ELSEVIER

Atherosclerosis 192 (2007) 211–217

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA)

Samia Mora[a,*], Moyses Szklo[b], James D. Otvos[c], Philip Greenland[d], Bruce M. Psaty[e], David C. Goff Jr.[f], Daniel H. O'Leary[g], Mohammed F. Saad[h], Michael Y. Tsai[i], A. Richey Sharrett[b]

[a] *Center for Cardiovascular Disease Prevention, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA, USA*
[b] *Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA*
[c] *LipoScience Inc., Raleigh, NC, USA*
[d] *Departments of Preventive Medicine and Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA*
[e] *Departments of Medicine, Epidemiology, and Health Services, University of Washington, SE, USA*
[f] *Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC, USA*
[g] *Department of Radiology, Tufts-New England Medical Center, Boston, MA, USA*
[h] *Department of Preventive Medicine, Stony Brook School of Medicine, Stony Brook, NY, USA*
[i] *Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA*

Received 26 January 2006; received in revised form 2 May 2006; accepted 4 May 2006
Available online 12 June 2006

Abstract

Background: Previous studies showing that smaller low-density lipoprotein (LDL) size is associated with greater atherosclerotic risk did not adequately control for small and large LDL particle correlation.
Methods and results: We studied the association of lipoproteins measured by proton nuclear magnetic resonance spectroscopy with carotid intima-media thickness (IMT) in apparently healthy individuals ($N = 5538$, 38% White, 28% African American, 22% Hispanic, 12% Chinese). Small and large LDL particle concentrations (LDL-p) were inversely correlated ($r = -0.63$, $P < 0.0001$). Controlling for risk factors but not for LDL subclass correlation, LDL size and small LDL-p separately were associated with IMT (−20.9 and 31.7 μm change in IMT per 1-S.D., respectively, both $P < 0.001$), but large LDL-p was not (4.9 μm, $P = 0.27$). When LDL subclasses were included in the same model, large and small LDL-p were both associated with IMT (36.6 and 52.2 μm higher IMT per 1-S.D., respectively, both $P < 0.001$; 17.7 and 11.6 μm per 100 nmol/L, respectively). LDL size was not significant after accounting for LDL subclasses and risk factors ($P = 0.10$).
Conclusion: Both LDL subclasses were significantly associated with subclinical atherosclerosis, with small LDL confounding the association of large LDL with atherosclerosis. Future studies of LDL size should account for the strong inverse correlation of LDL subclasses.

Keywords: Lipoproteins; Lipids; Subclinical atherosclerosis; Carotid arteries; Magnetic resonance spectroscopy

1. Introduction

Previous studies have shown that individuals with predominantly small LDL particles (pattern B) have greater cardiovascular risk than those with predominantly large LDL (pattern A) [1–5]. However, these studies examined only the distribution of LDL subclasses or LDL size phenotype (large or small) rather than particle concentrations of LDL subclasses. Thus, they did not adequately control for the inverse correlation between small and large LDL particle concentrations (LDL-p) and potential confounding due to their differing associations with other lipoproteins, lipids, and traditional cardiovascular risk factors [1–3]. Prior studies also

* Corresponding author at: Brigham and Women's Hospital, 900 Commonwealth Ave., Boston, MA 02215, USA. Tel.: +1 617 278 0783; fax: +1 617 264 9194.
E-mail address: smora2@partners.org (S. Mora).

0021-9150/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2006.05.007

did not directly compare the risk associated with small and large LDL particles on a per particle basis. This distinction is important because small LDL particles contain substantially less cholesterol than large ones, such that at the same serum concentration of LDL cholesterol (LDL-c), individuals with predominantly small LDL have greater total concentration of LDL particles than those with predominantly large LDL [6].

Nuclear magnetic resonance (NMR) spectroscopy enables quantification of concentrations of lipoprotein particles of varying size [7]. We sought to directly compare the associations of small and large LDL with carotid intima-media thickness (IMT), a measure of subclinical atherosclerosis, before and after accounting for the inverse correlation and potential confounding relationship of the two subclasses. In a subgroup of participants, we also examined whether fasting remnants of triglyceride-rich lipoproteins carrying apolipoprotein B48, which have been purported to be atherogenic, are associated with IMT. Carotid IMT, measured from ultrasound images, is a direct, non-invasive measure of atherosclerosis. It is closely related to all major cardiovascular risk factors and is a well-validated predictor of clinical cardiovascular disease [8].

2. Methods

2.1. Study population

Study participants were enrolled in the Multi-Ethnic Study of Atherosclerosis (MESA), a multi-center cohort initiated by the National Heart, Lung and Blood Institute (NHLBI) to elucidate the pathogenesis and progression of subclinical atherosclerosis [9]. Eligible participants were community-based men and women, ages 45–84 years and free of self-reported cardiovascular disease, recruited from four diverse racial/ethnic groups (African American, Hispanic, White, and Asian predominantly of Chinese descent) at six centers in the United States (Forsyth County, NC, Northern Manhattan and the Bronx, NY, and selected portions of Baltimore City and Baltimore County, MD, St. Paul, MN, Chicago and Maywood, IL, and Los Angeles County, CA).

The MESA study-wide exclusion criteria were pregnancy, cancer or other serious medical conditions, any recent chest computed tomographic scan, morbid obesity (>136 kg), nursing home residency, cognitive impairment, intention to leave the community within 5 years, or language barrier, resulting in a total of 6814 enrolled participants at baseline. For the purposes of this analysis, we also excluded participants who were on lipid-lowering medication ($N = 1187$), had triglycerides >400 mg/dL ($N = 82$), or did not have LDL-p or LDL-c measurements ($N = 7$). Of the remaining 5538 participants, 150 (2.7%) did not have IMT measurements and were included only in the descriptive analyses.

All data were collected at the first MESA examination (2000–2002), when participants gave informed consent, completed questionnaires on medical history, medications, and lifestyle factors, and had measurements of blood pressure, anthropometry, carotid ultrasound and fasting blood samples for lipids and lipoproteins. The study was approved by the institutional review boards of the participating institutions.

2.2. Anthropometric and clinical variables

Three resting blood pressure readings were recorded using an automated oscillometric method (Dinamap, Criticon Inc., Florida), and the mean of the second and third measurements was used. Hypertension was mean systolic and diastolic blood pressure $\geq$140 or $\geq$90 mmHg, respectively, or use of antihypertensive medication. Diabetes was fasting glucose >125 mg/dL (6.88 mmol/L) or use of antidiabetic medication. Body mass index (BMI) was calculated as weight divided by the square of the height (kg/m^2) [10].

2.3. Carotid ultrasound measurements

High-resolution B-mode ultrasound was used to measure carotid atherosclerosis. We used the mean of eight measurements of maximal IMT (right and left, near and far walls, common and internal carotid) [11]. All imaging studies were transmitted to a central reading center for interpretation. Quality assurance/control measures included standardized protocols, centralized training of technicians, routine calibration of equipment, and monthly reports on scan quality and protocol adherence.

2.4. Lipid and NMR lipoprotein measurements

Triglycerides, high-density lipoprotein cholesterol (HDL-c), and total cholesterol were measured in fasting plasma using CDC-standardized methods. The Friedewald equation was used to estimate LDL-c. Fasting remnant-lipoprotein particle cholesterol (RLP-c) was measured in a random subgroup of individuals ($N = 844$) using the RLP-® Cholesterol Immunoseparation Assay (Polymedco Inc., New York) [12].

Lipoprotein particle concentrations and size were measured on frozen plasma specimens (−70 °C) by proton NMR spectroscopy (LipoScience Inc., North Carolina) [7,13–15]. Particle concentrations of lipoprotein subclasses of different sizes were directly obtained from the measured amplitudes of their spectroscopically distinct lipid methyl group NMR signals. Weighted-average lipoprotein particle sizes are derived from the sum of the diameter of each subclass multiplied by its relative mass percentage based on the amplitude of its methyl NMR signal [7].

The concentrations (in nanomoles of particles/litre or nmol/L) of the following subclasses were measured: small LDL (diameter of 18.0–21.2 nm), large LDL (21.2–23.0 nm), intermediate-density lipoprotein or IDL (23.0–27.0 nm), large HDL (8.8–13.0 nm), medium HDL (8.2–8.8 nm), small HDL (7.3–8.2 nm), large very low-density lipoprotein or VLDL (>60 nm), medium VLDL (35.0–60.0 nm), and small VLDL (27.0–35.0 nm). The small LDL subclass encompassed both intermediate small (19.8–21.2 nm) and very

small particles (18.0–19.8 nm), which have nearly identical correlations with lipid and lipoprotein levels, so they were combined into one subclass, as previously reported [15]. Inter-assay reproducibility, determined from replicate analyses of plasma pools, is indicated by the following coefficients of variation: <2% for very low-density lipoprotein (VLDL) size and <0.5% for LDL and HDL size, <10% for VLDL-p subclasses, <4% for total LDL-p, <8% for large and small LDL-p, and <5% for large and small HDL-p, with higher variation (<30%) for medium HDL-p and IDL-p (the latter due to their typically low concentrations).

2.5. Statistical methods

Statistical analyses were performed using STATA 8.2, 2003. First, we calculated Spearman rank correlation coefficients (r) for lipoproteins and lipids. Next, multiple linear regression analyses adjusting for potential confounders were performed on the 5388 individuals who had both IMT and lipoprotein measurements, while regression analyses of RLP-c measurements were limited to the subgroup with RLP-c measurements ($N = 844$). Lipoprotein coefficients were expressed per 1-S.D. and per 100 nmol/L. Comparing lipoprotein effects on a 1-S.D. basis relates their effects to their population distributions, whereas the 100 nmol/L comparison compares their atherogenic effects per particle.

To examine the extent to which small and large LDL were associated with IMT, we initially considered each lipoprotein variable in a separate model that adjusted for traditional risk factors but did not include other lipid or lipoprotein measures. Then we included both small and large LDL-p subclasses together in the same model in order to determine if they were independently associated with IMT after accounting for their correlation. We also tested the association of large LDL-p with IMT in participants with low (<sex-specific median) or high (≥sex-specific median) levels of small LDL-p and also within four categories of small LDL-p. Tests of interaction were obtained from likelihood ratio tests comparing models with and without the interaction term.

Finally, small and large LDL were added to a model that included all other lipoprotein subclasses in order to estimate the associations of the LDL subclasses with IMT independent of all other lipoproteins. No correlations among subclasses (ranging from 0.4 to 0.7) were strong enough to affect the precision of their estimated effects.

Since our objective was to determine the LDL subclass effects on IMT independent of established risk factors, we adjusted on an *a priori* basis for age (years), race, sex, hypertension, and smoking. Also on an *a priori* basis, we additionally adjusted the models for traditional lipids, and for the effects of BMI and diabetes, two lipid-altering risk factors. Additional models not including hypertension were also examined. Study site was included in initial models, but was subsequently dropped because it did not affect lipid coefficients, nor did menopausal status or hormone replacement therapy use in women. Sex-stratified models showed no substantial differences or interactions by sex. Since a substantial proportion of the original MESA participants were on lipid lowering medication (17.4%), we also repeated all analyses without excluding those participants. P values were two-tailed and values <0.05 were considered significant.

3. Results

The mean age (±S.D.) was 61.4 (±10.3) years. The study population was multi-ethnic (38% White, 28% African American, 22% Hispanic, and 12% Chinese) with 53% women (Table 1).

Small and large LDL-p were inversely correlated ($r = -0.63$). They were both positively correlated with LDL-c ($r = 0.40$ and 0.27, respectively), but they correlated in oppo-

Table 1
Mean (±S.D.) values or percentage distributions of clinical, lipid, and lipoprotein variables

	Women ($N = 2930$)	Men ($N = 2608$)
Age (years)	61.2 (10.3)	61.7 (10.3)
Intima-media thickness (μm)	902 (317)	996 (356)
Total cholesterol (mg/dL)	201 (34)	190 (34)
LDL-c (mg/dL)	120 (32)	119 (31)
HDL-c (mg/dL)	57 (15)	45 (12)
Triglycerides (mg/dL)	120 (61)	128 (68)
RLP-c (mg/dL) ($N = 844$)	8.4 (4.4)	9.8 (5.5)
Body mass index (kg/m^2)	28.6 (6.3)	27.7 (4.5)
Current smoking (%)	12.0	15.3
Hypertension (%)	42.2	38.7
Diabetes (%)	10.2	13.1
Race (%)		
White	37.7	37.8
Chinese	11.6	12.5
African American	28.8	26.8
Hispanic	21.9	22.9
Lipoprotein particle concentration (nmol/L)		
LDL-p		
Total	1284 (376)	1358 (368)
Large	479 (206)	333 (179)
Small	786 (444)	1004 (427)
IDL-p	19.0 (23.6)	20.5 (22.5)
HDL-p		
Total	32956 (5853)	28671 (4874)
Large	8527 (4007)	5476 (3255)
Medium	5232 (4747)	3629 (3383)
Small	19195 (4970)	19566 (4171)
VLDL-p		
Total	68.5 (37.9)	78.0 (37.5)
Large	3.2 (4.2)	3.8 (4.9)
Medium	27.8 (21.8)	34.4 (24.7)
Small	37.6 (20.8)	39.7 (19.3)
Lipoprotein particle size (nm)		
LDL	21.1 (0.8)	20.6 (0.7)
HDL	9.3 (0.4)	9.0 (0.4)
VLDL	51.1 (9.1)	50.6 (8.5)

The small LDL subclass encompassed both intermediate small and very small particles. To convert mg/dL to mmol/L, multiply LDL-c, HDL-c, and total cholesterol values by 0.02586 and triglycerides by 0.011.

site directions with LDL size (−0.91 and 0.87, respectively), HDL-c (−0.65 and 0.67), and triglycerides (0.57 and −0.40). LDL-c and total LDL-p were positively correlated ($r = 0.69$).

In separate regression models which considered one lipoprotein or lipid variable at a time and adjusted for age, race, sex, hypertension, and smoking (Table 2), LDL size and small LDL-p were associated with IMT (−20.9 and 31.7 μm change in IMT per 1-S.D., respectively, both $P < 0.001$), but large LDL-p was not (4.9 μm, $P = 0.27$). Large HDL-p, but not the small- or medium-size HDL-p subclasses, was inversely related to IMT. LDL-c, HDL-c, and triglycerides were all individually associated with IMT in the expected direction. RLP-c showed no significant association with IMT.

In order to unmask the association of large LDL with IMT, we divided participants into those with low (<sex-specific median) and high (≥sex-specific median) levels of small LDL-p. In these stratified analyses, large LDL-p was now significantly associated with IMT in participants with low and high levels of small LDL-p (26.5 and 25.4 μm higher IMT per 1-S.D. increment in large LDL-p, $P < 0.001$ and 0.001, respectively). If we further cross-classified participants into four categories each of small and large LDL-p, increasing concentrations of large LDL were positively associated with IMT within any category of small LDL-p (Fig. 1).

Table 2
Association of individual lipoprotein and lipid variables with IMT

	1-S.D.	ΔIMT (SE) in μm per 1-S.D.	*P* value
Lipoprotein particle concentration (nmol/L)			
LDL-p			
Total	374	40.2 (4.1)	<0.001
Large	207	4.9 (4.4)	0.27
Small	449	31.7 (4.2)	<0.001
IDL-p	23.1	26.7 (4.1)	<0.001
HDL-p			
Total	5821	−23.2 (4.4)	<0.001
Large	3975	−21.4 (4.5)	<0.001
Medium	4236	−5.1 (4.2)	0.22
Small	4614	−5.0 (4.1)	0.23
VLDL-p			
Total	38.0	15.8 (4.1)	<0.001
Large	4.5	11.1 (4.1)	0.007
Medium	23.4	3.6 (4.1)	0.38
Small	20.1	22.8 (4.1)	<0.001
Lipoprotein particle size (nm)			
LDL	0.8	−20.9 (4.5)	<0.001
HDL	0.4	−20.3 (4.3)	<0.001
VLDL	8.8	0.9 (4.1)	0.82
Lipids (mg/dL)			
LDL-c	31.2	37.4 (4.1)	<0.001
HDL-c	15.0	−22.4 (4.5)	<0.001
Triglycerides	64.6	13.1 (4.1)	0.002
RLP-c ($N = 833$)	4.9	12.0 (9.1)	0.19

Values per 1-S.D. increase in lipid or lipoprotein variable were obtained from separate linear regression models that included only one lipid or lipoprotein variable at a time and adjusted for age, sex, race, hypertension, and smoking. Intermediate and very small LDL were combined into one variable (small LDL) due to the nearly identical correlations between intermediate and very small LDL.

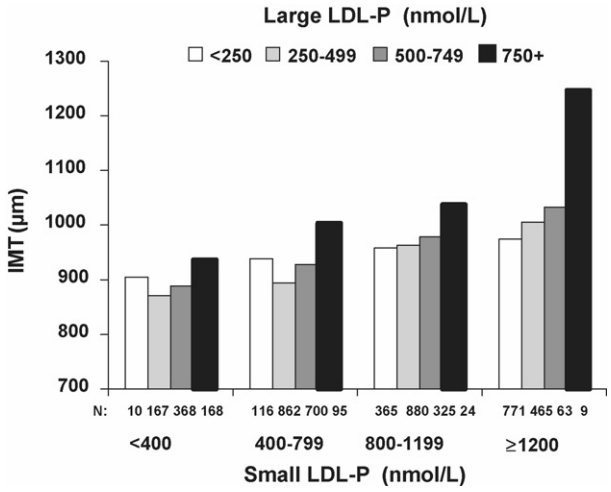

Fig. 1. Mean IMT (*y*-axis) for increasing levels of large LDL particle concentration (LDL-p) are shown across increasing levels of small LDL-p. *N* is the number of individuals in each category.

Fig. 2 shows the highly significant linear association of large LDL-p with IMT, which is evident only after accounting for its inverse correlation with small LDL-p. There was no association (*P* trend = 0.94) between large LDL-p and IMT when small LDL-p was not adjusted for in the linear regression model (Fig. 2 left panel). However, after additionally adjusting for small LDL-p in order to account for the inverse correlation between small and large LDL (Fig. 2 right panel), the highly significant association between large LDL-p and IMT became apparent (*P* trend < 0.001). There was no significant interaction between large and small LDL-p categories with respect to IMT (*P* for interaction = 0.83).

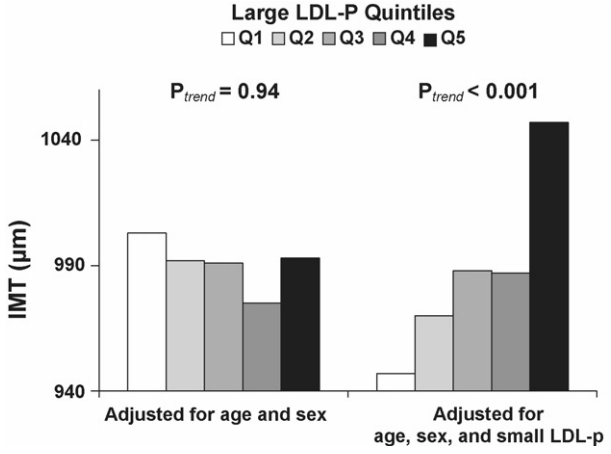

Fig. 2. Mean IMT (*y*-axis) for increasing quintiles of large LDL particle concentration (LDL-p) adjusting for age (years) and sex (left panel) but not for small LDL-p showed no significant association between large LDL-p and IMT. After additionally adjusting for small LDL-p to account for the correlation between small and large LDL (right panel), there was a significant linear association between large LDL-p and IMT. *P* values for linear trend were obtained from linear regression models. Mean IMT values in the figure represent those for an average age man (61.4 years). To obtain values for a 61.4-year-old woman subtract 80 μm.

Table 3
Association of LDL subclasses with IMT after adjusting for LDL subclass correlation

	ΔIMT (SE) in μm per 1-S.D.					
	Model 1[a]	*P* value	Model 2[b]	*P* value	Model 3[c]	*P* value
Large LDL-p	36.6 (5.4)	<0.001	23.8 (9.1)	0.009	30.3 (9.4)	0.001
Small LDL-p	52.2 (5.2)	<0.001	38.7 (9.2)	<0.001	34.8 (10.1)	0.001
LDL-c			13.5 (7.7)	0.08	11.8 (7.8)	0.13
HDL-c					−17.3 (5.7)	0.003
Triglycerides					−1.6 (5.1)	0.75

S.D. values for lipid and lipoprotein variables are shown in Table 2.
[a] Model 1 included the two LDL subclasses, age (years), sex, race, hypertension, and smoking.
[b] Model 2 included model 1 variables plus LDL-c.
[c] Model 3 included model 1 variables plus LDL-c, HDL-c, and triglycerides.

In a linear regression model that accounted for the LDL subclass correlation and also adjusted for age, race, sex, hypertension, and smoking (Table 3), both large and small LDL-p were independently associated with IMT (36.6 and 52.2 μm higher IMT per 1-S.D., respectively, both $P < 0.001$). Not shown in a table, when examined on a per particle basis (rather than per 1-S.D.), large LDL was associated with a greater difference in mean IMT than was small LDL (17.7 μm versus 11.6 μm higher IMT per 100 nmol/L increment, respectively). In the subgroup of participants with diabetes ($N = 597$), both large and small LDL-p were significantly associated with carotid IMT. Specifically, 1-S.D. increments in large and small LDL-p were associated with 41.7 and 46.1 μm higher IMT, $P = 0.025$ and 0.009, respectively, in diabetic participants, after adjusting for age, sex, race, hypertension and smoking. In participants without diabetes ($N = 4753$), 1-S.D. increments in large and small LDL-p were associated with 38.6 and 52.5 μm higher IMT, $P < 0.001$ for both.

Both LDL particle subclasses remained significantly associated with IMT after adjustment for LDL-c and traditional lipids (Table 3). There was no significant additional contribution of LDL-c to IMT once the two LDL subclasses were included in the model. Adding BMI and diabetes did not substantially alter the association of the LDL subclasses with IMT. LDL size was no longer significant ($P = 0.10$) after accounting for LDL subclass concentrations and risk factors (including BMI and diabetes). Excluding hypertension did not change our findings, nor did adjusting for hormone replacement use or menopause status in women. When total LDL-p was placed in model 2 instead of the two LDL-p subclasses, both LDL-c and total LDL-p were independently associated with IMT (18.1 and 27.1 μm higher IMT per 1-S.D., $P = 0.002$ and $P < 0.002$, respectively).

We then proceeded to examine the associations of small and large LDL-p in a model that accounted for correlations of the LDL subclasses with HDL and VLDL subclasses (Table 4). In this more extensive lipoprotein model, both large and small LDL-p remained significantly associated with IMT (42.9 and 39.1 μm higher IMT per 1-S.D., respectively, both $P < 0.001$), with no substantial change seen when also adjusting for BMI and diabetes or excluding hypertension and smoking. All HDL-p subclasses showed statistically significant inverse associations with IMT. Subclasses of VLDL-p showed mixed effects and lost statistical significance after additionally adjusting for BMI and diabetes. There was no significant additional contribution of LDL-c ($P = 0.49$) nor of LDL, HDL, or VLDL particle size ($P = 0.13$, 0.50, and 0.12, respectively) to the full lipoprotein model.

Table 4
Association of LDL subclasses with IMT after adjusting for other lipoproteins

Lipoprotein particle concentration	ΔIMT (SE) in μm per 1-S.D.	*P* value
Large LDL-p	42.9 (6.7)	<0.001
Small LDL-p	39.1 (7.1)	<0.001
IDL-p	10.9 (5.4)	0.045
Large HDL-p	−25.1 (7.0)	<0.001
Medium HDL-p	−16.6 (4.8)	<0.001
Small HDL-p	−13.2 (4.5)	0.003
Large VLDL-p	9.2 (5.3)	0.08
Medium VLDL-p	−11.8 (5.4)	0.03
Small VLDL-p	1.6 (5.4)	0.76

Values shown were obtained from one model containing all lipoproteins together and adjusted for sex, age, race, hypertension and smoking. S.D. values for lipid and lipoprotein variables are listed in Table 2. The highest correlation was found between large HDL-p and small LDL-p ($r = -0.69$) and large HDL-p and large LDL-p ($r = 0.74$). All other correlation coefficients were <0.70.

Finally, we repeated our analyses without excluding the 1187 (17.4%) participants who were taking lipid lowering medication. Similar results were obtained for all analyses except that LDL-c was no longer significantly associated with IMT in the model that included LDL-c together with total LDL-p. Our findings for the associations of small and large LDL subclasses with IMT remained essentially unchanged.

4. Discussion

In this large multi-ethnic cohort of asymptomatic individuals, small and large LDL particle concentrations were inversely correlated with each other and correlated in opposite directions with LDL size. Without accounting for LDL subclass correlation, small LDL and smaller LDL size were

associated with IMT but large LDL was not. However, after accounting for their inverse correlation, both LDL subclasses showed highly significant and independent associations with IMT, with a greater difference in IMT per large LDL particle compared with small LDL. Smaller LDL size was no longer significant after taking into account the particle concentrations of the two LDL subclasses and risk factors. Thus, small LDL was a strong confounder of the association of large LDL with subclinical atherosclerosis, which may explain the widely-held view that larger LDL size is less atherogenic.

In analyses that did not adjust for small LDL, we found that large LDL was only weakly associated with atherosclerosis, consistent with prior reports [1–3,16]. Correlations among lipoproteins are well understood. Small LDL predominate in a triglyceride-rich environment, because precursors of small LDL are secreted by the liver and large LDL particles are transformed into smaller particles by cholesterol ester transfer protein (CETP)-mediated transfer followed by lipolysis. After accounting for particle correlations, we demonstrated that the magnitude of association between small LDL and carotid atherosclerosis became equal to large LDL (on a per 1-S.D. basis) or less than large LDL (on a per particle basis). Failure to account for the strong negative correlation between small and large LDL and their different associations with other lipoproteins may underlie the belief that small LDL particles are a more potent atherogenic subclass than large LDL.

The literature addressing LDL particle size as a cardiovascular risk factor is mixed [4–6]. Certain studies found that small LDL size was associated with increased CVD risk [17–19], while others did not confirm the independence of the association [20,21]. Two small studies specifically relating to IMT found no independent relation with LDL particle size measures [22,23]. Other studies have suggested that large LDL size may be associated with CVD [24,25]. However, most reports relating to LDL size examined only the distribution of LDL subclasses or LDL size phenotype (large or small) rather than LDL subclass concentrations. We found that the atherogenicity of large LDL became apparent only when individuals were classified according to low or high levels of small LDL or after accounting for levels of small LDL by statistical adjustment. This may explain the variable strength of association found in prior studies for the association of LDL size with CVD risk, i.e. different population distributions of small and large LDL and potential confounding by levels of small LDL. Moreover, both large and small LDL were significantly associated with carotid atherosclerosis in our study participants, whether or not they had diabetes mellitus. Our findings regarding the atherogenicity of both LDL subclasses have been confirmed in the VA-HIT trial, where both subclasses were significantly associated with coronary events once their correlation was taken into account [26].

There are several mechanisms that may underlie the atherosclerotic effect of both large and small LDL [5]. At both extremes of LDL size, there is decreased receptor-binding affinity for LDL receptors [27]. Small LDL may be oxidized more rapidly and have been associated with endothelial dysfunction and metabolic dyslipidemia [28]. In comparison, large LDL predominate in patients with familial hypercholesterolemia [29] and those consuming high saturated fat diets. Large LDL have higher core cholesterol ester content, potentially delivering more cholesterol per particle to arterial walls [30], a speculation supported by our finding a greater IMT difference for large compared to small LDL on a per particle basis.

5. Limitations

One potential limitation to our study is its cross-sectional nature. However, substantial cross-sectional bias is unlikely in view of the subclinical nature of our measure of atherosclerosis (IMT). Since NMR is a relatively new lipoprotein measurement technique, limited comparisons exist with ultracentrifugation or other subfractionation methods. However, correlations between lipoprotein measures obtained from our NMR data were similar to those found in studies utilizing traditional separation techniques, and the unadjusted associations of lipoprotein subclasses and particle size with IMT were similar to other studies [1–3,14]. The large study size allowed us to parse out the effect of lipoprotein and lipid variables that were moderately correlated ($r \sim 0.4$–0.7), with little or no loss of precision, and we believe our nine-particle model gives a minimally biased estimate of the independent atherogenic effects of the individual lipoprotein subclasses. However, estimates from the model that added NMR-derived average particle size to lipoprotein subclass concentrations should be considered less reliable, given the high correlation ($r \sim 0.9$) of certain particle concentrations with average particle size.

6. Conclusion

Small LDL confounded the association of large LDL with IMT because of its strong inverse correlation with large LDL, which may underlie the widespread belief that large LDL confers less cardiovascular risk than small LDL. Contrary to current opinion, both small and large LDL were significantly associated with subclinical atherosclerosis independent of each other, traditional lipids, and established risk factors, with no association between LDL size and atherosclerosis after accounting for the concentrations of the two subclasses. This knowledge may contribute to our understanding of atherogenesis, and future studies examining LDL size and atherosclerosis should account for the significant inverse correlation between small and large LDL.

Acknowledgements

This research was supported by contracts N01-HC-95159 through N01-HC-95165 and N01-HC-95169 from the

National Heart, Lung, and Blood Institute. The authors thank the other investigators, staff, and participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org.

Conflicts of interest disclosures: Dr. James Otvos is Chief Scientific Officer of LipoScience Inc. None of the other authors have any potential conflicts of interest.

References

[1] 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;22:1175–80.
[2] Blake GJ, Otvos JD, Rifai N, Ridker PM. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. Circulation 2002;106:1930–7.
[3] 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-I) trial. Am J Cardiol 2002;90:89–94.
[4] Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res 2002;43:1363–79.
[5] Sacks FM, Campos H. Clinical review 163: cardiovascular endocrinology: low-density lipoprotein size and cardiovascular disease: a reappraisal. J Clin Endocrinol Metab 2003;88:4525–32.
[6] Cromwell WC, Otvos JD. Low-density lipoprotein particle number and risk for cardiovascular disease. Curr Atheroscler Rep 2004;6:381–7.
[7] Otvos JD. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. Clin Lab 2002;48:171–80.
[8] O'Leary DH, Polak JF. Intima-media thickness: a tool for atherosclerosis imaging and event prediction. Am J Cardiol 2002;90:18L–21L.
[9] Bild DE, Bluemke DA, Burke GL, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. Am J Epidemiol 2002;156:871–81.
[10] Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults—The Evidence Report. National Institutes of Health. Obes Res 1998;6(Suppl 2):51S–209S.
[11] O'Leary DH, Polak JF, Kronmal RA, et al. Thickening of the carotid wall. A marker for atherosclerosis in the elderly? Cardiovascular Health Study Collaborative Research Group. Stroke 1996;27:224–31.
[12] Schaefer EJ, McNamara JR, Tayler T, et al. Effects of atorvastatin on fasting and postprandial lipoprotein subclasses in coronary heart disease patients versus control subjects. Am J Cardiol 2002;90:689–96.
[13] Otvos JD, Jeyarajah EJ, Cromwell WC. Measurement issues related to lipoprotein heterogeneity. Am J Cardiol 2002;90:22i–9i.
[14] 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;18:1046–53.
[15] Festa A, Williams K, Hanley AJ, et al. Nuclear magnetic resonance lipoprotein abnormalities in prediabetic subjects in the Insulin Resistance Atherosclerosis Study. Circulation 2005;111:3465–72.
[16] St-Pierre AC, Cantin B, Dagenais GR, et al. Low-density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Quebec Cardiovascular Study. Arterioscler Thromb Vasc Biol 2005;25:553–9.
[17] Lamarche B, Tchernof A, Moorjani S, et al. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Circulation 1997;95:69–75.
[18] Rajman I, Kendall MJ, Cramb R, et al. Investigation of low density lipoprotein subfractions as a coronary risk factor in normotriglyceridaemic men. Atherosclerosis 1996;125:231–42.
[19] 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;33:1255–60.
[20] 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;276:882–8.
[21] Gardner CD, Fortmann SP, Krauss RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA 1996;276:875–81.
[22] Hulthe J, Wiklund O, Bondjers G, Wikstrand J. LDL particle size in relation to intima-media thickness and plaque occurrence in the carotid and femoral arteries in patients with hypercholesterolaemia. J Intern Med 2000;248:42–52.
[23] Tzou WS, Douglas PS, Srinivasan SR, et al. Advanced lipoprotein testing does not improve identification of subclinical atherosclerosis in young adults: the Bogalusa Heart Study. Ann Intern Med 2005;142:742–50.
[24] Campos H, Roederer GO, Lussier-Cacan S, Davignon J, Krauss RM. Predominance of large LDL and reduced HDL2 cholesterol in normolipidemic men with coronary artery disease. Arterioscler Thromb Vasc Biol 1995;15:1043–8.
[25] Campos H, Moye LA, Glasser SP, Stampfer MJ, Sacks FM. Low-density lipoprotein size, pravastatin treatment, and coronary events. JAMA 2001;286:1468–74.
[26] Otvos JD, Collins D, Freedman DS, et al. LDL and HDL particle subclasses predict coronary events and are changed favorably by gemfibrozil therapy in the veterans affairs HDL intervention trial (VA-HIT). Circulation 2006;113:1556–63.
[27] Campos H, Arnold KS, Balestra ME, Innerarity TL, Krauss RM. Differences in receptor binding of LDL subfractions. Arterioscler Thromb Vasc Biol 1996;16:794–801.
[28] Tribble DL, Rizzo M, Chait A, et al. Enhanced oxidative susceptibility and reduced antioxidant content of metabolic precursors of small, dense low-density lipoproteins. Am J Med 2001;110:103–10.
[29] Patsch W, Ostlund R, Kuisk I, Levy R, Schonfeld G. Characterization of lipoprotein in a kindred with familial hypercholesterolemia. J Lipid Res 1982;23:1196–205.
[30] Rudel LL, Parks JS, Hedrick CC, Thomas M, Williford K. Lipoprotein and cholesterol metabolism in diet-induced coronary artery atherosclerosis in primates. Role of cholesterol and fatty acids. Prog Lipid Res 1998;37:353–70.