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Relationship between low-density lipoprotein subclasses and asymptomatic atherosclerosis in subjects from the Atherosclerosis Risk in Communities (ARIC) Study

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Low-density lipoprotein (LDL) particle size has been associated with coronary heart disease, but an association between LDL size and preclinical atherosclerosis is less well established. Using gradient gel electrophoresis, large (A), intermediate (I) and small (B) LDL size subclasses were determined in 198 cases with asymptomatic carotid artery atherosclerosis (determined by B-mode ultrasonography) and 318 controls from the Atherosclerosis Risk in Communities (ARIC) Study. In Caucasians, a smaller LDL size was more prevalent in men and associated with a higher body mass index, hypertension prevalence, and plasma total- and LDL-cholesterol and triglycerides, but lower HDL-cholesterol. In African-Americans, a smaller LDL size was associated with higher triglycerides and lower HDL-cholesterol and hypertension prevalence. In Caucasians, Subclass B prevalence was 29.1% among cases and 14.8% among controls. The odds ratio (95% confidence interval) for Subclass B rather than Subclass A in Caucasian cases was 2.94 (1.67–5.17); the association remained significant after controlling for age, body mass index, smoking, and either plasma triglycerides or HDL-cholesterol. In African-Americans, however, there was no significant association between LDL subclass and case status. A predominance of smaller LDL particles is associated with asymptomatic carotid artery atherosclerosis in Caucasians, through mechanisms that remain to be elucidated.

Keywords: low-density lipoproteins, carotid atherosclerosis, particle size.

Introduction

Plasma low-density lipoprotein (LDL) concentrations are associated with a risk of coronary artery disease (Castelli *et al.* 1986), but LDL particles are not homogeneous (Lindgren *et al.* 1951). Such physical properties of LDL particles as size, flotation rate and density vary both within and among individuals, and particles of different sizes vary greatly in the relative amounts of triglycerides, cholesteryl esters, cholesterol and phospholipids they contain (Adams and Schumaker 1970, Lee and Alaupovic 1970). While several methods of measuring LDL particle sizes exist, gradient gel electrophoresis has been used to distinguish three common LDL subclasses (Austin *et al.* 1988a, 1990). Subclass A is characterized

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by a predominance of large, buoyant LDL particles, with a major peak on densitometric scans occurring at a particle diameter $\geq$25.5 nm. Subclass B is characterized by a predominance of small, dense LDL particles, with a major peak formed by particles $<$25.5 nm in diameter. LDL profiles of some individuals do not fit unambiguously in either subclass, forming the intermediate Subclass I.

Though genetic factors appear to influence LDL particle size, the genes involved have yet to be determined definitively (Austin *et al.* 1988b, 1993, 1998, 1999, Rotter *et al.* 1996, Allayee *et al.* 2000, Talmud *et al.* 2000, Bossé *et al.* 2003). Non-genetic factors, such as hormone use (Campos *et al.* 1993, De Graaf *et al.* 1993), pregnancy (Silliman *et al.* 1994), weight loss (Williams *et al.* 1990) and disease (Feingold *et al.* 1993) might also affect LDL particle size. Moreover, individuals' LDL subclass patterns may change over time (McNamara *et al.* 1992), sometimes in response to diet (Dreon *et al.* 2000, Krauss 2001).

Many studies have reported an association between a predominance of small, dense LDL particles and coronary heart disease (CHD) (Griffin *et al.* 1994, Rajman *et al.* 1996, Lamarche *et al.* 1997, 1998, Koba *et al.* 2002) and the progression of coronary atherosclerosis (Watts *et al.* 1993, Vakkilainen *et al.* 2003), although reported associations have not always been independent of plasma triglyceride (Crouse *et al.* 1985, Austin *et al.* 1988a, 1990, Coresh *et al.* 1993) or high-density lipoprotein (HDL) cholesterol levels (Campos *et al.* 1992). However, studies of subjects with symptomatic CHD cannot establish the role LDL size may play in subclinical atherosclerosis, since clinical management of CHD can alter plasma lipid profiles (Pasternak and Braunwald 1991) and may affect LDL size. We report here that in a sample of Caucasian men and women drawn from the multicentre Atherosclerosis Risk in Communities (ARIC) Study, a predominance of small, dense LDL is significantly associated with asymptomatic atherosclerosis defined by intimal-medial (I-M) thickening in the extracranial carotid arteries, even after fasting plasma triglyceride levels are taken into account.

Materials and methods

Subjects

Cases and controls were selected from the ARIC Study, a prospective multicentre investigation of atherosclerosis and its clinical outcomes in men and women aged 45–64 years. Nearly 16 000 residents were recruited, approximately 4000 from each of four communities: Forsyth County, NC; Washington County, MD; Jackson, MS; and the north-western suburbs of Minneapolis, MN. Except in Jackson, where only African-Americans were sampled, the cohort was selected to represent a probability sample of all residents. The design of the ARIC Study has been described elsewhere (The ARIC Investigators 1989).

Carotid artery thickness was measured by B-mode ultrasonography (Bond *et al.* 1991), using the protocol of Pignoli *et al.* (1986). Caucasian subjects classified as cases had a maximum carotid artery far wall thickness $>$2.5 mm or a bilateral wall thickness exceeding the approximate 95th percentile of the ARIC Study cohort distribution (1.7 mm in the internal carotid, 1.8 mm in the carotid bifurcation, 1.6 mm in the common carotid). African-Americans were classified as cases if bilateral wall thickness in the same segments exceeded the approximate 90th percentile (1.2, 1.675 and 1.0 mm, respectively), to increase the number of cases. These criteria were intended to identify a group of subjects having unusually severe, non-uniform carotid artery thickening more likely to indicate the presence of atherosclerotic plaques. For all controls, maximum far wall thickness of each carotid segment and the popliteal artery was below the 75th percentile (Heiss *et al.* 1991). Subjects were excluded if they showed evidence of symptomatic cardiovascular or cerebrovascular disease, type 1 diabetes mellitus, gall bladder disease, pancreatitis, intestinal malabsorption syndromes, or chronic renal or liver disease; had fasting triglyceride levels $>$400 mg dl^{-1}; used β-blockers or cholesterol-lowering drugs or thyroid preparations;

or were premenopausal women using contraceptive hormones. Approximately 20% of potential cases, but fewer than 10% of potential controls, were excluded for these possible confounders.

For each subject, three sitting blood pressure measurements were made after a 5-min rest, using a random-zero sphygmomanometer; the average of the second and third readings was used. Hypertension was defined as systolic blood pressure above 160 mmHg or diastolic blood pressure above 95 mmHg, or current use of antihypertensive medication. Body mass index (BMI, $kg\,m^{-2}$) was calculated from measurements of weight (to the nearest pound) and height (to the nearest centimetre).

Laboratory methods

Venous blood was collected in tubes containing ethylenediamine tetra-acetic acid (EDTA), following a 12-h fast. Plasma was separated by centrifugation at 4°C; aliquots were stored at −70°C and weekly shipped on dry ice to the ARIC Central Lipid Laboratory for storage at −70°C until analysed. Total plasma cholesterol (Siedel *et al.* 1983) and triglyceride (Nägele *et al.* 1984) levels were measured enzymatically on a Cobas-Fara centrifugal analyser (Riche Diagnostics, Montclair, NJ, USA), using kits (cat. nos 236 691 and 701 912; Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). HDL-cholesterol (HDL-C) was measured in the supernatant after treating the plasma with $MgCl_2$ and dextran sulfate (Warnick *et al.* 1982). LDL-cholesterol (LDL-C) levels were calculated (Friedewald *et al.* 1972).

LDL size was determined by gradient gel electrophoresis, using 2–16% non-denaturing polyacrylamide-agarose gels (PAA 2–16%; Pharmacia, Uppsala, Sweden). Plasma aliquots were mixed with a 40% sucrose, 0.1% bromophenol blue solution and electrophoresed in a Pharmacia GE 2/4 apparatus at 120 V for 18 h at 10°C in buffer (0.09 M Tris, 0.08 M boric acid and 0.003 M Na_2 EDTA, pH 8.3). Gels were stained with Sudan Black B for 20 h, destained in 50% Cellosolve solution for 2–3 days, stored in 25% Cellosolve to restore their size and shape, then scanned with an Ultrascan XL laser densitometer using GSXL software (LKB Instruments, Inc., Paramus, NJ, USA). Each assay included two plasma standards from separate donors, one with predominantly small, dense LDL, the other with predominantly large, buoyant LDL, as determined by zonal ultracentrifugation (Patsch and Patsch 1986). Each LDL sample was evaluated by three independent observers and assigned to one of the three subclasses, A, B or I (Austin *et al.* 1988a, 1990). Approximately 5% of the samples were reanalysed to verify the assignments; no discrepancies were found.

Statistical methods

Unmatched *t*-tests and non-parametric rank-sum tests were used in comparing cases and controls, while analysis of variance and the Kruskal–Wallis rank-sum test were used for comparisons among LDL subclasses. Frequencies were compared using standard χ^2-tests. Conditional logistic regression (Hosmer and Lemeshow 1989) was used to estimate odds ratios (OR) while controlling for the effects of covariates, with case and control strata matched by sex, ARIC study centre, age (<55 or ≥ 55 years), and examination period (within 6 months). In this cross-sectional study, the OR measures the odds of exposure in cases relative to controls at the time of examination, rather than the risk of developing atherosclerotic disease.

Results

Differences among race, sex and case groups

Cardiovascular disease risk factor levels in cases and controls are shown in table 1. By design, age and sex distributions were similar in the two groups. Among Caucasians, hypertension prevalence and the proportion of those who had ever smoked were higher in cases than controls. Caucasian cases also had significantly higher mean BMI and plasma glucose, total- and LDL-cholesterol, and triglyceride levels than controls, but significantly lower mean plasma HDL-C levels. Among Caucasians, mean carotid I-M thickness was 1.19 ± 0.26 mm in cases and 0.63 ± 0.08 mm in controls. Among African-Americans, mean carotid I-M thickness was 0.95 ± 0.22 mm in cases, and 0.62 ± 0.08 mm in controls, the smaller difference being attributable in part to the less stringent case criteria for African-Americans (see the Materials and methods). Apart from I-M thickness, there were no significant differences between African-American cases and controls in any of the measured physical, biochemical or lifestyle variables. There were no

Table 1. Cardiovascular risk factors in cases with asymptomatic atherosclerosis and controls, by race.

	Cases	Controls
Caucasians		
Sex, M/F (*n*)	102/49	155/82
Age (Years)	56.5±5.1	55.4±5.4
BMI (kg m^{-2})	27.3±4.5	25.7±3.8[a]
HBP prevalence (%)	24±3.5	10±2.0a
Smoking (% ever)	84±3.0	53±3.2a
Ethanol use (g week^{-1})	55±100.2	44±84.6
Glucose (mmol l^{-1})	5.64±0.56	5.49±0.54[b]
Cholesterol (mmol l^{-1})	5.70±1.01	5.27±0.98[a]
Triglycerides (mmol l^{-1})	1.52±0.74	1.17±0.64[a]
LDL-C (mmol l^{-1})	3.78±0.91	3.32±0.92[a]
HDL-C (mmol l^{-1})	1.23±0.40	1.41±0.45[a]
I-M thickness (mm)	1.19±0.26	0.63±0.08[a]
African–Americans		
Sex, M/F (*n*)	19/28	33/48
Age (Years)	53.7±6.2	54.1±5.8
BMI (kg m^{-2})	27.9±5.3	27.7±5.4
HBP prevalence (%)	36±7.0	37±5.4
Smoking (% ever)	51±7.3	48±5.6
Ethanol use (g week^{-1})	30±89.6	45±196.4
Glucose (mmol l^{-1})	5.37±0.49	5.53±0.63
Cholesterol (mmol l^{-1})	5.52±0.97	5.45±1.12
Triglycerides (mmol l^{-1})	1.10±0.65	1.21±0.54
LDL-C (mmol l^{-1})	3.49±0.92	3.36±1.09
HDL-C (mmol l^{-1})	1.53±0.44	1.53±0.47
I-M thickness (mm)	0.95±0.22	0.62±0.08

Continuous variables given as percentages or means±SD.

HBP, high blood pressure; I-M, intimal-medial; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Cases and controls were group-matched for race and sex.

Cases versus controls: [a]$p \leq 0.001$; [b]$p \leq 0.01$.

significant differences between cases and controls in menopausal status or prevalence of oestrogen use in either African-American or Caucasian women (data not shown).

There were no significant differences in LDL subclass distributions between African-Americans and Caucasians (table 2), either in controls ($p = 0.340$), who would more closely resemble the general population, or in the full sample ($p = 0.475$). In African-Americans, LDL subclass distributions varied little between the control group and the full sample. LDL subclass distributions did not differ significantly between males and females in either African-American ($p = 0.216$) or Caucasian ($p = 0.101$) controls (table 3). However, because cases and controls were selected to have similar proportions of males and females, we also examined LDL subclass distributions with cases and controls combined. Among Caucasians, LDL subclass distributions in the full group differed significantly between males and females ($p = 0.002$); the proportion of males with Subclass B (24.9%) was more than twice that of females (11.4%), for a male:female ratio of 2.2:1, virtually identical to that in controls (2.1:1). Among African-Americans, the proportion of Subclass B was higher in males than in females both in controls and in the full sample; the lack of statistical significance in either case likely reflects low statistical power.

Table 2. Distribution of LDL subclasses in African–American and Caucasian controls and in the full sample with cases and controls combined.

	LDL subclass					
	A		I		B	
	n	%	*n*	%	*n*	%
Controls						
African–American	41	50.6	27	33.3	13	16.1
Caucasian	141	59.5	61	25.7	35	14.8
Total	182	57.2	88	27.7	48	15.1
Cases and controls combined						
African–American	64	50.0	42	32.8	22	17.2
Caucasian	202	52.1	107	27.6	79	20.4
Total	266	51.5	149	28.9	101	19.6

In Caucasians, several cardiovascular disease risk factors differed significantly among LDL subclasses (table 3): BMI and plasma levels of total-cholesterol, LDL-C and triglycerides were highest in Subclass B, while HDL-C levels were lowest. The prevalence of hypertension in Subclasses I and B was higher than in Subclass A. In African-Americans, however, only HDL-C and triglyceride levels and hypertension prevalence differed significantly among subclasses (table 3). In contrast to Caucasians, African-Americans showed a much higher prevalence of

Table 3. Association of LDL subclass with plasma lipid levels and other risk factors, controls only, by race.

	LDL subclass		
	A	I	B
Caucasians			
Sex, M/F (*n*)	86/55	41/20	28/7
Age (Years)	55.2±5.7	55.6±5.4	55.7±4.5
BMI (kg m^{-2})	25.0±3.3	26.3±4.3	27.6±3.8[c]
Hypertension (%)	6.4±2.1	18.0±4.9	11.4±5.4[a]
Glucose (mmol l^{-1})	5.50±0.51	5.58±0.61	5.58±0.56
Cholesterol (mmol l^{-1})	5.15±0.94	5.37±1.02	5.55±0.99
Triglycerides (mmol l^{-1})	0.95±0.49	1.31±0.68	1.76±0.66[c]
LDL-C (mmol l^{-1})	3.19±0.91	3.46±0.89	3.61±0.90[a]
HDL-C (mmol l^{-1})	1.52±0.45	1.31±0.37	1.13±0.42[c]
I-M thickness (mm)	0.62±0.08	0.63±0.08	0.67±0.09[b]
African–Americans			
Sex, M/F (*n*)	14/27	11/16	8/5
Age (Years)	54.4±6.1	54.2±5.8	53.1±5.4
BMI (kg m^{-2})	27.5±5.6	28.3±6.0	27.2±3.2
Hypertension (%)	39.0±7.6	48.2±9.6	7.7±7.4[a]
Glucose (mmol l^{-1})	5.64±0.61	5.52±0.53	5.50±0.89
TC (mmol l^{-1})	5.58±0.92	5.11±1.25	5.74±1.34
TG (mmol l^{-1})	0.97±0.40	1.38±0.57	1.59±0.56[c]
LDL-C (mmol l^{-1})	3.37±0.94	3.14±1.19	3.80±1.25
HDL-C (mmol l^{-1})	1.76±0.50	1.33±0.27	1.22±0.34[c]
I-M thickness (mm)	0.61±0.08	0.64±0.08	0.62±0.06

Values for continuous variables are means or percentage±SD.
Test for difference among subclasses: [a]$p \leq 0.05$; [b]$p \leq 0.01$; [c]$p \leq 0.001$.

hypertension in Subclasses A and I than in B. In both African-Americans and Caucasians, the highest mean triglyceride and lowest mean HDL-C levels occurred in Subclass B.

Association of LDL subclass with carotid artery atherosclerosis

In Caucasians, LDL subclass and case status were significantly associated (table 4): 29.1% of cases and 14.8% of controls were in Subclass B, while 30.5% of cases and 25.7% of controls were in Subclass I. Relative to Subclass A, the odds ratio for cases in Subclass B, adjusted for sex, was 3.04 (95% confidence interval [CI]: 1.75–5.27); for Subclass I, the sex-adjusted odds ratio was 1.76 (95% CI: 1.08–2.88). In African-Americans, there was no significant association between LDL subclass and case status (table 4).

For the conditional logistic regression analyses in Caucasians, potential predictors in the models were age (years), BMI, cigarette and ethanol consumption, and plasma glucose, triglyceride, and HDL-C levels. Forward, backward and stepwise selection all yielded the same model, which included age (OR: 1.14 per year, 95% CI: 1.05–1.24), BMI (OR: 2.70 for BMI ≥ 30 kg m^{-2}; 95% CI: 1.34–5.45), smoking (OR: 5.01, ever smoked versus never smoked; 95% CI: 2.80–8.96), and triglyceride level (OR: 2.60 for ≥ 1.61 mmol l^{-1}; 95% CI: 1.48–4.56). When LDL subclass type was added to the model (table 5), Subclass B (OR: 2.05; 95% CI: 1.03–4.10) increased risk significantly relative to Subclass A, but Subclass I did not (OR: 1.30; 95% CI: 0.74–2.30). Removing triglyceride level from the model increased the effects of both Subclass B (OR: 2.81; 95% CI: 1.49–5.31) and Subclass I (OR: 1.43; 95% CI: 0.82–2.50), though the latter still fell short of statistical significance. In all combinations of these variables, the effect of Subclass B remained statistically significant (table 5). When we forced HDL-C into the full model, neither HDL-C (OR: 1.58 for <1.03 mmol l^{-1}; 95% CI: 0.85–2.94), triglycerides (OR: 1.79; 95% CI: 0.95–3.38), Subclass I (OR: 1.27; 95% CI: 0.72–2.24) nor Subclass B (OR: 1.86; 95% CI: 0.92–3.76) remained statistically significant predictors of

Table 4. Association for LDL subclass pattern with case/control status, by race.

	Cases		Controls	
LDL subclass	*n*	%	*n*	%
Caucasians				
A	61	40.4	414	59.5
I	46	30.5	61	25.7
B	44	29.1	35	14.8
Total	151		237[a]	
African–Americans				
A	23	48.9	41	50.6
I	15	31.9	27	33.3
B	9	19.1	13	16.0
Total	47		81[b]	

[a]Cases versus controls: $\chi^2 = 16.563$, 2 df; $p < 0.001$.
[b]Cases versus controls: $\chi^2 = 0.201$, 2 df; $p = 0.904$.

Table 5. Odds ratios (OR) for carotid artery atherosclerosis case status in LDL subclasses I and B relative to subclass A, Caucasians only.

	LDL subclass I		LDL subclass B	
Variables in model	OR	95% CI	OR	95% CI
LDL Subclass	1.74	1.04–2.91	2.94	1.67–5.17
LDL Subclass, Age	1.75	1.04–2.95	3.07	1.71–5.49
LDL Subclass, BMI	1.58	0.94–2.66	2.65	1.49–4.72
LDL Subclass, Smoking	1.56	0.91–2.66	2.95	1.61–5.41
LDL Subclass, TG	1.55	0.91–2.62	2.02	1.08–3.75
LDL Subclass, HDL-C	1.62	0.97–2.73	2.29	1.26–4.17
LDL Subclass, Age, BMI	1.58	0.93–2.70	2.80	1.55–5.06
LDL Subclass, Age, TG	1.56	0.91–2.66	2.14	1.13–4.05
LDL Subclass, Age, HDL-C	1.63	0.96–2.77	2.44	1.31–4.53
LDL Subclass, Age, Smoking	1.58	0.92–2.74	3.13	1.67–5.84
LDL Subclass, Age, Smoking, BMI	1.43	0.82–2.50	2.81	1.49–5.31
LDL Subclass, Age, Smoking, TG	1.40	0.80–2.46	2.15	1.09–4.27
LDL Subclass, Age, Smoking, HDL-C	1.47	0.85–2.56	2.49	1.29–4.81
LDL Subclass, Age, Smoking, BMI, TG	1.30	0.74–2.30	2.05	1.03–4.10
LDL Subclass, Age, Smoking, BMI, HDL-C	1.35	0.77–2.37	2.28	1.17–4.45
LDL Subclass, Age, Smoking, BMI, TG, HDL-C	1.27	0.72–2.24	1.86	0.92–3.76

Cases and controls were matched on sex, ARIC study centre, age (<55 or ≥ 55 years), and examination period within the ARIC Study.

CI, confidence interval.

case status. However, HDL-C and triglyceride levels are highly correlated (Spearman rank correlation $= -0.55$; $p < 0.001$), and LDL subclass is related to both (table 3). Substituting HDL-C for triglycerides in the models had little effect on the results for LDL subclasses (table 5).

Discussion

Because individuals were selected for the study based on carotid artery I-M thickness (see the Materials and methods), the sample was not random from the population. However, no significant difference overall was found in LDL subclass distributions between African-Americans and Caucasians either in controls, who are more nearly representative of the ARIC study populations, or in the full sample. In the Insulin Resistance Atherosclerosis Study, in which LDL size was quantified as the size of the LDL particles in the predominant peak for each individual, LDL size was significantly larger in African-Americans than in non-Hispanic Whites (Haffner *et al.* 1999). Despite the similar subclass distributions in the two groups in our sample, it would be possible for overall LDL size to be larger in African-Americans, if the peak LDL size in a given subclass tended to be larger in African-Americans than in Caucasians; we cannot now determine whether this is the case.

A significant association was found between small, dense LDL particles and asymptomatic carotid artery atherosclerosis only in Caucasians. The association of Subclass B with case status remained significant even after controlling for plasma triglyceride levels. Several previous studies have reported an association between small, dense LDL particles and clinically documented CHD (Crouse *et al.* 1985, Austin *et al.* 1988a, Griffin *et al.* 1994, Rajman *et al.* 1996, Lamarche *et al.* 1997,

1998, Koba *et al.* 2002), but there have been few studies relating LDL size to subclinical atherosclerosis in healthy individuals. In healthy 58-year-old Swedish men, smaller peak LDL particle size was associated with greater I-M thickness in the carotid and femoral arteries, but an association between Subclass B and greater I-M thickness did not reach statistical significance (Hulthe *et al.* 2000). In a study of healthy 50-year-old Swedish men that used a different system of LDL subclasses than the A/I/B system used in the present paper, plasma concentration of the major small LDL subfraction accounted for 10% of the variation in carotid artery I-M thickness even with plasma triglyceride and LDL-C levels included in the regression model (Skoglund-Andersson *et al.* 1999). It is important to study LDL subclasses in relation to preclinical atherosclerosis, because the onset or treatment of clinically evident cardiovascular disease may induce changes that could affect LDL particle size. For example, patients with diagnosed CHD may alter their dietary fat and carbohydrate intake significantly, possibly affecting LDL particle size (Campos *et al.* 1991, 1992). Some drugs used to treat atherosclerotic disease, such as β-blockers (Superko *et al.* 1993) and fibrates (Bruckert *et al.* 1993, Guérin *et al.* 1996), may also alter LDL size distributions. Our cases had no clinically diagnosed CHD, and the moderate atherosclerosis in their extracranial carotid arteries produced no symptoms, minimizing the likelihood of such confounding effects.

No association was found between LDL subclass and carotid artery atherosclerosis in African-Americans, however, in whom the crude odds ratios, relative to Subclass A, were 1.23 (95% CI: 0.46–3.33) for Subclass B and 0.99 (95% CI: 0.44–2.23) for Subclass I. The less stringent case criteria for African-Americans reduced the difference in I-M thickness between cases and controls and may have contributed to this result. This finding may also reflect the weaker associations between carotid artery atherosclerosis and many lipid measures, including plasma LDL-C, found for African-Americans than for Caucasians in the ARIC cohort. It has been suggested that lipid measurements in middle-aged adults may not reflect lipid levels over the course of a lifetime very well, and African-Americans in the ARIC cohort might have had lower LDL-C levels than Caucasians when younger (Sorlie *et al.* 1999).

In Caucasians, the odds ratio for case status in those with Subclass B rather than Subclass A appeared higher in men (3.35) than in women (2.24); however, these values were not significantly different. When controlled for sex, the odds ratio for carotid artery atherosclerosis in Caucasians with Subclass B was 3.04 (95% CI: 1.75–5.27), virtually identical to the risk of myocardial infarction associated with Subclass B reported in another study which included both sexes (Austin *et al.* 1988a). The present results may well underestimate the association of Subclass B with carotid artery atherosclerosis, since carotid I-M thickness is associated with prevalent CHD in the ARIC cohort (Burke *et al.* 1995), and CHD cases were excluded from our sample; it is estimated that this probably eliminated three times as many potential cases as controls. Among African-Americans, no significant association was found between LDL subclass and case status in either sex.

In agreement with several previous studies (Austin *et al.* 1988a, 1990, Feingold *et al.* 1992, McNamara *et al.* 1992), we found a strong association of small LDL

particles with high plasma triglyceride and low HDL-C levels. In our study, unlike some (Crouse *et al.* 1985, Campos *et al.* 1992, Coresh *et al.* 1993), the association between LDL size and case status remained significant when either triglyceride or HDL-C levels were included in the analyses. When both were included, neither triglycerides, HDL-C nor LDL subclass remained significant predictors of case status, suggesting complicated interrelationships among the three variables. Because the metabolism of triglyceride-rich lipoproteins may be a major determinant of LDL size (McNamara *et al.* 1992, Karpe *et al.* 1993, Berneis and Krauss 2002) the relationship between LDL subclass and plasma triglycerides may be most germane to understanding how small LDL particles are formed and why they are atherogenic, with HDL-C levels being less directly associated with LDL size. A high concentration of triglyceride-rich lipoproteins may drive the transfer of triglycerides to LDL in exchange for cholesteryl ester (Deckelbaum *et al.* 1984), with lipolysis acting to reduce the size of LDL particles as they become triglyceride-enriched. A complicated balance between lipid exchange and lipolysis probably determines individual LDL profiles (Lagrost *et al.* 1994, Guérin *et al.* 1996).

LDL size in itself may not be the principal determinant of LDL atherogenicity (Campos *et al.* 1991, Brunzell 1995), though conformational changes in apolipoprotein B associated with small LDL particles may reduce their affinity for the LDL receptor (Chen *et al.* 1994, Galeano *et al.* 1994). Small, dense LDL may be more susceptible to oxidation (De Graaf *et al.* 1991, Tribble *et al.* 1992), with oxidized LDL being more atherogenic (Steinberg *et al.* 1989). Small LDL particles have also been associated with impaired vascular endothelium function (Vakkilainen *et al.* 2000). A predominance of small LDL particles may be less a cause of atherosclerosis than an indicator of impaired lipoprotein metabolism that can promote atherosclerosis through multiple mechanisms. Our study adds to the preponderance of evidence indicating that smaller LDL particles are associated with atherosclerosis and may themselves be atherogenic, acting through mechanisms which remain to be determined completely.

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