Studies of low density lipoprotein molecular weight in human beings with coronary artery disease

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Abstract Low density lipoprotein molecular weight (LDL MW) correlates positively with coronary artery disease in cholesterol-fed nonhuman primates. To evaluate this in human beings with coronary artery disease (CAD) we measured LDL MW in 93 volunteers undergoing coronary angiography (47 controls and 46 CAD patients). LDL MW of CAD patients was less than that of controls (patients, 2.79 ± 0.17 g/μmol; controls, 2.93 ± 0.19 g/μmol; $P < 0.001$). However, LDL MW decreased as plasma triglyceride increased and concentrations of triglyceride were greater in CAD patients than in controls. Since decreased LDL MW is likely to result, in part, from increased plasma triglyceride concentrations, we attempted to determine the effect of triglyceride on the relation of LDL MW to CAD in this study. After covariance adjustment for triglyceride, there was no LDL MW difference between CAD patients and controls. Because LDL heterogeneity has been identified in other studies and was apparent on inspection of agarose column profiles of LDL of these volunteers, we sought differences in the profiles that might distinguish coronary disease cases from controls. No differences could be found. In addition, we used density gradient ultracentrifugation to characterize LDL in more detail in a subset of volunteers who had a wide range of plasma triglyceride concentrations (50 mg/dl to 900 mg/dl). LDL mean hydrated density was inversely related to LDL MW and increased as triglyceride increased. The increase in peak density was reflected in an increase in percent of total protein in LDL found to have d > 1.045 g/ml and a decrease in protein in LDL of d 1.035–1.040 g/ml. These interrelationships were not apparently influenced by coronary artery status. These observations suggest that the relationship between LDL MW, LDL density, and coronary disease is complex and dependent, in part, on plasma triglyceride concentration. When viewed in conjunction with the LDL MW measurements made by others in nonhuman primates, the assessments of LDL MW in men suggest that the high molecular weight (cholesteryl ester-rich) LDL and coronary atherosclerosis produced by feeding cholesterol to nonhuman primates results partly from their relatively low plasma triglyceride. Plasma triglyceride concentrations that are higher than those in nonhuman primates (but within the normal range for human beings) may diminish the potential for LDL cholesteryl ester accumulation and LDL enlargement with cholesterol feeding and thus may be "anti-atherogenic." Supranormal plasma triglyceride concentrations and diminished LDL MW, however, were both found to be associated with coronary disease in this study. — **Crouse, J. R., J. S. Parks, H. M. Schey, and F. R. Kahl.** Studies of low density lipoprotein molecular weight in human beings with coronary artery disease. *J. Lipid Res.* 1985. **26:** 566–574.

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Low density lipoprotein molecular weight (LDL MW) correlates with coronary disease in certain species of cholesterol-fed nonhuman primates (1). In one study, LDL MW alone explained 44% of the variability in coronary atherosclerosis (1). Increases in LDL MW were accompanied by preferential enrichment of particles with cholesteryl ester and were positively correlated with total plasma cholesterol. In addition, with LDL enlargement, saturated and monosaturated cholesteryl ester fatty acids were found to increase preferentially relative to polyunsaturated fatty acids (2). As a result, physical-chemical studies of LDL have revealed a transition in the core cholesteryl ester from an ordered smectic-like (layered) structure to a more disordered state that occurs above body temperature for large LDL (MW > 4 g/μmol) while the transition occurs below body temperature for normal LDL (MW < 4 g/μmol) (2). The high molecular weight LDL has been found to deliver more cholesterol to cells in culture than control LDL (3), and, in addition, ordered cholesteryl esters are removed less efficiently from cells than liquid (disordered) cholesteryl esters (4). These observations may partly explain the association between LDL MW and coronary atherosclerosis in nonhuman primates.

High molecular weight LDL has been sought in patients with familial hypercholesterolemia (5–7) and in cholesterol-fed volunteers (8) but no consistent relationship has been found. Hypertriglyceridemic diabetic patients with clinical evidence of atherosclerosis may be more likely to have "polydisperse" LDL than disease-free

Abbreviations: LDL, low density lipoprotein; CAD, coronary artery disease; LDL MW, low density lipoprotein molecular weight; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; HDL, high density lipoprotein; ILDL, intermediate size low density lipoprotein.

controls (7), but LDL MW has not been systematically related to coronary disease in volunteers referred for evaluation of their coronary status.

Studies of LDL MW and density have previously been carried out in healthy individuals, and both have been found to correlate with triglyceride concentrations (9, 10). However, patients with CAD have not been evaluated for possible perturbations in these interrelationships.

The present studies were undertaken to evaluate the role of LDL MW in patients with coronary disease and to determine whether coronary status affects correlations between LDL MW, LDL density, and plasma triglyceride concentrations.

METHODS

Patient selection

The male volunteers selected for this study were part of a case-comparison study of risk factors and coronary artery disease (CAD). Patients were accessed from April to December, 1982. For this study, all patients undergoing cardiac catheterization and selective coronary angiography at the North Carolina Baptist Hospital were evaluated and individuals were excluded who had any of the following conditions: *1*) use of certain medications (cortisone, heparin, lipid-lowering drugs); *2*) presence of certain diseases (myocardial infarction within the prior 6 weeks, New York Heart Association class IV status; hepatic, thyroid, or renal disease); *3*) presence of conditions that interfere with angiographic assessment (coronary bypass or angioplasty). Following angiography, volunteers were divided according to coronary status into those with obstructive coronary disease, those with non-obstructive coronary disease, and disease-free controls (see below). Because over 40% of all individuals undergoing catheterization are found to be males older than 50 with obstructive coronary disease, we also separated volunteers into those older than and those younger than 50 years of age. After excluding patients with non-obstructive disease (see below), we employed a random sampling strategy to each of the four groups (males with coronary disease >50 years of age, males free of disease >50, males with disease ≤ 50, males free of disease ≤ 50) so that only 10% of volunteer males with coronary disease older than 50 were chosen for the study, whereas for the other three groups the percents chosen were 100%, 35% and 80%, respectively. This led to the following numbers in each of the four groups: 19, 24, 27, 23 (46 CAD patients and 47 controls). The clinical diagnoses in the 47 controls were as follows: 16, valvular heart disease (7 aortic valve disease, 5 mitral valve disease, 4 combined valvular disease); and 31 noncoronary chest pain syndrome [21 Rose Questionnaire class 1 or 2 (exercise-related chest pain) and 10 Rose Questionnaire class 3 (non-exercise-related chest pain)]. Seven hundred and twenty-five individuals were screened and 300 were subjected to the random sampling process to obtain these 93 volunteers. Eighty-seven volunteers were excluded because of non-obstructive coronary artery disease. Three hundred and thirty-eight individuals were excluded for one of the clinical conditions listed above. Sixty-three percent of clinical exclusions were due to one of three conditions (refusals, 22%; recent infarct, 27%; coronary bypass or angioplasty, 14%); only 8 volunteers (1% of all individuals screened) were excluded because of use of lipid-lowering drugs. Clinical data for the 93 volunteers chosen for the study are presented in **Table 1.**

Evaluation of coronary angiograms

Coronary angiograms were in all instances evaluated independently on two occasions. The first evaluation was the clinical evaluation and the second was the "research" evaluation. For the second reading, angiograms were evaluated jointly by two experienced angiographers. For this study control patients were defined as those who had strictly normal angiograms; CAD patients were defined as those with $\geq 50\%$ stenosis of at least one major coronary artery. Patients with luminal irregularities or moderate diameter reduction of $<50\%$ were considered to have non-obstructive disease and were excluded from this study. On re-reading, 1% of patients had angiograms that were felt to be "uninterpretable" by the "research" evaluation and 9% of angiograms originally read as normal ("clinical" evaluation) were felt on re-reading to have non-obstructive coronary disease. No patients originally diagnosed as having obstructive disease were found on re-reading to have non-obstructive coronary disease. For patients with obstructive coronary disease, 22% had single vessel disease, 29% had double vessel disease, and 49% had triple vessel disease. "Clinical" and "research" readings agreed as to numbers of vessels involved in 91% of cases.

TABLE 1. Clinical data of volunteers

Clinical Class	n	Mean Age	Hypertension[a]	Diabetes[b]	Smoking[c]	Positive Family History[d]	Mean IBW[e]
		yr			%		
Control	47	50	40	4.2	59.6	27.6	118
Patient	46	57	43	13.0	76.1	26.1	120

[a]Percent of volunteers with a positive history of high blood pressure or blood pressure > 140/90.
[b]Percent of volunteers with a history of Type I or Type II diabetes.
[c]History of smoking > 5 pack-years.
[d]Positive history for coronary disease before age 55 in one or more first-degree relatives.
[e]Percent ideal body weight.

Analyses

Venous blood was drawn from patients who had fasted for 12 hr; blood was drawn on the morning of the catheterization and prior to removal to the catheterization laboratory. Patients who might have needed intravenous fluids prior to catheterization because of renal disease or a clinically unstable condition were excluded from the study (see above), and the morning's medications had not been administered at the time of venipuncture. Before initiating this study we carried out a pilot project in 23 patients to compare lipid concentrations in venous blood drawn in patients' rooms the morning of catheterization with arterial samples drawn in the cardiac catheterization laboratory at the time of catheterization. Lipid concentrations in venous samples drawn prior to catheterization were significantly higher than those found in arterial samples drawn at the time of catheterization. Although the differences were, on the average, not large (for total cholesterol the difference was 5%, $P < 0.01$), in isolated cases more marked differences were noted. For this reason, all samples for the study were drawn in patients' rooms the morning of catheterization. This way we also avoided problems that might have developed from patients undergoing catheterization late in the afternoon and thus fasting for a variable length of time.

Plasma samples were analyzed for total plasma cholesterol and triglyceride and lipoprotein cholesterol using routine AutoAnalyzer II and Lipid Research Clinics methodology (11). All plasma samples were ultracentrifuged at d 1.006 g/ml to float very low density lipoproteins (VLDL) prior to analysis of the infranate for LDL cholesterol and LDL MW.

Apparent LDL MW was measured after a second ultracentrifugation of the d > 1.006 g/ml fraction at d 1.225 g/ml as previously described (12). One to three ml of the material of 1.006 g/ml < d < 1.225 g/ml was mixed with 20 μl of an ^{125}I-labeled LDL standard (40,000 cpm) of known molecular weight. The LDL standard was isolated from rhesus monkey plasma, was iodinated by the iodine monochloride method, and molecular weight was determined by analytical ultracentrifugation (2.9 g/μmol). These procedures have previously been described in greater detail (13). Sample and standard were mixed and applied to a column (90 × 1.6 cm) of Bio-Gel A-15m (Bio-Rad; Richmond, VA). Lipoproteins were eluted with a 1.006 g/ml NaCl solution (0.01% EDTA, 0.01% sodium azide, pH 7.4) at a rate of 5 ml/hr. The elution pattern of whole plasma d < 1.225 g/ml lipoproteins of nonhuman primates typically consists of four peaks of decreasing molecular size (1): peak I, void volume material (VLDL); peak II, intermediate size low density lipoprotein (ILDL); peak III, low density lipoprotein (LDL); and peak IV, high density lipoprotein (HDL). The apparent LDL ["peak III" (1)] molecular weight was calculated from the relative elution ratio of the LDL and ^{125}I-labeled LDL peaks as described previously (12). This involved finding the peak of the LDL fraction by reading the absorbance at 280 nm of each tube across the peak. The ^{125}I-labeled LDL peak was located by counting tubes in a gamma counter.

In a subset of individuals (16 CAD patients and 13 controls) whose plasma lipid concentrations were comparable to those of the larger groups, the material that eluted from the agarose column in the region of LDL peak III) was pooled and refractionated. This peak contained 80–100% of LDL defined by standard density intervals (d 1.019–1.063 g/ml) (L. Rudel, personal communication). To refractionate this material we used a discontinuous density gradient ultracentrifugation method developed for this purpose as follows. The density gradient consisted of 12 ml of a d 1.060 g/ml solution overlayered with the sample in a volume of 19 ml at a density of 1.030 g/ml, which in turn was overlayered with 8 ml of a solution of density 1.006 g/ml. Samples were spun to equilibrium density in a Beckman VTi-50 vertical rotor for 6 hr at 50,000 rpm at 20°C. Tubes were then pierced and a solution of Fluorinert (d 1.9 g/ml; ISCO, Inc., Lincoln, NE) was pumped into the tube. The sample was drained through a UV monitor and fractions (0.9 ml/tube) were collected. The fractions were then divided arbitrarily into four density cuts at d < 1.035, d 1.035–1.040, d 1.040–1.045, and d > 1.045 g/ml. A tracer amount of the ^{125}I-labeled LDL standard was added to each sample to monitor the reproducibility of the gradient. Individual fractions were counted in a gamma counter to localize the ^{125}I-labeled LDL peak. The density of individual fractions was determined by pycnometry. Protein was quantified by a modified Lowry method using bovine serum albumin as a standard (14).

Qualitative differences in the agarose column elution profiles of the material of 1.006 g/ml < d < 1.225 g/ml could be observed. When a definite population of particles of size comparable to the "peak II" material of nonhuman primates was observed, it was collected and refractionated using the density gradient procedure described above for peak III material.

Statistical analysis

Patients were compared with controls using the standard *t* test or analysis of covariance (BMDP PlV). In cases where nonparametric testing was appropriate, chi square tests of association using 2 × 2 contingency tables were used.

RESULTS

Table 2 presents data for plasma lipid and lipoprotein cholesterol and triglyceride concentrations as well as

TABLE 2. Total plasma and lipoprotein lipid concentrations and low density lipoprotein molecular weight in volunteers

Clinical Class	Total Cholesterol	Total Triglyceride	LDL Cholesterol	HDL Cholesterol	LDL Molecular Wt
			mg/dl		
Control	188 ± 31[a] (95-246)[b]	164 ± 100 (50-552)	123 ± 28 (49-176)	36 ± 9 (21-65)	2.93 ± 0.18 (× 10^6) (2.66-3.33 × 10^6)
Patient	219 ± 44 (148-314)	266 ± 100 (100-1345)	140 ± 36 (39-218)	30 ± 5 (17-43)	2.79 ± 0.16 (× 10^6) (2.29-3.02 × 10^6)
P[c]	<0.001	<0.01	<0.025	<0.001	<0.001

[a]Mean ± SD.
[b]Range.
[c]*P*, Comparison of mean lipid value on controls and patients.

mean LDL MW in control patients and coronary disease (CAD) patients. In order to determine whether plasma lipid concentrations in the volunteers selected for study by the random sampling process were representative of the entire group of patients, mean concentrations of total plasma, LDL, and HDL cholesterol and of plasma triglyceride were compared for selected and nonselected volunteers. No statistically significant differences were found between mean values for selected volunteers and those individuals who volunteered and had blood drawn but were not selected by the random sampling process. For total plasma cholesterol concentration, 2% of controls and 21% of coronary disease patients fell in the top 5% of the Lipid Research Clinics population (15). For total triglyceride concentration, comparable percents were 8% (controls) and 21% (coronary disease patients). As has been shown by others, CAD patients had higher concentrations of LDL cholesterol and lower concentrations of HDL cholesterol than controls. In addition, coronary disease patients tended to have higher triglyceride concentrations than controls. The mean LDL MW of patients was significantly lower than that of controls [2.79 ± 0.18 vs 2.93 ± 0.18 g/μmol, $P < 0.001$]. For all volunteers the scatter diagram of LDL MW versus LDL cholesterol had no pattern, but LDL MW decreased as plasma triglyceride increased (**Fig. 1**). The plot of LDL MW versus triglyceride was not linear, and therefore a log transformation of triglyceride was used for regression versus LDL MW. The regression equation was [LDL MW = −0.47 (log) Tg + 3.9; $r = -0.64$, $P < 0.001$]. Since decreased LDL MW is likely to result, in part, from increased triglyceride concentrations (see discussion below), and since plasma triglyceride was higher in patients than controls, we compared LDL MW of patients and controls after covariance adjustment for log triglyceride. After covariance adjustment the LDL MW difference between patients and controls was no longer statistically significant. The mean covariance adjusted values were 2.89 ± 0.15 g/μmol for controls and 2.83 ± 0.15 g/μmol for CAD patients ($F = 2.85$; $0.075 < P < 0.10$).

Density gradient centrifugation was performed on LDL ["peak III" (ref. 1)] from subsets of 13 controls and 16 CAD patients with mean total plasma triglyceride, total plasma cholesterol, LDL cholesterol, and HDL cholesterol that were comparable to group means for controls and patients. The ranges for total plasma triglyceride in

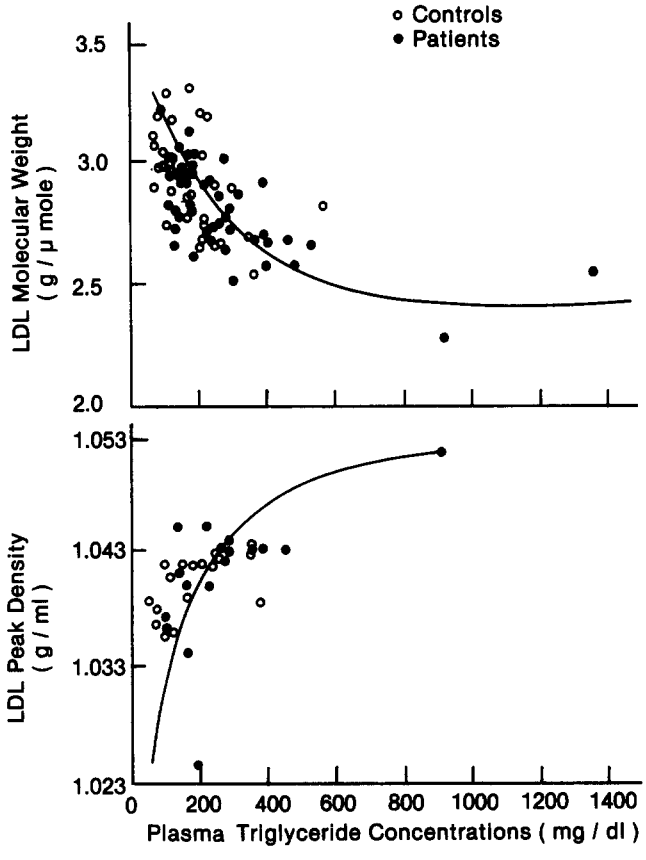

Fig. 1 Relationship of LDL size (MW) and LDL peak density to plasma triglyceride concentrations for controls (○) and patients with coronary artery disease (●). Values for LDL MW and density were obtained as described in Methods. The top panel contains data from 93 volunteers while the bottom panel contains data from a subset of them (n = 34).

these subsets were 50–383 mg/dl (controls) and 100–516 mg/dl (patients). Illustrative density gradient profiles are shown in **Fig. 2** for a control with elevated serum triglyceride concentration and a CAD patient with normal triglyceride concentrations. The LDL density gradient profile generally revealed apparently monodispersed LDL (Fig. 2, bottom) or a major peak with a shoulder (Fig. 2, top). In this subset of 29 volunteers no patient-control difference could be demonstrated in peak LDL density. The LDL peak density was 1.0410 ± 0.0036 g/ml (mean ± SD) for CAD patients versus 1.0406 ± 0.0025 g/ml for controls. A trace amount of ^{125}I-labeled LDL was included in each run as an internal standard and the ^{125}I count profile is shown in Fig. 2 also. The position of the tracer was very similar in all runs and averaged 1.038 ± 0.001 g/ml. Tubes from the density gradient run that fell between the indicated vertical marks on the absorbance profile were pooled into four fractions and saved for protein analysis. A comparison of LDL MW versus LDL peak density showed that as LDL MW increased the LDL peak density decreased. This relationship was similar for CAD patients and controls.

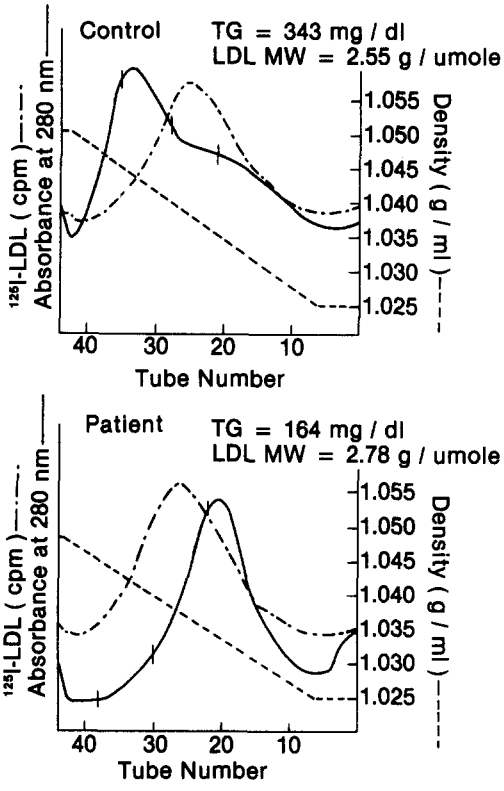

Fig. 2 Discontinuous density gradient centrifugation profile of LDL isolated from a control subject (top) and a patient with coronary artery disease (bottom). LDL were isolated from plasma by ultracentrifugation and agarose column chromatography. The isolated LDL (absorbance; —) along with a trace amount of ^{125}I-labeled LDL (cpm; - - -) were subfractionated by density gradient centrifugation as described in the Methods section. Vertical lines on the absorbance profile indicate where samples were pooled for protein quantitation.

The relationship of plasma triglyceride concentration to LDL peak density and protein distribution was examined also. As plasma triglyceride increased there was an increase in LDL peak density that corresponded to the decrease in LDL MW (Fig. 1). When plasma triglyceride concentrations were > 400 mg/dl the LDL peak density and LDL MW appeared to approach limiting values. The relationships between plasma triglyceride concentrations and LDL properties were similar for CAD patients and controls. The relationship of plasma triglyceride concentrations to the density distribution of LDL protein is shown in **Fig. 3.** Similar trends were seen for CAD patients and controls. As plasma triglyceride increased there was a significant decrease in the percentage of LDL protein distributed in the d 1.035–1.040 g/ml fraction and a corresponding increase in the d > 1.045 g/ml fraction. The percentage protein in the d < 1.035 and d 1.040–1.045 g/ml fractions did not vary with plasma triglyceride concentrations.

When isolated from an agarose column and respun in a density gradient, the peak II and peak III material (described above) each showed a certain degree of heterogeneity, and the designations ILDL and LDL serve to identify the major fraction in each peak. The "atherogenic" LDL most consistently observed in three species of nonhuman primates fed cholesterol elutes from the column as a large molecular weight particle in the peak III region (1). In addition, in certain other experiments, feeding cholesterol to nonhuman primates has led to accumulation of material of various densities (d < 1.006, 1.006 < d < 1.019, 1.019 < d < 1.050, and 1.050 < d < 1.100 g/ml) in the peak II (size) region (12, 13). In an effort to compare this to the situation in human beings, we evaluated the agarose column profiles in these volunteers. As described above, qualitative differences in the agarose column elution profiles of the material of 1.006 < d < 1.225 g/ml could be observed in our volunteers. The agarose column profile of the plasma material of 1.006 < d <1.225 g/ml of the majority of volunteers (52/93) was characterized by, in addition to the peak for HDL, a single symmetric peak in the region of LDL (peak III). In 32 volunteers, the material eluting in the peak III region was asymmetric, and 9 volunteers had, in addition to material in the region of peak III, a separate peak in the area corresponding to peak II in nonhuman primates. The agarose column, under the conditions of the present study, can detect a protein concentration of 2 mg/dl in the peak II region after isolation of plasma lipoproteins at 1.006 < d < 1.225 g/ml. By sequential ultracentrifugation of lipoproteins of other volunteers (n = 12, not included in this study) at d 1.006, d 1.019, and d 1.063 g/ml and measurement of cholesterol (as above) in each fraction, we have observed (unpublished observations), in agreement with others (16, 17), that the mass of cholesterol in intermediate density lipoprotein (IDL, 1.006

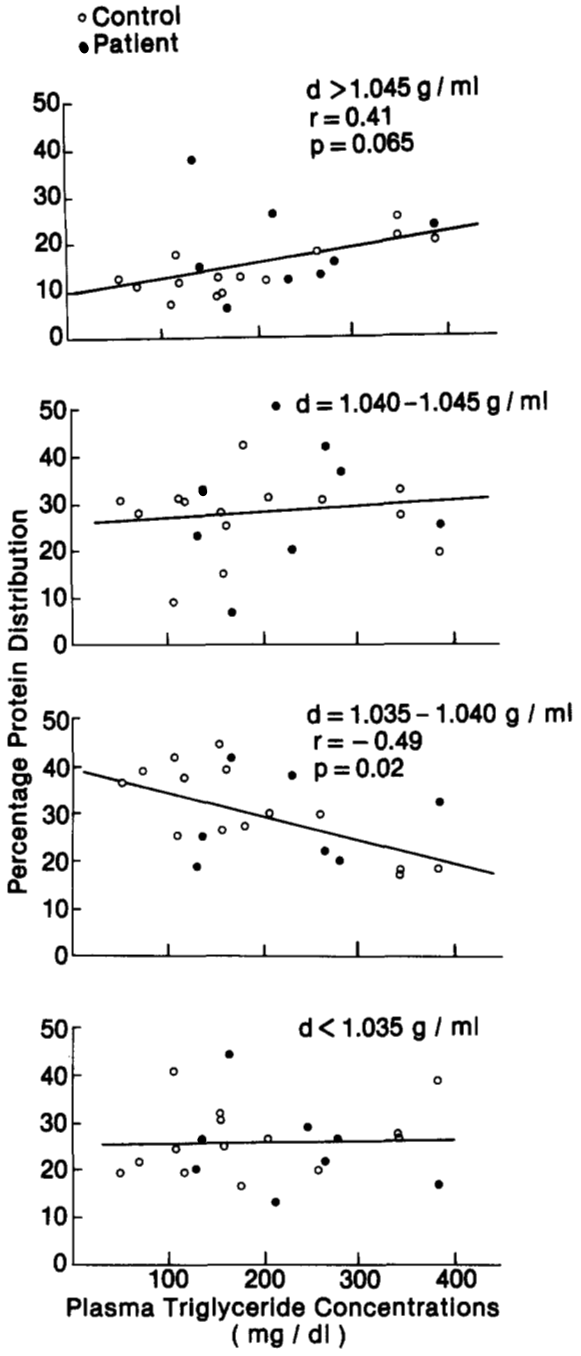

Fig. 3 Percentage protein distribution of LDL density gradient fractions versus plasma triglyceride concentrations of controls (○) and patients with coronary artery disease (●). Isolated LDL were subfractionated by density gradient centrifugation (see Methods), pooled into four density ranges and the protein was quantitated. The line of best fit is shown in each density panel. The equations for the d > 1.045 and 1.035–1.040 g/ml fractions were y = 0.0306X + 10 and y = – 0.0484 + 39, respectively.

< d < 1.019 g/ml) approximately equals 10–20% of that in LDL. A similar distribution of protein has also been found (16, 17). Consequently, it is very likely that IDL was not well separated from LDL in the majority of our volunteers and was responsible in part for the asymmetry of the peak III material found in the large minority of volunteers. No direct attempt was made to quantitate IDL in these studies. However, we did test for an association between peak asymmetry and coronary disease in these volunteers and found none (χ^2 test of a 2 × 2 contingency table). Small quantitative differences in IDL between CAD patients and comparison subjects would have been missed by this approach.

Because "peak II" material in nonhuman primates fed cholesterol consists partly of a discrete population of LDL (1.019 < d < 1.063 g/ml) of very large molecular size (12, 13), we refractionated "peak II" material when it eluted from the column in a separate peak in a very few volunteers. Density gradient ultracentrifugation of the peak II material revealed that it consisted of at least two populations of lipoproteins, one with density < 1.025 g/ml and one with density > 1.050 g/ml. Because the latter material had a pre-beta mobility on agarose electrophoresis, it presumably was Lp(a) (18); the d < 1.025 g/ml material was intermediate density lipoprotein. No material could be identified with density similar to LDL (1.019 < d < 1.063 g/ml).

DISCUSSION

Nonhuman primates characteristically develop a high molecular weight LDL when fed cholesterol. In one study the molecular weight of LDL of cholesterol-fed rhesus monkeys was 4.8 ± 0.2 g/μmol and that of controls was 3.2 ± 0.1 g/μmol (13). This LDL was enriched in cholesteryl ester out of proportion to protein, phospholipid, and nonesterified cholesterol (12), and the particle has been shown to deliver more cholesterol to cells in culture than LDL of normal size (3). In *Macaca fascicularis,* the LDL size correlated better with coronary artery disease than did LDL cholesterol (1, 19, 20). These observations have been corroborated in African green monkeys. In this species the effect is particularly apparent for LDL of molecular size greater than 3.4 g/μmol (21). Although LDL of large molecular size has been sought in patients with familial hypercholesterolemia (5–7) and in volunteers fed cholesterol (8), consistent increases have not been found.

LDL molecular size and heterogeneity have been evaluated in patients with and without coronary disease using analytical ultracentrifugation (7). In that study the molecular weight of monodisperse LDL of coronary disease patients was the same as that of controls. Polydisperse LDL was identified in plasma of volunteers when multiple peaks were found on analytical ultracentrifugation of lipoproteins isolated between density 1.006 g/ml and 1.063 g/ml. Polydisperse LDL was associated with hypertriglyceridemia and diabetes but not with athero-

sclerosis. Patients with polydisperse LDL were generally found to have three major LDL subpopulations: S_f 20, S_f 10, and S_f 4, of which the last (lowest molecular weight material) predominated.

The association between LDL MW and coronary disease in nonhuman primates provided the rationale for our investigation of LDL MW in a case-comparison study of volunteers undergoing cardiac catheterization. We anticipated that coronary disease cases would have LDL of higher molecular weight than controls. In fact the opposite was observed: patients had smaller LDL than controls. However, in this study as in several other case-comparison studies (reviewed in ref. 22), the mean plasma triglyceride concentration of coronary disease patients exceeded that of controls. There is now evidence for an inverse association between hypertriglyceridemia and LDL MW (9, 10, 23, 24). The concept has been proposed (25) that in human beings high plasma triglyceride affords a substrate for the cholesteryl ester exchange protein, and through it IDL and LDL become enriched in triglyceride and lose cholesteryl ester. The presence of triglyceride then allows continued particle size reduction through lipase action. We, therefore, used covariance analysis to control for the effect of triglyceride on LDL molecular size. Even after this adjustment we could not demonstrate large LDL in coronary disease patients, and, in fact, LDL MW of patients remained (insignificantly) less than controls. Although these data argue against the role for large LDL in angiographically demonstratable coronary disease in human beings, they do not eliminate the possibility of an opposite effect: that, in human beings, LDL of small rather than large molecular size may be associated with coronary disease. Although the difference between mean LDL MW in patients and controls disappeared after covariance adjustment for triglyceride, small LDL did relate to coronary atherosclerosis in the univariate analysis. Indeed the role of hypertriglyceridemia in coronary disease has been (22) and continues to be (26) the subject of much debate. Our studies suggest that the same relationships between hypertriglyceridemia and LDL MW previously described for normal human beings (9, 10, 23, 24) also pertain to patients with coronary artery disease. In the present study, patients were evaluated because of suspected heart disease and only eight (8.6%) individuals in whom we were able to measure LDL MW (two controls, six CAD patients) also had diabetes. This contrasts with the previous report (7) in which 27% of individuals tested had diabetes. Perhaps because of the small number of patients with diabetes and coronary disease, we were unable to define a relationship between LDL MW, coronary disease, and diabetes.

LDL of some individuals has recently been shown to consist of a heterogeneous family of particles (27, 28). In a previous publication (7) no relationship could be demonstrated between LDL heterogeneity and coronary disease. We evaluated LDL heterogeneity in our patients in two ways—by inspection of the profile of LDL and material larger than LDL that eluted from the agarose column and by density gradient ultracentrifugation of the LDL so isolated. In the present series we found qualitative differences in agarose column profiles of lipoproteins of 1.006 g/ml $<d<$ 1.221 g/ml. We were able to define three agarose column profiles: in one there was a distinct population of particles of larger size than LDL; in a second the LDL peak was asymmetric; and in the third the LDL peak was symmetric. In these volunteers, the presence of particles of larger size than LDL was not related to coronary disease, diabetes or hypertriglyceridemia, nor did the asymmetric LDL relate to coronary disease, hypertriglyceridemia, or diabetes. No specific attempt was made in these studies to quantitate intermediate density lipoprotein, and small quantitative differences in IDL that correlated with coronary disease would have been missed. In the few cases in which we fractionated "peak II" material we found it to consist partly of IDL, and we suspect that IDL was also partly responsible for asymmetry of the LDL peak where it was seen. In these volunteers "peak II" material also contained particles greater than LDL in density [possibly Lp(a)]. The "peak II" material was fractionated because, in nonhuman primates, although the characteristic "atherogenic" particle is a large LDL (peak III material), some studies have also shown a second population of particles of larger size than LDL but of LDL density [identified as II_{50} in previous publications (13)]. In nonhuman primates these particles are clearly distinct from the large LDL in peak III. Density gradient ultracentrifugation of the peak II material in human beings showed no material that corresponded to the II_{50} material of nonhuman primates.

Density gradient ultracentrifugation of LDL (peak III) was also undertaken in a subset of these volunteers in an attempt to evaluate LDL heterogeneity. We found a negative association between LDL density and molecular weight and a positive association between LDL density and plasma triglyceride. Our studies in this respect extend the observations of others (9, 10, 23, 24) to patients with coronary disease. Although LDL appeared heterogeneous in individual samples, mean peak densities for patients and controls were similar as were LDL density gradient protein distribution profiles. The similarity of these LDL protein distribution profiles suggests that, when LDL is defined and isolated in this fashion (agarose column chromatography), no heterogeneity that might distinguish CAD patients from controls is detectable by density gradient ultracentrifugation. We did not, however, perform gradient gel electrophoresis (27, 28) on the LDL so isolated, and subtle differences in LDL heterogeneity might not have been detected.

We (29) and others (23, 24) have found that the small LDL of hypertriglyceridemic individuals is rich in triglyceride and poor in cholesteryl ester, and has relatively more protein than LDL of normoglyceridemic individuals. These data fit with the concept alluded to above that, in humans beings, hypertriglyceridemia is partly responsible for LDL of small molecular size. These data in human beings thus bear on previous observations in nonhuman primates. Nonhuman primates have plasma triglyceride concentrations (25 mg/dl) that are lower than those found in human beings. This limits the potential for exchange of esterified cholesterol for triglyceride on IDL and LDL, lipolysis, and reduction of LDL size in monkeys. With less potential for triglyceride exchange, esterified cholesterol governs LDL size and, in cholesterol-fed nonhuman primates, LDL enlarges to produce a particle with potential for atherogenesis. Plasma triglyceride concentrations that are higher than those found in nonhuman primates but within the normal range for human beings may diminish the potential for production of large LDL and decrease the amount of cholesteryl ester per particle in patients fed cholesterol (8) or with familial hypercholesterolemia (5–7); in this sense plasma triglyceride concentrations within the normal range in human beings might be considered to be "anti-atherogenic." This concept is partly supported by studies in alcohol-fed nonhuman primates. Feeding alcohol to these animals leads to hypertriglyceridemia, small triglyceride-rich LDL, and diminished atherosclerosis (30).

Our preliminary studies (29) and those of others (5–7, 9, 23, 24) have additionally shown that, while cholesteryl ester is lost and the size of the LDL particle diminishes in patients with hypertriglyceridemia, there is minimal or no loss of apoprotein (apoB) from the particle. This accounts for the increased density of the particle that we and others have found.

Replacement of LDL cholesteryl ester by triglyceride with preservation of apoB on small dense particles of hypertriglyceridemic individuals may partly explain the observations made by ourselves (31) and others (32–40) that LDL apoB correlates better with coronary disease than does LDL cholesterol. Apparently, hypertriglyceridemic patients have increased numbers of triglyceride-rich cholesteryl ester-poor LDL particles. Studies of larger numbers of volunteers using multivariate analysis will be necessary to determine whether LDL apoB aids in definition of coronary status after adjustment for plasma triglyceride concentration.

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