Lipoprotein Lipase Enhances the Cholesteryl Ester Transfer Protein-mediated Transfer of Cholesteryl Esters from High Density Lipoproteins to Very Low Density Lipoproteins*

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These studies were undertaken to examine the effects of lipoprotein lipase (LPL) and cholesteryl ester transfer protein (CETP) on the transfer of cholesteryl esters from high density lipoproteins (HDL) to very low density lipoproteins (VLDL). Human or rat VLDL was incubated with human HDL in the presence of either partially purified CETP, bovine milk LPL or CETP plus LPL. CETP stimulated both isotopic and mass transfer of cholesteryl esters from HDL into VLDL. LPL caused only slight stimulation of cholesteryl ester transfer. However, when CETP and LPL were both present, the transfer of cholesteryl esters from HDL into VLDL remnants was enhanced 2- to 8-fold, compared to the effects of CETP alone. The synergistic effects of CETP and LPL on cholesteryl ester transfer were more pronounced at higher VLDL/HDL ratios and increased with increasing amounts of CETP. In time course studies the stimulation of cholesteryl ester transfer activity occurred during active triglyceride hydrolysis. When lipolysis was inhibited by incubating LPL with either 1 M NaCl or 2 mM diethyl paranitrophenyl phosphate, the synergism of CETP and LPL was reduced or abolished, and LPL alone did not stimulate cholesteryl ester transfer. These experiments show that LPL enhances the CETP-mediated transfer of cholesteryl esters from HDL to VLDL. This property of LPL is related to lipolysis.

Increased levels of plasma HDL1 are associated with a decreased incidence of atherosclerotic cardiovascular disease (1). A major hypothesis to account for this apparent protective effect of HDL is that HDL is involved in the process of "reverse cholesterol transport," i.e. movement of cholesterol from periphery to liver. Although centripetal cholesterol transport involving HDL has yet to be clearly demonstrated in vivo, several lines of experimental evidence suggest a series of steps which could achieve this process. Tissue cholesterol may diffuse into HDL where it is esterified by lecithin:cholesterol acyltransferase (2). HDL cholesteryl esters can be transferred to triglyceride-rich lipoproteins (VLDL or chylomicrons) as a result of the action of a plasma cholesteryl ester transfer protein (3–7). The triglyceride-rich lipoproteins may then be lipolyzed by lipoprotein and hepatic lipases, forming remnant particles which are taken up by the liver as a result of receptor-mediated endocytosis involving apo-E or apo-B,E receptors (8).

The present study examines the transfer of cholesteryl esters from HDL to VLDL. In vivo this transfer probably occurs as HDL is being lipolyzed. Although cholesteryl ester transfer has been well characterized in inclusions of plasma (3, 9) or isolated lipoproteins (10, 11), the effects of lipolysis on cholesteryl ester transfer protein activity have not been previously reported. Therefore, we have measured the transfer of cholesteryl esters from HDL into VLDL in incubations containing LPL and/or CETP.

MATERIALS AND METHODS

VLDL (d < 1.006 g/ml) and HDL (1.215-1.210 g/ml) were isolated from fresh human plasma by preparative ultracentrifugation. HDL containing 3H-cholesteryl esters was prepared as described previously (12); 94% of radioactivity was present in cholesteryl esters and 6% in cholesterol. Lipoprotein lipase was purified from bovine milk by the method of Bengtsson and Olivecrona (13). LPL (0.35 mg/ml) was stored at −20 °C in 1 M NaCl or 2 mM diethyl paranitrophenylphosphate, the synergism of CETP and LPL was reduced or abolished, and LPL alone did not stimulate cholesteryl ester transfer. These experiments show that LPL enhances the CETP-mediated transfer of cholesteryl esters from HDL to VLDL. This property of LPL is related to lipolysis.
To examine the effects of lipolysis on cholesteryl ester transfer, the following components were mixed (in order): VLDL, albumin (to achieve a molar ratio of albumin to triglyceride fatty acid of 0.12–0.18 (16)), heparin, 50 mM Tris, 0.01% NaN₃, pH 8.5 (buffer A), HDL, and CETP and/or LPL. In most experiments the ratio of CETP to HDL was 1:3 mg of CETP/mg of HDL cholesterol. Except for LPL, stock solutions of the different components had been dialyzed into or brought up in buffer A. The final incubation volume was 1 ml unless indicated otherwise. The mixtures were incubated in stoppered test tubes for 1 to 2 h at 37 °C in a metabolic shaker. Lipolysis was stopped by immersing test tubes in ice and by the addition of a concentrated NaBr solution to give a final solution density of 1.06 g/ml; in time course studies 2 mM diethyl paranitrophenyl-phosphate was also added to ensure rapid cessation of lipolysis (17). VLDL or VLDL remnants were separated from HDL by ultracentrifugation at 40,000 rpm in a Beckman Ti-50.3 rotor or in a Sorvall 45 rotor. To verify that radioactivity in the d < 1.06 fraction was present in VLDL remnants, the d < 1.06 fractions were analyzed by chromatography on a column (1.0 × 110 cm) of Sepharose CL6B and also on a column (1 × 110 cm) of Sepharose CL4B. In the latter instance the peak particle size was characterized by calculating the Kᵥ (Kᵥ = Vᵥ – V₀/Vₐ – V₀), where Vᵥ is the peak elution volume, V₀ the void volume (phenol red), and Vₐ the included volume (phenol red). The transfer of cholesteryl ester radioactivity was determined by liquid scintillation counting of the VLDL or VLDL remnant fraction. The recovery of radioactivity during centrifugal preparation of VLDL or VLDL remnants was greater than 90%. In order to evaluate cholesteryl ester mass transfer, VLDL or HDL were extracted by the method of Bligh and Dyer (18), and cholesteryl and cholesteryl ester mass were measured by gas-liquid chromatography (19). In experiments where cholesteryl ester transfer was related to triglyceride hydrolysis, the extracted lipids of the VLDL or VLDL remnant fraction were analyzed by thin-layer chromatography in hexane/ether/acetic acid (70:30:1); cholesteryl ester and triglyceride spots were scraped and counted. The percent of lipolysis was determined from the amount of radioactivity in the triglyceride spot. Unless otherwise indicated, the experimental points (Figs. 2–5) are the means of duplicate or triplicate values; the standard errors were about 5–10% of the mean values. The statistical significance of the differences of means was evaluated by the Student’s t test. The values shown in the tables are means ± S.E.

RESULTS

VLDL was incubated with HDL in the presence of CETP and/or LPL. VLDL or VLDL remnants were separated from HDL by ultracentrifugation at a density of 1.06 g/ml and then analyzed by chromatography on a Sepharose CL6B column (Fig. 1). Both VLDL (Fig. 1, A and B) and lipolyzed VLDL (Fig. 1, C and D) eluted in or shortly after the column void volume. In experiments containing LPL there was hydrolysis of about 60% of VLDL triglycerides, with a corresponding decrease in absorbance. The presence of CETP resulted in about 2-fold greater transfer of total cholesterol (unesterified cholesteryl ester plus cholesteryl ester) radioactivity into the VLDL fraction (cf. Fig. 1A with Fig. 1B), while LPL caused only slight stimulation of cholesterol transfer (cf. Fig. 1A with Fig. 1D). However, when LPL and CETP were both present there was about a 4-fold greater transfer of cholesterol radioactivity into the VLDL remnant fraction (cf. Fig. 1A and Fig. 1C), indicating a synergistic effect of LPL and CETP on cholesteryl ester transfer. In all incubations the major peak of cholesterol radioactivity conformed to the absorbance profile of VLDL or VLDL remnants. There was no peak of cholesterol radioactivity in the HDL region. To ensure that the void volume eluting fractions did not contain multiple peaks, the d < 1.06 fractions from an experiment similar to that shown in Fig. 1 were analyzed by chromatography on a 4% agarose column (not illustrated). Again, for each of the incubations there was a single peak of absorbance and cholesterol radioactivity; the VLDL remnants emerged later in the column profile (peak Kᵥ = 0.56) than the VLDL (Kᵥ = 0.49). These experiments show that the cholesterol radioactivity of the d < 1.06 fraction was present largely in VLDL or VLDL remnants and not in a modified HDL particle. Thus, in subsequent work VLDL or VLDL remnants were separated from HDL by a single ultracentrifugation at a density of 1.06 g/ml.

Fig. 1 shows the distribution of total cholesterol (i.e. unesterified cholesteryl ester plus cholesteryl ester) radioactivity in the d < 1.06 fraction. Since the radiolabeled HDL used in these experiments contained 94% of its radioactivity in cholesteryl esters and 6% in cholesterol, the distribution of radioactivity between cholesteryl ester and cholesterol esters was determined by thin-layer chromatography of the lipid extract of the void volume eluting peaks (Fig. 1); the per cent of VLDL or VLDL remnant radioactivity present in cholesterol/cholesteryl esters was 62/38% (Fig. 1A); 36/64% (Fig. 1B); 19/81% (Fig. 1C); and 48/52% (Fig. 1D). Thus, most of the small amount of radioactivity transferred from HDL to VLDL in the blank incubation (Fig. 1A) represented unesterified cholesterol; a similar absolute amount of unesterified cholesterol radioactivity was transferred from HDL to VLDL or VLDL remnants in Fig. 1, B–D, indicating that CETP did not influence the transfer of unesterified cholesterol radioactivity. When transfer of cholesteryl ester radioactivity was considered, after

FIG. 1. Analysis of the d < 1.063 fraction by agarose column chromatography, showing the distribution of cholesterol radioactivity and the absorbance at 280 nm. The incubations shown are VLDL + HDL (A), VLDL + HDL + CETP (B), VLDL + HDL + CETP + LPL (C), and VLDL + HDL + LPL (D). Each incubation (2 h, 37 °C) contained human VLDL (4.4 mg of triglycerides), human HDL₄ (190 μg of cholesterol with 120,000 cpm of cholesteryl ester and 8000 cpm of cholesterol radioactivity), 140 mg of albumin, 20 units of heparin, and, as indicated, 200 μg of CETP and/or 1.6 μg of LPL. V₀ and Vᵥ show the void and salt volumes of the column. The elution positions of control preparations of human LPL and HDL₄ are also indicated. Each fraction contained 1 ml. Absorbance values are only shown for fractions with values < 0.005.

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ment of cholesteryl ester transfer into the VLDL remnant fraction (Fig. 1). The values reported subsequently in this paper refer only to transfer of cholesteryl ester radioactivity.

Fig. 2 shows the time course of hydrolysis of VLDL triglycerides related to the transfer of HDL cholesteryl ester radioactivity into the \( d < 1.06 \) g/ml fraction. Hydrolysis of VLDL triglycerides was largely completed in the first 60 min. Lipolysis proceeded at a similar rate with or without CETP. In the absence of added CETP or LPL, 1.5% of HDL cholesteryl ester radioactivity was recovered in the \( d < 1.06 \) fraction; this amount did not change with time and has, therefore, been subtracted from the values shown in Fig. 2. In the presence of CETP, there was a continuous transfer of cholesteryl ester radioactivity into the \( d < 1.06 \) fraction, representing about 10% of HDL cholesteryl esters after 2 h (Fig. 2). LPL caused a lesser time-dependent stimulation of cholesteryl ester transfer from HDL to VLDL. When LPL and CETP were both present, cholesteryl ester transfer was considerably enhanced. The synergistic effects of CETP and LPL on cholesteryl ester transfer were most pronounced during the first 60 min of the incubation, resulting in 2- to 3-fold greater transfer of cholesteryl ester, compared to CETP alone. In the presence of CETP plus LPL, the most rapid cholesteryl ester transfer occurred during active lipolysis, raising the possibility that the synergism of LPL and CETP depends on lipolysis. The experiments shown in Fig. 2 employed rat VLDL biosynthetically radio-labeled in the triglyceride moiety. Similar results were obtained using human VLDL, which had been radiolabeled by introducing \(^3\)H-triglycerides dissolved in dimethyl sulfoxide (15); using conditions identical to those in Fig. 2, at 60 min there was 2.8-fold greater transfer of cholesteryl ester radioactivity from HDL into human VLDL using CETP plus LPL, compared to CETP alone.

Previous studies have demonstrated that cholesteryl ester transfer protein activity is greatly influenced by the ratio of donor to acceptor lipoprotein (10). Thus, in further experiments the effects of variable VLDL/HDL ratio on cholesteryl ester transfer were examined (Fig. 3). The amount of HDL was held constant, while VLDL, LPL, and albumin were increased. A constant ratio of LPL/VLDL was used in order that the per cent of lipolysis would be similar. As reported previously, the effects of CETP on cholesteryl ester transfer increased with increasing VLDL/HDL ratio (10), reaching a plateau at a 2:1 ratio of VLDL to HDL cholesterol. Significant effects of LPL alone were only observed at higher ratios of VLDL to HDL. Cholesteryl ester transfer in incubations containing CETP plus LPL also increased with increasing VLDL/HDL ratio throughout the range of ratios examined. There was a pronounced effect of LPL on CETP activity, resulting in 3- to 8-fold increase in cholesteryl ester transfer, compared to CETP alone. Although most pronounced at high ratios, this enhancement of cholesteryl ester transfer also occurred at VLDL/HDL ratios commonly encountered physiologically (VLDL/HDL cholesterol about 1.2 to 1:1).

Fig. 4 shows the effects of increasing CETP on cholesteryl ester transfer in incubations containing constant VLDL, HDL, and LPL. With increasing CETP there was increasing cholesteryl ester transfer from HDL to the \( d < 1.06 \) g/ml fraction for both CETP and CETP plus LPL. The presence of both CETP and LPL resulted in a 2- to 3-fold increase in cholesteryl ester transfer, compared to CETP alone. Although the synergistic effect of LPL and CETP was observed up to the highest amount of CETP employed, the -fold increase in cholesteryl ester transfer was somewhat greater with lower CETP masses. Plasma containing a mass of VLDL and HDL similar to that used in this experiment would give rise to...
Fig. 4. Transfer of HDL cholesteryl ester radioactivity into the $d < 1.063$ fraction as a function of increasing mass of CETP. Incubations contained VLDL plus HDL plus CETP (C) or VLDL plus HDL plus CETP plus LPL (A). Each incubation (2 h, 37°C) contained human VLDL (100 µg of cholesterol), HDL (60 µg of cholesterol), 20 mg of albumin, 4 units of heparin, HDL$_o$ (60 µg of cholesterol = 15,600 cpm of cholesteryl ester radioactivity), and the indicated amount of CETP or CETP plus LPL (0.05 µg). In incubations conducted with LPL alone the transfer of HDL cholesteryl ester radioactivity into the $d < 1.063$ fraction (1%) was identical to that in the blank (VLDL plus HDL).

about 10 to 20 µg of partially purified CETP (12). Thus, the lower range of masses shown in this experiment might have greatest potential relevance to possible physiological interactions of the activities of CETP and LPL.

In order to determine the effects of increasing amounts of LPL on cholesteryl ester transfer from HDL to the $d < 1.06$ fraction, a constant amount of VLDL and HDL was incubated with increasing amounts of LPL, with and without CETP (Fig. 5). In a 2-h incubation the amount of lipolysis increased with increasing amounts of LPL. The dose-response curves of lipolysis were similar, whether CETP was present or not. Increasing amounts of LPL were associated with increasing cholesteryl ester transfer, up to the maximum dose of LPL used. By contrast, in the presence of CETP, LPL caused a marked stimulation of cholesteryl ester transfer at much lower doses. The peak effect, which resulted in about 8-fold greater transfer of cholesteryl esters than CETP alone, occurred at a dose of LPL resulting in about 75% lipolysis. With further increase in the amount of LPL there was a decline in cholesteryl ester transfer. Similar results were obtained with human VLDL containing radiolabeled triglycerides.

The experiment shown in Fig. 5 was conducted at a high ratio of VLDL/HDL cholesterol (4:1). Similar experiments were conducted at VLDL/HDL ratios of 1:1 and 1:4. At the 1:1 ratio the effects of LPL increased with increasing dose, but the per cent of cholesteryl ester radioactivity transferred into VLDL was only about one-third of that shown in Fig. 5 at the same dose of LPL. Significant effects of LPL on CETP activity were observed with as little as 5% triglyceride hydrolysis. The maximum synergism of LPL and CETP was observed at a dose of LPL giving about 30% triglyceride hydrolysis. At the 1:4 ratio, LPL alone exerted no significant effects on cholesteryl ester transfer, even with doses of LPL 3 to 4 times those resulting in maximal lipolysis. The maximum effect of LPL on CETP activity was observed at a dose of LPL giving more than 95% triglyceride hydrolysis. These results indicate a complex relationship between dose of LPL, amount of lipolysis, and stimulation of cholesteryl ester transfer. In general, the synergism of CETP and LPL was observed with small amounts of lipolysis, increased with an increasing dose of LPL to a maximum value which occurred at a different per cent of lipolysis dependent on VLDL/HDL ratio, then decreased with further increase in LPL dose.

In the above experiments the transfer of cholesteryl esters has been inferred from the movement of cholesteryl ester radioactivity. To determine if the enhancement of transfer of cholesteryl ester radioactivity was due to net transfer or exchange processes, mass data was obtained for the $d < 1.063$ fraction. Table I shows the composition of the $d < 1.063$ fraction in an experiment conducted at a 3:1 ratio of VLDL/HDL cholesterol. There were significant increases in cholesteryl ester mass in the presence of both CETP and CETP plus LPL. In both cases the per cent of HDL cholesteryl ester mass transferred into the $d < 1.06$ fraction was similar to that of HDL cholesteryl ester radioactivity, indicating that under the conditions of this experiment the movement of radioactivity reflected mass transfer processes. Other significant compositional changes included a loss of triglyceride and protein mass in the VLDL remnants and a decrease in phospholipid mass in VLDL remnants prepared in the absence of CETP. Sodium dodecyl sulfate-gel analysis of the delipidated apoproteins of the $d < 1.06$ fraction showed a relative increase in chromogenicity of apo-B and a decrease in apo-E and apo-C in the VLDL remnants, compared to...
The experiments shown in Tables I and II were performed with a small amount of LPL, giving less than 50% hydrolysis of VLDL triglycerides. In a further experiment (Table III) mass analysis of the HDL fraction was performed under conditions of more complete lipolysis (80–90% of VLDL triglycerides). In the presence of CETP plus LPL the transfer of cholesteryl ester radioactivity (58%) was more pronounced than that of cholesteryl ester mass (38%). Thus, although the enhanced transfer of cholesteryl ester radioactivity was due to a major component of net mass transfer, there was also a contribution from exchange processes.

Some of the above experiments have suggested that LPL’s stimulation of cholesteryl ester transfer is related to its lipo-lytic effects. In order to address this question more directly experiments were performed in the presence of inhibitors of lipolysis (Fig. 6). In the first set of experiments (Fig. 6, I) LPL was preincubated in buffer or in buffer containing 1 M NaCl and then incubated with VLDL and HDL, with or without CETP. Incubation of VLDL with LPL in the high salt buffer resulted in almost complete inhibition of lipolysis. There was no significant change in triglyceride mass when VLDL was incubated with LPL in high salt buffer (Fig. 6, I, TG, bars C and D (+)); however, slight but significant (p < 0.05) increases in fatty acid in the d < 1.063 fraction (Fig. 6, I, FA, bars C and D (+)) indicated that some lipolysis had occurred. The high salt buffer did not affect the ability of the CETP to stimulate cholesteryl ester transfer (cf. Fig. 6, I, CE, bar B (−) with bar B (+)). However, the effect of LPL on CETP activity was reduced by about 2.3 (p < 0.05) (cf. bar C (−) with bar C (+)), and the small stimulation of cholesteryl ester transfer due to LPL alone was abolished (bar D). Thus, although inhibition of lipolysis by high salt buffer did not completely abolish the synergism of CETP with LPL, it significantly reduced this effect.

In a second set of experiments diethyl parainitrophenyl-phosphate was employed as an inhibitor of lipolysis (Fig. 6, II) (17). The presence of E600 resulted in complete inhibition of lipolysis, as judged both by preservation of triglyceride mass and by the lack of fatty acid formation. E600 abolished the synergism of LPL and CETP and also the stimulation of

### Table I

**Composition of d < 1.06 fraction**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Triglyceride</th>
<th>Phospholipid</th>
<th>Cholesterol</th>
<th>CE</th>
<th>Mass</th>
<th>Radioactivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. VLDL + HDL</td>
<td>180 ± 4</td>
<td>692 ± 35</td>
<td>200 ± 4</td>
<td>108 ± 8</td>
<td>198 ± 4</td>
<td>0</td>
</tr>
<tr>
<td>B. VLDL + HDL + CETP</td>
<td>176 ± 1</td>
<td>646 ± 20</td>
<td>210 ± 4</td>
<td>106 ± 8</td>
<td>208 ± 6</td>
<td>9.5 ± 4</td>
</tr>
<tr>
<td>C. VLDL + HDL + CETP + LPL</td>
<td>158 ± 5*</td>
<td>397 ± 22*</td>
<td>198 ± 5</td>
<td>114 ± 3</td>
<td>222 ± 8*</td>
<td>22.8 ± 5*</td>
</tr>
<tr>
<td>D. VLDL + HDL + LPL</td>
<td>153 ± 3*</td>
<td>371 ± 18*</td>
<td>182 ± 2*</td>
<td>100 ± 4</td>
<td>197 ± 7</td>
<td>0</td>
</tr>
</tbody>
</table>

*Significantly different from control (incubation A), p < 0.001.

### Table II

**Transfer of cholesteryl esters from HDL to the d < 1.06 fraction (low LPL)**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Cholesterol</th>
<th>Cholesteryl ester</th>
<th>ΔCE mass</th>
<th>ΔCE cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. VLDL + HDL</td>
<td>10.3 ± 0.6</td>
<td>94.7 ± 2.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B. VLDL + HDL + CETP</td>
<td>11.7 ± 0.4</td>
<td>86.8 ± 0.96*</td>
<td>−8.7 ± 1.0*</td>
<td>−9.3 ± 1.6*</td>
</tr>
<tr>
<td>C. VLDL + HDL + CETP + LPL</td>
<td>14.2 ± 0.3</td>
<td>73.3 ± 1.4*</td>
<td>−22.6 ± 1.5*</td>
<td>−22.1 ± 1.8*</td>
</tr>
<tr>
<td>D. VLDL + HDL + LPL</td>
<td>13.6 ± 0.4</td>
<td>91.3 ± 4.5</td>
<td>−3.5 ± 2.6</td>
<td>−1.1 ± 0.1</td>
</tr>
</tbody>
</table>

*Significantly different from control (incubation A), p < 0.001.
Cholesteryl ester transfer depends on its lipolytic properties. Cholesteryl ester transfer produced by LPL alone. These results strongly suggest that the ability of LPL to influence cholesteryl ester transfer depends on its lipolytic properties.

As shown in Fig. 6, II (cholesteryl esters, bar B), 2 mM E600 was found to enhance the activity of CETP. Since similar results were obtained with 2 mM phenylmethylsulfonyl fluoride, another serine protease inhibitor, we considered the possibility that these effects were due to contamination of the CETP preparation with bacterial proteases. However, identical effects of E600 on cholesteryl ester transfer were obtained using density > 1.21 fraction prepared from plasma collected in the presence of Na2EDTA (1 mg/ml), gentamicin (0.1 mg/ml), chloramphenicol (0.05 mg/ml), and NaN3 (0.01%.

The above results indicate that the effects of LPL on cholesteryl ester transfer are related to lipolysis. To determine if CETP must be present with active LPL for their synergism to occur, VLDL was incubated for 30 min in the presence of LPL; then lipolysis was stopped by addition of 2 mM E600. Next HDL and CETP were added, and the incubation was continued for another 2 h at 37 °C. Compared to native VLDL, the lipolyzed VLDL showed an enhanced ability to accept HDL cholesteryl esters in the presence of CETP (Table IV), even though LPL was completely inactivated by the preincubation with E600. In these experiments VLDL remnants were not reisolated prior to addition of HDL ± CETP. In further experiments VLDL was incubated with LPL, E600 was added, and then VLDL remnants were separated from the other components of the lipolysis system by chromatography on a 6% agarose column. Compared to control VLDL (i.e. VLDL treated identically except for the addition of LPL), isolated VLDL remnants showed an enhanced ability to accept cholesteryl esters in the presence of CETP. For incubations similar to those in Table IV, the per cent of HDL cholesteryl ester radioactivity transferred into VLDL or VLDL remnants was: A, 0.3 ± 0.1%; B, 4.5 ± 0.3%; C, 7.7 ± 0.3%; D, 1.1 ± 0.2%. These experiments show that the property of the lipolysis system enhancing cholesteryl ester transfer remained associated with the separated VLDL remnants. In a further experiment, aiming to determine if high salt buffers had an effect on the enhanced ability of lipolyzed VLDL to accept HDL cholesteryl esters, VLDL was lipolyzed, E600 was added, and then HDL and CETP were added, and a 2-h incubation was conducted in buffer A or in buffer B containing 1 mM NaCl. In the presence of the high salt buffer a 2-fold increase of cholesteryl ester radioactivity as VLDL (n = 3), while in the low salt buffer it accepted 2.2-fold as much cholesteryl ester radioactivity, indicating no significant effect of 1 mM NaCl on the synergistic

### Table III

<table>
<thead>
<tr>
<th></th>
<th>HDL mass</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesteryl</td>
<td>Cholesteryl</td>
</tr>
<tr>
<td></td>
<td>mass</td>
<td>mass</td>
</tr>
<tr>
<td>A. VLDL + HDL</td>
<td>7.5 ± 0.5</td>
<td>57.2 ± 1.5</td>
</tr>
<tr>
<td>B. VLDL + HDL + CETP</td>
<td>8.6 ± 0.2</td>
<td>50.1 ± 1.9a</td>
</tr>
<tr>
<td>C. VLDL + HDL + CETP + LPL</td>
<td>9.1 ± 0.3</td>
<td>35.3 ± 1.0ab</td>
</tr>
</tbody>
</table>
* Significantly different from A, p < 0.001.
* Significantly different from B, p < 0.001.

*The differences of the means of C and B were statistically significant, p < 0.01.

### Table IV

Transfer of HDL cholesteryl esters into VLDL remnants with inactive LPL (per cent of HDL cholesteryl esters transferred into d < 1.06 fraction)

VLDL (30 μg triglycerides) was incubated with saline (A and B) or with LPL (0.02 μg) (C and D) for 30 min at 37 °C, in the presence of 0.6 mg of albumin and 4 units of lipase in 1 ml of buffer A. Lipolysis was stopped by addition of 2 mM E600 (to A–D), with further incubation for 1 h at 37 °C. Next HDLs (30 μg of protein, 8000 cpm of cholesteryl ester radioactivity) was added to A–D and CETP (10 μg of protein) to incubations B and C, and samples were incubated a further 2 h at 37 °C. The results shown are mean ± S.E. values for an incubation conducted in pentuplicate.

<table>
<thead>
<tr>
<th></th>
<th>CETP (B)</th>
<th>CETP + LPL (C)</th>
<th>LPL (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A)</td>
<td>0.7 ± 0.2</td>
<td>3.0 ± 0.1</td>
<td>5.0 ± 0.3a</td>
</tr>
</tbody>
</table>

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interaction of lipolyzed VLDL with CETP. These results confirm that the inhibition of synergism noted in the earlier experiment with high salt buffer (Fig. 6, I) was a direct result of decreased lipolysis.

Since complete lipolysis of VLDL results in the formation of LDL-like particles (20), the above experiments raised the possibility that the enhanced ability of VLDL remnants to accept HDL cholesteryl esters resulted from their conversion to LDL-like particles. Thus, we compared VLDL, VLDL undergoing lipolysis, and LDL as acceptors of HDL cholesteryl esters. The acceptor lipoproteins were matched for cholesteryl ester content and incubated with HDL in the presence of CETP (Table V). These experiments revealed that both VLDL and LDL incubated with LPL were significantly better acceptors of HDL cholesteryl esters than LDL. In fact, VLDL incubated with LPL showed about 5-fold greater cholesteryl ester transfer than LDL. Furthermore, these experiments revealed that LPL did not increase the CETP-mediated transfer of cholesteryl esters from HDL to LDL, nor did LPL alone stimulate cholesteryl ester transfer from HDL to LDL. The greater transfer of cholesteryl ester radioactive into VLDL compared to LDL probably reflects the greater core lipid content of VLDL in these experiments.

Two observations suggest that very little lipolysis may result in an enhancement of the activity of CETP. First, in the LPL dose-response studies significant effects were observed with only 5-20% triglyceride hydrolysis. Second, LPL’s enhancement of CETP activity was relatively resistant to inhibition of lipolysis by high salt buffers, with some interaction evident with less than 5% triglyceride hydrolysis (Fig. 6, I). The effect of LPL on cholesteryl ester transfer (with or without CETP) increased with increasing amounts of lipolysis. This could be consistent with formation of a lipolytic product responsible for the stimulation of CETP activity. Formation of increased amounts of this product could also account for the greater cholesteryl ester transfer observed at high VLDL/HDL ratios (Fig. 3). Since LPL has significant phospholipase activity (21), the effects of lipolysis on cholesteryl ester transfer could be mediated either by triglyceride hydrolysis or by phospholipidolysis. The LPL dose-response curves (Fig. 5) also suggest the generation of an inhibitory effect with higher amounts of lipolysis.

The enhancement of transfer of cholesteryl ester radioactivity from HDL to VLDL by CETP plus LPL was found to be due to increased net transfer of cholesteryl esters or increased net transfer of cholesteryl esters plus increased exchange of cholesteryl esters, depending on the incubation conditions (Tables I-III). The enhancement of cholesteryl ester exchange became apparent when there was more extensive lipolysis of VLDL (Table III); the experimental errors were sufficiently large that a minor component of enhanced exchange at lower amounts of lipolysis might not have been detected. Previous work has shown that CETP promotes mass transfer of cholesteryl esters from HDL to VLDL by stimu-

### TABLE V

<table>
<thead>
<tr>
<th>Incubation</th>
<th>HDL CE (d &lt; 1.06)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. VLDL + HDL</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>B. VLDL + HDL + CETP</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>C. VLDL + HDL + CETP + LPL</td>
<td>10.1 ± 0.8*</td>
</tr>
<tr>
<td>D. VLDL + HDL + LPL</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>E. LDL + HDL</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>F. LDL + HDL + CETP</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>G. LDL + HDL + CETP + LPL</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>H. LDL + HDL + LPL</td>
<td>0.6 ± 0.5</td>
</tr>
</tbody>
</table>

* Significance of differences of means: C versus B, p < 0.001, B versus F, p < 0.01, C versus F, p < 0.001.

Discussion

These experiments demonstrate that bovine milk LPL enhances the CETP-mediated transfer of cholesteryl esters from HDL to VLDL. Under certain conditions LPL alone also caused slight stimulation of cholesteryl ester transfer. Several lines of evidence indicate that these properties of LPL are related to lipolysis. The time courses studies showed that the enhancement of cholesteryl ester transfer occurred during active lipolysis. Also, LPL did not increase the CETP-mediated transfer of cholesteryl esters from HDL to a nonlipolyzed lipoprotein, LDL. Most importantly, inhibition of lipolysis by incubation of LPL in buffers containing 1 M NaCl or 2 mM diethylparanitrophenyl phosphate (E600) resulted in a reduced or abolished effect of LPL on cholesteryl ester transfer. High salt conditions are thought to interfere with the apo-C-II enhancement of LPL activity (17), while E600 probably inhibits lipolysis by binding to a reactive serine in the active site of LPL (17). Thus, the inhibition of lipolysis by two separate mechanisms interfered with the stimulation of cholesteryl ester transfer activity by LPL. These results strongly suggest that LPL’s stimulatory effect results from lipolysis.

When lipolysis of VLDL preceded the addition of HDL and CETP, there was still enhanced cholesteryl ester transfer compared to native VLDL, indicating that VLDL remnants were more active as cholesteryl ester acceptors than native VLDL. In these experiments lipolysis was blocked by addition of E600, ensuring abolition of further LPL activity. Thus, the effects of lipolysis on cholesteryl ester transfer could be observed even though lipolysis was not ongoing. These results imply that the stimulation of cholesteryl ester transfer activity by LPL is a result of LPL activity but does not result from a direct interaction of CETP with LPL. Since enhanced transfer of cholesteryl esters into previously lipolyzed VLDL remnants occurred in the presence of either E600 or high salt buffers, the inhibition of synergism exerted by these agents when present during lipolysis (Fig. 6) must have resulted from their effects on lipolysis, rather than through some other mechanism. Complete lipolysis of VLDL results in the formation of LDL-like particles (20). However, LDL matched for cholesteryl ester dose with VLDL was inferior to VLDL or VLDL undergoing lipolysis as an acceptor of HDL cholesteryl esters in the presence of CETP (Table V). Thus, the effects of lipolysis on CETP activity does not simply reflect formation of particles of the same general size and shape as LDL. The greater transfer of cholesteryl ester radioactive into VLDL compared to LDL probably reflects the greater core lipid content of VLDL in these experiments.

The enhancement of transfer of cholesteryl ester radioactivity from HDL to VLDL by CETP plus LPL was found to be due to increased net transfer of cholesteryl esters or increased net transfer of cholesteryl esters plus increased exchange of cholesteryl esters, depending on the incubation conditions (Tables I-III). The enhancement of cholesteryl ester exchange became apparent when there was more extensive lipolysis of VLDL (Table III); the experimental errors were sufficiently large that a minor component of enhanced exchange at lower amounts of lipolysis might not have been detected. Previous work has shown that CETP promotes mass transfer of cholesteryl esters from HDL to VLDL by stimu-
lating the hetero-exchange of core lipids between HDL and VLDL, i.e. CETP enhances cholesteryl ester-triglyceride exchange, resulting in greater net transfer of cholesteryl esters from HDL to VLDL (11). The stimulation of lipid exchange or transfer is thought to reflect the same mechanism of action of CETP, with the proportion of exchange or transfer determined by the core lipid composition of the donor and acceptor particles (11). Assuming that CETP stimulates hetero-exchange of neutral lipids between VLDL remnants and HDL by a similar mechanism, it is likely that the stimulation of cholesteryl ester exchange at higher amounts of LPL (Table III) reflected the formation of more cholesteryl ester-rich VLDL remnants.

Two general mechanisms by which lipolysis may enhance CETP action can be suggested. In the first mechanism LPL might increase the effective concentration of substrate lipid available to CETP. It is possible that the rate of exchange is dependent on the concentration of neutral lipids in the lipoprotein surface. Lipolysis, by promoting the formation of more amphipathic lipids (monoglycerides, diglycerides, and fatty acid soaps) in the surface of VLDL (22) may increase the effective concentration of lipids available for exchange with HDL cholesteryl esters. However, it is presently unknown if these lipids can undergo exchange with HDL cholesteryl esters. A second possible mechanism might involve an enhancement of the catalytic efficiency of CETP. For example, this might involve enhanced binding of CETP to a lipolyzed VLDL particle or increased formation of a shuttle complex containing CETP due to the presence of lipids resulting from lipolysis; the fact that enhanced CETP activity was observed with isolated VLDL remnants would be more consistent with the former of these mechanisms.

LPL has been previously shown to enhance cholesteryl ester transfer in tissue culture (23-25) and organ perfusion experiments (26). Fielding (26) demonstrated transfer of cholesteryl esters from chylomicrons into perfused rat heart tissue; this effect was independent of the uptake of intact particles and was mediated by tissue-bound LPL. Chajek-Shaul et al. and Stein et al. (23-25) showed that bovine milk LPL enhanced cholesteryl ester transfer from chylomicrons into cultured endothelial and smooth muscle cells (23-25). Heparin abolished the effect of LPL, indicating that LPL must be bound to the cells to enhance cholesteryl ester transfer. It appeared that LPL mediated cholesteryl ester transfer independent of triglyceride hydrolysis or phospholipolysis, suggesting that LPL has a cholesteryl ester-binding site, separate from its lipolytic site (25). These experiments are to be contrasted with the findings of the present investigation. First, we have shown net transfer of cholesteryl esters into rather than out of triglyceride-rich lipoproteins. Second, in our study the cholesteryl ester transfer mediated by LPL was to a large extent an enhancement of the activity of CETP, which was presumably absent in the tissue culture studies. Third, in contrast to the tissue culture experiments (23-25), LPL’s enhancement of the CETP-mediated transfer of cholesteryl esters between lipoproteins was related to lipolysis. These considerations suggest that LPL’s stimulation of cholesteryl ester transfer between lipoproteins is mediated by a different mechanism than that enhancing cholesteryl ester transfer from triglyceride-rich lipoproteins into cells. On the other hand, we have shown that very small amounts of lipolysis can influence cholesteryl ester transfer, raising the possibility that similar mechanisms might influence cholesteryl ester transfer into cells.

The physiological relevance of the stimulation of CETP activity by lipolysis is unknown. In in vitro lipolysis experiments the products of lipolysis (fatty acids and partial glycerides) may accumulate in abnormal amounts, since they are either bound in large amounts to albumin or retained in the surface of the triglyceride-rich lipoprotein (16). However, since LPL’s stimulation of CETP activity was observed at VLDL/albumin and VLDL/HDL ratios and with amounts of CETP similar to those found in normal plasma, it is conceivable that lipolysis influences cholesteryl ester transfer activity in vivo. If so, our findings raise the possibility that cholesteryl ester transfer rates measured in incubated plasma (9) may underestimate those occurring in the intact organism where lipolysis is ongoing. Also, the activity of lipoprotein lipase, which is correlated with HDL levels (27), may be related to the activity of the cholesteryl ester transfer system. Since the enhancement of CETP activity by lipolysis is much greater at higher VLDL/HDL ratios, the physiological transfer of cholesteryl esters from HDL to VLDL or chylomicron remnants might be favored during alimentary lipemia or in hypertriglyceridemic states.

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