In pioneering studies more than 60 years ago, using the new technique of analytical ultracentrifugation, Gofman and colleagues identified and characterized the major classes of plasma lipoproteins as a function of their flotation rate in a salt solution, a property that is a function of both size and hydrated density of lipoprotein particles. They, and subsequently others, showed that low-density lipoproteins (LDLs), like the other lipoprotein fractions, comprise a spectrum of particles with differing flotation characteristics. However, despite compelling evidence presented by Gofman and colleagues for the relationship of plasma concentrations of LDLs, as well as lipoproteins of lower density, to coronary heart disease (CHD), analytical ultracentrifugation was not amenable to widespread implementation and replication in other laboratories. Interest in physicochemical heterogeneity within the LDL particle spectrum resurfaced when it was shown using density gradient ultracentrifugation that multiple discrete subclasses of LDLs could be identified in the plasma of healthy individuals, and that they could also be discriminated on the basis of particle diameter using nondenaturing gradient gel electrophoresis. Moreover, using gradient gel electrophoresis, it was found that a distinct phenotype characterized by smaller LDL peak particle diameter (designated LDL subclass pattern B) was associated with increased CHD risk. At the same time it was shown that smaller sized LDLs are significantly positively correlated with plasma triglyceride and inversely with high-density lipoprotein cholesterol, which together constitute a cluster originally designated atherogenic lipoprotein phenotype, and now referred to as atherogenic dyslipidemia, a key feature of metabolic syndrome.

More recently, other procedures have been developed to analyze the spectrum of LDLs and other lipoprotein particles, including nuclear magnetic resonance spectroscopy, vertical rotor ultracentrifugation, and ion mobility. Several classification schemes for LDL subclasses have been proposed based on their size and density as assessed by the various analytic procedures used. As summarized previously, these may be grouped into 4 main categories: LDL-I (large and buoyant), LDL-II (medium size and density), LDL-III (small and dense), and LDL-IV (very small and dense). Because LDL-IV is often present in relatively low concentrations and may not be well resolved from LDL-III, these fractions are often combined and designated small dense LDL or sdLDL. Despite several properties of sdLDL that might be predicted to confer heightened CVD risk compared with other LDL fractions, including reduced LDL receptor affinity, greater binding to arterial wall proteoglycans, and increased oxidative susceptibility, the clustering of sdLDL with other CHD risk factors, as well as their inverse association with large buoyant LDLs, have created challenges in assessing the specific contribution of sdLDL concentrations to CHD risk, as reviewed elsewhere.

As suggested by Hoogeveen et al in this issue, analyses based on sdLDL may refine the interpretation of genotype associations with the inter-related variables, high-density lipoprotein cholesterol and triglycerides, that have been linked to CHD risk. However, measurement of LDL subspecies may also provide greater specificity to genotype associations that have been reported exclusively for LDL cholesterol. A notable example is a common genetic variant at a locus on chromosome 1p13 that regulates hepatic sortilin and is strongly associated with both LDL cholesterol and risk of myocardial infarction. The major allele at this locus is preferentially associated with increased levels of very small LDL-IV. Sortilin has been shown to bind apolipoprotein (apoB) with high affinity and to affect its intracellular processing and secretion, as well as its internalization at the cell surface, thus providing functional evidence for a novel pathway that preferentially influences plasma levels of very small LDL particles and CHD risk. These observations highlight the need to consider the pathophysiological and clinical significance of heterogeneity within the category of sdLDL.

Another consideration with regard to heterogeneity of LDL particles is their content of specific proteins and perhaps lipids that could refine assessment of pathophysiologic properties of lipoproteins, as proposed originally by Alaupovic. Of particular interest is apoCIII, a small molecular weight protein that has been found consistently to be strongly associated with risk of CVD when present on apoB-containing lipoproteins, likely because of the combined effects of increased very low density lipoprotein (VLDL) secretion, impaired VLDL remnant catabolism, and proinflammatory activity. Recently, it has been reported that whereas LDL particles containing apoCIII are significantly associated with CHD risk, this relationship is substantially weaker for the majority of LDLs lacking apoCIII. Notably, apoCIII is particularly abundant in very small LDL particles. Other subspecies of sdLDL that may have particularly high atherogenic potential are those with increased content of glycated apoB and increased electronegative charge. Recently, based on nuclear magnetic resonance spectroscopy analyses in >20,000 participants in the Heart Protection
Study with 5.3 years of follow-up on simvastatin 40 mg/d, levels of small LDLs as defined by that method were significantly associated with both major occlusive coronary events (hazard ratio, 1.20; \( P < 10^{-5} \)) and revascularization procedures (hazard ratio, 1.14; \( P = 0.0003 \)), whereas larger LDL particles were not (1.08 and 1.02, respectively).\(^{29}\) Total LDL particle concentrations were strongly correlated with levels of small LDLs (\( r = 0.76 \)) but not large LDLs (\( r = 0.16 \)), and not unexpectedly, the relationships of small LDLs with major occlusive coronary events and revascularization procedures were no longer significant when adjusted for total LDL particle number. Thus, although measurements of small LDLs did not statistically improve risk assessment beyond conventional lipid measures, their preferential association with disease outcomes, as well as their pathological properties, suggests their potential use in monitoring efficacy of lipid-altering therapy for reducing CHD risk in individual patients.

The armamentarium of tools for assessing the clinical significance of LDL heterogeneity has been expanded recently by the availability of an automated homogeneous assay for measuring blood levels of cholesterol in sdLDL (sdLDL-C).\(^{30}\) The nomenclature used for this measurement, however, can lead to confusion in comparison with other methods, because it is designed to measure LDLs of density 1.044 to 1.063 g/mL, whereas a significant proportion of LDL-III particles have density <1.044 g/mL.\(^{5,12}\) Hence, the sdLDL-C measurement may represent relative enrichment of very small and dense LDL-IV. Very recently there have been 2 reports in this journal of the application of this method in large population cohort studies of CHD risk. Hoogeveen et al found that among 11,419 men and women in the Atherosclerosis Risk in Communities (ARIC) study followed up for \( \approx 11 \) years, sdLDL-C was significantly associated with incident CHD in models after adjusting for standard nonlipid CHD risk factors, even in individuals with LDL cholesterol levels <100 mg/dL.\(^{16}\) Tsai et al reported that in 4387 participants in the Multi-Ethnic Study of Atherosclerosis (MESA), elevated sdLDL-C was a risk factor for developing CHD during 8.5 years of follow-up after adjusting for standard CHD risk factors, as well as triglyceride and high-density lipoprotein cholesterol, in normoglycemic individuals.\(^{31}\) Notably, as in the ARIC study, the association of sdLDL-C with CHD risk in this subgroup remained significant.

![Figure. Correlations of small dense low-density lipoprotein cholesterol (sdLDL-C; top) and large buoyant low-density lipoprotein cholesterol (lbLDL-C; bottom) as determined by nondenaturing gradient gel electrophoresis (GGE) with measurements of corresponding particles by analytical ultracentrifugation (left) and ion mobility (right). Data were derived from measurements in 178 overweight and obese men in samples obtained after a standardized baseline diet as described in Ref. 32. For all methods, sdLDL is the sum of values for LDL-III and LDL-IV (cholesterol in mg/dL for GGE, total mass in mg/dL for analytical ultracentrifugation, and particle concentration in nmol/L for ion mobility). For GGE, lbLDL-C is the cholesterol concentration in LDL-I plus LDL-II. The cholesterol concentrations in the LDL subfractions measured by GGE were determined by multiplying the respective percent lipid-stained areas by plasma LDL cholesterol. GGE and analytical ultracentrifugation were performed as described in Ref. 32, and ion mobility was performed by the method of Caulfield et al.\(^{11}\) ](http://atvb.ahajournals.org/content/960-2995.long)
when LDL cholesterol of <100 mg/dL was included in a multivariable model. Although individuals in MESA with fasting blood glucose levels >125 mg/dL had higher sdLDL-C than those with lower glucose, there was not a significant association with CHD, perhaps reflecting limited statistical power.

In the MESA study population, measurement of small LDL by nuclear magnetic resonance spectroscopy showed only a modest correlation with sdLDL-C ($r=0.59$) and no association with CHD risk. This discrepancy was interpreted by both Tsai et al and Hoogeveen et al to suggest that nuclear magnetic resonance spectroscopy may be measuring physical and pathophysiological property(ies) of small LDLs that differ from those represented by sdLDL-C. In this regard, it is notable that sdLDL-C as determined by gradient gel electrophoresis is highly significantly correlated ($P<0.0001$; $n=178$) with measurements of corresponding particles by analytical ultracentrifugation as a function of total mass in mg/dL ($r=0.88$), and by ion mobility as a function of particle concentration in nmol/L ($r=0.86$; Figure). Thus, it seems unlikely that the cholesterol content of sdLDL is a measure of these particles that is more informative than their overall plasma concentrations. Nevertheless, the results of the studies reviewed here lend support to efforts aimed at determining whether there are specific pathophysiological properties of particles within the spectrum of sdLDL that merit the use of standardized assays for their measurement in the assessment and management of CHD risk.

Disclosures

Dr. Krauss has consulted for Merck, Janssen Pharmaceuticals, Madrigal Pharmaceuticals, Amarin, and Quest Diagnostics, has received grants from Sanofi-Regeneron and Quest Diagnostics, and is coinventor on several licensed patents for lipoprotein particle analyses.

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