VLDL-bound lipoprotein lipase facilitates the cholesteryl ester transfer protein-mediated transfer of cholesteryl esters from HDL to VLDL

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Abstract **In recent years, it has been established that lipoprotein lipase (LPL) is partly associated with circulating lipoproteins. This report describes the effects of physiological amounts of very low density lipoprotein (VLDL)-bound LPL on the cholesteryl ester transfer protein (CETP)-mediated cholesteryl ester transfer (CET) from high density lipoprotein (HDL) to VLDL. Three patients with severe LPL deficiency exhibited a strong decrease in net mass CET that was more than 80% lower than that of common hypertriglyceridemic subjects. Recombination experiments showed that this was due to an abnormal behavior of the VLDL fraction. Replacement of the latter by normal VLDL totally normalized net mass CET. We therefore prepared VLDL containing controlled amounts of bound LPL that we used as CE acceptors in experiments involving unidirectional radioisotopic CET measurements. These were carried out either in the absence or in the presence of inhibitors of LPL lipolytic activity. When LPL-induced lipolysis was totally blocked, the stimulating effect of the enzyme on the CETP-dependent CET was only reduced by about 50%, showing that it did not entirely result from its lipolytic action. These data were dependent upon neither the type of LPL inhibitor (E600 or THL) nor the source of CETP (delipidated plasma or partially purified CETP).** **Thus, in addition to the well-known stimulating effect of LPL-dependent lipolysis on CET, our work demonstrates that physiological amounts of VLDL-bound LPL may facilitate CET through a mechanism partially independent of its lipolytic activity.**—Pruneta, V., T. Pulcini, F. Lalanne, C. Marçais, F. Berthezène, G. Ponsin, and P. Moulin. **VLDL-bound lipoprotein lipase facilitates the cholesteryl ester transfer protein-mediated transfer of cholesteryl esters from HDL to VLDL.** ***J. Lipid Res.*** **1999.** 40: **2333–2339.**

Supplementary key words LPL-deficiency • hypertriglyceridemia

The continuous remodeling of plasma lipoproteins results from the combined actions of specific enzymes and lipid transfer proteins. In humans, cholesteryl ester transfer protein (CETP) promotes the net cholesteryl ester (CE) transfer (CET) from high density lipoproteins (HDL) to apoB-containing very low and low density lipoproteins (VLDL and LDL), a process which is accompanied by a reciprocal transfer of triglycerides (TG) (1, 2). These CETP-mediated lipid transfers constitute an important step in the reverse cholesterol transport from peripheral cells to the liver (3). Net CET is frequently elevated in situations at high risk for atherosclerosis (4–6), which emphasizes the necessity of a better understanding of the mechanisms that modulate the CETP activity. Net mass CET necessarily depends upon the concentration and composition of donor and acceptor lipoproteins (2, 7–9). In particular, it has been clearly established that net CET was increased during lipolysis in the postprandial state (10, 11). This effect was attributed to the lipolytic effect of lipoprotein lipase (LPL) which results in the accumulation of negatively charged non-esterified fatty acids (NEFA) that facilitate the binding of CETP to VLDL (12–16). The quantitative influence of LPL on net CET has been recently evaluated by Bagdade et al. (17) in three patients with LPL deficiency. On the basis of the works mentioned above, the lack of LPL might be expected to diminish net CET. Alternatively, a significant CET acceleration could have been predicted as well, as elevated concentrations of TG-rich lipoproteins, as CE acceptors, have been shown to facilitate the CETP-mediated net CET (18). In fact, the data indicated that, in LPL deficiency, net CET dramatically decreased by more than 80%, by comparison with that observed in common hypertriglyceridemia. Although net CET could be normalized upon addition of exogenous LPL, the magnitude of the phenomenon is intriguing

Abbreviations: LPL, lipoprotein lipase; VLDL, very low density lipoprotein; CETP, cholesteryl ester transfer protein; CET, cholesteryl ester transfer; HDL, high density lipoprotein; LDL, low density lipoprotein; CE, cholesteryl ester; E600, diethyl *p*-nitrophenyl phosphate (Paraoxon); THL, tetrahydrolipstatin (Orlistat™); TG, triglyceride; LCAT, lecithin: cholesterol acyltransferase; NEFA, non-esterified fatty acid; GuHCl, guanidine hydrochloride.

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in that it is incommensurate with respect to what could be expected from a simple lipolysis defect. Indeed, albeit clearly established, the facilitating effect of NEFA on CETP-dependent lipid transfers is rather moderate: a ten times difference in plasma NEFA concentration was associated with only a 40% difference in CET (14). Thus, one cannot rule out the possibility that, in addition to its lipolytic effect, LPL might stimulate CET through another mechanism. The latter is not easy to comprehend, taking into account that the greatest part of LPL is anchored at the surface of the capillary endothelium (19). However, recent studies have convincingly demonstrated that a substantial amount of LPL is associated with circulating lipoproteins in human plasma (20–24). Thus, one can now consider the possibility that VLDL-bound LPL might facilitate the action of CETP by improving the efficiency of VLDL as CE acceptors. This concept led us to investigate the putative effect of physiological amounts of VLDL-bound LPL on the CETP-mediated CET from HDL to VLDL and to determine to what extent it was due to the lipolytic action of LPL. The latter aspect is of particular interest inasmuch as the question as of whether or not the VLDL-bound LPL is lipolytically active has been a matter of controversies (22, 23). We therefore measured CET in various experiments where several types of VLDL were used as CE acceptors. These, isolated either from LPL-deficient patients or from normal subjects, were treated to contain controlled amounts of LPL. The relative importance of lipolysis in the LPL-facilitated CETP-mediated CE transfer was determined by measuring CET after complete inhibition of the enzyme lipolytic activity.

MATERIALS AND METHODS

All experiments were carried out using a buffer (TSE) containing 150 mm NaCl, 2 mm EDTA, 10 mm Tris-HCl, and 0.02% NaN_3, pH 7.4.

Materials

Bovine milk lipoprotein lipase (EC 3. 1. 1. 34), bovine serum albumin (BSA, fraction V, essentially fatty acid-free), diethyl *p*-nitrophenyl phosphate (Paraoxon, E600), were purchased from Sigma Chemicals (St. Louis, MO). Bolton and Hunter reagent was obtained from Amersham. Tetrahydrolipstatin (THL, Orlistat) was a kind gift from Dr. H. Lengsfeld (Hoffman-La Roche, Basel, Switzerland). [4-^{14}C]cholesterol (45–60 Ci/mmol) was purchased from New England Nuclear. Enzymatic kits for the determinations of total and free cholesterol, and that for NEFA measurements were from Boehringer-Mannheim, and from Oxoïd (France), respectively.

Subjects

Plasma were obtained after an overnight fast from eight normolipidemic controls, seven hypertriglyceridemic and three LPL-deficient subjects (**Table 1**). The mutations in the gene for LPL were characterized by sequencing after screening by SSCP. One patient was homozygous for the Gly 188 Glu missense mutation in exon 5 of the LPL gene and the second was homozygous for G→A mutation at position minus one of the acceptor splice site of intron 1 inducing a defect in the splicing. The third LPL deficiency was due to the presence of a circulating anti-LPL autoantibody and was previously characterized (25). The lipid compositions of VLDL and HDL of these patients are presented in **Table 2**. Informed consent was obtained from all subjects.

TABLE 1. Characteristics of subjects

	NormoTG (n = 8)	HyperTG (n = 7)	LPL-Deficient (n = 3)
		mmol/L	
TG	1.0 ± 0.3	3.7 ± 0.6	12.9 ± 5.8
TC	5.1 ± 1.8	6.4 ± 1.0	3.8 ± 0.6
HDL-C	1.2 ± 0.3	0.8 ± 0.1	0.4 ± 0.1

Values given as mean ± SD.

Lipoprotein isolation

Chylomicrons were removed from the plasma of LPL-deficient patients by ultracentrifugation at 30,000 rpm for 45 min at 4°C. VLDL ($d < 1.006$ g/mL) and HDL ($1.063 < d < 1.21$ g/mL) were isolated by sequential preparative ultracentrifugation at 4°C in a Beckman TL-100 table-top ultracentrifuge at 100,000 rpm for 4 h 40 min and 6 h 30 min, respectively, using a TLA 100.3 fixed-angle rotor. When desired, VLDL isolated from control plasma were cleared of endogenous LPL by an additional 6-h ultracentrifugation. All lipoprotein fractions were then extensively dialyzed at 4°C against TSE buffer.

Binding of exogenous LPL to LPL-free VLDL

Bovine milk LPL was radioiodinated using the Bolton-Hunter reagent (26). After gel filtration on a Sephadex G75 column, labeled LPL, that had a specific radioactivity of $1.5–2 \times 10^3$ cpm/ng, was stored in TSE buffer containing fatty acid-free albumin (2 g/L).

VLDL containing controlled amounts of LPL were prepared as follows. LPL-free VLDL (1.1 μmol TG) were incubated in the presence of bovine LPL (60 ng) and of trace amounts of radioiodinated LPL for 30 min at 4°C in a final volume of 250 μl. VLDL were then reisolated by precipitation using the PTA/$MgCl_2$ method, as recommended by others (21). The binding of LPL to VLDL was then calculated after counting the samples for iodine radioactivity. Under the conditions used, the LPL/apoB molar ratio in VLDL was of about 1/400 which approximately corresponds to that observed in normal plasma. When desired, LPL was monomerized in stock solution by incubation in the presence of 0.75 m guanidine hydrochloride (GuHCl) (27). Monomeric LPL was then incubated with VLDL as mentioned above, in conditions that brought the final GuHCl concentration to less than 0.03 m.

Determination of net CE transfer

The net mass CET from HDL to VLDL/LDL was measured in native plasma or after various recombinations of lipoprotein frac-

TABLE 2. Lipid composition of VLDL and HDL of LPL-deficient patients

	Patient 1		Patient 2		Patient 3	
	VLDL	HDL	VLDL	HDL	VLDL	HDL
			mmol/L			
TG	0.97	0.32	0.94	0.21	1.26	0.37
CE	0.07	0.02	0.07	0.02	0.11	0.03
FC	0.06	0.02	0.05	0.02	0.13	0.02
PL	0.20	0.11	0.16	0.08	0.31	0.15

Patient 1 had a Gly 188 Glu mutation; patient 2 had G→A intron 1 mutation, and patient 3 had an autoimmune LPL deficiency.

tions, during incubations at 37°C in the presence of 1.5 mm iodoacetate to inhibit LCAT activity, as previously described (28). After 1, 2, 4, and 6 h, aliquots were withdrawn and immediately chilled on ice. ApoB-containing lipoproteins were then precipitated. After centrifugation, the concentrations of free and total cholesterol were determined in the HDL-containing supernatants. The net mass CET was then calculated as the decrease in the CE content of HDL.

Purification of CETP

CETP was partially purified from human plasma according to the sequential procedure previously described (29). Briefly, pooled plasma (d 1.21 g/ml) was ultracentrifuged at 40,000 rpm for 48 h at 4°C and the clear middle zone was successively submitted to a hydrophobic interaction chromatography on a phenyl-Sepharose CL-4B column (Pharmacia) and to affinity chromatography on a Q2 column (Pharmacia). This procedure was partially automated by using a computer-driven fast protein liquid chromatography system (Bio-Rad). After dialysis, the partially purified CETP was aliquoted and stored at −80°C until use.

Unidirectional radioisotopic assay of CE transfer

[4-^{14}C]CE-labeled HDL (200 nmol of cholesterol) was incubated at 37°C with various VLDL preparations (1.1 μmol of TG) in the presence or in the absence of either delipidated plasma or partially purified CETP, in a final volume of 0.5 ml. VLDL were then separated from HDL by precipitation as described above. Unidirectional CET was determined by measuring the apparition of radioactivity in VLDL. It never exceeded 30%. The conditions mentioned above were specifically chosen to maintain VLDL at a limiting concentration, thereby permitting the determination of their efficiency as CE acceptors. When desired, the incubations were performed in the presence of various concentrations of heparin (0.1, 1, 10 IU/ml).

Lipolysis monitoring

The lipolytic activity of LPL was monitored by measuring the amount of NEFA generated during incubations of the samples concomitantly used to determine CET. The complete inhibition of LPL-mediated lipolysis was obtained when the incubations were performed in the presence of either 2 mm Paraoxon (E600), a known neurotoxic inhibitor of lipase activity (30), or tetrahydrolipstatin (THL) used at a THL/LPL molar ratio of 6 according to previously published data (31, 32).

Statistical analysis

Values are given as the mean ± SD. Statistical analyses were performed using the StatView 4.02 software. Comparisons between groups were made by ANOVA or Student's *t*-test when appropriate.

RESULTS

The kinetics of CETP-mediated net mass CET from HDL to VLDL/LDL determined in patients with severe LPL deficiency were compared to those obtained in control subjects and in patients with common hypertriglyceridemia (**Fig. 1A**). As commonly noticed, the latter group exhibited a CET more elevated than controls, consistent with the positive correlation observed between CET and plasma TG concentrations (Fig. 1B). In spite of their severe hypertriglyceridemia, LPL-deficient patients had extremely low net CET (87.0 ± 1.7% of decrease with respect to hypertriglyceridemic group after 6 h of incubation). To establish the cause of this defect, net CET was determined in a series of experiments involving various recombinations of plasma fractions previously separated on the basis of their density. Thus, fractions containing both HDL and CETP were recombined with fractions containing VLDL. These

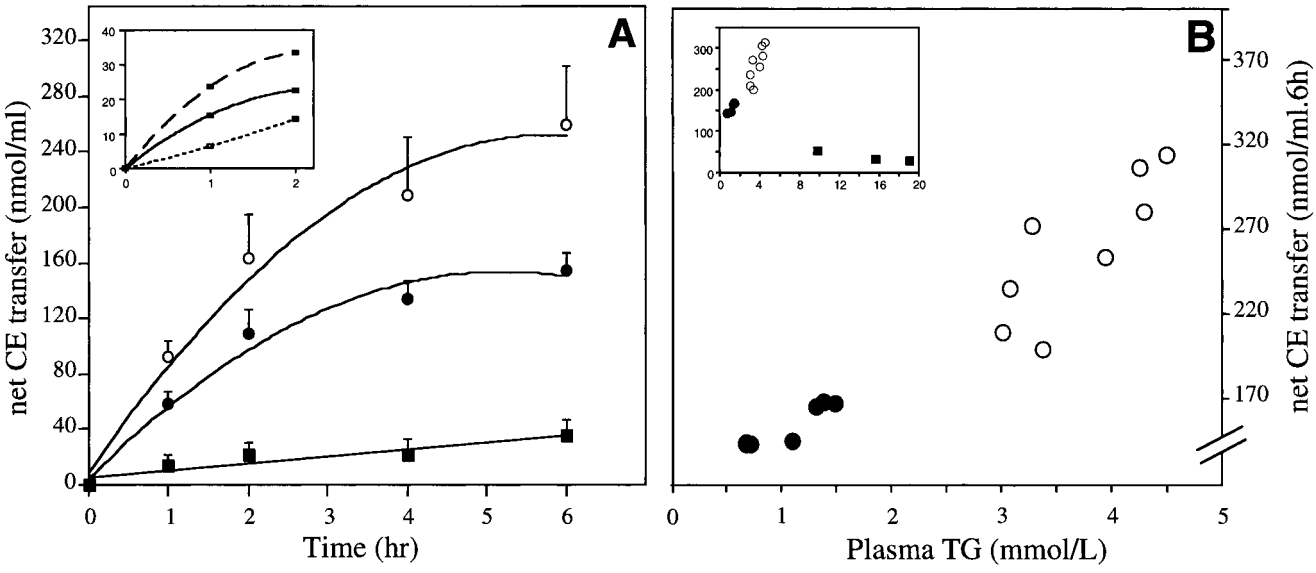

Fig. 1. A: Time course of net CE transfer from HDL to apoB-containing lipoproteins. Plasma samples from normolipidemic subjects (n = 8, ●), hypertriglyceridemic patients (n = 7, ○) and LPL-deficient patients (n = 3, ■) were incubated at 37°C in the presence of iodoacetate to inhibit LCAT activity. The inset shows the initial transfer velocities of each individual LPL-deficient patients (– – –, patient with Gly 188 Glu mutation; ·····, patient with G→A intron 1 mutation; ——, patient with anti-LPL autoantibody). B: Correlation between the net CE transfer measured after 6 h of incubation and plasma TG concentration in normolipidemic and hypertriglyceridemic subjects. The inset exhibits the same data at a reduced scale to show that the values of LPL-deficient patients were largely out of the correlation line.

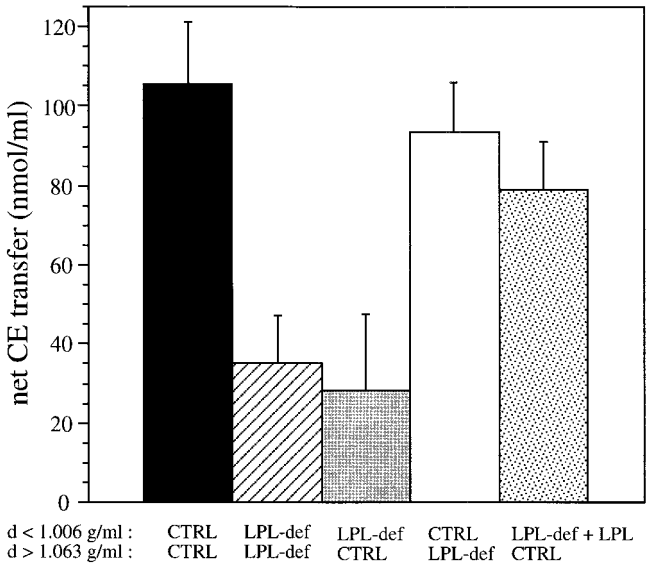

Fig. 2. Net CE transfer promoting capacity of VLDL ($d < 1.006$ g/ml) and HDL- and CETP-containing $d > 1.063$ g/ml plasma fraction of LPL-deficient patients or control subjects. After homologous or heterologous recombinations of plasma fractions, the samples were incubated for 90 min at 37°C. Net CE transfer was then measured as described in the Methods section. All recombination experiments were performed, using the same limiting concentration of VLDL (1.1 mm of TG). When desired (▨), the binding of bovine LPL to LPL-deficient VLDL was carried out during a pre-incubation at 4°C for 30 min. VLDL, re-isolated by precipitation, contained about one LPL molecule per 400 particles.

were used at a final TG concentration of 1.1 mm, which is a limiting acceptor concentration in CET measurements. The results clearly demonstrated that the HDL- and CETP-containing fractions induced no change in CET whether isolated from control or LPL-deficient plasma (**Fig. 2**). In contrast, when LPL-deficient VLDL was used as CE acceptor, CET decreased by about 70% by comparison with that obtained with control VLDL. When LPL-deficient VLDL was pretreated to contain physiological amounts of LPL (LPL/apoB molar ratio: 1/400), CET was almost completely normalized, reaching values not statistically different from those of controls (Fig. 2). Thus, the very low CET observed in intact plasma of LPL-deficient patients clearly resulted from an abnormal behavior of VLDL that could be normalized after binding of LPL.

To further investigate the mechanism of LPL effect, we used a radioisotopic assay designed to measure unidirectional CET from HDL to VLDL. VLDL, isolated from a patient with a complete LPL deficiency (homozygous for an intron 1 splicing defect) were used as acceptors, either directly or after pretreatment to contain about 1 LPL molecule per 400 VLDL particles. The presence of active LPL bound to the patient VLDL induced an unidirectional CET three times higher than that obtained with native patient VLDL (**Fig. 3A**). The measurements were also performed in the presence of E600 at a concentration that totally blocked LPL lipolytic activity without affecting its binding to VLDL (Fig. 3A, inset). In these conditions, the stimulating effect of the enzyme on CET clearly persisted

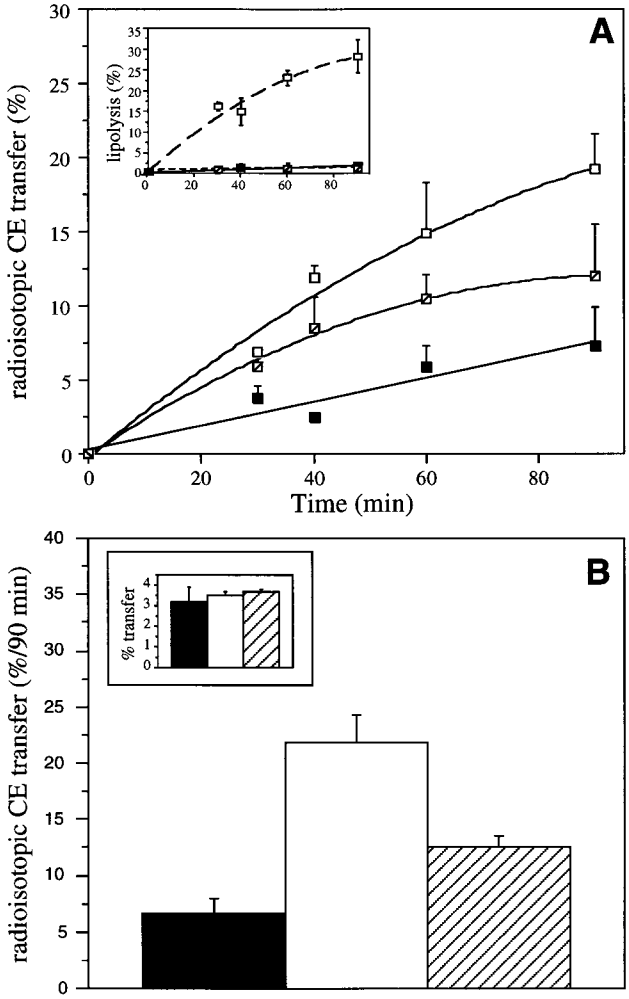

Fig. 3. A: Effect of VLDL-bound LPL on the time course of CETP-facilitated radioisotopic CE transfer from HDL to VLDL. Bovine LPL was bound to LPL-deficient VLDL (VLDL obtained from a patient with a complete LPL deficiency) as described in the legend of Fig. 2. When the inhibition of LPL lipolytic activity was desired, preincubations were performed in the presence of 2 mm E600. VLDL containing no LPL (■), active LPL (□), or inhibited LPL (▨), were then used as CE acceptors in experiments where the unidirectional transfer of radioactive CE from HDL to VLDL was measured in the presence of CETP-containing $d > 1.21$ g/ml plasma fraction. The inset shows the degree of lipolysis that was determined in all samples by measuring the time-course of apparition of free fatty acids. Binding of LPL was similar in all the experiments (one LPL per 408 ± 28 VLDL particles, mean ± SD). B: CETP-facilitated transfer obtained after 90 min of incubation when LPL-deficient VLDL were replaced by normal VLDL previously depleted of their endogenous LPL by ultracentrifugation. The inset shows that in the absence of CETP, the transfer values were similar, irrespective of the presence of active or inhibited LPL.

although it was significantly reduced by about 50%. Very similar results were obtained when naturally occuring LPL-deficient VLDL were replaced by normal VLDL previously cleared of endogenous LPL by ultracentrifugation (Fig. 3B). Thus, in the presence of normal lipoproteins, the stimulating action of VLDL-bound LPL on CET appeared to result from the combination of both lipolysis-dependent and lipolysis-independent effects. The latter

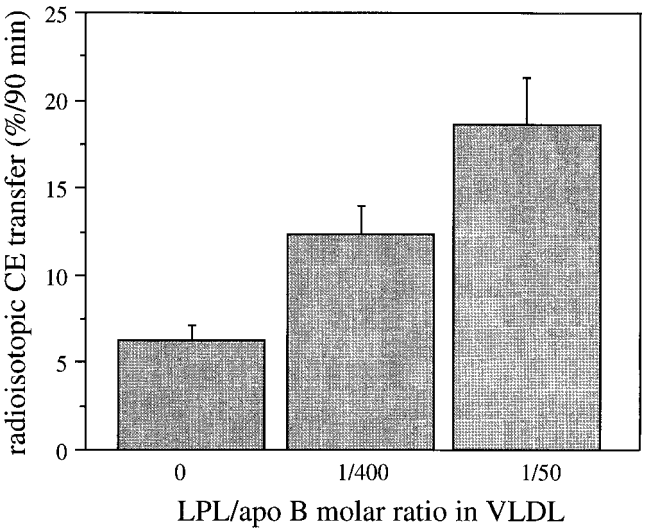

Fig. 4. Dose–response effect of VLDL-bound inhibited LPL on the CETP-facilitated radioisotopic CE transfer from HDL to VLDL, after 90 min of incubation. LPL was bound to VLDL during preincubations as described in the legend of Fig. 2. The lipolytic activity of LPL was inhibited by 2 mm E600, and the experimental conditions were chosen to obtain LPL/apoB molar ratios of 1/406 (±16) and 1/52 (±5), which are values approximately corresponding to normal and post-heparin plasma conditions, respectively (means of three independent experiments performed in duplicate).

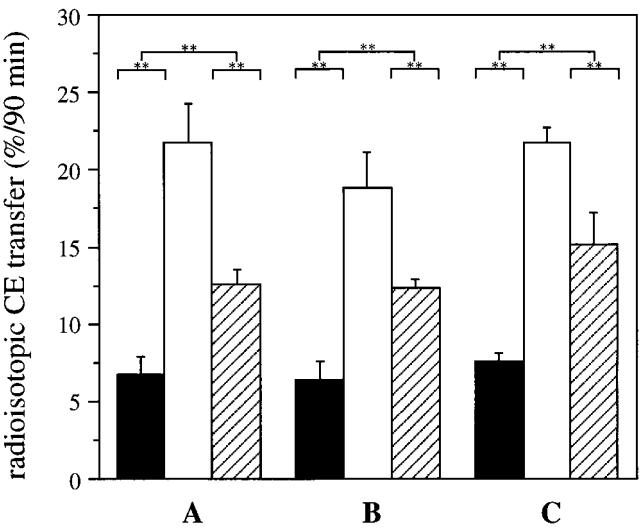

Fig. 5. Effects of two LPL inhibitors and two CETP sources on the CETP-facilitated radioisotopic CE transfer from HDL to VLDL, after 90 min of incubation. The CETP-facilitated CE transfers were measured in the absence (■) or in the presence of LPL either lipolytically active (□) or inhibited (▨). In A, the LPL lipolytic activity was inhibited by 2 mm E600 while a pool of delipidated plasma was used as the source of CETP. In B, E600 was replaced by THL at a THL/LPL molar ratio of 6, while in C, partially purified CETP was in addition used instead of delipidated plasma (same CETP activity). The values are shown as the mean ± SD of six independent experiments in duplicate. No statistical difference was found among either of the three conditions tested, while in each group of experiments the stimulating effect of LPL on the facilitated CE transfer was only reduced by about 50% after inhibition of the enzyme lipolytic activity (** $P < 0.0001$). In the absence of LPL, the transfer values respectively observed in the presence or in the absence of CETP (10.0 ± 1.1 and 4.2 ± 1.0%) were unaffected by either E600 (10.0 ± 0.2 and 4.4 ± 0.5%) or THL (10.2 ± 0.6 and 4.1 ± 0.6%), showing that these inhibitors had no unexpected effects.

enhanced when the LPL/apoB molar ratio in VLDL was increased from 1/400 to 1/50, values which approximately corresponded to normal and post-heparin plasma conditions, respectively (**Fig. 4**).

To determine whether monomeric LPL had any effect on CET, LPL preincubated in 0.75 m GuHCl was recombined with normal VLDL previously cleared of endogenous LPL. When these particles were used in CET assay, the lipolysis-dependent stimulating effect of LPL was largely decreased as compared to control conditions (19.1 ± 0.5 vs. 24.1 ± 0.5%, $P < 0.0001$), reaching values closed to those of the lipolysis-independent effect of LPL determined in the presence of E600 (17.0 ± 0.5%). Interestingly, the latter remained unaffected by LPL monomerization (16.2 ± 0.3%, NS). The molecular basis of LPL action was also studied by determining the effects of various concentrations of heparin on the LPL-stimulated CETP-dependent CET both in the absence or in the presence of E600. Even at a concentration of 10 IU/ml, heparin induced no change on the lipolysis-dependent (22.2 ± 1.9 vs. 23.9 ± 1.3%, NS) as well as the lipolysis-independent (15.4 ± 0.6 vs. 16.8 ± 1.8%, NS) effects of LPL.

To facilitate comparison between data, two methodological conditions were maintained in all the experiments described above. First a pool of delipidated plasma was used as the source of CETP, and second, when desired, the inhibition of LPL lipolytic activity was achieved by treatment with E600. To check that these conditions did not result in any artifactual data, we performed a series of control experiments (**Fig. 5**). No change in the data was noticed when THL was substituted for E600 to inhibit the enzyme lipolytic activity (A vs. B). Finally, when we compared the effects of LPL on CET, whether facilitated by delipidated plasma or by purified CETP (B vs. C), both the lipolysis-dependent and the lipolysis-independent stimulating effects of LPL were indistinguishable.

DISCUSSION

This work was undertaken to precisely determine the influence of VLDL-bound LPL on CET from HDL to apoB-containing lipoproteins. Our study in LPL-deficient patients confirms and extends the data recently reported by Bagdade et al. (17). In both cases, LPL-deficient patients exhibited a net mass CET that was more than 80% lower than that of common hypertriglyceridemic patients, even reaching values beneath those of control subjects.

In principle, besides CETP concentration, the magnitude of net CET may depend upon the concentration and composition of donor as well as acceptor lipoproteins. Plasma HDL concentrations of LPL-deficient patients were lowered. However, as judged from recombination experiments, the low level of net CET of these subjects could not be explained on the basis of CETP or HDL concentra-

tions. Rather, it appeared to be due to a defect in the VLDL fraction, although an elevated concentration of CE acceptor particles should have favored an increase of CET. Indeed, several studies have reported a positive correlation between CET and plasma TG concentration in various physiopathological situations, as predicted from the mass action law (5, 6, 18). Thus, the qualitative defect of VLDL induced by LPL deficiency was strong enough to overcome their putative positive concentration effect. The direct implication of LPL in this process was demonstrated by experiments exhibiting the normalization of CET after in vitro binding of physiological amounts of the enzyme either to LPL-deficient VLDL or to normal VLDL previously deprived of their endogenous LPL.

The experimental conditions described above suggest that circulating LPL is likely able to stimulate CET in physiological situations. In fact, the possibility of a stimulating effect of LPL on CET has been already considered for a long time, as net CET was reported to increase during lipolysis in the postprandial state in early 80s (10–12). In subsequent in vitro works performed by Tall and colleagues (13), it was shown that LPL might facilitate the CETP-dependent CET through the lipolysis-induced release of NEFA able to favor the binding of CETP to lipoproteins. The effect of NEFA on CETP has now been characterized in detail by others (14–16). While their reports confirmed an overall positive action of fatty acids on CET, they also showed that it was highly variable and in any case quantitatively moderate (14). On the basis of these data, one could not rule out the possibility that the profound lowering of net CET induced by LPL deficiency might not be entirely explained by the absence of LPL-dependent lipolysis. As it is now well established that a substantial amount of LPL is bound to circulating lipoproteins (20–24), we performed in vitro CET measurements, using VLDL that contained physiological amounts of bound LPL, whether lipolytically active or not (22, 23). The results unambiguously demonstrated that the stimulating action of LPL on CETP-mediated CET was only decreased by about 50% in conditions where lipolysis was totally blocked, either by E600 or by THL. One may notice that none of these inhibitors modified the binding of LPL to VLDL and that the stimulating effect of inactive LPL bound to VLDL was obtained at a concentration commonly reported in human plasma (22). Moreover, the dose response increase in facilitated CET suggest that the underlying mechanism might influence neutral lipid transfer in vivo (Fig. 4). From this view point, the LPL-deficient patient having no LPL protein should have a residual CET activity lower than that of patients whose LPL deficiency results from an inactive LPL protein. Consistent with this concept, our patient having a LPL gene-splicing defect was found to have the lowest CET velocity (Fig. 1A, inset) and the lowest HDL-TG content (Table 2). Although further studies involving more patients will be necessary to definitely establish the physiological relevance of our results, the present data strongly suggest that at physiological circulating concentrations, LPL may stimulate the CETP-facilitated CET by two different mechanisms. The first, well established, is lipolysis-dependent, while the second, previously unknown, is lipolysis-independent. The lipolysis-dependent effect of LPL was inhibited when VLDL-bound LPL was monomeric, which is consistent with the current concept that LPL dimer represents the catalytically active form of the enzyme (33). In contrast, LPL monomerization did not prevent the lipolysis-independent effect of LPL on CET, suggesting that it did not involve an LPL-mediated bridging between VLDL and HDL. This finding was confirmed by our CET experiments involving heparin as a bridging effect of LPL should have been affected by the latter, which was not the case.

The molecular basis of the lipolysis-independent LPL effect was not further studied in this work. One can, however, speculate that two different mechanisms, albeit non exclusive, are to be logically considered. Firstly, a protein–protein interaction between LPL and CETP might facilitate the binding of the latter to VLDL. Alternatively, the binding of LPL to VLDL might unstabilize the particle surface, thereby facilitating the lipid exchanges between VLDL and CETP (3) or putatively resulting in the displacement of the lipid transfer inhibitory protein recently identified as apoF (34).

Finally, our report describes a novel non-lipolytic function for lipoprotein-bound LPL that comes in addition to other functions recently described. Several works have indeed demonstrated that LPL may facilitate the interaction of different lipoproteins with various tissues by mechanisms involving heparan sulfate proteoglycans and/or membrane receptors (35–42). Thus LPL appears now clearly as a protein that exerts multiple functions in lipoprotein metabolism, functions that do not necessarily result from its lipolytic activity.

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REFERENCES

1. Tall, A. R. 1993. Plasma cholesteryl ester transfer protein. *J. Lipid Res.* **34:** 1255–1274.
2. Lagrost, L. 1994. Regulation of cholesteryl ester transfer protein (CETP) activity: review of in vitro and in vivo studies. *Biochim. Biophys. Acta.* **1215:** 209–236.
3. Bruce, C., R. A. Chouinard, Jr., and A. R. Tall. 1998. Plasma lipid transfer proteins, high-density lipoproteins, and reverse cholesterol transport. *Annu. Rev. Nutr.* **18:** 297–330.
4. Bagdade, J. D., M. C. Ritter, and P. V. Subbaiah. 1991. Accelerated cholesteryl ester transfer in plasma of patients with hypercholesterolemia. *J. Clin. Invest.* **87:** 1259–1265.
5. Bagdade, J. D., M. C. Ritter, and P. V. Subbaiah. 1991. Accelerated cholesteryl ester transfer in patients with insulin-dependent diabetes mellitus. *Eur. J. Clin. Invest.* **21:** 161–167.
6. Tall, A., E. Granot, R. Brocia, I. Tabas, C. Hesler, K. Williams, and M. Denke. 1987. Accelerated transfer of cholesteryl esters in dys-

lipidemic plasma. Role of cholesteryl ester transfer protein. *J. Clin. Invest.* **79:** 1217–1225.
7. Morton, R. E. 1988. Free cholesterol is a potent regulator of lipid transfer protein function. *J. Biol. Chem.* **263:** 12235–12241.
8. Quig, D. W., and D. B. Zilversmit. 1990. Plasma lipid transfer activities. *Annu. Rev. Nutr.* **10:** 169–193.
9. Sparks, D. L., and P. H. Pritchard. 1989. Transfer of cholesteryl ester into high density lipoprotein by cholesteryl ester transfer protein: effect of HDL lipid and apoprotein content. *J. Lipid Res.* **30:** 1491–1498.
10. Castro, G. R., and C. J. Fielding. 1985. Effects of post-prandial lipemia on plasma cholesterol metabolism. *J. Clin. Invest.* **75:** 874–882.
11. Tall, A., D. Sammet, and E. Granot. 1986. Mechanisms of enhanced cholesteryl ester transfer from high density lipoproteins to apolipoprotein B-containing lipoproteins during alimentary lipemia. *J. Clin. Invest.* **77:** 1163–1172.
12. Sammett, D., and A. R. Tall. 1985. Mechanisms of enhancement of cholesteryl ester transfer protein activity by lipolysis. *J. Biol. Chem.* **260:** 6687–6697.
13. Tall, A. R., D. Sammett, G. M. Vita, R. Deckelbaum, and T. Olivecrona. 1984. Lipoprotein lipase enhances the cholesteryl ester transfer protein-mediated transfer of cholesteryl esters from high density lipoproteins to very low density lipoproteins. *J. Biol. Chem.* **259:** 9587–9594.
14. Lagrost, L., E. Florentin, V. Guyard-Dangremon, A. Athias, H. Gandjin, C. Lallemant, and P. Gambert. 1995. Evidence for nonesterified fatty acids as modulators of neutral lipid transfers in normolipidic human plasma. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1388–1396.
15. Lagrost, L., and P. J. Barter. 1991. Effects of various non-esterified fatty acids on the transfer of cholesteryl esters from HDL to LDL induced by the cholesteryl ester transfer protein. *Biochim. Biophys. Acta.* **1085:** 209–216.
16. Barter, P. J., L. B. F. Chang, and O. V. Rajaram. 1990. Sodium oleate dissociates the heteroexchange of cholesteryl esters and triacylglycerol between HDL and triacylglycerol-rich lipoproteins. *Biochim. Biophys. Acta.* **1047:** 294–297.
17. Bagdade, J. D., M. C. Ritter, H. Lithell, D. Bassett, F. Mailly, P. Talmud, and M. R. Hayden. 1996. Reduced cholesteryl ester transfer in plasma of patients with lipoprotein lipase deficiency. *J. Lipid Res.* **37:** 1696–1703.
18. Mann, C. J., F. T. Yen, A. M. Grant, and B. E. Bihain. 1991. Mechanism of plasma cholesteryl ester transfer in hypertriglyceridemia. *J. Clin. Invest.* **88:** 2059–2066.
19. Olivecrona, T., and G. Bengtsson-Olivecrona. 1987. Lipoprotein lipase from milk: the model enzyme in lipoprotein lipase research. *In* Lipoprotein Lipase. J. Borensztajn, editor. Evener Publishers, Inc., Chicago, IL. 15–77.
20. Goldberg, I. J., J. J. Kandel, C. B. Blum, and H. N. Ginsberg. 1986. Association of plasma lipoproteins with postheparin lipase activities. *J. Clin. Invest.* **78:** 1523–1528.
21. Carrero, P., D. Gómez-Coronado, G. Olivecrona, and M. A. Lasunción. 1996. Binding of lipoprotein lipase to apolipoprotein B-containing lipoproteins. *Biochim. Biophys. Acta.* **1299:** 198–206.
22. Vilella, E., J. Joven, M. Fernández, S. Vilaró, J. D. Brunzell, T. Olivecrona, and G. Bengtsson-Olivecrona. 1993. Lipoprotein lipase in human plasma is mainly inactive and associated with cholesterol-rich lipoproteins. *J. Lipid Res.* **34:** 1555–1564.
23. Zambon, A., I. Schmidt, U. Beisiegel, and J. D. Brunzell. 1996. Dimeric lipoprotein lipase is bound to triglyceride-rich plasma lipoproteins. *J. Lipid Res.* **37:** 2394–2404.
24. Vilella, E., and J. Joven. 1991. Lipoprotein lipase binding to plasma lipoproteins. *Med. Sci. Res.* **19:** 111–112.
25. Pruneta, V., P. Moulin, F. Labrousse, P-J. Bondon, G. Ponsin, and F. Berthezène. 1997. Characterization of a new case of autoimmune Type I hyperlipidemia: long-term remission under immunosuppressive therapy. *J. Clin. Endocrinol. Metab.* **82:** 791–796.
26. Bolton, A. E., and W. M. Hunter. 1973. The labelling of proteins to high specific raioactivities by conjugation to a 125I-containing acylating agent. *Biochem. J.* **133:** 529–539.
27. Osborne, J. C., Jr., G. Bengtsson-Olivecrona, N. S. Lee, and T. Olivecrona. 1985. Studies on inactivation of lipoprotein lipase: role of dimer to monomer dissociation. *Biochemistry.* **24:** 5606–5611.
28. Elchebly, M., B. Porokhov, T. Pulcini, F. Berthezène, and G. Ponsin. 1996. Alterations in composition and concentration of lipoproteins and elevated cholesteryl ester transfer in non-insulin-dependent diabetes mellitus (NIDDM). *Atherosclerosis.* **123:** 93–101.
29. Lagrost, L., A. Athias, P. Gambert, and C. Lallemant. 1994. Comparative study of phospholipid tansfer activities mediated by cholesteryl ester transfer protein and phospholipid transfer protein. *J. Lipid Res.* **35:** 825–835.
30. Zambon, A., S. I. Hashimoto, and J. D. Brunzell. 1993. Analysis of techniques to obtain plasma for measurement of levels of free fatty acids. *J. Lipid Res.* **34:** 1021–1028.
31. Hadváry, P., W. Siedler, W. Meister, W. Vetter, and H. Wolfer. 1991. The lipase inhibitor tetrahydrolipstatin binds covalently to the putative active site serine of pancreatic lipase. *J. Biol. Chem.* **266:** 2021–2027.
32. Lookene, A., N. Skottova, and G. Olivecrona. 1994. Interactions of lipoprotein lipase with the active-site inhibitor tetrahydrolipstatin (Orlistat®). *Eur. J. Biochem.* **222:** 395–403.
33. Peterson, J., W. Y. Fujimoto, and J. D. Brunzell. 1992. Human lipoprotein lipase: relationship of activity, heparin affinity, and conformation as studied with monoclonal antibodies. *J. Lipid Res.* **33:** 1165–1170.
34. Xinxing W., D. M. Driscoll, and R. E. Morton. 1999. Molecular cloning and expression of lipid transfer inhibitor protein reveals its identity with apolipoprotein F. *J. Biol. Chem.* **274:** 1814–1820.
35. Obunike, J. C., I. J. Edwards, S. C. Rumsey, L. Curtiss, W. D. Wagner, R. J. Deckelbaum, and I. J. Goldberg. 1994. Cellular differences in lipoprotein lipase-mediated uptake of low density lipoproteins. *J. Biol. Chem.* **269:** 13129–13135.
36. Felts, J. M., H. Itakura, and R. T. Crane. 1975. The mechanism of assimilation of constituents of chylomicrons, very low density lipoproteins and remnants: A new theory. *Biochem. Biophys. Res. Commun.* **66:** 1467–1475.
37. Mulder, M., P. Lombardi, H. Jansen, T. J. C. van Berkel, R. R. Frants, and L. M. Havekes. 1993. Low density lipoprotein receptor internalizes low density lipoproteins and very low density lipoproteins that are bound to heparan sulfate proteoglycans via lipoprotein lipase. *J. Biol. Chem.* **268:** 9369–9375.
38. Beisiegel, U., W. Weber, and G. Bengtsson-Olivecrona. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc. Natl. Acad. Sci. USA.* **88:** 8342–8346.
39. Niemeier, A., M. Gafvels, J. Heeren, N. Meyer, B. Angelin, and U. Beisiegel. 1996. VLDL receptor mediates the uptake of human chylomicron remnants in vitro. *J. Lipid Res.* **37:** 1733–1742.
40. Saxena, U., M. G. Klein, T. M. Vanni, and I. J. Goldberg. 1992. Lipoprotein lipase increases low density lipoprotein retention by subendothelial cell matrix. *J. Clin. Invest.* **89:** 373–380.
41. Rutledge, J. C., and I. J. Goldberg. 1994. Lipoprotein lipase (LpL) affects low density lipoprotein (LDL) flux through vascular tissue: evidence that LpL increases LDL accumulation in vascular tissue. *J. Lipid Res.* **35:** 1152–1160.
42. Rinninger, F., T. Kaiser, W. A. Mann, N. Meyer, H. Greten, and U. Beisiegel. 1998. Lipoprotein lipase mediates an increase in the selective uptake of high density lipoprotein-associated cholesteryl esters by hepatic cells in culture. *J. Lipid Res.* **39:** 1335–1348.