Atherogenic Lipoprotein Particles in Atherosclerosis

Rafael Carmena, Patrick Duriez and Jean-Charles Fruchart

Circulation. 2004;109:III-2-III-7
doi: 10.1161/01.CIR.0000131511.50734.44
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539

Atherogenic Lipoprotein Particles in Atherosclerosis

Rafael Carmena, MD; Patrick Duriez, PhD; Jean-Charles Fruchart, PhD

Abstract—The importance of low-density lipoprotein (LDL) cholesterol in the development of atherosclerosis has long been recognized, and LDL cholesterol remains the primary target of therapy for the prevention of coronary heart disease. Nevertheless, increasing research attention over the past decade has been devoted to the heterogeneity of LDL particles and the atherogenicity of lipids and lipoproteins other than LDL. Particularly atherogenic forms of LDL include small, dense LDL particles and oxidized LDL. All lipoproteins that contain apolipoprotein B, such as LDL, very-low-density lipoprotein, and intermediate-density lipoprotein, tend to promote atherosclerosis; however, these particles differ in their apolipoprotein and triglyceride content. High levels of plasma triglycerides increase the risk of acute coronary events. Lipoprotein(a) is now considered an independent risk factor in both men and women. Ultimately, better understanding of the roles of these lipid particles and subfractions in the initiation and progression of atherosclerosis may affect treatment decisions. **(*Circulation*. 2004;109[suppl III]:III-2–III-7.)**

Key Words: apolipoprotein B ■ lipoprotein(a) ■ low-density lipoprotein ■ triglyceride-rich lipoproteins ■ triglycerides

Epidemiological studies have shown a positive relationship between total cholesterol concentrations and mortality from coronary heart disease (CHD). Total cholesterol does not accurately predict the risk of CHD in many patients, however, because it is the sum of all cholesterol carried not only by atherogenic lipoproteins (eg, very-low-density lipoprotein [VLDL], low-density lipoprotein [LDL], intermediate-density lipoprotein [IDL]) but also by antiatherogenic lipoproteins (ie, high-density lipoprotein [HDL]). Therefore, the decision to treat is based on LDL-cholesterol values. Yet, treatment decisions may also need to take into account LDL heterogeneity, which has been recognized for many years. Small, dense LDL particles are more atherogenic than large, buoyant LDL particles, and oxidation of LDL also increases its atherogenicity. In addition, LDL belongs to the group of lipoproteins that contain apolipoprotein (apo) B-100. Some of the particles in this highly heterogeneous group contain other apolipoproteins, such as apo C-II, apo C-III, and apo E. Furthermore, some particles are larger and rich in triglycerides (large VLDL), whereas others are smaller and rich in cholesteryl esters (small VLDL, IDL). It is now known that remnant lipoproteins containing apo C-III are highly atherogenic, as is lipoprotein(a) [Lp(a)], another member of the apo B-100 group. This article reviews recent studies involving LDL subclasses and atherogenic lipoproteins, many of which used novel methods of lipoprotein subfractioning.

Small, Dense LDL

Analytical Methods

LDL heterogeneity is characterized according to different physical properties of the LDL particles: lipoprotein size, flotation rate, and density. Features of some common assays used to characterize LDL subfractions (as well as to measure other lipids and lipoproteins) are summarized in the Table. Gradient gel electrophoresis using nondenaturing conditions is commonly used to characterize the distribution of LDL particles by size.[1] LDL subclass phenotype A is characterized by a predominance of large LDL particles, whereas LDL subclass phenotype B is characterized by a predominance of small LDL particles. A majority (85% to 90%) of subjects can be classified as having either LDL subclass phenotype A or B, whereas the remainder have an intermediate phenotype.[2]

High-performance gel-filtration chromatography[3] and nuclear magnetic resonance (NMR) spectroscopy[4] have recently been applied to determinations of LDL particle size. Several different methods of density gradient ultracentrifugation have been used to characterize LDL flotation rate (Svedberg flotation unit) using the analytical ultracentrifuge. Methods for characterizing LDL subclasses based on flotation rates are modifications of a procedure developed by Chung et al[5] using a vertical rotor in a preparative ultracentrifuge (Vertical Auto Profile [VAP]).[6] When subjects are classified as LDL subclass phenotype A or B, there are significant differences in LDL relative flotation (Rf).[7] Several methods based on discontinuous salt gradients are used to determine LDL subclasses based on density.[8] These various

From the Department of Endocrinology and Nutrition (R.C.), Facultad de Medicina y Hospital Clínico Universitario, Valencia, Spain; Faculté de Pharmacie (P.D., J.-C.F.), Université du Droit et de la Santé de Lille 2, and the Département de Recherche sur les Lipoprotéines et l'Athérosclérose, Inserm U545 (P.D., J.-C.F.), Institut Pasteur de Lille, Lille, France.

Correspondence to Professor Jean-Charles Fruchart, Département de Recherche sur les Lipoprotéines et l'Athérosclérose, Inserm U545 - Institut Pasteur, 1 rue du Pr Calmette, BP245 59019 Lille, France. E-mail Jean-Charles.Fruchart@pasteur-lille.fr

Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/01.CIR.0000131511.50734.44**

Methods Used to Measure Lipid/Lipoprotein Subfractions

Name of Test	Technology Used	Lipids/Lipoproteins Measured
Vertical Auto Profile (VAP) (Atherotech)	Vertical ultracentrifugation technique — spins down plasma using centrifuge	LDL density (pattern A or B) by rate of descending particles; IDL and HDL subtypes; VLDL density and Lp(a). TGs are estimates, so LDL not accurate when TGs >400 mg/dL
Segmented Gradient Gel Electrophoresis (sGGE) (Berkeley HeartLab)	Electrophoresis gradient gel — calibrated against the analytical ultracentrifugation (ANUC)	Basic Panel: apo B; LDL (calculated peak particle size and % distribution); 5 subclasses of LDL, 2 subclasses of IDL, and 3 subclasses of VLDL Advanced Panel: above plus 6 subtypes of HDL
Lipoprint LDL System (Quantimetrix Corp)	High-resolution 3% polyacrylamide gel tubes	Three subclasses of IDL; 7 subclasses of LDL (LDL particle size and phenotype)
Nuclear Magnetic Resonance (NMR) (LipoMed Inc)	Measures the spin or electrical resonance of various particles	No., quantity, and size of LDL particles (calculated measurements of 4 LDL subclasses); HDL (large and small); VLDL particle sizes (low, medium and large)

apo indicates apolipoprotein; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); TG, triglyceride; VLDL, very-low-density lipoprotein.

methods of determining LDL subclasses show a high degree of correlation, despite the fact that they measure different physical properties of LDL.

Clinical Significance

Several cross-sectional studies have reported differences in LDL particle size, density, and composition between patients with CHD and healthy controls. Prospective, nested case-control studies have since confirmed that the presence of small, dense LDL particles is associated with a more than 3-fold increase in CHD risk.

In the Québec Cardiovascular Study, men with an LDL particle size <25.6 nm had a significant 2.2-fold increase in the 5-year rate of ischemic heart disease (IHD) compared with men having an LDL particle size >25.6 nm.[9] Multivariate and subgroup analyses indicated that small, dense LDL particles predicted the rate of IHD independently of LDL cholesterol, triglycerides, HDL cholesterol, apo B, and the total cholesterol–HDL cholesterol ratio. The magnitude of the increase in IHD risk attributed to lipid risk factors was modulated to a significant extent by variations in LDL particle size. This study suggests that information on LDL diameter may improve the ability to predict IHD risk accurately over traditional lipid variables.[9]

On the other hand, data from the Physicians' Health Study suggest that small, dense LDL may not be an independent risk factor.[10] A nested case-control study with prospectively collected samples examined whether a predominance of small, dense LDL particles and elevated triglyceride levels are independent risk factors for myocardial infarction (MI). Cases had a significantly smaller LDL diameter (mean [SD], 25.6 [0.9] nm) than did controls (mean [SD], 25.9 [8] nm; $P<0.001$). Cases also had higher median triglyceride levels (1.9 versus 1.5 mmol/L [168 versus 132 mg/dL]; $P<0.001$).[10] The LDL diameter had a high inverse correlation with triglyceride level ($r=-0.71$), and a high direct correlation with HDL-cholesterol level ($r=0.60$). After simultaneous adjustment for lipids and a variety of coronary risk factors, LDL particle diameter was no longer a statistically significant risk indicator. However, triglyceride levels remained significant. The total-cholesterol level, but not HDL-cholesterol level, also remained significant. These findings indicate that nonfasting triglyceride levels appear to be a strong and independent predictor of future risk of MI, particularly when total cholesterol is also elevated. In contrast, LDL particle diameter was associated with risk of MI, but not after adjustment for triglyceride level.[10]

Proton NMR spectroscopy was used to simultaneously quantify levels of subclasses of VLDL, LDL, and HDL in men to examine whether NMR-derived lipoprotein subclass levels improve the prediction of coronary artery disease (CAD).[11] It was found that a global measure of CAD severity was positively associated with levels of large VLDL and small HDL particles and inversely associated with intermediate-sized HDL particles. Men with relatively high (higher than the median) levels of either small HDL or large VLDL particles were 3 to 4 times more likely to have extensive CAD than were men with lower levels; men with high levels of both large VLDL and small HDL were 15 times more likely to have extensive CAD. In contrast, adjustment for levels of triglycerides or HDL cholesterol greatly reduced the relation of small LDL particles to CAD. These findings suggest that large VLDL and small HDL particles may play important roles in the development of occlusive disease.[11]

Relationships between incident cardiovascular disease and lipoprotein subclass measurements by NMR spectroscopy were evaluated in the Cardiovascular Health Study (CHS)[4] in a nested case-cohort analysis. By univariate analysis, the median levels for healthy participants versus participants with incident MI and angina pectoris were 0 versus 7 mg/dL for small LDL, 1501 versus 1680 nmol/L for the number of LDL particles, and 21.6 versus 21.3 nm for LDL size; these values were significantly different between healthy participants and those with incident MI and angina for women but not for men. The level of less dense LDL, which represents most of the LDL cholesterol in women, was not related to incident MI and angina. For men and women, levels of total and VLDL triglycerides were higher in the case group than in the healthy group. In multivariate models for women that included triglycerides and HDL cholesterol, the number of LDL particles (but not LDL size) remained significantly related to MI and angina. This study showed that the size of LDL

particles and the greater number of LDL particles are related to incident CHD among older women.[4]

A prospective, nested case-control study among healthy middle-aged women was conducted to assess LDL particle size and concentration (NMR) as risk factors for future MI, stroke, or death from CHD.[12] Median baseline levels of LDL particle concentration (NMR) were higher and LDL particle size (NMR) was lower (21.5 versus 21.8 nm; $P=0.046$) among women who subsequently had cardiovascular events than among those who did not. Of these factors, LDL particle concentration (NMR) was the stronger predictor.[12]

It has been shown recently that LDL particle size, but not susceptibility of LDL to oxidation in vitro, is independently associated with carotid intima-media thickness in asymptomatic familial combined hyperlipidemia family members.[13]

Insulin Resistance, Diabetes, and Small, Dense LDL

The small, dense LDL phenotype rarely occurs as an isolated disorder. It is most frequently accompanied by hypertriglyceridemia, reduced HDL-cholesterol levels, abdominal obesity, and insulin resistance (all of which are components of the metabolic syndrome) and by a series of other metabolic alterations predictive of an impaired endothelial function and increased susceptibility to thrombosis.

Results from the Québec Cardiovascular Study have indicated that persons displaying elevated plasma concentrations of insulin and apo B together with small, dense LDL particles showed a remarkable increase in CHD risk.[14]

NMR spectroscopy was used to define the effects of insulin resistance on lipoprotein subclasses in insulin-sensitive, insulin-resistant, and untreated subjects with type 2 diabetes mellitus.[15] In the group as a whole, insulin resistance had profound effects on lipoprotein size and subclass particle concentrations for VLDL, LDL, and HDL. When compared with insulin-sensitive subjects, the insulin-resistant and diabetes subgroups exhibited a 2- to 3-fold increase in concentrations of large VLDL particles (no change in medium or small VLDLs), which resulted in an increase in serum triglycerides. They also showed a decrease in LDL size (as a result of a marked increase in small LDL particles and reduced large LDL) plus an increase in overall LDL particle concentration. Taken together, these latter changes led to no difference (insulin sensitive versus insulin resistant) or a minimal difference (insulin sensitive versus diabetes) in LDL-cholesterol levels. In type 2 diabetes, these alterations could be attributed primarily to the underlying insulin resistance. These changes in the NMR lipoprotein subclass profile predictably increased the risk of cardiovascular disease but were not fully apparent in the conventional lipid panel.[15]

A recent study examined whether NMR lipoprotein spectroscopy improves the prediction of CAD in patients with type 1 diabetes, independently of conventional lipid and other risk factors. In a prospective, nested case-control study of subjects with childhood-onset type 1 diabetes (Pittsburgh Epidemiology of Diabetes Complications Study),[16] univariate analyses showed that both lipid mass and particle concentrations (NMR spectroscopy) of all VLDL subclasses, small LDL, medium LDL, and medium HDL were increased in CAD cases compared with controls, whereas large HDL was decreased. Mean LDL and HDL particle sizes were less in CAD cases.[16]

The Diabetes Atherosclerosis Intervention Study (DAIS)[17] showed that lipid-modifying treatment decreased the angiographic progression of coronary atherosclerosis in subjects with type 2 diabetes. This effect was related in part to the correction of lipoprotein abnormalities. Compared with placebo, fenofibrate treatment significantly increased LDL particle size and HDL cholesterol and decreased plasma total cholesterol, LDL cholesterol, and triglyceride concentrations. The final LDL particle size was inversely correlated with the increase in percentage diameter stenosis.[17]

Oxidized LDL

It has been suggested for 20 years that oxidative stress, and particularly LDL oxidation, could induce atherosclerosis[18] and that markers of LDL oxidation in plasma (circulating oxidized LDL, autoantibodies against oxidized LDL) could be used to assess the development of atherosclerosis in patients. Circulating oxidized LDL is additive to the global risk score based on age, sex, total and HDL cholesterol, diabetes mellitus, hypertension, and smoking as a useful marker for identifying persons at risk for CAD.[19,20] In one study, circulating oxidized LDL was associated with both subclinical atherosclerosis (clinically silent ultrasound-assessed atherosclerotic changes in the carotid and femoral arteries) and inflammatory variables (C-reactive protein and the inflammatory cytokines interleukin-6 and tumor necrosis factor-α), supporting the concept that oxidatively modified LDL may play a major role in atherosclerosis development, although no causality could be shown.[21] It has been proposed that, because of the antigenic properties of oxidized LDL, the anti-oxidized LDL antibody titer could represent a useful index of in vivo LDL oxidation. Autoantibodies against oxidized LDL have been reported to be associated with atherosclerosis. However, the data are not consistent: some studies have reported a positive relationship between autoantibodies against oxidized LDL and CHD,[22,23] whereas another did not.[24] There is a strong cross-reactivity between autoantibodies against oxidized LDL and anticardiolipin antibodies, which have been positively associated with CHD.[23]

Despite the plausible role of oxidative stress in atherogenesis, results of controlled, prospective trials examining the effects of antioxidant supplements on clinical end points have been disappointing.[25] Recently, Steinberg and Witztum, who were early proponents of the role of oxidized LDL in the development of atherosclerosis,[26] have argued for further research, suggesting that the failure of these clinical trials does not disprove the role of lipoprotein oxidation in atherogenesis and citing a number of limitations. For example, the clinical trials conducted to date may not have used the right antioxidants at the right dosages. In addition, the trials may not have been started early enough in the disease process or continued long enough to demonstrate a favorable effect.[27] This subject will undoubtedly remain controversial.

Apolipoprotein B

As first suggested by Lee and Alaupovic,[28] lipoprotein particles can be differentiated on the basis of their constituent

apolipoproteins. Apo B-100 is the major apolipoprotein component of the atherogenic lipoproteins (VLDL, LDL, IDL). Although epidemiological data relating apo B concentrations to CHD are limited, some case-control studies in patients with CHD have found plasma apo B levels to be more discriminating than other plasma lipids and lipoproteins.[29] Nevertheless, the extent to which plasma apo B levels were better predictors of risk varied.[30] Some of these discrepancies may have resulted from difficulties in standardizing assays for measuring plasma apo B levels; however, the World Health Organization–International Federation of Clinical Chemistry (IFCC) has now developed standardized methods for measurement of apo B and apo A-I,[31] and population results have subsequently been reported.[32] The prospective Québec Cardiovascular Study[33] and other investigations have demonstrated the importance of apo B in estimating coronary risk. Recently, the large prospective Apolipoprotein-related Mortality Risk (AMORIS) study[34] showed that the age-adjusted values of apo B and the apo B/apo A-I ratio were strongly and positively related to increased risk of fatal MI in men and women. In multivariate analyses, apo B was a stronger predictor of risk than LDL-cholesterol in both men and women. This study suggests that apo B and the apo B/apo A-I ratio should be regarded as highly predictive in evaluating cardiac risk. Apo B might be of greatest value in the diagnosis and treatment of persons with normal or low concentrations of LDL cholesterol.[34] For example, patients with type 2 diabetes mellitus frequently have hypertriglyceridemia together with hyper-apo B, an atherogenic lipid profile that is often unappreciated because of concomitant low or normal levels of LDL cholesterol.[35]

Triglyceride-Rich Lipoproteins

A meta-analysis of 17 prospective population-based studies found hypertriglyceridemia to be an independent risk factor for cardiovascular disease.[36] Nevertheless, it is possible that, rather than being actual atherogenic agents in themselves, elevated triglycerides merely serve as a marker for increases in triglyceride-rich remnant lipoproteins and that it is these latter particles that are involved in the development of atherosclerosis.[37,38]

Triglyceride-rich lipoproteins comprise a great variety of nascent and metabolically modified lipoprotein particles differing in size, density, and lipid and apolipoprotein composition. Studies have shown an inverse relationship between the size of lipoproteins and their ability to cross the endothelial barrier to enter the arterial intima. Chylomicrons and large VLDLs (Svedberg flotation unit [S_f] 60 to 400) are probably not capable of entering the arterial wall. On the other hand, small VLDLs (S_f 20 to 60) and IDLs (S_f 12 to 20) can enter the arterial intima. Therefore, certain triglyceride-rich lipoproteins are atherogenic, whereas others are not. A large body of evidence suggests that small VLDLs and IDLs are independently associated with atherosclerosis.

A study[39] in 174 patients with type 2 diabetes mellitus who were not receiving lipid-lowering therapy concluded that the severity of CHD (as examined by angiography) was related to the number of circulating fasting triglyceride-rich lipoprotein particles. This relationship was stronger in women than in men and was independent of HDL and LDL. Remnant-like particles (RLPs) can be isolated from the plasma or serum by an antibody-based assay. In the Framingham Offspring Study,[40] mean RLP cholesterol and RLP triglycerides were highly significantly ($P<0.0001$) increased in diabetic women and significantly ($P<0.001$) increased in diabetic men compared with respective controls.

Apo C-III in VLDL is associated with denser, smaller VLDL subclasses and is believed to be particularly atherogenic. Remnants associated with apo C-III are more related to the development of atherosclerosis than are triglycerides per se. Fasting remnant lipoproteins could reflect postprandial remnant lipoproteins and predict future clinical coronary events independently of other risk factors.[41] The concentration of apo C-III in VLDL and IDL particles has been significantly correlated with development of coronary stenosis.[42]

Results of the Monitored Atherosclerosis Regression Study (MARS)[43] provide evidence for a strong positive relationship between the progression of CAD and concentrations of triglyceride-rich lipoprotein remnants. Lipoproteins in the S_f 12 to 60 range (IDLs and small VLDLs) were independently correlated with development of coronary atherosclerosis. In MARS, triglyceride-rich lipoproteins such as small VLDLs and particles rich in apo C-III were related to the progression of mild or moderate (<50% diameter stenosis) coronary artery lesions rather than severe (≥50% diameter stenosis) lesions.[43] Lesions of <50% diameter stenosis appear to highly predict clinical coronary events.

In a randomly selected subgroup of 63 MARS subjects, lipoprotein particles were isolated by column immunoaffinity techniques.[44] Subjects who showed progression of coronary artery lesions had significantly higher levels of LP-B_c (a summed measure of triglyceride-rich lipoproteins) and LP-A-II:B:C:D:E particles of small VLDL and/or IDL-like sizes.[44] In a prospective study (Etude Cas Temoins sur Infarctus du Myocarde [ECTIM]), Lp B:C-III was significantly increased in French and Irish MI survivors compared with controls.[45]

Atherogenic lipoprotein particles were evaluated in a prospective, nested case-control substudy[46] of the Cholesterol and Recurrent Events (CARE) trial, a randomized, placebo-controlled trial of pravastatin in patients with previous MI and average LDL concentrations at baseline (3 to 4.5 mmol/L, mean 3.6 mmol/L [115 to 174 mg/dL, mean 139 mg/dL]). Baseline concentrations of VLDL-apo B (the VLDL particle concentration), VLDL lipids, and apo C-III and apo E in VLDL+LDL and in HDL were compared in patients who experienced either a recurrent MI or coronary death with concentrations in patients who did not have a cardiovascular event during the 5 years of follow-up. Results showed that plasma concentrations of VLDL particles and apo C-III in VLDL and LDL were more specific measures of CHD risk than were plasma triglycerides.[46]

Lipoprotein(a)

Lp(a) particles contain apo(a) and apo B in a 1:1 molar ratio. Apo(a) contains a kringle domain and a carboxyl-terminal domain with 85% amino acid identity with the plasminogen

protease domain. The molecular mass of apo(a) protein varies from 187 kDa for an apo(a) that contains 12 kringle 4 domains, to 662 kDa for an apo(a) that contains 50 kringle 4 domains.[47] Among numerous retrospective case-control studies, virtually all have shown a strong association between elevated Lp(a) levels and CHD. On the other hand, the results of prospective studies have been somewhat discordant—a total of 9 prospective studies concluded that Lp(a) is an independent risk factor for MI or CHD in men, but 4 studies reached the opposite conclusion. These inconsistencies may relate to a lack of standardization and the failure of some immunoassays to measure all apo(a) isoforms equally.[48] Nevertheless, a meta-analysis of prospective studies indicated that plasma Lp(a) concentration is an independent risk factor for CHD in both men and women.[49] Recently, the Prospective Epidemiological Study of Myocardial Infarction (PRIME), a cohort study that included 9133 men from France and Northern Ireland with no history of CHD or use of hypolipidemic drugs, confirmed Lp(a) as a predictor of CHD risk.[50]

Conclusions

Elevated LDL-cholesterol levels are associated with a high risk of CHD, and LDL cholesterol continues to be the primary target of therapy for the prevention of CHD. However, the heterogeneity of LDL particles and the increasing recognition of the atherogenicity of other lipoproteins, remnant lipoprotein particles, and certain apolipoproteins, demands attention. Physicians need to be aware of these other atherogenic lipoproteins because they may become the targets of therapeutic intervention in the future. Small, dense LDL particles are highly atherogenic, and high levels of circulating oxidized LDL increase the risk of CHD. Lipoproteins that contain apo B are highly heterogeneous in terms of chemical composition and size. Further fundamental and clinical studies are needed to characterize these lipoproteins and their capacity to induce atherosclerosis. For the time being, apo B might be of greatest value in the diagnosis and treatment of men and women with some common lipid abnormalities but normal or low concentrations of LDL cholesterol. The apo B/apo A-I ratio should also be regarded as highly predictive in evaluating cardiac risk. Elevated plasma triglyceride levels increase the risk of acute coronary events and are an independent risk factor, but the concentrations of remnant particles associated with apo C-III are more related to the development of atherosclerosis than are triglycerides per se. Lipoprotein(a) is now considered an independent risk factor for CHD in both men and women. An important challenge is to develop standardized and simple analytical methods to generalize the measurement of different atherogenic lipoprotein particles in clinical biochemistry and in clinical practice.

References

1. Rainwater DL, Andres DW, Ford AL, et al. Production of polyacrylamide gradient gels for the electrophoretic resolution of lipoproteins. *J Lipid Res*. 1992;33:1876–1881.
2. Austin MA, King MC, Vranizan KM, et al. Atherogenic lipoprotein phenotype: a proposed genetic marker for coronary heart disease risk. *Circulation*. 1990;82:495–506.
3. Scheffer PG, Bakker SJ, Heine RJ, et al. Measurement of LDL particle size in whole plasma and serum by high performance gel-filtration chromatography using a fluorescent lipid probe. *Clin Chem*. 1998;44:2148–2151.
4. Kuller L, Arnold A, Tracy R, et al. Nuclear magnetic resonance spectroscopy of lipoproteins and risk of coronary heart disease in the Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol*. 2002;22:1175–1180.
5. Chung BH, Segrest JP, Cone JT, et al. High resolution plasma lipoprotein cholesterol profiles by a rapid, high volume semi-automated method. *J Lipid Res*. 1981;22:1003–1014.
6. Kulkarni KR, Garber DW, Marcovina SM, et al. Quantification of cholesterol in all lipoprotein classes by the VAP-II method. *J Lipid Res*. 1994;35:159–168.
7. Swinkels DW, Hak-Lemmers HL, Demacker PN. Single spin density gradient ultracentrifugation method for the detection and isolation of light and heavy low-density lipoprotein subfractions. *J Lipid Res*. 1987;28:1233–1239.
8. Skoglund-Andersson C, Tang R, Bond MG, et al. LDL particle size distribution is associated with carotid intima-media thickness in healthy 50-year-old men. *Arterioscler Thromb Vasc Biol*. 1999;19:2422–2430.
9. Lamarche B, St Pierre AC, Ruel IL, et al. A prospective, population-based study of low density lipoprotein particle size as a risk factor for ischemic heart disease in men. *Can J Cardiol*. 2001;17:859–865.
10. Stampfer MJ, Krauss RM, Ma J, et al. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *JAMA*. 1996;276:882–888.
11. Freedman DS, Otvos JD, Jeyarajah EJ, et al. Relation of lipoprotein subclasses as measured by proton nuclear magnetic resonance spectroscopy to coronary artery disease. *Arterioscler Thromb Vasc Biol*. 1998;18:1046–1053.
12. Blake GJ, Otvos JD, Rifai N, et al. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. *Circulation*. 2002;106:1930–1937.
13. Liu ML, Ylitalo K, Nuotio I, et al. 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;33:1255–1260.
14. Lamarche B, Lemieux I, Despres JP. The small, dense LDL phenotype and the risk of coronary heart disease: epidemiology, pathophysiology and therapeutic aspects. *Diabetes Metab*. 1999;25:199–211.
15. Garvey WT, Kwon S, Zheng D, et al. Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. *Diabetes*. 2003;52:453–462.
16. Soedamah-Muthu SS, Chang YF, Otvos J, et al. Lipoprotein subclass measurements by nuclear magnetic resonance spectroscopy improve the prediction of coronary artery disease in type 1 diabetes: a prospective report from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetologia*. 2003;46:674–682.
17. Vakkilainen J, Steiner G, Ansquer JC, et al. Relationships between low-density lipoprotein particle size, plasma lipoproteins, and progression of coronary artery disease: the Diabetes Atherosclerosis Intervention Study (DAIS). *Circulation*. 2003;107:1733–1737.
18. Steinbrecher UP, Parthasarathy S, Leake DS, et al. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc Natl Acad Sci U S A*. 1984;81:3883–3887.
19. Holvoet P, Mertens A, Verhamme P, et al. Circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2001;21:844–848.
20. Toshima S, Hasegawa A, Kurabayashi M, et al. Circulating oxidized low density lipoprotein levels: a biochemical risk marker for coronary heart disease. *Arterioscler Thromb Vasc Biol*. 2000;20:2243–2247.
21. Hulthe J, Fagerberg B. Circulating oxidized LDL is associated with subclinical atherosclerosis development and inflammatory cytokines (AIR Study). *Arterioscler Thromb Vasc Biol*. 2002;22:1162–1167.
22. Sherer Y, Tenenbaum A, Praprotnik S, et al. Coronary artery disease but not coronary calcification is associated with elevated levels of cardiolipin, β-2-glycoprotein-I, and oxidized LDL antibodies. *Cardiology*. 2001;95:20–24.
23. Erkkila AT, Narvanen O, Lehto S, et al. Autoantibodies against oxidized low-density lipoprotein and cardiolipin in patients with coronary heart disease. *Arterioscler Thromb Vasc Biol*. 2000;20:204–209.
24. Leinonen JS, Rantalaiho V, Laippala P, et al. The level of autoantibodies against oxidized LDL is not associated with the presence of coronary

heart disease or diabetic kidney disease in patients with non-insulin-dependent diabetes mellitus. *Free Radic Res*. 1998;29:137–141.
25. Yusuf S, Dagenais G, Pogue J, et al, for the Heart Outcomes Prevention Evaluation Study Investigators. Vitamin E supplementation and cardiovascular events in high-risk patients. *N Engl J Med*. 2000;342:154–160.
26. Yla-Herttuala S, Palinski W, Rosenfeld ME, et al. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J Clin Invest*. 1989;84:1086–1095.
27. Steinberg D, Witztum JL. Is the oxidative modification hypothesis relevant to human atherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis? *Circulation*. 2002;105:2107–2111.
28. Lee DM, Alaupovic P. Composition and concentration of apolipoproteins in very-low- and low-density lipoproteins of normal human plasma. *Atherosclerosis*. 1974;19:501–520.
29. Kwiterovich PO Jr, Coresh J, Smith HH, et al. Comparison of the plasma levels of apolipoproteins B and A-1, and other risk factors in men and women with premature coronary artery disease. *Am J Cardiol*. 1992;69:1015–1021.
30. Sigurdsson G, Baldursdottir A, Sigvaldason H, et al. Predictive value of apolipoproteins in a prospective survey of coronary artery disease in men. *Am J Cardiol*. 1992;69:1251–1254.
31. Marcovina SM, Albers JJ, Kennedy H, et al. International Federation of Clinical Chemistry standardization project for measurements of apolipoproteins A-I and B, IV: comparability of apolipoprotein B values by use of International Reference Material. *Clin Chem*. 1994;40:586–592.
32. Jungner I, Marcovina SM, Walldius G, et al. Apolipoprotein B and A-I values in 147576 Swedish males and females, standardized according to the World Health Organization-International Federation of Clinical Chemistry First International Reference Materials. *Clin Chem*. 1998;44(8, pt 1):1641–1649.
33. Lamarche B, Moorjani S, Lupien PJ, et al. Apolipoprotein A-I and B levels and the risk of ischemic heart disease during a five-year follow-up of men in the Quebec Cardiovascular Study. *Circulation*. 1996;94:273–278.
34. Walldius G, Jungner I, Holme I, et al. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. *Lancet*. 2001;358:2026–2033.
35. Sniderman AD, Scantlebury T, Cianflone K. Hypertriglyceridemic hyperapoB: the unappreciated atherogenic dyslipoproteinemia in type 2 diabetes mellitus. *Ann Intern Med*. 2001;135:447–459.
36. Cullen P. Evidence that triglycerides are an independent coronary heart disease risk factor. *Am J Cardiol*. 2000;86:943–949.
37. Havel RJ. Role of triglyceride-rich lipoproteins in progression of atherosclerosis. *Circulation*. 1990;81:694–696.
38. Krauss RM. Atherogenicity of triglyceride-rich lipoproteins. *Am J Cardiol*. 1998;81:13B–17B.
39. Tkac I, Kimball BP, Lewis G, et al. The severity of coronary atherosclerosis in type 2 diabetes mellitus is related to the number of circulating triglyceride-rich lipoprotein particles. *Arterioscler Thromb Vasc Biol*. 1997;17:3633–3638.
40. Schaefer EJ, McNamara JR, Shah PK, et al. Elevated remnant-like particle cholesterol and triglyceride levels in diabetic men and women in the Framingham Offspring Study. *Diabetes Care*. 2002;25:989–994.
41. Kugiyama K, Doi H, Takazoe K, et al. Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. *Circulation*. 1999;99:2858–2860.
42. Phillips NR, Waters D, Havel RJ. Plasma lipoproteins and progression of coronary artery disease evaluated by angiography and clinical events. *Circulation*. 1993;88:2762–2770.
43. Hodis HN, Mack WJ, Azen SP, et al. Triglyceride- and cholesterol-rich lipoproteins have a differential effect on mild/moderate and severe lesion progression as assessed by quantitative coronary angiography in a controlled trial of lovastatin. *Circulation*. 1994;90:42–49.
44. Alaupovic P, Mack WJ, Knight-Gibson C, et al. The role of triglyceride-rich lipoprotein families in the progression of atherosclerotic lesions as determined by sequential coronary angiography from a controlled clinical trial. *Arterioscler Thromb Vasc Biol*. 1997;17:715–722.
45. Luc G, Fievet C, Arveiler D, et al. Apolipoproteins C-III and E in apoB- and non-apoB-containing lipoproteins in two populations at contrasting risk for myocardial infarction: the ECTIM study. Etude Cas Temoins sur Infarctus du Myocarde. *J Lipid Res*. 1996;37:508–517.
46. Sacks FM, Alaupovic P, Moye LA, et al. VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. *Circulation*. 2000;102:1886–1892.
47. Durrington PN. Lipoprotein (a). *Baillieres Clin Endocrinol Metab*. 1995;9:773–795.
48. Seman LJ, DeLuca C, Jenner JL, et al. Lipoprotein(a)-cholesterol and coronary heart disease in the Framingham Heart Study. *Clin Chem*. 1999;45:1039–1046.
49. Craig WY, Neveux LM, Palomaki GE, et al. Lipoprotein(a) as a risk factor for ischemic heart disease: metaanalysis of prospective studies. *Clin Chem*. 1998;44:2301–2306.
50. Luc G, Bard JM, Arveiler D, et al. Lipoprotein (a) as a predictor of coronary heart disease: the PRIME Study. *Atherosclerosis*. 2002;163:377–384.