

Clinical Consequence of the Physicochemical Properties of LDL Particles in Type 2 Diabetes

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ABSTRACT Atherosclerosis is the major cause of death in type 2 diabetes. LDL cholesterol and atherosclerosis are correlated, both in nondiabetes people and those with diabetes, but people with diabetes are more given to atheroma, even though their LDL cholesterol levels are similar. This review analysed the evidence that modification of physicochemical properties of LDL play a role in the accelerated athero-sclerosis associated with type 2 diabetes.

KEY WORDS *atherosclerosis, diabetes, LDL oxidation, LDL glycation, LDL size*

Introduction

Diabetes and cardiovascular risk

Atherosclerosis is the leading cause of death in type 2 diabetes. LDL cholesterol and atherosclerosis are related, both in healthy people and those with diabetes; people with diabetes are more prone to atheroma, even though their LDL cholesterol levels are similar to those in their non-diabetic persons. This is because LDL particles are modified in the presence of diabetes to become more atherogenic. These modifications include glycation in response to high plasma glucose levels; oxidative reactions mediated by increased oxidative stress; and transfer of cholesterol ester, which makes the particles smaller and denser. The latter modification is strongly associated with hypertriglyceridaemia. Oxidatively and non-oxidatively modified LDL is involved in plaque formation, and may thus contribute to the accelerated atherosclerosis.

Atherosclerosis begins with endothelial dysfunction, accumulation of lipids in macrophages, and an inflammatory response, and results in plaque formation and narrowing of the lumen. The more vulnerable plaques are prone to rupture, which may lead to myocardial infarction (MI) or stroke. Atherosclerotic cardiovascular disease is the leading cause of morbidity and mortality in patients with type 2 diabetes, and the risk of developing the disease is two to four times higher than in non-diabetic subjects [1]. In addition, the risk of cardiac morbidity and mortality in individuals with type 2 diabetes

without previous MI has been shown to be similar to that in non-diabetic subjects with a history of MI. Although cardiovascular morbidity and mortality are increased in patients with diabetes, no more than 25% of the excess risk of cardiovascular disease can be explained by the co-existence of traditional risk factors such as hypertension, dyslipidaemia and central obesity [2].

Lipid disorders in diabetes

LDL cholesterol is one of the strongest predictors of CHD, both in individuals with and without diabetes type 2. It might be anticipated that plasma levels would be increased in diabetes; however, the *Heart Protection Study* found that plasma concentrations of LDL cholesterol are similar in control subjects and those with well-controlled type 2 diabetes [3]. This study also provided conclusive evidence that lowering cholesterol is beneficial to people with diabetes. Dyslipidaemia, characterised by hypertriglyceridaemia (fasting and postprandial) and a reduced HDL cholesterol level, is common in patients with the metabolic syndrome and type 2 diabetes, and is an independent predictor of cardiovascular disease. In the *Skaraborg Hypertension and Diabetes Project*, HbA1c and duration of diabetes were positively associated with plasma triglyceride concentrations [4], and the subjects with poor glucose control have higher concentrations of serum triglyceride —a

phenomenon that is often attributed to insulin resistance (Fig. 1).

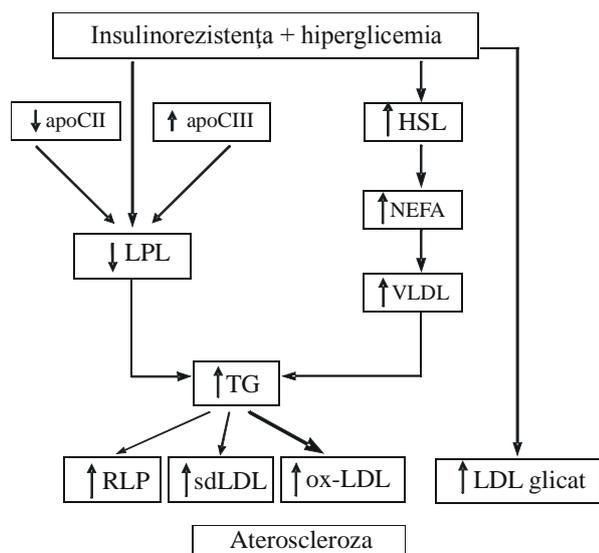


Figura 1 – Mechanism for the development of atherosclerosis in type 2 diabetes.

Lipoprotein lipase (LPL), which hydrolyses triglycerides into monoglycerides and fatty acids, is inhibited by apolipoprotein CIII and is activated by apolipoprotein CII and insulin. Reduced insulin action may thus increase plasma triglycerides by lowering their clearance rate. Increased hepatic production of triglyceride-rich VLDL also contributes to the increase in triglyceride. In central obesity, which is considered to play an important role in insulin resistance and the metabolic syndrome, a process mediated by hormone-sensitive lipase increases the nonesterified fatty acid (NEFA) concentration. This adipocyte-associated enzyme stimulates the hydrolysis of stored triglyceride and is inhibited by insulin. The NEFA flux from adipose tissue is thus increased in insulin-resistant subjects and will, in turn, contribute to increased synthesis and secretion of VLDL by the liver.

Since chylomicrons have to compete with VLDL for entry to the lipolytic system, which delays their clearance, insulin resistance is also associated with postprandial hypertriglyceridaemia. High postprandial concentrations of triglyceride-rich lipoproteins affect endothelial function, promote atherogenesis, and are associated with coronary artery disease (CAD) [5]. Remnants of these triglyceride-rich lipoproteins are now considered to be atherogenic since, compared with LDL particles, they can deliver more cholesterol to macrophages. High concentrations of remnant-like particles (RLP) have recently been found in type 2 diabetic patients [6].

LDL particle size

Each LDL particle (average 2.5 million) contains one molecule of apolipoprotein B100 (apoB100) and approximately 3000 lipid molecules. The core of the particle consists of cholesterol ester and triglyceride. Surrounds this core exists shell of protein, free unesterified cholesterol and phospholipids, and some 3300 fatty acids are bound in different lipids, approximately 50% of which are polyunsaturated (specifically, linoleic and arachidonic acid) (Table 1).

Molecule type	Number of molecules per LDL particle	Weight (%)
ApoB100	1	20.5±1.1
Triglycerides	191±42	6.7±1.6
Free cholesterol	605±66	9.4±0.8
Phospholipides	751±62	23.5±0.9
Cholesterol esters	1534±143	39.9±2.2
Fatty acids		
C16:0 (acid palmitic)	873±103	
C18:0 (acid stearic)	261±64	
C18:1 (acid oleic)	616±101	
C18:2 (acid linoleic)	1328±213	
C20:4(acid arahidonic)	207±5	

Table 1. Composition of LDL, expressed as the number of lipid molecules per LDL particle and as a percentage of the weight of the particle, determined in 94 individuals with well-controlled, normolipidaemic type 2 diabetes [8]

LDL is very heterogeneous with to lipid composition, charge, density, and particle size and shape and the strongest determinant of LDL particle size in type 2 diabetes is the free cholesterol content of the shell of the particle. A number of studies have shown that LDL particle size is negatively correlated with plasma triglyceride concentrations and positively correlated with HDL cholesterol levels. In contrast to plasma triglyceride, the triglyceride content of LDL is not associated with LDL particle size [7]. Triglyceride in the LDL particle is hydrolysed by hepatic lipase, making it smaller and denser. In response to elevated concentrations of plasma triglyceride-rich lipoproteins, as is typically the case in patients with type 2 diabetes, the rate of transfer is increased.

An alternative, complementary theory for the formation of small LDL is based on the existence of multiple LDL precursors. There are two pools of VLDL, large VLDL1 and small VLDL2, each of which has a different metabolic fate. Hepatic synthesis of VLDL1 is increased at fasting triglyceride concentrations exceeding 1.5 mmol/l. Paradoxically, large VLDL1 is preferentially metabolised into small, dense LDL particles. Multiple genes may contribute to LDL particle size, and heritability investigations have suggested

that genetic influences account for one-third to one-half of the variation in LDL peak particle diameter in humans.

LDL particle size and association with atherosclerosis and diabetes

Small LDL particles differ from normal-sized LDL particles in terms of metabolism and atherogenicity. Smaller LDL particles penetrate more easily into the arterial intima exhibit increased binding to arterial wall proteoglycans [9] and are more prone to oxidative stress. These small and dense LDL particles also have a prolonged plasma half-life because of their lower binding affinity for the LDL receptor. The presence of small, dense LDL particles in plasma is therefore considered pro-atherogenic [10-12].

LDL particle size may be used in conjunction with plasma LDL cholesterol measurements to provide an indication of the number of circulating LDL particles. Two individuals may have the same LDL cholesterol concentration, but the one with predominantly smaller particles will require more LDL particles to carry the same load of cholesterol. The observation that those with diabetes have smaller LDL particles but normal levels of plasma LDL cholesterol suggests that the number of particles is increased in diabetes. A rough estimate of the number of LDL (and VLDL) particles may be obtained by assessing the concentration of apoB100, because these lipoproteins contain one molecule of the protein. *The Insulin Resistance Atherosclerosis Study* found that apoB100 outperformed LDL cholesterol in the assessment of cardiovascular risk [13], and *Health Professionals' Follow-up Study* showed that, compared with LDL cholesterol, apoB100 was a stronger predictor of cardiovascular disease among diabetic men.

Assessment of LDL particle number by measurement of LDL size or apoB100 concentration will thus enhance the precision of the risk estimates based on LDL cholesterol.

LDL oxidation and oxidative stress

Oxidation of LDL initiates a series of events that ultimately lead to the enhanced uptake of LDL by macrophages, foam cell formation and plaque development.

Oxidised LDL and antibodies against the modified form of the lipoprotein have been found in human atherosclerotic lesions [14], but not in

normal arteries or veins. Several characteristics of oxidised LDL play a role in the development of atherosclerotic plaques: oxidised LDL is toxic to endothelial cells, recruits leucocytes to atherosclerotic lesions, and promotes the proliferation of macrophages within plaques.

The rate of LDL oxidation in the intima depends on multiple factors, including LDL concentration, endothelial barrier function, the intrinsic resistance of LDL to oxidation, and the local concentration of free radicals; the latter will depend upon the balance between the production of free radicals and the scavenging capacity of the antioxidant defence system. Although the initiating stimulus for LDL oxidation remains unknown, several potential mechanisms have been identified, including reactions with reactive oxygen species (ROS), haem proteins, and enzymes such as lipoxygenase and myeloperoxidase (Fig. 2).

There is good evidence for the involvement of enhanced oxidative stress in the pathogenesis of cardiovascular disease in diabetes. Free radicals can damage the double bonds of polyunsaturated fatty acids in the cell membrane, leading to a chain of chemical reactions called lipid peroxidation, during which aldehydes are formed. The measurement of malondialdehyde (MDA) by the thiobarbituric acid test is an indirect way of quantifying oxidative stress. Although this method lacks specificity and selectivity, it is widely used to estimate the level of lipid peroxidation.

Plasma MDA concentrations are increased in subjects with type 2 diabetes compared with those in type 1 diabetic subjects and healthy control subjects [15]. F₂-isoprostanes, prostaglandin F₂-like compounds formed by the nonenzymatic oxidation of arachidonic acid, are currently considered the most reliable biomarkers of in vivo oxidative stress, and several studies have reported that the level of isoprostanes is increased in type 2 diabetic patients.

The antioxidant capacity of diabetic patients, both enzymatic and non-enzymatic, has also been examined in detail. Plasma vitamin E concentrations are lower in diabetic patients than in control subjects; leucocytes from type 2 diabetic patients have reduced levels of vitamin C; and erythrocyte superoxide dismutase activity is reduced in diabetes.

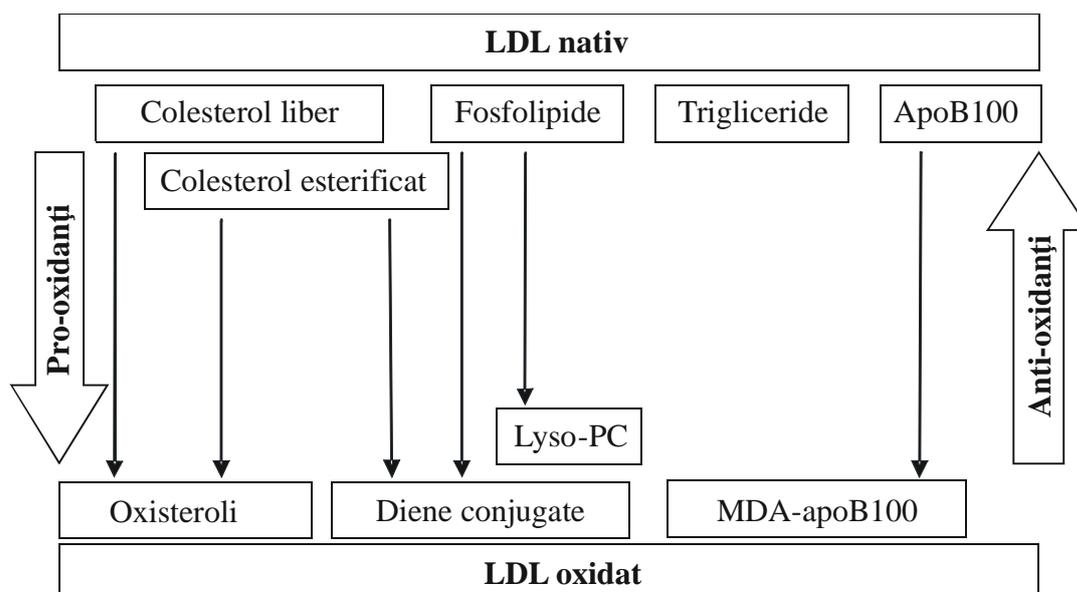


Fig.2. Schematic diagram of the oxidation products formed during free radical-mediated LDL oxidation. Several oxysterols are formed during oxidation of cholesterol; phosphatidylcholine is converted into lyso-phosphatidylcholine; and lipid hydroperoxides are cleaved to form shorter aldehydes (e.g. MDA) which, in turn, may react with apoB100. Pro-oxidants are myeloperoxidase, lipoxygenase, haem proteins, peroxynitrite, metal ions, ROS, NO; endogenous and plasma antioxidants are Co enzyme Q10, vitamin E, vitamin C, β -carotene, flavonoids, albumin.

LDL glycation

The initial products generated by glycation, otherwise known as non-enzymatic glycosylation, undergo intramolecular rearrangements over time and transform into AGE (*Advanced Glycation End products*). Since AGE form predominantly on long-lived proteins such as collagen, their clinical significance is beyond the scope of this review. However, the initial glycation products that arise from the reaction of glucose with the lysine residues of apoB100 represent an important modification of LDL. The LDL of diabetic patients is more glycated than that of non-diabetic individuals.

This is especially true in those with poor glycaemic control. Glycation of LDL affects its biological function. For example, compared with normal LDL, glycated LDL is catabolised more slowly. Oxidation may also damage glucose directly, with concomitant generation of free radicals; consequently, hyperglycaemia may lead to oxidative stress. The combination of glycation and oxidation is termed 'glycooxidation', and Wolff and Dean have demonstrated that, under diabetic conditions, ROS are produced via glucose auto-oxidation. Consistent with this, it has been shown that hyperglycaemia results in an increased oxidative load and that glucose intake stimulates ROS generation [16]. Some studies have shown that glycation accelerates the oxidisability of LDL *in vitro*. For example, when native LDL was

glycated with different concentrations of glucose (0, 5, 10 and 20 mmol/l) and then oxidised by copper ions, the amount of thiobarbituric acid reactive substances (TBARS) in LDL—a parameter of lipid oxidation—increased with increasing glucose concentrations in a dose-dependent mode. Consistent with this, the glycation level of native LDL is positively correlated with LDL oxidation, as assessed by measuring TBARS during a 4-h oxidation period.

In summary, apoB100 glycation is approximately twice as high in the LDL of diabetic patients than in the LDL of non-diabetic subjects. Hyperglycaemia is associated with enhanced glycation of LDL and an increase in free radical production. It therefore seems reasonable to assume that this contributes to the accelerated atherosclerosis associated with diabetes, and this assumption should be explored in longitudinal studies.

Conclusions and perspectives

There is growing evidence that modifications of LDL enhance its atherogenicity. Since modified LDL is not a single homogeneous entity, there is no single diagnostic or prognostic marker that adequately reflects the risk of cardiovascular disease associated with modified LDL. However, there is no conclusive evidence of enhanced *in vitro* oxidisability of LDL in type 2 diabetic subjects. This may be because *in vitro* oxidisability of LDL merely reflects its

susceptibility to oxidation, which is only one of many factors that determine the rate of LDL oxidation in the vascular wall. Other factors include the permeability of the endothelium, retention of LDL by the components of the extracellular matrix, and local oxidant stress. Following oxidation in the vascular wall, some oxidised LDL particles may escape scavenging by resident macrophages and return to the circulation. Unlike *in vitro* measures of LDL oxidisability, circulating oxidised LDL therefore probably reflects the overall process of LDL oxidation. Circulating levels of *in vivo* oxidised LDL are higher in patients with type 2 diabetes than in control subjects, and further studies are needed to determine whether assessment of oxidised LDL is of value for the identification of patients at high risk of cardiovascular disease.

The presence of small, dense LDL particles in plasma is associated with hypertriglyceridaemia and an increased risk of CAD. Small LDL particles predominate in individuals with the insulin resistance syndrome, and an inverse relationship has been observed between LDL size and circulating *in vivo* oxidised LDL in type 2 diabetic patients. LDL particle size can be favourably affected by establishing good blood glucose control. This supports the importance of tight glycaemic control in diabetes, above and beyond its established benefits in microvascular disease. LDL particle size is increased by fenofibrate, whereas statin therapy, which strongly reduces the total amount of LDL cholesterol, does not affect LDL particle size [18]. Glycation of LDL is enhanced in individuals with type 2 diabetes, and this may contribute to accelerated atherosclerosis via an increase in free radical production and possibly by rendering LDL more susceptible to oxidation.

In conclusion, LDL cholesterol is a poor predictor of the CAD risk associated with type 2 diabetes. Several oxidative and non-oxidative LDL modifications, such as oxidation and glycation, contribute to the accelerated atherosclerosis associated with this condition. Measurement of these LDL modifications is technically demanding, and therefore unsuitable for routine practice. Plasma triglyceride concentrations are closely linked to a preponderance of small LDL particles and increased concentrations of oxidized LDL. In addition, the number of LDL particles is positively related to the risk of CAD. This can be assessed by measuring LDL size or estimated by measuring apoB100, but cannot be measured by determination of the LDL cholesterol

concentration. Prediction of future CAD in type 2 diabetic patients could thus be improved by the routine measurement of triglyceride and apoB100 in addition to LDL cholesterol.

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