking the Niemann–Pick C1-like 1 protein (NPC1L1) protein in the brush border of jejunal enterocytes, an integral part of the uptake of micelles from the intestinal lumen to the enterocyte (124). Ezetimibe reduces enterocyte cholesterol absorption, chylomicron formation and secretion, and cholesterol reflux from bile, and increases LDL receptor expression on the surface of hepatocytes, resulting in reductions in serum LDL-C levels (124). In subjects with T2DM, combined ezetimibe and statin therapy reduced LDL-C and TC more than statin alone (125). Intestinal cell cultures also demonstrated that high glucose levels increased the expression of NPC1L1 and, consequently, cholesterol uptake (126, 127). According to these findings, diabetic patients have a higher expression of NPC1L1 mRNA than those without diabetes (128) (Figure 2).

The Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) showed that the benefit of adding ezetimibe to statin therapy was beneficial in patients with T2DM; there was a more significant incremental reduction in median LDL-C over time (by 3 mg/dL) (129). The best clinical outcome was in the group of individuals with a lower mean dose of simvastatin and whose additional LDL-C reduction was driven by ezetimibe (130).

**Figure 2: Effect of increased glucose on LDL-C metabolism and insulin secretion.** In enterocytes, the increase in plasma glucose causes a decrease in cholesterol excretion consecutive to the reduction in the expression of ABCG5 and ABCG8. On the other hand, there is an increase in cholesterol absorption as a result of the high expression of NPC1L1. Both mechanisms contribute to an increase in plasma LDL-C levels. The increase in plasma glucose also reduces expression of LDLR in hepatocytes, another mechanistic pathway contributing to the increased plasma concentration of LDL-C. Such elevated plasma LDL-C levels together with the high expression of LDLR in pancreatic beta cells stimulate insulin secretion (2).

*LDLR: LDL receptor, ABCG5/8: ATP Binding Cassette Subfamily G Member 5 / ABCG8: ATP Binding Cassette Subfamily G Member 8, NPC1L1: Niemann–Pick C1-like 1 protein, LDL-C: LDL cholesterol.*

* 1. *Anti-PCSK9 (proprotein convertase subtilisin/kexin 9) antibody (ab)*

This injectable monoclonal antibody therapy has been indicated to treat adults with heterozygous familial hypercholesterolemia or those with clinical atherosclerotic cardiovascular disease who require further LDL-lowering (131).

When added to a background statin treatment, anti-PCSK9ab resulted in a 42% decrease in total plasma cholesterol and a profound, near 80% decrease in LDL-C with a 53% reduction in total plasma apoB100 (132). Plasma levels of ApoC-III, a physiological inhibitor of LPL activity, have been reported to be markedly higher in T2DM patients. Glucose induces the transcription of ApoC-III, a mechanism that links hyperglycemia, hypertriglyceridemia, and cardiovascular disease in patients with T2DM (133). However, the addition of anti-PCSK9ab moderately reduced plasma ApoC-III levels (15%), and there was a more pronounced decrease in apoE concentration (33%) (132). In order to investigate the effect of adding anti-PCSK9ab to Sodium-glucose cotransporter 2 inhibitors (SGLT2i) therapy (which are reported to increase LDL levels) (134) on LDL subfractions, the prospective randomized clinical trial EXCEEDBHS3 was designed, but the trial is still ongoing (135). In an animal model, treatment with anti-PCSK9ab reduced the size of atherosclerotic plaques and infiltration of pro-inflammatory macrophages, in addition to increasing circulating endothelial progenitor cells and angiogenic cells, suggesting that these results are secondary effects of LDL-C reduction (136).

* 1. *Increase LDL-C with SGLT2 Inhibitors*

SGLT2i is associated with a decrease in cardiovascular events and the progression of chronic kidney disease (137). They act by reducing renal tubular reabsorption of glucose without inducing insulin release. One study showed that empagliflozin was associated with small increases in LDL-C in T2DM patients at high risk for cardiovascular events (138). Furthermore, a meta-analysis involving 38 studies with SGLT2i showed that canagliflozin increased LDL-C to a greater extent compared to other inhibitors at other different doses (139). This LDL-C increase with canagliflozin was also seen over 104 weeks in patients with inadequately controlled T2DM (140). Conversely, in Japanese patients with a 16-week treatment regimen, LDL-C levels were not different between placebo and canagliflozin groups (141). Dapagliflozin also slightly increased LDL-C akin to the other SGLT2i, despite having no change in the LDL:HDL ratio, which according to the authors, is considered unlikely to yield any clinical significance (142).

* 1. *Insulin treatment*

Insulin therapy is widely used in patients at an advanced stage of T2DM and inefficient glycemic control, having beneficial effects on triacylglycerol and HDL cholesterol (HDL-C) levels (143). Thus, to precisely analyze the effect of insulin treatment on the metabolism of lipoproteins containing apoB100, they performed a kinetic study of a stable isotope *in vivo*. ApoB-containing lipoprotein metabolism was impaired in T2DM patients before insulin therapy (144). Interestingly, the LDL catabolic rate was significantly decreased in patients, resulting in longer intravascular time. However, insulin therapy restored this catabolism to average, leading to a standard LDL particle residence time (144). As already mentioned in this review, the residence time of LDL particles in plasma increases the probability of becoming oxidized and glycated lipoproteins.

The use of intensive insulin therapy administered to poorly controlled diabetic patients using sulfonylureas caused an increase in LDL particle size and significantly reduced sdLDL levels. There was also a reduction in ApoC-III (145). In diabetic patients with poor metabolic control, the LDLR on the surface of mononuclear cells was reduced by 41% before insulin treatment. After three months of therapy, LDLR expression increased by 57% in these individuals (146).

* 1. *Thiazolidinediones and sdLDL in T2DM*

Thiazolidinediones (TZDs) are insulin-sensitizing drugs and ligands for the nuclear receptor transcription factor PPARγ. PPARγ is expressed at high levels in adipose tissue, where it functions as a master regulator of adipocyte differentiation, and at much lower levels in other tissues (147), and it is also a key regulator of glucose homeostasis (148).

Sixty overweight T2DM patients without lipid-lowering therapy were randomized to metformin, pioglitazone, or gliclazide. LDL subfraction '3' mass and the LDL '3'-to-LDL ratio decreased with pioglitazone and metformin, with no change with gliclazide (149). These LDL '3' reductions were associated with reciprocal LDL '1' increases. Changes were independent of body weight, glycemic control, and TG (149). Pioglitazone combination treatment produced significant increases from baseline for mean and peak LDL particle size. Pioglitazone plus metformin reduced apoB100 levels (150).

However, drugs from the same class had different effects on lipoprotein concentrations and sizes (151). These differences are mainly driven by the partial activation of PPARα obtained with pioglitazone but not with rosiglitazone (152). In patients with T2DM and dyslipidemia, pioglitazone reduced the total concentration of LDL particles, whereas treatment with rosiglitazone increased. Both treatments increased LDL particle size, but treatment with pioglitazone had a more significant effect (151).

* 1. *Glucagon-Like Peptide-1 (GLP-1) Receptor Agonists, Liraglutide*

Glucagon-like peptide-1 (GLP-1) is an incretin hormone, inactivated by dipeptidyl peptidase-4 (DPP-4), capable of stimulating insulin secretion after an oral glucose administration (153). In parallel, the use of GLP-1 agonists in diabetic individuals favors inhibition of glucagon production and decreases apoptosis of pancreatic β cells while promoting their proliferation (153).

In individuals with T2DM, after six months of treatment with liraglutide, a decrease in the fasting plasma concentration of apoB100 and TG was observed (154). The kinetic study with stable isotopes showed an increase in the catabolism of TG-rich lipoproteins, including LDL (154). In mice, this therapy increased lipoprotein lipase (LPL) gene expression, reduced PCSK9 gene expression, and increased LDLR protein expression in the liver (154). However, in clinical studies, the change in the plasma lipid profile is mild or absent (155).

1. **The intriguing inverse relationship between LDL-C and T2DM** 
   1. *The inverse relationship between LDL-C and T2DM risk*

Although the association between dyslipidemia and T2DM is well known, the causal relationship remains debatable. The evaluation of genetic variants investigating the relationships between circulating lipid fractions and T2DM showed a strong association between genetically lower circulating LDL-C levels and the risk of T2DM (156). Furthermore, in an observational study with individuals not using antihypertensive or lipid-lowering therapies, low LDL-C concentrations may be associated with or causing an increased risk of diabetes (157). Regardless of lipid-lowering therapies, low LDL-C concentrations have been associated with a 2-fold increased risk of T2DM, and this association is proportional to the lifetime exposure to low LDL-C concentrations (158). Incident diabetes did not increase when the target LDL-C level was higher than 2.59 mmol/L, meaning the lower LDL-C target statin level contributed to the higher risk of diabetes (159).

In the Justification for the Use of Statins in Primary Prevention (JUPITER) trial, the risk of T2DM was higher in patients who achieved LDL-C <30 mg/dL compared to those with higher levels (160). In a meta-analysis with 13 randomized controlled trials, there was a 9% increased risk for incident DM (161). A similar result was observed by our consideration of the trials with anti-PCSK9ab (162). An analysis of 5 studies showed that intensive-dose statin therapy was associated with an increased risk of newly acquired diabetes compared with moderate-dose statin therapy (163). The same was found in an analysis involving 8 trials with intensive statin therapy, there was an 18% higher risk of incident DM (164). Indeed, high-dose statin increased the incidence of new-onset diabetes among patients with 2 to 4 risk factors for diabetes (165). Despite this slight increase of incident T2DM, this risk is outweighed by reducing the incidence of coronary events (161).

The molecular mechanisms that mediate the association between the use of statins and the incidence of DM remain unclear, especially the increased risk with greater reductions in LDL-C. One possible explanation involves the relationship between β-cell inflammation, oxidation and apoptosis (166) due to the internalization of cholesterol in pancreatic β cells resulting in impaired insulin secretion (167). It is possible that the effect of statins on incident diabetes may originate from impaired insulin secretion, resulting in altered glucose metabolism (168), since high levels of intracellular cholesterol are detrimental to the function of pancreatic β cells (167). This was verified in *in vitro* studies which demonstrated that prolonged exposure of isolated rat islet β cells to LDL caused necrosis (169).

In adipocytes, insulin stimulates glucose uptake by activating the insulin receptor tyrosine kinase, which in turn phosphorylates insulin receptor substrate-1, resulting in the recruitment of insulin-sensitive solute carrier family 2, member 4 (SLC2A4) to the plasma membrane (170). In an animal model of T2DM, treatment with atorvastatin at clinical doses inhibited adipocyte differentiation and decreased SLC2A4 expression, impairing insulin sensitivity (170). The reduction in SLC2A4 expression favors insulin resistance and this mechanism could explain the incidence of DM with the reduction of LDL.

* 1. *PCSK9 and HMGCR variants associated with LDL-C reduction but an increased risk of diabetes*

The effect of anti-PCSK9 in increasing the risk of diabetes, as it markedly lowers LDL-C was assessed using genetic scores. The study included 112,772 participants from 14 prospective cohort or case-control studies (171). Genetic variants that mimic the effect of PCSK9 inhibitors had a very similar effect on the risk of cardiovascular events and diabetes. Also, when statin variants were present, they showed independent and additive effects on both risks (171).

In a Mendelian randomized study, selection of four SNPs in or near *PCSK9* was associated with reductions in LDL-C and increased risk of diabetes together with higher circulating glucose concentration, body weight, and waist-to-hip ratio (172). Consistently, the presence of a defective allele in the gene of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), which encodes the target of statins, was associated with an increased risk of T2DM, at least partially mediated by increasing body weight (173).

The effect of DNA sequence variations that reduce plasma LDL-C levels on the incidence of coronary events was evaluated over a 15-year interval (174). The data showed that average lifetime LDL-C reduction was associated with a substantial reduction in the incidence of coronary events, even in populations with a high prevalence of non-lipid-related cardiovascular risk factors (174). Despite the increased risk of diabetes, the benefit of decreasing the risk of cardiovascular disease outweighs the other effects.

* 1. *Reduced risk of diabetes in familial hypercholesterolemia*

To verify whether the prevalence of T2DM is decreased in patients with familial hypercholesterolemia (FH), an observational study involving the Dutch national FH screening program registry found that the prevalence of T2DM was significantly lower in FH patients compared to unaffected relatives (167). The prevalence of T2DM in patients with heterozygous FH was 40% lower than that observed in the general population, and LDL-C concentrations were shown not to be risk factors (175). An inverse dose-response relationship has also been observed between the severity of familial mutation-causing hypercholesterolemia and the prevalence of T2DM (167).

1. **Conclusion**

LDL is a denomination for a set of lipoproteins that differ in size, composition, density and atherogenicity, whose phenotype is determined by the presence of several clinical conditions. Hyperglycemia promotes increased enteral cholesterol absorption and reduces LDLR expression in the liver. The subsequent increase in LDL-C plasma concentration stimulates glucose-mediated insulin secretion. Thus, in the short term, variations in plasma LDL-C concentration are expected when there is a disruption in glucose homeostasis. In contrast, in T2DM, the chronic exposition to hyperglycemia and insulin resistance triggers a wide range of changes in LDL, in particular the formation of sdLDL, which potentiate LDL’s pro-atherogenic action. In addition, sdLDL have reduced affinity for the LDLR and, therefore, present a prolonged residence time in plasma, being subject to constant oxidation and glycation, which makes them more atherogenic.

Given the above, it is evident that the progression of atherosclerotic disease occurs earlier in individuals with T2DM and that changes in LDL metabolism contribute to this predisposition. In this sense, LDL-lowering therapies have been emphasized in patients with T2DM with the aim of achieving intensive LDL-C goals, including drug combinations, capable of reducing levels that were until recently impossible. However, the residual risk remains high in T2DM patients, which may at least in part reflect a suboptimal change in the concentration of modified LDL particles, a condition not inferred from the usual LDL-C dosage. In this review we present T2DM-mediated modifications in the LDL phenotype and its potential clinical impacts. The evidence presented indicates that there is room for investigation of new parameters related to LDL as possible markers of residual risk and even potential therapeutic targets.

**Conflict of interest**

The authors declare no conflict of interest.

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