Abnormalities of low density lipoproteins in normolipidemic type II diabetic and nondiabetic patients with coronary artery disease

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Abstract The characteristics of low density lipoproteins (LDL) of ten non-insulin-dependent diabetic (NIDDM) and ten nondiabetic patients with coronary artery disease (CAD) were investigated and compared to LDL of ten NIDDM patients without CAD and ten healthy persons. All subjects had LDL cholesterol below 160 mg/dl and serum triglycerides below 200 mg/dl. The mean LDL particle size and particle distribution profiles were analyzed by using nondenaturing polyacrylamide gradient gel electrophoresis. The LDL composition and hydrated density distribution were investigated by using density gradient ultracentrifugation. Both NIDDM and nondiabetic CAD patients tended to have larger LDL particles than NIDDM patients without CAD and healthy subjects. The increase of LDL particle size of CAD patients was due to marked enrichment of triglycerides (TG) in their LDL. The percentage content of TG in LDL of NIDDM patients with CAD was 14.5% and in LDL of nondiabetic CAD patients 13.4% compared with 7.9% in LDL of NIDDM patients without CAD and 7.2% in normal-LDL ($P < 0.05$ or less between either CAD group and NIDDM without CAD or normals). The LDL TG/apolipoprotein (apo) B weight ratio was significantly higher in both CAD groups compared with LDL of the two groups without CAD (0.70 and 0.68 vs. 0.38 and 0.34, respectively, $P < 0.05$, $P < 0.05$ and $P < 0.01$, $P < 0.01$). The LDL total lipid to apoB weight ratio was similar in all four groups. Consistent with this, the hydrated density distributions of LDL in the four groups were similar, the average peak densities being 1.0346 g/ml, 1.0331 g/ml, 1.0331 g/ml, and 1.0331 g/ml, respectively. The findings of this study demonstrate that normolipidemic patients with CAD may have marked abnormalities in their LDL composition and these anomalies are present in both diabetic and nondiabetic patients.—**Tilly-Kiesi, M., M. Syvänne, T. Kuusi, S. Lahdenperä, and M-R. Taskinen.** Abnormalities of low density lipoproteins in normolipidemic type II diabetic and nondiabetic patients with coronary artery disease. *J. Lipid Res.* 1992. **33:** 333–342.

Supplementary key words normolipidemia • gradient gel electrophoresis • density gradient ultracentrifugation • LDL size • LDL density • LDL composition

Low density lipoproteins are considered to be the most atherogenic of lipoproteins based on the close association between LDL cholesterol concentration and increased risk for coronary heart disease (CHD) (1). However, one-third of the persons with premature coronary atherosclerosis have apparently normal plasma cholesterol and LDL cholesterol levels. Hamsten et al. (2) reported that 31% of young survivors of acute myocardial infarction with angiographically verified coronary artery disease (CAD) had lipid and lipoprotein levels within normal range. Similarly, Nieminen et al. (3) observed that 31.5% of middle-age CAD patients exhibit normal lipoprotein phenotype.

In non-insulin-dependent diabetes mellitus (NIDDM), the leading cause of death is CAD and management of dyslipidemia in diabetics is of special importance (4). LDL cholesterol level is generally within normal range or mildly elevated, whereas moderate elevations of plasma and VLDL triglycerides and reduced HDL cholesterol concentration are characteristic for NIDDM (5). In general, changes of LDL cholesterol and VLDL triglycerides correlate with the metabolic state and are markedly improved if proper glycemic control is achieved. In contrast, the changes of HDL cholesterol level are less clearly associated with glycemic control (5). In NIDDM the association of serum lipids, lipoproteins, and apolipoproteins with prevalence of myocardial infarction (MI) is qualitatively similar to nondiabetics (6). However, NIDDM patients with CHD appear to have somewhat higher plasma triglyceride and lower HDL cholesterol concentrations compared to nondiabetic CHD patients (6). Despite this, concentrations of plasma triglyceride, LDL, and HDL cholesterol are not significant predictors of MI (7). This raises the question whether lipoproteins also exhibit potentially atherogenic compositional changes.

Abbreviations: LDL, low density lipoproteins; NIDDM, non-insulin-dependent diabetes; CHD, coronary heart disease; CAD, coronary artery disease; TG, triglyceride; VLDL, very low density lipoproteins; HDL, high density lipoproteins; IDL, intermediate density lipoproteins.

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Recently the search for atherogenic properties of low density lipoproteins has revealed two LDL phenotypes (hyperapoB and LDL subclass pattern B) (8–13), in which plasma lipid and LDL cholesterol levels may be normal. In these disorders the LDL composition, size, and metabolism are abnormal (8–10) or the LDL particle size distribution is typical for the phenotype (11–13). However, these LDL abnormalities appear only in a part of normolipidemic patients with CHD or CAD (12, 14). In contrast to several studies on the quantative changes of serum total and LDL cholesterol in NIDDM heretofore, there was no information on compositional abnormalities of LDL and their role as a risk factor for CAD in NIDDM.

Therefore, the purpose of the present study was to evaluate and compare the particle size, hydrated density distribution, and composition of low density lipoproteins of normolipidemic NIDDM patients and nondiabetic patients with angiographically verified CAD in relation to LDL properties of NIDDM patients without CAD and of healthy persons.

SUBJECTS AND METHODS

Subjects

LDL analyses were performed using blood samples obtained from 40 fasting male subjects. Twenty men had CAD, 10 with NIDDM (DM+, CAD+) and 10 nondiabetics (DM–, CAD+). They were referred for coronary angiography to I Department of Medicine in Helsinki University Central Hospital because of severe angina. The CAD diagnosis was based on ≥ 50% stenosis of at least one major coronary artery in a performed coronary angiography. Six of the DM+, CAD+ patients had three-vessel disease, one two-vessel disease, and three one-vessel disease. All the DM–, CAD+ patients had three-vessel disease. Sixteen of the CAD patients underwent later coronary artery bypass surgery, two were treated with percutaneous transluminal coronary angioplasty (PTCA) and two conservatively. None of the patients had a history of myocardial infarction in the 3 months prior to the lipid determinations. The two control groups included ten male patients with NIDDM, but no symptoms or signs of CAD (DM+, CAD–) and ten healthy men (DM–, CAD–). To be eligible for the study, the participants had serum triglycerides ≤ 2.30 mmol/l (≤ 200 mg/dl) and LDL cholesterol concentration ≤ 4.10 mmol/l (≤ 160 mg/dl). None of the subjects were taking lipid-lowering drugs. Diabetics with significant microalbuminuria (> 30 μg/min) and/or nephropathy were excluded. All subjects had normal hepatic and thyroid function tests.

In NIDDM patients with CAD the median duration of diabetes was 4.5 years (range 1–15 yr). Four were on sulfonylurea therapy, one received biguanide, one subcutaneus insulin, and four patients were treated with diet only. The concentration of fasting C-peptide averaged 0.88 ± 0.56 nM (mean ± SD). The patients were in stable glycemic control: the mean ± SD for glycosylated hemoglobin (HbAlc) value was 7.3 ± 1.3% and for fasting blood glucose 8.7 ± 3.2 mmol/l. Ten nondiabetic CAD patients had normal fasting blood glucose concentration 4.8 ± 0.4 mmol/l (mean ± SD) and normal HbAlc values 5.4 ± 0.4% (mean ± SD). The use and doses of beta blockers and long-acting nitrates in diabetic and non-diabetic CAD subjects were similar, but number of users of calcium channel blockers differed in these two groups (**Table 1**).

The ten normolipidemic NIDDM males without symptoms or signs of cardiovascular disease were participants of a larger clinical study performed in the

TABLE 1. Clinical characteristics of study groups

Subjects	n[a]	Age	BMI[b]	Beta-Blockers[c]	Ca Channel Blockers	Nitrate[d]
		yr	*kg/m²*			
DM+ CAD+ (a)	10	57 ± 2.8	28.4 ± 3.8	7/10	9/10	9/10
DM– CAD+ (b)	10	56 ± 4.8	27.9 ± 2.1	8/10	5/10	9/10
DM+ CAD– (c)	10	59 ± 4.8	27.0 ± 3.5	2/10	0/10	0/10
Normals (d)	10	49 ± 6.3	26.4 ± 2.5			
a vs b		ns	ns			
a vs c		ns	ns			
a vs d		$P < 0.01$	ns			
b vs c		ns	ns			
b vs d		$P < 0.01$	ns			
c vs d		$P < 0.01$	ns			
Variance analysis		$P < 0.01$	ns			

Abbreviations: DM, diabetes; CAD, coronary artery disease; ns, not significant.
[a]Number of subjects.
[b]Body mass index calculated from weight (kg)/height (m) exp2.
[c]Beta-blockers: metoprolol or atenolol in DM+, CAD+, and DM–,CAD+ patients and pindolol in DM+,CAD– persons.
[d]Long acting nitrate.

Helsinki University Central Hospital. All proved to have a normal finding in a thallium exercise test. The median duration of diabetes was 6.8 years (range 1–15 yr). The concentration of fasting C-peptide averaged 0.84 ± 0.26 nM. One patient was treated with sulfonylurea, three received sulfonylurea and biguanide, two subcutaneus insulin, two subcutaneus insulin plus sulfonylurea, and two were treated with diet only. Their glycemic control was comparable to NIDDM patients with CAD: glycosylated hemoglobin averaged 7.0 ± 1.6% and fasting blood glucose was 8.0 ± 3.1 mmol/l. Additionally, LDL samples of ten healthy normolipidemic males were analyzed. The donors were employees of the Cultor company (Kantvik, Finland) participating in a plasma lipid survey arranged by the health department of the company and Helsinki University Central Hospital. The subjects were in good physical health, had no history of cardiovascular disease, and fulfilled the criteria for lipid values of the study (serum TG ≤ 2.30 mmol/l and LDL cholesterol ≤ 4.10 mmol/l).

The clinical parameters of all participants are presented in Table 1; serum lipid and lipoprotein values are shown in **Table 2**.

Methods

Serum lipoproteins were isolated by sequential ultracentrifugation (15). Three ml serum and 2.5 ml KBr solution (d 1.006 g/ml) were ultracentrifuged at 35,000 rpm for 18 h at 2°C in a Beckman L8-70 ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA) in a Beckman Type 50.4TI rotor. After centrifugation, the very low density lipoprotein (VLDL) fraction was removed by the tube slicing and the infranate was adjusted to d 1.063 g/ml and ultracentrifuged (35,000 rpm, 24 h, 2°C). The fraction containing the intermediate density lipoproteins (IDL) and low density lipoproteins (LDL) was removed by tube slicing and HDL remained in the infranate. The LDL + IDL sample was dialyzed for 8 h against NaBr solution, d 1.0340 g/ml. The efficacy of dialysis was checked in preliminary studies with LDL + IDL preparations prepared in the same way as the samples in which a DMA 46 Digital Density Meter (Anton Paar, Graz, Austria) was used.

LDL density gradient ultracentrifugation

The LDL density gradient ultracentrifugation was carried out in a Beckman L8-70 ultracentrifuge with a SW 40TI swinging bucket rotor using Beckman Ultraclear 9/16 × 33/14 13-ml centrifuge tubes. The method was based on the same principles as the method reported by Shen et al. (16) with slight modifications in preparing the discontinuous gradient of sodium bromide solutions and with a direct density control in each sample tube as described elsewhere (17). The mean density range in the tubes after centrifugation was from d 1.0162 to 1.0656 g/ml. Of the 26 0.5-ml fractions gathered, the 4 lightest 0.5-ml fractions (d 1.0162–1.0193 g/ml) contained IDL. The remaining 22 fractions were analyzed for concentrations of cholesterol, free cholesterol, triglyceride, phospholipid, and apoB in each subject. The LDL mass in every 0.5-ml fraction of each subject was calculated by adding the measured concentrations of apoB and lipid components. The density of the fraction containing the greatest LDL mass was designated "peak density."

LDL gradient gel electrophoresis

The nondenaturing polyacrylamide gradient gel electrophoresis of LDL was performed from serum samples of 20 patients and 20 controls according to the method described by Nichols, Krauss, and Musliner (18) using commercial Pharmacia PAA 2%–16% gels stained for lipids with Sudan black. The stain was

TABLE 2. Mean (± SD) serum lipid and lipoprotein cholesterol concentrations in CAD patients with NIDDM and without NIDDM, in NIDDM patients without CAD, and in healthy persons

Subjects	n	Chol	TG	LDL-Chol	HDL-Chol	VLDL-Chol	VLDL-TG
				mmol/l			
DM+ CAD+ (a)	10	4.88 ± 0.76	1.68 ± 0.31	3.02 ± 0.76	1.14 ± 0.25	0.45 ± 0.09	1.08 ± 0.34
DM– CAD+ (b)	10	5.32 ± 0.57	1.53 ± 0.28	3.44 ± 0.53	1.19 ± 0.21	0.46 ± 0.19	0.86 ± 0.25
DM+ CAD– (c)	10	5.19 ± 0.76	1.53 ± 0.49	3.22 ± 0.72	1.37 ± 0.50	0.43 ± 0.19	1.03 ± 0.42
Normals (d)	10	5.89 ± 0.78	1.71 ± 0.32	3.45 ± 0.59	1.69 ± 0.36	0.53 ± 0.19	1.08 ± 0.41
a vs b		ns	ns	ns	ns	ns	ns
a vs c		ns	ns	ns	ns	ns	ns
a vs d		$P < 0.01$	ns	ns	$P < 0.01$	ns	ns
b vs c		ns	ns	ns	ns	ns	ns
b vs d		$P < 0.05$	ns	ns	$P < 0.01$	ns	ns
c vs d		$P < 0.05$	ns	ns	ns	ns	ns
Variance analysis		$P < 0.01$	ns	ns	$P < 0.01$	ns	ns

Abbreviations: n, number of subjects; Chol, total cholesterol; TG, triglycerides; LDL-Chol, low density lipoprotein cholesterol; HDL-Chol, high density lipoprotein cholesterol; VLDL-Chol, very low density lipoprotein cholesterol; VLDL-TG, very low density triglycerides; DM, non-insulin-dependent diabetes; CAD, coronary artery disease; ns, not significant.

prepared by dissolving 2 g of Sudan black B in a solvent mixture containing 30% methanol, 30% 2-propanol, and 40% distilled water (total volume, 300 ml). The solution was stirred with care with warming in a water bath close to the boiling temperature and then filtered using 595 S&S Rundfilter (Schleicher & Schuell, Dassel, Germany). The stain was allowed to cool before adding 0.2 mg/100 ml Zn acetate with gentle stirring. The mixture was then filtered a second time.

Stained gels were scanned at 595 nm with a computer-assisted scanning densitometer (Cliniscan 2, Helena Laboratories, Beaumont, TX) capable of superimposing on the screen with each sample a calibrated reference LDL preparation. This was run in each gel to enable the evaluation of the particle diameter of the main peak of the LDL sample.

Laboratory determinations

The concentrations of cholesterol and triglycerides in whole serum and in VLDL, LDL, and HDL isolated by sequential ultracentrifugation were determinated by enzymatic colorimetric methods (Boehringer Mannheim GmbH, Diagnostica, Germany). Enzymatic colorimetric methods were also used to measure the concentrations of cholesterol, free cholesterol (FC), triglycerides, and phospholipids (PL) in the fractions of LDL obtained by density gradient ultracentrifugation. The cholesteryl ester concentration was calculated by subtracting free cholesterol concentration from total cholesterol. Apolipoprotein B was measured in LDL subfractions by a commercial immunoturbidimetric assay (Orion Diagnostica, Finland). Blood glucose determination was performed using a glucose oxidase method (Auto-Analyzer, Technicon, Tarrytown, NY). Glycosylated hemoglobin (HbAlc) was measured by use of the Diamat Analyzer System (Bio-Rad, Clinical Division, Richmond, CA). The normal range was 4.0–6.0%.

Statistical analyses

The statistical significances between two means were assessed with an unpaired *t* test; multiple comparisons were computed with one-way analysis of variance; and LDL density distribution profiles of the groups were compared by multivariate analysis of variance using BMDP statistical software (University of California Press, 1988).

RESULTS

LDL hydrated density distribution

The LDL hydrated density distributions of the four groups were very similar (**Fig. 1**). The mean peak densities were 1.0346 g/ml, 1.0331 g/ml, 1.0331 g/ml, and 1.0331 g/ml in NIDDM with CAD, in nondiabetic CAD patients, in NIDDM without CAD, and in healthy persons, respectively. The shapes of the LDL mass distribution curves resembled each other and statistical analysis showed that the four groups did not differ from each other in respect to the percentage of total LDL mass found in separate density fractions (Fig. 1). One to three individuals in each group displayed polydisperse LDL density distribution, but this LDL profile was not a characteristic finding for any of the study groups (Fig. 1).

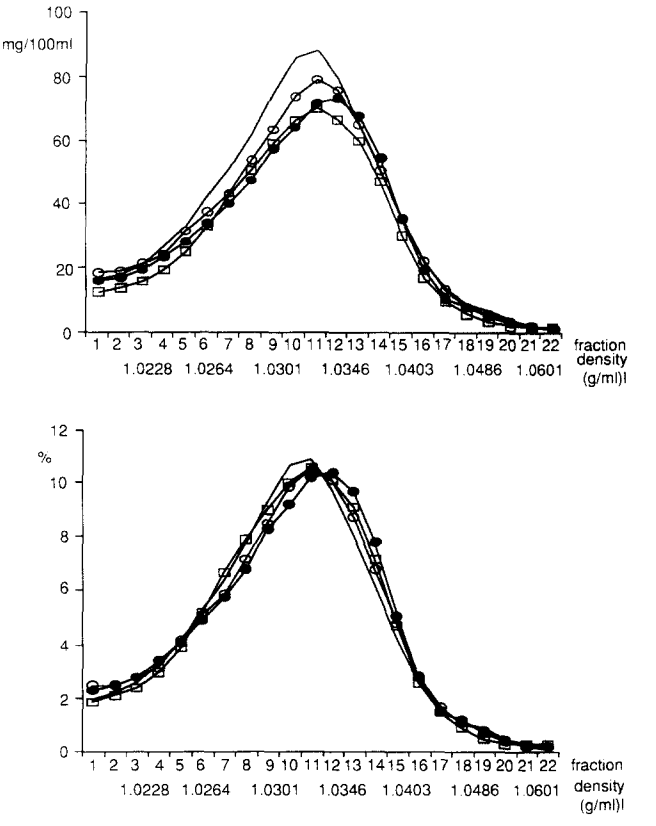

Fig. 1. Top: Hydrated density distribution of low density lipoprotein mass in CAD patients with NIDDM (●), in nondiabetic patients with CAD (——), in NIDDM patients without CAD (○), and in healthy subjects (□). The LDL mass was calculated from the concentration of apoB, cholesterol, triglyceride, and phospholipid measured in 22 fractions obtained by density gradient ultracentrifugation. Each point represents the mean value of the patients in each group of CAD patients with NIDDM, or nondiabetic CAD patients, or NIDDM subjects without CAD, or healthy persons (10 subjects per group). Bottom: The percentage of total LDL mass in separate density fractions of NIDDM patients with CAD (●), nondiabetic CAD patients (——), NIDDM subjects without CAD (○), and in healthy persons (□).

LDL particle size

The distribution of LDL particles by size and the particle diameter of the main peak was determined by use of nondenaturing polyacrylamide gradient gel electrophoresis. In diabetic CAD patients, 8/10 had at

least two distinct peaks and in 8/10 the major peak size was larger than 255 Å. In nondiabetic CAD patients, two or more peaks were observed in 8/10, and in 6/10 the major peak size was larger than 255 Å. In NIDDM without CAD, one major peak and a minor peak were verified in 5/10, and in three subjects the major peak was larger than 255 Å. In the normal group, two or more peaks were seen in 5/10 and in five persons the major peak size was larger than 255 Å. The densitometric scans for eight representative subjects (two in each group) are presented in **Fig. 2.** The particle diameters of the major LDL peak averaged 259 ± 5 Å, 259 ± 8 Å, 253 ± 8 Å, and 254 ± 7 Å in NIDDM with CAD, in nondiabetic CAD patients, in NIDDM without CAD, and in normals, respectively. Differences in means of major peak diameters of the four groups were not statistically significant. However, the LDL peak diameter of all CAD patients combined (259 ± 7) was, on average, greater compared to that of all non-CAD subjects combined (253 ± 8) ($P < 0.05$).

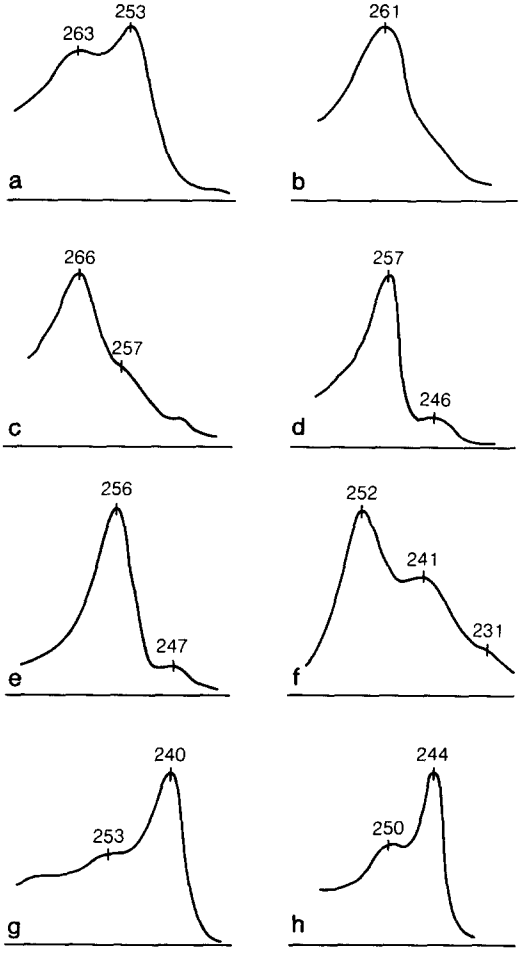

Fig. 2. Demonstration of plasma LDL particle subclasses separated by 2–16% polyacrylamide gradient gel electrophoresis. Scans of two CAD patients with NIDDM; peak particle diameters 253 Å (a) and 261 Å (b). Scans of two nondiabetic CAD patients having peak particle diameters 266 Å (c) and 257 Å (d). Scans of two NIDDM patients without CAD with peak particle diameters 256 Å (e) and 252 Å (f). Scans of two healthy, normolipidemic persons with the peak particle diameters 240 Å (g) and 244 Å (h).

LDL composition

The content of apoB and the lipid to apoB weight ratio in LDL of the four groups were similar (**Table 3, Table 4**). This is consistent with the observed density distribution of LDL, since the density of LDL particles is determined by the total lipid to apoB ratio. However, the groups differed from each other in respect to LDL lipid composition. In diabetic CAD patients, the percentage of triglycerides in LDL was increased by 83.5% and 101.4% compared to diabetics without CAD and to normals, respectively (Table 3). Similarly, in nondiabetic CAD patients the percentage of triglycerides in LDL was increased by 69.6% and 86.1% compared to diabetics without CAD or to normals, respectively (Table 3). Also, the triglyceride to apoB weight ratio in LDL was increased in diabetic CAD patients by 84.2% and 105.9% and in nondiabetic CAD patients by 78.9% and 100.0% in comparison to diabetics without CAD and to normals, respectively, (Table 4). The LDL CE/TG weight ratio in the diabetic and nondiabetic CAD groups was abnormally low [2.79 in diabetics and 2.81 in nondiabetics vs. 4.47 in NIDDM without CAD ($P < 0.05$ for both) and vs. 5.18 in normals ($P < 0.01$ for both) Table 4]. The findings indicate that LDL composition of our CAD patients, diabetic or nondiabetic, was characterized by a substantial enrichment of LDL with triglycerides, whereas depletion of cholesteryl esters was less pronounced. The percentage of free cholesterol and phospholipid in LDL of both diabetic and nondiabetic CAD patients was reduced compared to NIDDM patients without CAD or to normals (Table 3). The compositional changes shared by both CAD groups compared to either group without CAD are in line with the slightly larger particle diameter of the main LDL peak of CAD patients, since the surface area/core volume ratio of a spherical particle is indirectly related to its radius.

DISCUSSION

Recent findings have demonstrated that not only the elevated concentration of LDL but also compositional abnormalities of these lipoproteins are associated with increased risk for CHD. Some individuals with normal plasma lipid levels may have hyperapobetalipoproteinemia, a lipoprotein phenotype characterized by small, dense LDL with abnormal composition and association with CAD (8–10). According to the study by Vega and Grundy (14), approximately

TABLE 3. Mean diameter of the major LDL peak, and the composition of LDL in CAD patients (with or without NIDDM), in NIDDM patients without CAD, and in healthy persons

Subjects	n	Diameter[a]	CE	FC	TG	PL	ApoB
		nm			%		
DM+ CAD+ (a)	10	259 ± 5	30.5 ± 3.8	9.5 ± 1.2	14.5 ± 7.6	23.9 ± 2.2	21.6 ± 2.5
DM– CAD+ (b)	10	259 ± 5	32.1 ± 1.4	9.4 ± 1.6	13.4 ± 4.6	24.8 ± 1.2	20.3 ± 1.8
DM+ CAD– (c)	10	253 ± 8	32.8 ± 3.4	11.0 ± 1.9	7.9 ± 2.1	26.7 ± 2.1	21.5 ± 4.2
Normals (d)	10	254 ± 8	34.8 ± 2.2	10.8 ± 1.2	7.2 ± 1.8	26.0 ± 1.0	21.2 ± 1.2
a vs b		ns	ns	ns	ns	ns	ns
a vs c		ns	ns	$P < 0.05$	$P < 0.05$	$P < 0.01$	ns
a vs d		ns	$P < 0.01$	$P < 0.05$	$P < 0.01$	$P < 0.05$	ns
b vs c		ns	ns	$P < 0.05$	$P < 0.01$	$P < 0.05$	ns
b vs d		$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.01$	$P < 0.05$	ns
c vs d		ns	ns	ns	ns	ns	ns
Variance analysis		ns	$P < 0.05$	$P < 0.05$	$P < 0.01$	$P < 0.01$	ns

Values given as mean ± SD. Abbreviations: n, number of subjects; CE, cholesteryl ester; FC, free cholesterol; TG, triglycerides; PL, phospholipid; apoB, apolipoprotein B; DM, diabetes; CAD, coronary artery disease; ns, not significant.
[a]The diameter of the major peak in gradient gel electrophoresis.

10% of normolipidemic CHD or CAD patients have this lipoprotein disorder. However, none of our patients fulfilled the established criteria for hyperapobetalipoproteinemia having LDL apoB over 120 mg/dl and LDL cholesterol lower than 200 mg/dl (8). The infrequency of hyperapobetalipoproteinemia is consistent with earlier reports on the low prevalence of this lipoprotein disorder in Finns (19, 20). Austin et al. (11–13) have described an LDL subclass pattern B that is characterized by predominance of small, dense LDL particles with mostly normal composition. This phenotype appears to be inherited as a single-gene trait at an estimated frequency of 15% expressed after age of 40 (12, 13). LDL subclass pattern B associates strongly with increased risk for myocardial infarction and its prevalence is closely and directly associated with increases of plasma triglycerides as well as with variations of other lipid levels (12, 13). However, some of the subjects with this phenotype may have normal lipid levels. We verified a pattern B type profile in one-third of the tested subjects (12/40) with similar presentation in all four groups.

The present results reveal three major findings. First, in the patients with angiographically verified CAD, diabetic or nondiabetic, the LDL particle size, density distribution, and composition remarkably resembled each other. Second, the LDL composition of CAD patients (both diabetic and nondiabetic) differed strikingly from that of subjects without CAD (both diabetic and healthy persons) in spite of similar concentrations of serum triglycerides and LDL cholesterol. Third, qualitative differences existed between LDL of diabetics having CAD and diabetics not having CAD. The similarity in LDL hydrated density distribution in the four groups is in agreement with the similar LDL lipid to apoB ratio and with the previously demonstrated connection between serum triglyceride concentration and the distribution of LDL particles within the LDL density range (17, 21–24). Appreciating this close link between serum triglyceride level and the distribution of LDL particles, we intentionally selected the groups with similar levels of triglyceride. This selection process may explain why our CAD patients unlike those of Austin et al. (12, 13) did not exhibit "small-size" LDL. In fact Crouse et al. (24) found no difference in LDL molecular weight between CAD patients and controls after covariance adjustment for triglyceride. Similarly, Richards, Grundy, and Cooper (25) did not observe a greater number of CAD patients with abnormally dense LDL particles compared with normal subjects, independent of triglyceride concentration. Notably, LDL particle size seems to be sensitive to variations in serum triglycerides even within the normal range (25). This may explain the somewhat low diameter of the major LDL peak in our two control groups.

Despite similar density distribution, the LDL particle size tended to be increased due to a prominent enrichment with core triglycerides in both diabetic and nondiabetic patients with CAD. Tornvall et al. (26) verified that in all lipoprotein fractions only the triglyceride level in both light and heavy LDL correlated significantly with the severity of coronary atherosclerosis, and both the light and heavy LDL were richer in triglycerides in the patients with more severe CAD.

In the present study we did not observe differences in LDL size, density distribution, or composition between NIDDM patients and nondiabetic subjects. This finding agrees well with the data of Klein, Lyons, and Lopes-Virella (27). Similarly, James and Pometta (28) reported no gross differences in the relative composition of LDL in NIDDM patients versus controls. In contrast, Bagdade et al. (29) have described that in NIDDM patients the TG/CE core lipid ratio is increased in LDL but it is decreased in VLDL. Notably,

TABLE 4. Mean lipid/apoB weight ratios in LDL, CE/TG in LDL core lipid, PL/FC in surface lipid, and total lipid to apoB ratios in CAD patients (with or without NIDDM), in NIDDM patients without CAD, and in healthy persons

Subjects	n	CE/ApoB	FC/ApoB	TG/ApoB	PL/ApoB	CE/TG	PL/FC	Lipid/ApoB
DM+ CAD+ (a)	10	1.42 ± 0.18	0.44 ± 0.07	0.70 ± 0.35	1.12 ± 0.13	2.79 ± 1.65	2.53 ± 0.20	3.68 ± 0.55
DM− CAD+ (b)	10	1.59 ± 0.10	0.46 ± 0.07	0.68 ± 0.26	1.23 ± 0.10	2.81 ± 1.42	2.71 ± 0.44	3.96 ± 0.41
DM+ CAD− (c)	10	1.59 ± 0.33	0.53 ± 0.13	0.38 ± 0.10	1.29 ± 0.25	4.47 ± 1.48	2.46 ± 0.32	3.78 ± 0.68
Normals (d)	10	1.65 ± 0.16	0.51 ± 0.06	0.34 ± 0.08	1.23 ± 0.10	5.18 ± 1.46	2.43 ± 0.29	3.72 ± 0.28
a vs b		ns	ns	ns	ns	ns	ns	ns
a vs c		ns	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$	ns	ns
a vs d		$P < 0.05$	$P < 0.05$	$P < 0.05$	ns	$P < 0.01$	ns	ns
b vs c		ns	ns	$P < 0.01$	ns	$P < 0.05$	ns	ns
b vs d		ns	ns	$P < 0.01$	ns	$P < 0.01$	ns	ns
c vs d		ns	ns	ns	ns	ns	ns	ns
Variance analysis		ns	ns	$P < 0.01$	ns	$P < 0.01$	ns	ns

Values given as mean ± SD. Abbreviations: n, number of subjects; CE, cholesteryl ester; apoB, apolipoprotein B; FC, free cholesterol; TG, triglycerides; PL, phospholipid; DM, diabetes; CAD, coronary artery disease; ns, not significant.

these abnormalities were not corrected by 2 months intensive insulin treatment. Barakat et al. (30) observed a clear shift toward lighter density LDL and greater LDL particle size after gastric bypass surgery and improved metabolic control in initially obese, non-insulin-dependent patients with poor glucose tolerance and hyperinsulinemia. Their results indicate that small dense LDL in NIDDM is associated with obesity, poor glucose tolerance, hyperinsulinemia, and increased plasma triglyceride levels.

Although the mean values of serum triglycerides were within upper normal range, the groups did not diverge from each other with regard to Chol/TG ratio in VLDL, indicating that abnormal LDL composition of CAD patients is obviously not a consequence of the precursor defect transferred to LDL through the VLDL - IDL - LDL cascade. How can we explain the triglyceride enrichment of LDL? In general, LDL may accumulate triglycerides in the exchange of cholesteryl esters by means of plasma cholesteryl ester transfer protein (CETP) (31). Normally, excess triglycerides are hydrolyzed by the action of lipoprotein lipase (LPL) and/or hepatic lipase (HL) (23, 32–35). The depletion of core lipids leads to a sequential conversion of particle size to smaller LDL particles (32). Low lipolytic activity (LPL or HL) would result in the accumulation of triglycerides in LDL. Since the observed abnormalities are best explained by a defective lipolytic system, our tentative hypothesis is that the qualitative differences of low density lipoproteins in CAD patients compared to others could result from reduced LPL or HL activity or disturbances in their action. Regardless of a normal fasting plasma triglyceride concentration, the LPL activity may be subnormal and insufficient to clear postprandial lipemia. In NIDDM patients LPL activity in adipose tissue is frequently within low normal range (36–39) and in CAD patients Breier et al. (40) have demonstrated an inverse correlation between the LPL activity and the severity of arterial lesions.

The abnormal LDL composition was a common characteristic for both diabetic and nondiabetic CAD patients. Additionally, LDL properties of the diabetics seemed to depend on whether they had CAD or not. This suggests that the defect may be directly related to CAD but not to NIDDM. Alternatively, these compositional anomalies may also be secondary and caused by factors shared by the two CAD patient groups but not present in NIDDM males without CAD or in healthy controls. Recently it has been documented that the density distribution, composition, and size of LDL can be changes resulting from medication, i.e., hypolipidemic drugs (41, 42). Although none of our patients were taking hypolipidemic drugs, a common factor for both patient groups was the extensive medication. The majority of the CAD patients (15/20) were treated with beta-adrenergic agents (atenolol and metoprolol), which are known to produce a slight elevation in plasma triglyceride and to reduce HDL cholesterol concentration (43–45), while IDL concentration is reported to increase (46). Among patients with acute myocardial infarction, beta-blocker users have a decreased Chol/TG ratio in LDL compared to nonusers (47). Although no significant effects of these drugs on LDL composition were observed in the study of Tornvall et al. (26), there were somewhat higher concentrations of triglycerides in LDL fractions of beta-blocker users. According to Superko and Krauss (48), more dense LDL particles are present in patients receiving beta-blockers, which was not seen in our patients probably because of similar triglyceride levels. Beta-blockers have not been confirmed to cause changes in lipoprotein lipase activity and the mechanism behind changes of lipids and lipoproteins during beta-blocker therapy is unsolved (46, 49, 50). Due to severe angina, the patients were physically less active than healthy controls. There is convincing evidence that lipoprotein lipase activity in adipose tissue and skeletal muscle (51–53) as well as in plasma correlates with physical exercise capacity (54, 55). Con-

sequently, we cannot conclude whether the structural changes of LDL are due to CAD per se or to related factors.

The present findings demonstrate distinct abnormalities in LDL of both diabetic and nondiabetic CAD patients not apparent from measurements of serum total and LDL cholesterol or triglyceride levels. The observed alterations in LDL core lipids are similar to those produced by modifying LDL in vitro by varying LPL, HL, or CETP activity. These changes lead to less effective LDL catabolism via LDL receptors (56–58). On the other hand, normalization of LDL composition in vivo has improved the uptake and intracellular degradation of LDL (59, 60). Clearly, more studies are necessary to unveil the mechanism behind the compositional abnormalities of LDL and to prove the relevance of these findings with respect to the metabolism of LDL and atherogenesis.

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REFERENCES

1. Wilson, P. W. F., W. P. Castelli, and W. B. Kannel. 1987. Coronary risk prediction in adults (the Framingham Heart Study). *Am. Heart J.* **59:** 91G–94G.
2. Hamsten, A., G. Walldius, A. Szamosi, G. Dahlen, and U. de Faire. 1986. Relationship of angiographically defined coronary artery disease to serum lipoproteins and apolipoproteins in young survivors of myocardial infarction. *Circulation.* **73:** 1097–1110.
3. Nieminen, M. S., K. J. Mattila, K. Aalto-Setälä, T. Kuusi, K. Kontula, R. Kauppinen-Mäkelin, C. Ehnholm, M. Jauhiainen, M. Valle, and M -R. Taskinen. 1992. Lipoproteins and their genetic variation in subjects with and without angiographically verified coronary artery disease. *Arterioscler. Thromb.* **12:** In press.
4. Garg, A., and S. M. Grundy. 1990. Management of dyslipidemia in NIDDM. *Diabetes Care* **13:** 153–169.
5. Taskinen, M-R. 1990. Hyperlipidaemia in diabetes. *In* Bailliere's Clinical Endocrinology and Metabolism. Vol. 4. D. J. Betteridge, editor. Baillière, Tindall, London. 743–775.
6. Rönnemaa, T., M. Laakso, V. Kallio, K. Pyörälä, J. Marniemi, and P. Puukka. 1989. Serum lipids, lipoproteins, and apolipoproteins and the excessive occurrence of coronary heart disease in non-insulin-dependent diabetic patients. *Am. J. Epidemiol.* **130:** 632–645.
7. Uusitupa, M. I. J., L. K. Niskanen, O. Siitonen, E. Voutilainen, and K. Pyörälä. 1990. Five-year incidence of atherosclerotic vascular disease in relation to general risk factors, insulin level, and abnormalities in lipoprotein composition in non-insulin-dependent diabetic and nondiabetic subjects. *Circulation.* **82:** 27–36.
8. Sniderman, A., S. Shapiro, D. Marpole, B. Skinner, B. Teng, and P. O. Kwiterovich, Jr. 1980. The association of coronary atherosclerosis and hyperapobetalipoproteinemia (increased protein but normal cholesterol content in human plasma low density (b) lipoproteins). *Proc. Natl. Acad. Sci. USA.* **97:** 604–608.
9. Teng, B., A. D. Sniderman, A. K. Soutar, and G. R. Thompson. 1986. Metabolic basis of hyperapobetalipoproteinemia. Turnover of apolipoprotein B in low density lipoprotein and its precursors and subfractions compared with normal and familial hypercholesterolemia. *J. Clin. Invest.* **77:** 663–672.
10. Kwiterovich, P. O., Jr. 1988. HyperapoB: a pleiotropic phenotype characterized by dense low-density lipoproteins and associated with coronary artery disease. *Clin. Chem.* **34:** B71–B77.
11. Austin, M. A., and R. M. Krauss. 1986. Genetic control of low-density-lipoprotein subclasses. *Lancet.* **2:** 592–595.
12. Austin, M. A., J. L. Breslow, and C. H. Hennekens. 1988. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *JAMA.* **260:** 1917–1921.
13. Austin, M. A., M. C. King, K. M. Vranizan, and R. M. Krauss. 1990. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary disease risk. *Circulation.* **82:** 495–506.
14. Vega, G. L., and S. M. Grundy. 1984. Comparison of apolipoprotein B to cholesterol in low density lipoproteins of patients with coronary heart disease. *J. Lipid Res.* **25:** 580–592.
15. Havel, J. R., H. A. Eder, and J. H. Bragdon. 1955. Distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
16. 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.
17. Tilly-Kiesi, M. 1991. The effect of lovastatin treatment on low density lipoprotein hydrated density distribution and composition in patients with intermittent claudication and primary hypercholesterolemia. *Metabolism.* **40:** 623–628.
18. Nichols, A. V., R. M. Krauss, and T. A. Musliner. 1986. Nondenaturing polyacrylamide gradient gel electrophoresis. *Methods Enzymol.* **128:** 417–431.
19. Kauppinen-Mäkelin, R., and E. A. Nikkilä. 1988. Serum lipoproteins in patients with myocardial infarction. *Atherosclerosis.* **74:** 65–74.
20. Kuusi, T., M-R. Taskinen, T. Solakivi, and R. Kauppinen-Mäkelin. 1988. Role of apolipoproteins E and C in type V hyperlipoproteinemia. *J. Lipid Res.* **29:** 293–298.
21. Nelson, C. A., and M. D. Morris. 1977. The ultracentrifugal heterogeneity of serum low density lipoproteins in normal humans. *Biochem. Med.* **18:** 1–9.
22. Nelson, C. A., and M. D. Morris. 1983. Human low density lipoprotein structure: correlations with serum lipoprotein concentrations. *Lipids.* **18:** 553–557.
23. Eisenberg, S., D. Gavish, Y. Oschry, M. Fainaru, and R. J. Deckelbaum. 1984. Abnormalities in very low, low, and high density lipoproteins in hypertriglyceridemia. Reversal toward normal with bezafibrate treatment. *J. Clin. Invest.* **74:** 470–482.

24. Crouse, J. R., J. S. Parks, H. M. Schey, and F. R. Kahl. 1985. Studies of low density lipoprotein molecular weight in human beings with coronary artery disease. *J. Lipid Res.* **26:** 566–574.
25. Richards, E. G., S. M. Grundy, and K. Cooper. 1989. Influence of plasma triglycerides on lipoprotein patterns in normal subjects and in patients with coronary artery disease. *Am. J. Cardiol.* **63:** 1214–1220.
26. Tornvall, P., F. Karpe, L. A. Carlson, and A. Hamsten. 1991. Relationship of low density lipoprotein subfractions to angiographically defined coronary artery disease in young survivors of myocardial infarction. *Atherosclerosis.* **90:** 67–80.
27. Klein, R. L., T. J. Lyons, and M. F. Lopes-Virella. 1990. Metabolism of very low- and low-density lipoproteins isolated from normolipidaemic type 2 (non-insulin-dependent) diabetic patients by human monocyte-derived macrophages. *Diabetologia.* **33:** 299–305.
28. James, R. W., and D. Pometta. 1991. The distribution profiles of very low density and low density lipoproteins in poorly controlled male, type 2 (non-insulin-dependent) diabetic patients. *Diabetologia.* **34:** 246–252.
29. Bagdade, J. D., W. E. Buchanan, T. Kuusi, and M-R. Taskinen. 1990. Persistent abnormalities in lipoprotein composition in non-insulin-dependent diabetes after intensive insulin therapy. *Arteriosclerosis.* **10:** 232–239.
30. Barakat, H. A., J. W. Carpenter, V. D. McLendon, P. Khazanie, N. Leggett, J. Heath, and R. Marks. 1990. Influence of obesity, impaired glucose tolerance, and NIDDM on LDL structure and composition. *Diabetes.* **39:** 1527–1533.
31. Tall, A. R. 1986. Plasma lipid transfer proteins. J. *Lipid Res.* **27:** 361–367.
32. Deckelbaum, R. J., S. Eisenberg, Y. Oschry, E. Butbul, I. Sharon, and T. Olivecrona. 1982. Reversible modification of human plasma low density lipoproteins toward triglyceride-rich precursors. *J. Biol. Chem.* **257:** 6509–6517.
33. Breckenridge, W. C., J. A. Little, P. Alaupovic P, C. S. Wang, A. Kuksis, G. Kakis, F. Lindgren, and G. Gardiner. 1982. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis.* **45:** 161–179.
34. Levy, E., R. J. Deckelbaum, R. L. Thibault, E. Seidman, T. Olivecrona, and C. C. Roy. 1990. In vitro remodelling of plasma lipoproteins in whole plasma by lipoprotein lipase in primary and secondary hypertriglyceridemia. *Eur. J. Clin. Invest.* **20:** 422–431.
35. Auwerx, J. H., C. A. Marzetta, J. E. Hokanson, and J. D. Brunzell. 1988. Large buoyant LDL-like particles in hepatic lipase deficiency. *Arteriosclerosis.* **9:** 319–325.
36. Pykälistö, O. J., P. H. Smith, and J. D. Brunzell. 1975. Determinants of human adipose tissue lipoprotein lipase. Effect of diabetes and obesity on basal- and diet-induced activity. *J. Clin. Invest.* **56:** 1108–1117.
37. Taskinen, M-R., E. A. Nikkilä, T. Kuusi, and K. Harno. 1982. Lipoprotein lipase activity and serum lipoproteins in untreated type 2 (insulin-independent) diabetes associated with obesity. *Diabetologia.* **22:** 46–50.
38. Pfeifer, M. A., J. D. Brunzell, J. D. Best, R. G. Judgewitsch, J. B. Halter, and D. Porte, Jr. 1983. The response of plasma triglyceride, cholesterol, and lipoprotein lipase to treatment in non-insulin-dependent diabetic subjects without familial hypertriglyceridemia. *Diabetes.* **32:** 525–531.
39. Taskinen, M-R., W. F. Beltz, I. Happer, R. M. Fields, G. Schonfeld, S. M. Grundy, and B. V. Howard. 1986. Effects of NIDDM on very-low-density lipoprotein triglyceride and apolipoprotein B metabolism. Studies before and after sulfonylurea therapy. *Diabetes.* **35:** 1268–1277.
40. Breier, C. H., V. Mühlberger, H. Drexel, M. Herold, H-J. Lisch, E. Knapp, and H. Braunsteiner. 1985. Essential role of postheparin lipoprotein lipase activity and of plasma testosterone in coronary artery disease. *Lancet.* **i:** 1242–1244.
41. Shepherd, J., B. Griffin, M. Caslake, A. Gaw, and C. Packard. 1991. The influence of fibrates on lipoprotein metabolism. *Atheroscler. Rev.* **22:** 163–169.
42. Tilly-Kiesi, M., and M. J. Tikkanen. 1991. Low density lipoprotein density and composition in hypercholesterolemic men treated with HMG-CoA reductase inhibitors and gemfibrozil. *J. Intern. Med.* **229:** 427–434.
43. Weinberger, M. H. 1985. Antihypertensive therapy and lipids: evidence, mechanisms, and implications. *Arch. Intern. Med.* **145:** 1102–1105.
44. Miller, N. E. 1987. Effects of adrenoreceptor-blocking drugs on plasma lipoprotein concentrations. *Am. J. Cardiol.* **60:** 17E–23E.
45. Frick, H., D. A. Cox, P. Himanen, M. Huttunen, T. Pitkäjärvi, P. Pörsti, L. Pöyhönen, M-L. Pyykönen, P. Reinikainen, P. Salmela, and M. Saraste. 1987. Serum lipid changes in a one-year, multicenter, double-blind comparison of doxazosin and atenol for mild to moderate essential hypertension. *Am. J. Cardiol.* **59:** 61G–67G.
46. Superko, H. R., P. D. Wood, and R. M. Krauss. 1989. Effect of alpha- and selective beta-blockade for hypertension control on plasma lipoproteins, apoproteins, lipoprotein subclasses, and postprandial lipemia. *Am. J. Med.* **86(suppl 1B):** 26–31.
47. Kauppinen-Mäkelin, R. 1985. Serum lipoproteins and postheparin plasma lipase activities in patients with myocardial infarction. Academic Dissertation. Third Department of Medicine, University of Helsinki, Helsinki
48. Superko, H. R., and R. M. Krauss. 1988. LDL and HDL particle subclass differences in CHD patients treated with selective and nonselective beta-blocking medications. Proceedings of the 8th International Symposium of Atherosclerosis, Rome, October 9–13.
49. Ferrara, L. A., T. Marotta, P. Rubba et al. 1986. Effects of alpha-adrenergic and beta-adrenergic receptor blockade on lipid metabolism. *Am. J. Med.* **80(suppl 2A):** 104–108.
50. Sacks, F. M., and V. J. Dzau. 1986. Adrenergic effects on plasma lipoprotein metabolism: speculation on mechanisms of action. *Am. J. Med.* **80(suppl 2A):** 71–81.
51. Nikkilä, E. A., M-R. Taskinen, S. Rehunen, and M. Härkönen. 1978. Lipoprotein lipase activity in adipose tissue and skeletal muscle of runners: relation to serum lipoproteins. *Metabolism.* **27:** 1661–1671.
52. Taskinen, M-R., E. A. Nikkilä, S. Rehunen, and A. Gordin. 1980. Effect of acute vigorous exercise on lipoprotein lipase activity of adipose tissue and skeletal muscle in physically active men. *Artery.* **6:** 471–483.
53. Nikkilä, E. A. 1984. HDL in relation to the metabolism of triglyceride-rich lipoproteins. *In* Clinical and Metabolic Aspects of High Density Lipoproteins. N. E. Miller and G. J. Miller, editors. Elsevier Science Publishers B. V., Amsterdam. 217–245.
54. Krauss, R. M. 1982. Regulation of high density lipoprotein levels. *Med. Clin. North Am.* **66:** 403–430.
55. Rönnemaa, T., J. Marniemi, P. Puukka, and T. Kuusi. 1988. Effects of long-term physical exercise on serum lipids, lipoproteins and lipid metabolizing enzymes in

type 2 (non-insulin-dependent) diabetic patients. *Diabetes Res.* **7:** 79–84.
56. Chait, A., S. Eisenberg, A. Steimetz, J. J. Albers, and E. L. Bierman. 1984. Low-density lipoproteins modified by lipid transfer protein have altered biological activity. *Biochim. Biophys. Acta.* **795:** 314–325.
57. Aviram, M., E. L. Bierman, and A. Chait. 1988. Modification of low density lipoprotein by lipoprotein lipase or hepatic lipase induces enhanced uptake and cholesterol accumulation in cells. *J. Biol. Chem.* **263:** 15416–15422.
58. Aviram, M., S. Lund-Katz, M. C. Phillips, and A. Chait. 1988. The influence of the triglyceride content of low density lipoprotein on the interaction of apolipoprotein B-100 with cells. *J. Biol. Chem.* **263:** 16842–16848.
59. Kleinman, Y., S. Eisenberg, Y. Oschry, D. Gavish, O. Stein, and Y. Stein. 1985. Defective metabolism of hypertriglyceridemic low density lipoprotein in cultured human skin fibroblasts. Normalization with bezafibrate therapy. *J. Clin. Invest.* **75:** 1796–1803.
60. Lopes-Virella, M. F., G. K. Sherer, A. M. Lees, H. Wohltmann, R. Mayfield, J. Sagel, E. C. LeRoy, and J. A. Colwell. 1982. Surface binding, internalization and degradation by cultured human fibroblasts of low density lipoproteins isolated from type I (insulin-dependent) diabetic patients: changes with metabolic control. *Diabetologia.* **22:** 430–436.