Comparison of apolipoprotein B to cholesterol in low density lipoproteins of patients with coronary heart disease

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Abstract This study was carried out to determine whether patients with coronary heart disease (CHD) have an unusually high level of apolipoprotein B (apoB) relative to cholesterol (C) in low density lipoproteins (LDL). Seven groups of men were studied. Seventy-two with normolipidemia (NLP) had CHD documented on clinical grounds; another 34 NLP patients had proven coronary artery disease (CAD) by angiography (greater than 50% occlusion of two or three coronary arteries). Another group of 37 with documented CHD had hypertriglyceridemia (HTG), and still another 25 with HTG had proven CAD. Three normolipidemic control groups consisted of 30 healthy young men, 40 healthy middle-aged men, and 35 hypertensive men. In normolipidemic CHD and CAD patients, plasma LDL-C averaged 142 ± 37 (SD) and 136 ± 32 mg/dl, respectively; in HTG patients with CHD and CAD, LDL-C levels were 137 ± 37 and 127 ± 34 mg/dl, respectively. These values were near those of hypertensive controls (141 ± 31 mg/dl), but higher than middle-aged and younger healthy controls (118 ± 32 and 106 ± 26 mg/dl, respectively). Levels of LDL-apoB followed a similar pattern: CHD-NLP (88 ± 25 mg/dl), CAD-NLP (83 ± 25 mg/dl), CHD-HTG (94 ± 30 mg/dl), CAD-HTG (89 ± 25 mg/dl), hypertensive controls (89 ± 24 mg/dl), middle-aged controls (80 ± 25 mg/dl) and younger controls (58 ± 14 mg/dl). Normolipidemic patients with CHD and CAD did not have higher LDL-C and LDL-apoB levels than hypertensive and normotensive controls. HTG patients with CHD and CAD however tended to have higher LDL-apoB levels, and their LDL-apoB/C ratios were higher on average than normal. Nevertheless, among all coronary groups, there were no sizeable subgroups with elevated LDL-apoB; only about 11% of all coronary patients had LDL-apoB levels over 120 mg/dl (compared to 8% for normo- and hypertensive controls of middle age).■ The data of this study therefore suggest that LDL-apoB may not prove to be a better indicator of coronary risk in normolipidemic people, but LDL-apoB could be a superior predictor of risk in HTG patients.—**Vega, G. L., and S. M. Grundy.** Comparison of apolipoprotein B to cholesterol in low density lipoproteins of patients with coronary heart disease. *J. Lipid Res.* 1984. **25:** 580–592.

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Plasma levels of total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) are correlated with the prevalence of coronary heart disease (CHD) (1). High levels of LDL-C clearly predispose to premature CHD (2). Still, many patients with CHD do not have elevated LDL-C or other detectable abnormalities in lipoprotein-lipid levels. This fact has led to a search for other defects in plasma lipoproteins that might explain accelerated atherosclerosis in some individuals. Recently, concentrations of LDL-apolipoprotein B (apoB) have been reported to differentiate patients with CHD from unaffected people better than do TC, triglycerides (TG), or LDL-C. For instance, Sniderman et al. (3) reported that many patients with advanced coronary atherosclerosis have elevated levels of LDL-apoB, but normal LDL-C. These workers suggested that abnormalities in the metabolism of apoB, which are not necessarily revealed by concentrations of LDL-C, may cause acceleration of atherosclerosis. In another report, Kesaniemi and Grundy (4) described a group of patients with premature CHD who had overproduction of LDL-apoB and yet had normal concentrations of LDL-C; they speculated that this enhanced production of LDL-apoB may have contributed to their accelerated atherosclerosis. Also, we recently reported on a patient with severe tendon xanthomatosis who had overproduction of LDL-apoB but normal levels of LDL-C (5). Therefore, in the present study, we have attempted to determine whether normolipidemic and hypertriglyceridemic patients with coronary disease have abnormally high concentrations of LDL-apoB or a disproportionate increase in apoB relative to cholesterol in plasma LDL.

METHODS

Patients

This study was carried out in a total of 282 male subjects. Most of the subjects were selected from inpatients and outpatients of the Veterans Administration Medical Center at Dallas, Texas. A group of medical students was also recruited from Southwestern Medical School in Dallas. Seven groups of subjects were studied, and their clinical characteristics are listed in **Table 1.**

Group 1 consisted of 30 young adult-normolipidemic (YNG-NLP) men. They were medical students and none were on medication. None had plasma lipids or lipoproteins exceeding the 95th percentile cutpoints for their age and sex according to the Lipid Research Clinic (LRC) Population Study (6, 7). Group 2 included 40 normotensive-normolipidemic (NTN-NLP) middle-aged men recruited from inpatients with minor illnesses at the VA Medical Center. Most were obtained from Orthopedics, Ophthalmology, and Otolaryngology Services. None of the patients were acutely ill nor were they malnourished. Group 3 consisted of 35 hypertensive-normolipidemic (HTN-NLP) men without evidence of ischemic heart disease. They were obtained from the Hypertension Clinic at the VA Medical Center. Twenty nine percent of these patients were smokers. Fifty three percent were taking diuretics, 32% beta-adrenergic blockers, and 15% other antihypertensive medications. Some of the patients were on combined drug therapy for hypertension.

Group 4 included 72 normolipidemic men with coronary heart disease (NLP-CHD). These men were recruited mainly from the outpatient Cardiology Clinic at the VA Medical Center. All patients had a history of myocardial infarction documented on clinical grounds. No patient had undergone coronary artery bypass graft surgery during the last 6 months before the study. In all patients of this group, concentrations of plasma total TG were below the 90th percentile for their age according to the Lipid Research Clinic (LRC) Population Study (6). Patients whose LDL-C exceeded the 95th percentile were also excluded from this group. Their plasma HDL-C averaged 40 ± 11 mg/dl. A history of hypertension was present in 23%, and 23% also were smokers at time of study. Forty four percent of patients with hypertension were smokers. None had diabetes mellitus nor were they taking anticoagulants or lipid-lowering drugs. Many of the patients were taking cardiovascular medication, as described below. Group 5 consisted of 37 patients with a history of documented myocardial infarction and concomitant hypertriglyceridemia (plasma TG over the 90th percentile by LRC criteria) (CHD-HTG). For groups 4 and 5 combined, the percentage of patients taking cardiovascular drugs included 21% on diuretics, 13% on beta-adrenergic blockers, 32% on nitrates, 5% on procainamide or quinidine, 7% on nefedipine, and 20% on digoxin.

Group 6 included 43 normolipidemic inpatients undergoing coronary angiography for clinical evidence of ischemic heart disease. All patients were reported to have greater than 50% occlusion of two or three major coronary arteries. They were designated CAD-NLP. Group 7 consisted of 25 men with greater than 50% occlusion of two or three coronary arteries, documented by angiography. All patients in this group had plasma TG over the 90th percentile, hence the designation CAD-HTG. For these two groups, 22% of patients were smokers and 20% had a history of hypertension. Percentage of patients taking cardiovascular drugs included 19% on diuretics, 11% on beta-adrenergic blockers, 35% on nitrates, 2% on procainamide or quinidine, 8% on nefedipine, and 20% on digoxin.

Sample collection and storage

Blood was collected after an overnight fast (14 hr) into tubes containing (1 mg/ml) Na_2EDTA. After removal

TABLE 1.   Clinical characteristics of study groups

Group	Number	Age	% Ideal Body Wt.	Total Cholesterol	Total Triglycerides	HDL Cholesterol
		yrs ± SD	% ± SD		mg/dl ± SD	
YNG-NLP	30	26 ± 4[a]	97 ± 7[b]	166 ± 29[c]	92 ± 32[c]	47 ± 11[d]
NTN-NLP	40	52 ± 9[a]	120 ± 18	184 ± 34[c]	146 ± 68	37 ± 11
HTN-NLP	35	56 ± 7	116 ± 22	206 ± 31	134 ± 51	41 ± 14
CHD-NLP	72	57 ± 6	115 ± 13	203 ± 41	128 ± 45	40 ± 11
CHD-HTG	37	57 ± 6	119 ± 13	232 ± 35[d]	390 ± 178[d]	29 ± 7[c]
CAD-NLP	43	58 ± 8	105 ± 18[b]	195 ± 36	127 ± 38	42 ± 17
CAD-HTG	25	57 ± 9	118 ± 21	209 ± 32	300 ± 110[d]	28 ± 10[c]

[a] Two groups (YNG-NLP and NTN-HTP) were significantly younger than all other groups ($P < 0.05$).
[b] Two groups (YNG-NLP and CAD-NLP) had significantly lower % ideal body weights than all other groups ($P < 0.05$).
[c] These groups had significantly lower values than other groups in each category ($P < 0.05$).
[d] These groups had significantly higher values than other groups in each category ($P < 0.05$).

of red blood cells by centrifugation at 4°C, 5 ml of plasma was taken directly for measurement of lipids and lipoproteins. Another 10 ml of plasma was transferred into dry tubes containing dinitrothiobenzoic acid (DTNB) (2 mM), chloramphenicol (0.15 mM), and sodium azide (1.5 mM) (8). Both tubes were stored at 4°C until analysis, which was done within the week of collection.

Plasma total lipids

From the first 5 ml of plasma, TC and TG were measured by enzymatic methods (9, 10). Cholesterol was determined by the procedure of Roeschlau, Bernt, and Gruber (9) using a Gilford System 203 autoanalyzer. The standard supplied by Gilford Diagnostics was calibrated using a pooled reference plasma standard. The cholesterol in the latter was determined by gas–liquid chromatography (Hewlett-Packard model 5880A) on a microcapillary column (10 m SE-30; 0.25 mm i.d, and 0.25 micron filter, Supelco). Pure cholesterol for standard was obtained from Supelco.

Lipoprotein-cholesterol concentrations

Cholesterol in VLDL, LDL, and HDL was estimated by modifications of Lipid Research Clinic procedures (11). When plasma TG was less than 200 mg/dl, analyses were carried out without ultracentrifugation along lines described previously by Friedewald, Levy, and Fredrickson (12), Wilson and Spiger (13), and Wilson et al. (14). When TG levels were greater than 200 mg/dl, samples were subjected to ultracentrifugation to remove lipoproteins of density <1.006 g/ml before proceeding with analyses. A description of the method used to obtain each lipoprotein-cholesterol level and the operational definition for each follows. Subsequently, an asterisk will be used to denote a cholesterol value estimated either by an equation or by indirect measurement.

VLDL-cholesterol* (VLDL-C*). The term VLDL-C* is used for the estimate of cholesterol in the VLDL fraction obtained in either of two ways. When TG levels were below 200 mg/dl, VLDL-C* was estimated by the equations of Myers, Phillips, and Havel (15). These equations take into account differences in C/TG ratios in VLDL of men and women at different TG concentrations. They appear to provide a somewhat better estimate of VLDL-C* than the value of TG/5 proposed by Friedewald et al. (12). When plasma TG exceeded 200 mg/dl, VLDL-C* was estimated as the difference between total cholesterol (TC) and infranatant cholesterol after ultracentrifugal removal of the d < 1.006 g/ml fraction.

HDL-cholesterol (HDL-C). On both whole plasma (TG < 200 mg/dl) and the 1.006 g/ml infranatant (TG > 200 mg/dl), apoB-containing lipoproteins were precipitated with heparin-manganese. Manganese chloride (92 mM) was used to achieve optimum precipitation of apoB-containing lipoproteins (16). Cholesterol was estimated on the supernatant and was designated HDL-C.

LDL-cholesterol* (LDL-C*). The term LDL-C* was calculated in the following way: LDL-C* = (TC) − (VLDL-C*) − (HDL-C) where VLDL-C* was estimated by the equations of Myers et al. (15) (when TG < 200 mg/dl) or by difference (when TG > 200 mg/dl). In either case, LDL-C* should closely approximate IDL − C + LDL − C (d 1.006–1.063 g/ml). Myers et al. (15) reported a high correlation ($r = 0.93$) between estimated LDL-C* and IDL − C + LDL − C determined directly by ultracentrifugal analysis.

Isolation of VLDL and LDL for compositional analysis

Four ml of plasma from each patient was used to isolate VLDL and LDL for compositional analysis. To this plasma was added 2 ml of a salt solution of 0.432 M NaBr and 0.195 M NaCl according to the method of Lindgren and Jensen (17). This mixture was made in 6-ml Ultra-Clear Beckman centrifuge tubes. The resulting solution had a density of 1.019 g/ml. The tube was spun in a fixed-angle Beckman 50.3 Ti rotor, at 40,000 rpm for 20 hr at 15°C (8). The top 2 ml was aspirated and designated VLDL.

The infranatant was brought to a density of 1.070 g/ml in the same tube by addition of 2 ml of 1.815 M NaBr and 0.195 M NaCl. LDL was then isolated by ultracentrifugation as described above for VLDL; the top 2 ml (33% of tube volume) was aspirated again. Because of the gradient produced in the tube during ultracentrifugation, the fraction isolated in the top 2 ml should include all lipoproteins of d < 1.067 g/ml. This upper limit was chosen rather than 1.063 g/ml to insure isolation of all LDL. Previous work of Anderson et al. (18) has shown that this lower limit (1.067 g/ml) would not include any HDL-cholesterol. To minimize losses of apoproteins, neither VLDL nor LDL were washed by recentrifugation. The infranatants of density greater than 1.067 g/ml were saved for further analysis.

Lipoproteins were dialyzed against 0.15 M NaCl containing 0.15 mM chloramphenicol, 1.5 mM sodium azide, and 0.27 mM disodium ethelenediamine-tetraacetate (EDTA) at pH 7.4 (8). Dialysis was carried out shortly after lipoprotein isolation.

Estimation of apoB in 1.067 g/ml infranatant

To test the possibility that some apoB was lost into 1.067 g/ml plasma infranatant, the apoB content of this infranatant was estimated by radial immunodiffusion (RID) in 62 normolipidemic patients with CHD and in 28 young controls. RID was conducted as described by Sniderman, Teng, and Jerry (19) except that a 0.025 M Tris-tricine buffer, pH 8.6, was used (20). Briefly, agarose

(1.5%, low electroendosmosis, 0.16–0.19 EEO units) mixed with buffer and anti-LDL-apoB (0.2%) (8) was casted into plates (200 × 100 × 1 mm). LDL isolated from plasma of a donor was used as standard, and apoB was determined by the Lowry et al. procedure (see below). Samples were diluted (1:4) with buffer. Plates were incubated for 72 hr; thereafter, plates were immersed in 0.17 mM phosphotungstic acid to facilitate visualization of precipitated rings for measurement (21). Ring diameters were measured with a Polaron 7-X magnification lens containing a vernier scale in 0.1-mm units. Small amounts of immunoreactive material were present in almost all the samples. The mean apoB level in the 62 CHD-NLP patients was 4.9 ± 0.8 (SD) mg/dl, and for the 28 young controls it was essentially the same, 4.3 ± 0.5 mg/dl. The range in both groups was 4.0 to 6.0 mg/dl.

Determination of LDL-apoB/C and VLDL-apoB/C ratios

Ratios of apoB to cholesterol (apoB/C ratios) were determined in LDL and VLDL isolated as described above. The term LDL denotes lipoproteins of density 1.019–1.067 g/ml, and VLDL indicates lipoproteins of d < 1.019 g/ml. The LDL-apoB/C and VLDL-apoB/C (without asterisks) thus were from isolated LDL (d 1.019–1.067 g/ml) and VLDL (d < 1.019 g/ml) and should be differentiated from LDL* and VLDL* defined operationally before. Cholesterol in isolated LDL and VLDL was determined after dialysis as described above, and apoB was estimated as follows.

ApoB mass by Lowry procedure. Total protein in LDL and VLDL was measured by a modification (22) of the procedure of Lowry et al. (23). ApoB concentration in the lipoproteins was calculated as the difference between total protein in the fractions and soluble proteins after selective precipitation of apoB by isopropyl alcohol (24, 25).

Bovine serum albumin (BSA), obtained from the National Bureau of Standards, was used as a protein standard. The concentration of this standard had been determined by biuret and Keldahl methods (26, 27) and by measurement of absorbance at 278 nm (28). The stability of the standard was monitored routinely by measuring its absorbance at 280 nm and using $E_{cm}^{1\%} = 6.6$ (28).

Reagents for the Lowry procedure were those described by Markwell et al. (29). The reagents included *a*) 0.283 M Na_2CO_3 containing 0.052 M sodium dodecyl sulfate (SDS), 0.010 M sodium tartrate, and 0.15 M NaOH, *b*) 0.240 M $CuSO_4 \cdot 5H_2O$, and *c*) 1 N phenol (Folin). SDS was added to remove lipid turbidity as described by Helenius and Simon (30), Bradley et al. (31), Kashyap, Hynd, and Robinson (32), and by Markwell et al. (29). According to the latter (29), SDS is preferable to 1.0 N NaOH for

removal of lipid turbidity because NaOH can cause autooxidation of lipid and generate products that give a color reaction with the reagents. We compared SDS for removal of turbidity with solvent extraction as described by Sata, Havel, and Jones (22). The comparisons are shown in **Table 2.** In Part A (Table 2), duplicate measurements were made on each of seven different fractions of LDL; aliquots were left untreated, treated with SDS, or extracted with chloroform or ether, the latter according to Sata et al. (22). Removal of lipids by ether requires *a*) 0.377 M Na_2CO_3 dissolved in 0.2 N NaOH, *b*) 0.125 M $CuSO_4 \cdot 5H_2O$, and *c*) 0.142 M sodium potassium tartrate. Untreated samples in some cases gave higher readings due to the presence of turbidity. Results with SDS were similar to those with solvent extraction. Part B (Table 2) shows results for multiple measurements of two LDL samples, one with a low concentration and another with a high level. Again, untreated samples tended to give higher values, while the other three gave essentially the same results.

The BSA standard curve used in the procedure of Lowry et al. (23) ranged from 7 to 50 μg of protein per ml of reagent. Concentrations of VLDL-protein and LDL-protein routinely ranged between 10 to 30 μg. These concentrations were chosen because they were within the linear portion of the standard curve.

The procedure for precipitation of apoB with isopropyl alcohol is essentially that described by Holmquist et al. (24, 25). One hundred to 150 μg of VLDL-protein and 175 to 200 μg of LDL-protein were used for precipitation (24). A chromogenicity factor of 1.0 was used to compare BSA to apoB; this factor was reported by Kane et al. (33), and it was obtained by amino acid analysis of both proteins. The chromogenicity of isopropyl alcohol-soluble proteins in LDL has never been determined. A ratio of 1.0 compared to BSA therefore was used. Since the quantity of soluble protein in LDL was small, inexactitude in chromogenicity of this fraction should have little effect on calculation of LDL-apoB. LDL-apoB ranged from 90 to 100% (mean 94 ± 2% SD) of LDL-protein. For isopropyl alcohol-soluble proteins of VLDL, a chromogenicity factor of 1.16 was used; this factor also was reported by Kane et al. (33).

Effect of ultracentrifugation on LDL composition

LDL from plasma of 30 coronary patients was isolated by ultracentrifugation as described above; it was taken from the top 2 ml recovered in the isolation; 1 ml of each sample of LDL was analyzed for apoB and cholesterol content. The remaining 1 ml was resuspended in the 1.067 g/ml infranatant and recentrifuged for 20 additional hours. The purpose of this recentrifugation was to determine whether prolonged ultracentrifugation results in loss of apoB relative to cholesterol in LDL. After

TABLE 2. LDL protein measurements (comparison of delipidation methods)

Sample	Not Delipidated	Delipidation Reagent		
		SDS[a]	Chloroform[b]	Ether[b]
		mg/dl		
Part A. Multiple samples[c]				
1	1.7	1.6	1.6	2.0
2	1.9	1.7	1.9	1.9
3	3.8	3.5	3.3	4.8
4	6.5	7.2	6.3	6.1
5	7.7	7.3	6.7	6.4
6	12.4	9.3	8.4	9.0
7	13.2	14.0	11.9	12.5
Mean ± SEM	6.7 ± 1.9	6.4 ± 1.7	5.7 ± 1.4	6.1 ± 1.4
Part B. Single samples (multiple measurements)				
		mg/dl ± SD		
Patient 1[d]	3.5 ± 0.1	2.5 ± 0.2	2.6 ± 0.2	2.5 ± 0.2
Patient 2[e]	13.3 ± 0.5	12.4 ± 0.3	12.1 ± 0.3	12.8 ± 0.8

[a] Procedure of Markwell et al. (29).
[b] Procedure of Sata et al. (22).
[c] For each sample, duplicate measurements were made for each treatment.
[d] Eighteen measurements.
[e] Ten measurements.

recentrifugation, LDL was recovered into 2 ml and analyzed for apoB and cholesterol. Mean concentration of LDL-apoB in the first lipoprotein preparation was not significantly different from that of the second (0.911 ± 0.30 mg/ml for the first vs. 0.817 ± 0.20 mg/ml for the second); mean LDL-C was significantly higher after the first LDL spin than after the second (1.574 ± 0.60 mg/ml vs. 1.252 ± 0.40 mg/ml, respectively; $P < 0.025$). These differences also resulted in a significantly higher LDL-apoB/C ratio (0.656 ± 0.090) in the second LDL compared to the first (0.591 ± 0.06) ($P < 0.005$). Thus, prolonged ultracentrifugation does not cause losses of LDL-apoB relative to LDL-C; instead, LDL-C is lost relative to LDL-apoB.

Lipoprotein-apoB concentrations

LDL-apoB*. The entity LDL-apoB* represents the apoB concentration in the fraction corresponding to LDL-C*, i.e., essentially the 1.006–1.063 g/ml fraction. This value was calculated as:

$$LDL\text{-}apoB* = LDL\text{-}C* \times LDL\text{-}apoB/C.$$

The equation assumes that LDL-apoB*/C* = LDL-apoB/C. This may not be precisely true because the apoB/C ratio in IDL may not be exactly that in pure LDL. On the other hand, the available data from Hammond and Fisher (34) and Shen et al. (35) indicate that apoB/C ratios in IDL closely approximate those in LDL. It might be noted that this equation has been used routinely to estimate LDL-apoB levels in most LDL turnover studies (36). For reasons to be described in the Discussion

section, the upper normal limit of LDL-apoB* was set arbitrarily at 120 mg/dl.

VLDL-apoB*. The term VLDL-apoB* denotes the concentration of apoB corresponding to VLDL-C* and is calculated as:

$$VLDL\text{-}apoB* = VLDL\text{-}C* \times VLDL\text{-}apoB/C.$$

This value of VLDL-apoB* suffers the limitation that VLDL-apoB/C was determined on the d < 1.019 g/ml fraction, and not on the d < 1.006 g/ml fraction. Since IDL-apoB/C tends to be higher than pure VLDL-apoB/C, the value for VLDL-apoB* may slightly overestimate the apoB concentration in the d < 1.006 g/ml fraction. Some degree of accuracy in this determination was sacrificed for two reasons: *a*) a major aim of the study was the accurate measurement of LDL-apoB/C, and *b*) the VLDL-apoB contributes much less to the plasma total apoB concentration than does LDL-apoB.

Nomenclature

The terms LDL-C*, LDL-apoB*, LDL-apoB/C, VLDL-C*, VLDL-apoB*, and VLDL-apoB/C will be employed in the Results section, tables, and figures according to the operational definitions described above. The asterisks will be omitted in the Discussion section where our data are compared to other data obtained by a variety of methods.

Statistical analysis

Linear statistical procedures available as Interactive Statistical Programs (ISP) were used for data analysis.

The analyses were carried out at the Medical Computing Resources Center, University of Texas Health Science Center at Dallas. One-way analysis of variance (ANOVA) was used for multiple comparisons of all parameters for all groups (Tables 1 and 3–6).

For a comparison of LDL-apoB* and LDL-C* concentrations for each group (Figs. 1–3), correlation coefficients were determined by the Pearson method (37), and statistical significance compared to zero was set up at a P value of 0.001. For comparison of slopes and intercepts among groups, the Newman-Keuls critical Q tables for a significance of P value of 0.05 were used (37).

Descriptive statistical analysis was used for the distribution of LDL-apoB/C ratios (Table 4). A statistical program was employed to generate a histogram from the observed data. This program plots the data in a step-wise fashion by selecting a series of ratios around which subgroups of ratios cluster. The critical ratios are called the Midpoint Class Interval. This histogram was tested for the presence of normal (Gaussian) distribution according to Shapiro and Wilk (38). Significance was set at $P < 0.05$.

One-way analysis of covariance with age as a covariate also was used in the analysis of LDL-apoB/C ratios among the groups (37).

RESULTS

Low density lipoproteins (LDL)

Concentrations of LDL-C*, LDL-apoB*, and LDL-apoB/C ratios are given in **Table 3.** The lowest levels of LDL-C* were in young and middle-aged men without coronary disease. In both, concentrations of LDL-C* were significantly lower than in hypertensive patients with nor-

mal lipids. The latter group had LDL-C* levels similar to all four groups of coronary patients; among the latter, no statistical differences were found.

The lowest concentrations of LDL-apoB* were found in young, normolipidemic subjects. Middle-aged men without elevated lipids or high blood pressure had significantly higher levels of LDL-apoB* than younger men even though LDL-C* concentrations were not different for the two groups. There were no significant differences in plasma LDL-apoB* among middle-aged groups except for CHD patients with hypertriglyceridemia; the latter had a mean LDL-apoB* level that was significantly higher than any of the other groups.

Young normolipidemic subjects had lower LDL-apoB/C ratios than any other group. There were no other differences in LDL-apoB/C ratios among any other normolipidemic groups, whether with or without coronary disease. On the other hand, both hypertriglyceridemic groups with coronary disease had significantly higher ratios than all the other groups.

In Figs. 1–3, concentrations of LDL-apoB* are plotted against LDL-C* levels for each of the seven groups. Comparisons for the three groups of control subjects are shown in **Fig. 1.** For each control group there was a high and statistically significant correlation between levels of LDL-apoB* and LDL-C* (YNG-NLP, $r = 0.763$; NTN-NLP, $r = 0.888$; and HTN-NLP, $r = 0.816$).

A statistical comparison of the regression lines showed that the slopes of the lines were not statistically different among the three groups. There were no distinct subgroups in these three categories with disproportionately high LDL-apoB* levels; however, each middle-aged group had three patients with LDL-apoB* concentrations exceeding 120 mg/dl.

The same comparisons for normolipidemic patients with CHD and CAD are given in **Fig. 2.** For patients with CHD and CAD, there were highly significant correlations between levels of LDL-apoB* and LDL-C* ($r = 0.867$ and 0.757, respectively). There were no significant differences between the slopes of these regression lines and those of the control groups. Ten patients with CHD had LDL-apoB* concentrations exceeding 120 mg/dl; these patients also had relatively high concentrations of LDL-C*, but the latter did not exceed the 95th percentile cutoff value. Only two patients with CAD had levels of LDL-apoB* exceeding 120 mg/dl.

Results for the hypertriglyceridemic patients are given in **Fig. 3.** The correlation coefficients between LDL-apoB* and LDL-C* were highly significant for both groups (CHD-HTG, $r = 0.758$; CAD-HTG, $r = 0.859$). Compared to the other groups, the slopes of regression lines for hypertriglyceridemic patients were not significantly different. For 37 patients with CHD, five had a plasma LDL-apoB* exceeding 120 mg/dl. Only two of
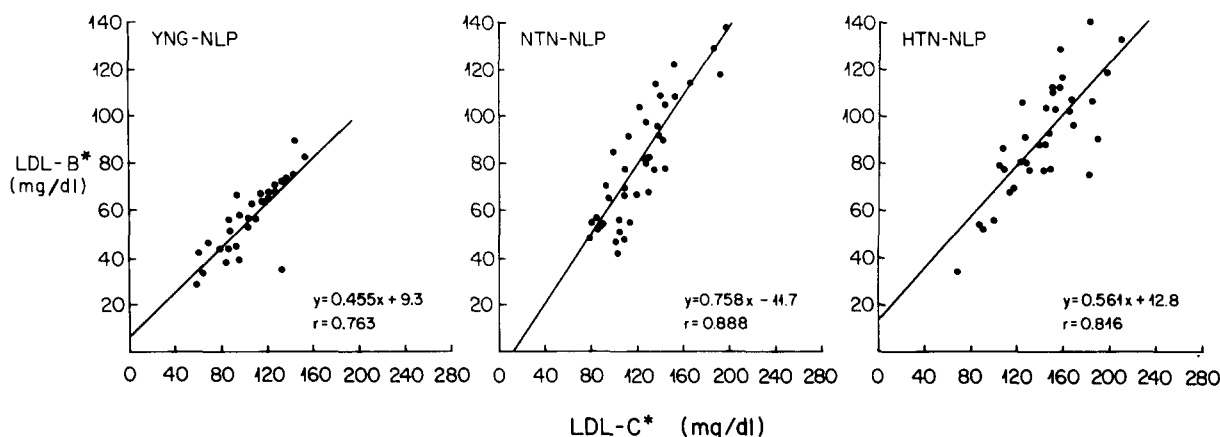
TABLE 3. Concentrations of LDL-C* and LDL-apoB* and ratios of apoB to C in LDL

Group (n)	LDL-C*	LDL-apoB*	LDL-apoB/C
	mg/dl ± SD		ratio ± SD
YNG-NLP (30)	106 ± 26[a]	58 ± 14[a]	0.56 ± 0.08[a]
NTN-NLP (40)	118 ± 32[a]	80 ± 25[b]	0.65 ± 0.11
HTN-NLP (35)	141 ± 31	89 ± 24	0.64 ± 0.12
CHD-NLP (72)	142 ± 37	88 ± 25	0.62 ± 0.09
CAD-NLP (43)	136 ± 32	83 ± 17	0.62 ± 0.10
CHD-HTG (37)	137 ± 37	94 ± 30[c]	0.69 ± 0.15[c]
CAD-HTG (25)	127 ± 34	89 ± 25	0.70 ± 0.10[c]

[a] Significantly lower than all groups; $P < 0.0001$ by ANOVA.
[b] Significantly higher than YNG-NLP and lower than other middle-aged groups; $P < 0.0001$.
[c] Significantly higher than all groups; $P < 0.0001$.

Fig. 1. Plasma concentrations of LDL-apoB* vs. LDL-C* in three control groups: young normolipidemic (YNG-NLP), middle-aged normotensive normolipidemic (NTN-NLP), and middle-aged hypertensive normolipidemic (HTN-NLP) subjects. For YNG-NLP subjects, LDL-apoB* levels ranged from 30 to 90 mg/dl, and their cholesterol concentrations from 60 to 160 mg/dl. The correlation was high ($r = 0.763$). LDL-apoB* concentrations for NTN-NLP and HTN-NLP groups ranged from 50 to 140 mg/dl, while the cholesterol levels ranged from 80 to 200 mg/dl. The two parameters also correlated highly ($r = 0.888$ and 0.816 in NTN and HTN-NLP, respectively).

25 patients with CAD had an LDL-apoB* level over 120 mg/dl.

The distributions of LDL-apoB/C ratios for each group are summarized in **Table 4.** All groups were tested for Gaussian distribution of ratios (38). The young normolipidemic controls were shown to have a normal distribution with a peak ratio in the range of 0.50 and 0.59. None of the other groups had a normal distribution. Furthermore, there was a skewing towards higher ratios in all groups including middle-aged controls. It seemed particularly pronounced in patients with hypertriglyceridemia. Thus, increasing age and hypertriglyceridemia

appeared to contribute to higher LDL-apoB/C ratios in some patients.

In **Table 5,** LDL-apoB/C ratios are compared for obese and nonobese subjects; obesity was defined as weight greater than 125% ideal body weight (% IBW). There were no obese subjects among young normolipidemics. Only a small number of middle-aged normolipidemic subjects (NTN-NLP) were obese, and no effect was noted. However, in hypertensive controls, the obese subgroups did have a higher mean ratio. In normolipidemic, coronary patients (CHD-NLP and CAD-NLP), no effect of obesity was detected. On the other hand, obesity in hy-
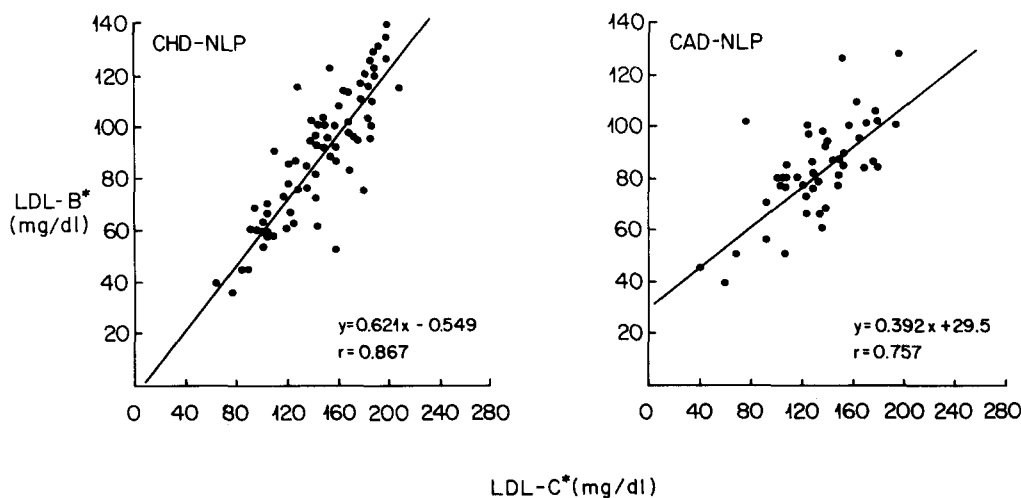

Fig. 2. Concentrations of LDL-apoB* vs. LDL-C* of two normolipidemic groups of patients with coronary disease (CHD-NLP and CAD-NLP). LDL-apoB* concentrations ranged between 40 and 140 mg/dl, and LDL-C* levels from 60 to 200 mg/dl. There was a high correlation between LDL-apoB* and LDL-C* ($r = 0.867$ and $r = 0.757$) in CHD and CAD, respectively.
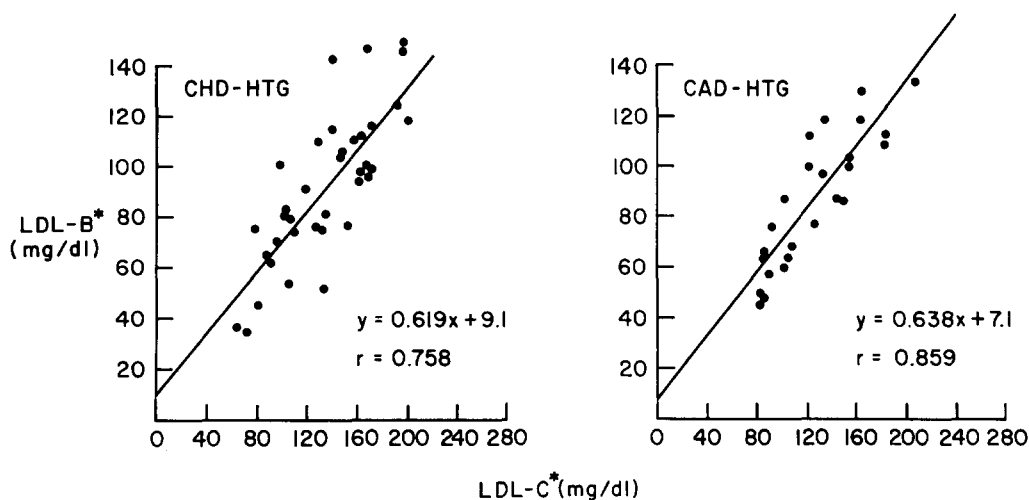
Fig. 3. Concentrations of LDL-apoB* vs. LDL-C* in two groups with hypertriglyceridemia and coronary disease (CHD-HTG and CAD-HTG). LDL-apoB* concentrations in both groups ranged from 40 to 140 mg/dl, and LDL-C* ranged from 80 to 200 mg/dl. LDL-apoB* and LDL-C* levels correlated highly (r = 0.785 and r = 0.859) for CHD and CAD groups, respectively.

pertriglyceridemic patients was associated with a higher ratio. This was most evident in hypertriglyceridemic patients with CHD ($P < 0.001$), but a significant difference also was present when the two hypertriglyceridemic groups were combined ($P = <0.02$).

Very low density lipoproteins (VLDL)

Levels of VLDL-C*, VLDL-apoB*, and VLDL-apoB/C ratios are shown for the seven groups in **Table 6.** Concentrations of VLDL-C* were similar in the two normolipidemic groups with CHD and the two older control groups. Young normolipidemic subjects had somewhat lower levels. Mean VLDL-C* levels were higher in hypertriglyceridemic patients than in corresponding groups with normal plasma TG. Essentially the same pattern was noted for VLDL-apoB* levels. There was some variability in VLDL-apoB/C ratios throughout all the

groups, but no striking differences were noted among any except that young subjects with normolipidemia had the lowest ratios.

DISCUSSION

Numerous epidemiological studies have shown a positive correlation between plasma TC and risk for CHD (2). This correlation also extends to LDL-C levels. It is thus reasonable that a similar positive correlation would exist between levels of LDL-apoB and CHD. Several studies indeed support the latter, and some reports go further to suggest that LDL-apoB levels are more highly correlated with occurrence of CHD than LDL-C. Several examples can be cited. Wayne et al. (39) reported that a group of 89 patients with proven CAD had a mean

TABLE 4. Distribution of LDL-apoB/C ratios for each group[a]

LDL-apoB/C Ratio	YNG	NTN	HTN-NLP	CHD-NLP	CAD-NLP	CHD-HTG	CAD-HTG
range				*percent*			
0.40–0.44	3.4	4.7	5.3	2.4	2.5	2.5	0
0.45–0.49	6.9	9.3	0	5.9	10.1	2.5	0
0.50–0.54	38.1	6.9	7.9	14.2	12.5	4.9	0
0.55–0.59	31.3	4.7	10.6	20.4	27.5	29.3	20.8
0.60–0.64	10.4	18.6	36.8	16.7	7.5	7.3	16.7
0.65–0.69	3.4	23.3	13.1	29.7	12.5	12.2	16.7
0.70–0.74	6.9	16.3	15.8	5.9	14.9	17.1	16.7
0.75–0.79	0	6.9	2.7	2.4	12.5	12.2	8.3
0.80>	0	9.3	7.9	2.4	0.0	12.2	20.8

[a] Number in each group shown in Table 2.

TABLE 5. LDL-apoB/C ratios in obese and nonobese subjects

Group	Nonobese			Obese		
	n	%IBW	LDL-apoB/C	n	%IBW	LDL-apoB/C
		mean ± SD			*mean ± SD*	
YNG-NLP	30	97 ± 8	0.57 ± 0.13			
NTN-NLP	34	111 ± 9	0.64 ± 0.10	6	151 ± 12[a]	0.66 ± 0.15
HTN-NLP	26	113 ± 9	0.61 ± 0.11	9	139 ± 14[a]	0.71 ± 0.10[b]
CHD-NLP	56	109 ± 8	0.62 ± 0.09	16	134 ± 10[a]	0.61 ± 0.09
CAD-NLP	37	100 ± 16	0.63 ± 0.09	6	136 ± 5	0.56 ± 0.11
CHD-HTG	20	111 ± 9	0.61 ± 0.09	17	131 ± 8[a]	0.75 ± 0.16[b]
CAD-HTG	18	107 ± 11	0.71 ± 0.11	7	149 ± 14[a]	0.75 ± 0.15
Both HTG	38	109 ± 10	0.66 ± 0.10	24	136 ± 10[a]	0.75 ± 0.16[b]

[a] Obese patients had a significantly higher %IBW than nonobese ($P < 0.001$).
[b] Obese higher than nonobese ($P < 0.02$).

LDL-C level 34% higher than 60 normolipidemic controls; in these same patients, LDL-apoB concentrations in the CAD group were 41% higher. In another study, Avogaro et al. (40) reported that LDL-C in CHD patients was 13% higher than normal, while LDL-apoB was 46% higher. In both of these studies LDL-apoB was estimated by electroimmunoassay (EIA). Fager et al. (41), using the same basic methodology, could not confirm a difference between LDL-C and LDL-apoB as they related to coronary disease. In their group of CHD patients, plasma LDL-C was only 3% higher than control, and LDL-apoB was only 5% higher. On the other hand, De Backer, Rosseneu, and Deslypere (42), using immunonephelometry to estimate LDL-apoB, found LDL-C to be increased by 13% in CHD patients while LDL-apoB was 39% higher than control. These few reports thus suggest the possibility that levels of LDL-apoB may be a better indicator of CHD risk than LDL-C.

Two other studies of this type have been reported by Sniderman et al. (3, 43). These workers estimated LDL-apoB levels by radial immunodiffusion (RID). With this

TABLE 6. Concentrations of VLDL-C* and VLDL-apoB* and ratios of apoB to C in VLDL

Group[a]	VLDL-C*	VLDL-apoB*	VLDL-apoB/C
	mg/dl ± SD		*ratio ± SD*
YNG-NLP	15 ± 7	6 ± 3	0.38 ± 0.15
NTN-NLP	20 ± 10	10 ± 5	0.46 ± 0.11
HTN-NLP	23 ± 15	9 ± 5	0.42 ± 0.19
CHD-NLP	21 ± 10	8 ± 5	0.42 ± 0.13
CAD-NLP	21 ± 7	12 ± 6	0.53 ± 0.17
CHD-HTG	59 ± 35[b]	19 ± 14[b]	0.42 ± 0.14
CAD-HTG	49 ± 23[b]	22 ± 8[b]	0.49 ± 0.13

[a] See Table 2 for numbers (n).
[b] Significantly higher than other groups ($P < 0.005$) by ANOVA.

procedure the concentration of apoB is directly proportional to the diameter of the circle of immunoprecipitate. Measurements of LDL-apoB were made on whole plasma; the technique depends on the assumption that VLDL-apoB does not contribute to the diameter of the RID ring, while LDL-apoB does (19). In a first report (3), these workers selected patients on the basis of angiographic findings of coronary atherosclerosis. Thirty-one control patients, who were shown to be without coronary atherosclerosis, had a mean LDL-C of 112 mg/dl and an LDL-apoB of 82 mg/dl (LDL-apoB/C ratio = 0.732). In contrast, 59 patients with significant atherosclerosis had higher levels of all (mean LDL-C = 134 mg/dl, LDL-apoB = 118 mg/dl, and LDL-apoB/C = 0.881). More recently, Sniderman et al. (43) have reported that high LDL-apoB levels (and high LDL-apoB/C ratios) in patients with advanced CAD are frequently related to hypertriglyceridemia. In a group of 47 patients with hypertriglyceridemia and proven CAD, LDL-apoB levels averaged 144 mg/dl (LDL-apoB/C = 0.918). These workers thus used the term "hyperapobetalipoproteinemia" to represent an increased plasma LDL-apoB in the presence of a normal LDL-C; they suggest that hyperapobetalipoproteinemia is responsible for accelerated atherosclerosis in many patients with CAD.

The investigations reported above raise three questions that must be distinguished, although they are related. The first is: are levels of LDL-apoB a better predictor of CHD risk than concentrations of LDL-C? Second, is there a subgroup of patients with CHD who have elevated LDL-apoB levels but normal LDL-C; if so, such patients can be said to have hyperapobetalipoproteinemia. And third, do some patients with CHD have an abnormal composition of LDL that renders this lipoprotein unusually atherogenic? Patients with hyperapobetalipoproteinemia might have LDL particles of this type, but abnormal LDL could also occur in patients with normal levels of LDL-

apoB. Each of these three questions can be addressed separately.

LDL-apoB as a risk factor for CHD

None of the studies described above proves that levels of LDL-apoB are better indicators of CHD risk than are concentrations of LDL-C. The present report, furthermore, does not resolve the issue. To determine whether LDL-apoB is a better predictor of risk than LDL-C, a large number of patients must be surveyed. It should be pointed out that even when thousands of patients have been screened, the degree to which LDL-C levels predict CHD risk has been a matter of dispute (44). Not until LDL-apoB levels are accurately measured along with LDL-C in a large population will it become clear whether LDL-apoB is a better index of CHD risk than LDL-C. Prospective surveys, such as those done in the Framingham study (2) and the Pooling Project (45), are needed rather than currently available retrospective investigations.

Hyperapobetalipoproteinemia

Next, let us ask whether any of the coronary patients of the present study had hyperapobetalipoproteinemia. By definition such patients must have normal levels of LDL-C; to test the prevalence of this condition we eliminated all patients from the study who had LDL-C over the 95th percentile by Lipid Research Clinic criteria (6, 7). In addition, to have hyperapobetalipoproteinemia, patients must have an elevated LDL-apoB level. But what constitutes an abnormally high level of LDL-apoB? The upper limit of normal of LDL-apoB has not been defined rigorously. According to Sniderman et al. (3, 43), an LDL-apoB level over 120 mg/dl is elevated. The value is compatible with our data. In the two older non-coronary groups (NTG-NLP and HTN-NLP), the percentage of patients with LDL-apoB levels over 120 mg/dl were 7.5% and 8.7%, respectively, which for our relatively small groups are not far from the 95th percentile cutoff value. Furthermore, the 120 mg/dl outer limit approximates the value obtained by multiplying the 95th percentile for LDL-C from the LRC study by LDL-apoB/C ratios obtained in normal subjects of the present investigation.

If we assume a value of 120 mg/dl as the upper normal limit of LDL-apoB concentration for middle-aged men, the following percentages of patients in each group with coronary disease had hyperapobetalipoproteinemia (i.e., high plasma LDL-apoB and normal LDL-C): CHD-NLP = 14%, CAD-NLP = 5%, CHD-HTG = 14%, and CAD-HTG = 8%. For all four coronary groups combined, 19 of 177 patients (11%) could be called hyperapobetalipoproteinemic.

Although some of the coronary patients fit the criteria of hyperapobetalipoproteinemia, the fraction was considerably less than reported by Sniderman et al. (3). They studied 59 patients with angiographically proven, significant CAD all of whom had normal LDL-C levels below the 95th percentile (7); of these, 34 (58%) were reported to have LDL-apoB levels over 120 mg/dl. How then can we account for the marked difference between the results of Sniderman et al. (3, 43), and somewhat similar results reported by others (39–42), and those of the present study?

One possibility is that the patient populations were different. In the report of Sniderman et al. (3), patients were studied in Montreal; these patients may have come from a relatively homogeneous population in which the incidence of genetic disorders of lipoprotein metabolism is high. Our patients could have been more heterogeneous with fewer genetic disorders. Beyond any possible differences in population, however, methodology could have been a factor. Most previous reports have employed immunological techniques for estimating LDL-apoB, whereas the present work measured LDL-apoB mass by a modification of the procedure of Lowry et al. (22, 23). This latter procedure should provide a direct measurement of apoB mass while immunological procedures infer mass from immunoreactivity; for the large insoluble apoB embedded into a lipoprotein particle, immunoreactivity may not always be identical to mass. For example, in the study of Sniderman et al. (3), some of the patients could have apparently elevated levels of LDL-apoB because of differences in immunoreactivity of apoB among different individuals. According to these workers (3), VLDL-apoB does not contribute to the LDL-apoB mass as measured in their RID system; they claim that 1.5% agarose excludes VLDL particles (19). However, recent studies have shown that many patients (46), particularly those with familial combined hyperlipidemia (47), have VLDL with particle sizes near to those of LDL. These particles, therefore, might diffuse in 1.5% agarose. Since Sniderman et al. (43) reported that a significant proportion of patients with hyperapobetalipoproteinemia have elevated plasma TG, relatively high levels of VLDL-apoB in these patients could have increased their apparent LDL-apoB to levels over 120 mg/dl if diffusible VLDL were present.

Abnormal composition of LDL

By definition, patients with hyperapobetalipoproteinemia have an abnormal composition of LDL (i.e., a high LDL-apoB/C ratio). This has been documented in a recent report by Teng et al. (48). Since the proportion of our patients with categorical hyperapobetalipoproteinemia was relatively small, we might ask whether many of

the remaining patients with coronary disease had a high ratio of apoB to C in LDL. If so, such lipoproteins might be unusually atherogenic. In the first report of Sniderman et al. (3), a sizable number of patients with proven CAD apparently had unusually high ratios of apoB to C in LDL in spite of having normal levels of both LDL-apoB and LDL-C.

To determine whether the same was true for our patients, it is necessary first to define the normal LDL-apoB/C ratio. Most young controls in our study had ratios below 0.60. Older control subjects on the average had higher ratios. Seemingly, LDL-apoB/C ratios frequently rise with age. In Table 4 the distribution of LDL-apoB/C ratios is given for each group; the results suggest that ratios exceeding 0.75 to 0.80 are abnormally high, although a study of many more subjects would be required to define a normal distribution curve for the adult population.

For normotriglyceridemic patients with CHD and CAD, the mean LDL-apoB/C ratio was not higher than the two control groups of older normolipidemic patients. For hypertriglyceridemic patients with coronary disease, the mean ratio of LDL-apoB/C was higher than that of other groups, whether coronary or control (Table 2). However, the ratios in both groups with high TG were distributed widely; some patients clearly had unusually high ratios, but others had relatively low ratios (Table 4). In patients with elevated TG levels, high ratios appeared to be related in part to obesity (Table 5).

The studies of Hammond and Fisher (34), Fisher (49), and Fisher, Hammond, and Warmke (50) have demonstrated that many patients with hypertriglyceridemia have "polydisperse" LDL. On analytical ultracentrifugation there is a bimodal distribution of LDL particle size. Some particles are larger than normal; others are smaller. Their data indicate that two factors can cause an increase in the LDL-apoB/C ratios in these patients: *a*) replacement of cholesteryl ester by triglyceride in larger LDL, and *b*) deficiency of total core lipids in small LDL. Redgrave and Carlson (51) have also shown that patients with hypertriglyceridemia often have an increase in apoB/C ratios in LDL. Teng et al. (48) have recently confirmed the presence of multiple species of LDL in a group of patients with hypertriglyceridemia, and these patients also showed increased amounts of small, "heavy" LDL with a high LDL-apoB/C ratios. In addition, they observed a group of normotriglyceridemic patients having high LDL-apoB levels and normal LDL-C concentrations who had a similar abnormal pattern in LDL.

The major purpose of this study was to examine the possibility that measurement of apoB concentrations in LDL may uncover a metabolic defect in patients with coronary disease that cannot be detected by determination of LDL-C levels alone. For the vast majority of our coronary patients with normolipidemia, LDL-apoB levels were not increased disproportionally to LDL-C; the term hyperapobetalipoproteinemia was applicable to only about 11% of this group compared to about 8% for non-coronary patients. Also, most coronary patients with hypertriglyceridemia did not have a definite elevation of LDL-apoB levels, and again, only about 10% could be said to have hyperapobetalipoproteinemia. On the other hand, many hypertriglyceridemic patients did have high LDL-apoB/C ratios suggestive of a defect in apoB metabolism. This high ratio may reflect an increased turnover rate of LDL-apoB observed frequently in hypertriglyceridemic patients with CHD (52).

REFERENCES

1. Wilson, P. W., R. J. Garrison, W. P. Castelli, U. Feinleib, P. M. McNamara, and W. B. Kannel. 1980. Prevalence of coronary heart disease in the Framingham offspring study: study of lipoprotein cholesterols. *Am. J. Cardiol.* **46:** 649–654.
2. Gordon, T., W. P. Castelli, M. C. Hjortland, W. B. Kannel, and T. R. Dawber. 1977. Predicting coronary heart disease in middle-aged and older persons. The Framingham study. *J. Am. Med. Assoc.* **238:** 497–499.
3. Sniderman, A., S. Shapiro, D. Marpole, B. Skinner, B. Teng, and P. O. Kwiterovich, Jr. 1980. Association of coronary atherosclerosis with hyperapobetalipoproteinemia (increased protein but normal cholesterol levels in human plasma low density (β) lipoproteins). *Proc. Natl. Acad. Sci. USA.* **77:** 604–608.
4. Kesaniemi, Y. A., and S. M. Grundy. 1983. Overproduction of low density lipoproteins associated with coronary heart disease. *Atherosclerosis.* **3:** 40–46.
5. Vega, G. L., D. R. Illingworth, S. M. Grundy, F. T. Lindgren, and W. E. Connor. 1983. Normocholesterolemic tendon xanthomatosis with overproduction of apolipoprotein B. *Metabolism.* **32:** 118–125.
6. The Lipid Research Clinics Program Epidemiology Committee. 1979. The Lipid Research Clinic Study. Plasma lipid distributions in selected North American populations: The Lipid Research Clinics Program Prevalence Study. *Circulation.* **60:** 427–439.
7. Heiss, G., I. Tamir, C. E. Davis, H. A. Tyroler, B. M. Rifkind, G. Schonfeld, D. Jacobs, and I. D. Frantz. 1980. Lipoprotein-cholesterol distribution in selected North American populations: The Lipid Research Clinics Program Prevalence Study. *Circulation.* **61:** 302–315.
8. Vega, G. L., E. Groszek, R. Wolf, and S. M. Grundy. 1982.

Influence of polyunsaturated fats on composition of plasma lipoproteins and apolipoproteins. *J. Lipid Res.* **23:** 811–822.

9. Roeschlau, P., E. Bernt, and W. Gruber. 1974. Enzymatic determination of total cholesterol in serum. *Z. Klin. Chem. Klin. Biochem.* **12:** 226.

10. Wahlefeld, A. W. 1974. Methods of Enzymatic Analysis. H. U. Bergmeyer, editor. Academic Press, New York. 1831.

11. Lipid and Lipoprotein Analysis. Manual of Laboratory Operations, Lipid Research Clinic Program. 1974. DHEW publication No. NIH/75-628.

12. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentrations of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.

13. Wilson, D. E., and M. Spiger. 1973. A dual precipitation method for quantitative plasma lipoprotein measurement without ultracentrifugation. *J. Lab. Clin. Med.* **82:** 473–475.

14. Wilson, P. W., R. D. Abbott, R. J. Garrison, and W. P. Castelli. 1981. Estimation of very-low density lipoprotein cholesterol from data on triglyceride concentration in plasma. *Clin. Chem.* **27:** 2008–2010.

15. Myers, L. H., N. R. Phillips, and R. J. Havel. 1976. Mathematical methods for estimation of the concentration of the major lipid components of human serum lipoproteins. *J. Lab. Clin. Med.* **88:** 491–505.

16. Albers, J. J., G. R. Warnick, D. Wiebe, P. King, P. Steiner, L. Smith, E. Breckenridge, A. Chow, K. Kuba, S. Weidman, H. Arnett, P. Wood, and A. Shlagenhoft. 1978. Multi-laboratory comparison of three heparin-Mn^{2+} precipitation procedures for estimating cholesterol in high density lipoprotein. *Clin. Chem.* **24:** 853–856.

17. Lindgren, F. T., and L. C. Jensen. 1972. Isolation and quantitative analysis of serum lipoproteins. *In* Blood Lipids and Lipoproteins: Quantitation, Composition and Metabolism. G. L. Nelson, editor. Wiley-Interscience, New York. 181–270.

18. Anderson, D. W., A. V. Nichols, S. S. Pan, and F. T. Lindgren. 1978. High density lipoprotein distribution. Resolution and determination of three major components in a normal population sample. *Atherosclerosis.* **29:** 161–179.

19. Sniderman, A. D., B. Teng, and M. Jerry. 1975. Determination of B protein of low density lipoprotein directly in plasma. *J. Lipid Res.* **16:** 465–467.

20. Monothony, J. F., E. G. Wallace, and D. M. Allen. 1978. A non-barbital buffer for immunoelectrophoresis and zone electrophoresis in agarose gels. *Clin. Chem.* **24:** 1825–1827.

21. Renn, D. W., and E. Evans. 1975. Use of heteropoly acids as immunological precipitation brightness. *Anal. Biochem.* **64:** 620–623.

22. Sata, T., R. J. Havel, and A. L. Jones. 1972. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. *J. Lipid Res.* **13:** 757–768.

23. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.

24. Holmquist, L., K. Carlson, and L. A. Carlson. 1978. Comparison between the use of isopropanol and tetramethyl urea for the solubilization and quantitation of human serum very low density apolipoproteins. *Anal. Biochem.* **88:** 457–460.

25. Holmquist, L., and K. Carlson. 1977. Selective extraction of human serum very low density apolipoproteins with organic solvents. *Biochim. Biophys. Acta.* **493:** 400–409.

26. Doumas, B. T. 1975. Standards for total serum protein assays: a collaborative study. *Clin. Chem.* **21:** 1159–1166.

27. Kirk, P. L. 1947. The chemical determination of proteins. *Adv. Protein Chem.* **3:** 139–167.

28. Tanford, C., and C. L. Roberts, Jr. 1952. Phenolic hydroxyl ionization in proteins. I. Bovine serum albumin. *J. Am. Chem. Soc.* **74:** 2509–2515.

29. Markwell, M. A. K., S. M. Haas, N. E. Tolbert, and L. L. Bieber. 1981. Protein determination in membranes and lipoprotein samples: manual and automated procedures. *Methods Enzymol.* **72:** 296–302.

30. Helenius, A., and R. Simon. 1971. Removal of lipids from human plasma low-density lipoproteins by detergents. *Biochemistry.* **10:** 2542–2547.

31. Bradley, W. A., E. G. Gilliam, A. M. Gotto, Jr., and S. H. Gianturco. 1982. Apolipoprotein-E degradation in human very low density lipoproteins by plasma protease(s): chemical and biological consequences. *Biochem. Biophys. Res. Commun.* **109:** 1360–1367.

32. Kashyap, M. L., B. A. Hynd, and K. Robinson. 1980. A rapid and simple method for measurement of total protein in very low density lipoproteins by the Lowry assay. *J. Lipid Res.* **21:** 491–495.

33. Kane, J. P., T. Sata, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins in human serum. *J. Clin. Invest.* **56:** 1622–1634.

34. Hammond, M. G., and W. R. Fisher. 1971. The characterization of a discrete series of low density lipoproteins in the disease, hyper-pre-β-lipoproteinemia. *J. Biol. Chem.* **246:** 5454–5465.

35. Shen, M. M. S., R. M. Krauss, F. T. Lindgren, and T. M. Forte. 1981. Heterogeneity of serum low density lipoproteins in normal human subjects. *J. Lipid Res.* **22:** 236–244.

36. Kesaniemi, Y. A., G. L. Vega, and S. M. Grundy. 1982. Kinetics of apolipoprotein B in normal and hyperlipidemic man: review of current data. *In* Lipoprotein Kinetics and Modeling. M. Berman, S. M. Grundy, and B. V. Howard, editors. Academic Press, New York. 182–203.

37. Zar, J. H. 1974. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ.

38. Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality. *Biometrika.* **52:** 591–611.

39. Wayne, T. F., P. Alaupovic, M. D. Curry, E. G. Lu, P. S. Anderson, and E. Schectin. 1981. Plasma apolipoprotein B and VLDL-, LDL-, and HDL-cholesterol as risk factors in the development of coronary artery disease in male patients examined by angiography. *Atherosclerosis.* **39:** 411–424.

40. Avogaro, P., G. Bittolo Bon, G. Cazzolato, G. B. Quinci, and F. Belussi. 1978. Plasma levels of apolipoprotein A-I and apolipoprotein B in human atherosclerosis. *Artery.* **4:** 385–394.

41. Fager, G., O. Wiklund, S. O. Olofsson, L. Wilhelmsen, and G. Bondjers. 1981. Multivariate analyses of serum apolipoproteins and risk factors in relation to acute myocardial infarction. *Arteriosclerosis.* **1:** 273–279.

42. De Backer, G., M. Rosseneu, and J. P. Deslypere. 1982. Discriminative value of lipids and apoproteins in coronary heart disease. *Atherosclerosis.* **42:** 197–203.

43. Sniderman, A., C. Wolfson, B. Teng, F. A. Franklin, P. S. Bachorik, and P. O. Kwiterovitch. 1982. Association of

hyperapobetalipoproteinemia with endogenous hypertri-glyceridemia and atherosclerosis. *Ann. Intern. Med.* **97:** 833–839.

44. Carlson, L. A. 1982. Serum lipids and atherosclerosis. *In* Metabolic Risk Factors in Ischemic Cardiovascular Disease. L. A. Carlson and B. Pernow, editors. Raven Press, New York. 1–16.

45. The Pooling Project Research Group. 1978. Relationship of blood pressure, serum cholesterol, smoking habit, relative weight, and ECG abnormalities to incidence of major coronary events: final report of the pooling project. *J. Chronic Dis.* **31:** 201–306.

46. Groszek, E., and S. M. Grundy. 1978. Electron microscopic evidence of particles smaller than 250 Å in very low density lipoproteins of human plasma. *Atherosclerosis* **32:** 241–250.

47. Brunzell, J. D., J. J. Albers, A. Chait, S. M. Grundy, E. Groszek, and G. B. McDonald. 1983. Plasma lipoproteins in familial combined hyperlipidemia and monogenic familial hypertriglyceridemia. *J. Lipid Res.* **24:** 147–155.

48. Teng, B., G. R. Thompson, A. D. Sniderman, T. M. Forte, R. M. Krauss, and P. O. Kwiterovich, Jr. 1983. Composition and distribution of low density lipoprotein fractions in hyperapobetalipoproteinemia, normolipidemia, and familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA.* **80:** 6662–6666.

49. Fisher, W. R. 1970. The characterization and occurrence of an S_f20 serum lipoprotein. *J. Biol. Chem.* **245:** 877–884.

50. Fisher, W. R., M. G. Hammond, and G. L. Warmke. 1972. Measurements of molecular weight variability of plasma low density lipoproteins among normals and subjects with hyper-β-lipoproteinemia. Demonstration of molecular heterogeneity. *Biochemistry.* **11:** 519–525.

51. Redgrave, T. G., and L. A. Carlson. 1979. Changes in plasma very low density and low density lipoprotein content, composition, and size after a fatty meal in normo- and hypertriglyceridemic man. *J. Lipid Res.* **20:** 217–229.

52. Vega, G. L., W. Beltz, and S. M. Grundy. 1983. Overproduction of LDL in hypertriglyceridemic patients with coronary heart disease. *Circulation.* **68:** III–189.