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Clinical significance of the physicochemical properties of LDL in type 2 diabetes

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Abstract 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; however, people with diabetes are more prone to atheroma, even though their LDL cholesterol levels are similar to those in their non-diabetic peers. 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. This review discusses the techniques currently used to determine the physicochemical properties of LDL, and examines the evidence that modification of these properties plays a role in the accelerated atherosclerosis associated with type 2 diabetes.

Keywords Atherosclerosis · Diabetes · LDL · LDL diameter · LDL glycation · LDL oxidation · LDL size

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Abbreviations apoB100: apolipoprotein B100 · CAD: coronary artery disease · CETP: cholesterol ester transfer protein · LPL: lipoprotein lipase · MDA: malondialdehyde · MI: myocardial infarction · NMR: nuclear magnetic resonance · RLP: remnant-like particles · ROS: reactive oxygen species · TBARS: thiobarbituric acid reactive substances

Introduction

Diabetes and cardiovascular risk

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 [2]. 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 [3].

Lipid disorders in diabetes

LDL cholesterol is one of the strongest predictors of CHD, both in individuals with and without diabetes [4]. It might therefore 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 [5]. 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 (particularly HDL2), is common in patients with the metabolic syndrome and type 2 diabetes [6, 7], and is an independent predictor of cardiovascular disease. In the Skaraborg Hypertension and Diabetes Project, HbA_{1c} and duration of diabetes were positively associated with plasma triglyceride concentrations [8], consistent with the finding that those with poor glucose control have higher concentrations of serum triglyceride—a phenomenon that is often attributed to insulin resistance (Fig. 1). 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. Enhanced hepatic production of triglyceride-rich VLDL also contributes to the increase in triglyceride. In central obesity, which is considered to play a crucial role in insulin resistance and the metabolic syndrome, a process mediated by hormone-sensitive lipase increases the 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.

Fig. 1 Schematic diagram of the proposed mechanism for the development of atherosclerosis in type 2 diabetes. Reduced insulin action and/or an increased concentration of apolipoprotein CIII may lead to elevated plasma triglyceride through inhibition of LPL. In central obesity, which is common in type 2 diabetes, the NEFA concentration is increased because hormone-sensitive lipase is not inhibited by insulin. This results in hepatic overproduction of triglyceride-rich VLDL, particularly VLDL1. High plasma triglyceride concentrations are associated with RLP, oxidised LDL and small, dense LDL. Together with glycated LDL, these features are at least partly responsible for the accelerated atherosclerosis observed in individuals with type 2 diabetes. *HSL* hormone-sensitive lipase, *ox-LDL* oxidized LDL, *sdLDL* small dense LDL, *TG* triglyceride

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) [9]. 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 [10].

LDL particle size

Each LDL particle (average M_r ~2.5 million) contains one molecule of apolipoprotein B100 (apoB100) and approximately 3,000 lipid molecules [11]. The core of the particle consists of cholesterol ester and triglyceride. A shell of protein, free (unesterified) cholesterol and phospholipids surrounds this core, and some 3300 fatty acids are bound in different lipids, approximately 50% of which are polyunsaturated (specifically, linoleic and arachidonic acid) (Table 1). LDL is very heterogeneous with respect to lipid composition, charge, density, and particle size and shape (Fig. 2), and the strongest determinant of LDL particle size in type 2 diabetes is the free cholesterol content of the shell of the particle [12]. A number of studies have shown that LDL particle size is negatively correlated with plasma triglyceride concentrations (Fig. 3) and positively correlated with HDL cholesterol levels [13, 14]. In contrast to plasma triglyceride, the triglyceride content of LDL is not associated with LDL particle size [13]. The association between LDL particle size and the concentration of fasting plasma triglyceride can be explained by changes in the metabolism of triglyceride-rich particles that result in the

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

	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
Phospholipids	751±62	23.5±0.9
Cholesterol esters	1534±143	39.9±2.2
Fatty acids		
C16:0 (palmitic acid)	873±103	
C18:0 (stearic acid)	261±64	
C18:1 (oleic acid)	616±101	
C18:2 (linoleic acid)	1328±213	
C20:4 (arachidonic acid)	207±5	

Data are means±SD. Fatty acids that contribute less than 5% are not listed

Fig. 2 Electron micrograph (taken with a CM 100 Bio Electron Microscope; Philips, Eindhoven, The Netherlands) of human LDL negatively stained with methylamine tungstate. LDL was isolated by ultracentrifugation with a density between 1.019 and 1.063 kg/l and then dialysed for 24 h against 0.1 mmol/l PBS containing 1% ammonium acetate. The image was recorded at a magnification of $\times 280,000$

remodelling of lipoprotein particles. Briefly, the transfer of cholesterol ester from HDL and LDL to triglyceride-rich lipoproteins (VLDL, chylomicrons and RLP), and the transfer of triglyceride in the opposite direction, is mediated by cholesterol ester transfer protein (CETP), the activity of which is increased in type 2 diabetes [15]. 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. Synthesis of VLDL1 by the liver is increased at fasting triglyceride concentrations exceeding 1.5 mmol/l. Paradoxically, large VLDL1 is preferentially metabolised into

Fig. 3 Association between LDL particle diameter, as estimated by high performance gel-filtration chromatography, and plasma triglyceride in 788 subjects with normal glucose metabolism, impaired glucose metabolism or type 2 diabetes. The strength of the association ($r = -0.64$, $p < 0.001$) was assessed by Spearman's rank correlation test (unpublished data)

small, dense LDL particles [6]. Multiple genes may contribute to LDL particle size, and heritability analyses have suggested that genetic influences account for one-third to one-half of the variation in LDL peak particle diameter in humans [16].

Measurement of LDL particle size

LDL particle size is traditionally measured by non-denaturing PAGE [17]. Alternatively, electron microscopy of negatively stained LDL may be used (Fig. 2) [18]. Both methods are time consuming and may be unsuitable for large series of samples. Easier methods of measuring LDL particle size are currently being developed, including light scattering [19], high-performance gel-filtration chromatography [13, 20], and nuclear magnetic resonance (NMR) spectroscopy [21]. LDL particle diameter may also be determined from particle volume, which, in turn, may be calculated from its lipid composition, based on the fact that each LDL particle contains a single molecule of apoB100. This calculation is only valid if LDL particle shape is spherical. However, this assumption may be false, since recent studies have shown that LDL particles may be discoid in shape rather than spherical [12, 22]. If confirmed, this would affect the interpretation of data obtained by techniques such as dynamic light scattering and NMR, in which particle volume is the variable that is actually measured, and particle diameter is derived under the assumption of spherical geometry. This observation may explain the recently described discrepancy between LDL particle size as measured by NMR and that determined by gradient gel electrophoresis, which was found to be especially pronounced in women and those with diabetes [23].

Measurement of LDL particle size is technically demanding; various efforts have therefore been made to estimate this variable indirectly. Wagner and coworkers have shown that the ratio of apoB100 : LDL cholesterol in plasma is a good predictor of LDL phenotype B (characterised by a predominance of small, dense LDL particles) in type 2 diabetes [24]; however, Tallis and coworkers could not confirm this finding [25]. A possible explanation for this discrepancy is that the percentage of plasma apoB100 carried in VLDL particles rather than in LDL particles increases at higher triglyceride concentrations (> 2 mmol/l). Consequently, the apoB100 : LDL cholesterol ratio is only a good predictor of LDL particle size at plasma triglyceride concentrations up to 2 mmol/l.

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 [26], exhibit increased binding to arterial wall proteoglycans [27], and are more prone to oxidative stress [28–30]. Small, dense LDL particles also have a prolonged plasma

half-life because of their lower binding affinity for the LDL receptor [31]. The presence of small, dense LDL particles in plasma is therefore considered pro-atherogenic. Several cross-sectional and prospective studies have shown that a preponderance of smaller LDL particles is associated with an increased risk of CAD, and the Diabetes Atherosclerosis Intervention Study (DAIS) reported that small LDL particle size is significantly associated with its progression [32]. A study conducted by Campos and coworkers found that the prevalence of small LDL particles was higher in those with CAD than in control subjects [33]. The Stanford Coronary Risk Intervention Project (SCRIP) reported that the annual progression of stenosis over a 4-year period was more rapid in men with smaller LDL particles than in men with large LDL particles [34]. The prospective Quebec Cardiovascular Study reported that men with small, dense LDL particles had an increased risk of CAD compared with men with normal-sized LDL particles, independent of LDL cholesterol, triglyceride and the total cholesterol : HDL cholesterol ratio [35]. However, others have failed to find an independent association between particle size and MI [36]. People with abnormal glucose metabolism have significantly smaller average particle diameters than non-diabetic individuals [37, 38]. A study of Japanese men found that 50% of those with glucose intolerance had small LDL particles, as compared with 10% of controls [39]. Furthermore, the risk of developing type 2 diabetes is more than doubled in individuals with a preponderance of small LDL particles [14].

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 [40], and the Health Professionals' Follow-up Study showed that, compared with LDL cholesterol, apoB100 was a stronger predictor of cardiovascular disease among diabetic men [41]. 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 [42]. Oxidised LDL and antibodies against the modified form of

the lipoprotein have been found in human atherosclerotic lesions [43], 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 [44], recruits leucocytes to atherosclerotic lesions [45], and promotes the proliferation of macrophages within plaques [46].

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. 4).

There is good evidence for the involvement of enhanced oxidative stress in the pathogenesis of cardiovascular disease in diabetes [47]. 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 [48]. F2-isoprostanes, prostaglandin F2-like compounds formed by the non-enzymatic 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 [49].

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 [50]; leucocytes from type 2 diabetic patients have reduced levels of vitamin C [51]; and erythrocyte superoxide dismutase activity is reduced in diabetes [48].

Measurement of LDL oxidation

A number of products are formed by the peroxidation and fragmentation of lipids and by the modification and degradation of the protein moiety (apoB100) during the oxidation of an LDL particle (Fig. 4). Conjugated dienes are initially formed from unsaturated fatty acids by free radical-mediated hydrogen abstraction and the rearrangement of double bonds [52]. Phosphatidylcholine is degraded into lysophosphatidylcholine, and cholesterol is converted into a large number of different oxysterols. LDL protein is modified by reactions between secondary oxidation products (e.g. MDA) and lysine residues on apoB100. Measurement of any of these compounds may thus provide an

Fig. 4 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 shown on the left-hand side of the figure; endogenous and plasma antioxidants are indicated on the right-hand side. *Lyso-PC* lyso-phosphatidylcholine, *NO* nitric oxide

index of *in vivo* LDL oxidation [53]. Much research has focused on determining the susceptibility of LDL to *in vitro* oxidation, based on the assumption that this measure reflects the *in vivo* oxidisability of LDL. The most widely accepted procedure involves monitoring the conversion of polyunsaturated fatty acids into conjugated dienes, as originally described by Esterbauer et al. [52]. The time course shows three consecutive phases (Fig. 5).

In vivo oxidation of LDL has also been studied by measuring the subfraction that has a relatively more negative electrical charge. This subfraction is formed by the reaction of positively charged amino groups with aldehydes and thus represents the oxidatively modified form of LDL [54]. Autoantibodies directed against oxidised LDL are also used as an indirect measure of the *in vivo* oxidation of LDL [55], and ELISAs have been developed for direct measurement of oxidised apoB100 in LDL [43, 56].

Fig. 5 Kinetics of *in vitro* oxidation of LDL determined by absorbance at 234 nm (corresponding to absorbance by conjugated dienes). The time course is divided into three phases: (1) a lag phase, during which the LDL particle is protected from oxidation; (2) a propagation phase, which occurs when the antioxidants are depleted; and (3) a decomposition phase, during which double bonds are cleaved and aldehydes are formed

LDL oxidation and association with atherosclerosis and diabetes

Small, dense LDL particles are considered more susceptible to oxidation than larger particles, and at least three studies have found an inverse relationship between the lag time of LDL oxidation and LDL density [28–30]. Using fractionated LDL, these studies showed that small, dense LDL particles are less resistant to *in vitro* oxidation than large, buoyant LDL particles taken from the same individual. However, we were unable to demonstrate a relationship between LDL oxidisability and LDL particle diameter in cross-sectional studies including individuals with type 2 diabetes [38, 57].

Several studies have found inverse relationships between the lag time of *in vitro* LDL oxidation and the severity and progression of coronary atherosclerosis [58], suggesting that enhanced susceptibility to oxidation may underlie the excess vascular disease observed in patients with diabetes. This is consistent with studies showing that the mean lag time of LDL oxidation is lower in patients with diabetes than in controls [50, 59]. This issue is still under debate, however, since other studies found no difference between healthy control subjects and patients with diabetes [38, 60]. Ahotupa and colleagues developed a simple technique for evaluating *in vivo* LDL oxidation based on the determination of baseline levels of conjugated dienes in LDL [61], and we and others found no differences between these levels in patients and control subjects [53, 62].

The susceptibility of an LDL particle to oxidation is largely determined by the number and type of fatty acids it contains. Polyunsaturated fatty acids are particularly susceptible to peroxidation by free radicals, whereas LDL particles enriched with monounsaturated fatty acids are less prone to oxidative injury [63]. In agreement with these *in vitro* findings, patients with type 2 diabetes treated with a diet enriched with monounsaturated fatty acids show an increased LDL lag time [64]. We investigated the contri-

butions made by the various constituents of LDL in 94 normolipidaemic patients with well-controlled type 2 diabetes and found that the cholesterol, phospholipid and triglyceride content of LDL had little influence on its oxidisability [65]. Arachidonic acid was inversely correlated with lag time, whereas linoleic acid was not. Consistent with this, fatty acids with three or more double bonds were the strongest determinants of LDL lag time ($r=-0.56$, $p<0.001$), suggesting that LDL rich in highly unsaturated fatty acids is more prone to oxidation. Linoleic acid, the most abundant fatty acid in LDL, was positively correlated with conjugated diene production, so both linoleic acid and arachidonic acid were associated with increased LDL oxidisability. Tomkin and coworkers have demonstrated that type 2 diabetic patients have an increased amount of linoleic acid and arachidonic acid in LDL as compared with non-diabetic individuals [59]. One relatively small study reported that levels of total plasma oxysterols and 7-ketocholesterol tend to be higher in type 2 diabetic patients than in control subjects [66]. In accordance with this, we recently demonstrated that free (unesterified) 7-ketocholesterol levels are significantly higher in the LDL of patients with well-controlled type 2 diabetes than in the LDL of healthy subjects [53]. Furthermore, it has been shown that the percentage of electronegative LDL in plasma is higher in microalbuminuric diabetic patients than in normoalbuminuric diabetic patients or control subjects [67].

Plasma levels of oxidised LDL, as measured by sandwich ELISA, were increased in individuals with IGT compared with control subjects after adjusting for age and BMI [68], and are higher in type 2 diabetic patients with macroalbuminuria than in patients with normo- or microalbuminuria or healthy control subjects [69]. Patients with well-controlled diabetes have higher levels of circulating oxidised LDL than control subjects, when adjusted for LDL cholesterol concentration [38, 53]. Moreover, an inverse relationship has been observed between circulating levels of oxidised LDL and LDL size in patients with type 2 diabetes [38]. An independent association has been observed in middle-aged men between the plasma concentration of oxidised LDL and subclinical atherosclerosis, as measured by the intima media thickness of the common carotid artery [70]. In a prospective study of the role of oxidised LDL in the development of atherosclerosis it was shown that, among type 2 diabetic patients with angiographically proven CAD, those with elevated levels of oxidised LDL had a significantly higher prevalence of cardiac events during the follow-up period of 52 months [71]. It has also been demonstrated that circulating oxidised LDL is associated with the progression of ultrasound-assessed atherosclerosis in clinically healthy middle-aged men, independently of conventional risk factors [72].

The significance of autoantibodies against oxidised LDL remains controversial. One study found a non-significant reduction in levels of autoantibodies against oxidised LDL in patients with type 2 diabetes or CAD than in control subjects [73], whereas another study reported significantly higher levels in diabetes [74]. Antibodies against oxidised LDL are increased in type 2 diabetic patients with

macrovascular disease, but not in those without [75]. An unexpected inverse association has been found between carotid artery intima media thickness and IgM autoantibody titres to MDA-modified LDL [76]. Increased titres of autoantibodies against oxidised LDL are therefore hard to interpret; they may simply reflect enhanced LDL oxidation or, conversely, might be considered beneficial in that they indicate an adequate response by the immune system.

In summary, there is strong evidence for increased oxidative stress and accelerated LDL oxidation in type 2 diabetes, which may contribute to the increased cardiovascular risk associated with this disease. Measurement of oxidised LDL appears better than the determination of *in vitro* oxidation or measurement of autoantibodies against oxidised LDL for assessment of cardiovascular risk in patients with type 2 diabetes.

LDL glycation

The initial products generated by glycation, otherwise known as non-enzymatic glycosylation, undergo intramolecular rearrangements over time and transform into so-called AGE. 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 [77]. The LDL of diabetic patients is more glycated than that of non-diabetic individuals [78]. 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 [79]. Oxidation may also damage glucose directly, with concomitant generation of free radicals; consequently, hyperglycaemia may lead to oxidative stress [80]. The combination of glycation and oxidation is termed 'glycoxidation', and Wolff and Dean have demonstrated that, under diabetic conditions, ROS are produced via glucose auto-oxidation [81]. Consistent with this, it has been shown that hyperglycaemia results in an increased oxidative load and that glucose intake stimulates ROS generation [82]. 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 manner [83]. 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 [84]. 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 [38]. LDL particle size can be favourably affected by establishing good blood glucose control [85, 86]. 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 [32], whereas statin therapy, which strongly reduces the total amount of LDL cholesterol, does not affect LDL particle size [87]. 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 oxidised 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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