Associations of hepatic and lipoprotein lipase activities with changes in dietary composition and low density lipoprotein subclasses

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Abstract To test whether lipoprotein lipase or hepatic lipase activities are associated with lipoprotein subclasses, and to assess the effects of dietary manipulations on these associations, enzyme activities were measured in postheparin plasma (75 U heparin/kg) from 43 healthy men who were randomly allocated to a low-fat (24% fat, 60% carbohydrate) and a high-fat (46% fat, 38% carbohydrate) diet for 6 weeks each in a cross-over design. The high-fat diet significantly increased both lipoprotein lipase (+20%, $P = 0.02$) and hepatic lipase (+8%, $P = 0.007$) activities. On both diets, hepatic lipase activity was significantly positively correlated ($P < 0.01$) with plasma apolipoprotein (apo)B concentrations, and with levels of small dense low density lipoprotein (LDL) III, measured by analytic ultracentrifugation as mass of lipoproteins of flotation rate (S_f^0) 3–5, while lipoprotein lipase activity was inversely associated with levels of LDL III ($P < 0.05$). Despite the cross-sectional correlations, increased hepatic lipase activity was not significantly correlated with the reduction in LDL III mass observed on the high-fat diet. Rather, changes in hepatic lipase were correlated inversely with changes in small very low density lipoproteins (VLDL) of S_f^0 20–40, and small intermediate density lipoproteins (IDL) of S_f^0 10–16. Moreover, changes in lipoprotein lipase activity were not significantly correlated with changes in small LDL, but were positively associated with changes in small IDL of S_f^0 10–14, and large LDL I of S_f^0 7–10. Thus, while increased levels of small dense LDL are associated with a metabolic state characterized by relatively increased hepatic lipase and decreased lipoprotein lipase activity, changes in these enzymes do not appear to be primary determinants of diet-induced changes in levels of this LDL subfraction. On the other hand, increased lipoprotein lipase activity induced by high-fat feeding may contribute to the accumulation in plasma of both large LDL I and small IDL, whereas increased hepatic lipase may promote catabolism or clearance of triglyceride-rich lipoprotein remnants.—**Campos, H., D. M. Dreon, and R. M. Krauss.** Associations of hepatic and lipoprotein lipase activities with changes in dietary composition and low density lipoprotein subclasses. *J. Lipid Res.* 1995. **36:** 462–472.

Supplementary key words dietary fat • triglyceride • cholesterol • VLDL • apoB

Low density lipoproteins (LDL) comprise of a series of particles of varying buoyant density and particle size (1, 2). Differences among LDL particles in physical and chemical properties (3, 4), metabolic characteristics (5), oxidative susceptibility (6, 7), carbohydrate content (8), and cellular uptake (9) may be related to differing roles in the development of coronary artery disease (CAD) (10–12). Several metabolic factors such as lipoprotein and hepatic lipase and cholesteryl ester transfer protein are involved in the formation of LDL from triglyceride-rich lipoprotein precursors (12–14). In addition, there is evidence that hepatic lipase activity plays a key role in metabolic processing of LDL (13).

Lipoprotein lipase is needed for effective hydrolysis of circulating chylomicrons and large very low density lipoproteins (VLDL) (15, 16), and also enhances cellular uptake of lipoproteins (17). Postheparin plasma lipoprotein lipase activities have been positively associated with high density lipoprotein (HDL) cholesterol and negatively with VLDL cholesterol concentrations (18–20). Severe lipoprotein lipase deficiency states are generally characterized by marked reductions in HDL cholesterol and LDL cholesterol concentrations and increases in levels of triglyceride-rich lipoproteins (21, 22).

Hepatic lipase hydrolyzes both triglyceride and phospholipid in lipoprotein particles (16). Absence of hepatic lipase results in accumulation of triglyceride-rich lipoproteins, and buoyant, large LDL particles (13, 23). Data from population studies have shown a strong negative association between postheparin plasma hepatic lipase activity and HDL, particularly HDL_2 (19, 24). Several factors such as age, gender, hormones, exercise, and obesity are known to affect lipoprotein and hepatic lipase activities (15, 25–29). In addition, it has been reported that very

Abbreviations: LDL, low density lipoprotein; CAD, coronary artery disease; VLDL, very low density lipoprotein; HDL, high density lipoprotein; FFA, free fatty acid; TG, triglyceride; IDL, intermediate density lipoprotein; apo, apolipoprotein.

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low-fat diets (<8% calories from fat) significantly reduce lipase activities, particularly lipoprotein lipase (30, 31). Furthermore, increased saturated fat and cholesterol intakes in monkeys have been reported to increase both hepatic and lipoprotein lipase activities (32, 33). A rise in lipoprotein lipase activity in response to an oral fat load has been associated with the accumulation of chylomicron-derived free fatty acids (FFA), suggesting that FFA may play a role in regulating lipoprotein lipase in humans (34).

The purpose of this study was to test whether heparin-releasable hepatic and lipoprotein lipase activities are associated with plasma levels of LDL subclasses, and whether these relationships are influenced by dietary manipulations. We therefore analyzed plasma lipoproteins and postheparin plasma lipase activities from 43 healthy men who were randomly allocated to a low-fat (24% fat, 60% carbohydrate) and a high-fat (46% fat, 38% carbohydrate) diet for 6 weeks each in a cross-over design (35).

METHODS

Subjects

Subjects were 43 free-living, healthy, normocholesterolemic men, who responded to advertisements and agreed to participate in the study. Subjects were eligible if they: *1)* had no history of CAD, cerebrovascular disease, or peripheral vascular disease, and of cancer other than skin cancer; *2)* were not taking lipid-lowering drugs or antihypertensive medications; *3)* had blood pressure less than 160/105; *4)* were under 130% ideal body weight using the Metropolitan tables (36); and *5)* had total cholesterol concentration less than the 95th percentile for age decile (total cholesterol levels ≤260 mg/dl in all subjects), and triglyceride (TG) concentrations less than 500 mg/dl (5% of subjects had TG 273–392 mg/dl). All subjects who participated in the study signed a consent form approved by the Committee for the Protection of Human Subjects at our institution.

Diet protocol

Subjects were randomized to a low-fat or a high-fat diet period for 6 weeks each in a cross-over design. All subjects attended a 1-h educational session where the dietary protocols and test diets were reviewed. Handouts were provided with detailed calorie-controlled menus, shopping lists, and recipes, a chart to monitor body weight, and a diet deviations sheet to record additional or deleted foods. Body weight was monitored throughout the study and caloric requirements were adjusted as necessary to maintain body weight. The two experimental diets were designed to achieve a comparison of high-fat and low-fat intake by substitution of carbohydrate without significant change in other major nutrient parameters. On the high-fat phase the prescribed diet consisted of 38% carbohydrate and 46% total fat (18% saturated 12% polyunsaturated), and on the low-fat diet phase 60% carbohydrate and 24% total fat (6% saturated and 4% polyunsaturated). Total calories (as needed to maintain weight), dietary cholesterol (150 mg/1000 kcal), fiber (4–5 g/1000 kcal), protein (16%), and the P:S ratio (0.7) were maintained constant throughout the study. Outpatient dietary adherence was monitored by completion of 4-day food records at the end of each diet period. Dietary adherence was promoted by provision of meal plans and food scales to weigh items on the menus, telephone contacts, and by recording of weight fluctuations. We and others have shown (37–39) that video-taped instruction combined with follow-up counseling by a dietitian is an effective way to promote diet adherence. We provided this instruction at screening, therefore eliminating those who were unwilling to comply with the basic requirements of the study. It has been reported (40) that 4-day records have high correlation coefficients with 7-day records for most nutrients, with the advantage that it decreases participant burden and enhances reliability (40). **Table 1** shows the mean dietary intake on both diets as determined by 4-day records. These data indicate that, on average, dietary compliance was achieved.

Specimen collection

Blood samples were obtained from each subject after an overnight fast during the last week of each diet phase. Blood samples for lipid and lipoprotein analysis were collected in 0.15% EDTA. Plasma was separated by low-speed centrifugation and kept at 4°C for lipid analysis. The d < 1.063 g/ml fraction of plasma was isolated by ultracentrifugation (41) for analytic ultracentrifugation and gradient gel electrophoresis. Samples for determination of hepatic and lipoprotein lipase activities were obtained 10 min after intravenous heparin administration (75 U heparin/kg) and postheparin plasma was stored at −70°C before enzyme assay.

TABLE 1. Dietary intake during the low-fat and high-fat dietary intervention

Nutrient	Low-Fat Diet	High-Fat Diet
	% caloric intake	
Protein	16.8 ± 1.9	16.3 ± 0.9
Carbohydrate	58.8 ± 3.1	39.2 ± 2.5
Total fat	24.2 ± 3.3	45.2 ± 2.4
Saturated F.A.	6.0 ± 1.2	18.1 ± 1.3
Monounsaturated F.A.	11.6 ± 1.9	12.4 ± 1.2
Polyunsaturated F.A.	4.3 ± 1.1	11.8 ± 1.8

F.A. indicates fatty acids, n = 43. Values are given as mean ± SD.

Laboratory procedures

Lipase activities were determined by the method of selective inhibition with protamine sulfate as previously described (31, 42). All determinations were run in triplicate and a control sample was run with each batch of test samples. Between assay and within assay coefficients of variation for a control sample were 8.1% and 2.8%, respectively. Activities were expressed in units of μmol FFA/ml per h.

LDL particle diameters were determined on whole plasma at baseline, and d < 1.063 g/ml fraction during the dietary intervention, by electrophoresis in nondenaturing 2–16% polyacrylamide gradient gels as previously described (3, 43). Gels were stained with either Oil Red O for whole plasma or Coomassie Brilliant Blue R250 for the d < 1.063 g/ml fraction. Stained gels were scanned using a Transidyne RFT scanning densitometer (Transidyne Corp., Ann Arbor, MI), and LDL particle diameters were estimated from calibration curves using latex beads and Pharmacia high molecular weight standards for reference. For comparison with other studies using LDL patterns, classification of subjects as having pattern A or B was carried out by three independent observers as previously described (44). Because the LDL patterns were influenced by the dietary intervention, for some analyses subjects were classified by LDL subclass group based on their LDL peak diameter on the low-fat and the high-fat diet. Subjects with pattern A on both diets were classified as stable A pattern (n = 21, 49%). Subjects with pattern B on both diets were classified as stable B pattern (n = 11, 25.5%) and those with pattern A on the high-fat diet and pattern B on the low-fat diet were classified as changed pattern (n = 11, 25.5%). No subjects exhibited pattern B on the high-fat diet and pattern A on the low-fat diet.

Plasma concentrations of lipoprotein mass were measured as a function of S_f^o by analytic ultracentrifugation as previously described (41). The flotation intervals determined were S_f^o 100–400 for large VLDL, S_f^o 20–100 for small VLDL, S_f^o 14–20 for large intermediate density lipoprotein (IDL), and S_f^o 10–14 for small IDL (41). LDL mass was measured in four intervals (3, 4, 45, 46): LDL I (S_f^o 7–10), LDL II (S_f^o 5–7), LDL III (S_f^o 3–5), and LDL IV (S_f^o 0–3). Peak LDL S_f^o rate and peak LDL density were determined by analytic ultracentrifugation as the values at the maximum of the LDL particle distribution (41). Cholesterol and triglyceride concentrations were measured enzymatically on a Gilford Impact 400E analyzer, using Gilford reagents. Our laboratory is standardized through the Centers for Disease Control standardization program. HDL cholesterol was measured after heparin–manganese precipitation of apoB-containing lipoproteins (47). Plasma apoB levels were measured by single radial immunodiffusion (48) using plates and standards from Tago, Inc. (Burlingame, CA) over the range of 25–150 mg/dl, or higher by dilution. Assays were carried out in duplicate or triplicate with standards, controls, and unknowns on the same plate, and with a coefficient of variation of ± 5%.

Statistical analyses

All changes reported are high-fat minus low-fat values. Diet-induced changes in lipoproteins and lipase activities were determined by two-tailed paired *t*-test analysis. Spearman rank partial correlation coefficients were calculated to test cross-sectional associations between lipase activities and change in lipoprotein subfractions. For these analyses we used the CORR procedure available in the Statistical Analysis Systems (SAS) (Cary, NC, SAS Institute, Inc.). Within the CORR procedure, we used the options available for partial correlation analyses to adjust cross-sectional associations for age and body mass index (BMI = kg/m^2). This adjustment was done to establish that the correlations observed with hepatic and lipoprotein lipase activities were not confounded by data from individuals who were older and/or heavier. Secondary cross-sectional analyses included the calculation of partial correlation coefficients after adjustment for plasma triglyceride levels. Since the diet-induced change in plasma triglyceride was associated with change in lipase activities, we calculated the partial correlation coefficients between diet-induced changes in lipase activities and change in lipoprotein subfractions, after adjustment for triglyceride change using the procedures described above. Age and BMI adjustment was not necessary in this analysis because each subject served as his own control.

The association between lipase activities and lipoprotein subfractions was also examined by comparing age- and BMI-adjusted mean lipase activities by LDL pattern group, using the general linear analysis of covariance model procedure available in SAS. Logarithmic transformations were used for triglyceride concentrations to improve normality in all our analyses.

TABLE 2. Subject characteristics at baseline

Parameter (n = 43)	Mean ± SD
Age (years)	50 ± 11
Body mass index (kg/m^2)	25.9 ± 2.9
Total triglyceride (mg/dl)	135 ± 73
Total cholesterol (mg/dl)	206 ± 27
LDL cholesterol (mg/dl)	134 ± 24
HDL cholesterol (mg/dl)	45 ± 9
LDL diameter (Å)	261 ± 6
% Pattern B	33%

TABLE 3. Plasma hepatic and lipoprotein lipase activities and lipoprotein concentrations after low-fat and high-fat dietary intervention

Parameter (n = 43)	Low-Fat	High-Fat	Change Low to High	% Change Low to High	*P* Value
Hepatic lipase (μmol FFA/ml/h)	15.4 ± 5.3	16.6 ± 5.5	1.3 ± 2.3	8	0.007
Lipoprotein lipase (μmol FFA/ml/h)	4.1 ± 2.6	4.9 ± 3.1	0.8 ± 2.1	20	0.02
Total triglyceride (mg/dl)	154 ± 85	104 ± 59	−50 ± 53	−32	0.0001
Total cholesterol (mg/dl)	194 ± 36	212 ± 38	18 ± 19	9	0.0001
LDL cholesterol (mg/dl)	124 ± 35	145 ± 32	20 ± 20	16	0.0001
HDL cholesterol (mg/dl)	39 ± 8	47 ± 10	8 ± 4	21	0.0001
Apo B (mg/dl)	107 ± 24	112 ± 25	4 ± 14	4	0.06

Concentrations are given as mean ± SD.

RESULTS

The subjects' general characteristics and plasma lipoprotein parameters at baseline are shown in **Table 2.** All subjects were normolipidemic and the prevalence of pattern B at baseline (33%) is in accordance with previous reports in healthy male populations (10, 49).

Plasma lipoprotein concentrations and lipase activities after the low-fat and high-fat dietary interventions in all subjects are shown in **Table 3.** Overall, an increase in total fat in the diet, from 24% to 45%, and a reduction in carbohydrate, from 59% to 39%, significantly increased both hepatic (+8%, $P = 0.007$) and lipoprotein lipase (+20%, $P = 0.02$) activities. As reported previously for the entire study cohort (35), total cholesterol (+9%), LDL cholesterol (+16%), and HDL cholesterol (+21%) concentrations were also significantly ($P = 0.0001$) increased, and total triglyceride (−32%) concentrations were significantly ($P = 0.0001$) reduced with the high-fat diet. Changes in apoB concentrations (+4%) were of borderline significance ($P = 0.06$) after the high-fat diet period.

Diet-induced changes in VLDL, IDL, and LDL mass subfractions for the entire group are shown in **Table 4.** Increased dietary fat consumption significantly increased mean peak LDL particle diameter from 257 to 263 Å and peak flotation rate from 5.3 to 6.0, and decreased calculated LDL peak density from 1.034 to 1.032 g/ml (all $P = 0.0001$). The effects of the high-fat diet on lipoprotein mass were to increase small IDL (+16%, $P = 0.0009$), and large, buoyant LDL, particularly LDL I (+45%, $P = 0.0001$), and to a lesser extent LDL II (+10%, $P = 0.03$). No significant changes in large IDL mass ($P = 0.6$) were noted. Large (−53%) and small (−38%) VLDL, small, dense LDL III (−19%), and LDL IV (−36%) mass were significantly ($P < 0.005$) reduced with the high-fat diet.

Table 5 shows age- and BMI-adjusted cross-sectional correlations among hepatic and lipoprotein lipase activities and plasma lipoprotein concentrations and lipoprotein mass subfractions. The age and BMI adjustments were carried out because of significant inverse correlations of age and positive correlations of BMI with hepatic lipase, and inverse correlations of BMI with lipoprotein lipase (data not shown). On both diets, positive correlations with hepatic lipase activity and inverse correlations with lipoprotein lipase activity were observed for triglyceride and apoB, mass of large VLDL and LDL III, and LDL density. Peak S_f^o was inversely correlated with hepatic lipase and positively correlated with lipoprotein lipase. In addition, on the low-fat diet only, LDL IV mass was positively correlated with hepatic lipase and inversely

TABLE 4. Lipoprotein subfraction mass (mg/dl), and LDL size and density after low-fat and high-fat dietary intervention

Parameter (n = 43)	Low-Fat	High-Fat	Change Low to High	% Change Low to High	*P* Value
Large VLDL mass (S_f^o 100–400)	40.2 ± 40.9	18.7 ± 22.3	−21.5 ± 30.2	−53	0.0001
Small VLDL mass (S_f^o 20–100)	106.1 ± 57.5	66.0 ± 55.9	−40.1 ± 36.4	−38	0.0001
Large IDL mass (S_f^o 14–20)	20.8 ± 11.7	21.5 ± 12.6	0.7 ± 11.4	3	0.6
Small IDL mass (S_f^o 10–14)	31.2 ± 8.8	36.3 ± 9.4	5.0 ± 9.2	16	0.0009
LDL I mass (S_f^o 7–10)	75.2 ± 42.6	108.9 ± 51.8	33.7 ± 25.2	45	0.0001
LDL II mass (S_f^o 5–7)	111.7 ± 40.2	122.9 ± 34.9	11.3 ± 34.1	10	0.03
LDL III mass (S_f^o 3–5)	86.4 ± 42.0	69.7 ± 45.4	−16.6 ± 35.6	−19	0.004
LDL IV mass (S_f^o 0–3)	22.1 ± 19.1	14.1 ± 13.7	−8.0 ± 14.5	−36	0.0008
LDL peak flotation rate (S_f^o)	5.3 ± 1.0	6.0 ± 1.1	0.7 ± 0.5	13	0.0001
LDL density (g/ml)	1.034 ± 0.004	1.032 ± 0.004	−0.002 ± 0.002	−0.2	0.0001
LDL particle diameter (Å)	257 ± 7	263 ± 8	6 ± 6	2	0.0001

Values are given as mean ± SD.

TABLE 5. Age- and BMI-adjusted partial Spearman correlation coefficients with (r_s) hepatic and lipoprotein lipase activities after low-fat and high-fat dietary intervention

	Hepatic Lipase		Lipoprotein Lipase	
	Low-Fat Diet	High-Fat Diet	Low-Fat Diet	High-Fat Diet
Plasma parameters				
Total triglyceride	0.39[b]	0.40[b]	−0.30[a]	−0.41[a]
Total cholesterol	0.31[a]	0.28	−0.28	−0.22
LDL cholesterol	0.29	0.34[a]	−0.28	−0.21
HDL cholesterol	−0.21	−0.30[a]	0.15	0.24
ApoB	0.49[c]	0.43[b]	−0.40[b]	−0.30[a]
Lipoprotein subfraction mass				
Large VLDL (S_f^o 100–400)	0.34[a]	0.37[b]	−0.31[a]	−0.47[b]
Small VLDL (S_f^o 20–100)	0.36[a]	0.29	−0.29	−0.40[b]
Large IDL (S_f^o 14–20)	0.14	0.08	−0.02	−0.08
Small IDL (S_f^o 10–14)	0.15	0.03	−0.02	0.02
LDL I (S_f^o 7–10)	−0.22	−0.28	0.26	0.32[a]
LDL II (S_f^o 5–7)	0.01	0.27	−0.26	−0.11
LDL III (S_f^o 3–5)	0.53[c]	0.51[c]	−0.63[c]	−0.39[b]
LDL IV (S_f^o 0–3)	0.53[c]	0.28	−0.53[c]	−0.18
LDL size and density				
Particle diameter	−0.14	−0.28	0.16	0.33[a]
Peak density	0.44[b]	0.44[b]	−0.45[b]	−0.42[b]
Peak flotation rate	−0.42[b]	−0.47[b]	0.38[b]	0.45[b]

[a] $P \leq 0.05$; [b] $P \leq 0.01$; [c] $P \leq 0.001$; n = 43.

correlated with lipoprotein lipase. There were weaker correlations of hepatic lipase with total cholesterol and small VLDL mass on the low-fat diet and with LDL cholesterol on the high-fat diet. Finally, on the high-fat diet, lipoprotein lipase was inversely correlated with small VLDL mass and weakly positively correlated with mass of LDL I and peak LDL diameter.

Because of strong correlations of plasma triglyceride levels with LDL subfraction levels (50), the correlations in Table 5 were examined after further adjustment for triglyceride concentrations (data not shown). Associations that remained statistically significant included the positive correlations of hepatic lipase and inverse correlations of lipoprotein lipase with apoB and mass of LDL III and LDL IV on the low-fat diet (all $P < 0.01$) and the positive correlation of hepatic lipase with LDL III mass on the high-fat diet ($P < 0.05$). In addition, on the low-fat diet, LDL cholesterol was correlated positively with hepatic lipase and inversely with lipoprotein lipase (both $P < 0.05$).

Figure 1 shows correlations of diet-induced changes in hepatic and lipoprotein lipase activities with changes in lipoprotein subfractions after adjusting for changes in plasma triglyceride concentrations. Changes in hepatic lipase activity were significantly inversely correlated with change in mass of small VLDL of S_f^o 20–40 and small IDL of S_f^o 10–16. In addition, hepatic lipase change was positively correlated with change in total LDL cholesterol concentration ($r = 0.31$, $P = 0.04$, data not shown), and nonsignificantly with change in level of apoB ($r = 0.25$, $P = 0.1$). In contrast, diet-induced increases in lipoprotein lipase activity were correlated with increases in mass of small IDL (S_f^o 10–14) and large LDL I (significant for S_f^o 7–8 and 9–10). Correlations similar to those shown in Fig. 1 were observed for unadjusted lipase and lipoprotein values (data not shown).

To further examine the association between lipase activities and LDL subclasses, and for comparison with previous studies using LDL subclass patterns (described as patterns A and B), we divided subjects into three groups based on their LDL pattern on both the high-fat and the low-fat diet (see Methods). **Figure 2** shows the LDL particle size distribution for each LDL group. In the stable pattern A group (49% of subjects), peak LDL size was significantly greater ($P = 0.0001$) on the high-fat (268 ± 4 Å) compared to the low-fat diet (264 ± 4 Å). Subjects in the stable pattern B group (25.5%) had no significant changes in LDL particle diameter on the two diets peak (LDL size = 250 ± 4 Å on the low-fat vs. 252 ± 5 Å on the high-fat diet). By definition, the changed pattern group (25.5%), showed the largest difference in LDL size between the low-fat (252 ± 2 Å) versus the high-fat diet (265 ± 5 Å).

Table 6 shows age- and BMI-adjusted lipase activities by LDL subclass group on the low-fat and the high-fat diets and the percent change with the diet intervention. Before adjusting for age and BMI, hepatic lipase activities were significantly higher ($P < 0.05$) in subjects with stable pattern B compared to stable pattern A on both diets (data not shown). After adjusting for age and BMI, hepatic lipase activities were 25% and 21% higher in sub-

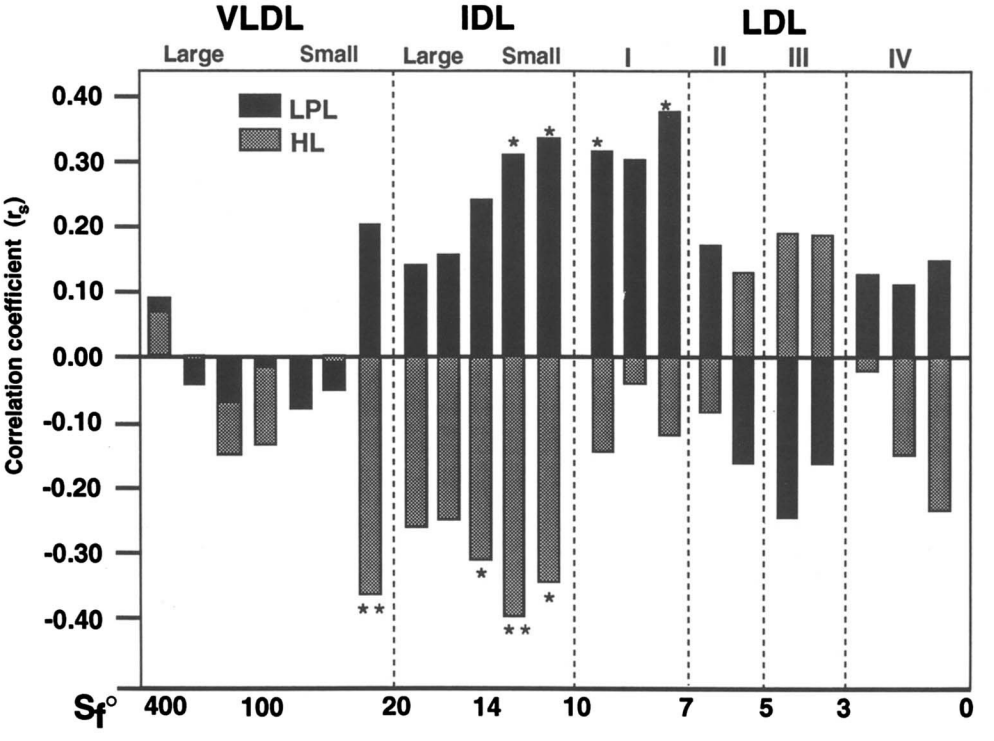

Fig. 1. Spearman correlation coefficients (r_s), adjusted for age, BMI, and triglyceride, between diet-induced changes in lipoprotein and hepatic lipase activities and lipoprotein mass, n = 43. *$P \leq 0.05$, **$P \leq 0.01$.

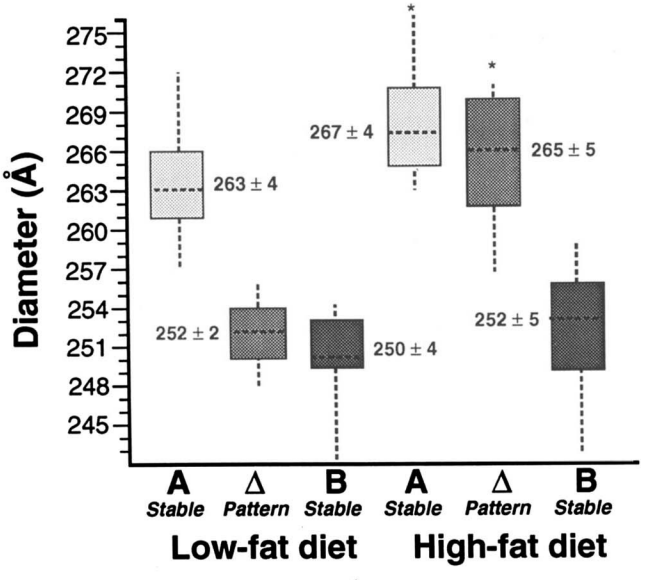

Fig. 2. LDL diameter distribution (Å) 25th, 50th, and 75th percentiles, as well as the mean ± SD in subjects classified as stable pattern A, n = 21 (pattern A on both the low-fat and high-fat diet), Δ pattern, n = 11 (pattern A on the high-fat diet and pattern B on the low-fat diet) and stable pattern B, n = 11 (pattern B on both the high-fat and low-fat diet). *Different from the low-fat diet, $P = 0.0001$.

jects with stable pattern B on the low-fat and high-fat, respectively, compared to stable pattern A, but these differences did not reach significance at $P < 0.05$. Differences in lipoprotein lipase activities among the groups were of relatively greater magnitude than the differences in hepatic lipase activities. Before adjusting for age and BMI, lipoprotein lipase activities were significantly ($P < 0.01$) lower in stable pattern B compared to stable pattern A subjects on both diets (data not shown). After adjusting for age and BMI, subjects with stable pattern B showed lower lipoprotein lipase activities on the low-fat (−31%, $P = 0.1$), and significantly lower lipoprotein lipase activities on the high-fat (−44%, $P = 0.03$), than those with stable pattern A.

Changes in hepatic and lipoprotein lipase activities induced by the high-fat diet in the LDL subclass pattern groups are also shown in Table 6. Subjects in the stable pattern A group increased both hepatic (+12%, $P = 0.004$) and lipoprotein lipase (+19%, $P = 0.06$) activities with the high-fat diet. Hepatic lipase activity was increased (+8%, $P = 0.07$) and lipoprotein lipase activity was not changed (+3%, $P = 0.9$) with the high-fat diet in the stable pattern B group. The greatest increase in lipoprotein lipase (+37%, $P = 0.05$) was found in subjects with changed pattern, while no change in hepatic lipase (+2%, $P = 0.6$) was found in this group. It should be

TABLE 6. Age- and BMI-adjusted lipoprotein and hepatic lipase activities by LDL subclass pattern stable A, stable B, and changed pattern

	Low-Fat Diet	High-Fat Diet	Change Low to High	% Change Low to High	*P* Value
	Hepatic Lipase				
Stable pattern A, n = 21	13.9 ± 1.1	15.6 ± 1.2	1.7 ± 0.5	12	0.004
Changed pattern, n = 11	16.1 ± 1.5	16.4 ± 1.6	0.4 ± 0.7	2	0.6
Stable pattern B, n = 11	17.4 ± 1.6	18.8 ± 1.7	1.4 ± 0.8	8	0.07
	Lipoprotein Lipase				
Stable pattern A, n = 21	4.8 ± 0.5	5.7 ± 0.6	0.9 ± 0.5	19	0.06
Changed pattern, n = 11	3.5 ± 0.7	4.8 ± 0.8	1.3 ± 0.6	37	0.05
Stable pattern B, n = 11	3.3 ± 0.7	3.2 ± 0.9[a]	0.1 ± 0.7	3	0.9

Adjusted to age = 50 years and BMI = 25.9 kg/m^2. Lipase activities are given as mean ± SEM in μmol free fatty acid/ml plasma per h.
[a]Different from stable pattern A, $P = 0.03$.

noted that subjects in the changed pattern group also had the largest increase in LDL I mass (+77%, data not shown).

DISCUSSION

The present study extends information gained from earlier reports of the effects of high-fat diets on hepatic and lipoprotein lipase activities (30–33), as well as studies of cross-sectional relationships between these enzyme activities and defined lipoprotein subpopulations (13, 51–53). It has been demonstrated in monkeys that high-fat atherogenic diets significantly increase both hepatic and lipoprotein lipase activities (32, 33). The effect of dietary intake on lipase activities in humans is limited to studies containing unusual dietary composition (30, 31). These studies indicate that diets containing <8% of calories from fat significantly decreased lipase activities, particularly lipoprotein lipase (31). In the present study, increased dietary fat intake, from 24% to 45% calories, and a reduction in carbohydrate, from 59% to 39%, significantly increased both hepatic lipase (+8%) and particularly lipoprotein lipase (+20%) activities.

The dietary intervention also increased total cholesterol, LDL cholesterol, and HDL cholesterol and decreased triglyceride concentrations, as described by others (54). Moreover, LDL particle diameter was significantly increased, LDL density was significantly decreased, and plasma apoB concentrations remained unchanged on the high-fat diet. These findings are accounted for by alterations of the LDL particle distribution. Large LDL mass was significantly increased, particularly LDL I mass (+45%) compared to LDL II mass (+10%), while small LDL III (−19%) and LDL IV mass (−36%) were significantly reduced. The increase in levels of larger LDL particles is consistent with studies in monkeys indicating that diets high in saturated fat increase LDL particle size (55). In addition, in humans, cross-cultural and observational studies indicate that large LDL particle size is associated with consumption of diets that are higher in saturated fat (49, 56). In monkeys, large LDL particles are increased in cholesteryl ester content and are strong predictors of atherosclerosis (57). A relation of large LDL particles to atherosclerosis has not been established in humans. In fact, in humans, increased levels of small LDL particles have been associated with higher triglyceride, lower HDL cholesterol, and increased CAD risk (10–12).

In the present study, cross-sectional analyses revealed significant inverse relationships of lipoprotein lipase activity with plasma levels of triglyceride, apoB, large VLDL mass, and LDL III. After adjustment for plasma triglyceride level, the correlations with apoB and LDL III remained significant, but only on the low-fat diet. It is possible that the weaker relationships on the high-fat diet were due to the influence of additional metabolic factors or to diet-induced changes in lipoprotein physical and chemical properties that may have altered their reactivity with lipoprotein lipase.

The increases in lipoprotein lipase induced by the high-fat diet were positively correlated with increases in small IDL and large LDL I mass and inversely, but not significantly, related to changes in small LDL III mass. These results, and the significant reciprocal changes between LDL I and LDL III mass observed in this study ($r = -0.70$, $P = 0.0001$, data not shown) and in a previous report (50), suggest that lipoprotein lipase contributes to the coordinate regulation of these LDL subfractions. As it has been reported that the conversion of VLDL to LDL is completely inhibited when lipoprotein lipase activity is blocked in the monkey (58), it may be that small IDL and LDL I are products of lipoprotein lipase-mediated catabolism of triglyceride-rich precursors.

In contrast to the cross-sectional relationships observed with lipoprotein lipase, hepatic lipase was significantly positively correlated with plasma triglyceride, apoB, and

mass of large VLDL and LDL III. After adjustment for plasma triglyceride level, the association of hepatic lipase activity with levels of LDL III remained significant on both diets, suggesting that in addition to reduced lipoprotein lipase, increased hepatic lipase activity is a component of the metabolic profile associated with increased levels of small, dense LDL particles. As buoyant, triglyceride-rich LDL particles accumulate in patients with hepatic lipase deficiency (13, 59) and after acute inhibition of hepatic lipase activity in the cynomolgus monkey (60), our findings are consistent with the hypothesis that hepatic lipase is involved in the conversion of such triglyceride-enriched LDL precursors to small, dense products (53). We did not, however, observe a significant inverse correlation of hepatic lipase activity with levels of buoyant LDL particles, as has recently been reported (53). Although methodological differences for LDL subclass measurements might be responsible for the differing results, they may also be due to differences in the range of distribution of LDL subclass levels and hepatic lipase activities in the subject populations, in that women, who typically have higher levels of large LDL (49) and lower hepatic lipase activity (31) than men, were not included in the present study.

In contrast to the positive cross-sectional association of hepatic lipase activity with levels of small, dense LDL, the high-fat diet induced increases in hepatic lipase activity together with reductions in small LDL, and there were no significant correlations of changes in hepatic lipase with changes in LDL subfractions. It is possible that reduced levels of small LDL on the high-fat diet resulted primarily from other metabolic changes, such as a lower rate of production of precursor particles or diminished lipolytic degradation of such particles due to reduced transfer of triglyceride from VLDL (61).

On the other hand, diet-induced changes in hepatic lipase activity were inversely correlated with changes in levels of small VLDL and IDL particles. These findings are consistent with studies that have shown a role for hepatic lipase in the catabolism of small VLDL and IDL (58, 59, 62). Moreover, recent studies have indicated that hepatic lipase facilitates the initial uptake of chylomicron remnants by the liver (63). It may be that enhanced catabolism and/or clearance of triglyceride-rich lipoprotein remnants by hepatic lipase served to attenuate the accumulation of IDL induced by the high-fat diet.

Hepatic lipase activity also was significantly associated with total and LDL cholesterol concentrations on the high-fat diet, and changes in hepatic lipase induced by the high-fat diet were positively correlated with changes in LDL cholesterol. Therefore, diet-induced increases in hepatic lipase activity may contribute to increases in LDL cholesterol concentrations observed with high-fat feeding. Alternatively, it is possible that changes in hepatic cholesterol metabolism induced by the high-fat diet lead to increased hepatic lipase activity (64). Recently it has been reported that infusion of cholesterol-rich chylomicron remnant and β-VLDL particles in rabbits can induce increases in hepatic lipase activity (65), raising the additional possibility that increased remnant formation on the high-fat diet and the accumulation of smaller IDL may have mediated the elevation in hepatic lipase activity observed here.

Our data indicate that the high-fat diet changed LDL subclass patterns in approximately 25% of the subjects from a predominance of small LDL particles (pattern B) (10–12, 40, 66) to a predominance of larger LDL (pattern A). The subjects that changed their LDL pattern also had the greatest change in lipoprotein lipase activity (+37%) but did not exhibit a change in hepatic lipase activity (+2%). Therefore, the higher prevalence of pattern A on the high-fat diet (49%) versus the low-fat diet (23.5%) could be related, in part, to changes in lipoprotein lipase but not to changes in hepatic lipase activity. This observation is supported by the positive correlation observed between diet-induced increases in LPL and increased LDL I. Among the subjects that did not change their LDL pattern with the dietary intervention, lipoprotein lipase activities were 38% lower in subjects with stable pattern B compared to those with stable pattern A, after adjusting for age and BMI. Furthermore, lipoprotein lipase activities did not change with the high-fat diet in stable pattern B subjects. These findings support the notion that reduced lipoprotein lipase activity contributes to the lipoprotein profile observed in pattern B subjects. They are also consistent with a recent report that coronary disease patients with LDL subclass pattern B have relatively reduced lipoprotein lipase and increased hepatic lipase activities compared with pattern A patients, although these differences were no longer significant after adjusting for plasma triglyceride and HDL-cholesterol levels (67).

Previous studies have shown that lipoprotein lipase deficiency is caused by the inheritance of two defective alleles of the lipoprotein lipase gene (22). While homozygotes are characterized by extremely high levels of triglyceride-rich lipoproteins, fasting chylomicronemia, and marked reductions in HDL and LDL (22), heterozygotes are difficult to detect due to their normal lipase activities and fasting plasma lipids when other factors such as obesity or hyperinsulinemia are not present (21, 68, 69). Heterozygous carriers of the lipoprotein lipase defective gene have been reported to have impaired triglyceride tolerance (51), and their plasma lipoprotein characteristics resemble those of pattern B in this study, and other previous reports (10, 49, 66). While several factors such as increased age, gender, physical activity, and dietary intake have been associated with LDL particle size in some studies (45, 56, 70) and with lipoprotein lipase activity in other independent observations (15, 25, 27, 29–31), we found that even after taking a number of these factors into

consideration, subjects with stable pattern B had lower lipoprotein lipase activities compared to stable pattern A. Therefore, some subjects with pattern B in our study and in the general population could be heterozygous carriers of a lipoprotein lipase defective gene (51). However, because of the low estimated prevalence (approximately 1 in 500) of heterozygous lipoprotein lipase deficiency (68), it is unlikely to account for a major proportion of pattern B in the general population (approximately 33% in men, 10% in women) (49).

In summary, our data indicate that on both high- and low-fat diets, increased levels of small, dense LDL III and the presence of LDL subclass B are related to increased hepatic lipase and reduced lipoprotein lipase activity. However, the increases in both hepatic and lipoprotein lipase activities that were induced by the high-fat diet were not significantly associated with the reductions in levels of small, dense LDL observed with this diet, suggesting that other metabolic factors were primarily responsible for this diet-induced lipoprotein change. On the other hand, the correlations of increased lipoprotein lipase activity with the increases in small IDL and large LDL induced by the high-fat diet suggest a possible role for this enzyme in the generation of these particles from lipolytic precursors. Finally, while diet-induced increases in hepatic lipase activity did not appear to contribute to net changes in LDL subclass distributions on the high-fat diet, the inverse correlations of changes in hepatic lipase with changes in levels of small VLDL and IDL are consistent with the hypothesis that this enzyme promotes the catabolism and hepatic clearance of lipolytic remnants of triglyceride-rich lipoproteins.

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