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Original Contributions

Effect of Hepatic Lipase on LDL in Normal Men and Those With Coronary Artery Disease

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Hepatic triglyceride lipase (HL) is thought to play a role in the formation of low density lipoproteins (LDLs) from small very low density lipoproteins (VLDLs) and intermediate density lipoproteins (IDLs). To analyze the possible physiological role of HL in determining LDL buoyancy, size, and chemical composition, HL activity and LDL were studied in 21 patients with coronary artery disease (CAD) and 23 normolipidemic subjects. In both groups, LDL buoyancy and size were inversely associated with HL activity levels. The effect of HL on LDL size was comparable in CAD patients and in normolipidemic subjects. HL appeared to influence LDL lipid composition primarily by affecting the surface lipid components. The free cholesterol content of LDL particles was highly correlated with HL activity in both CAD and normolipidemic individuals. The free cholesterol to phospholipid ratio in LDL particles correlated with HL in both CAD and normolipidemic subjects. When the individuals were separated according to their LDL subclass patterns, pattern B subjects had significantly higher HL than pattern A subjects in both CAD and normolipidemic groups. The analysis of the cholesterol distribution profiles across the lipoprotein density gradient confirmed that LDL buoyancy is affected by HL. These data support the hypothesis that HL modulates the physical and compositional properties of LDL and contributes to the expression of the LDL subclass phenotype, suggesting a physiological role for HL in LDL metabolism. (*Arteriosclerosis and Thrombosis* 1993;13:147–153)

KEY WORDS • **hepatic lipase** • **LDL** • **coronary heart disease** • **lipoprotein size** • **lipoprotein density**

The association between low density lipoprotein (LDL) cholesterol and coronary artery disease (CAD) is now believed to be causal.[1–4] LDLs have been characterized as heterogeneous lipoproteins that include particles differing in size, density, and protein and lipid composition.[5–7] A number of different intravascular enzymes are involved in the lipoprotein cascade leading to LDL formation. These include lipoprotein lipase (LPL), hepatic triglyceride lipase (HL), and lipid transfer protein. Although HL seems to be involved in the conversion of remnant particles initially formed by LPL action to LDL,[8] the physiological role of HL in LDL metabolism is not clear. HL appears to be synthesized by the liver cells and bound to the luminal surface of the endothelial cells of the liver where the remodeling of small very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL) to LDL may take place.[9] The strongest evidence supporting the role of HL in LDL formation comes from studies performed on human and animal models with HL deficiency.[8,10–14] In these studies, low HL activity was associated with larger and more buoyant LDL particles, often extending into the IDL density range.

To further examine the possible involvement of HL in determining the physical characteristics of LDL, the compositional and physical properties of LDL were examined in relation to HL activity levels in CAD patients and normolipidemic subjects.

Methods

Subjects

Twenty-one men aged 50.2±9 (mean±SEM) years and with elevated apolipoprotein (apo) B plasma levels (>125 mg/dl), a family history of CAD, and evidence of coronary atherosclerosis by coronary arteriography[3] were selected. All subjects were participants in a clinical intervention trial of intensive drug therapy for CAD; blood samples for the present study were drawn at a protocol time point 6 weeks after discontinuing lipid-lowering therapy, at which time all LDL and apo B–related measurements had returned to pretreatment baseline levels.[3] Two subjects were taking β-blockers at the time of the present study. Dietary advice was given according to the American Heart Association's step I and II diets.[3] Patients with diabetes; severe hypertension; cancer; or liver, thyroid, or kidney diseases were excluded.

An additional 23 Caucasian men aged 33±2 years were recruited by advertisement and were paid to

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participate in the present study. Plasma triglycerides (TGs), LDL cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and apo B values were between the fifth and 95th percentiles as reported in the Lipid Research Clinics Population Study (North America) for white men.[15,16] None of these subjects was taking any medication known to alter lipid metabolism nor was following particular dietary restrictions.

The present study was approved by the human subjects review committee of the University of Washington. Informed consent was obtained from all subjects.

Blood Collection

Blood samples were collected in 0.1% EDTA after a 12-hour fast. An intravenous heparin bolus (LyphoMed Inc., Rosemont, Ill.) of 60 IU/kg was then administered, and after 10 minutes blood was collected in iced lithium-heparin tubes for the measurement of HL activity. Plasma was immediately processed by centrifugation at 4°C and stored at either 4°C for subsequent lipoprotein analysis or at −70°C for later determination of HL activity.

Single Vertical-Spin Density Gradient Ultracentrifugation for Apo B–Containing Particles

This technique[17] was developed as a variation of the single vertical-spin ultracentrifugation method by Chung et al[18] to specifically improve the resolution of apo B–containing lipoproteins. By layering 2 ml of plasma adjusted to a density of 1.080 g/ml underneath 12 ml of a 1.006 g/ml NaCl solution, a discontinuous salt gradient was produced in a Sorvall TV-865B (DuPont Co., Wilmington, Del.) rotor. Samples were centrifuged at 65,000 rpm for 90 minutes (total $\omega t^2 = 2.36 \times 10^{11}$) at 10°C; the tubes were then fractionated from the bottom at a flow rate of 1.7 ml/min, and 38 0.45-ml fractions were collected. Total cholesterol was measured in each fraction. The relative flotation rate (Rf), which characterized LDL peak buoyancy, was obtained by dividing the fraction number containing the LDL-C peak by the total number of fractions collected. To evaluate the intra-assay coefficient of variation, density gradient ultracentrifugation (DGUC) of paired samples was performed on plasma from nine subjects. The plasma from each subject had been frozen with ethanol/dry ice and stored at −70°C for 1–29 months for replicate analysis. The Rf of the LDL peak was obtained as described above. The coefficient of variation of the Rf values obtained by replicate analyses[19] of the same samples was 0.2%. Thus, the LDL Rf number determined by single vertical-spin DGUC was found to be highly reproducible. The fractions selected to contain the material defining the sharp peak of LDL were pooled after DGUC. The ranges characterizing these fractions varied among subjects and were based on the cholesterol profile of each sample to ensure that the pooled samples contained the entire lipoprotein peak.

Nondenaturing Gradient Gel Electrophoresis

Six microliters of whole plasma was diluted 4:1 in tracking solution (50% sucrose and 0.01% bromophenol blue) and electrophoresed at 12–14°C in 2–16% polyacrylamide gradient gels (Pharmacia, Uppsala, Sweden) at 125 V for 24 hours using tris(hydroxymethyl)aminomethane (0.09 M)/boric acid (0.08 M)/Na_2EDTA (0.003 M) buffer, pH 8.3. Samples were then electrophoresed as described.[5] Gels were stained for lipid with oil red O and scanned at 490 nm. The molecular diameter of peaks was determined using a calibration curve based on standards of known diameters. The standards included ferritin and thyroglobulin (Pharmacia) with molecular diameters of 12.2 and 17.0 nm, respectively, and thyroglobulin dimer and carboxylated latex beads (Dow Chemical) with diameters of 23.6 and 38.0 nm, respectively. To determine the intra-assay coefficient of variation, fasting plasma was obtained from 13 individuals with TG values ranging from 46 to 602 mg/dl. These samples were collected in refrigerated tubes containing 1 mg/ml EDTA, spun at 2,500 rpm to remove cells, and separated into several aliquots. One aliquot was stored at 4°C for <1 week before electrophoresis. The remaining aliquots were immediately frozen using ethanol/dry ice and stored at −70°C for replicate analysis performed within 1–8 weeks. The mean diameter of the major LDL subspecies of the 13 fresh samples was 26.0 nm. Replicate analysis of the same samples that were frozen and then electrophoresed after thawing showed a mean diameter of 26.91 nm. The coefficient of variation of the diameter of the major LDL subspecies by replicate analysis[19] of 13 samples was 0.8%. Thus, the determination of LDL peak particle diameter by gradient gel electrophoresis was found to be highly reproducible.

Postheparin Plasma Lipase Activity

Total lipolytic activity was measured in postheparin plasma as described previously.[20] The substrate containing glycerol tri[1-^{14}C]oleate (Amersham, Arlington Heights, Ill.) and lecithin was incubated with aliquots of postheparin plasma for 60 minutes at 37°C, and the liberated free fatty acid (FFA) radioactivity was extracted and counted. Lipase activity is expressed as nanomoles of fatty acid released per minute per milliliter of plasma (nmol FFA/min per ml). LPL activity was selectively eliminated by incubation with a specific monoclonal antibody (5D2) against LPL; HL activity was defined as the remaining activity detected in the postheparin sample after incubation with the antibody.[21] For each assay, a bovine milk LPL standard and a human postheparin plasma standard were included to adjust for interassay variation.

Other Analyses

Lipoproteins were isolated using standard sequential ultracentrifugation.[22] Plasma total cholesterol and VLDL cholesterol (VLDL-C), LDL-C, and HDL-C were determined by enzymatic methods (Boehringer Mannheim, Indianapolis, Ind.).[23] Plasma TG and LDL TGs were measured by use of an enzymatic kit (Sigma Chemical Co., St. Louis, Mo.). LDL free cholesterol, esterified cholesterol, and phospholipid analyses were performed by the Northwest Lipid Research Laboratories following published procedures.[24] Apos B and A-I were analyzed by immunonephelometry (Behring Diagnostic, Sommerville, N.J.), as described.[25] Briefly, each assay was calibrated using frozen serum reference material with an assigned value traceable to primary reference material. Frozen-serum quality-control pools at three different levels (low, normal, and high) were included in each run. The average intra-assay and

TABLE 1. Clinical and Lipoprotein Characteristics of CAD Patients (n=21)

	Mean±SEM	Range
Age (years)	50±2	35–65
BMI (kg/m^2)	27±0.6	23–30
HL (nmol FFA/min per ml)	264±31	35–563
LPL (nmol FFA/min per ml)	210±23	106–476
Total cholesterol (mg/dl)	311±18	118–448
Total triglycerides (mg/dl)	202±22	64–410
VLDL cholesterol (mg/dl)	42±5	6–77
LDL cholesterol (mg/dl)	223±18	90–381
HDL cholesterol (mg/dl)	45±2	29–67
Apolipoprotein B (mg/dl)	209±14	129–334
Apolipoprotein AI (mg/dl)	136±5	100–179

CAD, coronary artery disease; BMI, body mass index; HL, hepatic lipase; FFA, free fatty acids; LPL, lipoprotein lipase; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

interassay coefficient of variation for both apo A-I and apo B was <3%.

Statistical Analysis

Statistical analyses were performed with STATISTIX, PC DOS version 2.0 (NH Analytical Software) and SIGMAPLOT version 4.1 (Jandel Scientific) computer programs. A linear regression model was used to determine correlation coefficients for the association of HL activity with LDL physicochemical indices. Where appropriate, an unpaired two-tailed Student's t test was applied. The results are expressed as mean±SEM. The level of statistical significance for these experiments was $p<0.05$.

Results

In CAD patients (Table 1), mean TG ($p<0.001$), total cholesterol ($p<0.001$), VLDL-C ($p<0.001$), LDL-C ($p<0.001$), and apo B ($p<0.001$) values were significantly higher than in normolipidemic subjects (Table 2), whereas HDL-C and apo A-I measurements were not significantly different between the two groups. No significant differences were observed between CAD patients and normolipidemic subjects for LPL activity (210±23 versus 170±11 nmol FFA/min per ml, respectively) and HL activity (264±31 versus 271±20 nmol FFA/min per ml). The range of HL activity was similar in CAD patients and normolipidemic subjects (Tables 1 and 2). Age and body mass index (BMI) were significantly higher ($p<0.005$) in CAD patients than in normolipidemic subjects. Although these groups were different primarily because of the disease status, the difference in age may also have contributed to the variation in various parameters. No significant association was found for either group between HL activity and age (CAD patients: $r=-0.006$, $p=0.98$; normolipidemic subjects: $r=-0.19$, $p=0.38$) or between HL and BMI (CAD patients: $r=0.07$, $p=0.76$; normolipidemic subjects: $r=0.34$, $p=0.11$).

TABLE 2. Clinical and Lipoprotein Characteristics of Normolipidemic Subjects (n=23)

	Mean±SEM	Range
Age (years)	33±2	21–59
BMI (kg/m^2)	23±0.7	20–33
HL (nmol FFA/min per ml)	271±20	154–555
LPL (nmol FFA/min per ml)	170±11	79–284
Total cholesterol (mg/dl)	181±6	122–223
Total triglycerides (mg/dl)	88±9	42–244
VLDL cholesterol (mg/dl)	17±2	8–49
LDL cholesterol (mg/dl)	113±6	58–160
HDL cholesterol (mg/dl)	50±2	30–67
Apolipoprotein B (mg/dl)	100±5	56–142
Apolipoprotein AI (mg/dl)	135±3	117–162

BMI, body mass index; HL, hepatic lipase; FFA, free fatty acids; LPL, lipoprotein lipase; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

In CAD patients, a significant inverse linear relation between HL activity and both the diameter of the major LDL subclass (Figure 1A; $r=-0.46$, $p=0.036$) and the LDL buoyancy (Figure 1B; $r=-0.80$, $p<0.001$) was found. When the same linear regression was performed without the two patients treated by β-blockers, the inverse relation between HL and LDL physical properties was even more evident (HL versus LDL diameter: $r=-0.51$, $p=0.026$; HL versus Rf: $r=-0.83$, $p<0.001$). In normolipidemic subjects a similar, inverse linear trend between HL activity and the diameter of the major LDL subclass was seen (Figure 2A; $r=-0.60$, $p<0.0025$). A significant, strong inverse relation also was observed between HL and LDL buoyancy (Figure 2B; $r=-0.72$, $p<0.001$).

Possible LDL compositional modifications related to HL activity and accounting for changes in size and buoyancy were subsequently analyzed in the CAD patients and normolipidemic subjects. Linear regression analysis was performed and correlation coefficients calculated between HL activity and LPL activity and 1) the lipid composition of each LDL fraction, 2) the LDL lipid composition per particle (obtained by dividing each lipid value of the LDL fraction by the same LDL fraction's apo B content), and 3) the relative lipid distribution within the surface of the LDL particle and between the surface and core (Table 3).

In CAD patients, HL activity was inversely and significantly associated with free cholesterol, cholesteryl ester, TG, and phospholipids of the LDL fraction. In normolipidemic subjects the free cholesterol of the LDL fraction was significantly associated with HL activity while cholesteryl ester, TG, and phospholipids did not show significant associations. There was no association between LPL activity and any lipid component of the LDL fraction in either the CAD or normolipidemic group.

In CAD patients as well as in the normolipidemic group, HL activity was strongly and inversely related to the free cholesterol content per LDL particle. In individuals with CAD, the phospholipid content per LDL particle was also significantly and inversely associated with HL activity, whereas neither group showed a relation between HL activity and cholesteryl ester and TG content per LDL particle. LPL activity in both the CAD and normolipidemic groups was not associated with the lipid composition per LDL particle. In CAD patients and in normolipidemic subjects, a marked inverse association was also seen between HL activity

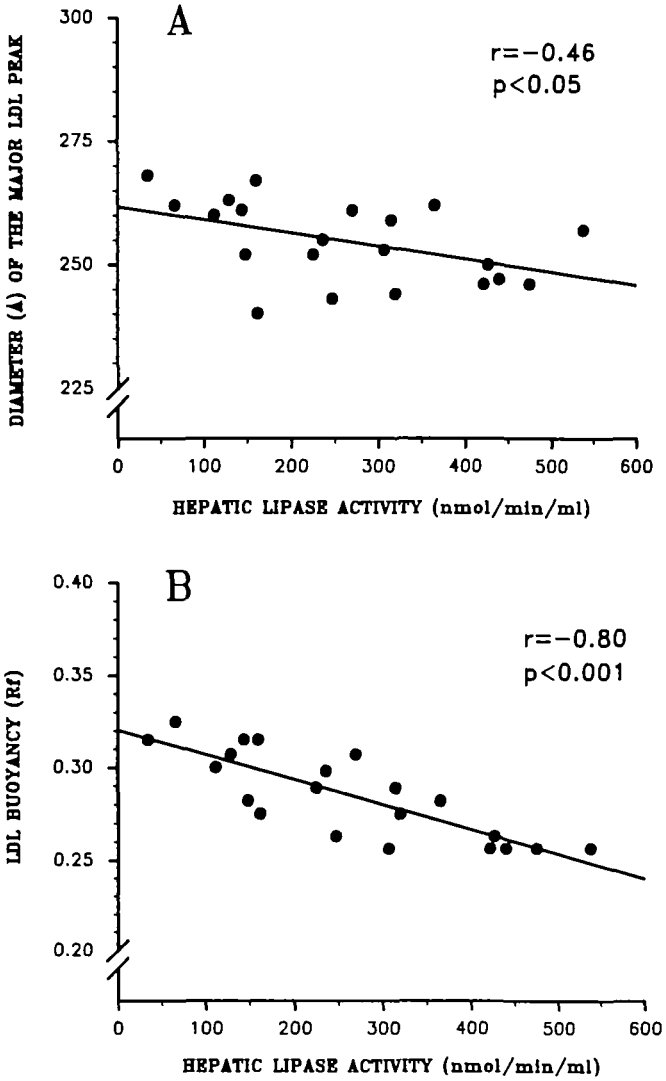

FIGURE 1. *Plots show correlation by linear regression analysis between (panel A) hepatic lipase (HL) activity (nmol/min per ml) and low density lipoprotein (LDL) size (Å) and (panel B) HL activity and LDL buoyancy (relative flotation [Rf] value) in coronary artery disease patients.*

and the free cholesterol to phospholipid ratio per LDL particle ($r=-0.81$, $p<0.005$, CAD group; $r=-0.58$, $p<0.005$, normolipidemic group). Conversely, LPL activity was not associated with the free cholesterol to phospholipid ratio of LDL particles.

The HL activity affected the overall lipid distribution between surface lipids (free cholesterol and phospholipids) and core lipids (TG and cholesteryl ester) of LDL particles in individuals of both groups. A statistically significant inverse relation was found between the HL activity and the LDL lipid surface to core ratio ($r=-0.60$, $p<0.025$, CAD patients; $r=-0.59$, $p<0.005$, normolipidemic subjects). The LPL activity was not associated with the LDL lipid surface to core ratio in either the CAD or normolipidemic group.

Gradient gel electrophoresis analysis of LDL was used to classify subjects into two subgroups based on the LDL subclass patterns A and B according to criteria previously reported.[26,27] In the present study both CAD patients and normolipidemic subjects characterized by LDL subclass distribution pattern B had significantly higher HL activity than pattern A individuals (HL activity, 328 versus 160 nmol/min per ml, $p<0.005$, CAD patients; 303 versus 197, $p<0.05$, normolipidemic subjects).

Discussion

Previous studies have mainly examined the effect of HL on VLDL and HDL metabolism,[28-32] whereas the possible effect of HL on LDL physical properties and composition is less clear. The present study supports the hypothesis that HL is involved in determining LDL physicochemical properties,[10-12] suggesting also that this finding is observed despite marked differences in clinical characteristics and lipid levels of the subjects examined.

We studied two populations, normolipidemic men and those with CAD, with different clinical characteristics and lipoprotein values, to evaluate the physiological effect of HL action on LDL particles. In both populations, subjects with low HL activity had relatively large (by gradient gel electrophoresis) and buoyant (by DGUC) LDLs, whereas high values of HL activity were associated with LDL particles that were smaller and denser. In particular, linear regression analyses showed that 64% and 52%, in CAD patients and normolipidemic subjects, respectively, of the variation (calculated as r^2) in the LDL buoyancy was accounted for by differences in HL activity. The changes in LDL size were accounted for by differences in HL activity to the extent of 26% in CAD patients and 36% in normolipi-

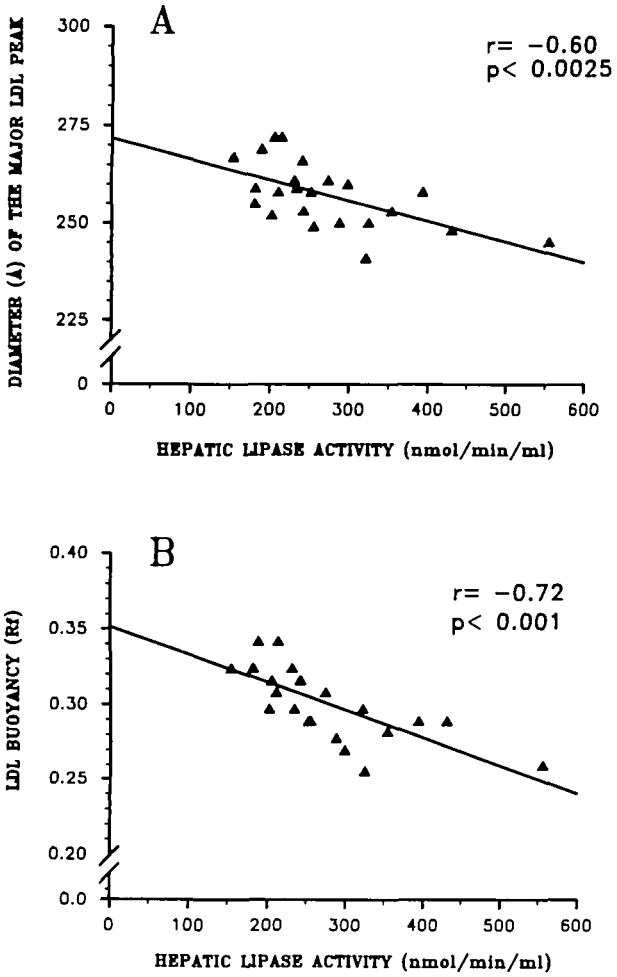

FIGURE 2. *Plots show correlation by linear regression analysis between (panel A) hepatic lipase (HL) activity (nmol/min per ml) and low density lipoprotein (LDL) size (Å) and (panel B) HL activity and LDL buoyancy (relative flotation [Rf] value) in normolipidemic subjects.*

demic subjects. These data confirm previous studies that showed the presence of more buoyant and larger LDLs in both familial[10,11] and acquired[12] forms of HL deficiency. The low coefficients of correlation *(r)* obtained for the association between HL activity and LDL size in both populations indicate that other factors, genetically and environmentally modulated,[26,27,33] also play an important role in accounting for changes in LDL size. LPL activity was not associated with the buoyancy or the size of LDL particles in either CAD patients or normolipidemic subjects.

Compositional studies were performed on the LDL fraction to explain the physical changes associated with HL activity. HL activity was significantly and inversely associated with the four lipid components of the LDL fraction in CAD patients. In normolipidemic subjects the LDL free cholesterol content was significantly and inversely associated with HL activity. In CAD patients low HL activity was associated with a significant increase in TG, phospholipids, and free and esterified cholesterol content of the LDL fraction, confirming previous studies on animal models.[34,35] Changes in the distribution of the lipid components in the LDL fraction, reported as absolute lipid values, provide a general insight into the effect of HL activity on this lipoprotein subclass. To specifically examine the compositional modifications per LDL particle, the apo B value of the fraction containing the LDL peak was determined in each subject, and the LDL lipid to apo B ratio for each lipid component was calculated. Notably, in CAD patients, high HL activity resulted in LDL particles that were relatively lipid depleted; in particular, a statistically significant decrease in free cholesterol and phospholipid content per LDL particle was observed. In normolipidemic subjects the free cholesterol content showed the same statistically significant trend. Jansen et al[34] demonstrated that inhibition of HL activity by use of a specific antibody did not affect the disappearance of labeled LDL protein from plasma. The present data suggest that the HL-related removal of phospholipids and free cholesterol from LDL particles occurs independently of the degradation of their protein moieties. Because of the phospholipase A_1 activity possessed by HL, the decrease in phospholipids induced by high levels of this enzyme is easily understood. The decrease in free cholesterol content per LDL particle associated with high levels of HL activity in both the CAD and

TABLE 3. Linear Regression Analysis (*r* Values) Between Hepatic Lipase and Lipoprotein Lipase and LDL Lipid Composition

	CAD patients				Normolipidemic subjects			
	HL		LPL		HL		LPL	
LDL	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Free cholesterol	−0.64	<0.005	0.29	NS	−0.5	<0.025	−0.12	NS
Cholesteryl ester	−0.51	<0.025	0.32	NS	0.02	NS	−0.13	NS
Triglycerides	−0.57	<0.025	0.11	NS	0.3	NS	−0.01	NS
Phospholipids	−0.61	<0.005	0.32	NS	−0.02	NS	−0.18	NS
Free cholesterol/apo B	−0.72	<0.005	−0.01	NS	−0.59	<0.005	0.04	NS
Cholesteryl ester/apo B	−0.34	NS	−0.34	NS	0.19	NS	−0.02	NS
Triglycerides/apo B	−0.4	NS	−0.35	NS	0.36	NS	−0.13	NS
Phospholipids/apo B	−0.45	<0.05	−0.42	NS	0.06	NS	−0.06	NS
Free cholesterol/phospholipids	−0.81	<0.005	0.26	NS	−0.58	<0.005	−0.07	NS
Surf/core	−0.6	<0.025	0.18	NS	−0.59	<0.005	0.11	NS

LDL, low density lipoprotein; CAD, coronary artery disease; HL, hepatic lipase; LPL, lipoprotein lipase; apo, apolipoprotein; Surf, LDL surface lipids; core, LDL core lipids. Lipids and apolipoprotein in milligrams per deciliter and lipolytic activity in nanomoles per minute per milliliter were used for regression analyses.

normolipidemic groups is likely consequent to the HL action on LDL phospholipids, with increased efflux of free cholesterol from LDL to HDL particles. Indeed, a modification of the relative free cholesterol to phospholipid molar ratio of LDL particles was demonstrated as a major determinant of the free cholesterol net transfer from LDL to HDL particles.[36,37] The statistically significant, inverse association between HL activity and the LDL free cholesterol to phospholipid ratio observed in both groups can also be explained by this metabolic model.

The free cholesterol to phospholipid ratio measured in whole plasma has been reported to be positively correlated with the risk for ischemic vascular diseases.[38] Whether HL activity affects the risk for ischemic vascular diseases by modifying the free cholesterol to phospholipid ratio of LDL particles must be confirmed. HL activity appeared primarily to influence the surface lipid components of LDL particles. A statistically significant relation between this enzyme and the LDL surface to core lipid ratio was found in CAD patients and normolipidemic subjects.

The analysis of the DGUC cholesterol profiles of the two populations confirmed that LDL buoyancy is affected by HL activity. These results confirm previous studies that used DGUC to analyze the lipoprotein profile in subjects with HL deficiency.[10,11] Decreased buoyant LDLs with an increase in dense LDL particles characterized the lipoprotein profile of subjects with high HL activity in both populations.

The LDL subclass size distribution appears to be genetically influenced, as shown by Austin et al.[26,27] In those studies, gradient gel electrophoresis analysis of LDL was used to classify subjects into two subgroups based on the LDL subclass patterns A and B according to criteria previously reported.[26,27] In the present study both patients and control subjects with pattern B had higher HL activity than pattern A individuals, suggesting that HL may be involved in the expression of the LDL subclass patterns.

In summary, we have described studies of the effect of HL on LDL physical and compositional characteristics and on LDL-C distribution in two populations with markedly different clinical and lipid characteristics. In both groups HL seems to affect both LDL size and buoyancy, and its action mainly involves the surface lipid components of LDL particles. The cholesterol distribution within the LDL density range also seems to be influenced by HL activity, with increased dense LDLs and a reduction of buoyant LDL-C associated with high levels of HL activity. These data suggest that HL, among other genetic and environmental factors, is important in determining LDL physical and compositional properties in both CAD and normolipidemic populations, supporting the hypothesis of a physiological role for HL in LDL metabolism.

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