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Comparison of four methods of analysis of lipoprotein particle subfractions for their association with angiographic progression of coronary artery disease

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Abstract

Background—Compare gradient gel electrophoresis (GGE), vertical auto profile (VAP-II), nuclear magnetic resonance spectroscopy (NMR), and ion mobility for their ability to relate low- (LDL), intermediate- (IDL), very-low-density (VLDL) and high-density lipoprotein (HDL) subfraction concentrations to atherosclerotic progression.

Methods and Results—Regression analyses of 136 patients who received baseline and follow-up coronary angiographies and subfraction measurements by all four methods in the HDL Atherosclerosis Treatment Study. Prior analyses have shown that the intervention primarily affected disease progression in proximal arteries with <30% stenoses at baseline.

Three-year increases in percent stenoses were consistently associated with higher on-study plasma concentrations of small, dense LDL as measured by GGE (LDLIIIb, $P=10^{-6}$; LDLIVa, P=0.006; LDLIVb, P=0.02), VAP-II (LDL4, P=0.002), NMR (small LDL, P=0.001), and ion mobility (LDL

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IIb, P=0.04; LDLIIIa, P=0.002; LDLIIIb, P=0.0007; LDLIVa, P=0.05). Adjustment for triglycerides, HDL-cholesterol, and LDL-cholesterol failed to eliminate the statistical significance for on-study GGE estimated LDLIIIb ($P=10^{-5}$) and LDLIVa (P=0.04); NMR-estimated small LDL (P=0.03); or ion mobility estimated large LDLIIIa (P=0.04) or LDLIIIb (P=0.02). All methods show that the effects were significantly greater for the on-study than the baseline small, dense LDL concentrations, thus establishing that the values concurrent to the progression of disease were responsible. The rate of disease progression was also related to individual VLDL, IDL, and HDL subclasses to differing extents among the various analytic methods.

Conclusion—Four methodologies confirm the association of small, dense LDL with greater coronary atherosclerosis progression, and GGE, NMR, and ion mobility confirm that the associations were independent of standard lipid measurements.

Clinical Trial Registration—clinicaltrials.gov/ct2/show/NCT00000553

Keywords

Lipoprotein subfractions; low-density lipoproteins; very low density lipoproteins; high density lipoproteins; angiography; coronary artery disease; prevention

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Plasma low density lipoprotein (LDL) cholesterol, along with high density lipoprotein (HDL) cholesterol and triglyceride concentrations, are recommended by European and North American guidelines for assessing and managing risk of cardiovascular disease (CVD) [1–3]. However, lipoproteins are comprised of multiple subclasses that can be distinguished by their physicochemical properties. For example, LDL-cholesterol is the sum of the cholesterol levels in at least seven particle subclasses that differ in size and density [4,5]. Although there is substantial evidence that increased levels of smaller, denser LDL particles are associated with greater risk of coronary heart disease [6], an independent effect of small dense LDL has not been established, in part due to concomitant elevations in triglycerides and total LDL particle numbers and reductions in HDL-cholesterol [6–9].

Recognition that lipoprotein heterogeneity could potentially improve risk prediction spurred technological development for its analysis. In addition to GGE, the methods include nuclear magnetic resonance spectroscopy (NMR), vertical auto profile ultracentrifugation (VAP-II), and ion mobility. The latter three methods use different principles to provide quantitative estimates of subfraction concentrations across the full spectrum of lipoprotein particles. NMR estimates are obtained from the mathematical deconvolution of spectroscopically distinct lipid methyl group NMR signals whose amplitudes are directly proportional to the numbers of subclass particles emitting the signal, irrespective of variation in particle lipid composition [10,11]. VAP-II is based on the deconvolution into subfractions of the direct cholesterol quantitation of lipoproteins separated by flotation rate (a function of size and hydrated density) [12]. Ion mobility uses an electrospray procedure to obtain direct lipoprotein particle counts as a function of particle size [13]. It is based on the principle that particles of a given size and uniform charge behave in a predictable manner when carried in a laminar airflow subjected to an electric field. GGE, VAP-II, NMR, and ion mobility may also be used to characterize relative distribution of LDL particles as a function of their particle diameter by the peak (mode) or mean of the LDL size distribution.

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GGE, VAP-II, NMR, and ion mobility have been used for relating lipoprotein subfractions to various measures of CVD. However, a direct comparison of all four methods for their associations with angiographically measured coronary disease progression within the same group of patients has not been previously reported. To this end, angiographically measured coronary disease progression may be more closely related to the atherogenic properties of lipoproteins than cardiovascular events because the latter represent the consequences of both atherogenesis and factors that promote plaque rupture and thrombosis [14]. We therefore assessed whether GGE, VAP-II, NMR, and ion mobility measurements are consistent in identifying associations of specific lipoprotein subfractions with angiographically measured changes in coronary artery stenosis in the HDL-Atherosclerosis Treatment Study (HATS), a randomized placebo controlled clinical trial of simvastatin plus niacin and/or antioxidant supplements in patients with reduced HDL cholesterol [15,16]. We have reported previously that, within the LDL particle spectrum, higher on-study concentrations of LDLIIIb and LDLIVa as measured by GGE were significantly associated with greater rates of progression of coronary stenosis in HATS [16]. The current analyses test whether measurements of LDL subfractions by VAP-II, NMR, and ion mobility provide results comparable to GGE, and for the three latter methods, whether levels of subfractions within very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), or HDL also show significant associations with changes in coronary artery disease progression. For this purpose, analyses were restricted to coronary artery regions with <30% stenosis since these showed the greatest benefit from intervention with drug treatment in the clinical trial [15], and also showed the strongest association with on-study LDL subfraction concentrations in subsequent analyses [16].

Methods

Study

The design and results of the original clinic trial have been described in detail elsewhere [15]; their summary to follow includes only those details relevant to the current analyses. The study included 160 men and women under the ages of 63 and 70 years old, respectively, who were recruited between 1995 and 1997, and who had clinical coronary disease (previous myocardial infarction, coronary interventions, or confirmed angina) and at least three stenoses ≥ 30 percent of the luminal diameter or one stenosis ≥ 50 percent. The participants were recruited for low HDL cholesterol (≤0.91 mmol/L if male and ≤ 1.03 mmol/L if female), LDL-cholesterol ≤3.75 mmol/L and triglycerides ≤ 4.52 mmol/L [15]. The current analyses are restricted to the associations of lipoprotein concentrations vs. coronary disease progression, and therefore the random assignment of the patients to four different treatment arms (simvastatin-niacin with antioxidants or antioxidant placebo, antioxidants vs. simvastatin-niacin placebo, and placebos alone) is only relevant in producing variation in on-study lipoprotein subfraction concentrations. The protocol was approved by the human-subjects committee and the patients provided signed consent.

Arteriography

Eight views of the left and right coronary arteries at baseline and follow-up were compared side-by-side to measure the minimal luminal diameter ($Diameter_{minimum}$) and nearby normal

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diameters ($Diameter_{normal}$) in millimeters using the catheter for the calibration. Stenosis was expressed as a percentage (i.e., $100*(Diameter_{normal} - Diameter_{minimum})/(Diameter_{normal})$. The prespecified primary end point was the mean change per patient from the initial arteriogram to the final arteriogram in the percent stenosis caused by the most severe lesion in each of the nine proximal coronary segments. Those arteries exhibiting <30% stenosis accounted for most of intervention-related and LDL-related disease progression during the trial [15,16]

Laboratory measurements

Fasting plasma concentrations of triglycerides, total, HDL, and LDL cholesterol, and apolipoprotein B were determined by Northwest Lipid Research Laboratories [17].

Gradient gel estimates of LDL peak diameter and LDL subclass cholesterol concentrations were determined from fresh whole plasma using 2%–14% non-denaturing polyacrylamide gradient gel electrophoresis [4], The cholesterol concentrations of the subclasses were estimated by multiplying percent of the total stained areas for each subclass by the cholesterol measured in ultracentrifugally isolated LDL fractions [18].

Vertical Auto Profile (VAP-II) measurements of lipoprotein subclass cholesterol concentrations were performed at Atherotech Diagnostics Lab (Birmingham, AL) [12,20,21] on −80° C frozen samples taken 3 to 6 years earlier. Plasma lipoproteins were separated by single vertical spin density-gradient ultracentrifugation of diluted plasma samples adjusted to a density of 1.21 kg/L. A density gradient was prepared by first pipetting 1.4 ml of density-adjusted diluted plasma in each tube which were then overlayed with 3.9 ml of saline/EDTA and centrifuged in tandem with a tube containing calibration plasma with a known total cholesterol concentration. A VAP-II analyzer used the direct cholesterol measurements from eluted fractions to obtain profiles of digitized absorbance units (Y-coordinate) vs. relative gradient position from the sample drain time (X-coordinate) which were decomposed into component curves corresponding to Lp(a), five HDL, four LDL, two IDL, and 3 VLDL subclasses. The analyses presented herein corresponds to HDL2, HDL3, four LDL subclasses, IDL, and buoyant (VLDL1 and VLDL2) and dense VLDL (VLDL3) concentrations. Peak LDL flotation rate was measured in time (seconds) to the mode of the LDL cholesterol concentration distribution.

NMR spectra of frozen plasma specimens were acquired at LipoScience (Raleigh, NC) in 2001 and the digitized data subsequently analyzed for lipoprotein particle concentrations and sizes using the current LipoProfile-3 spectral deconvolution algorithm. VLDL, LDL, and HDL subclass particle concentrations were directly obtained from the derived amplitudes of their spectroscopically distinct lipid methyl group NMR signals, and mean LDL particle diameter was calculated from a weighted average of each subclass diameter multiplied by its relative mass percentage based on the amplitude of its methyl NMR signal [10]. The following subclass catgegories were investigated: large VLDL including remnants (>60 nm), intermediate VLDL (42–60 nm), small VLDL (29–42 nm), IDL (23–29 nm), large LDL (20.5–23 nm), small LDL (18–20.5 nm), large HDL (9.4–14 nm), intermediate HDL (8.2–9.4 nm), and small HDL (7.3–8.2 nm). Total LDL particle concentration was calculated as the sum of small LDL, large LDL, and IDL concentrations.

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Ion mobility estimated of lipoprotein subfractions were made on frozen samples in 2012. The method uses gas-phase differential electrophoretic macromolecular mobility to directly measure of lipoprotein particle concentrations [13]. The electrospray chamber was set to a 1.6 L/min airflow containing approximately 5% CO_2, and lipoprotein particles ranging from 1.7 to 54.2 nm were counted in 0.1-s bins that were grouped to subclasses, and the predominant LDL particle size (modal diameter) was determined. The values used in this report include refinements made subsequent to the methods original description [19].

For NMR it has been reported that measurements of LDL and HDL subfractions are not affected by frozen storage or by multiple freeze-thaw cycles, although variation in VLDL subclasses has been observed [11]. Similarly, IM measurements have been shown to be unaffected by frozen storage (Krauss, R.M., unpublished data) or multiple freeze-thaw cycles [13].

Statistics

Baseline and on-study lipoprotein subfraction measurements were included simultaneously as independent variables in multiple regression analyses to assess the significance of their associations with three-year change in percent stenoses of the proximal arteries when adjusted for baseline age, sex, BMI, and smoking status. The baseline and on-study coefficients were compared directly to assess whether evidence of causality could be strengthened by showing that coronary disease progression was more closely related to the concurrent (i.e., on-study) lipoprotein levels than to baseline levels. Multiple regression analyses were also used to compare methodologies directly for their abilities to identify relationships between lipoprotein concentrations and disease progression. Additional adjustment for treatment group assignment (a 2×2 design of simvastatin-niacin treatment and antioxidants) was also included to ensure that the observed association were not secondary to effects of simvastatin-niacin, or antioxidants on both disease progression and lipoproteins, however, this may represent over-adjustment given that much of the lipoprotein change is in response to the interventions. Partial correlations were adjusted for baseline age, sex, BMI and smoking status.

Results

Of the 160 patients included in the original HATS trial, we excluded 14 for missing angiographic data, eight for missing ion mobility measurements, and two for missing gradient gel subfractions, leaving 136 (119 men, 17 women) for analyses, whose characteristics and lipoprotein concentrations are presented in Table 1. Five patients had no proximal arterial segments with <30% stenosis at baseline and were therefore excluded from the analyses of lipoproteins vs. disease progression. The annual changes in percent stenosis in these segments in the remaining patients increased by 3.18±6.11% (mean ± SE) per year.

Correlation of subfractions with traditional lipoprotein measurements and within methods

All methodologies show that baseline small LDL is concordantly related to plasma triglyceride concentrations and inversely correlated with plasma HDLcholesterol, whereas large LDL shows the opposite relationships. On-study measurements show somewhat

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weaker associations (Supplementary Table 1). Supplementary tables S2–S5 show that: 1) LDLI correlates strongly with LDLIIa and more weakly with LDLIIb; 2) there is concordance for LDLIIIb, LDLIVa and LDLIVb that is greater for ion mobility than GGE; and 3) there is little concordance between LDLIIIa and the larger subfractions.

Correlations of subfraction measurements between methods

Table 2 shows that the correlations between GGE and ion mobility estimates of LDL subfractions were strongest for LDLI and somewhat diminished for subfractions of decreasing particle size, especially for the baseline measurements. The NMR measurement of large LDL showed the strongest correlation with both GGE and ion mobility measurements of LDLI and LDLIIa. With respect to peak or mean LDL diameter (not displayed in the tables), the ion mobility measurement correlated significantly with those obtained by GGE (baseline/onstudy: r=0.85/r=0.88) and NMR (r=0.74/r=0.63), as did the results for GGE vs. NMR (r=0.74/r=0.63). VAP-II LDL peak flotation rate also correlated significantly with peak diameter measured by ion mobility (r=0.64/r=0.76), and GGE (r=0.62/r=0.75), and mean LDL diameter determined by NMR (r=0.71/r=0.45).

Percent stenosis vs. traditional lipoprotein measurements

The change in percent stenosis was significantly related to on-study LDL cholesterol (2.11±0.79% per mmol/L, P=0.008), HDL cholesterol (−8.87±3.93% per mmol/L, P=0.03) and apoB concentrations (0.06±0.02% per mg/dL, P=0.01) and the total/HDLcholesterol ratio (0.99±0.38% per unit increment, P=0.01), but not to on-study triglyceride levels (0.35±0.46% per mmol/L, P=0.46). The on-study regression slope was significantly greater than the baseline slope for LDL cholesterol (difference in slope±SE: 2.64±1.30% per mmol/L, P=0.04), and HDL cholesterol (−19.97±8.81% per mmol/L, P=0.03), but not triglyceride (P=0.75) or apoB concentrations (P=0.07), or the total/HDL cholesterol ratio (P=0.06).

Percent stenosis vs. plasma concentrations of specific subclasses

Table 3 shows that increased stenosis was consistently associated with higher on-study plasma concentrations of smaller LDL. This included LDLIIIb ($P=10^{-6}$), LDLIVa (P=0.006), and LDLIVb for GGE (P=0.02); LDL4 (P=0.002) for VAP-II; small LDL for NMR (P=0.001; and LDLIIb (P=0.04), LDLIIIa (P=0.002), LDLIIIb (P=0.0007), and LDLIVa (P=0.05) for ion mobility,. Moreover, the effects were significantly greater for the on-study than the baseline concentration for the GGE estimate of LDLIIIb and LDLIVb, the VAP-II estimate of LDL4, the NMR estimate of small LDL, and the ion mobility estimates of LDLIIIa and LDLIIIb, thus establishing that the values concurrent to the progression of disease, rather than at baseline, were responsible.

Greater disease progression was also significantly associated with higher plasma concentrations of VAP-II estimated dense VLDL (P=0.02), and IDL (P=0.003), and lower plasma concentrations of VAP-II estimated HDL2 (P=0.02); and for ion mobility estimated VLDL subfractions (large: P=0.001; medium: P=0.003; small: P=0.01), Moreover, the effects were significantly greater for the on-study than the baseline concentration for the

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VAP-II estimates of dense VLDL, IDL, and HDL2, and the ion mobility estimates of small VLDL.

Adjustment for traditional lipoprotein risk factors

Adjustment for the on-study standard lipoprotein risk factors (triglycerides, HDL cholesterol, and LDL cholesterol) failed to eliminate the statistical significance for on-study: 1) GGE estimated LDLIIIb ($P=10^{-5}$) and LDLIVa (P=0.04); 2) NMR-estimated small LDL (P=0.03); or 3) ion mobility estimated large VLDL (P=0.02), LDLIIIa (P=0.04), or LDLIIIb (P=0.02); Neither did adjustment for on-study total/HDL cholesterol or apoB (an estimate of low-to-very low density particle number) eliminate the statistical significance for: 1) GGE-estimated LDLIIIb ($P<10^{-5}$) and LDLIVa ($P\leq0.04$); 2) NMR-estimated small LDL ($P\leq0.03$); or 3) ion mobility measurements of large VLDL (P=0.02), medium VLDL (P=0.05), LDLIIIa (P=0.04), or LDLIIIb (P=0.02). VAP-II LDL4 concentrations significantly predicted the rate of stenosis when adjusted for total/HDL cholesterol (P=0.04), but not plasma apoB (P=0.09) or triglyceride, HDL cholesterol and LDL cholesterol concentrations (P=0.13). The VAP-II LDL1 concentrations no longer predicted the rate of stenosis when adjusted for total/HDL cholesterol (P=0.35), plasma apoB (P=0.76) or triglyceride, HDL cholesterol and LDL cholesterol concentrations (P=0.90).

Adjustment for treatment assignment and other subfractions

Table S6 shows that with the exception of small LDL, the other associations between lipoprotein subfraction concentrations and disease progression became non-significant when adjusted for treatment assignment. Disease progression remained significantly associated with LDLIIb and LDLIVa from GGE, small LDL from NMR and LDLIIIb from ion mobility. Table S7 shows that NMR-estimated small LDL, VAPII-estimated LDL4, and GGE-estimated LDLIIIb remained predictive of significantly greater increases in percent stenoses when adjusted for the other subfractions. The regression identified no significant independent associations of other lipoprotein fractions with disease progression by any of the methods.

Discussion

These analyses demonstrate the positive association between plasma concentrations of small, dense LDL particles and increase of coronary artery stenosis using four independent laboratory methodologies. For GGE, NMR, and ion mobility the associations were statistically independent of traditional lipoprotein measurements, apoB concentrations, and total/HDL cholesterol. Comparable results for GGE, VAP-II, NMR, and ion mobility measures of small LDL suggest flexibility in the choice of methodologies for research and clinical assessment, and indicate the desirability of standardization of the results among these methods. The analyses also confirmed the relationship of coronary disease progression and GGE estimated LDLIVb (concordant) [23] and VAP-estimated HDL2 (inverse) [1]. Ion mobility appears to provide greater resolution of the pro-atherogenic effects of VLDL than other methodologies, whereas the VAP-II was best at identifying the inverse relationship between HDL and disease progression.

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Our analyses showed that the three-year increase in percent stenosis of the proximal arteries were significantly associated with NMR estimates of small LDL (concordant relationship) and that this relationship was significant when adjusted for traditional lipid and lipoprotein risk factors. In contrast, the published summary by Ip et al. [7] reported that none of the four lipid-adjusted associations between NMR estimated LDL particle size and incident cardiovascular disease achieved statistical significance and among the three unadjusted studies only one showed that smaller particle size was significantly related to greater CHD risk using NMR. Of the seven studies that compared NMR estimates of small LDL concentrations to incident CVD, none of the studies that adjusted for lipids showed significant associations with either large or small LDL concentrations, four reported greater risk for small LDL unadjusted for other lipids or adjustment unspecified, and two reported greater risk for large LDL unadjusted for other lipids. One study showed that both concentrations of smaller LDL and smaller LDL size were significantly associated with CVD severity when adjusted for other lipids.

More recently, NMR estimates of LDL subclasses were not found to significantly improve the prediction of incident cardiovascular disease during 11-year follow-up of the Women's Health Study when adjusted for traditional lipoprotein risk factors [24]. In the Heart Protection Study of 20,000 participants followed for 5.3 years for incident vascular events, examination of the LDL subclasses in separate regression models showed that concentrations of small LDL particles were significantly associated with major occlusive coronary events whereas large LDL particles were not [8]. However, when examined in the same regression model to eliminate any confounding resulting from their inverse correlation, both large and small LDL concentrations were comparably associated with clinical events. Findings were similar for associations of NMR-measured large and small LDL with incident CHD events in the Veterans Affairs HDL Intervention trial (VA-HIT) [11] and with carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA) [30].

Ion mobility has only been recently introduced for the study of lipoprotein subfractions and disease. In a prospective follow-up of 4368 men and women from the Malmö Diet and Cancer Study Cardiovascular Cohort, small LDL (20.8–21.4 nm) and medium-sized LDL (21.4–22.0 nm) were predictive of cardiovascular events (myocardial infarction, stroke, and death from coronary heart disease) [25]. Neither very small LDL (18.0–20.8 nm) or large LDL (22.0–23.3 nm) were significantly related to cardiovascular events, nor were any of the IDL or VLDL subfractions, whereas large HDL predicted substantially lower risk. Unlike the current results or previous studies, adjustment for HDL cholesterol in the Malmö Diet and Cancer Study Cardiovascular Cohort eliminated the statistical significance of the measures of LDL subclass concentrations.

Higher on-study large, less-dense LDL concentrations were significantly associated with greater disease progression when estimated by VAP-II but not by the other methodologies (Table 3). The observation is important given that several studies have associated large LDL with increased CHD risk [26–29] in contrast to the majority of studies assigning increased CHD risk to small LDL or LDL pattern B [6]. With respect to the current study, on-study VAP-II LDL1 concentration showed no association with plasma triglyceride ($r=-0.04$) or HDL cholesterol measurements ($r=-0.03$), in contrast to its expected concordant relationship

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to HDL cholesterol and inverse relationship to plasma triglycerides. In fact, large LDL was concordantly related to HDLcholesterol and inversely correlated with triglycerides for VAP-II LDL1 at baseline (r=0.48 and r=−0.39, respectively), and for both on-study and baseline large LDL as measured by GGE, ion mobility, and NMR (Table S1). Thus the LDL1-disease relationship could be due to density or compositional characteristics of large LDL during the trial that are not reflected by the other measurements. For example, shifts in the ratio of 1ipids to proteins could result in lipoprotein flotation changes in the absence of any detectable alteration in particle size [4]. In the current study large LDL was not an independent risk factor for disease progression by any of the methods. In this regard, in contrast to previous reports [24,30], multivariable adjustments accounting for inverse relationships between large and small LDL subclasses did not yield significant positive associations of large LDL with coronary disease by any of the methods (Table S7)

A major consideration in the current analysis is that lipoprotein particle associations with angiographic progression of coronary artery disease may represent effects on atherogenesis that are clinically relevant but less evident in studies in which the incidence of major cardiovascular events is strongly influenced by other processes, such as inflammation and thrombosis. Nevertheless, we have recently shown that GGE estimates of LDL3b concentrations in the HATS trial were significantly associated with clinical cardiovascular events independent of standard lipids [16], suggesting that refined lipoprotein subfraction analyses may enhance the strength of associations with CVD.

We found that coronary artery disease progression had a significantly stronger relationship to on-study than baseline lipoprotein levels. The results are not inconsistent with recent meta-analyses of statin users showing that on-treatment LDL cholesterol, non-HDL cholesterol and apoB were predictive of major cardiovascular events in statin users [31], but differ from the finding of the Treating to New Targets (TNT) study which reported that on-study HDL, LDL, and triglyceride levels were unrelated to major cardiovascular events when adjusted for baseline lipids [32]. However, the current analysis differs from that in the TNT study in its focus on lipoprotein subfractions and angiographic progression as a disease endpoint.

The Heart Protection Study 5.3-year follow-up of 20,000 participants reported that NMR estimates of small LDL particle concentrations were significantly associated with both major occlusive coronary events (hazard ratio: 1.20 per 0.34 μmol/L, $P<10^{-4}$) and revascularization procedures (hazard ratio: 1.14 per 0.34 μmol/L, P=0.0003), whereas large LDL particles were not (1.08 and 1.02 per 0.34 μmol/L, respectively) in the statin-treated patients [8]. Total LDL particle concentrations were strongly correlated with small LDL particle concentrations (r=0.76) but not large LDL particle concentrations (r=0.16), and not unexpectedly, the relationships of small LDL with major occlusive coronary events and revascularization procedures lost their significances when adjusted for total LDL particle number.

We also included analyses that adjusted for treatment group assignment (see online supplement), which are expected to be conservative given that the changes in lipoprotein subclasses were largely a direct consequence of the intervention. They show that even when

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adjusted for treatment assignment, on-study concentrations of LDLIIIb and LDLIVa as measured by GGE, small LDL as measured by NMR, and LDLIIIb measured by ion mobility were significantly associated with greater coronary disease progression. The finding that small LDL is the principal lipoprotein determinant of atherosclerosis risk with statin plus niacin treatment may be of relevance to the findings of the AIM-HIGH study, in which there was failure of niacin to reduce CVD event rates when added to a regimen in which maximum LDL lowering had been achieved with statin ± ezetimibe treatment [33].

In conclusion, these analyses establish that in this population of patients with coronary artery disease and low HDL cholesterol, there are relationships of specific lipoprotein subclasses, particularly small, dense LDL, that are concurrent to coronary atherosclerosis progression, and are independent of the metabolic and genetic processes affecting lipoprotein levels prior to the intervention. The effects are also shown to be independent of traditional risk factors. Consistency of the relationships of coronary atherosclerosis progression with measures of small LDL by four independent methods supports their use for assessing the pathophyiologic effects of these particles. and for managing risk for coronary artery disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

GGE	gradient gel electrophoresis
HDL	high density lipoprotein
IDL	intermediate density lipoprotein
LDL	low-density lipoprotein
NMR	nuclear magnetic resonance spectroscopy
VAP-II	vertical auto profile
VLDL	very low density lipoprotein

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- 136 patients received baseline and follow-up coronary angiographies
- LDL were measured by 4 different laboratory procedures.
- Greater percent stenosis was consistently associated with small, dense LDL levels.
- The association remained significant when adjusted for standard lipoproteins.
- Disease progression was also related to individual VLDL, IDL, and HDL subclasses.

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Table 1

Baseline characteristics and mean lipoprotein concentrations (±SD).

	Males		Females	
	Baseline	**On-study**	**Baseline**	**On-study**
Age	53.19±7.39		57.19±12.38	
Current smokers	22.69%		23.53	
BMI (kg/m^2)	29.57±3.72		29.27±5.80	
Triglycerides (mmol/L)	2.44±1.16	2.08±1.48	2.46±1.19	1.77±0.93
HDL cholesterol (mmol/L)	0.80±0.11	0.90±0.15	0.87±0.12	1.11±0.27
LDL cholesterol (mmol/L)	3.19±0.75	2.47±0.71	3.45±0.74	2.35±0.90
Apolipoprotein B (mg/dL)	114.73±22.39	91.58±22.79	126.25±17.80	83.97±26.58
Gradient gel electrophoresis				
LDLpeak diameter (nm)	25.61±0.73	25.87±0.77	25.69±0.68	26.24±0.92
LDLI	0.42±0.27	0.40±0.18	0.59±0.35	0.41±0.21
LDLIIa	0.44±0.32	0.43±0.23	0.60±0.37	0.42±0.23
LDLIIb	0.69±0.35	0.64±0.33	0.80±0.27	0.51±0.29
LDLIIIa	0.58±0.31	0.49±0.31	0.64±0.31	0.38±0.36
LDLIIIb	0.15±0.12	0.13±0.12	0.16±0.11	0.12±0.09
LDLIVa	0.12±0.07	0.12±0.05	0.12±0.04	0.12±0.04
LDLIVb	0.11±0.05	0.11±0.04	0.12±0.04	0.10±0.03
Vertical spin centrifugation (VAP-II)				
LDLpeak flotation rate (sec)	109.05±4.78	110.41±4.23	110.76±3.79	111.18±5.60
VLDLbuoyant	0.28±0.17	0.28±0.22	0.32±0.14	0.21±0.21
VLDLdense	0.31±0.14	0.29±0.18	0.37±0.11	0.23±0.18
IDL	0.42±0.15	0.35±0.15	0.49±0.13	0.29±0.13
LDL1	0.44±0.15	0.39±0.13	0.54±0.18	0.33±0.16
LDL2	0.27±0.24	0.29±0.19	0.44±0.43	0.35±0.21
LDL3	1.12±0.47	1.06±0.39	1.33±0.35	0.94±0.49
LDL4	0.69±0.33	0.59±0.33	0.73±0.31	0.44±0.32
HDL3	0.65±0.11	0.75±0.12	0.72±0.10	0.87±0.20
HDL2	0.16±0.06	0.17±0.06	0.16±0.05	0.23±0.12
Nuclear magnetic resonance (NMR)				
LDLmean diameter (nm)	20.21±0.64	20.21±0.56	20.54±0.94	20.47±0.64
VLDLlarge	12.59±8.14	10.21±8.18	12.00±7.50	7.48±5.09
VLDLintermediate	36.76±18.77	28.98±21.08	35.67±24.41	20.86±28.38
VLDLsmall	27.48±18.56	26.50±17.37	24.66±19.96	22.25±16.22
IDL	346.94±180.66	281.75±152.07	460.24±156.87	278.82±168.06
Large LDL	299.99±269.04	255.18±212.61	412.41±332.88	270.53±186.29
Small LDL	1159.14±382.62	938.15±399.68	1080.35±454.08	739.82±519.36
HDL-small	15.47±5.31	16.73±6.06	15.20±4.78	15.00±6.80

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	Males		Females	
	Baseline	**On-study**	**Baseline**	**On-study**
HDL-intermediate	7.70±5.66	8.40±5.72	8.91±5.23	11.55±5.02
HDL-large	2.58±0.95	3.48±1.63	3.29±0.93	5.46±2.59
Ion mobility				
LDLpeak diameter (nm)	21.49±0.48	21.67±0.51	21.57±0.50	21.90±0.87
VLDLlarge	30.81±9.17	24.81±9.79	31.74±13.16	16.88±8.97
VLDLmedium	77.97±20.61	63.19±21.12	84.63±24.56	44.70±19.91
VLDLsmall	84.32±26.57	70.60±22.66	95.33±29.80	58.20±23.64
IDL	307.22±69.05	327.90±87.19	335.81±76.74	298.75±86.97
LDLI	275.11±134.37	254.73±111.28	339.74±223.22	258.86±104.33
LDLIIa	271.11±131.61	236.72±93.35	300.44±132.04	216.49±98.17
LDLIIb	392.05±144.26	325.36±125.64	429.13±114.30	265.77±158.50
LDLIIIa	418.24±181.07	324.64±173.99	462.47±189.41	244.64±198.24
LDLIIIb	144.40±88.24	108.52±71.27	150.35±85.20	90.67±68.71
LDLIVa	128.73±73.82	106.59±57.28	126.06±50.93	107.67±51.12
LDLIVbc	154.44±69.01	143.47±75.17	159.69±54.63	151.62±125.50
HDL3&2a	4570.93±2202.40	4330.64±1516.59	6058.70±3733.66	5135.92±1841.91
HDL2b	1107.46±403.57	1193.11±562.84	1257.78±520.72	1996.91±1455.66

Ion mobility estimated lipoprotein particle concentrations are given in nmol/L, GGE-estimated LDL concentrations are given in mmol/L of cholesterol; NMR particle concentrations are given in nmol/L for VLDL, IDL, and LDL, and μmol/L for HDL, and all VAP-II-estimated lipoprotein concentrations are given in mmol/L of cholesterol.

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Table 2

Correlations between ion mobility, GGE, NMR, and vertical spin untracentrifugation estimates of LDL subfraction concentrations (baseline/on-study)

	Ion mobility						
	Large LDL		**Medium LDL**	**Small LDL**	**Very small LDL**		
	LDLI	**LDLIIa**	**LDLIIb**	**LDLIIIa**	**LDLIIIb**	**LDLIVa**	**LDLIVbc**
NMR							
Large LDL	0.76/0.61	0.76/0.70	0.37/0.37	−0.37/−0.12	−0.53/−0.37	−0.42/−0.43	−0.30/−0.38
Small LDL	−0.54/−0.38	−0.44/−0.14	0.07/0.41	0.77/0.82	0.82/0.85	0.68/0.71	0.52/0.46
Vertical spin centrifugation							
LDL1	0.55/0.28	0.56/0.49	0.50/0.60	0.01/0.39	−0.30/0.17	−0.33/0.03	−0.27/−0.05
LDL2	0.79/0.71	0.59/0.37	0.05/−0.13	−0.46/−0.30	−0.42/−0.30	−0.28/−0.32	−0.20/−0.35
LDL3	0.59/0.61	0.65/0.83	0.54/0.64	−0.12/0.14	−0.47/−0.16	−0.49/−0.27	−0.38/−0.27
LDL4	−0.42/−0.31	−0.28/0.05	0.26/0.63	0.68/0.78	0.52/0.62	0.35/0.42	0.30/0.23
GGE							
LDLI	0.78/0.73	0.67/0.59	0.27/0.19	−0.32/−0.20	−0.45/−0.35	−0.40/−0.40	−0.31/−0.42
LDLIIa	0.80/0.78	0.73/0.80	0.28/0.32	−0.41/−0.22	−0.55/−0.43	−0.49/−0.44	−0.41/−0.39
LDLIIb	0.37/0.39	0.54/0.74	0.61/0.70	−0.03/0.15	−0.45/−0.27	−0.50/−0.27	−0.38/−0.23
LDLIIIa	−0.37/−0.26	−0.29/−0.04	0.21/0.49	0.65/0.78	0.45/0.66	0.22/0.38	0.13/0.14
LDLIIIb	−0.36/−0.30	−0.38/−0.24	−0.12/0.14	0.45/0.61	0.66/0.73	0.63/0.59	0.49/0.35
LDLIVa	−0.23/−0.21	−0.27/−0.19	−0.15/0.06	0.21/0.40	0.43/0.58	0.56/0.67	0.56/0.57
LDLIVb	−0.12/−0.11	−0.16/−0.02	−0.05/0.14	0.16/0.29	0.23/0.41	0.33/0.56	0.44/0.67

	GGE						
	Large LDL		**Medium LDL**	**Small LDL**	**Very small LDL**		
	LDLI	**LDLIIa**	**LDLIIb**	**LDLIIIa**	**LDLIIIb**	**LDLIVa**	**LDLIVb**
NMR							
Large LDL	0.77/0.55	0.80/0.70	0.59/0.67	−0.28/−0.03	−0.34/−0.25	−0.07/−0.23	0.04/−0.17
Small LDL	−0.42/−0.26	−0.47/−0.27	−0.23/0.03	0.61/0.76	0.59/0.66	0.40/0.50	0.25/0.38
Vertical spin centrifugation							
LDL1	0.72/0.44	0.65/0.43	0.62/0.64	0.14/0.50	−0.15/0.16	−0.05/0.09	0.11/0.21

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	GGE						
	Large LDL		**Medium LDL**	**Small LDL**	**Very small LDL**		
	LDLI	**LDLIIa**	**LDLIIb**	**LDLIIIa**	**LDLIIIb**	**LDLIVa**	**LDLIVb**
LDL2	0.69/0.68	0.71/0.60	0.20/0.14	−0.39/−0.22	−0.22/−0.18	0.02/−0.18	0.09/−0.16
LDL3	0.67/0.61	0.75/0.78	0.78/0.83	0.10/0.26	−0.25/−0.05	−0.08/−0.07	0.11/0.01
LDL4	−0.19/−0.12	−0.25/−0.13	0.17/0.37	0.69/0.78	0.34/0.48	0.18/0.37	0.18/0.27

Baseline and on-study values adjusted for sex, age, and smoking status.

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Table 3

Regression analyses of change in percent stenosis in the <30% stenosis arteries (dependent variable) vs. baseline and on-study lipoprotein subfractions (coefficients±SE).

	On-study	Baseline	$Coefficient_{on\text{-}study}$- $Coefficient_{baseline}$	
			Difference	Significance
Gradient gel electrophoresis				
LDLpeak diameter	−0.137±0.081	−0.026±0.089	−0.112±0.150	0.46
LDLI	0.120±3.423	−2.333±2.229	2.453±4.980	0.62
LDLIIa	1.363±2.718	−2.559±1.950	3.922±4.098	0.34
LDLIIb	2.171±1.917	−1.081±1.804	3.252±3.185	0.31
LDLIIIa	2.944±1.846	0.377±1.994	2.567±3.248	0.43
LDLIIIb	25.538±4.905§	−3.301±4.742	28.839±8.404	0.0008
LDLIVa	32.513±11.647†	−0.170±8.463	32.684±16.907	0.06
LDLIVb	35.333±15.176*	−9.986±12.320	45.319±22.181	0.04
Vertical spin centrifugation				
LDL peak flotation rate (sec)	−0.224±0.149	0.121±0.139	−0.346±0.255	0.18
VLDLbuoyant	4.349±2.588	−2.854±3.481	7.203±5.015	0.15
VLDLdense	7.887±3.238*	−5.878±4.205	13.765±6.266	0.03
IDL	11.555±3.786†	−6.494±3.664	18.049±6.084	0.004
LDL1	8.549±4.340*	−1.821±3.518	10.371±6.248	0.10
LDL2	−0.345±3.472	−1.643±2.367	1.297±5.165	0.80
LDL3	1.750±1.495	−0.211±1.318	1.961±2.377	0.41
LDL4	5.258±1.677†	−2.225±1.743	7.484±2.781	0.008
HDL3	−5.892±4.681	2.772±5.730	−8.664±9.057	0.34
HDL2	−20.063±8.704*	11.884±10.075	−31.946±15.735	0.04
Nuclear magnetic resonance				
LDLmean diameter	−1.641±1.191	0.598±0.994	−2.239±1.949	0.25
VLDLlarge	0.107±0.080	−0.023±0.080	0.129±0.138	0.35
VLDLintermediate	0.046±0.029	0.002±0.033	0.044±0.055	0.43
VLDLsmall	0.027±0.035	−0.034±0.032	0.061±0.055	0.27
IDL	0.005±0.004	0.001±0.003	0.003±0.006	0.58
Large LDL	0.000±0.003	−0.001±0.002	0.001±0.005	0.86
Small LDL	0.005±0.001‡	−0.002±0.002	0.007±0.002	0.008
HDL-small	−0.022±0.100	0.035±0.119	−0.057±0.185	0.76
HDL-intermediate	0.008±0.102	0.045±0.106	−0.037±0.168	0.82
HDL-large	−0.558±0.343	0.177±0.628	−0.735±0.830	0.38
Ion mobility				
LDLpeak diameter	−0.146±0.111	−0.052±0.130	−0.094±0.209	0.65

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	On-study	Baseline	Coefficient$_{on\text{-}study}$- Coefficient$_{baseline}$	
			Difference	Significance
VLDLlarge	0.183±0.055‡	0.025±0.055	0.158±0.087	0.07
VLDLintermediate	0.078±0.026†	0.000±0.026	0.078±0.040	0.06
VLDLsmall	0.065±0.025*	−0.023±0.021	0.088±0.039	0.02
IDL	0.011±0.007	−0.004±0.008	0.015±0.012	0.23
LDLI	−0.002±0.006	−0.002±0.005	−0.001±0.010	0.96
LDLIIa	0.005±0.006	−0.001±0.004	0.006±0.009	0.48
LDLIIb	0.009±0.004*	0.001±0.004	0.008±0.007	0.23
LDLIIIa	0.010±0.003†	−0.002±0.003	0.012±0.005	0.02
LDLIIIb	0.028±0.008‡	−0.010±0.007	0.039±0.013	0.003
LDLIVa	0.022±0.011*	−0.007±0.009	0.029±0.017	0.09
LDLIVbc	0.003±0.008	0.000±0.009	0.002±0.015	0.88
HDL3&2a	0.001±0.000	0.000±0.000	0.000±0.000	0.93
HDL2b	−0.001±0.001	0.001±0.001	−0.002±0.002	0.21

Ion mobility estimated lipoprotein particle concentrations are given in nmol/L, GGE-estimated LDL concentrations are given in mmol/L of cholesterol; NMR particle concentrations are given in nmol/L for VLDL, IDL, and LDL, and μmol/L for HDL, and all VAP-II-estimated lipoprotein concentrations are given in mmol/L of cholesterol. Adjusted for age, sex, smoking status, and BMI.

Significance levels coded:

* P≤0.05;

† P≤0.01;

‡ P≤0.001;

§ P≤0.0001.