



## Cholesteryl ester transfer activity in lipoprotein lipase deficiency and other primary hypertriglyceridemias

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### Abstract

Cholesteryl ester transfer protein (CETP) activity was measured in  $d > 1.21$  g/ml plasma from hypertriglyceridemic patients and compared with normolipidemic subjects. The assay consisted in measuring the specific transfer of [<sup>3</sup>H]cholesteryl oleate from a prelabelled, apo E-poor HDL fraction to VLDL after incubation at 37°C in the presence of the  $d > 1.21$  g/ml plasma sample: the lipoproteins were then separated by precipitation with dextran sulfate/Mg<sup>2+</sup> solution. Increasing the volume of  $d > 1.21$  g/ml plasma or purified human CETP in the assay produced linear responses in measured activity, whereas, either during incubation at 4°C or in the presence of rat plasma instead of human plasma, the transfer of [<sup>3</sup>H]cholesteryl oleate to VLDL was not stimulated. Thus, the assay reflects changes in CETP in the sample and appears to be suitable for measuring CETP activity in  $d > 1.21$  g/ml plasma. CETP activity was very similar in the two groups of normolipidemic subjects considered: adolescents ( $203 \pm 11$  nmol esterified cholesterol transferred per 8 h/ml plasma) and adults ( $215 \pm 5$ ). Patients were grouped into lipoprotein-lipase (LPL)-deficient and non-LPL-deficient according to their enzyme activity in postheparin plasma. CETP activity was highly increased in LPL-deficient, severe hyperchylomicronemic patients ( $430 \pm 42$ ) and was directly correlated with VLDL levels in the non-LPL-deficient individuals. Marked differences were observed in the lipid composition of HDL and apolipoprotein A-I levels among patients and controls. In the control group, CETP activity was correlated only with HDL-triglyceride and HDL-triglyceride/apo A-I mass ratio, which is compatible with the physiological role of CETP in transferring triglyceride to HDL from other lipoprotein particles. When all hyper-

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triglyceridemic patients were considered together, CETP activity was inversely correlated with apo A-I and HDL-cholesterol, whereas it was directly correlated with HDL-triglyceride/HDL-cholesterol and HDL-triglyceride/apo A-I mass ratios. The results indicate that the enhanced CETP activity associated with hypertriglyceridemia contributes to the compositional change of HDL, which in turn may be responsible for the reduction of HDL levels in this condition.

*Key words:* Cholesteryl ester transfer protein; Lipoprotein lipase deficiency; Hyperchylomicronemia; Hypertriglyceridemia; HDL; VLDL

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## 1. Introduction

One of the processes that delineates the highly dynamic metabolism of lipoproteins in human plasma is the exchange of neutral lipids between lipoproteins, mediated by the cholesteryl ester transfer protein (CETP). This process consists of the heteroexchange of one molecule of triglyceride for one of esterified cholesterol between VLDL and HDL [1,2]. The other lipoprotein classes, namely chylomicrons and LDL, may also participate in this process [3,4] and a homoexchange of esterified cholesterol between lipoproteins [5] may also occur. As a result of the action of CETP, cholesteryl esters formed in HDL by the action of LCAT are transferred to VLDL. This lipoprotein becomes progressively enriched in cholesteryl ester while HDL becomes richer in triglyceride [6,7]. In this way, CETP participates in the reverse cholesterol transport, by diverting cholesteryl esters to VLDL (and chylomicrons) that can be taken up by the liver when these lipoproteins have been transformed to their respective remnants or LDL.

Recent studies have shown that every HDL subfraction may act as the donor particle for the CETP reaction [8] and, similarly, every VLDL subfraction is the acceptor of cholesteryl esters [3], even though differences in their maximum capacity can be distinguished among these subfractions [3,8]. The net transfer of neutral lipids depends on the CETP mass or activity, as well as on the amount and relative proportion of donor to acceptor lipoproteins [8-10]. Species which lack active CETP (i.e. rat, pig) [11,12] and human CETP-deficient subjects [13,14] have elevated HDL-cholesterol levels, whereas the administration of human CETP to rats [15] or the expression of CETP in transgenic mice [16,17] results in a reduction of HDL-cholesterol, illustrating the physiological role of CETP.

Little is known about the regulation of CETP expression although cholesteryl ester transfer activity has been observed to be altered in different pathological states. Secretion of neutral lipid transfer protein by human CaCo-2 enterocytes is enhanced by the presence of fatty acids in the luminal side [18], but this factor does not affect CETP secretion in hepatocytes [18]. Cholesterol feeding in the rabbit also produces a significant rise in liver CETP mRNA levels [19], which is in accordance with the increased plasma CETP in the hypercholesterolemic rabbit [19-21]. In humans, CETP mass and activity are also increased in primary hypercholesterolemia and dysbetalipoproteinemia [20,22,23]. On the other hand, the relationship between CETP and triglyceride levels is not clear, since CETP mass is highly increased in hyperchylomicronemic patients but is normal in moderately hypertriglyceridemic subjects [23]. As for secondary dyslipidemic states, a defective cholesteryl ester trans-

fer activity in non-insulin dependent diabetics was first found [24,25]; however, more recently this transfer activity has been observed to be accelerated in insulin-dependent diabetics [26,27], probably due to the presence of altered VLDL which favors this process rather than a change in CETP mass [27]. On the contrary, cholesteryl ester transfer activity is decreased in hypothyroidism, which appears to contribute to the elevation of HDL-cholesterol concentration observed in this condition [28]. Finally, in normolipidemic subjects, by use of monoclonal antibodies it has recently been found that CETP mass is positively correlated with HDL-cholesterol, HDL-triglyceride, apo A-I and also apo E [29].

In the present work we were interested in determining CETP activity in several hypertriglyceridemic states in the human, in whom there is a great imbalance between the plasma concentration of donor and acceptor lipoprotein particles. To avoid the interference of endogenous lipoproteins, the CETP assay was performed in the  $d > 1.21$  g/ml plasma fraction, by measuring the transfer of [ $^3$ H]cholesteryl oleate from prelabelled-HDL to exogenous VLDL.

## 2. Patients and methods

### 2.1. Human subjects

Four patients with familial lipoprotein lipase (LPL) deficiency and 15 patients with primary hypertriglyceridemia, non-deficient in lipoprotein lipase, were studied. The patients were attending the Servicio de Pediatría or Servicio de Dietética y Nutrición Clínica, Hospital Ramón y Cajal, Madrid. The patients with LPL deficiency (2 female, 2 male, aged 3–16 years) had been previously diagnosed with hyperchylomicronemia and a clinical history compatible with type I hyperlipidemia (i.e. abdominal pain, pancreatitis, eruptive xanthomata). The molecular defect of LPL in two of these patients has been published elsewhere [30]. Patients with primary hypertriglyceridemia (11 male, 4 female, aged 21–65 years) were also previously diagnosed by routine analyses and selected according to the criterion of serum triglyceride concentrations greater than 500 mg/dl. None had elevated fasting glucose levels, hypothyroidism, overt obesity, or other known causes of secondary hyperlipidemia, nor clinical signs of cardiovascular disease. The patients received dietary recommendations and were referred to the Unidad de Dislipemias for thorough lipid analyses, while not taking any lipid lowering medication. The adult control group consisted of 71 (44 female, 27 male) normolipidemic healthy volunteers from the medical center personnel, aged 18–65 years. The child control group consisted of 16 (8 male, 8 female) normolipidemic subjects, aged 7–14 years, who came to this Unit for a family study of dyslipidemia. All the patients and controls gave informed consent. After an overnight fast, two blood samples were collected: one in basal conditions and the other 10 min after administration of heparin (50 IU/kg body wt., i.v.). Blood was collected in Na<sub>2</sub>EDTA-containing tubes (1 mg/ml), cooled in ice and the plasma was separated promptly by low-speed centrifugation.

### 2.2. Isolation of lipoproteins

Lipoproteins were isolated from a 10-ml plasma aliquot by sequential ultracentrifugation with a Beckman 50 Ti rotor in a Beckman L5–50 ultracentrifuge

(Beckman Instruments, Palo Alto, CA), at the following density ranges: VLDL,  $d < 1.006$  g/ml; LDL,  $d = 1.006$ – $1.063$  g/ml; HDL,  $d = 1.063$ – $1.21$  g/ml. The infranant of  $d < 1.21$  g/ml (lipoprotein-free plasma) was used for cholesteryl ester transfer assay. The lipoprotein-containing supernatants were used for lipid analysis without any further manipulation. The CETP-containing fraction was exhaustively dialyzed against 0.15 mol/l NaCl, 1 mmol/l Na<sub>2</sub>EDTA, brought to the initial plasma volume and stored at  $-70^{\circ}\text{C}$  until processing.

### 2.3. CETP activity assay

CETP was determined in lipoprotein-free plasma as the activity which transfers [<sup>3</sup>H]cholesteryl oleate from prelabelled HDL to exogenous VLDL. For this, VLDL and HDL were isolated from pooled human serum by ultracentrifugation as above and the HDL was labelled with [1,2,6,7-<sup>3</sup>H]cholesteryl oleate (New England Nuclear, Bad Homburg, Germany) by incubation for 15 h at  $37^{\circ}\text{C}$ , in the presence of lipoprotein-deficient human serum, as previously described [8]. The <sup>3</sup>H-HDL was re-isolated by ultracentrifugation and then subjected to heparin-Sepharose CL-6B (Pharmacia Fine Chemicals, Uppsala, Sweden) chromatography as described [8]. The tubes corresponding to the non-bound fraction were collected, pooled and dialyzed against 0.15 mol/l NaCl, 1 mmol/l Na<sub>2</sub>EDTA. This fraction, called HDL-A, was chemically characterized elsewhere [8,31] and contains apo E-poor lipoprotein particles. This <sup>3</sup>H-HDL-A preparation was finally passed through 0.45  $\mu\text{m}$ -pore-size filters, kept at  $4^{\circ}\text{C}$  and used within 1 month.

For the assay, 20  $\mu\text{l}$  of sample (lipoprotein-free plasma) was mixed with 150  $\mu\text{l}$  of saline (0.15 mol/l NaCl), 25  $\mu\text{l}$  of Tris 0.25 mol/l containing dithionitrobenzoic acid 18 mmol/l, 50  $\mu\text{l}$  of VLDL (153  $\mu\text{g}$  triglyceride) and 50  $\mu\text{l}$  of <sup>3</sup>H-HDL-A (13.5  $\mu\text{g}$  esterified cholesterol, radioactivity  $> 70,000$  dpm). In some tubes, VLDL was omitted and substituted by an equal volume of 0.15 mol/l NaCl. The tubes were incubated in a shaking bath at  $37^{\circ}\text{C}$ , 60 cycles per min, for 8 h. For control, other tubes were not incubated but immediately processed after the addition of <sup>3</sup>H-HDL-A. For the isolation of the newly formed <sup>3</sup>H-VLDL, the tubes were placed in ice, 200  $\mu\text{l}$  of human serum were added to each tube and then 50  $\mu\text{l}$  of a solution containing 10 g/l dextran sulfate (MW 50,000, from Sochibo, Vélizy Villaloubly, France) and 0.32 mol/l MgCl<sub>2</sub>. The tubes were mixed by vortex, incubated for 15 min at room temperature and centrifuged at  $1,500 \times g$  for 30 min. The clear supernatant, containing no VLDL, was transferred carefully to scintillation vials, mixed with OptiPhase HiSafe II cocktail (Pharmacia LKB Biotechnology) and used for <sup>3</sup>H-radioactivity counting in a Beckman 3800 liquid-scintillation counter. In the tubes containing no VLDL, as well in those not incubated or containing rat, instead of human, lipoprotein-deficient plasma, radioactivity in the supernatant was always greater than 80% of total added. Radioactivity incorporated into VLDL was calculated as the difference between the value obtained in the supernatant of the tube that contained no VLDL and that containing VLDL. Cholesteryl ester transfer activity was expressed as nmol cholesteryl ester transferred to VLDL per ml plasma, correcting for specific radioactivity of <sup>3</sup>H-HDL-A. As internal control we used both a concentrated (1:2.5 vs. initial volume) lipoprotein-deficient fraction that was obtained from pooled human sera, with an activity of  $575 \pm 4$  nmol esterified cho-

lesterol per 8 h/ml (mean  $\pm$  standard error) and a lipoprotein-deficient fraction, non-concentrated, from a normal subject ( $193 \pm 6$  nmol esterified cholesterol per 8 h/ml). The interassay coefficients of variation (C.V.) were 6.2% ( $n = 16$ ) with the former and 9.1% ( $n = 9$ ) with the latter sample.

In other instances, the lipoprotein-deficient fraction was from pooled plasmas from male rats. CETP was partially purified from the  $d = 1.21$ – $1.24$  g/ml fraction from pooled human sera as described [32]. Basically, 44 ml of that fraction, coming from 1 l of pooled human sera approximately, were loaded directly, without prior dialysis, into a  $2.5 \times 30$  cm phenyl-Sepharose CL-4B column. After the bulk of the protein was eluted with 0.15 mol/l NaCl, 10 mmol/l Tris, 1 mmol/l Na<sub>2</sub>EDTA (pH 7.4), the transfer activity was eluted with distilled water; the tubes with the highest 280-nm absorbances were pooled, exhaustively dialyzed against 0.15 mol/l NaCl, 1 mmol/l Na<sub>2</sub>EDTA and frozen in aliquots until used. CETP activity recovered in this fraction was approximately 68% of that present in the  $d = 1.21$ – $1.24$  g/ml plasma fraction.

#### 2.4. Lipid analysis and lipoprotein lipase assay

Lipids were measured in whole plasma and in the lipoprotein fractions separated by ultracentrifugation. Total cholesterol, free cholesterol, triglyceride and phosphatidylcholine were measured enzymatically in a Technicon RA-1000 autoanalyzer (Technicon Ltd., Dublin, Ireland). Esterified cholesterol was calculated as the difference between total and free cholesterol. Glycerol was measured in deproteinized plasma [33] and this value was subtracted from those of triglyceride in total plasma and in lipoprotein fractions, taking into account the dilution of plasma through the ultracentrifugation procedure. For apolipoprotein analysis, VLDL were delipidated and applied to electrofocusing gels, pH 4–6.5 (as in Ref. 34). Apo A-I was determined by means of the Array Beckman nephelometer (Beckman Instruments, Palo Alto, CA) using the respective reagent kit.

Hepatic lipase activity in postheparin plasma was determined according to Huttunen et al. [35]. Lipoprotein lipase activity was measured in post-heparin plasma with human VLDL prelabelled with [<sup>3</sup>H]triolein as substrate. For labelling, 200  $\mu$ Ci of glycerol tri(8,9n-H<sup>3</sup>)oleate (New England Nuclear, Bad Homburg, Germany) dissolved in 200  $\mu$ l of dimethylsulfoxide were injected into 50 ml of  $d > 1.006$  g/ml human plasma fraction and, after incubation at 37°C for 1 h, VLDL from 500 ml of human plasma was added. The mixture was incubated at 37°C for 17 h in a shaking bath at 60 cycles per min and the <sup>3</sup>H-labelled VLDL was isolated by ultracentrifugation at  $d = 1.006$  g/ml as above. Recovery of the radioactivity in VLDL was greater than 10% in every case. The <sup>3</sup>H-VLDL was thoroughly dialyzed against 0.15 mol/l NaCl, 1 mmol/l Na<sub>2</sub>EDTA, frozen in aliquots and used within 1 month. For the assay, 10  $\mu$ l of sample (postheparin plasma) was mixed with 50  $\mu$ l of <sup>3</sup>H-VLDL (final concentration, 1.5 mmol/l VLDL-triglyceride, radioactivity >60,000 dpm), 100  $\mu$ l of 0.2 mol/l Tris-HCl (pH 8.2) containing 0.15 mol/l NaCl and 18% fatty acid-free bovine serum albumin and 50  $\mu$ l of saline. In other tubes, to inhibit LPL, saline was substituted by 4.2 mol/l NaCl (final concentration, 1 mol/l). The tubes were incubated for 30 min in a shaker bath at 37°C, 60 cycles per min and the <sup>3</sup>H-free fatty acids formed were extracted and the radioactivity

counted as described [36]. LPL activity was calculated as the difference of total  $^3\text{H}$ -VLDL hydrolysis and that observed in the presence of 1 mol/l NaCl and expressed in pKat of triglyceride hydrolyzed/ml plasma by considering the specific radioactivity of  $^3\text{H}$ -VLDL and the amount of triglyceride present in the sample. A pool of postheparin human plasmas was used as internal control (interassay C.V., 4.2%).

### 2.5. Statistical analysis

Statistical analyses were performed by one-way analysis of variance and multiple range analysis (Newman-Keuls test). Linear regressions between two variables were estimated by using Pearson's correlation coefficient ( $r$ ). All calculations used the Statgraphics statistical package (Statistical Graphics Corporation, STSC Inc., Rockville, MD).

## 3. Results

### 3.1. CETP activity assay

Figure 1 shows the time course of radioactivity in the supernate during incubation of  $^3\text{H}$ -HDL-A at 37°C in the absence or presence of VLDL and lipoprotein-deficient plasma as source of CETP. In the absence of lipoprotein-deficient plasma, either in the absence or in the presence of VLDL, most of the radioactivity remained in the supernate and remained constant for up to 24 h incubation. After incubation in the presence of lipoprotein-deficient plasma it remained mostly unaffected when no VLDL was present. On the contrary, in the presence of VLDL, radioactivity in the supernate disappeared as incubation progressed. This phenomenon occurred at 37°C but not at 4°C and the magnitude of this change depended on the VLDL concentration (data not shown). With 10  $\mu\text{l}$  of the concentrated lipoprotein-deficient plasma, the decrease in radioactivity in supernate was linear up to 24 h incubation. With 25  $\mu\text{l}$  of sample, the decrease was exponential (Fig. 1).

The results corresponding to incubations up to 16 h were plotted against the volume of lipoprotein-deficient plasma in Fig. 2. Radioactivity in supernate decreased linearly as a function of the volume of sample added when assayed at 8 h incubation. Shorter incubation (2 h) resulted in small decreases of radioactivity, whereas incubation for 16 h produced greater changes but the assay was not satisfactorily linear (Fig. 2). Taking into account the transfer activity of this pooled lipoprotein-deficient plasma, the assay was estimated to be linear for activities per tube of up to 14.5 nmol esterified cholesterol transferred per 8 h. Therefore, an incubation of 8 h and a sample volume of 20  $\mu\text{l}$  were chosen.

To study further the specificity of the assay, both a partially purified human CETP preparation and rat lipoprotein-deficient plasma were incubated with  $^3\text{H}$ -HDL-A and VLDL as before. Purified CETP produced a pronounced decrease in the radioactivity in the supernate when VLDL was present in the media (Fig. 3) whereas rat lipoprotein-deficient plasma did not produce this effect; rather, supplementation of purified CETP with rat lipoprotein-deficient plasma did not modify the effect of CETP alone (Fig. 3). These results are in accordance with zero or extremely low CETP activity in rat plasma [11] and demonstrate the specificity of our assay for CETP activity.

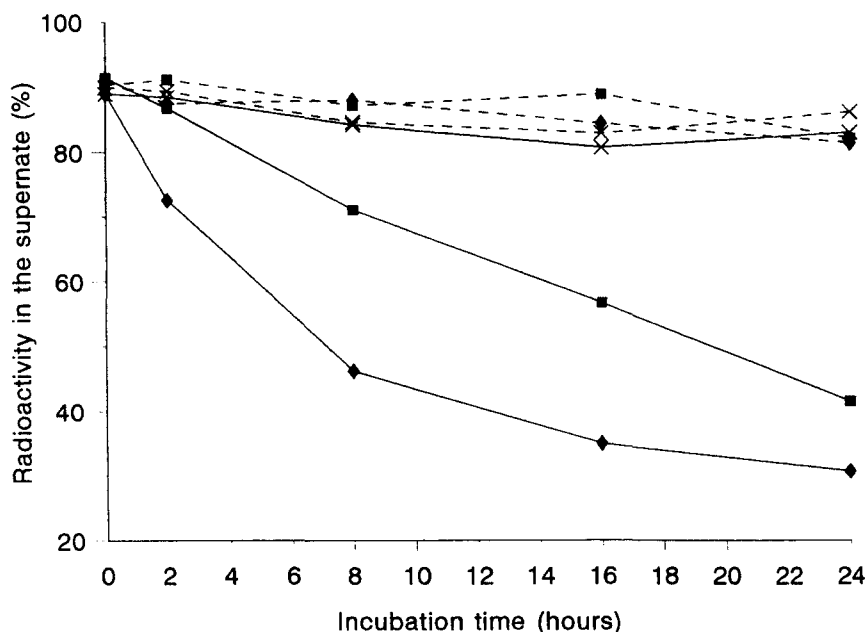


Fig. 1. Effect of incubation at 37°C on the transfer of [<sup>3</sup>H]cholesteryl oleate from prelabelled HDL to VLDL by  $d > 1.21$  g/ml human plasma. Human  $d > 1.21$  g/ml plasma (concentrated 1:2.5 vs. initial volume) was mixed with 50  $\mu$ l of <sup>3</sup>H-HDL-A (13.5  $\mu$ g esterified cholesterol, radioactivity > 70,000 dpm), 25  $\mu$ l of Tris 0.25 mol/l and either 0 (dashed lines) or 153  $\mu$ g (continuous lines) of VLDL-triglyceride in a final volume of 300  $\mu$ l (0.15 mol/l NaCl, final concentration). After incubation at 37°C for the times shown, 200  $\mu$ l of human serum and 50  $\mu$ l of dextran sulfate (10 g/l), MgCl<sub>2</sub> (0.32 mol/l) were added to each tube to precipitate VLDL. The radioactivity in the supernate was counted and was expressed as percentage of initial radioactivity in the tube. Sample volume: +, 0  $\mu$ l; ■, 10  $\mu$ l; ◆, 25  $\mu$ l. Data correspond to the mean of duplicates from a representative experiment out of two.

We found that VLDL itself did not affect the extent of radioactivity precipitation in samples not incubated (Fig. 1, time 0). Therefore, the decrease of <sup>3</sup>H-radioactivity in the supernate of incubated samples reflects the transfer of [<sup>3</sup>H]cholesteryl oleate to VLDL by the action of CETP present in human plasma. On the basis of these results, CETP activity in samples from patients was calculated as the radioactivity in the supernate observed in the absence of VLDL, minus that in the presence of VLDL and corrected by the specific activity of the <sup>3</sup>H-HDL-A. We used this assay to measure the CETP activity in plasma from different hyperlipidemic patients.

### 3.2. CETP transfer activity in hyperlipidemic patients and controls

Table 1 shows the plasma lipid levels and related parameters in the LPL-deficient subjects and a control group of children. All the patients showed intense hypertriglyceridemia, with very elevated triglyceride content in every lipoprotein and diminished cholesterol in HDL and LDL. As demonstrated by agarose electrophoresis, chylomicrons were the most abundant lipoprotein in their plasma (data

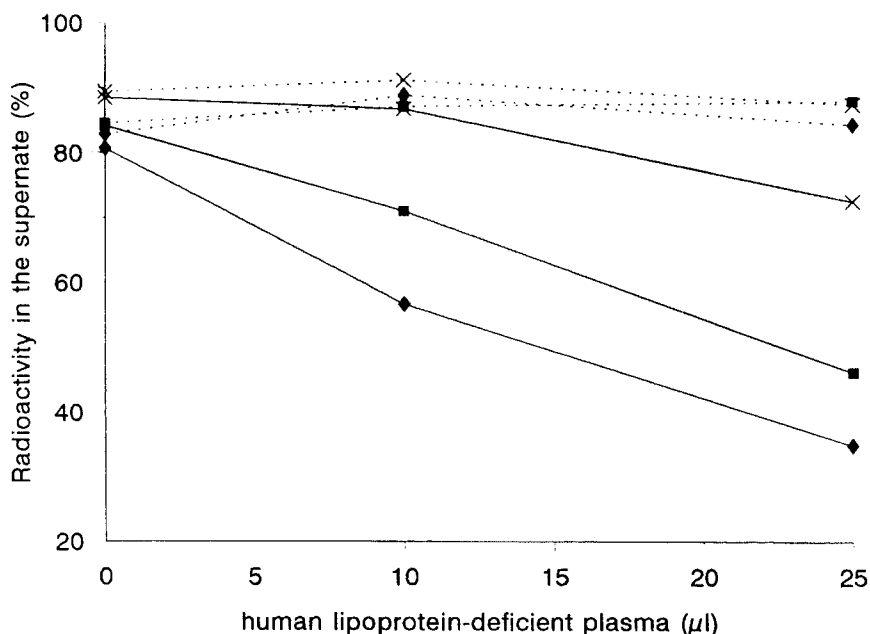


Fig. 2. Linearity of the transfer of [ $^3\text{H}$ ]cholesteryl oleate from prelabelled HDL to VLDL by  $d > 1.21$  g/ml human plasma. Human  $d > 1.21$  g/ml plasma (concentrated 1:2.5 vs. initial volume) was mixed with 50  $\mu\text{l}$  of  $^3\text{H}$ -HDL-A (13.5  $\mu\text{g}$  esterified cholesterol, radioactivity  $> 70,000$  dpm), 25  $\mu\text{l}$  of Tris 0.25 mol/l and either 0 (dotted lines) or 153  $\mu\text{g}$  (continuous lines) of VLDL-triglyceride in a final volume of 300  $\mu\text{l}$  (0.15 mol/l NaCl, final concentration). After incubation at 37°C for 2 (+), 8 (■) or 16 h (◆), 200  $\mu\text{l}$  of human serum and 50  $\mu\text{l}$  of dextran sulfate (10 g/l),  $\text{MgCl}_2$  (0.32 mol/l) were added to each tube to precipitate VLDL. The radioactivity in the supernate was counted and was expressed as percentage of initial radioactivity in the tube. Data correspond to the mean of duplicates from a representative experiment out of two.

not shown) and all of them could be diagnosed as having type I hyperlipoproteinemia (hyperchylomicronemia). LPL activity in post-heparin plasma was undetectable in three of the patients and was extremely low in the other (23 pKat/ml). Hepatic lipase activity was slightly but not significantly reduced compared with controls. All the subjects had apo C-II as demonstrated by electrofocusing of apo-VLDL (data not shown). CETP activity was very elevated in all the cases, the mean value being two times higher than in controls (Table 1).

The hypertriglyceridemic group consisted of 15 patients with primary hyperlipidemia who were selected according to the criteria of having serum triglyceride concentration greater than 500 mg/dl in a previous analysis. The apo E phenotypes were: 8 E3/E3, 3 E3/E2, 3 E4/E4 and 1 E4/E3. All had elevated VLDL-triglyceride and four had some chylomicrons as evidenced by agarose electrophoresis (data not shown). LDL-cholesterol was slightly but not significantly reduced compared with controls, whereas LDL-triglyceride was elevated. HDL-cholesterol was moderately diminished and HDL-triglyceride was elevated in every case. None had LPL deficiency; LPL and hepatic lipase activities were normal (Table 1). In this group CETP



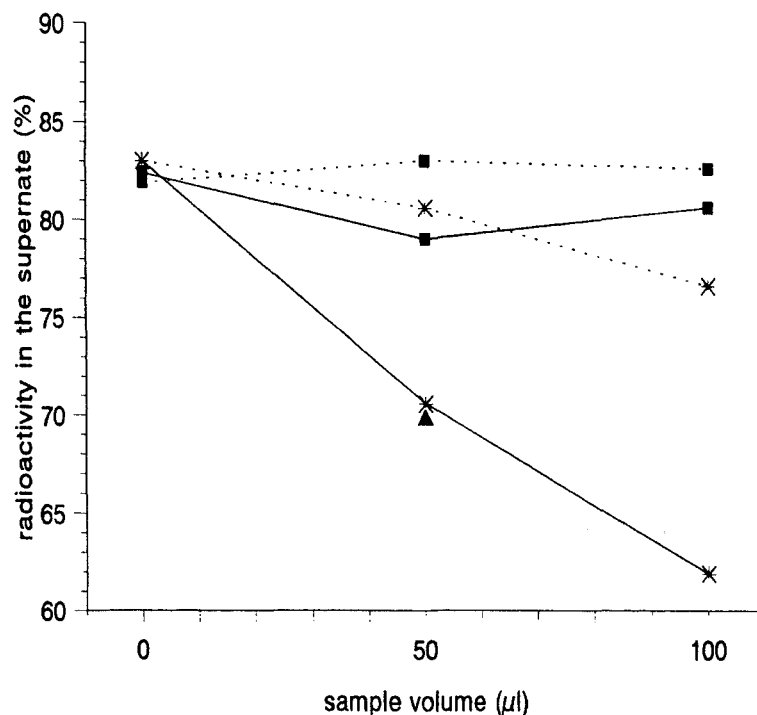


Fig. 3. Effect of purified human CETP and rat  $d > 1.21$  g/ml plasma on the transfer of [ $^3$ H]cholesteryl oleate from prelabelled HDL to VLDL. Purified CETP from human  $d = 1.21$ – $1.24$  g/ml plasma (\*) or rat  $d > 1.21$  g/ml plasma (■) were mixed with  $50 \mu\text{l}$  of  $^3\text{H}$ -HDL-A ( $9 \mu\text{g}$  esterified cholesterol, radioactivity  $> 55,000$  dpm),  $25 \mu\text{l}$  of Tris  $0.25$  mol/l and either  $0$  (dotted lines) or  $150 \mu\text{g}$  (continuous lines) of VLDL-triglyceride, in a final volume of  $300 \mu\text{l}$  ( $0.15$  mol/l NaCl, final concentration). ▲, corresponds to  $50 \mu\text{l}$  of purified CETP plus  $50 \mu\text{l}$  of rat  $d > 1.21$  g/ml plasma. After incubation at  $37^\circ\text{C}$  for  $8$  h,  $200 \mu\text{l}$  of human serum and  $50 \mu\text{l}$  of dextran sulfate ( $10$  g/l),  $\text{MgCl}_2$  ( $0.32$  mol/l) were added to each tube to precipitate VLDL. The radioactivity in the supernate was counted and was expressed as percentage of initial radioactivity in the tube. Data correspond to the mean of duplicates.

activity ranged from 126 to 333, with a mean value of 215, which was statistically lower than in LPL-deficient patients but similar to controls. No significant differences in lipoprotein levels, lipolytic enzymes or CETP activity between adolescent controls and adult controls were found (Table 1). When separated by sex, CETP activity in men was very similar to that in women ( $214 \pm 7$  vs.  $215 \pm 8$ , not significant).

Major alterations in HDL composition could be detected in both patient groups (Table 2). In correlation with HDL-cholesterol levels, apo A-I plasma concentration was slightly diminished in the hypertriglyceridemic patient group compared with controls and more significantly in the LPL-deficient patients. Apo A-I/HDL-cholesterol mass ratio was significantly higher in LPL-deficient patients than in controls, whereas the ratios A-I/HDL-triglyceride, A-I/HDL-phospholipid and HDL-esterified cholesterol/HDL-free cholesterol were significantly lower (Table 2). In the

Table 1  
Plasma lipids, lipolytic activities and cholesteryl ester transfer activity in lipoprotein lipase-deficient patients, other hypertriglyceridemic subjects and controls

	Lipoprotein lipase-deficient (n = 4)	Hypertriglyceridemic (n = 15)	Adolescent controls (n = 16)	Adult controls (n = 71)
Total triglyceride <sup>1</sup>	5232 ± 302 <sup>b</sup>	1185 ± 199 <sup>c</sup>	56 ± 6 <sup>a</sup>	88 ± 6 <sup>a</sup>
Total cholesterol	54 ± 26 <sup>b</sup>	285 ± 16 <sup>c</sup>	200 ± 10 <sup>a</sup>	203 ± 5 <sup>a</sup>
VLDL-triglyceride	4966 ± 383 <sup>b</sup>	1050 ± 195 <sup>c</sup>	27 ± 6 <sup>a</sup>	51 ± 5 <sup>a</sup>
VLDL-cholesterol	507 ± 27 <sup>b</sup>	139 ± 17 <sup>c</sup>	4 ± 1 <sup>a</sup>	9 ± 1 <sup>a</sup>
LDL-triglyceride	19 ± 2 <sup>a</sup>	45 ± 11 <sup>b</sup>	14 ± 1 <sup>a</sup>	22 ± 1 <sup>a</sup>
LDL-cholesterol	7 ± 1 <sup>b</sup>	83 ± 12 <sup>a</sup>	117 ± 9 <sup>a</sup>	122 ± 4 <sup>a</sup>
HDL-triglyceride	24 ± 1 <sup>b</sup>	22 ± 2 <sup>b</sup>	7 ± 1 <sup>a</sup>	9 ± 1 <sup>a</sup>
HDL-cholesterol	9 ± 1 <sup>b</sup>	30 ± 3 <sup>c</sup>	59 ± 3 <sup>a</sup>	57 ± 2 <sup>a</sup>
LPL activity <sup>2</sup>	7 ± 5 <sup>b</sup>	526 ± 76 <sup>a</sup>	417 ± 50 <sup>a</sup>	413 ± 35 <sup>a</sup>
HL activity <sup>2</sup>	692 ± 130 <sup>a</sup>	774 ± 167 <sup>a</sup>	869 ± 163 <sup>a</sup>	944 ± 67 <sup>a</sup>
CETP-activity <sup>3</sup>	430 ± 42 <sup>b</sup>	215 ± 18 <sup>a</sup>	203 ± 11 <sup>a</sup>	215 ± 5 <sup>a</sup>

Statistical comparisons between groups by ANOVA and Newman Keuls: different letters denote statistically significant differences ( $P < 0.05$ ).

<sup>1</sup>mg/dl plasma.

<sup>2</sup>pKat/ml postheparin plasma.

<sup>3</sup>nmol cholesteryl ester transferred per 8 h/ml plasma.

**Table 2**  
 Apoprotein A-I plasma levels and composition of HDL separated by ultracentrifugation in lipoprotein lipase-deficient patients, other hypertriglyceridemia subjects and controls

	Lipoprotein lipase-deficient (n = 4)	Hypertriglyceridemic (n = 15)	Adolescent controls (n = 16)	Adult controls (n = 71)
Plasma apo A-I <sup>1</sup>	37 ± 7 <sup>b</sup>	97 ± 11 <sup>c</sup>	141 ± 7 <sup>a</sup>	153 ± 4 <sup>a</sup>
A-I/HDL-cholesterol <sup>2</sup>	4.3 ± 0.8 <sup>b</sup>	3.3 ± 0.3 <sup>a</sup>	2.4 ± 0.1 <sup>a</sup>	2.8 ± 0.1 <sup>a</sup>
A-I/HDL-triglyceride	1.6 ± 0.3 <sup>b</sup>	5.6 ± 1.3 <sup>b</sup>	23.8 ± 2.3 <sup>a</sup>	19.5 ± 0.1 <sup>a</sup>
A-I/HDL-phospholipid	1.2 ± 0.2 <sup>b</sup>	1.6 ± 0.2 <sup>a</sup>	1.5 ± 0.1 <sup>a</sup>	1.6 ± 0.1 <sup>a</sup>
HDL-esterified cholesterol/HDL-free cholesterol	0.8 ± 0.2 <sup>b</sup>	3.3 ± 0.4 <sup>a</sup>	4.0 ± 0.2 <sup>a</sup>	4.1 ± 0.1 <sup>a</sup>

Statistical comparisons between groups by ANOVA and Newman Keuls. different letters denote statistically significant differences ( $P < 0.05$ ).

<sup>1</sup>mg/dl plasma.

<sup>2</sup>mg/mg.

Table 3  
Pearson correlation coefficients for CETP activity versus other lipoprotein variables in patients and controls

	Lipoprotein lipase-deficient and hypertriglyceridemic (n = 19)		Hypertriglyceridemic (n = 15)		All controls (n = 87)	
	r	P	r	P	r	P
Total triglyceride	0.8923	<0.0001	0.6658	<0.01	-0.1439	NS
Total cholesterol	0.7187	<0.001	0.1000	NS	0.0868	NS
VLDL-triglyceride	0.8923	<0.0001	0.6260	<0.05	-0.1982	NS
VLDL-cholesterol	0.8102	<0.0001	0.2964	NS	-0.1573	NS
LDL-triglyceride	0.0167	NS	0.4889	NS	0.1024	NS
LDL-cholesterol	-0.5917	<0.01	-0.2590	NS	0.1284	NS
HDL-triglyceride	0.2582	NS	0.3266	NS	0.2205	<0.05
HDL-cholesterol	-0.6722	<0.01	0.4162	NS	0.0133	NS
LPL activity	-0.6700	<0.01	-0.3927	NS	0.1996	NS
Plasma apo A-I	-0.6096	<0.01	-0.3216	NS	0.0505	NS
A-I/HDL-cholesterol	0.0973	NS	-0.0167	NS	0.0209	NS
A-I/HDL-triglyceride	-0.4529	<0.05	-0.3145	NS	-0.2266	<0.05
A-I/HDL-phospholipid	-0.4474	NS	-0.3256	NS	-0.0570	NS
HDL-esterified cholesterol/HDL-free cholesterol	-0.7972	<0.001	-0.6329	<0.05	-0.0801	NS
HDL-triglyceride/HDL-cholesterol	0.8660	<0.0001	0.6801	<0.005	0.2378	NS

NS:  $P > 0.05$ .

hypertriglyceridemic group the only alteration corresponded to the A-I/HDL-triglyceride mass ratio, which was significantly lower than in controls (Table 2). These concentrations and mass ratio values, indicate that LPL-deficient patients had a very reduced number of HDL particles, which were proportionally richer in triglyceride, phospholipids and free cholesterol and poorer in cholesteryl esters than those in controls. A similar tendency was observed in the hypertriglyceridemic patient group, though the only significant change was the enrichment in triglycerides.

Pearson correlation coefficients between CETP activity and several lipidic parameters are shown in Table 3. Because of the absence of significant differences between the two control groups, they were considered together for these calculations (all controls in Table 3); two patient groups were made, one with all the patients together and the other with only the hypertriglyceridemic patients not deficient in LPL; LPL-deficient patients were not studied separately because of the few cases. When all patients were considered together, many significant correlations were observed. As a whole, CETP activity appeared to be directly correlated with total triglyceride and VLDL levels, but inversely with LDL, HDL and apo A-I levels. The best correlated mass ratios were those affecting HDL-triglyceride, as well as the HDL-esterified/HDL-free cholesterol ratio which was inversely correlated with CETP. When only the hypertriglyceridemic, non-LPL-deficient patients were considered, total triglyceride, VLDL-triglyceride and HDL-triglyceride/HDL-cholesterol were directly correlated and the HDL-esterified cholesterol/HDL-free cholesterol mass ratio was inversely correlated with CETP activity (Table 3). In the control group, CETP activity was weakly but significantly correlated with both HDL-triglyceride levels (directly) and the A-I/HDL-triglyceride mass ratio (inversely) (Table 3).

#### 4. Discussion

We describe here the pronounced elevation of cholesteryl ester transfer activity in type I hyperchylomicronemic patients, compared with other hypertriglyceridemic individuals and document the relationships between CETP activity and HDL composition in these patients and two control groups.

The sensitive and specific assay for CETP activity that we have developed appears suitable for CETP estimation in samples from hyperlipidemic patients. Since the samples we used were  $d > 1.21$  g/ml density plasma fractions, transfer measurements were not interfered with by the presence of endogenous lipoproteins. This is particularly convenient when hypertriglyceridemic samples have to be compared with normolipemic ones, since both VLDL and chylomicrons are acceptors of cholesteryl esters from HDL [3]. Previous experiments in our laboratory demonstrated that HDL subpopulations were not homogeneously labelled *in vitro* with [ $^3$ H]cholesteryl oleate and that the polyanion-based reagents precipitate the HDL subfractions to different extents, in relation to their apolipoprotein composition [8]. According to these results, for this CETP assay we used a [ $^3$ H]cholesteryl oleate labelled, apo E-poor fraction instead of total HDL as the donor lipoprotein: this fraction was minimally precipitated by the dextran sulfate/Mg $^{2+}$  reagent and show-

ed the highest capacity to deliver cholesteryl esters to VLDL by the action of CETP, among the different HDL subfractions [8]. In our assay conditions, precipitation of  $^3\text{H}$ -HDL was not affected by the addition of exogenous VLDL except when they were incubated together at  $37^\circ\text{C}$  in the presence of either human lipoprotein-deficient plasma or purified CETP. In these cases, the disappearance of radioactivity from the supernate was proportional to the volume of human lipoprotein-deficient plasma or CETP solution added (Figs. 2 and 3). This effect is interpreted as the result of the action of CETP transferring [ $^3\text{H}$ ]cholesteryl oleate from  $^3\text{H}$ -HDL to VLDL, this latter lipoprotein being precipitated by the dextran sulfate-based reagent. Beside this, the assay seems specific for CETP since the transfer of [ $^3\text{H}$ ]cholesteryl oleate to VLDL did not take place at  $4^\circ\text{C}$  or when human lipoprotein-deficient plasma was substituted by rat plasma. The relative proportions of donor and acceptor (VLDL) lipoproteins in the assay were those favorable to the transfer of [ $^3\text{H}$ ]cholesteryl oleate to VLDL [8], so that the appearance of [ $^3\text{H}$ ]cholesteryl oleate in VLDL reflects mass transfer of cholesteryl esters. The transfer of [ $^3\text{H}$ ]cholesteryl oleate to VLDL was linear with the incubation time for activities in tube up to 14.5 nmol esterified cholesterol transferred per 8 h, that is equivalent to 725 nmol esterified cholesterol transferred per 8 h/ml plasma, which is much higher than the usual CETP transfer activity in plasma.

One of the most relevant findings of this present work is the increased CETP activity in all the LPL-deficient patients studied. In the other hypertriglyceridemic patients, not deficient in LPL, the mean CETP activity was not statistically different from that in controls, but CETP values significantly correlated with both total triglyceride and VLDL-triglyceride, which suggests that the intensity of the hypertriglyceridemia rather than the LPL deficiency condition itself is causally related to the increase in CETP activity. These results are in accordance with those of McPherson et al. [23], who found increased CETP mass in both hyperchylomicronemic and hypertriglyceridemic patients. The reason why CETP activity is elevated in highly hypertriglyceridemic patients is not obvious. Other studies revealed that dietary treatment of hyperchylomicronemic or highly hypertriglyceridemic, dysbetalipoproteinemic patients resulted in normalization of CETP plasma concentration [23]. These results together with the significant correlation between CETP activity and plasma triglyceride or VLDL-triglyceride levels observed herein, suggest that the increased CETP activity is secondary to the intense elevation of triglyceride-rich lipoproteins. Increases in CETP activity have also been found both during alimentary lipemia in humans [37] and after feeding fat-rich diets to laboratory animals [38,39]. These effects could be related to the observation that CETP synthesis in CaCo-2 cells is activated by the entry of fatty acids through the apical border membrane [18]; thus, fat feeding could stimulate CETP synthesis and secretion by the absorptive intestinal cells. However, this appears not to be pertinent with our hyperchylomicronemic patients, who suffered from endogenous hyperlipidemia and certainly were not on a fat-rich diet; on the other hand, plasma CETP probably comes from multiple cell types [40] and the regulation of CETP synthesis may differ between them.

The increased CETP activity in highly hypertriglyceridemic patients may have important metabolic consequences. We have found that CETP activity is inversely cor-

related with the HDL-esterified cholesterol/HDL-free cholesterol mass ratio in these patients. Since the physiological action of CETP is to remove cholesteryl esters from HDL [1,2], the variation of esterified cholesterol content as related to free cholesterol in HDL observed in the highly hypertriglyceridemic patients could be attributed in part to CETP. As a result of CETP action, HDL-triglyceride would be expected to increase. Actually, both HDL-triglyceride levels and the relative triglyceride content in HDL were elevated in the two hypertriglyceridemic groups and the HDL-triglyceride/HDL-cholesterol mass ratio was significantly correlated with plasma CETP activity. Furthermore, in the control group, both HDL-triglyceride and the A-I/HDL-triglyceride mass ratio correlated significantly with CETP activity. All this suggests that CETP is one of the determinants of HDL triglyceride content. Obviously, factors such as the number of HDL particles, VLDL-triglyceride levels, and plasma lipolytic activities will also contribute to HDL-triglyceride levels. In the highly hypertriglyceridemic state, both the augmented VLDL levels and the increased CETP will be responsible for the accelerated acquisition of triglyceride by HDL. When lipoprotein lipase is active, as in the hypertriglyceridemic group studied, the increase of triglyceride in HDL is partially prevented; when lipoprotein lipase is absent or inactive, HDL are maximally enriched in triglyceride. Since the triglyceride content is a major determinant of the fractional catabolic rate of HDL [41], we propose that the increased CETP activity in highly hypertriglyceridemic patients contributes to the decreased HDL levels generally observed in these patients.

In conclusion, by using a sensitive and specific assay we have found that CETP activity in  $d > 1.21$  g/ml plasma is elevated in primary hypertriglyceridemia, this being clearly detected in highly hypertriglyceridemic patients, such as LPL-deficient subjects. This alteration together with the increased levels of triglyceride-rich lipoproteins are probably the major determinants of the changes of HDL composition and HDL levels that are characteristic of this disease. In normolipidemic subjects, HDL triglyceride content may be causally related to plasma CETP activity as indicated by the significant correlation found between these two parameters. The transfer of triglyceride to HDL promoted by CETP may be an important determinant of the metabolic fate of HDL.

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