Who needs to care about small, dense low-density lipoproteins?

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SUMMARY

Background: Increasing evidence suggest that the ‘quality’ rather than only the ‘quantity’ of low-density lipoprotein (LDL) exerts a great influence on the cardiovascular risk. Small, dense LDL seem to be an important predictor of cardiovascular events and progression of coronary artery disease (CAD) and their predominance has been accepted as an emerging cardiovascular risk factor by the National Cholesterol Education Program Adult Treatment Panel III. Discussion: Some studies showed in past years that small, dense LDL are usually elevated in patients at very high cardiovascular risk, such as those with CAD and type 2 diabetes. More recently elevated levels of these particles have been found in other categories of patients at high cardiovascular risk, such as those with non-coronary forms of atherosclerosis (e.g. with carotid artery disease, aortic abdominal aneurysm and peripheral arterial disease) and metabolic diseases (with polycystic ovary syndrome and growth hormone deficiency); notably, in most of them, the predominance of small, dense LDL characterised their type of dyslipidaemia, alone or in combination with elevated triglycerides and reduced high-density lipoproteins cholesterol concentrations. Conclusions: The therapeutical modulation of small, dense LDL have been shown to significantly reduce cardiovascular risk and weight reduction and increased physical activity may constitute first-line therapy. In addition, lipid-lowering drugs are able to favourably alter these particles and nicotinic acid seem to be the most effective agents. Promising data are also available with the use of rosvastatin, the latest statin introduced in the market, and ezetimibe, a cholesterol absorption inhibitor.

Introduction

Low-density lipoproteins (LDL) particles do not comprise an homogenous population but multiple subclasses with discrete size and density, different physicochemical composition, metabolic behaviour and atherogenicity (1), with at least four major subclasses: large LDL-I, medium LDL-II, small LDL-III and very small LDL-IV (2,3) (Figure 1). LDL size correlates positively with plasma high-density lipoprotein cholesterol (HDL-C) levels and negatively with plasma triglyceride levels and the combination of small, dense LDL, decreased HDL-C and increased triglycerides has been defined as the ‘atherogenic lipoprotein phenotype’ (4,5). Inverse correlations of changes in large and small LDL and of changes of medium-sized and very small LDL in dietary intervention studies raise the possibility of precursor-product relationships between distinct subclasses (6).

The prevalence of small, dense LDL is 30–35% in adult men, 5–10% in men < 20 years and in premenopausal women and 15–25% in postmenopausal women. It has been shown that LDL size is genetically influenced with a heritability ranging from 35% to 45% based on an autosomal dominant or codominant model with varying additive and polygenic effects (7). Dietary factors are of importance too. It has been shown that a very low fat, high carbohydrate diet can induce the predominance of small, dense LDL-B in persons genetically predisposed (8); in fact, there is evidence for heritable effects on diet-induced subclass changes and for the involvement of specific genes (9). In addition, the predominance of small, dense LDL is commonly found in conjunction with familial disorders of lipoprotein metabolism that are associated with increased risk of premature coronary artery disease (CAD), including familial combined hyperlipidaemia, hyper-beta-lipoproteinaemia.
and hypo-alpha-lipoproteinaemia (10–12). Thus, non-genetic and environmental factors influence the expression of this phenotype and an increase of small, dense LDL has been shown for abdominal adiposity and oral contraceptive use (13–15).

Formation of small, dense LDL

Low-density lipoprotein size is related to the activity of lipolytic enzymes (e.g. lipoprotein lipase and hepatic lipase): reduced activity of lipoprotein lipase and increased activity of hepatic lipase have been shown in subjects with a predominance of small, dense LDL (16). Hepatic lipase has a greater affinity for LDL than lipoprotein lipase and is positively correlated with plasma triglycerides, apolipoprotein B, mass of large very low-density lipoproteins (VLDL) and small, dense LDL, but not with the mass of large LDL (17). We also showed in animal models a central role for hepatic lipase in regulating total plasma LDL concentrations, as well as in the production of small, dense LDL from larger, more buoyant precursors (18).

The strong relationship of LDL size with triglycerides is based on their importance as substrates for the size reduction of LDL particles: LDL (and also HDL) can become triglyceride enriched and can be further processed by lipases by exchange of cholesterol esters with triglycerides. It has been shown that profound changes in the physicochemical composition of both LDL and HDL particles occur with increasing triglyceridaemia, while core cholesterol esters is progressively depleted and replaced by triglycerides (19).

In addition, the production of large triglyceride-rich VLDL is dependent on triglycerides availability and is associated with smaller, denser LDL particles [reviewed in (6)]; in fact, it has been suggested that there are parallel metabolic pathways for the production of the major LDL subclasses (Figure 2), based on kinetic and dietary intervention studies (6,20). Cholesteryl ester transfer protein has an important role in the remodelling of larger-to-smaller LDL particles by mediating triglycerides enrichment of intermediate density lipoproteins and large LDL (19). In patients with type 2 diabetes it has been shown that cholesteryl ester transfer protein contributes significantly to the increased levels of small, dense LDL by preferential transfer of cholesteryl esters from HDL to small, dense LDL, as well as through an indirect mechanism involving enhanced transfer of cholesteryl esters from HDL to larger VLDL (21).

Atherogenicity of small, dense LDL

Low-density lipoprotein size seems to be an important predictor of cardiovascular events and progression of CAD and the predominance of small, dense LDL have been accepted as an emerging cardiovascular risk factor by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) (22). The predominance of small, dense LDL has been associated with an approximately three to
sevenfold increased risk for CAD and several reasons have been suggested to explain the enhanced atherogenicity of small, dense LDL. Smaller, denser LDL are taken up more easily by arterial tissue than larger LDL (23), suggesting greater transendothelial transport of smaller particles. In addition, smaller LDL particles may also have decreased receptor-mediated uptake and increased proteoglycan binding (24). Sialic acid, perhaps because of its exposure at the LDL surface, plays a determinant role in the in vitro association of LDL with the polyanionic proteoglycans (25) and it has been shown that sialic acid content of LDL particles of subjects with a predominance of small, dense LDL is reduced. Further, it has been shown that oxidative susceptibility increases and antioxidant concentrations decreases with decreasing LDL size (26). Altered properties of the surface lipid layer associated with reduced content of free cholesterol (27) and increased content of polyunsaturated fatty acids (28) might contribute to enhanced oxidative susceptibility of small, dense LDL.

To date, the association of LDL size with cardiovascular diseases has been tested in over 50 studies, including cross-sectional and prospective epidemiologic as well as clinical intervention trials (29). The vast majority of these studies showed a strong and significant association of small, dense LDL with increased CAD risk at univariate analyses (2,3). As LDL size is rarely a significant and independent predictor of CAD risk after multivariate adjustments for confounding variables (e.g. plasma triglyceride levels and HDL-C concentrations), it is still on debate if the increased atherogenic potential of small, dense LDL may be a consequence of the broader pathophysiology of which these particles are a part of (30).

Which subjects have elevated levels of small, dense LDL?

With CAD or type 2 diabetes

It is well known that patients at very high cardiovascular risk, such as those with CAD and type 2 diabetes, have increased levels of atherogenic small, dense LDL (as reviewed in 31,32). In addition, in recent years, it has been tested whether other categories of subjects at high cardiovascular risk may have increased levels of these particles too. Some authors have studied patients with vascular diseases; in fact, it has been stated by the NCEP ATP III that clinical forms of non-coronary atherosclerosis carry a risk for CAD equal to those with established CAD (22). These conditions include carotid artery disease, peripheral arterial disease and abdominal aortic aneurysm (22).

With carotid artery disease

Preliminary, Landray et al. (33) first showed an association between small, dense LDL and asymptomatic carotid atherosclerosis and this has been confirmed by other similar studies in healthy individuals (34–36). Other authors found a significant relationship between LDL size and the occurrence of preclinical and clinical carotid atherosclerosis (37–41) and we recently demonstrated (42) that LDL
size is significantly associated with carotid intima media thickness in patients with type 2 diabetes; notably, in our study multivariate analysis revealed that LDL size was the strongest predictor of intima media thickness within all lipid parameters and the second strongest predictor (after smoking) among all traditional cardiovascular risk factors. Regarding clinical trials, van Tits et al. (41) showed an association between intima media thickness regression and baseline LDL size (by statin therapy withatorvastatin and simvastatin) and this was confirmed by Wallenfeldt et al. (43) in a 3 years follow-up of 313 58-year-old subjects. These findings are consistent with a role of LDL size in modulating carotid atherosclerosis regression.

**With peripheral arterial disease**

Regarding subjects with peripheral arterial disease Lupattelli et al. (44) did not find any difference in LDL size between normolipaemic non-diabetic patients with peripheral arterial disease and controls, matched for gender, age and body mass index (BMI); by contrast, O’Neal et al. (45) performed a similar study but on a larger sample size and they found that smaller LDL size was associated with the presence of peripheral arterial disease in the absence or presence of diabetes. These findings were confirmed by our recent study performed on patients with intermittent claudication (46): in relation to age-BMI-matched controls, patients with peripheral arterial disease had lower LDL size with decreased larger subclasses and increased small, dense particles. Notably, multivariate analysis suggested an independent association between small, dense LDL and peripheral arterial disease, beyond those already established for smoking, diabetes and hypertension.

**With abdominal aortic aneurysm**

Limited data is available on cardiovascular risk factors in subjects with atherosclerotic abdominal aortic aneurysm. Gorter et al. (47) found an elevated prevalence of the metabolic syndrome [as defined by the NCEP ATP III (22)] in patients with manifest atherosclerotic vascular diseases, including patients with abdominal aortic aneurysm. We recently showed that patients with abdominal aortic aneurysm have a smaller LDL size and a different subclass distribution towards increased levels of small, dense LDL (48).

**With metabolic diseases**

In addition to these findings from subjects with non-coronary forms of atherosclerosis, other recent studies showed elevated levels of small, dense LDL in patients with different metabolic diseases, including polycystic ovary syndrome and growth hormone deficiency (49,50), and reduced LDL size in women with gestational diabetes (51); notably, in most of them, the predominance of small, dense LDL characterised their type of dyslipidaemia, alone or in combination with elevated triglycerides and reduced HDL-C concentrations. These three lipid abnormalities constitute the so-called lipid triad or ‘atherogenic lipoprotein phenotype’ (4,5); this phenotype is highly atherogenic and its prevalence may suggest higher overall burden of atherosclerotic disease than that associated with hypercholesterolaemia (52). As stated by the NCEP ATP III (22), there is evidence that each component of the lipid triad is individually atherogenic but the relative contribution of each component cannot be easily determined. For this reason, it has been suggested to consider this trait as a whole as a ‘risk factor’. This is supported by data from epidemiological studies considering high-risk populations, which showed that the contribution to cardiovascular risk of each individual component of atherogenic dyslipidaemia cannot be dissected from the sum of all lipid risk factors (22,53).

**With the metabolic syndrome**

Therefore, it cannot surprise that the predominance of small, dense LDL is a feature of the metabolic syndrome (54). Haffner et al. (55) already showed in 1995 that LDL size was decreased in subjects with multiple metabolic disorders. As no exact definition was available at that time regarding the metabolic syndrome, the authors examined in 488 non-diabetic subjects the association of LDL size with different clinical and biochemical variables (including insulin, proinsulin, increased triglycerides, decreased HDL-C, hypertension and impaired glucose tolerance). Hulthe et al. (38) assessed the prevalence of metabolic syndrome in a population-based sample of clinically 58-year-old healthy men, using the WHO definition (56), showing that LDL size was significantly smaller in subjects with the metabolic syndrome. These findings were confirmed by a recent study performed on 105 patients with the metabolic syndrome (57), as assessed by the ATP III criteria (22): patients with the metabolic syndrome exhibited higher concentrations of small, dense LDL than individuals without it. The authors also showed that this increase was directly related to the number of components of the metabolic syndrome and mainly determined by triglyceride concentrations.

**The clinical impact of the modulation of small, dense LDL**

Weight reduction and increased physical activity may constitute first-line therapy; in addition, hypolipidae-
mic agents are able to favourably alter LDL size and subclasses (58). Particularly, medications with triglyceride-lowering effects shift LDL peak size from smaller, more dense to larger, more buoyant particles; in fact, reduced availability of triglyceride-rich particles lead to reduced production of small, dense LDL. This has been shown for fibrates and niacin: these substances lower preferentially small, dense LDL (59). Statins potentially lower large, medium and small LDL particles, but a strong variation has been noticed among the different agents. Pravastatin and simvastatin showed a limited net effect on LDL subclasses, while treatment with fluvastatin and atorvastatin resulted more frequently in a beneficial effect; promising data were also recently published on the use of rosuvastatin (60).

Other studies have more interestingly investigated if the therapeutic modification of LDL size may be significantly associated with reduced cardiovascular risk. Such investigations used arteriographic changes as outcome variables and have reported that benefit was concentrated in patients with a predominance of small, dense LDL who received treatment that tended to lower small, dense LDL. These studies included the ‘Stanford Coronary Risk Intervention Project (SCRIP)’, the ‘Familial Atherosclerosis Treatment Study (FATS)’ and the ‘Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I)’ trial (61–63). Lovastatin was administered in the SCRIP (with bile acid-binding resins, niacin or fibrates) and in the FATS (with colesterol, vs. niacin and colestipol), pravastatin was used in the PLAC-I.

The therapeutical modulation of LDL size was significantly associated with reduced cardiovascular risk at univariate analysis. In addition, at multivariate analyses with adjustments for confounding factors, changes in LDL size by drug therapy were the best correlates of changes in coronary stenosis in FATS (63). In PLAC-I, using a logistic regression models that adjusted for lipid levels and other confounding factors, elevated levels of small LDL were associated with a ninefold increased risk of CAD progression in the placebo group (61). All these data seem to suggest that the therapeutic modification of LDL size may be significantly associated with reduced cardiovascular risk, even after multivariate adjustment for confounding factors. In addition, as already reported (64), although not directly demonstrated, the modulation of LDL size with fibrates probably contributed to the reduction of cardiovascular risk in two clinical trials, the ‘Helsinki Heart Study’ and the ‘Veterans Affairs High-density Lipoprotein Cholesterol Intervention Trials Study Group (VA-HIT)’ (65–67).

Yet, although fibrates are more powerful than statins in improving LDL quality, existing evidence suggest that statins are more powerful agents in reducing cardiovascular morbidity and mortality. Fenofibrate seems to be very effective in lowering small, dense LDL, but the FIELD study (68,69) showed no significant reduction in primary endpoint in type 2 diabetics (randomised to receive fenofibrate or placebo). In this study triglycerides were reduced from 1.95 to 1.47 mmol/l; as it is expected that LDL distribution improves below the triglyceride threshold of 1.5 mmol/l, the findings of the FIELD study may argue against the concept that increasing LDL size is a major modulator of cardiovascular risk. In addition, in a subset statin-free cohort of the FIELD study it as been recently showed that fenofibrate produced a clear shift in HDL subspecies towards smaller more atherogenic particles (70).

Rizos and Mikhailidis (71) made an interesting comparison between the Bezafibrate Infarction Prevention Trial (BIP) and the VA-HIT trials. In the BIP, bezafibrate did not significantly reduce cardiovascular risk, while in the VA-HIT gemfibrozil reduced it. However, because of the baseline mean LDL values, the BIP population may have been more effectively treated with a statin and in the VA-HIT study LDL levels were close to those recommended in USA and UK for secondary prevention. Also, in the Lipid Coronary Angiography Trial, gemfibrozil therapy retarded the progression of coronary atherosclerosis and the formation of bypass-graft lesions after coronary bypass surgery in men with low HDL cholesterol (72). Combined therapy remains an option.; fibrate + statin treatment may be particularly beneficial in higher-risk individuals (59).

Conclusions

Besides traditional studies that reported high levels of atherogenic small, dense LDL in patients with CAD and type 2 diabetes, recent findings suggest the presence of elevated levels of these particles in other categories of patients at high cardiovascular risk, such as those with non-coronary forms of atherosclerosis (e.g. with carotid artery disease, aortic abdominal aneurysm and peripheral arterial disease) and metabolic diseases, including subjects with growth hormone deficiency and women with polycystic ovary syndrome. Notably, in most of them, the predominance of small, dense LDL characterised their type of dyslipidaemia, alone or in combination with elevated triglycerides and reduced HDL-C concentrations (atherogenic lipoprotein phenotype).

The small, dense phenomenon applies to all lipoprotein particles so that small, dense chylomicron remnants as well as small, dense HDL may
contribute to the atherogenic nature of the profile, but there is also evidence that large cholesterol-rich particles contribute to cardiovascular risk [e.g. large LDL particles, as reviewed in (24)]. This is further complicated by the fact that at the same level of LDL-C individuals with a predominance of small, dense LDL have significantly more particles than those with a predominance of larger, more buoyant LDL. The number of LDL particles in plasma is potentially important, because the arterial walls are exposed to these particles and an increased number might increase atherogenicity independently of particle size (73). Also, it cannot be excluded from available evidence that the predominance of small, dense LDL may be associated with abnormalities in coagulation, fibrinolysis or platelets (74).

Is higher risk of individuals with a predominance of small, dense LDL attributable to the fact that they have more LDL particles in total, or does the smaller size contribute independently to CHD risk? As LDL size is seldom a significant and independent predictor of cardiovascular risk after multivariate adjustment for confounding variables (particularly plasma triglyceride levels and HDL-C concentrations), a clear causal relationship between small, dense LDL and increased cardiovascular risk cannot be fully proven, based on our present knowledge. Yet, the methodology used to assess LDL size and subclasses represents a crucial point, as there is general agreement on sizing based on gradient gels electrophoresis or nuclear magnetic resonance, while the conclusions are far less clear for the other techniques (75).

The therapeutic modulation of small, dense LDL have been shown to significantly reduce cardiovascular risk; lipid-lowering drugs are able to favourably alter these particles and fibrates and nicotinic acid seem to be the most effective agents. Statins potentially lower all LDL subclasses and their net effect is often limited; rosvuastatin, the latest statins’ molecule introduced in the market, seems to be strongly effective in modulating plasma lipids and LDL subclasses too (60). Promising data are also available with the use of ezetimibe, a cholesterol absorption inhibitor (76,77). As recently suggested (78,79), because of the strong relationships between small, dense LDL, triglycerides and HDL-C, the therapeutic modification of the atherogenic lipoprotein phenotype probably represent one of the most effective methods of reducing cardiovascular risk.

References
1 Krauss RM, Blanche PJ. Detection and quantitation of LDL subfractions. Curr Opin Lipidol 1992; 3: 377–83.
2 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: 59–67.
3 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: 241–53.
4 Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. Circulation 1990; 82: 495–506.
5 Rizzo M, Berneis K. Lipid triad or atherogenic lipoprotein phenotype: a role in cardiovascular prevention? J Atheroscler Thromb 2005; 12: 237–9.
6 Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res 2002; 43: 1563–79.
7 Austin MA. Genetic epidemiology of low-density lipoprotein subclass phenotypes. Ann Med 1992; 24: 477–81.
8 Dreon DM, Fernstrom HA, Williams PT, Krauss RM. LDL subclass patterns and lipoprotein response to a low-fat, high-carbohydrate diet in women. Arterioscler Thromb Vasc Biol 1997; 17: 707–14.
9 Krauss RM. Dietary and genetic probes of atherogenic dyslipidemia. Arterioscler Thromb Vasc Biol 2005; 25: 2265–72.
10 Austin MA, Brunzell JD, Fitch WL, Krauss RM. Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipidemia. Arteriosclerosis 1990; 10; 520–30.
11 Teng B, Thompson GR, Sniderman AD, Forte TM, Krauss RM, Kwitterovich PO Jr. Composition and distribution of low density lipoprotein fractions in hyperapobetalipoproteinemia, normolipidemia, and familial hypercholesterolemia. Proc Natl Acad Sci USA 1983; 80: 6662–6.
12 Genest J Jr, Bard JM, Fruchart JC, Ordovas JM, Schaefer EJ, Familial hyperalphalipoproteinemia in premature coronary artery disease. Arterioscler Thromb 1993; 13: 1728–37.
13 Terry RB, Wood PD, Haskell WL, Stefanick ML, Krauss RM. Regional adiposity patterns in relation to lipids, lipoprotein cholesterol, and lipoprotein subclass mass in men. J Clin Endocrinol Metab 1989; 68: 191–9.
14 Rizzo M, Barbagallo CM, Severino M et al. Low-density-lipoprotein peak particle size in a Mediterranean population. Eur J Clin Invest 2003; 33: 126–33.
15 de Graaf J, Swinkels DW, Demacker PN, de Haan AF, Stalenhoef AF. Differences in the low density lipoprotein subclass profile between oral contraceptive users and controls. J Clin Endocrinol Metab 1993; 76: 197–202.
16 Jansen H, Hop W, van Tol A, Bruschke AV, Birkenhager JC. Hepatic lipase and lipoprotein lipase are not major determinants of the low density lipoprotein subclass pattern in human subjects with coronary heart disease. Atherosclerosis 1994; 107: 45–54.
17 Campos H, Dreon DM, Krauss RM. Associations of hepatic and lipoprotein lipase activities with changes in dietary composition and low density lipoprotein subclasses. J Lipid Res 1995; 36: 462–72.
18 Rizzo M, Taylor JM, Barbagallo CM, Berneis K, Blanche PJ, Krauss RM. Effects on lipoprotein subclasses of combined expression of human hepatic lipase and human apolipoprotein in transgenic rabbits. Atherosclerosis 1994; 24: 141–6.
19 Deckelbaum RJ, Granot E, Oshry Y, Rose L, Eisenberg S. Plasma triglyceride determines structure-composition in low and high density lipoproteins. Arteriosclerosis 1984; 4: 225–31.
20 Krauss RM, Hailerstein MK, Neese RA, Blanche PJ, La Belle M, Shames DM. Altered metabolism of large low density lipoproteins in subjects with predominance of small low density lipoproteins. Circulation 1995; 92: 1–102.
21 Guerin M, Le Goff W, Lassell TS, Van Tol A, Steiner G, Chapman MJ. Atherogenic role of elevated CE transfer from HDL to VLDL(1) and dense LDL in type 2 diabetes: impact of the degree of triglyceridemia. Arterioscler Thromb Vasc Biol 2001; 21: 282–8.
22 National Cholesterol Education Program. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002; 106: 3143–42.

23 Bjornheden T, Baby A, Bondjers G, Wiklund O. Accumulation of lipoprotein fractions and subfractions in the arterial wall, determined in an in vitro perfusion system. Atherosclerosis 1996; 123: 43–56.

24 Galeano NF, Al-Haideri M, Keyserman F, Rumsey SC, Deckelbaum RJ. 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: 1263–73.

25 Camejo G, Lopez A, Lopez F, Quinones J. Interaction of low density lipoproteins with arterial proteoglycans. The role of charge and sialic acid content. Atherosclerosis 1985; 55: 93–105.

26 Tribble DL, Rizzo M, Chait A, Lewis DM, Blanche PJ, Krauss RM. Enhanced oxidative susceptibility and reduced antioxidant content of metabolic precursors of small, dense low-density lipoproteins. Am J Med 2001; 110: 103–10.

27 Tribble DL, Holl LG, Wood PD, Krauss RM. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. Atherosclerosis 1992; 93: 189–99.

28 de Graaf J, Hak-Lemmers HL, Hectors MP, Demacker PN, Hendriks JC, Stalenhoef AF. Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. Arterioscler Thromb 1991; 11: 298–306.

29 Rizzo M, Berneis K. Low-density lipoprotein size and cardiovascular risk assessment QJM. Int J Med 2006; 99: 1–14.

30 Sacks FM, Campos H. Low-density lipoprotein size and cardiovascular disease: a reappraisal. J Clin Endocr Metab 2003; 88: 4525–32.

31 Lamarche B, Lemieux I, Després JP. The small, dense LDL phenotype and the risk of coronary heart disease: epidemiology, pathophysiology and therapeutic aspects. Diab Metab 1999; 25: 199–211.

32 Krauss RM. Lipids and lipoproteins in patients with type 2 diabetes. Diabetes Care 2004; 27: 1496–504.

33 Landray MJ, Sagar G, Muskin J, Murray S, Holder RL, Lip GYH. Association of atherogenic low-density lipoprotein subfractions with carotid atherosclerosis. Q J Med 1998; 91: 345–51.

34 Skoglund-Andersson C, Tang R, Bond MG, de Faire U, Hamsten A, Karpe F. LDL particle size distribution is associated with carotid intima-media thickness in healthy 50-year-old men. Arterioscler Thromb Vasc Biol 1999; 19: 2422–30.

35 Bokemark L, Wikstrand J, Attvall S, Hultth J, Wedel H, Fagerberg B. insulin resistance and intima-media thickness in the carotid and femoral arteries of clinically healthy 58-year-old men. The Atherosclerosis and Insulin Resistance Study (AIR). J Intern Med 2001; 249: 59–67.

36 Hallman DM, Brown SA, Ballantyne CM, Sharrett AR, Boerwinkle E. Relationship between low-density lipoprotein subclasses and asymptomatic atherosclerosis in subjects from the Atherosclerosis Risks in Communities (ARIC) Study. Biomarkers 2004; 9: 190–202.

37 Hultth J, Wiklund O, Olsson G et al. Computerized measurement of LDL particle size in human serum. Reproducibility studies and evaluation of LDL particle size in relation to metabolic variables and the occurrence of atherosclerosis. Scand J Clin Lab Invest 1999; 59: 649–61.

38 Hultth J, Bokemark L, Wikstrand J, Fagerberg B. The metabolic syndrome, LDL particle size, and atherosclerosis: the Atherosclerosis and Insulin Resistance (AIR) study. Arterioscler Thromb Vasc Biol 2000; 20: 2140–7.

39 Liu ML, Yitiao K, Nuotio I, Salonen R, Salonen JT, Taskinen MR. Association between carotid intima-media thickness and low-density lipoprotein size and susceptibility of low-density lipoprotein to oxidation in asymptomatic members of familial combined hyperlipidemia families. Stroke 2002; 33: 1255–60.

40 Watanabe T, Koba S, Kasumura M et al. Small dense low-density lipoprotein and carotid atherosclerosis in relation to vascular dementia. Metabolism 2004; 53: 476–82.

41 van Titt L, Smilde TJ, van Wissen S, de Graaf J, Kastelein JJ, Stalenhoef AF. Effects of atorvastatin and simvastatin on low-density lipoprotein subfraction profile, low-density lipoprotein oxidizability, and antibodies to oxidized low-density lipoprotein in relation to carotid intima media thickness in familial hypercholesterolemia. J Investig Med 2004; 52: 177–84.

42 Berneis K, Jeaneret C, Muser J, Felix B, Miserez AR. Low-density lipoprotein size and subclasses are markers of clinically apparent and non-apparent atherosclerosis in type 2 diabetes. Metabolism 2005; 54: 227–34.

43 Wallenfeldt K, Bokemark L, Wikstrand J, Hultth J, Fagerberg B. Apolipoprotein B/apolipoprotein A-I in relation to the metabolic syndrome and change in carotid artery intima-media thickness during 3 years in middle-aged men. Stroke 2004; 35: 2248–52.

44 Lupattelli G, Pasqualini L, Piepi D et al. Increased postprandial lipemia in patients with normolipemic peripheral arterial disease. Am Heart J 2002; 143: 733–8.

45 O’Neal DN, Lewicki J, Ansari MZ, Matthews PG, Best JD. Lipid levels and peripheral vascular disease in diabetic and non-diabetic subjects. Atherosclerosis 1998; 136: 1–8.

46 Rizzo M, Pernice F, Fraschetti A, Berneis K. Atherogenic lipoprotein phenotype and LDL size and subclasses in patients with peripheral arterial disease. Atherosclerosis 2007 [Epub ahead of print].

47 Gorter PM, Olhjoek J, van de Graaf Y, Algra A, Rabelink TJ, Visseren JL; SMART Study Group. Prevalence of the metabolic syndrome in patients with coronary heart disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm. Atherosclerosis 2004; 173: 363–9.

48 Rizzo M, Berneis K. The role of small, dense low-density lipoproteins in non-coronary forms of atherosclerosis. Vasc Dis Prev 2006; 3: 269–74.

49 Berneis K, Rizzo M, Frazzetti F, Lazzaroni V, Carmina E. Atherogenic lipoprotein phenotype and low-density lipoproteins size and subclasses in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2007; 92: 186–91.

50 Rizzo M, Trepp R, Berneis K, Christ ER. Atherogenic lipoprotein phenotype and LDL size and subclasses in patients with growth hormone deficiency before and after short-term replacement therapy. Eur J Endocr 2007; 156: 361–7.

51 Qiu C, Rudra C, Austin MA, Williams MA. Association of gestational diabetes mellitus and low-density lipoprotein (LDL) particle size. Physiol Res 2007 [Epub ahead of print].

52 Superko HR. Beyond LDL cholesterol reduction. Circulation 1996; 94: 2351–6.

53 Packard CJ. Small dense low-density lipoprotein and its role as an independent predictor of cardiovascular disease. Curr Opin Lipidol 2006; 17: 412–7.

54 Grundy SM, Cleeman JT, Daniels SR et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005; 112: 2735–52.

55 Haffner SM, Mykkanen L, Robbins D et al. A preponderance of small dense LDL is associated with specific insulin, proinsulin and the components of the insulin resistance syndrome in non-diabetic subjects. Diabetologia 1995; 38: 1328–36.

56 World Health Organization. Definition, Diagnosis, and Classification of Diabetes Mellitus and its Complications: Report of a WHO Consultation. Geneva: World Health Organization, 1999.

57 Gazi I, Tsimihydrimos V, Filippatos T, Biairakati E, Tslepis AD, Efiss E. Concentration and relative distribution of low-density lipoprotein subfractions in patients with metabolic syndrome defined according to the National Cholesterol Education Program criteria. Metabolism 2006; 55: 885–91.

58 Davidson MH, Todh PP. Comparative effects of lipid-lowering therapies. Prog Cardiovasc Dis 2004; 47: 73–104.
59 Rizzo M, Berneis K. The clinical significance of the size of low-density-lipoproteins and the modulation of subclasses by fibrates. *Curr Med Res Opin* 2007; 23: 1103–11.

60 Rizzo M, Berneis K. The clinical relevance of low-density-lipoproteins size modulation by statins. *Cardiovasc Drug Ther* 2006; 20: 205–17.

61 Rosenson RS, Otvos JD, Freedman DS. Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-1) trial. *Am J Cardiol* 2002; 90: 89–94.

62 Miller BD, Alderman EL, Haskell WL, Fair JM, Krauss RM. Predominance of dense low-density lipoprotein particles predicts angiographic benefit of therapy in the Stanford Coronary Risk Intervention Project. *Circulation* 1996; 94: 2146–53.

63 Zambon A, Hokanson JE, Brown BG, Brunzell JD. Evidence for a new pathophysiological mechanism for coronary artery disease regression: hepatic lipase-mediated changes in LDL density. *Circulation* 1999; 99: 1959–64.

64 Marais AD. Therapeutic modulation of low-density lipoprotein size. *Curr Opin Lipidol* 2000; 11: 597–602.

65 Manninen V, Tenkanen L, Koskinen P et al. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. *Circulation* 1992; 85: 37–45.

66 Tenkanen L, Manttari M, Manninen V. Some coronary risk factors related to the insulin resistance syndrome and the treatment with gemfibrozil. Experience from the Helsinki Heart Study. *Circulation* 1995; 92: 1779–85.

67 Rubins HB, Robins SI, Collins D et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high density lipoprotein cholesterol: Veterans Affairs High-density Lipoprotein Cholesterol Intervention Trials Study Group. *N Engl J Med* 1999; 341: 410–8.

68 Keech A, Simes RJ, Barter P et al.; FIELD study investigators. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 2005; 366: 1849–61.

69 Wierzbicki AS. Fibrates after the FIELD study: some answers, more questions. *Diab Vasc Dis Res* 2006; 3: 166–71.

70 Hiukka A, Leinonen E, Jauhiainen M et al. Long-term effects of fenofibrate on VLDL and HDL subclasses in participants with type 2 diabetes mellitus. *Diabetologia* 2007; 50: 2067–75.

71 Rizos E, Mihailidis DP. Are high-density lipoprotein and triglyceride levels important in secondary prevention – impressions from the BIP and VA-HIT trials. *Int J Cardiol* 2002; 82: 199–207.

72 Frick MH, Syvanne M, Nieminen MS et al. Prevention of the angiographic progression of coronary and vein-graft atherosclerosis by gemfibrozil after coronary bypass surgery in men with low levels of HDL cholesterol. *Lipid Coronary Angiography Trial (LOCAT) Study Group. Circulation* 1997; 96: 2137–43.

73 Lada AT, Rudel LL. Associations of low density lipoprotein particle composition with atherogenicity. *Curr Opin Lipidol* 2004; 15: 19–24.

74 Halle M, Berg A, Keul J, Baumstark MW. Association between fibrinogen concentrations and HDL and LDL subclass phenotypes in healthy men. *Arterioscler Thromb Vasc Biol* 1996; 16: 144–8.

75 Stein EA. Are measurements of LDL particles ready for prime time? *Clin Chem* 2006; 52: 1643–4.

76 Geiss HC, Otto C, Parhofer KG. Effect of ezetimibe on low-density lipoprotein subtype distribution: results of a placebo-controlled, double-blind trial in patients treated by regular low-density lipoprotein apheresis and statins. *Metabolism* 2006; 55: 599–604.

77 Kalogirou M, Tsimihodimos V, Gazi I et al. Effect of ezetimibe monotherapy on the concentration of lipoprotein subfractions in patients with primary dyslipidaemia. *Curr Med Res Opin* 2007; 23: 1169–76.

78 Nesto RW. Beyond low-density lipoprotein: addressing the atherogenic lipid triad in type 2 diabetes mellitus and the metabolic syndrome. *Am J Cardiacc Drugs* 2005; 5: 379–87.

79 Barter P. Options for therapeutic intervention: how effective are the different agents? *Eur Heart J Suppl* 2006; 8: F47–53.

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