Relationship between lipoprotein lipase and high density lipoprotein cholesterol in mice: modulation by cholesteryl ester transfer protein and dietary status[1]

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Abstract. Plasma lipoprotein lipase (LPL) activity correlates with high density lipoprotein (HDL) cholesterol levels in humans. However, in several mouse models created either through transgenesis or targeted inactivation of LPL, no significant changes in HDL cholesterol values have been evident. One possible explanation for this species difference could be the absence of plasma cholesteryl ester transfer protein (CETP) activity in mice. To explore this possibility and further investigate interactions between LPL and CETP modulating HDL cholesterol levels in vivo, we examined the relationship between LPL activity and HDL levels in mice expressing the simian CETP transgene, compared with littermates not carrying the CETP gene. On a chow diet, increasing LPL activity was associated with a trend towards increased HDL levels (51 ± 29 vs. 31 ± 4 mg/dL highest vs. lowest tertiles of LPL activity, $P = 0.07$) in mice expressing CETP, while no such effects were seen in the absence of CETP (65 ± 12 vs. 61 ± 15 mg/dL). Furthermore, in the presence of CETP, a significant positive correlation between LPL activity and HDL cholesterol was evident ($r = 0.15$, $P = 0.006$), while in the absence of CETP no such correlation was detected ($r = 0.15$, $P = 0.36$), highlighting the interactions between LPL and CETP in vivo. When mice were challenged with a high fat, high carbohydrate diet, strong correlations between LPL activity and HDL cholesterol were seen in both the presence ($r = 0.45$, $P = 0.03$) and absence ($r = 0.73$, $P < 0.001$) of CETP. Therefore, under altered metabolic contexts, such as those induced by dietary challenge, the relation between LPL activity and HDL cholesterol may also become evident. Here we have shown that both genetic and environmental factors may modulate the association between LPL activity and HDL cholesterol, and provide explanations for the absence of any changes in HDL values in mice either transgenic or with targeted disruption of the LPL gene.—**Clee, S. M., H. Zhang, N. Bissada, L. Miao, E. Ehrenborg, P. Benlian, G. X. Shen, A. Angel, R. C. LeBoeuf, and M. R. Hayden.** Relationship between lipoprotein lipase and high density lipoprotein cholesterol in mice: modulation by cholesteryl ester transfer protein and dietary status. *J. Lipid Res.* 1997. **38:** 2079–2089.

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Lipoprotein lipase is a key enzyme in lipoprotein metabolism, which, anchored to the vascular endothelium via heparan sulphate proteoglycans, catalyzes the hydrolysis of core triglycerides (TGs) from triglyceride-rich lipoproteins (TGRL), such as chylomicrons and very low density lipoproteins (VLDL), generating free fatty acids (FFA) (1). After the hydrolysis of TGRL by LPL, surface remnants such as free cholesterol, phospholipids, and apolipoproteins are produced, which provide a key substrate for the maturation of HDL particles (1).

Positive correlations between LPL activity and HDL cholesterol concentrations have been observed by numerous investigators, in many different normo- and dyslipidemic human populations (1). Individuals with complete LPL deficiency, due to homozygosity or compound heterozygosity for mutations in the LPL gene, typically manifest with lipid abnormalities including fasting chylomicronemia and markedly decreased low density lipoprotein (LDL) and HDL cholesterol con-

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Abbreviations: LPL, lipoprotein lipase; HDL, high density lipoprotein; CETP, cholesteryl ester transfer protein; TG, triglyceride; TGRL, triglyceride-rich lipoprotein; VLDL, very low density lipoprotein; FFA, free fatty acids; LDL, low density lipoprotein; CE, cholesteryl ester; apo, apolipoprotein; SD, standard deviation; HL, hepatic lipase; chol, cholesterol.

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centrations (1). Heterozygous carriers have also been shown to have increased plasma TGs and decreased HDL cholesterol levels (2–4). Correlations between LPL activity and HDL cholesterol have further been extended, in both men and women, to both hypo- and hyperalphalipoproteinemic populations (5). Thus, alterations of LPL catalytic activity clearly affect both the plasma TG and HDL concentrations in humans.

These correlations have not, however, been detected in the genetically engineered mouse models of LPL. In transgenic mouse models overexpressing LPL, while the predicted effects of increased LPL activity on decreasing TGs have been evident, significant increases in HDL cholesterol have not been observed (6–8). Similarly, in the two LPL gene targeted models, while decreasing LPL activity was associated with increased TGs, no significant changes in HDL cholesterol were noted in the heterozygous offspring (9, 10). Given that these relations are well documented in humans, the mild effect of LPL on HDL in these mouse models may be attributable to species differences in lipid metabolism.

Several differences in lipoprotein metabolism between mice and humans have been documented, some of which are of direct importance to HDL metabolism. Specifically, mice possess higher circulating hepatic lipase (HL) levels compared with humans (11), and lack plasma cholesteryl ester transfer protein (CETP) activity (12, 13). These differences between the species might, therefore, be of particular importance to consider when examining models of human HDL metabolism in mice.

CETP is found in the plasma, carried to a large extent on HDL particles (12, 14), primarily LpAI (14). It binds to lipoproteins ionically through negatively charged residues on the lipoprotein surface, and catalyzes the exchange of neutral lipids between lipoprotein fractions (12). Transfer often occurs down the concentration gradient of each component (12, 15), resulting in an equilibration of components between particles (12). Thus, cholesteryl esters (CEs) are transferred from relatively CE-rich/TG-poor particles, such as HDL, to TG-rich/CE-poor acceptors such as VLDL, while triglycerides may be exchanged in the opposite direction, from VLDL to HDL (14, 15). One-way transfer of CEs without reciprocal TG exchange and transfer of CEs to LDL may also occur (14, 15). Individuals with genetic CETP deficiency have lipoprotein profiles that more closely resemble those of CETP-deficient animals, including increased HDL, and decreased VLDL, IDL, and LDL (16), and have been shown to possess large apoE-rich HDL (12, 17). Thus, a significant portion of the differences in lipoprotein profiles between humans and mice may be attributable to the absence of plasma CETP activity in mice.

Evidence has accumulated that interactions between LPL and CETP may coordinately regulate plasma levels of HDL in vivo. LPL and CETP are expressed in a similar tissue distribution pattern (18), and have common substrates in TGRL and HDL. In vivo, in the absence of plasma LPL activity, CETP activity in the plasma is very low (19), despite increased transfer activity towards exogenous substrates (20), suggesting a need for lipolysis of endogenous TGRL before transfer activity can proceed. VLDL lipolyzed by LPL have indeed been shown to be better acceptors of CE than non-lipolyzed particles in vitro (16). However, beyond this initial requirement, it is unclear how alterations in LPL activity may modulate the transfer process and thereby affect HDL cholesterol concentrations.

In this study we sought to further examine the relationship between LPL and CETP in regulating HDL cholesterol levels in vivo. Transgenic mice overexpressing human LPL (6) were used to obtain a group of mice expressing a wider range of LPL activities than obtained with only non-transgenic mice. This line of transgenic mice containing the human LPL cDNA driven by the CMV promoter have been shown to express at high levels in heart, muscle, and adipose tissue, as well as stomach, and at lower levels in the kidney (6), and provide a constitutive increase in LPL, above that from the endogenous mouse gene, which maintains its natural regulation. These mice were bred with mice expressing the simian CETP gene (21), resulting in mice expressing CETP with a broad distribution of LPL activities. Under these conditions, the relationship between plasma LPL activity and HDL cholesterol levels was examined. Here we show that in mice hemizygous for CETP, HDL cholesterol levels were significantly affected by LPL activity, while no such relation was seen in the absence of CETP on a chow diet. After high fat/high carbohydrate feeding, however, strong correlations between LPL and HDL cholesterol were noted in both the presence and absence of CETP, suggesting that the relationship between LPL and HDL cholesterol may also be influenced by diet. These data provide further in vivo evidence of the interaction of CETP and LPL in the regulation of HDL cholesterol levels, and also show how environmental changes may influence the relationship between LPL and HDL cholesterol values in mice.

MATERIALS AND METHODS

Animal housing and diets

The parent LPL and CETP transgenic strains previously described (6, 21) were bred for at least five generations onto the C57BL/6 strain by successive backcrossing. The mice were housed individually or in small

groups in microisolator cages, with 12-h light and dark cycles in an environmentally controlled facility. Mice were fed a standard rodent chow (Laboratory Rodent Diet, PMI Feeds, 5001) consisting of 23.4% protein, 4.5% fat, with no more than 270 PPM cholesterol, and were provided food and water ad libitum. For high fat/high carbohydrate feeding studies, male mice were fed a semi-synthetic diet consisting of 50% sucrose, 15% corn oil, and 21.9% protein, with no added cholesterol (Harlan Teklad, TD 96202).

Blood samples of approximately 500 μL were withdrawn retro-orbitally after light anesthesis (Halothane, MTC Pharmaceuticals, Cambridge ON). Samples for plasma LPL and HL measurements were withdrawn after injection of an intravenous (tail vein) bolus of heparin (Liquemin, Hoffman-La Roche, 49800) at a standard dose of approximately 100 U/kg. Lipoprotein assessment was performed on samples collected without the administration of heparin, into tubes containing 1 mM EDTA. For chow diet measurements, pre- and post-heparin samples were taken on two separate occasions spanning at least 2 weeks. For measurements in male mice consuming the high fat/high carbohydrate diet, pre- and post-heparin samples were measured on the same day, with the total blood volume withdrawn not exceeding 500 μL. All samples were taken after an overnight fast of approximately 14 h, and placed immediately on ice. Plasma was removed after centrifugation at 14000 rpm (Eppendorf, 5415C) for 10 min, and immediately frozen at −70°C until analysis. All work was approved by the UBC Animal Care Committee.

Identification of genotypes by polymerase chain reaction

The presence of each cDNA transgene was identified by polymerase chain reaction (PCR) using primers spanning more than one exon, such that endogenous genes would not amplify. Both sets of reactions contained 1.5 mM Mg^{2+} and 200 μM of each dNTP. For determination of the LPL transgene, 20 pmol of the upstream primer LPL65 (5′GTGGGACAGGATGTGGC), located in exon 3, and the downstream primer LPL55 (5′AAGTCCTCTCTCTGCAATCAC), in exon 5 were used in each 50 μL reaction. Thermal cycle conditions were 5 min at 96°C, followed by 30 cycles of 96°C for 1 min, 58°C for 1 min, 72°C for 45 sec, and a final 5-min extension at 72°C. The CETP transgene was assessed using 15 pmol each of primer CETP1 (5′CCTGAAGTATGGCTACACCAC) in exon 3, and primer CETP2 (5′GTGGAAGACTTGCTCGGAGAAC) in exon 9, with cycle conditions consisting of 5 min at 96°C followed by 30 cycles of 96°C for 1 min, 51°C for 30 sec, 72°C for 45 sec, with a final extension of 5 min at 72°C. Products, approximately 400 bp for LPL and 525 bp for CETP, were visualized after electrophoresis on a 1% agarose gel.

Post-heparin lipase activities

Total plasma lipase activity was measured in duplicate using a radiolabeled triolein emulsion as previously described (22). Hepatic lipase was measured after inhibition of LPL with 1 M NaCl (6), and LPL was measured by subtraction. One milliunit (mU) of activity is defined as the amount that generates 1 nmol free fatty acids per min at 37°C, and plasma activities are expressed as mU per milliliter of plasma.

Cholesteryl ester transfer activity

LDL (d 1.024–1.063 g/mL), HDL_3 (d 1.125–1.210 g/mL) and d >1.125 g/mL fractions of human plasma were isolated by sequential ultracentrifugation. The density >1.125 g/mL plasma fraction was incubated with [^{14}C]cholesterol (Amersham, Oakville, ONT) at 37°C overnight (23). The remaining free [^{14}C]cholesterol was removed by an incubation with excess LDL. Over 95% of radioactivity was found in the chemical form of CE determined by thin-layer chromatography, as previously described (24).

Aliquots of plasma were incubated with 20 μg of [^{14}C]CE-HDL_3 and 100 μg of LDL at 37°C for 16 h in a final volume of 0.7 mL. After the incubation, LDL in the incubation mixture was precipitated by 50 mM sodium phosphate (pH 7.4) and 16 mM $MnCl_2$ as previously described (24). Pellets containing LDL generated from a centrifugation (9000 *g* for 3 min) were washed using the buffer and then precipitated again by the same procedure. The radioactivity in the pellets after the second precipitation was counted using a liquid scintillation system. The amount of [^{14}C]CE in LDL indicated CETP activity. The values for CETP activity were corrected by subtracting the incubation blank. CETP activity in plasma was expressed in nmol CE/h/mL.

Lipid and lipoprotein assessment

Plasma total cholesterol and triglycerides were measured by enzymatic colorimetric procedures using commercially available kits (Boehringer Mannheim, Nos. 1442350 and 450032, respectively.) HDL cholesterol was measured after precipitation of apolipoprotein B-containing lipoproteins with an equal volume of a 20% polyethylene glycol solution, as previously described (6), and non-HDL cholesterol values were obtained by subtraction of this value from the total cholesterol measurement.

FPLC analysis of plasma samples

FPLC (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) separation of plasma lipoproteins was performed using two Superose™ 6 columns in series, as previously described (6). Briefly, equal volumes of plasma from each mouse in each group were pooled and fil-

TABLE 1. Baseline characteristics of male and female mice

	CETP Present		CETP Absent	
	LPL Low	LPL High	LPL Low	LPL High
Males				
n	9	17	16	11
Age (weeks)	29.2 ± 6.1	28.5 ± 6.4	26.7 ± 1.3	30.8 ± 6.9
CETP activity (nmol/h/mL)	13.9 ± 4.8[a]	13.0 ± 7.3[a]	0.5 ± 1.0	0.7 ± 1.2
LPL activity (mU/mL)	469 ± 97	888 ± 92[a]	478 ± 61	868 ± 96[a]
HL activity (mU/mL)	90 ± 22[b,c]	105 ± 20[d]	115 ± 20	128 ± 21
Females				
n	10	9	14	17
Age (weeks)	27.4 ± 1.8	27.2 ± 5.3	28.8 ± 2.7	31.9 ± 7.3
CETP activity (nmol/h/mL)	15.4 ± 5.9[a]	13.1 ± 8.7[a]	0.4 ± 0.8	0.8 ± 1.1
LPL activity (mU/mL)	394 ± 122	878 ± 70[a]	398 ± 109	893 ± 78[a]
HL activity (mU/mL)	115 ± 20	136 ± 23	135 ± 26	142 ± 36

Values are mean ± SD. All statistics were performed on values corrected for age except female CETP activities.

[a] All comparisons for low vs. high activity are significant at $P < 0.001$.

[b] $P = 0.04$, CETP Present-LPL Low vs. CETP Absent-LPL Low.

[c] $P = 0.001$, CETP Present-LPL Low vs. CETP Absent-LPL High.

[d] $P = 0.03$, CETP Present-LPL High vs. CETP Absent-LPL High.

tered through a 0.22-μm filter. Filtered plasma (200 μL) was loaded onto the columns and eluted at a flow rate of 0.5 mL/min in a buffer consisting of 0.15 M NaCl, 1 mM EDTA, and 0.02% NaN_3, pH 8.2. Cholesterol and TG content in each 0.5-mL fraction were assessed using commercially available enzymatic kits (Boehringer Mannheim, Nos. 1442350 and 701904, respectively).

Statistical analysis

Males and females were analyzed separately. Mice were grouped according to LPL activity in the highest (high) or lowest (low) tertiles, and the presence or absence of plasma CETP activity, creating four study groups. Values are presented as mean ± standard deviation. Between group differences were measured using an analysis of variance (ANOVA). As there was a wide distribution of ages within each group, age was included as a covariate within all analyses, except those noted in the tables, where covariate analysis was not possible. Individual pairwise comparisons between groups were made using the Tukey procedure. Correlations reported are Pearson correlation coefficients. *P*-values less than 0.05 were considered statistically significant. All analysis was performed using the Systat analysis program (Systat for Windows, version 5.0, SPSS).

RESULTS

Baseline characteristics

Transgenic mice overexpressing human LPL (6) were bred with transgenic mice expressing simian CETP (21), producing mice hemizygous for the CETP transgene or lacking CETP activity. As these LPL transgenic mice have activities that overlap with their nontransgenic littermates, when combined, this resulted in mice with a broad distribution of LPL activities. Thus, mice were divided into tertiles of LPL activity, and those in the highest and lowest groups were chosen for study. This resulted in four study groups: those within the lowest and highest tertiles of LPL activity, either expressing CETP or littermate controls without CETP, respectively.

Baseline group characteristics are presented in **Table 1.** Within both male and female groups, the presence of the CETP transgene was associated with significant levels of plasma CETP activity ($P < 0.001$), and did not differ with LPL status. Similarly, plasma LPL activity of mice in the highest tertile was increased approximately 1.8-fold over those in the lowest tertile ($P < 0.001$) and was unaffected by the presence of the CETP transgene. Thus the activity of either enzyme, as measured towards exogenous substrates, does not appear to be directly influenced by the activity of the other. There were no other differences in baseline characteristics except that male mice with CETP tended to have lower HL activities than those not expressing CETP.

Lipoprotein profiles

Lipoprotein profiles were then examined for each of the groups, and are presented for males and females in **Table 2** and **Table 3,** respectively. In males, the presence of plasma CETP activity had the predicted effects on the lipoprotein profile when compared with mice matched for LPL activity within the lowest tertile, but lacking CETP (Table 2, first vs. third columns). HDL cholesterol was significantly decreased in mice express-

TABLE 2. Lipoprotein profiles of male mice consuming standard rodent chow

	CETP Present		CETP Absent	
	LPL Low	LPL High	LPL Low	LPL High
n	9	17	16	11
Age (weeks)	31.9 ± 7.0	29.2 ± 5.1	31.3 ± 7.5	32.5 ± 6.9
Total chol (mg/dL)	45 ± 7[c]	65 ± 35	69 ± 15	76 ± 14
TG (mg/dL)	60 ± 13	39 ± 16	57 ± 21	56 ± 26
HDL chol (mg/dL)	31 ± 4[a,d]	51 ± 29	61 ± 16	65 ± 12
Non-HDL chol (mg/dL)	14 ± 5	14 ± 9	8 ± 7	11 ± 8
Total/HDL chol	1.45 ± 0.20[b,c]	1.32 ± 0.20	1.17 ± 0.23	1.18 ± 0.13

Values are mean ± SD. All statistics were performed on values corrected for age.
[a]$P = 0.005$; [b]$P = 0.008$, CETP Present-LPL Low vs. CETP Absent-LPL Low.
[c]$P = 0.02$; [d]$P = 0.003$, CETP Present-LPL Low vs. CETP Absent-LPL High.

ing CETP (31 ± 4 vs. 61 ± 16 mg/dL, $P = 0.005$, CETP present vs. absent), contributing to decreased plasma total cholesterol levels. This led, overall, to a significantly increased total cholesterol to HDL cholesterol ratio (1.45 ± 0.20 vs. 1.17 ± 0.23, $P = 0.008$, CETP present vs. absent). Triglycerides and non-HDL cholesterol were not significantly affected by the addition of CETP.

When LPL activity was increased from the lowest to highest tertile, HDL cholesterol levels were restored to values not significantly different from those seen in the absence of CETP (51 ± 29 mg/dL) (Table 2, second column). This increase nearly reached significance compared to those in the lowest tertile of LPL activity ($P = 0.07$, likely due to the large standard deviation) and contributed to elevated total cholesterol levels. Furthermore, in the presence of CETP, LPL activity and HDL cholesterol were significantly correlated (**Fig. 1,** $r = 0.43$, $P = 0.006$).

In contrast to the results in mice expressing CETP, in the absence of CETP there were no significant differences in lipoprotein profiles of mice between the lowest or highest tertiles of LPL activity (Table 2, third and fourth columns). No correlation between LPL activity and HDL cholesterol was evident (Fig. 1, $r = 0.15$, $P = 0.36$).

Profiles similar to the males were seen in the female mice (Table 3). The addition of the CETP transgene had the predicted effects on the lipoprotein profiles (Table 3, first and third columns). Comparing mice within the lowest tertile of LPL activity with and without CETP, total and HDL cholesterol levels were significantly decreased (46 ± 14 vs. 63 ± 12 mg/dL, $P = 0.02$ and 33 ± 12 vs. 53 ± 12 mg/dL, $P = 0.002$, respectively, in the presence vs. absence of CETP), while the total cholesterol to HDL cholesterol ratio was significantly increased (1.42 ± 0.19 vs. 1.21 ± 0.12, $P = 0.03$). Increasing LPL within the CETP expressing group (Table 3, second column) was again associated with increased HDL cholesterol levels (33 ± 12 vs. 45 ± 13 mg/dL, lowest vs. highest tertiles of LPL activity) although the correlation between the two parameters did not reach significance ($r = 0.29$, $P = 0.12$, data not shown). Additionally, increased total cholesterol and a decreased total cholesterol to HDL cholesterol ratio were noted. As in the males, in the absence of CETP, increasing LPL activity had little effect on the HDL cholesterol levels (Table 3, third and fourth columns), and no correlation was noted.

FPLC analysis

These differences in lipoprotein profiles are also clearly illustrated in the FPLC profiles of these animals

TABLE 3. Lipoprotein profiles of female mice consuming standard rodent chow

	CETP Present		CETP Absent	
	LPL Low	LPL High	LPL Low	LPL High
n	10	9	14	17
Age (weeks)	27.4 ± 7.7	28.1 ± 3.5	28.3 ± 7.7	30.4 ± 7.6
Total chol (mg/dL)	46 ± 14[a]	59 ± 17	63 ± 12	58 ± 12
TG (mg/dL)	42 ± 13	36 ± 11	33 ± 11	35 ± 11
HDL chol (mg/dL)	33 ± 12[b,d]	45 ± 13	53 ± 12	49 ± 12
Non-HDL chol (mg/dL)	13 ± 6	14 ± 12	10 ± 5	9 ± 5
Total/HDL chol	1.42 ± 0.19[c,d]	1.34 ± 0.27	1.21 ± 0.12	1.21 ± 0.14

Values are mean ± SD. All statistics were performed on values corrected for age.
[a]$P = 0.02$; [b]$P = 0.002$; [c]$P = 0.03$; CETP Present-LPL Low vs. CETP Absent-LPL Low.
[d]$P = 0.02$, CETP Present-LPL Low vs. CETP Absent-LPL High.

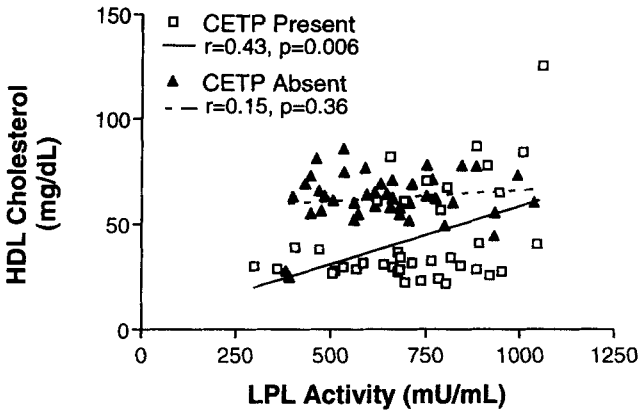

Fig. 1. Correlations between LPL activity and HDL cholesterol concentrations in male mice fed a standard rodent chow diet. Mice expressing CETP are represented by open squares and the solid line. Mice not expressing CETP are represented by solid triangles and a dashed line.

(**Fig. 2A and B,** for males and females, respectively) where the major effects of the genetic manipulations appear manifest in the HDL peak. In mice expressing CETP (Fig. 2A, top left), the HDL peak is characterized by a shift towards smaller particles. Furthermore, the addition of CETP was associated with apparently increased HDL and LDL triglyceride, and VLDL cholesterol, as would be predicted by the cholesteryl ester transfer process. Increasing LPL within this group (bottom left panel) led to a shift towards larger sized particles, resulting overall in dramatically elevated plasma HDL cholesterol levels. HDL and LDL-TG levels also appeared reduced. As these lipoproteins are not generally substrates for LPL, this suggests an inhibition of the CE transfer process within the plasma by increasing LPL. In the absence of CETP (Fig. 2A, right panels) the HDL peak is large, unimodal, and consists of particles relatively large in size. This profile does not change with increasing LPL activity.

In female mice expressing CETP, increasing LPL activity was associated with a restoration of the HDL cholesterol peak and decreased HDL-TG (Fig. 2B, left panels). In contrast, in the absence of CETP, few differences in the profiles between the lowest and highest LPL tertiles were evident (Fig. 2B, right panels).

Response to a high fat/high carbohydrate diet

As the concentration of plasma TG rich acceptor particles, such as VLDL, has been suggested to be an important modulator of the transfer process (12, 16), the relation between LPL and CETP was further explored under dietary conditions designed to favor VLDL synthesis. Male mice were fed a high fat/high carbohydrate diet (15% corn oil, 50% sucrose) for a period of 5 weeks, and lipoprotein profiles were examined. After high fat/high carbohydrate feeding, plasma LPL activities increased dramatically in all groups, consistent with results seen in humans after a fat-rich meal (25) and in LPL transgenic mice fed a high fat/cholesterol-enriched diet (8), such that there were no longer significant differences between the groups (716 ± 161 vs. 918 ± 203; 1048 ± 479 vs. 968 ± 519 mU/mL for lowest vs. highest tertiles, with and without CETP, respectively). Plasma triglycerides increased by approximately 50%, and total and HDL cholesterol increased by approximately 130% in all groups compared with pre-diet values (data not shown). Non-HDL cholesterol increased, though not significantly due to large standard deviations in all groups.

However, when levels of LPL activity are examined without grouping by tertiles, an association between LPL and HDL cholesterol is evident. After high fat/high carbohydrate feeding, plasma LPL activity is positively correlated with HDL cholesterol levels in both the presence (**Fig. 3,** $r = 0.45$, $P = 0.03$) and absence (Fig. 3, $r = 0.73$, $P < 0.001$) of CETP. This suggests that when mice consume standard low fat/low carbohydrate rodent chow, the relationship between LPL activity and HDL cholesterol is not evident in the absence of CETP. However, the effects of LPL on HDL cholesterol levels may become apparent when the system is challenged by a high fat/high carbohydrate diet, irrespective of the presence or absence of CETP.

Representative post-diet FPLC profiles of groups of mice matched for LPL activity, with and without CETP, are shown in **Fig. 4.** After high fat/high carbohydrate diet feeding, changes in the lipoprotein profiles were evident, compared with those seen on the rodent chow diet (Fig. 2A). In the absence of CETP, an accumulation of small LDL particles, which were overlapping with the HDL cholesterol range, was evident, while the addition of CETP resulted in a much clearer distinction between the LDL and HDL fractions.

DISCUSSION

Plasma levels of HDL cholesterol have generally been inversely correlated with an individual's risk of developing CAD (16). As such, understanding factors regulating the concentrations of this lipoprotein are of prime importance. Several genetic and metabolic factors have been implicated in this process (16). Four key enzymes in lipoprotein metabolism, HL, LPL, LCAT, and CETP, have been shown to account for almost 50% of the variability of HDL cholesterol levels within a hypertriglyceridemic cohort (26). However, while correlations between plasma LPL activity and HDL cholesterol have been observed in humans (2–5), such relationships have not been observed in the genetically altered

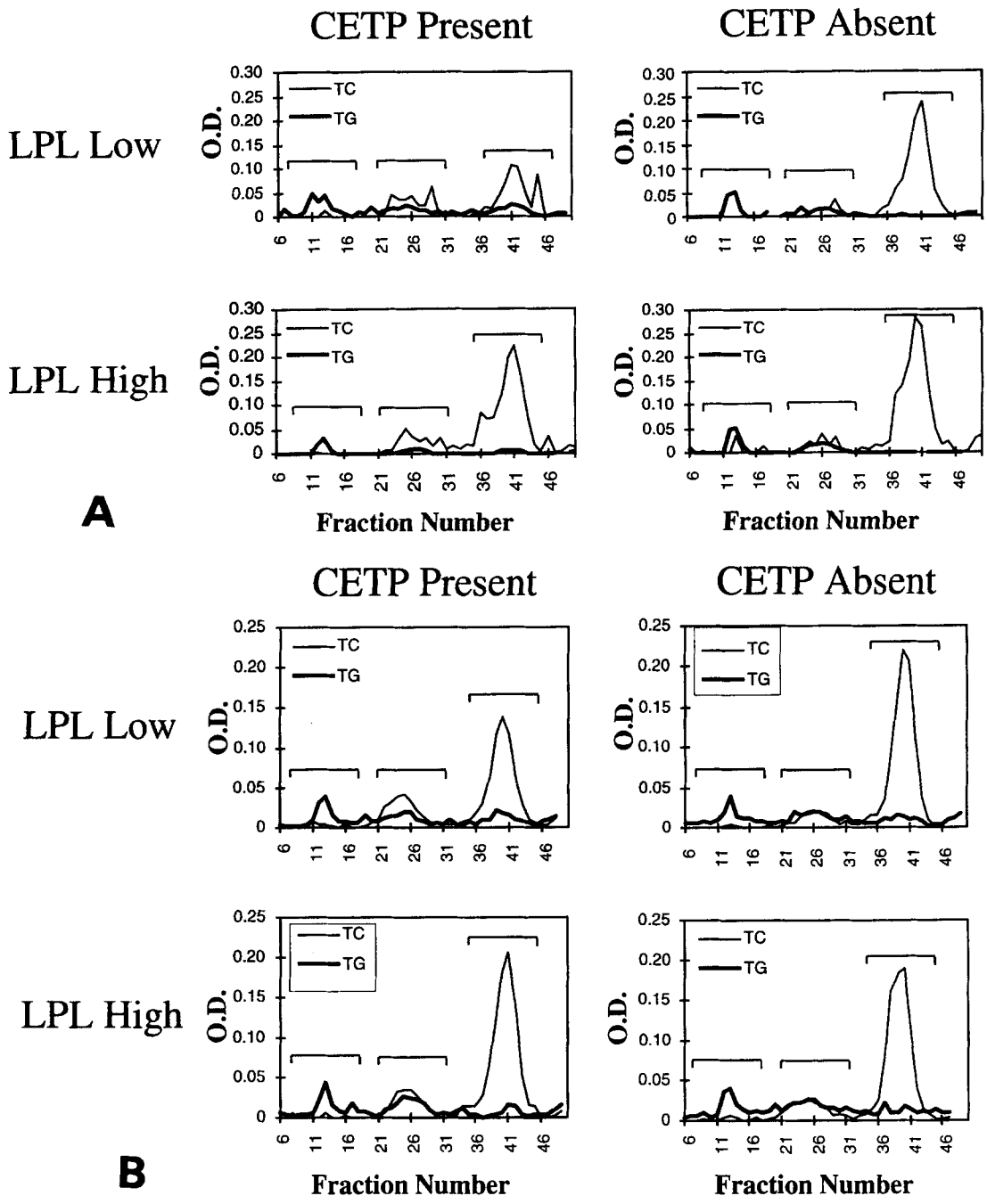

Fig. 2. A: FPLC profiles of male mice fed a standard rodent chow diet. Cholesterol in each fraction is shown by the thin line, and triglycerides in each fraction are shown by the thick line. From left to right, peak areas depicted represent VLDL, LDL, and HDL, respectively. B: FPLC profiles of female mice fed a standard rodent chow diet. Cholesterol in each fraction is shown by the thin line, and triglycerides in each fraction are shown by the thick line. From left to right, peak areas depicted represent VLDL, LDL, and HDL, respectively.

mouse models (6–10). Thus, for mice to be useful models in which to study the effects of LPL on lipids and atherosclerosis in relation to those seen in humans, these differences must be better understood. In this study we have examined whether species differences in lipid metabolism, such as the absence of plasma CETP activity in mice, might contribute to these findings. With mice on a standard rodent chow diet, we show that an increase in LPL activity is associated with increased HDL cholesterol levels only in the presence of CETP activity.

Two possible mechanisms may account for the interaction between LPL and CETP in regulating HDL cholesterol levels. One factor that plays a major role in de-

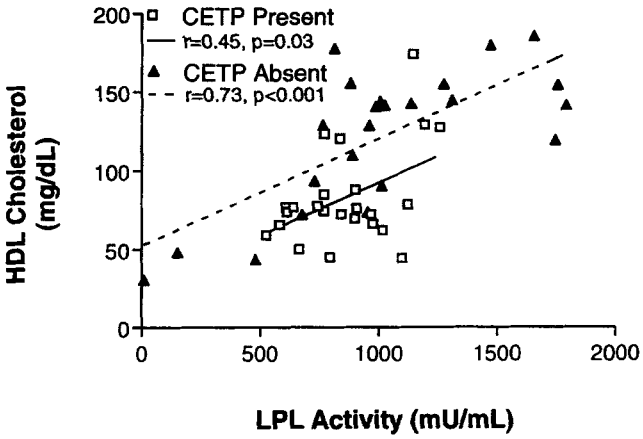

Fig. 3. Correlations between LPL activity and HDL cholesterol concentrations in male mice after high fat/high carbohydrate feeding. Mice expressing CETP are represented by open squares and the solid line. Mice not expressing CETP are represented by solid triangles and a dashed line.

termining the rate and extent of lipid transfer between particles is the concentration of TG-rich acceptor particles (16, 27). Cholesteryl ester transfer from HDL to VLDL and LDL fractions is correlated with plasma cholesterol concentrations in apoB-containing lipoproteins, and with plasma TG levels (28). Furthermore, VLDL triglyceride levels have also been shown to be a major predictor of CE transfer rate (29), and increased transfer of CE from HDL to TGRL is seen in hypertriglyceridemic individuals (16, 30). The size of the TG-rich acceptor pool has thus been suggested to be rate-limiting for transfer of CE from HDL to VLDL (12). Therefore, a decreased VLDL concentration, due to increased hydrolysis by LPL, leads to a decreased concentration of TG-rich acceptors. This decreased acceptor concentration is likely to impair the ability of CETP to transfer CE's from HDL to this fraction, resulting in increased HDL cholesterol concentrations. This is the probable mechanism of interaction between LPL and CETP in the regulation of HDL cholesterol levels.

However, another possible mechanism linking lipolysis and CE transport is that altered surface composition of VLDL or HDL, resulting from increased lipolysis, may affect the binding of CETP to, and interactions with, these particles (16). Lipolyzed VLDL may be a better acceptor of CE than non-lipolyzed VLDL (19, 27), due to enhanced binding of CETP to the particles through an increase in charged products on their surface (31). These data suggest that surface alterations induced by lipolysis may directly affect the ability of CETP to interact with its target lipoproteins. It could thus also be suggested that large increases in lipolysis may alter surface composition to the extent that CETP may bind less effectively, providing another potential mechanism underlying the interaction between LPL and CETP seen in this study.

Previous studies have shown that an interaction between hypertriglyceridemia and CETP in mice further decreases HDL concentrations (32). To further examine the relationship between LPL and CETP under a TG challenge, mice were fed a high fat/high carbohydrate diet intended to increase plasma TG and thus CE acceptor concentrations. Interestingly, feeding of this diet increased both LPL activities and HDL cholesterol concentrations, and resulted in striking correlations between LPL and HDL cholesterol independent of CETP status.

Several alterations in lipoprotein metabolism may occur upon high carbohydrate and high fat feeding (33, 34) that may help to explain the correlations between LPL and HDL regardless of CETP status. Consumption of a high sucrose diet increases hepatic secretion of TGRLs (33), while high fat feeding increases apoA-I

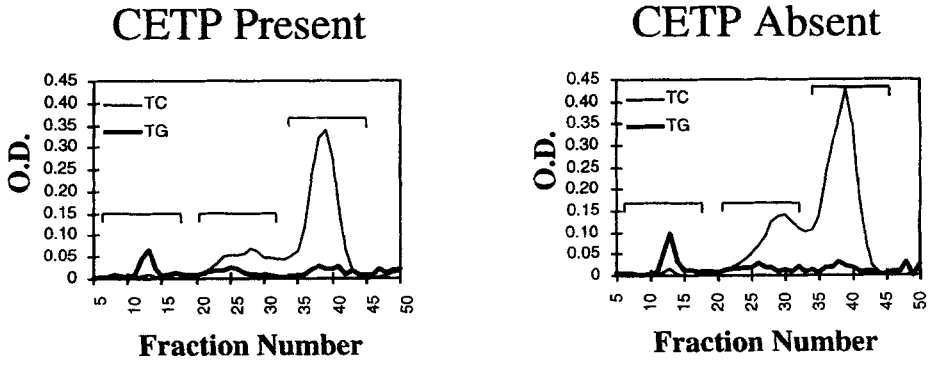

Fig. 4. Post-diet FPLC profiles of male mice. Groups were matched for LPL activity (918 ± 203 and 968 ± 519 mU/mL for CETP present and absent, respectively). Cholesterol in each fraction is shown by the thin line, and triglycerides in each fraction are shown by the thick line. From left to right, peak areas depicted represent VLDL, LDL, and HDL, respectively.

concentrations (34). Taken together with the changes observed in this study, these metabolic alterations may be sufficient to account for the relation between LPL and HDL cholesterol, independent of CETP status. We have observed an accumulation of small LDL (Fig. 4) that may result from increased hepatic secretion of TGRL combined with increased LPL activities, as shown in this manuscript. This suggests that lipolysis is increased in response to the dietary challenge. Therefore, we propose that consumption of a normal chow diet results in insufficient hydrolysis of TG-rich particles and formation of surface remnants such that a strong correlation between LPL activity and HDL cholesterol levels is not evident in the absence of CETP. However, after high fat/high carbohydrate feeding, increased lipolysis generates sufficient surface remnants, perhaps in conjunction with increased apoA-I concentrations to aid HDL formation, such that the association between HDL cholesterol and LPL activity independent of CETP status becomes apparent.

A potential confounding factor in this study is the significant difference in HL activities among the male mice at baseline. Hepatic lipase is a heparin-releasable enzyme whose main function is the hydrolysis of core TGs and phospholipids in the less TG-rich lipoprotein particles such as intermediate density lipoproteins (IDL), LDL, and HDL (35). One prime role for this enzyme is in the HDL conversion process, where it hydrolyzes core TGs in HDL_2, which, in conjunction with loss of CE's through CETP action, leads to the formation of HDL_3 (5, 16, 35). Thus, HL activity has been shown to correlate negatively with plasma HDL cholesterol (5, 16, 35). Differences in HL levels between groups could therefore affect comparisons of HDL. However, both groups of mice expressing CETP, which resulted in decreased HDL cholesterol concentrations, had the lowest HL activities. Decreased HL activity would be expected to increase HDL cholesterol within these groups. In addition, the effect of increasing LPL within the CETP expressing group, which was associated with increased HDL, would be opposed by the higher HL activity in this group. Furthermore, in the female mice, there were no significant differences in HL activities between groups, yet the same HDL trends were maintained. Thus, it seems unlikely that the findings of this study could be attributed to altered HL activities between the groups.

Interestingly, while there were no consistently significant differences between male and female mice, females tended to have both increased HL activities, and decreased plasma TG's compared to males. The combination of increased hydrolysis of TG's in the less TG-rich particles and decreased TG-rich acceptor pool concentrations may partly explain the milder effects of CETP in the females compared to the males.

Surprisingly, no differences in plasma TG levels with increasing LPL activity at baseline were observed in mice lacking CETP. This may be due to the altered timing of the fasting period in this study. Specifically, as the initiation of fasting in this study coincided with the onset of the dark cycle, the effective fasting time may have been longer if mice did not consume much during their less active light cycle period. Such prolonged fasting may have been of sufficient duration for most TGRL to be hydrolyzed, even with lower LPL activities. Thus, a failure to measure differences in plasma TGs may not necessarily indicate that there was no difference in LPL activities between the groups.

Here we have shown that under normal metabolic conditions in both male and female mice, plasma LPL activity was only correlated with HDL cholesterol in the presence of CETP, providing additional in vivo evidence of the interaction between LPL and CETP in the regulation of HDL cholesterol levels, likely due to changes in triglyceride-rich acceptor pool concentrations. Whereas, after the metabolic alterations induced by high fat, high carbohydrate feeding, strong correlations between LPL and HDL were demonstrated regardless of the CETP status. These data highlight the importance of gene-diet interactions in lipoprotein metabolism, and suggest that dietary factors also regulate the relationship between LPL and HDL cholesterol. The insights into the relationship between LPL and HDL cholesterol in mice gained here will improve the utility of such mouse models in enabling us to understand the role of LPL in lipid metabolism and atherosclerosis. Furthermore, in the presence of CETP, as in many species including humans, irrespective of dietary status, LPL activity is an important predictor of HDL cholesterol levels, capable of overcoming some of the detrimental effects of CETP. Thus, measurements of LPL activity are strong predictors of HDL cholesterol levels, and may therefore be important determinants of atherosclerosis susceptibility.

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