Association of plasma triglyceride concentration and LDL particle diameter, density, and chemical composition with premature coronary artery disease in men and women

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Abstract Low density lipoprotein (LDL) physical-chemical characteristics were studied as nontraditional risk factors of coronary artery disease (CAD) in a well-characterized population of 98 men aged ≤50 and 100 women aged ≤60 who underwent elective diagnostic coronary arteriography. The average LDL diameter was determined by gradient gel electrophoresis, chemical composition (%w/w) was measured, and the density of the major LDL peak was determined by equilibrium density gradient ultracentrifugation. Logistic regression was used to examine the association of various LDL characteristics with CAD before and after adjustment for other covariates. Smaller, cholesterol-poor LDL particles were associated with CAD independently of traditional risk factors (age, sex, smoking, diabetes, LDL and HDL cholesterol concentrations), other than the plasma triglyceride concentration. These characteristics were generally more strongly associated with CAD when measured on the major LDL subfraction (defined as the density gradient ultracentrifugation fraction with the highest LDL concentration) than the average characteristics of the more heterogeneous parent LDL (d 1.019–1.063 g/ml). The associations with CAD among men and women were generally similar. These data show that a broad range of LDL characteristics are associated *with CAD before, but not after, adjustment for the plasma tri*glyceride concentration. These data further indicate the importance of hypertriglyceridemia and LDL heterogeneity in premature CAD.—**Coresh, J., P. O. Kwiterovich, Jr., H. H. Smith, and P. S. Bachorik.** Association of plasma triglyceride concentration and LDL particle diameter, density, and chemical composition with premature coronary artery disease in men and women. *J. Lipid Res.* 1993. **34:** 1687–1697.

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Elevated plasma concentrations of total cholesterol and its major component, low density lipoprotein (LDL) cholesterol, hypertension, cigarette smoking, diabetes, and a low level of high density lipoprotein (HDL) cholesterol are strongly associated with coronary artery disease (CAD) (1–4). However, whether elevated concentrations of plasma triglyceride and its major carrier, very low density lipoprotein (VLDL), are independently associated with CAD has been controversial (5). Recent reports (5), including one from this study population (6), indicate that hypertriglyceridemia may impart risk for CAD. This risk is, in part, related to the atherogenic lipoproteins that may accompany hypertriglyceridemia, such as the presence of VLDL remnants, intermediate density lipoproteins (IDL), and small, dense LDL particles.

We and others reported that increased numbers of small, dense LDL particles, found in hyperapobetalipoproteinemia (hyperapoB) were strongly associated with premature CAD in studies of patients undergoing coronary arteriography (7, 8), post-myocardial infarction (9), and post-coronary artery bypass surgery (10). However, these reports did not examine separately the association of CAD with elevated apoB (LDL particle concentration) and small, dense LDL. We have reported that hyperapoB, at least in some families, reflects the presence of the disorder familial combined hyperlipidemia (FCH), and that the presence of small, dense LDL reflected the presence of an LDL molecule depleted in core cholesteryl ester, and relatively enriched in apoB (11). Compared to normal LDL, such LDL had a smaller diameter by electron microscopy, a lower S_f^0 by analytical ultracentrifugation, and a higher average density after equilibrium density gradient ultracentrifugation (11).

Fisher and co-workers (12) earlier suggested that the molecular weight of LDL may be under genetic control, and heterogeneity in LDL size may be important in the

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; CAD, coronary artery disease; FCH, familial combined hyperlipidemia; GGE, gradient gel electrophoresis; EDGU, equilibrium density gradient ultracentrifugation; RID, radial immunodiffusion; IDL, intermediate density lipoproteins.

development of CAD. Austin and co-workers (13), using gradient gel electrophoresis (GGE), identified three LDL phenotypes: pattern A, characterized by larger more buoyant LDL; pattern B, in which small, dense LDL particles predominate (peak diameter < 25.5 nm); and an intermediate pattern. Pattern B was accompanied by higher levels of plasma triglyceride, IDL, and apolipoprotein B (apoB), but lower levels of HDL cholesterol and apolipoprotein A-I (apoA-I) (13). Pattern B appears to be inherited as a Mendelian dominant trait (14, 15) and was associated with myocardial infarction in a case control study (16).

Campos et al. (17) reported that men with premature CAD had smaller LDL particles than controls. This association was independent of beta-blocker use, smoking, diabetes, hypertension, and LDL cholesterol. However, the association was not significant when further adjusted for HDL cholesterol, apoA-I, or plasma triglyceride level. Swinkles et al. (18) found the LDL cholesterol to apoB ratio to be lower in CAD cases than controls, but this difference did not persist after adjustment for HDL levels.

These reports show a high prevalence of small dense LDL among patients with premature CAD. However, it is unclear whether LDL particle characteristics are independent risk factors for CAD or merely a result of other atherogenic lipid abnormalities and offer no independent risk or predictive value. We report here an evaluation of the particle diameter, molecular weight, density, and chemical composition of LDL, and its major subfraction isolated by equilibrium density ultracentrifugation, in men and women undergoing elective coronary arteriography for premature CAD. We hypothesized that subjects with CAD would have LDL particles of smaller diameter, lower molecular weight, lower cholesterol and cholesteryl ester content, higher apoB content, and lower relative position on equilibrium density ultracentrifugation, reflecting a higher peak density. Measurement of multiple physical-chemical characteristics of LDL in a single study population was done to allow for a comparison of the predictive value of each of the measures. The comparison of the same measures on the total parent LDL to the major LDL subfraction isolated by equilibrium density ultracentrifugation was of particular interest. In addition, previous reports have focused on the association of LDL characteristics with CAD among men, while this study population contained a similar number of men and women allowing for a comparison of the associations in men with those in women.

METHODS

Patient population

The characteristics of the patient population have been previously reported (6). Briefly, the Johns Hopkins Coronary Artery Disease Study consisted of 99 white men ≤50 years of age and 103 white women ≤60 years of age undergoing elective diagnostic coronary arteriography. Coronary arteriograms were reviewed by a panel of three cardiologists who had no prior knowledge of the clinical history or laboratory data of the patients. The presence of stenosis in the 15 coronary artery segments designated by the American Heart Association was determined (19). CAD was considered present if at least one lesion narrowed the lumen diameter of any of the 15 coronary arterial segments by 50% or more. CAD was considered absent if no such lesions were present. Cigarette smoking, a history of high blood pressure or diabetes, and medication use were determined using an interview-administered questionnaire. Height and weight were measured and used to calculate the body mass index. Diabetes was defined as treatment with medication for diabetes or a fasting blood glucose above 140 mg/dl. Five patients with plasma triglyceride concentrations above 500 mg/dl were excluded as severe hypertriglyceridemia is known to cause abnormal LDL composition (5), and these patients have been excluded from previous studies of the association of LDL characteristics with CAD (16, 17). Thus, the final study population included 98 men and 100 women.

Isolation of LDL and the major LDL subfraction

Parent LDL (d 1.019–1.063 g/ml) was isolated by sequential preparative ultracentrifugation as described previously (11). LDL was then dialyzed to d 1.050 g/ml and subjected to equilibrium density gradient ultracentrifugation (EDGU) (11). The samples were centrifuged in an SW 40 Ti swinging bucket rotor at 202,000 *g* for 40 h at 10°C. The relative position, R_p, defined as the distance from the bottom of the gradient to the midpoint of the visible major LDL band divided by the total length of the gradient, was calculated. The sample was then pumped from the bottom of the tube and 0.5-ml fractions were collected. The absorbance of each fraction was measured at 280 nm. The tube with the highest absorbance and the two neighboring tubes (one before and one after) were combined and constituted the major component of LDL (11).

Characterization of LDL and the major LDL subfraction

Particle diameter by gradient gel electrophoresis. The diameter of LDL and the major LDL subfraction were determined by gradient gel electrophoresis (GGE), on 2–16% polyacrylamide gels, using a method modified after Krauss and Burke (20). The gels were fixed and stained for lipid with Oil Red O and the center of the most prominent band was marked on the gel. The gels were then counterstained with Coomassie Brilliant Blue R250 and photographed. The migration of the bands from the gel wells was measured to the nearest 0.5 mm. Three standards of

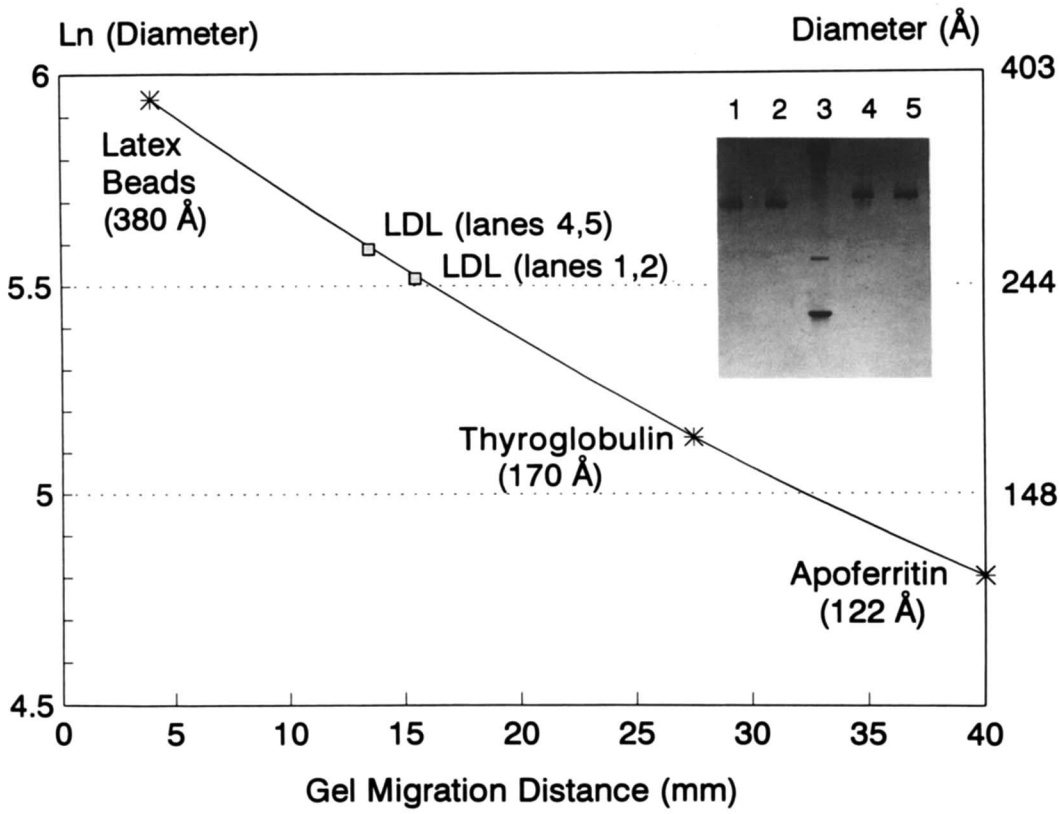

Fig. 1. Gradient gel calibration using a quadratic interpolation of natural log of particle diameter versus gel migration distance. Inset gradient gel contains parent LDL (d 1.006–1.019 g/ml, lane 1) and major LDL (isolated by gradient gel ultracentrifugation, lane 2) from subject number 260, latex beads, thyroglobulin, and ferritin (lane 3), and parent (lane 4) and major (lane 5) LDL from subject number 261.

known diameter, apoferritin (12.2 nm), thyroglobulin (17.0 nm), and carboxylated latex beads (38.0 ± .75 nm), were included on each gel. The average LDL particle diameter was estimated from a quadratic interpolation of a plot of the logarithm of the diameter of the standards versus the migration distance of the standards (**Fig. 1**). Twenty two gels were read a second time without knowledge of the first set of measurements. The absolute value of the difference in particle diameter between the two readings had a mean of 3.5 nm for the parent LDL and 4.0 nm for the major LDL diameter showing that this method of measuring gradient gels was relatively precise.

Chemical composition. The apoB content of LDL and the major LDL subfraction was determined by radial immunodiffusion (RID) as previously described (11). Total cholesterol and free (unesterified) cholesterol were determined enzymatically as described before (11), and the content of cholesteryl ester was computed by subtracting the free cholesterol from the total cholesterol and multiplying by 1.67 (11). The triglyceride content of LDL and its major LDL subfraction was determined enzymatically (11). Phospholipid content was determined by measuring the phosphorus content of LDL lipids using the method of Bartlett (21). This value was multiplied by 25 to convert the value to total phospholipid mass. Composition data were used to calculate the percentage by weight (%w/w) of each LDL component.

Molecular weight of LDL and major LDL subfraction. The masses of LDL and the major LDL subfraction were determined by adding the masses of their respective lipid and apoB components. The molecular weight of LDL and its major subfraction were then computed by dividing the mass of LDL by the mass of apoB, and multiplying by 550,000, the molecular weight of apoB.

Plasma lipids and lipoprotein cholesterol concentration. Separate aliquots of plasma were subjected to preparative ultracentrifugation either at d 1.006 g/ml or d 1.019 g/ml for 18 h at 105,000 *g*. The cholesterol concentration of the plasma and the d 1.006 g/ml and 1.019 g/ml ultracentrifugal infranates were measured enzymatically as described previously (11). LDL cholesterol was calculated as the difference between the d 1.019 g/ml infranate and the HDL cholesterol. Plasma triglyceride was determined enzymatically as described previously (11).

Plasma apolipoprotein A-I and B concentrations. The concentrations of apolipoprotein B and apolipoprotein A-I were determined by RID as described previously (6).

Statistical methods. Univariate distributions of all varia-

bles were examined, and all outliers were examined for internal consistency and data entry errors. LDL composition was censored for four outliers with a calculated LDL molecular weight above 3×10^6 daltons, and for two outliers with implausible phospholipid composition in order to avoid undue influence by these implausible values. Plasma triglyceride concentrations were $\log_{10}$-transformed for all analyses as they were markedly skewed. Pearson product moment correlations between the different LDL characteristics of the parent and major LDL were calculated. The Spearman rank correlations were examined as well and yielded similar results (data not shown). The LDL particle characteristics among subjects with and without CAD were compared using *t*-tests as well as analysis of variance, which allowed for adjustment for covariates. The adjustment covariates were those most strongly associated with CAD and LDL characteristics. Logistic regression was used to examine the odds ratio of CAD associated with different LDL characteristics. Odds ratios were calculated for the 75th minus 25th percentile (interquartile difference) of each LDL characteristic. In order to simplify comparisons between different characteristics, all odds ratios are presented in the direction expected a priori to show an odds ratio greater than one. For example, the odds ratio for LDL particle diameter is quoted as the odds of having CAD of subjects with smaller LDL (25th percentile) compared to subjects with larger LDL (75th percentile), as smaller LDL is expected to be positively associated with CAD. This allows for a comparison of the magnitude of the association of each LDL characteristic with CAD. All statistical analyses were conducted using SAS (22).

RESULTS

Clinical, lipoprotein, and apolipoprotein characteristics are shown in **Table 1.** Clinically significant CAD was present in 107 (54%) of the subjects and absent in 91 (46%) of the subjects; 70% of the 91 subjects without clinically significant CAD had no coronary lesions and none of these subjects had any 41–49% obstructive lesions. Subjects with coronary disease were more likely to smoke, have diabetes, and use beta-blockers. In addition, subjects with CAD were older and had lower HDL and apoA-I levels, and substantially higher apoB and triglyceride levels. The increased prevalence of a history of high blood pressure and higher LDL level among subjects with clinically significant CAD compared to those without clinically significant CAD was of borderline statistical significance ($P < 0.10$). A more detailed analysis of the association of these factors with CAD has been published (6). In this study population, the use of beta-blocker medications was not associated with any of the LDL particle characteristics after adjusting for the presence of CAD using either stratification or a linear regression model.

Correlations between different LDL particle characteristics are shown in **Table 2.** As expected, the different LDL particle characteristics were correlated. However, the correlation between the different measures was only moderate (r = 0.3–0.6 for most size measures). The LDL particle diameter measured by GGE, molecular weight calculated from particle composition, and the free cholesterol plus cholesteryl ester to apoB ratio were moderately intercorrelated for both the parent LDL and the major LDL subfraction (r = 0.45–0.83). The LDL particle diameter

TABLE 1. Clinical, lipoprotein, and apolipoprotein characteristics of 198 subjects[a] in the JH-CAD Study

	Coronary Artery Disease				
	Absent (n = 91)		Present (n = 107)		
Characteristic	N	%	N	%	*P* Value[b]
Sex, female	53	58%	51	46%	0.12
Smoking, ever	42	46%	93	85%	0.001
Diabetes	5	5%	22	20%	0.003
High blood pressure	39	43%	61	56%	0.08
Beta blockers	28	31%	60	55%	0.001
	Mean	SD	Mean	SD	
Age	47.4	7.8	49.5	6.0	0.04
Body mass index, kg/m²	27.1	5.3	27.3	4.3	0.79
LDL, mg/dl	115.9	40.1	127.5	45.2	0.06
HDL, mg/dl	56.6	17.0	51.3	14.3	0.02
ApoA-I, mg/dl	156.0	36.4	138.5	39.4	0.001
ApoB, mg/dl	130.1	36.8	156.2	36.7	0.0001
$\log_{10}$ (Triglycerides), mg/dl	2.03[c]	0.28	2.24[c]	0.25	0.0001

[a]At least 195 subjects were included for each variable except for body mass index (n = 188).
[b]*t*-Test for continuous variables and Fisher-exact test for categorical variables.
[c]Corresponding triglyceride levels are 107 mg/dl and 174 mg/dl, respectively.

TABLE 2. Correlation coefficients between different LDL particle characteristics among 198 subjects[a] in the JH-CAD Study

LDL	EDGU Rp	Diameter	MW	FC + CE / ApoB
Parent LDL				
Diameter (nm)			0.45	0.54
Molecular mass (Da)				0.83
Composition (% w/w)				
ApoB		−0.46	−0.99	−0.82
TG		−0.31	−0.14	−0.30
FC + CE		0.38	0.64	0.58
FC		0.60	0.43	0.57
CE		0.13	0.50	0.36
PL		0.24	0.27	0.35
(FC + CE)/apoB				
Major LDL				
EDGU Rp		0.61	0.52	0.54
Diameter (nm)			0.48	0.48
Molecular mass (Da)				0.74
Composition (% w/w)				
ApoB	−0.53	−0.48	−0.98	−0.72
TG	−0.01	−0.23	0.10	−0.06
FC + CE	0.34	0.47	0.64	0.54
FC	0.54	0.53	0.47	0.45
CE	0.01	0.16	0.37	0.28
PL	0.28	0.22	0.34	0.31
(FC + CE)/apoB				

Abbreviations: apoB, apolipoprotein B; TG, triglycerides; FC, free cholesterol; CE, cholesteryl ester; PL, phospholipid; MW, molecular weight.

[a]At least 175 subjects were included for all parent LDL correlations ($P < 0.05$ for $r > 0.15$; $P < 0.01$ for $r > 0.19$) and at least 147 subjects for all major LDL correlations ($P < 0.05$ for $r > 0.16$; $P < 0.01$ for $r > 0.22$).

and molecular weight were associated with their composition. The high correlation between the percent apoB in LDL and the LDL molecular weight is due to the fact that the latter was calculated based on the former. The measure of composition most tightly correlated with the diameter of both the parent and major LDL was the free cholesterol content. This is particularly striking given the fact that, on average, free cholesterol comprised less than 10% of the LDL mass. The largest source of variation in the average molecular weight of the LDL particle was the percent free and esterified cholesterol. The triglyceride content in LDL particles was negatively correlated with the LDL particle diameter.

LDL particle characteristics and CAD. **Table 3** shows the mean LDL particle characteristics for subjects by the presence or absence of CAD. Before adjustment, most LDL characteristics were associated with the presence of CAD. On average, patients with CAD had slightly smaller LDL particles of a lower molecular weight, which had a higher apoB and triglyceride content but a lower cholesterol content. The free cholesterol plus cholesteryl ester to apoB ratio was lower in the LDL particles of patients with CAD than of patients without CAD. The relative position (R_p) of the major LDL among patients with CAD was lower, indicating that the major LDL band was denser. The major LDL subfraction characteristics were associated with CAD in a way similar to the parent LDL characteristics.

Many factors associated with CAD confound the association between LDL particle characteristics and CAD. Smoking was the non-lipid factor most strongly associated with CAD. Male sex was an important confounder as it was associated with both CAD and smaller, more cholesterol-depleted LDL particles. Table 3 shows the significance of the association between each LDL characteristic and CAD after adjustment for age, sex, and smoking, as well as further adjustment for diabetes and a history of high blood pressure.

Correlations between LDL characteristics and other variables among subjects with no clinically significant CAD are shown in **Table 4.** The correlations were limited to subjects without CAD in order to avoid confounding by CAD. The select nature of this study population means that the correlations measured here are not necessarily representative of correlations in the general population. However, the correlations with LDL particle diameter are similar to those reported by Campos et al. (17) and are very important for assessing the possible confounding of the association between LDL particle characteristics and CAD.

None of the LDL characteristics showed a strong association with age. Women had different LDL characteristics than men. On average, women's LDL particles were larger and had a more bouyant major LDL band that contained less cholesteryl ester and more free cholesterol. Individuals with a higher LDL cholesterol concentration had LDL particles that were poor in triglycerides but enriched in cholesteryl ester. Individuals with lower HDL cholesterol tended to have smaller LDL particles with a lower molecular weight and lower free cholesterol content. The correlations between the plasma apoA-I level and LDL characteristics were similar to the correlations with HDL cholesterol. Individuals with higher apoB levels tended to have smaller LDL particles of a lower molecular weight and lower free cholesterol.

The associations between the plasma triglyceride level and LDL particle characteristics were particularly strong. The plasma triglyceride concentration was strongly positively correlated with the parent LDL triglyceride content ($r = 0.56$), and strongly negatively correlated ($r < -0.4$) with the parent LDL particle diameter, free cholesterol plus cholesteryl ester content, free cholesterol content, and major LDL R_p, particle diameter, and free cholesterol content. These associations were present at all levels of plasma triglyceride and were not limited to hypertriglyceridemic patients. It is of further interest that although the plasma triglyceride level was correlated with the LDL triglyceride content, this association was weaker

TABLE 3. LDL particle characteristics by presence of coronary artery disease among 198 subjects in the JH-CAD Study

Particle and Characteristic	Coronary Artery Disease: Absent (n = 91) (mean)	Coronary Artery Disease: Present (n = 107) (mean)	*P* Value: Crude[a]	*P* Value: Adj[b]	*P* Value: Adj[c]
Parent LDL					
Diameter (nm)	25.60	25.16	0.004	0.03	0.20
Molecular mass (10^6 Da)	2.22	2.13	0.002	0.02	0.45
Composition (% w/w)					
ApoB	24.9	26.0	0.001	0.01	0.39
TG	4.5	5.2	0.03	0.02	0.13
FC + CE	49.0	47.6	0.002	0.001	0.07
FC	8.4	7.7	0.0007	0.003	0.04
CE	40.6	39.9	0.08	0.02	0.31
PL	21.5	21.2	0.37	0.80	0.64
(FC + CE)/apoB	1.32	1.22	0.002	0.01	0.23
Major LDL					
EDGU Rp	0.74	0.71	0.0007	0.01	0.42
Diameter (nm)	25.56	25.05	0.0007	0.005	0.08
Molecular mass (10^6 Da)	2.05	1.91	0.0004	0.004	0.05
Composition (% w/w)					
ApoB	27.1	29.2	0.0002	0.002	0.03
TG	3.9	4.5	0.15	0.23	0.31
FC + CE	47.5	44.8	0.0001	0.001	0.002
FC	7.0	5.8	0.002	0.02	0.14
CE	40.5	39.0	0.009	0.006	0.002
PL	21.5	21.5	0.95	0.41	0.40
(FC + CE)/apoB	1.22	1.11	0.001	0.02	0.10

Abbreviations: apoB, apolipoprotein B; TG, triglycerides; FC, free cholesterol; CE, cholesteryl ester; PL, phospholipid.

[a] *P* values from a *t*-test.

[b] Adjusted for age and sex in ANOVA.

[c] Adjusted for age, sex, smoking, history of diabetes and blood pressure in ANOVA.

than the negative association with the LDL free cholesterol content. The strong correlation of plasma triglyceride level is consistent with the findings of Eisenberg et al. (23) and Deckelbaum et al. (24) who reported that the plasma triglyceride level was strongly associated with LDL particle characteristics. In general, the major LDL subfraction characteristics showed associations with the variables in Table 4 similar to the parent LDL characteristics.

Increased body mass index was associated with smaller parent LDL with less cholesterol and a smaller, denser major LDL band ($P < 0.05$). These associations were all dependent on the plasma triglyceride level. Diabetes was associated with a lower parent LDL molecular weight, free cholesterol content, and cholesterol to apoB ratio, and a lower major LDL relative position and free cholesterol content ($P < 0.05$). The associations with diabetes (except with the parent LDL cholesterol to apoB ratio) were independent of the plasma triglyceride concentration and the presence of CAD. The use of beta medications was not significantly associated with any of the LDL characteristics after adjusting for the presence of CAD (data not shown).

Table 5 presents the best multiple linear regression model for parent LDL particle diameter. LDL particle diameter was strongly associated with the plasma triglyceride and HDL level, and moderately associated with the plasma apoB and LDL level. Higher apoB levels were associated with smaller particle diameter while higher LDL cholesterol levels were associated with larger particle diameter once the apoB level was controlled for. Age, gender, and CAD status were forced into the model. However, it is clear that they were not associated with LDL particle diameter after adjustment for the other variables.

Multivariate analysis of LDL characteristics associated with CAD is presented in **Table 6.** The first odds ratio column shows the odds ratio of CAD associated with each LDL particle characteristic measured after adjustment for the traditional CAD risk factors (age, sex, smoking, diabetes, LDL, and HDL). Lower free cholesterol plus cholesteryl ester content was associated with CAD independently of the traditional risk factors whether measured in the par-

TABLE 4. Correlation coefficients between LDL particle characteristics and other characteristics among 91 subjects[a] without clinically significant CAD

Particle and Characteristic	Age	Sex[b]	LDL	HDL	ApoA-I	ApoB	log(TG)
Parent LDL							
Diameter (nm)	0.17	0.35	−0.06	0.51	0.33	−0.37	−0.45
Molecular mass (Da)	0.08	0.15	0.07	0.35	0.42	−0.23	−0.32
Composition (% w/w)							
ApoB	−0.08	−0.14	−0.07	−0.34	−0.42	0.21	0.32
TG	0.05	0.20	−0.33	−0.22	−0.23	0.14	0.56
FC + CE	0.10	−0.06	0.27	0.33	0.35	−0.13	−0.51
FC	0.15	0.17	0.04	0.48	0.35	−0.39	−0.62
CE	0.03	−0.16	0.28	0.12	0.21	0.06	−0.26
PL	−0.12	0.08	−0.05	0.05	0.13	−0.16	−0.05
(FC + CE)/apoB	0.08	0.14	0.15	0.43	0.38	−0.23	−0.43
Major LDL							
EDGU Rp	0.09	0.30	−0.23	0.52	0.33	−0.55	−0.57
Diameter (nm)	0.17	0.34	−0.05	0.52	0.36	−0.39	−0.54
Molecular mass (Da)	0.01	0.19	−0.15	0.19	−0.03	−0.31	−0.31
Composition (% w/w)							
ApoB	−0.01	−0.22	0.13	−0.20	0.04	0.32	0.36
TG	0.12	0.17	−0.18	−0.16	−0.26	0.09	0.28
FC + CE	−0.03	−0.00	0.09	0.22	0.08	−0.23	−0.38
FC	0.14	0.38	0.10	0.24	−0.02	−0.26	−0.44
CE	−0.14	−0.29	0.01	0.06	0.10	−0.05	−0.07
PL	−0.07	0.18	−0.17	0.13	0.10	−0.23	−0.24
(FC + CE)apoB	−0.06	0.12	−0.14	0.27	0.11	−0.28	−0.39

Abbreviations: apoB, apolipoprotein B; log(TG), $\log_{10}$ of triglycerides; FC, free cholesterol; CE, cholesteryl ester; PL, phospholipid.

[a]At least 84 subjects were included for all parent LDL correlations ($P < 0.05$ for $r > 0.21$; $P < 0.01$ for $r > 0.28$) and at least 70 subjects for all major LDL correlations ($P < 0.05$ for $r > 0.23$; $P < 0.01$ for $r > 0.30$).

[b]Female sex coded as 1; male sex coded as 0.

ent or major LDL. In addition, a higher triglyceride content and lower free cholesterol content for the parent LDL, and a smaller diameter ($P = 0.08$), smaller molecular weight, and lower cholesteryl ester content in the major LDL were associated with CAD independent of the traditional CAD risk factors. Generally, the major LDL characteristics were more strongly associated with CAD than the characteristics measured on the more heterogeneous parent LDL.

Adjustment for apoB decreased the magnitude of all associations, suggesting that some of the predictive value of LDL characteristics is due to their correlation with the apoB concentration. However, the total cholesterol content of the major LDL was still significantly associated with CAD and the magnitude of the odds ratios of many of the other LDL characteristics remained substantial despite their significance decreasing to the borderline range ($P \leq 0.10$). Adjustment for the plasma triglyceride level markedly reduced all of the associations leaving none with a P value less than 0.05. The plasma triglyceride level, however, remained significant in all of these models. Thus, the association between all of the LDL particle characteristics measured and CAD was dependent on the plasma triglyceride level. Adjustment for apoA-I level gave results similar to the adjustment for HDL level; the odds ratio for the association between the free cholesterol plus cholesteryl ester content of the major LDL and CAD remained at 1.8 (95% CI 1.0–3.2) after adjustment for age, sex, smoking, diabetes, apoB, apoA-I, and plasma triglyceride levels simultaneously.

TABLE 5. Best multiple linear regression model explaining variation in LDL diameter ($r^2 = 0.41$)

				Partial r^2	
Variable[a]	Beta	S.E.	P	Type I[b]	Type II
Age, years	0.02	0.12	0.89	0.018	0.0001
Sex, female	2.47	1.68	0.14	0.126	0.012
CAD, yes/no	0.47	1.36	0.73	0.038	0.0007
log TG, $\log_{10}$	−12.37	3.04	0.0001	0.262	0.086
HDL, mg/dl	0.18	0.04	0.0001	0.073	0.091
ApoB, mg/dl	−0.07	0.02	0.005	0.014	0.044
LDL, mg/dl	0.05	0.02	0.02	0.032	0.032

[a]Age, sex, and CAD were forced to remain in model.

[b]Partial r^2 is calculated in the order listed for Type I and with each variable being the last one in the model for Type II.

Association of LDL characteristics with CAD in men and women separately. The direction of the association of LDL characteristics with CAD was the same in men as in women for all LDL characteristics. Overall, the magnitude of the association between the different LDL characteristics and CAD was similar for men and women. The association of LDL diameter with CAD was somewhat

TABLE 6. Logistic regression of CAD on LDL particle characteristics among subjects in the JH-CAD Study

Particle and Characteristic	Direction	IQR	Odds Ratio (95% Confidence Interval)[a]: Variables Adjusted for in Each Logistic Regression Model: Age Sex Smoking Diabetes LDL HDL		Age Sex Smoking Diabetes apoB		Age Sex Smoking Diabetes Log(TG)	
Parent LDL								
Diameter (nm)	–	1.60	1.46	(0.8–2.6)	1.22	(0.7–2.2)	0.92	(0.5–1.7)
Molecular mass (10^6 Da)	–	0.25	1.29	(0.8–2.1)	1.23	(0.8–2.0)	1.08	(0.7–1.8)
Composition (% w/w)								
ApoB	+	3.0	1.36	(0.8–2.2)	1.28	(0.8–2.1)	1.11	(0.7–1.9)
TG	+	2.5	1.69*	(1.0–2.8)	1.48*	(0.9–2.3)	1.01	(0.6–1.7)
FC + CE	–	4.2	1.84*	(1.1–3.1)	1.60*	(1.0–2.6)	1.21	(0.7–2.1)
FC	–	2.0	1.92*	(1.1–3.3)	1.63*	(1.0–2.7)	1.18	(0.6–2.2)
CE	–	3.6	1.42	(0.9–2.3)	1.37	(0.9–2.1)	1.13	(0.7–1.8)
PL	+	2.6	1.08	(0.6–1.8)	1.14	(0.7–1.9)	1.20	(0.7–2.1)
(FC + CE)/apoB	–	0.26	1.49	(0.9–2.5)	1.34	(0.8–2.2)	1.02	(0.6–1.8)
Major LDL								
EDGU Rp	–	0.11	1.22	(0.7–2.2)	0.90	(0.5–1.7)	0.80	(0.4–1.6)
Diameter (nm)	–	1.45	1.68*	(0.9–3.0)	1.32	(0.7–2.3)	0.94	(0.5–1.8)
Molecular mass (10^6 Da)	–	0.34	1.75*	(1.0–3.0)	1.54	(0.9–2.7)	1.52	(0.9–2.6)
Composition (% w/w)								
ApoB	+	4.9	1.89*	(1.1–3.3)	1.67*	(0.9–2.9)	1.60	(0.9–2.8)
TG	+	3.6	1.43	(0.9–2.4)	1.34	(0.8–2.2)	0.99	(0.6–1.7)
FC + CE	–	5.4	2.62**	(1.4–4.8)	2.09*	(1.2–3.7)	1.79*	(1.0–3.2)
FC	–	3.6	1.59	(0.9–2.9)	1.32	(0.7–2.5)	1.17	(0.6–2.2)
CE	–	4.3	1.82*	(1.1–2.9)	1.73*	(1.1–2.8)	1.51*	(0.9–2.4)
PL	+	2.6	1.12	(0.7–1.8)	1.17	(0.7–1.9)	1.26	(0.7–2.1)
(FC + CE)/apoB	–	0.27	1.61*	(0.9–2.7)	1.62*	(0.9–2.8)	1.32	(0.8–2.3)

Abbreviations: IQR, inter-quartile range; apoB, apolipoprotein B; log(TG), $\log_{10}$ of triglycerides; FC, free cholesterol; CE, cholesteryl ester; PL, phospholipid.

[a]Odds ratios were calculated for the 75th minus 25th percentile for LDL characteristics positively (direction +) associated with CAD and for the 25th minus the 75th percentile for LDL characteristics negatively (direction –) associated with CAD.

*$0.05 < P \leq 0.10$; **$0.01 < P \leq 0.05$; ***$P \leq 0.01$.

stronger in women than in men; the odds ratio associated with major LDL diameter adjusted for the traditional risk factors was 2.1 (95% CI 1.0–4.5) in women compared to 1.2 (95% CI 0.5–2.8) in men. However, the characteristics based on composition were somewhat more strongly associated with CAD among men than women; the odds ratio of CAD associated with free cholesterol plus cholesteryl ester in the major LDL adjusted for the traditional risk factors was 3.0 (95% CI 1.3–7.1) for men and 2.3 (95% CI 1.1–5.2) for women. Neither of these differences between men and women was statistically significant. As there was no evidence for different associations in men than in women, combining the two genders in a common analysis was reasonable, particularly in view of the relatively small number of observations and strong confounders involved.

DISCUSSION

This study measured a wide range of LDL characteristics. The LDL composition was generally similar to that reported in smaller study populations (23–25). The LDL particle diameters measured visually were comparable to the scanned data reported by Austin et al. (16) in which patients with CAD had predominantly pattern B LDL (diameter < 25.5 nm) and controls had predominantly pattern A LDL (diameter ≥ 25.5 nm). The different LDL characteristics calculated were moderately intercorrelated. The fact that the correlation between the different measures is only moderate, rather than high, is partially due to the limited precision inherent in these measures. However, it also suggests that LDL particles vary along more than one dimension between individuals. Thus, different LDL characteristics contribute different information about the LDL particles.

The LDL characteristics associated with CAD in unadjusted analysis, namely, smaller particle diameter, lower molecular weight, higher apoB and triglyceride composition lower cholesterol and cholesteryl ester content, and a lower cholesterol plus cholesteryl ester to apoB ratio, were also highly associated with other patient characteristics that are known to be associated with CAD. Age was not strongly associated with LDL characteristics.

Women had larger LDL particles and a less dense major LDL band (Table 4). The larger LDL particle diameter among women was mainly related to their higher HDL and lower triglyceride levels compared to men. After adjustment for these variables, gender was no longer associated with LDL size (Table 5). This is similar to the findings of Swinkles et al. (26) who studied 146 healthy blood donors and found that gender was not significantly associated with the LDL subfraction distribution after adjustment for the serum triglycerides or HDL cholesterol level. LDL particle characteristics were also associated with the subject's HDL, apoA-I, and apoB levels, and very strongly associated with the plasma triglyceride levels. The latter association was present for the entire range of triglyceride concentrations, and was not limited to patients with hypertriglyceridemia. Other studies have reported this strong association across the range of triglyceride concentrations as well (11, 18, 26, 27). Furthermore, Eisenberg et al. (23) have shown that hypertriglyceridemic individuals have smaller LDL and more triglyceride-enriched particles than normolipidemic controls and these abnormalities reverted toward normal after treatment with bezafibrate. Deckelbaum et al. (24) further suggest that high levels of plasma triglyceride-rich lipoproteins serve as acceptors for cholesteryl esters and other constituents from LDL in an exchange for triglycerides mediated by lipid transfer proteins. The triglyceride in the LDL particles can then be removed by the action of lipoprotein lipase which would finally result in lipid-poor, protein-rich, dense LDL particles.

After adjustment for the major CAD risk factors (age, sex, smoking, diabetes, LDL, and HDL) the patients with CAD had LDL particles with a higher triglyceride and lower free cholesterol and free cholesterol plus cholesteryl ester content. Adjusted for the same variables the major LDL species of patients with CAD was smaller (P = 0.08), had a lower molecular weight (i.e., higher apoB content), a lower cholesteryl ester and cholesteryl ester plus free cholesterol content. The association of the parent LDL triglyceride content with CAD is consistent with the findings of Aviram et al. (28) that LDL triglyceride content influences the interaction of apoB on LDL with cells. However, this association completely disappeared after adjustment for the plasma triglyceride concentration.

Among the different LDL characteristics measured, the percent free cholesterol plus cholesteryl ester by mass was the characteristic most strongly and consistently associated with CAD. This can be explained by the fact that the percent total cholesterol includes in its numerator the free cholesterol and cholesteryl ester, both of which were negatively associated with CAD, while its denominator contains the triglyceride level which was positively related to CAD; the protein content per particle is constant and the phospholipid content was unrelated to CAD. Other than this mathematical explanation, the biological reason that this characteristic was the one most strongly associated with CAD is not clear at the present time. However, it must be noted that the confidence intervals for the association of LDL cholesterol plus cholesteryl ester content and LDL molecular weight with CAD were widely overlapping, which suggests the differences in the magnitude of association with CAD between the two measures could be due to chance.

The direction of the association with CAD was negative for both the cholesteryl ester and the free cholesterol content despite the fact that the former is largely located in the core of the LDL particle while the latter is largely located on the surface of the particle. LDL particles with less cholesteryl ester, which composes most of the LDL particle core lipid, should have a smaller particle diameter that may be important in atherogenesis, as will be discussed later. However, it is not clear why LDL with a lower free cholesterol content would be more atherogenic. The free cholesterol composes less than 10% of the total LDL mass and is therefore unlikely to play a major role in determining the LDL molecular weight. Vakakis et al. (29) found that red blood cells and LDL particles from hypertriglyceridemic patients have a lower ratio of unesterified cholesterol to phospholipid than normal. They suggest that this is due to the fact that newly secreted triglyceride-rich lipoproteins are poor in cholesterol and their excess production may prevent maintenance of the normal cholesterol content of red blood cells and LDL particles. Therefore, the lower free cholesterol content in the LDL of subjects with CAD may be secondary to increased VLDL synthesis. The marked drop in the strength of association between free cholesterol content of LDL and CAD after adjustment for the plasma triglyceride level (odds ratio of CAD dropped from 1.92 to 1.18 for the parent LDL and from 1.59 to 1.17 for the major LDL) suggests that the lower cholesterol to phospholipid ratio itself does not play a direct role in atherogenesis.

Adjustment for the plasma triglyceride level reduced the magnitude of all of the associations substantially and left none that was significant at the 0.05 level. This finding is consistent with previous studies of LDL particle diameter and myocardial infarction (16, 17, 30). Austin et al. (16) reported LDL subclass B, characterized by a preponderance of small, dense LDL particles, to be associated with myocardial infarction, (OR 3.0, 95% CI 1.7–5.3) after adjustment for age, sex, and body mass index. However, further adjustment for plasma triglyceride level decreased the odds ratio to 1.6 (95% CI 0.8–3.2). Crouse et al. (30) reported that LDL molecular weight estimated using ultracentrifugation was lower among 46 cases with CAD (documented arteriographically) than among 47 controls, but the difference was no longer present after adjustment for plasma triglyceride level. Campos et al. (17) reported that LDL diameter measured

by GGE was associated with CAD before but not after adjustment for HDL or apoA-I.

It is clear that many patients with CAD have an elevated plasma triglyceride level combined with certain LDL characteristics. However, none of the LDL characteristics measured in this study was more closely associated with CAD than the plasma triglyceride level. The strong dependency of the association of LDL characteristics and CAD on the plasma triglyceride level is intriguing as the mechanism and magnitude of association of triglyceride levels and CAD is controversial and poorly understood. Studies by Austin and colleagues (14, 15) have shown that LDL particle subclass pattern B may be under control of a dominant major gene. Recent work by Nishina et al. (31) has indicated that the atherogenic lipoprotein phenotype, LDL subclass pattern B, is linked to the LDL and insulin receptors on the short arm of chromosome 19. However, other loci may be involved in influencing LDL particle size in the population (32). Characterization of the molecular defect or defects causing the segregation of LDL particle subclass may allow further dissection of the connection between LDL particle characteristics, triglyceride concentration and CAD.

Recent studies (33–35) have pointed out that smaller LDL particles are more easily oxidized suggesting they may be more atherogenic. Teng et al. (36) found that smaller, denser LDL particles did not react as well as light LDL with monoclonal antibodies to the portion of apoB that is recognized by the high-affinity LDL receptor. Arad et al. (37) presented findings suggesting that LDL has an optimal size for maximal binding to monoclonal antibodies as well as the LDL receptor of fibroblasts. These in vitro observations are consistent with in vivo studies in which small, dense LDL did not appear to be cleared from the circulation as quickly as lighter, more buoyant LDL in both normal and hyperapoB subjects (38). These data suggest that small dense LDL is less likely to be cleared from the circulation by the high-affinity LDL receptor, and more likely to have a longer plasma residence time, to be oxidized, and to be taken up by macrophages in atheroma. This evidence for the biologic plausibility of the hypothesis that LDL particle characteristics are important in mediating the association between hypertriglyceridemia and CAD suggests that this association deserves further examination despite its lack of independence of the plasma triglyceride level. Furthermore, the relatively high variability in the detailed measures of LDL particle characteristics, which involve multiple steps prior to final measurement, compared to the simpler measurement of the plasma triglyceride level would tend to reduce their relative strength of association with CAD.

This study measured many LDL characteristics simultaneously in order to determine their association with CAD. None of the characteristics was associated with CAD independently of the plasma triglyceride level. CAD was clearly associated with the combined phenotype of hypertriglyceridemia and small dense, cholesterol and cholesteryl ester-depleted LDL particles. This phenotype often reflects over-production of VLDL, and accompanying hypertriglyceridemia (38). The pathogenetic link between the hypertriglyceridemia and abnormal LDL characteristics makes it difficult to separate their relative contribution to the pathogenesis of CAD using statistical techniques.

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