LDL particle size: an important drug target?

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Introduction

Low density lipoprotein (LDL) is the main carrier of plasma cholesterol and a major component of atherosclerotic plaque [1]. Lowering LDL cholesterol reduces coronary events and mortality from coronary artery disease (CAD) [2–4], however, the relation between LDL cholesterol concentration and (CAD) is complex. Many patients with CAD have plasma LDL cholesterol concentrations in the normal range for the general population [5]. Thus, it could be that coronary risk goes beyond LDL cholesterol concentration to the characteristics of the LDL particles themselves. The purpose of this communication is to address the issue of whether LDL particle size and density influences its atherogenecity and how this might be modified by drug therapy.

Physico-chemical properties of low density lipoproteins (LDL)

Human LDL particles can be isolated by density gradient centrifugation in the density range of 1.019–1.063 g ml^{-1} and contain approximately 50% cholesterol (free and esterified), 25% proteins, 20% phospholipids and 5% triglycerides. Over 95% of the LDL protein mass is apolipoprotein B-100 (apo B-100, 549 kDa) [6, 7], each LDL particle containing only one molecule of apo B-100. The molecular mass of LDL is in the range from 2.4–3.9 MDa [8]. The particles are usually described as spherical, containing a central core of non-polar cholesteryl esters and triglycerides while free cholesterol intercalates between the phospholipid fatty acid chains providing a degree of rigidity to the phospholipid monolayer that is the LDL outer coat which interfaces with plasma. ApoB-100 is exposed at the surface allowing receptor recognition [9]. More recent studies using cryoelectron microscopy suggest that human LDL is discoidal with diameter 21.4 ± 1.3 nm, height 12.1 ± 1.1 nm and average volume of 4352 nm^3 [10].

The LDL particle population is heterogeneous with the respect to size, density and composition. Distinct subpopulations vary in isoelectric point, electrical charge, hydrodynamic properties and immunoreactivity [11]. This heterogeneity has been identified through the use of density gradient, rate zonal and analytical ultracentrifugation as well as with nondenaturing gradient gel electrophoresis [12]. Depending on the methodology used, from 2 to 38 LDL subfractions have been separated [13].

Measurements of LDL subfraction diameters using negative staining electron microscopy have established that mean particle diameter decreases with increasing density [14]. The structure of LDL particles of different densities varies with respect both to the size of the core and the width of the surface shell [15].

LDL subfraction composition varies between individuals. Of the various phenotypic classifications of LDL subfraction patterns, two of the most widely used are those of Musliner & Krauss [16] and Austin *et al.* [17]. The former divide subjects into one of four main groups (LDL I to LDL IV) with properties shown in Table 1. In an alternative classification based on particle diameter Austin *et al.* [17] suggested two major patterns of LDL profile, pattern A (particle diameter 25.5 nm or greater) and pattern B (particle diameter less than 25.5 nm). In kinetic turnover studies, where the rate of urinary excretion of radioactive products of labelled LDL were

Table 1 Classification of LDL particles by Musliner & Krauss [16].

Subfraction	*Density*	*Particle diameter*
LDL I	1.025–1.035 g ml^{-1}	26–27 nm
LDL II	1.032–1.038 g ml^{-1}	25.5–26 nm
LDL III A	1.038–1.050 g ml^{-1}	24.7–25.6 nm
LDL III B		24.2–24.6 nm
LDL IV A	1.048–1.065 g ml^{-1}	23.3–24.2 nm
LDL IV B		21.8–23.2 nm

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measured, two LDL pools were demonstrated. A rapidly cleared pool A (probably consisting of large LDL particles) and a slowly cleared pool B (small LDL particles) [18].

The chemical composition of LDL subfractions

LDL subfractions share several common features. Cholesteryl ester is the principal lipid (38.3–42.8%) and free cholesterol (8.5–11.6%) tends to diminish as density increases. Triglycerides are a minor component (3–5%). Density increases with increasing protein content. ApoB-100 is the major protein in all subfractions. ApoE constitutes 0.1–1.3% and 0.2–1.9% of LDL proteins in subfractions of low and high density, respectively. The ratio of apoE to apoB changes from 1 : 60 to a maximum of 1 : 8 in denser subfractions possibly accounting for differences in binding affinities for LDL receptors. Apo C-III is present in subfractions with densities greater than 1.0358 g ml^{-1}. Calculation of the number of each chemical component per LDL subspecies showed the presence of one molecule of apoB per particle in association with decreasing amount of cholesteryl esters, free cholesterol and phospholipids [11]. The diameter of human LDL particles correlates positively with the molar ratio of phospholipid/apo B in LDL but not with the molar ratio of either cholesterol/apoB or triglyceride/apo B suggesting that phospholipid content is also an important determinant of LDL size [19].

There are distinct and constant differences in the electrical charge of LDL subfractions at neutral pH of 7.4 arising as a result of either dissimilarities in the relative proportions of charged phospholipids or of sialytion of associated proteins [11, 20]. Negative charge increases with increasing density of LDL particles. Small LDL particles have significantly lower neutral carbohydrate and sialic acid content [20, 21]. LDL particles with lower sialic acid content have greater affinity for proteoglycans in the arterial wall and could be preferentially involved in the development of atherosclerosis [21, 22].

Factors that influence LDL subfractions profile

The biochemical processes that underlie the formation of distinct LDL subfractions are incompletely understood. Most LDL particles originate from larger triglyceride rich apo-B containing particles such as VLDL that are secreted from the liver. However some kinetic studies suggest that LDL particles are also normally secreted from the liver [23]. Lipoprotein lipase (LPL) progressively removes triglycerides from the core of VLDL to form intermediate density lipoprotein (IDL) particles which can be either degraded directly by the liver via receptor-mediated binding or further metabolised by LPL and hepatic lipase (HL) to LDL particles. Some of the surface constituents (cholesterol, phospholipids, apo-C and apoE) are released and transferred to HDL. Cholesteryl ester remains and the remnant lipoprotein is a cholesteryl ester-enriched large LDL. Cholesterol ester transfer protein (CETP) transfers cholesteryl esters from the LDL back to VLDL in exchange for triglycerides. During lipolysis VLDL loses much of its apo-C, so the proportion of apo-E increases which is of importance as hepatic LDL receptors have a particularly strong affinity for apo-E [24].

The triglyceride content of the precursor lipoproteins is a major determinant of the size of the LDL product formed by lipolysis [25], larger triglyceride-rich VLDL particles giving rise to smaller LDL particles. This apparent paradox is explained by the fact that large triglyceride rich VLDL particles provide a ready substrate for the CETP. It transfers cholesteryl esters from LDL particles in exchange for triglycerides from VLDL. Triglyceride enriched LDL has its acquired triglycerides removed by the actions of the enzymes LPL and hepatic lipase (HL) leading to continued particle size reduction. High HL activity is associated with an increased concentration of small LDL even at lower plasma triglyceride levels [23, 25]. Accordingly, deficiency of HL is associated with increased large LDL particles whereas raised HL activity is associated with a predominance of smaller LDL [26].

The distribution of LDL particle size is determined by both genetic and environmental factors. Phenotype B (predominance of small LDL particles) is found in 30-35% of adult Caucasian men but is less prevalent in men younger than 20 years and in premenopausal women. The data are consistent with either an autosomal dominant or codominant model for inheritance of the pattern B phenotype with additional polygenic effects of variable magnitude. Pattern B is linked to the LDL receptor gene locus on chromosome 19 [27]. Estimates of heritability of LDL particle size range from 30-50% confirming the importance of environmental influences in determining the LDL profile [12]. Such environmental factors include diet, obesity, exercise and drugs (lipid lowering drugs, beta adrenergic receptor antagonists) as well as age and hormonal status.

The pattern B phenotype correlates strongly with insulin resistance [28]. The explanation for this association is not fully known. It is possible that failure of insulin to suppress free fatty acid release from adipose tissue, in subjects with insulin resistance, causes increased influx of free fatty acids to the liver. This would result in an increased secretion of VLDL and transfer of its triglycerides to LDL. Furthermore, insulin activation of LPL is suppressed in insulin resistance affecting hydrolysis of

triglyceride-rich lipoproteins including large VLDL, leading to further LDL particle size reduction [28].

LDL subfractions and CAD risk

The predominance of small, dense LDL particles is correlated with an increased risk for CAD [17, 29], and small LDL subfractions are more prevalent among patients with CAD. The predominance of small LDL subfractions is generally associated with increased triglyceride concentrations and often with low HDL cholesterol concentrations. Hence the risk associated with small LDL subfractions is reduced after adjusting for these parameters in multivariate analyses. However, three recent studies have shown an increased risk of CAD associated with the predominance of small LDL particles independent of other lipid parameters, including triglycerides [30–32].

Griffin and colleagues (1994) were first to demonstrate that the predominance of small, dense LDL particles in patients with CAD was independent of triglyceride concentrations. However, in their study patients with CAD had significantly higher triglyceride concentrations than the control subjects [30]. The only study of LDL subfraction profiles in normotriglyceridaemic men with established CAD, showed that LDL particles were significantly smaller in men with CAD than in controls, regardless of other plasma lipid parameters, including triglycerides and HDL cholesterol. Furthermore, LDL subfraction profile was the strongest predictive factor for the presence of CAD when compared to other lipid parameters [31]. Finally, in the first large prospective study of LDL subfractions followed over 5 years, LDL particle size was predictive of CAD independently of other lipid parameters including triglyceride concentrations [32]. Taken together these studies suggest that triglyceride concentration is not the only factor in determining LDL particle size. LDL subfraction analysis may further define risk of CAD, particularly in men with relatively normal lipid profiles.

Although the cited cross-sectional studies suggest that small, dense LDL particles are especially atherogenic, there are additional possibilities to be considered. For example, this atherogenic lipoprotein phenotype often clusters with insulin resistance which may be an etiological factor leading to enhanced CAD risk in many patients with the small dense LDL phenotype [33]. The predominance of small LDL particles over other LDL particles, is also strongly correlated with high plasma fibrinogen concentrations in men. The reason for this association (which is independent of cholesterol, triglycerides, body mass index, age and insulin resistance) is unknown but since hyperfibrinogenaemia is an independent risk factor for CAD this could account for some of the effects of small, dense LDL particles on CAD [34].

Atherogenic/thrombogenic characteristics of small, dense LDL particles

There are several mechanisms by which small dense LDL is likely to play a causal role in promoting atherosclerosis and thrombosis. These are discussed below.

a) Actions on the endothelium

Native LDL increases superoxide generation (O_2^-) from the endothelium and decreases basal nitric oxide (NO) production [35] and stimulated NO production [36]. It is likely that small dense LDL is more potent in this regard. Inhibition of NO production is atherogenic [37] and O_2^- inactivates NO [38] and can oxidise LDL (see below).

b) Increased trans-endothelial filtration

The filtration rate of LDL particles into subendothelium is inversely proportional to particle size, thus small LDL particles are transported more effectively from the circulation to the subendothelial space of artery wall than are large LDL particles [39].

c) Susceptibility to oxidation

Oxidized LDL plays an important role in atherogenesis since it is taken up by scavenger receptors on macrophages leading to cholesterol accumulation and foam cell formation in the evolving fatty streak [40]. Small, dense LDL particles are more susceptible to oxidation *in vitro* than large LDL particles [41]. This is attributable to several factors. The content of antioxidants including vitamin E and ubiquinol-10 is lower in small than in large LDL particles. The structure of small, dense LDL may expose their polyunsaturated fatty acids (PUFA) to free radical attack and lipid peroxidation [40]. Small, dense LDL particles have a higher content of PUFA, including arachidonic acid, than do large LDL particles. PUFA are degraded to conjugated dienes and other oxidation products during oxidation [42]. Non-enzymatic oxidation of arachidonic acid yields the isoprostanes, some of which are biologically active (e.g. 8-epi-$PGF_{2\alpha}$) and may contribute to atherogenesis as well as providing an *in vivo* measure of oxidative stress [43]. Lipid peroxidation starts by oxidation of the PUFA component of the phospholipids in the particle surface and propagates towards the particle core. Free cholesterol of the particle limits access of oxidants to PUFA in the particle surface thus stabilising LDL particles against initial oxidative attack. Small LDL particles are relatively depleted of free cholesterol and may therefore be less protected by this mechanism [44].

d) Reduced affinity for the LDL receptor

In the kinetic turnover studies, two LDL pools were demonstrated. Rapidly cleared pool A (probably consisting of large LDL particles) and slowly cleared pool B (small LDL particles) [18]. This observation is consistent with *in vitro* studies demonstrating that small, dense LDL particles have a lower affinity for LDL receptors than do larger LDL particles [45]. This results in reduced hepatic clearance and a longer residence time in plasma of small versus large LDL particles, increasing the likelihood that small LDL particles will be filtered into the arterial wall followed by oxidation and uptake via scavenger receptors [45]. This lower affinity of small, dense LDL particles for the LDL receptor is independent of their triglyceride content [46]. ApoB-100 in small, dense LDL particles has additional cleavage sites and different accessibility to protease attack, suggesting that the conformation of apoB-100 in small, dense LDL particles differs from that in other LDL particles. This may reduce their affinity for LDL receptors [47].

e) Increased binding to intimal proteoglycans

Small, dense LDL particles have greater affinity for intimal proteoglycans than do other LDL particles [48]. This may be related to their lower sialic acid content and to different exposures of the apoB region that influences interactions with proteoglycans. Binding to intimal proteoglycans leads to extracellular lipid accumulation which is an important component of atherogenesis [49].

f) Formation of proaggregatory/vasoconstrictor mediators

LDL particle size is related to endothelial vasodilator dysfunction in patients with CAD, independent of other lipoprotein variables [50]. Small, dense LDL particles stimulate thromboxane (TX) A_2 synthesis *in vitro*, more than large LDL particles [51]. Since TXA_2 stimulates platelet aggregation and is a potent vasoconstrictor this could contribute to the progression of CAD. Production of 8-epi-$PGF_{2\alpha}$ as a result of non-enzymatic oxidation of arachidonic acid in small, dense LDL particles could also promote vasoconstriction and platelet aggregation.

Methods for separation and identification of LDL subfractions

Generally, heterogeneity of LDL is investigated using either density gradient ultracentrifugation or polyacrylamide gel electrophoresis [29].

Density gradient ultracentrifugation (DGUC)

Several DGUC procedures have been developed to characterise LDL subfractions [29, 52]. The procedures differ both in the construction of the gradient and in the fractionation profile and no single method has yet assumed general usage.

The main disadvantages of all DGUC procedures for investigation of LDL subfractions are: a) expensive equipment (ultracentrifuges and appropriate rotors), b) time required for separation to be completed is very long (except when vertical rotors are used), c) lipoprotein degradation during ultracentrifugation (lipids and proteins tend to separate during centrifugation), d) complicated gradient preparation, e) large volumes of plasma are required for an adequate analysis of lipoprotein distributions, f) fractionation requires special gradient fractionator or punching a hole at the bottom of the tube and collecting subfractions; both techniques are time consuming and require great care as the gradient is easily disturbed. However, DGUC allows good resolution between lipoprotein subfractions and analysis of the chemical composition of each subfraction.

Electrophoresis

Electrophoresis method is relatively inexpensive, faster than centrifugation and can be used for analyses of small amounts of material. Furthermore, it enables different subfractions of LDL and HDL to be separated directly and with better resolution than when using other techniques. Electrophoresis separates lipoproteins according to their charge and size. Lipoproteins have an isoelectric point at about pH 5.5 above which they are negatively charged.

Analysis of LDL subfractions is usually performed by using nondenaturing polyacrylamide gradient gel electrophoresis. Lipoproteins migrate in an electric field through a gradient of increasing polyacrylamide concentration. The pore size of the matrix progressively decreases as the concentration of acrylamide increases. Migration of the particles stops when they reach their exclusion limit. Gradient slab gels of 2–16% polyacrylamide have commonly been used [53]. Electrophoresis for separation of LDL subfractions using these gels requires less than 25 ml of plasma and may last for up to 24 h depending on voltage used. After electrophoresis gels need to be stained and destained. So the whole procedure lasts for more than 24 h and is time consuming and labour intensive. Continuous polyacrylamide disc gels were introduced to simplify the method. These gels are a modification of the disc gels as described by Naito *et al.* [54] and Muniz [55]. In order to achieve desirable separation of LDL subfractions they were modified by

increasing the gel length and optimising the electrolyte buffers and gel composition. These gels allow separation of up to 7 LDL subfractions within 70 min using prestained serum samples [56].

The advantages of separation of LDL subfractions by using electrophoretic methods compared with DGUC are as follows: a) less expensive and less complicated equipment is required, b) generally, separations is achieved in shorter time, particularly if the prestained disc gels are used (still over 24 h if staining and destaining procedures applied), c) lipoprotein degradation does not occur during electrophoresis, d) technique is simpler to perform, e) small volume of sample is needed for the analysis (not more than 25 μl).

Interventions to influence LDL particle size

In view of the strong relationship between elevated plasma triglycerides and the small dense LDL phenotype, triglyceride lowering therapies could be expected to have a greater impact on LDL size and density than predominantly cholesterol lowering therapies. The HMG CoA reductase inhibitors (statins) lower LDL cholesterol substantially and their value in reducing CAD mortality and morbidity has been demonstrated conclusively [2–4]. These drugs have little effect on particle size when tested in patients with the small dense LDL phenotype. Simvastatin caused a decrease in both large and small LDL particles in combined hyperlipidaemic patients, with no overall improvement in the subclass phenotype [57]. Pravastatin reduced total and LDL cholesterol in combined hyperlipidaemic patients but LDL particle size was either unchanged or became even smaller [58, 59]. In familial hypercholesterolaemia, lovastatin and simvastatin decrease cholesterol more in the light LDL than in dense particles [60]. These statins cause little or no decrease in plasma triglycerides in the combined and familial hyperlipidaemic patients, which may explain why there is generally no reduction of small, dense LDL particles. Any apparent worsening of LDL phenotype by statins may be due to up-regulation of LDL receptors, preferentially increasing clearance of larger LDL particles which have a higher affinity for LDL receptors. As a result, small LDL particles come to dominate the plasma LDL subfraction profile. Potentially adverse effects of statins on LDL density profiles are clearly more than offset by the beneficial effects of reducing the total plasma LDL cholesterol pool, as evidenced by the reduction of CAD events which has been demonstrated in recent clinical trials [2–4]. A new member of the HMG CoA reductase inhibitor, atorvastatin, lowers plasma triglycerides more than other marketed statins at licensed doses [61]. As a result it may have greater beneficial effects on LDL density profiles than other currently licensed statins.

The impact of aggressive lipid lowering on CAD progression and the relationship to small dense LDL was evaluated in a retrospective analysis of data from the Familial Atherosclerosis Treatment Study, FATS [62]. Patients treated with nicotinic acid plus cholestyramine or lovastatin plus cholestyramine experienced a significant reduction in coronary stenosis severity compared to controls. There was a strong inverse relationship between the changes in LDL density and coronary stenosis. The reduction of small, dense LDL was a stronger predictor of decreased disease progression than was reduction of LDL cholesterol. Combinations of nicotinic acid plus cholestyramine and lovastatin plus cholestyramine decreased plasma triglycerides [63, 64], which probably contributed to the improvement in the small dense LDL phenotype. Cholestyramine alone tended to increase the level of small, dense LDL [63, 64]. This is probably due to up-regulation of LDL receptors. These preferentially bind (and hence clear) larger more buoyant LDL particles. Nicotinic acid alone reduces the concentration of small dense LDL [63, 65]. Nicotinic acid is more effective at lowering plasma triglycerides than cholesterol and in hypertriglyceridaemic patients the change in LDL phenotype caused by nicotinic acid is both correlated with baseline triglyceride levels and the reduction in triglycerides after treatment [65]. It causes only a modest reduction of LDL particle diameter in individuals with normal plasma triglycerides but a more marked reduction in particle size in subjects with hypertriglyceridaemia [63, 65].

Currently, the most widely used triglyceride lowering agents are fibrates. Several of these agents, including gemfibrozil, fenofibrate, bezafibrate and ciprofibrate, decrease small dense LDL in patients with combined hyperlipidaemia [66–68]. Gemfibrozil increased LDL particle size and decreased particle density in patients with triglycerides in the approximate range of 3.5–9.0 mmol l^{-1} [68]. The effect was strongly correlated with the reduction of triglycerides. Gemfibrozil had no effect on LDL density profile in hypercholesterolaemic patients with normal triglyceride levels (1.3 mmol l^{-1}), in whom LDL particles were larger and less dense [68]. In hypercholesterolaemic patients with somewhat higher triglycerides (2.0 mmol l^{-1}), gemfibrozil shifted LDL to the larger and less dense phenotype in association with reduced triglycerides [66]. Thus, the effect of gemfibrozil and the other fibrates on LDL size and density depends on the baseline triglyceride levels. Elevated plasma triglycerides favor the transfer of VLDL triglycerides to LDL by CETP. The subsequent hydrolysis of LDL triglyceride generates small dense LDL [69]. By reducing plasma triglycerides, fibrates limit the amount of substrate available for CETP-mediated transfer to LDL and thereby decrease the formation of small dense LDL. In addition,

fenofibrate was found to decrease CETP mass and transfer activity, which further limits the formation of small dense LDL [70].

Despite having only a modest effect on LDL-cholesterol, bezafibrate may reduce progression of coronary atherosclerosis and coronary events in young men following myocardial infarction [71]. Likewise, a subgroup analysis of patients in the Helsinki Heart study demonstrated a reduced number of ischaemic events in patients randomised to gemfibrozil [72]. Their pharmacological effects suggest that combination therapy with a statin and fibrate could be of particular benefit in dyslipidaemic patients with a preponderance of small dense LDL, a hypothesis that needs to be tested by clinical trials.

As discussed above, small dense LDL profile is associated with insulin resistance. Interventions that improve insulin sensitivity include exercise [73], thioziodolinediones [74] and possibly imidazoline receptor agonists [75] while reports on fibrates remain controversial. Insulin resistance, hypertension, hypertriglyceridaemia and small dense LDL particles coexist and together form the metabolic syndrome which is strongly associated with atherosclerosis ('syndrome X'). Interventions on these factors could increase LDL particle size. The thiazoledinedione, troglitazone, causes a small increase in LDL cholesterol in obese individuals [76] due to an increase in large, less dense LDL. This may explain the observation that troglitazone increases the resistance of LDL particles to oxidation [77, 78]. It is possible that troglitazone is protective against atherosclerosis. The shift in the LDL particle density is associated with a statistically insignificant decrease in plasma triglycerides, although larger effects on triglycerides are generally observed in patients treated with troglitazone [79]. Since the small, dense LDL profile is associated with insulin resistance, the improvement caused by troglitazone may be related to its ability to improve insulin sensitivity. Further studies will be required to determine the relative roles of enhanced insulin sensitivity and of reducing plasma triglyceride in the effects of troglitazone on LDL density.

Troglitazone and other thiazolidinediones exert their pharmacological effects by binding to the peroxisome proliferator activated receptor (PPAR) type γ found predominantly in adipocytes [80]. The precise mechanism by which they improve insulin sensitivity is not fully known, but is at least partially attributable to increased expression of a variety adipocyte genes involved in fatty acid metabolism [81]. The triglyceride lowering effects of thiazolidinediones also seems to involve PPARγ-mediated effects on adipocyte gene expression. Interestingly, triglyceride lowering fibrates exert their major pharmacologic activity by binding to a PPAR, in this case PPARα, which is expressed primarily in the liver [82]. Both the PPARγ (troglitazone) and PPARα ligands (fibrates) decrease plasma concentrations of small dense LDL. Their mechanisms overlap at the level of plasma triglycerides but there may be additional means by which they affect LDL density including effects on CETP. Recently, compounds were described that bind both PPAR and PPARα [83, 84]. These compounds decrease plasma triglycerides and increase insulin sensitivity in animal models. It is conceivable that such compounds may have greater effects on small dense LDL than thiazolidinediones or fibrates.

Additional therapeutic approaches that decrease plasma concentrations of triglycerides or transfer triglycerides between lipoprotein classes may influence the formation of small dense LDL. Thus, inhibitors of CETP and microsomal triglyceride transfer protein (MTP) which are currently under development may decrease small dense LDL. The value of these or other therapeutic approaches to modulate LDL size and density profiles still must be determined. Finally, studies in patients with differing degrees of insulin resistance and hypertriglyceridaemia should allow effects on particle size to be differentiated from effects on other factors. Such studies are needed to determine whether LDL particle size plays a direct role in atherogenicity. If so, evaluating the effects of different drug classes on particle size will play an increasing part in clinical cardiovascular pharmacology, influencing choice of therapy not only in dyslipidaemic states but in hypertension and diabetes.

References

1 Saxena U, Goldberg IJ. Endothelial cells and atherosclerosis: lipoprotein metabolism, matrix interaction and monocyte recruitment. *Curr Opin Lipidol* 1994; **5**: 316–322.

2 Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1995; **344**: 1383–1389.

3 Shepherd J, Cobbe S, Ford I, *et al*. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med* 1995; **333**: 1301–1307.

4 Sacks FM, Pfeffer MA, Moye LA, *et al*. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. *N Engl J Med* 1996; **335**: 1001–1009.

5 Kannel WB. Range of serum cholesterol values in the population developing coronary artery disease. *Am J Cardiol* 1995; **76**: 69C–77C.

6 Havel RS, Eder HA, Bradgon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 1955; **34**: 1345–1353.

7 McNamara JR, Campos H, Ordovas JM, Peterson J, Wilson PWF, Schaefer EJ. Effect of gender, age and lipid status on low density lipoprotein subfraction distribution. Results from the Framingham Offspring Study. *Arteriosclerosis* 1987; **7**: 483–490.

8 Fisher W, Hammond MG, Mengel MC, Warmke GL. A genetic determinant of the phenotypic variance of the molecular weight of low density lipoprotein. *Proc Natl Acad Sci USA* 1975; **72**: 2347–2351.

9 Fisher WR. Heterogeneity of plasma low density lipoproteins manifestation of the physiologic phenomenon in man. *Metabolism* 1983; **32**: 283–291.

10 Van Antwerpen R, Gilkey JC. Cryo-electron microscopy reveals low density lipoprotein substructure. *J Lipid Res* 1994; **35**: 2223–2231.

11 Chapman MJ, Laplaud PM, Luc G, *et al*. Further resolution of the low-density lipoprotein spectrum in normal human plasma: physicochemical characteristics of discrete subspecies separated by density gradient ultracentrifugation. *J Lipid Res* 1988; **29**: 442–458.

12 Krauss RM. Heterogeneity of plasma low-density lipoproteins and atherosclerosis risk. *Curr Opin Lipidol* 1994; **5**: 339–349.

13 Austin MA, Hokanson JE, Brunzell JD. Characterisation of low density lipoprotein subclasses: methodologic approaches and clinical relevance. *Curr Opin Lipidol* 1994; **5**: 395–403.

14 Shen MMS, Krauss RM, Lindgren FT, Forte TM. Heterogeneity of serum low density lipoproteins in normal human subjects. *J Lipid Res* 1981; **22**: 236–244.

15 Baumstark MW, Kreutz W, Berg A, Frey I, Keul J. Structure of human LDL subfractions determined by X ray small angle scattering. *Biochim Biophys Acta* 1990; **1037**: 48–57.

16 Musliner TA, Krauss RM. Lipoprotein subspecies and risk of coronary disease. *Clin Chem* 1988; **34**: B78–B83.

17 Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA* 1988; **26**: 1917–1921.

18 Caslake MJ, Packard CJ, Gaw A, *et al*. Fenofibrate and metabolic heterogeneity in hypercholesterolaemia. *Arterioscler Thromb* 1993; **13**: 702–711.

19 Barter PJ, Rajaram OV, Liang HQ, Rye KA. Relationship between the size and phospholipid content of LDL lipoproteins. *Biochim Biophys Acta* 1993; **1166**: 135–137.

20 La Belle M, Krauss RM. Differences in carbohydrate content of LDL lipoproteins associated with LDL subclass patterns. *J Lipid Res* 1990; **31**: 1577–1588.

21 Jaakkola O, Solakivi T, Tertov VV, *et al*. Characteristics of low density lipoprotein subfractions from patients with coronary artery disease. *Coron Artery Dis* 1993; **4**: 379–385.

22 Chappey B, Myara I, Benoit MO, *et al*. Human native LDL-no evidence of peroxidative modifications. *Biochim Biophys Acta* 1995; **1259**: 261–270.

23 Packard C. Plasma lipid and lipoprotein metabolism in the 1990s—what we know and what we need to know. In *Lipids: Current Perspective* ed Betteridge DJ. 1996; **1**: 1–19.

24 Rajman I, Maxwell S, Cramb R, Kendall M. Particle size: the key to the atherogenic lipoprotein? *Q J Med* 1994; **87**: 709–720.

25 McNamara JR, Jenner JL, Li Z, Wilson PWF, Schefer EJ. Change in LDL particle size is associated with change in plasma triglyceride concentrations. *Arterioscler Thromb* 1992; **12**: 1284–1290.

26 Griffin BA, Packard CJ. Metabolism of VLDL and LDL subclasses. *Curr Opin Lipidol* 1994; **5**: 200–206.

27 Nishina PM, Johnson JP, Naggeret KJ, Krauss RM. Linkage of atherogenic lipoprotein phenotype to the low density lipoprotein receptor locus on the short arm of chromosome 19. *Proc Natl Acad Sci USA* 1992; **89**: 708–712.

28 Hamsten A, Karpe F. Triglycerides and coronary heart disease—has epidemiology given us the right answer? In *Lipids: Current Perspective*, Vol. 1, ed. Betteridge DJ, 1996: 43–63.

29 Austin MA, Hokanson JE, Brunzell JD. Characterisation of low density lipoprotein subclasses: methodologic approaches and clinical relevance. *Curr Opin Lipidol* 1994; **5**: 395–403.

30 Griffin BA, Freeman DJ, Tait GW, *et al*. Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small, dense LDL to coronary heart disease risk. *Atherosclerosis* 1994; **106**: 241–253.

31 Rajman I, Kendall MJ, Cramb R, Holder R, Salih M, Gammage MD. Investigation of low density lipoprotein subfractions as a coronary risk factor in normotriglyceridaemic men. *Atherosclerosis* 1996; **125**: 231–242.

32 Lamarche B, Tchernof A, Moorjani S, *et al*. Small dense low density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. *Circulation* 1997; **95**: 69-75.

33 Reaven GM, Chen YD, Jeppesen J, Maheux P, Krauss RM. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. *J Clin Invest* 1993; **92**: 141–146.

34 Halle M, Berg A, Frey I, *et al*. Relationship between obesity and concentration and composition of LDL subfractions in normoinsulinemic men. *Metabol Clin Exp* 1995; **44**: 1384–1390.

35 Pritchard KA, Groszek L, Smalley DM, *et al*. Native low-density lipoprotein increases endothelial cell nitric oxide synthase generation of superoxide anion. *Circ Res* 1995; **77**: 510–518.

36 Andrews HE, Bruckdorfer KR, Dunn RC, Jacobs M. Low-density lipoproteins inhibit endothelium-dependant relaxation in rabbit aorta. *Nature* 1987; **327**: 237–239.

37 Cooke JP, Tsao PS. Is NO an endogenous antiatherogenic molecule? *Arteriosclerosis Thromb* 1994; **14**: 653–655.

38 Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived factor. *Nature* 1986; **320**: 454–456.

39 Nordestgaard BG, Nielsen LB. Atherosclerosis and arterial influx of lipoproteins, *Curr Opin Lipidol* 1994; **4**: 252–257.

40 Steinberg D, Parsatharathy S, Carew T, Khoo J, Witztum J. Beyond cholesterol: Modifications of low-density lipoprotein that increase its atherogenecity. *N Engl J Med* 1989; **320**: 915–924.

41 Dejager S, Bruckert E, Chapman MJ. Dense LDL subspecies with diminished oxidative resistance predominate in combined hyperlipidaemia. *J Lipid Res* 1993; **34**: 295–308.

42 De Graaf J, Hak-Lemmers HLM, Hectors MPL, Demacker PNM, Hendriks JCM, Stalenhoef AFH. Enhanced susceptibility to *in vitro* oxidation in healthy subjects. *Arteriosclerosis Thrombosis* 1991; **11**: 298–306.

43 Morrow JD, Roberts LJ. The isoprostanes. Current knowledge and directions for future research. *Biochem Pharmacol* 1996; **51**: 1–9.

44 Tribble DL. Lipoprotein oxidation in dyslipidaemia: insights

into general mechanisms affecting lipoprotein oxidative behaviour. *Curr Opin Lipidol* 1995; **6**: 196–208.
45 Nigon E, Lesnik P, Rouis M, Chapman MJ. Discrete subspecies of human LDL are heterogeneous in their interactions with the cellular LDL receptor. *J Lipid Res* 1991; **32**: 1741–1753.
46 Galeano NF, Milne R, Marcel YL, *et al*. Apoprotein B structure and receptor recognition of triglyceride rich low density lipoprotein is modified in small LDL but not in triglyceride rich LDL of normal size. *J Biol Chem* 1994; **269**: 511–519.
47 Chen GC, Liu W, Duchateu P, *et al*. Conformational differences in human apolipoprotein B-100 among subspecies of LDL. Association of altered proteolytic accessibility with decreased receptor binding of LDL subspecies from subjects. *J Biol Chem* 1994; **269**: 29 121–29 128.
48 Anber V, Millar JS, McConnell M, Shepherd J, Packard CJ. Interaction of very low density, intermediate density and low density lipoproteins with human arterial wall proteoglycans. *Arterioscler Thromb Vasc Biol* 1997; **17**: 2507–2514.
49 Galeano NF, Al-Haideri M, Keyserman F, Rumsey SC, Deckelbaum J. Small dense low density lipoprotein has increased affinity for LDL receptor-independent cell surface binding sites: a potential mechanism for increased atherogenicity, *J Lipid Res* 1998; **39**: 1263–1273.
50 Dyce MC, Anderson TJ, Yeung AC, *et al*. Indices of LDL particle size closely relate to endothelial dysfunction. *Circulation* 1993; **88**: I 466.
51 Weisser B, Locher R, de Graaf J, Moser R, Sachinidis A, Vetter W. LDL subfractions increase thromboxane formation in endothelial cells. *Biochim Biophys Res Commun* 1993; **192**: 1245–1250.
52 Griffin BA, Caslake MJ, Yip B, Tait GW, Packard CJ, Shepherd J. Rapid isolation of low density lipoprotein (LDL) subfractions from plasma by density gradient ultracentrifugation. *Atherosclerosis* 1990; **83**: 59–65.
53 Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J Lipid Res* 1982; **23**: 97–104.
54 Naito HK, Wada M, Erhart A, Lewis LA. Polyacrylamide gel disc electrophoresis as a screening procedure for serum lipoprotein abnormalities. *Clin Chem* 1973; **19**: 228–234.
55 Muniz N. Measurement of plasma lipoproteins by electrophoresis on polyacrylamide gel. *Clin Chem* 1977; **10**: 1826–1833.
56 Naito H, Hirano T, Yoshino G, *et al*. LDL particle size decreases in non-insulin dependant diabetic patients with nephropathy. *J Japan Diab Soc* 1995: **38**: 615–624.
57 Bredie SJH, de Bruin TWA, Demacker PNM, *et al*. Comparison of gemfibrozil versus simvastatin in familial combined hyperlipidemia and effects on apolipoprotein-B-containing lipoproteins, LDL subfraction profile and LDL oxidizability. *Am J Cardiol* 1995: **75**: 348–353.
58 Zambon S, Cortella A, Sartore G, Baldo-Enzi G, Manzato E, Crepaldi G. Pravastatin treatment in combined hyperlipidemia: effect on plasma lipoprotein and size. *Eur J Clin Pharmacol* 1994: **46**: 221–224.
59 Franceschini G, Cassinotti M, Vecchio G, *et al*. Pravastatin effectively lowers LDL cholesterol in familial combined hyperlipidemia without changing LDL subclass pattern. *Arterioscler Thromb* 1994: **14**: 1569–1575.
60 Tilly-Kiesi M, Tikkanen MJ. Low density lipoprtein density and composition in hypercholesterolaemic men treated with HMG CoA reductase inhibitors and gemfibrozil. *J Int Med* 1991; **229**: 427–434.
61 Bakker-Arkema RG, Davidson MH, Goldstein RJ, *et al*. Efficacy and safety of a new HMG CoA reductase inhibitor, atorvastatin, in patients with hypertriglyceridemia. *JAMA* 1996; **275**: 128–133.
62 Zambon A, Brown BG, Hokanson JE, Brunzell JD. Hepatic lipase mediated changes in LDL density predict coronary stenosis regression in the familial atherosclerosis treatment study. *Atherosclerosis* 1997; **134**: 28.
63 Griffin BA, Caslake MJ, Gaw A, Yip B, Packard CJ, Shepherd J. Effects of cholestyramine and acipimox on subfractions of plasma low density lipoprotein. Studies in normolipidaemic and hypercholsterolaemic subjects. *Eur J Clin Invest* 1992; **22**: 383–390.
64 Brown BG, Zambon A, Poulin D, *et al*. Use of niacin, statins and resins in patients with combined hyperlipidemia. *Am J Cardiol* 1998; **81** (4A): 52B–59B.
65 Superko HR, Krauss RM. Differential effects of nicotinic acid in subjects with different LDL subclass patterns. *Atherosclerosis* 1992; **95**: 69–76.
66 Tsai MY, Yuan J, Hunninghake DB. Effects of gemfibrozil on composition of lipoproteins and distribution of LDL subspecies. *Atherosclerosis* 1992; **95**: 35–42.
67 Bruckert E, Dejager S, Chapman MJ. Ciprofibrate therapy normalises the atherogenic low density lipoprotein subspecies profile in combined hyperlipidaemia. *Atherosclerosis* 1993; **100**: 91–102.
68 Yuan J, Tsai MY, Hunninghake DB. Changes in composition and distribution of LDL subspecies in hypertriglyceridemic and hypercholesterolemic patients during gemfibrozil treatment. *Atherosclerosis* 1994; **110**: 1–11.
69 Tsai MY, Yuan J, Hunninghake DB. Effect of gemfibrozil on composition of lipoproteins and distribution of LDL subspecies. *Atherosclerosis* 1992; **95**: 35–42.
70 Guerin M, Bruckert E, Dolphin PJ, Turpin G, Chapman MJ. Fenofibrate reduces plasma cholesterol ester transfer from HDL to VLDL and normalizes the atherogenic, dense LDL profile in combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 1996; **16**: 763–772.
71 Ericsson CG, Hamsten A, Nilsson J, *et al*. Angiographic assessment of effects of bezafibrate on progression of coronary artery disease in young male postinfarction patients. *Lancet* 1996; **347**: 849–853.
72 Huttunen JK, Manninen V, Manttari M, *et al*. The Helsinki Heart Study: Central findings and clinical implications. *Ann Int Med* 1991; **23**: 155–165.
73 Perseghin G, Price TB, Petersen KF, *et al*. Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin resistant subjects. *N Engl J Med* 1996; **335**: 1357–1362.
74 Nolan JJ, Ludvik B, Beerdsen P, Joyce M, Oletsky J. Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. *N Engl J Med* 1994; **331**: 1188–1193.
75 Krentz AJ, Evans AJ. Selective imidazoline receptor agonists for metabolic syndrome. *Lancet* 1998; **351**: 152–153.

76 Tack CJJ, Smits P, Demacker PNM, Stalenhoef AFH. Troglitazone decreases the proportion of small, dense LDL and increases the resistance of LDL to oxidation in obese subjects. *Diabetes Care* 1998; **21**: 796–799.

77 Cominacini L, Garbin U, Pastorino AM, *et al*. Effects of troglitazone on *in vitro* oxidation of LDL and HDL induced by copper ions and endothelial cells. *Diabetologia* 1997; **40**: 165–172.

78 Noguchi N, Sakai H, Kato, *et al*. Inhibition of oxidation of LDL by troglitazone. *Atherosclerosis* 1996; **123**: 227–234.

79 Ghazzi MN, Perez JE, Antonucci TK, *et al*. Cardiac and glycemic benefits of troglitazone treatment in NIDDM. *Diabetes* 1997; **46**: 433–439.

80 Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator receptor g (PPARg). *J Biol Chem* 1995; **270**: 12 953–12 956.

81 Martin G, Schoonjans K, Staels B, Auwerx J. PPARg activators improve glucose homeostasis by stimulating fatty acid uptake in the adipocytes. *Atherosclerosis* 1998; **137**: (Suppl.) S75–S80.

82 Schoonjans K, Staels B, Auwerx J. Role of peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J Lipid Res* 1996; **37**: 907–925.

83 Kliewer SA, Sundseth SS, Jones SA, *et al*. Fatty acids and eicosinoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors a and g. *Proc Natl Acad Sci USA* 1997; **94**: 4318–4323.

84 Murakami K, Tobe K, Ide T, *et al*. A novel insulin sensitizer acts as a coligand for PPAR-α and PPAR-γ: effect of PPAR-γ activation on abnormal lipid metabolism in liver of Zucker fatty rats. *Diabetes* 1998; **47**: 1841–1847.