Preferential Cholesteryl Ester Acceptors Among Triglyceride-Rich Lipoproteins During Alimentary Lipemia in Normolipidemic Subjects

Taous S. Lassel, Maryse Guérin, Silvy Auboiron, M. John Chapman, Bernard Guy-Grand

Abstract—Triglyceride-rich lipoproteins (TRLs), namely chylomicrons (CMs), VLDL, and their remnants, are implicated in the atherogenic features of postprandial lipemia. In human plasma, cholesteryl ester transfer protein (CETP) mediates the heterogeneous exchange of neutral lipids, ie, triglycerides (TG) and cholesteryl esters (CE), between distinct subpopulations of apoB- and of apoAI-containing lipoproteins. In fasting normolipidemic plasma, CETP plays an antiatherogenic role by promoting preferential CE redistribution from HDL to LDL particles of intermediate subclass with optimal binding affinity for the cellular LDL receptor. While the relative proportions and chemical compositions of donor and acceptor lipoproteins are known to influence CETP activity, elevated levels of TRL present during alimentary lipemia have been proposed to be associated with enhanced CETP activity. To identify the preferential CE acceptor particles among postprandial TRL subfractions, we investigated the effects of a typical Western meal (1200 kcal, 14% protein; 38% carbohydrate; and 48% fat, monounsaturated/polyunsaturated ratio 4:1) on the rates of postprandial CE transfer from HDL to apoB-containing lipoproteins in normolipidemic subjects (n=13). Two hours postprandially, plasma levels of TRL were significantly elevated (140 versus 51 mg/dL at baseline, P=.0001). Total rates of CE transferred (88±7 μg·h⁻¹·mL⁻¹) from HDL to apoB-containing lipoproteins were not significantly modified by alimentary lipemia over a period of 8 hours. Quantitatively, LDL accepted 64±5 μg CE per hour per milliliter plasma from HDL, whereas CM (Sf>400), VLDL1 (Sf 60 to 400), VLDL2 (Sf 20 to 60), and IDL (Sf 12 to 20) accepted 5±3, 16±3, 1.4±0.3, and 1.5±0.2, respectively. Quantitatively, VLDL1 was the major CE acceptor among TRLs (P=.0001); thus, VLDL1, but not CMs, represented the major CE acceptor among TRLs. Qualitatively however, VLDL2 and IDL displayed a higher capacity to accept CE from HDL (51.6±4.1 and 46.3±2.8 μg CE transferred per hour per milligram lipoprotein, respectively; P<.005) compared with CM, VLDL1, and LDL (12.6±2.8, 34.7±4.2, and 22.7±2.0 μg CE transferred per hour per milligram lipoprotein, respectively). In conclusion, elevated postprandial TRL levels are not associated with enhanced total CE transfer to these particles. Furthermore, the qualitative features of postprandial CE transfer from HDL to CM and VLDL1 were not related to the relative TG content of these particles. The CETP-facilitated enrichment of VLDL1 in CE therefore identifies them as potentially atherogenic particles during the postprandial phase. (Arterioscler Thromb Vasc Biol. 1998;18:65-74.)

Key Words: CETP activity ■ chylomicrons ■ VLDL subspecies

Several studies have provided data to support the hypothesis that postprandial hypertriglyceridemia represents an independent risk factor for CAD.1,2 In patients with CAD, postprandial TG levels are higher and remain elevated over a longer period in comparison with patients without CAD.1,2 The potential atherogenic role of postprandial TRLs, namely CMs, VLDL, and their remnants, was first proposed by Zilversmit.4,5 and has also been studied by Havel.6 Indeed, it has been demonstrated that VLDL and its remnants accumulate in human atherosclerotic lesions.7 Moreover, TG concentrations during the postprandial phase are more closely related to cardiovascular risk than those during the postabsorptive state.8 TRLs represent a heterogenous population of particles possessing potentially distinct atherogenic properties. CMs and their remnants appear to leave the circulation rapidly before they attain an optimal particle size for penetration of the arterial wall.9 In addition, and as a result of the efficient lipolysis of CM-TG by lipoprotein lipase, a predominance of VLDL containing apo-B100 of hepatic origin has been observed during the alimentary period.9 Thus, the accumulation of hepatogenous VLDL and its remnants in plasma after meal intake underlies the potential atherogenicity of these postprandial lipoproteins.

In plasma, the net transfer and exchange of neutral lipids, ie, TG and CE, between donor/acceptor lipoprotein particles is catalyzed by CETP.10 Plasma lipid transfer activity has a major effect on the composition, concentration, and size of both apoB- and apoAI-containing lipoprotein subspecies.11,12 CETP plays a central role in the reverse cholesterol transport process by which cholesterol from peripheral tissues is taken up by...
HDL and transported back to the liver. In fasting normolipidemic subjects, CETP promotes the preferential redistribution of CE from HDL to LDL, the latter representing the major quantitative CE acceptor among the apoB-containing lipoproteins. The transfer of CE from HDL to LDL is targeted by CETP to the intermediate LDL subspecies ($d=1.029$ to $1.039$ g/mL) as the major acceptors. Moreover, intermediate LDL subfractions display an elevated affinity for the cellular LDL receptor, which facilitates their rapid removal from plasma.

Under these conditions, CETP appears to play a protective rather than a proatherogenic role in normolipidemic subjects by promoting efficient CE removal from plasma via the LDL receptor pathway. Additionally, the relative proportions of both donor and acceptor lipoprotein particles have been demonstrated to play a determinant role among the several factors known to influence plasma CETP activity.

In dyslipidemic states, however, CETP activity is consistently elevated and appears to play a proatherogenic role. In primary hypertriglyceridemia, Mann et al reported an increased CE transfer rate from HDL to VLDL, probably as a result of elevation in VLDL concentrations. Similar observations have been made in combined hyperlipidemic subjects in whom the accelerated mass transfer of CE from HDL to VLDL is associated with a deficit in CE mass transfer from HDL to LDL. Other studies have demonstrated that the capacity of apoB-containing lipoproteins to accept CE from HDL is closely correlated with the relative TG content of the lipoprotein acceptor particles. In this context, it has been proposed that the elevated levels of TRL present during alimentary lipemia might be associated with enhancement of CETP activity.

There is a paucity of information concerning the potential relationships between postprandial lipemia and plasma CETP activity and concentration. Several investigations have reported an increase in plasma CETP activity during postprandial lipemia in normolipidemic subjects.

In a previous report, Marcel et al described a mean increase of 11% in plasma CETP mass concentration after a fatty meal intake. These authors postulated that the enhanced CE transfer from HDL to apoB-containing lipoproteins during alimentary lipemia might result from an increase in plasma TRL mass, from an increase in plasma CETP levels, or both. More recently, however, Lottenberg et al have demonstrated that plasma CETP concentration is not modified significantly after a lactose-free milkshake liquid meal providing 50 g fat.

To identify the preferential CE acceptor particles among postprandial TRL subfractions, we determined the effect of a typical Western meal (1200 kcal, 14% protein, 38% carbohydrate, and 47% fat, monounsaturated/polyunsaturated ratio 4:1) on the rates of CE transfer from HDL to apoB-containing lipoproteins in healthy, normolipidemic subjects. Quantitatively, VLDL1 ($S_f$ 60 to 400) was the major CE acceptor among TRLs throughout the postprandial phase of 8 hours. Moreover, on a quantitative basis, LDL remained the major CE acceptor particle. Qualitatively, however, VLDL2 ($S_f$ 20 to 60) and IDL ($S_f$ 12 to 20) exhibited the highest particle-acceptor affinity for CE among apoB-containing lipoproteins during postprandial lipemia. These studies establish that CM particles are minor postprandial CE acceptors.

### Methods

#### Subjects and Test Meal

This study was approved by the Medical Ethics Committee (Hôtel-Dieu, Paris, CCPPRB No. DGS 950435, France), and written informed consent was obtained from participants. Thirteen healthy young men with plasma levels of TG, LDL cholesterol, and HDL cholesterol (Table 1) within the normal range for their age were selected for the study; all displayed the apoE3/E3 genotype with the exception of two subjects (E3/E4). All subjects exhibited low levels of lipoprotein(a); none had diabetes, liver, renal, or thyroid disease; and they were nonsmokers. Before inclusion, the subjects were interviewed by registered dietitians, and a 3-day food record was established before the experiment to evaluate their alimentary behavior.

Subjects with total energy intake of more than 2800 kcal/d were excluded. The test meal consisted of freshly prepared commercially available foods: instant mashed potatoes mixed with oil (2/3 sunflower oil and 1/3 rapeseed oil; monounsaturated to polyunsaturated ratio 4:1), beef steak, cheese, bread, and apple. The nutrient and energy content of the meal is given in Table 2. This meal represented a total of 1200 kcal and consisted of 14% protein, 38% carbohydrate, and 48% fat, providing 66 g of fat and 142 mg of cholesterol. The subjects were asked to abstain from alcohol and vigorous exercise for 24 hours before the day of the test.

#### Blood Samples

For each subject, a baseline blood sample was collected at 8 AM after a 12- to 14-hour fasting period. After a standardized breakfast (300 kcal, containing 12% protein, 70% carbohydrate, and 18% fat) at 8:30 AM, the subjects consumed the test meal at 11:30 AM. Blood samples were collected in sterile EDTA-containing tubes (final concentration 1 mg/mL). Plasma was then immediately separated by low-speed centrifugation at 4°C and maintained at this temperature until fractionation on the same day. Plasma samples were obtained before the test meal (0 h), and at 1 hour, 2 hours, 3 hours, 4 hours, and 8 hours after meal consumption.
TABLE 2. Composition of the Typical Western Meal

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Carbohydrates, g</th>
<th>Protein, g</th>
<th>Fat, g</th>
<th>Fiber, g</th>
<th>Water, mL</th>
<th>Cholesterol, mg</th>
<th>Weight, g</th>
<th>Energy, kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mashed potatoes</td>
<td>81</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>365</td>
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<tr>
<td>White bread</td>
<td>23</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>12</td>
<td>0</td>
<td>40</td>
<td>104</td>
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<tr>
<td>Apple</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>102</td>
<td>0</td>
<td>120</td>
<td>56</td>
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<td>Water</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>300</td>
<td>0</td>
<td>300</td>
<td>0</td>
</tr>
<tr>
<td>Steak</td>
<td>0</td>
<td>28</td>
<td>8</td>
<td>1</td>
<td>63</td>
<td>62</td>
<td>100</td>
<td>184</td>
</tr>
<tr>
<td>Cheese</td>
<td>1</td>
<td>5</td>
<td>9</td>
<td>1</td>
<td>12</td>
<td>80</td>
<td>28</td>
<td>105</td>
</tr>
<tr>
<td>Oils*</td>
<td>0</td>
<td>0</td>
<td>48</td>
<td>0</td>
<td>48</td>
<td>48</td>
<td>432</td>
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<tr>
<td>Total</td>
<td>119</td>
<td>44</td>
<td>66</td>
<td>5</td>
<td>489</td>
<td>142</td>
<td>736</td>
<td>1246</td>
</tr>
</tbody>
</table>

*Two thirds sunflower oil and 1/3 rapeseed oil; monounsaturated to polyunsaturated ratio 4:1.

Isolation of TRL

All steps of ultracentrifugation were performed with a Kontron ultracentrifuge and a Kontron TST 41.14 swinging bucket rotor. All density solutions contained NaCl-KBr, sodium azide (0.01%), and gentamicin (0.005%) at pH 7.4. CMs (SF>400) were isolated by centrifugation at 20,000 rpm for 44 minutes at 15°C. Each plasma sample (5 mL) was overlaid with 7 mL of a d=1.006-g/mL solution in a 12-mL cellulose nitrate tube. After flotation, CMs were collected in two fractions each of 1 mL and were pooled. VLDL and LDL fractions were then isolated from CM-free plasma by a nonequilibrium density-gradient ultracentrifugation at 40,000 rpm for 6 hours at 15°C by a slight modification of the method of Musliner et al. Briefly, the density of CM-free plasma (4.5 mL) was increased to d=1.21 g/mL by addition of dry, solid KBr. The nonequilibrium density gradient was constructed as follows: 4.5 mL of CM-free plasma (d=1.21 g/mL), 3 mL of density 1.010 g/mL, and 1.5 mL of density 1.006 g/mL. After centrifugation, VLDL and IDL were collected from the top of the tube in 10 successive fractions of 0.5 mL; the two first fractions were pooled and corresponded to VLDL1 (SF 60 to 400; 1 mL), the third fraction of 0.5 mL corresponded to VLDL2 (SF 20 to 60). Fractions 4 through 9 were pooled and represented the IDL fraction (SF 12 to 20; 3 mL). The LDL and HDL fractions were subsequently isolated from the d<1.019 g/mL infranatant by precipitation of the apoB-containing lipoproteins with phosphotungstic acid and magnesium (Boehringer Mannheim kits). After centrifugation, HDL was recovered from the supernatant and the pellets were dissolved in 200 μL of Na2CO3 (0.5 mol/L).

Lipid and Protein Analyses

The lipid content in plasma and isolated lipoprotein fractions was quantified enzymatically by using Boehringer Mannheim kits for total and free cholesterol. CE mass was calculated as (total cholesterol-free cholesterol)×1.67 and thus represents the sum of the esterified cholesterol and fatty acid moieties. Bio-Mérieux kits were used for determination of TG and phospholipids. Bicinchoninic acid assay reagent (Pierce) was used for protein quantification. Lipoprotein mass was calculated as the sum of the mass of the individual components for each lipoprotein fraction. The coefficients of variation (intra-assay and interassay) for chemical analyses of cholesterol, TGs, phospholipids, and protein were 1.9%, 3.6%, 3.8%, and 5%, respectively. Plasma HDL cholesterol was determined after precipitation of apoB-containing lipoproteins with phosphotungstic acid and magnesium (Boehringer Mannheim kits). Plasma LDL cholesterol was calculated using the Friedewald formula.

Preparation of [3H]CE-labeled HDL

HDL (d=1.063 to 1.21 g/mL) obtained from fasting normolipidemic plasma was labeled as previously described by us and used for all CE transfer determinations. Briefly, HDL was obtained from the d=1.063 g/mL fraction of fasting plasma by ultracentrifugal flotation; it was subsequently incubated for 18 hours at 37°C in the presence of 4 μCi [1,2,6,7-3H]cholesterol in an ethanolic solution (specific activity 71 Ci/nmmol) to allow endogenous lecithin:cholesterol acyltransferase to esterify the radioactive cholesterol. HDL containing radionabeled CE was then isolated by adjusting the density of the incubation mixture to 1.21 g/mL by adding dry solid KBr, followed by centrifugation at 100,000 rpm for 5 hours and 30 minutes at 15°C. Radiolabeled HDL preparations displayed specific radioactivities of approximately 9500 cpm/μg CE; more than 95% of total radioactive free cholesterol added was transformed into labeled CE.

Determination of CE Transfer From HDL to ApoB-Containing Lipoproteins

Determination of CE transfer from HDL to apoB-containing lipoproteins was assayed by a slight modification of the method of Quemeneur et al. Briefly, radiolabeled HDL (equivalent to 1% of the total HDL CE mass present in 1 mL of the subject's plasma) and iodoacetamide (final concentration 1.5 mmol/L) were added to 5 mL of each subject's plasma. After 2 hours of incubation at 37°C, each TRL fraction (CM, VLDL1, VLDL2, and IDL) was isolated by ultracentrifugation; both LDL and HDL fractions were subsequently isolated by precipitation as described above. The radioactive CE and neutral lipid content of individual density fractions were determined as described above. Radioactivity was quantified with a Pharmacia Rack Beta 5/09 for liquid scintillation spectrometry. The recovery of radioactivity was more than 95% in all experiments. In normolipidemic subjects, CE mass transfer into apoB-containing lipoproteins, determined by measuring the increase in CE mass or obtained by calculation from the known specific radioactivity of labeled HDL, allows an equivalent determination during the first 4 hours of incubation. The time course of CE mass transferred during the initial period (5 to 6 hours) of incubation was determined.29 Similar observations were made in a control experiment using postprandial plasma (data not shown). Thus, the rate of CE net mass transfer was determined on the basis of radioactivity after 2 hours of incubation and is expressed in micrograms CE transferred per hour per milliliter plasma. The specific radioactivity expressed in cpm per microgram CE was calculated for each lipoprotein fraction from the cpm number divided by the CE content measured enzymatically after plasma incubation.

CETP-Dependent CE Transfer Assay

To determine whether any significant variation occurred in plasma CETP concentration during postprandial lipemia, we used an exogenous assay of CETP activity that has been previously shown to accurately reflect plasma CETP mass.28-30 Experiments were conducted at each of the three major postprandial points, ie, before ingestion of the test meal, at maximal TG level (2 hours), and at the end of the postprandial period (8 hours). Briefly, 0.5 mL of each plasma sample was adjusted to a density of 1.21 g/mL by addition of dry solid KBr. After centrifugation at 100,000 rpm for 6 hours at 15°C, plasma lipoproteins were removed and the bottom fraction of 1 mL was extensively dialyzed in Spectrapor membrane tubing at 4°C against a buffer containing 150 mmol/L NaCl, 10 mmol/L Tris base, 1 mmol/L EDTA, and 1 mmol/L sodium azide at pH 7.4. This bottom fraction was used as a source of CETP. Labeled HDL–CE (100 nmol) was mixed with 400 nmol of VLDL/LDL–CE in the presence or absence of 400 μL of the CETP-containing bottom fraction in a
CETP Activity During Postprandial Lipemia

Taq polymerase. The PCR product was then digested with 5 U Hha I restriction enzyme protocol as previously described by Hixson and Vernier. Briefly, genomic DNA isolated from blood by use of the Puregene kit (Gentra Systems) was amplified by PCR using specifically synthesized oligonucleotide primers and Taq polymerase. The PCR product was then digested with 5 U Hha I enzyme at 37°C for 5 hours. The digestion product was electrophoresed on a 5% MetaPhor agarose gel. DNA was stained with ethidium bromide and visualized by UV illumination. The pBR 322 plasmid digested by Hae III was used as a base-pair marker.

Statistical Analysis

The statistical significance of differences in lipoprotein concentration or composition at each postprandial time point compared with the fasting period was tested by ANOVA-ANOVA. All statistical analyses were performed on a PC computer using the BMDP program.

Results

Evolution of Plasma Lipids During Alimentary Lipemia

The effects of the test meal on postprandial plasma lipid parameters in normolipidemic subjects (n=13) are shown in Fig 1. Plasma TG levels peaked at 2 hours after meal intake (141 mg/dL versus 71 mg/dL at 2 hours and 0 hours, respectively, \(P<.0001\)). A progressive decrease in plasma TG levels followed, returning to baseline at 8 hours. In addition, plasma free cholesterol levels had increased by 20% (\(P<.0001\)) at 2 hours, whereas plasma phospholipid and CE concentrations were unchanged during the postprandial phase.

Concentration and Composition of Plasma TRL During Postprandial Lipemia

The evolution of total plasma TRL mass during postprandial lipemia is shown in Fig 2A. After meal intake, we observed a significant increase (2.7-fold) in total plasma TRL levels at 2 hours in comparison with the baseline value at 0 hours (\(P=.0001\)). Thereafter, plasma TRL concentrations returned to fasting levels at 8 hours after meal intake. Fig 2B represents the variation in concentration of each plasma TRL subfraction over the postprandial period. Maximal CM levels were attained at 2 hours (77±7 mg/dL), after which time they progressively decreased to 8 hours (19±3 mg/dL). The maximal postprandial concentration of VLDL1 (61±6 mg/dL) corresponded to that of CMs, but was broader, with a poorly defined peak, occurring over the period from 1 to 3 hours. No significant modification in the low levels (<5 mg/dL) of VLDL2 and IDL was observed during the postprandial period. Interestingly, CMs represented ~8% of total TRL mass at 0 time, whereas VLDL1 accounted for at least 80% of total TRL at this time point and thus represented the predominant TRL subfraction in the fasting state. By contrast, CMs represented 37% and 55% of total TRL mass at 1 hour and 2 hours, respectively. Simultaneously, the relative proportion of VLDL1 decreased (57% and 44% at 1 hour and 2 hours, respectively). Moreover, the absolute concentration and the relative proportion of CMs (19±3 mg/dL, 42%) remained elevated 8 hours postprandial, suggesting that the fasting state was not completely restored, even in these normolipidemic subjects.

The mean weight chemical compositions of each TRL subfraction (expressed as percent of the total TRL mass) during postprandial lipemia are presented in Table 3. CM (Sc>400 particles) isolated just before meal intake displayed a higher CE content (8.2%) and lower TG content (73.7%) than those
isolated during the postprandial phase (mean; 2.4% CE; 80.7% TG). These observations suggested that the fasting Sf>400 lipoproteins were closely equivalent to either CM remnants or to large VLDL remnant particles rather than newly secreted CMs. VLDL1 (Sf 60 to 400) and VLDL2 (Sf 20 to 60) represented two distinct subfractions of VLDL, as shown by the significant differences observed in their chemical composition. Thus, the relative proportion of TG in VLDL1 was 18% lower (P=.001) in comparison with VLDL2, whereas its protein and CE contents were significantly higher (+20% and +32%, respectively, P=.001). The meal intake induced no significant alteration in the chemical compositions of CMs, VLDL subfractions, and IDL at any point of the postprandial time course. This observation is consistent with the lack of variation in CE/TG ratio in each TRL subspecies. Nonetheless, each TRL subfraction displayed a tendency for the relative TG content to increase through the postprandial phase.

**TABLE 3. Chemical Composition of Plasma TRL in Normolipidemic Subjects During Postprandial State**

<table>
<thead>
<tr>
<th>Hours</th>
<th>Free Cholesterol</th>
<th>Cholesterol Ester</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
<th>Protein</th>
<th>CE/TG, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM, Sf &gt; 400</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>4.3±0.8</td>
<td>8.2±1.4</td>
<td>73.7±2.3</td>
<td>11.8±1.4</td>
<td>&lt;2</td>
<td>.111</td>
</tr>
<tr>
<td>1</td>
<td>1.2±0.2</td>
<td>2.2±0.3</td>
<td>84.0±1.2</td>
<td>10.7±0.9</td>
<td>&lt;2</td>
<td>.026*</td>
</tr>
<tr>
<td>2</td>
<td>0.7±0.1</td>
<td>1.3±0.1</td>
<td>78.9±1.3</td>
<td>17.1±1.2</td>
<td>&lt;2</td>
<td>.016*</td>
</tr>
<tr>
<td>3</td>
<td>1.1±0.2</td>
<td>1.9±0.3</td>
<td>81.4±0.6</td>
<td>13.7±0.7</td>
<td>&lt;2</td>
<td>.023*</td>
</tr>
<tr>
<td>4</td>
<td>1.9±0.4</td>
<td>3.6±0.8</td>
<td>79.3±1.2</td>
<td>13.3±0.9</td>
<td>&lt;2</td>
<td>.045*</td>
</tr>
<tr>
<td>8</td>
<td>1.6±0.5</td>
<td>2.8±0.9</td>
<td>80.1±1.6</td>
<td>13.4±0.7</td>
<td>&lt;2</td>
<td>.035*</td>
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<tr>
<td>VLDL 1, Sf 60-400</td>
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<tr>
<td>0</td>
<td>3.9±0.2</td>
<td>10.6±0.6</td>
<td>56.8±1.1</td>
<td>16.2±0.4</td>
<td>12.4±0.5</td>
<td>.187</td>
</tr>
<tr>
<td>1</td>
<td>3.7±0.2</td>
<td>10.0±0.3</td>
<td>58.3±0.8</td>
<td>16.2±0.3</td>
<td>11.7±0.8</td>
<td>.171</td>
</tr>
<tr>
<td>2</td>
<td>5.1±0.3</td>
<td>9.0±0.3</td>
<td>58.8±0.6</td>
<td>16.5±0.2</td>
<td>10.5±0.4</td>
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<tr>
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<td>17.0±0.2</td>
<td>10.4±0.5</td>
<td>.166</td>
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<tr>
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<td>5.2±0.3</td>
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<td>59.1±0.5</td>
<td>16.7±0.4</td>
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<tr>
<td>8</td>
<td>4.0±0.3</td>
<td>6.6±0.4†</td>
<td>62.0±0.7</td>
<td>15.6±0.5</td>
<td>11.8±0.8</td>
<td>.106†</td>
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<tr>
<td>VLDL 2, Sf 20-60</td>
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<tr>
<td>0</td>
<td>7.0±0.6</td>
<td>11.7±1.1</td>
<td>45.7±2.1</td>
<td>20.5±1.8</td>
<td>15.0±1.5</td>
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</tr>
<tr>
<td>1</td>
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<td>17.3±1.3</td>
<td>15.1±1.0</td>
<td>.237</td>
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<tr>
<td>2</td>
<td>6.8±0.5</td>
<td>12.0±1.2</td>
<td>48.4±1.2</td>
<td>17.3±1.1</td>
<td>15.4±1.1</td>
<td>.248</td>
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<tr>
<td>3</td>
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<td>12.5±1.2</td>
<td>48.8±1.7</td>
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<td>12.2±1.2</td>
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<tr>
<td>4</td>
<td>6.6±0.6</td>
<td>13.0±1.6</td>
<td>48.1±2.6</td>
<td>19.0±1.2</td>
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<td>9.0±1.2</td>
<td>13.2±2.3</td>
<td>49.7±2.2</td>
<td>18.5±2.1</td>
<td>9.6±0.8</td>
<td>.265</td>
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<td>IDL, Sf 12-20</td>
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<tr>
<td>0</td>
<td>8.6±0.6</td>
<td>16.4±1.5</td>
<td>35.5±2.1</td>
<td>17.6±0.7</td>
<td>21.9±1.5</td>
<td>.462</td>
</tr>
<tr>
<td>1</td>
<td>8.6±0.5</td>
<td>15.3±1.2</td>
<td>38.6±2.2</td>
<td>17.6±1.1</td>
<td>19.8±1.6</td>
<td>.396</td>
</tr>
<tr>
<td>2</td>
<td>8.9±0.5</td>
<td>15.8±1.2</td>
<td>39.1±1.8</td>
<td>17.8±1.3</td>
<td>18.4±2.0</td>
<td>.404</td>
</tr>
<tr>
<td>3</td>
<td>9.0±0.5</td>
<td>15.1±0.8</td>
<td>39.2±1.7</td>
<td>20.4±1.8</td>
<td>16.3±1.2</td>
<td>.385</td>
</tr>
<tr>
<td>4</td>
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<td>16.9±1.3</td>
<td>39.6±1.7</td>
<td>17.8±1.1</td>
<td>16.6±1.2</td>
<td>.427</td>
</tr>
<tr>
<td>8</td>
<td>10.0±0.7</td>
<td>16.8±1.2</td>
<td>36.3±2.3</td>
<td>20.1±1.6</td>
<td>16.7±1.6</td>
<td>.463</td>
</tr>
</tbody>
</table>

Values are means±SEM and are expressed as percent mean weight; n=13 subjects.
*P<.02 for all times vs zero time.
†P<.0002 for VLDL1 at 8 hours vs other time points.

meal intake, showed that the specific radioactivity of CE in apoB-containing lipoproteins increased (+18.2±1.5 cpm/µg CE), whereas those in HDL decreased (−21.0±7.0 cpm/µg CE) after 2 hours of incubation. These variations in CE specific radioactivities in acceptor and donor particles are consistent with a net mass transfer of CE from HDL to apoB-containing acceptor lipoproteins. It is important to note that the negative specific activity of HDL-CE is due to loss of CE occurring on incubation. Measurement of CE mass transfer from HDL to apoB-containing lipoproteins at 2 hours postprandially revealed no significant difference in the specific radioactivities of CE in apoB-containing lipoproteins (18.7±1.8 cpm/µg CE) and in HDL (−17.5±7.6 cpm/µg CE) in comparison with the baseline value. Moreover, throughout the postprandial phase, CE specific radioactivities in acceptor and donor particles were constant and ranged from 14 to 18 cpm/µg CE in apoB-containing lipoproteins and from −22 to −15 cpm/µg CE in HDL. The absence of significant modification in total CE transferred from HDL to apoB-containing lipoproteins observed at each time point of the postprandial phase indicated that no major variation in total plasma CETP activity occurred during the postprandial period. When individual acceptor particles within the apoB-containing lipoproteins were con-

**CE Mass Transfer From HDL to ApoB-Containing Lipoproteins During Alimentary Lipemia**

Determination of CE mass transfer from HDL to apoB-containing lipoproteins in normal fasting plasma, ie, before
sidered, we observed that both TRL and LDL acted as CE acceptors. Indeed, after 2 hours' incubation of either fasting or postprandial plasma, mean CE specific radioactivities in CM (55.2±10.9 cpm/µg CE), VLDL1 (44±2.5 cpm/µg CE), VLDL2 (38.4±2.9 cpm/µg CE), IDL (30.1±3.1 cpm/µg CE), and LDL (10.9±0.8 cpm/µg CE) were observed. The differences in specific radioactivities of CE observed between each apoB-containing lipoprotein reflect the wide range in particle content of CE and thus do not necessarily indicate a higher CE mass transfer from HDL to TRL. In addition, postprandial lipemia did not significantly modify the capacity of each apoB-containing lipoprotein species to accept CE from HDL, as shown by the quasi constant specific radioactivity of CE determined in each TRL subfraction at each time point of the postprandial period: specific radioactivities of CE ranged from 40 to 68 cpm/µg CE in CM, 40 to 47 cpm/µg CE in VLDL1, 33 to 40 cpm/µg CE in VLDL2, 26 to 36 cpm/µg CE in IDL, and 10 to 12 cpm/µg CE in LDL.

Rates of CE mass transfer from HDL to individual TRL subfractions and LDL over the postprandial phase are presented in Table 4. After meal consumption, no significant variation in the total rate of CE transfer from HDL to apoB-containing lipoproteins was observed. Indeed, over the postprandial period, minor variations (10%) in CE transfer rate were detected, but these variations did not attain significance. In agreement with our previous studies, in normolipidemic subjects, the rate of CE mass transfer to LDL (mean 65±5 µg CE transferred per hour per milliliter) exceeded that to the total TRL subfractions (22±6 µg CE transferred per hour per milliliter) by at least threefold and thus represents the major CE acceptor. Among TRLs, CMs accounted for at least 30% of the total CE transferred from HDL at 2 hours and 3 hours after meal intake, corresponding to a marked increase (10- to 15-fold) in particle mass. However, VLDL1 represented the major CE acceptor among TRL subfractions, accounting for 60% to 80% of total CE transferred from HDL to TRL at all time points. It is important to note that the lower proportion of CE transferred to VLDL1 corresponded to the predominance of CMs in plasma at 2 hours after meal intake. Correlation analysis indicated that a direct relationship existed between total plasma lipoprotein acceptor concentration and the net CE mass transferred from HDL (r=.955; P<.0001). A similar positive correlation was obtained when apoB-containing lipoproteins (without CMs) was considered (r=.959; P<.0001). By contrast, the quantitative features of CE transfer from HDL to CMs do not appear to be determined by their plasma concentration (r=.719; P=.1171).

When the total CE mass transferred from HDL to apoB-containing lipoproteins is expressed as a function of plasma lipoprotein concentration, the capacity of each lipoprotein subspecies to accept CE from HDL can be estimated (Fig 3). Thus, VLDL1, VLDL2, and IDL particles displayed a superior affinity for CE transferred from HDL in comparison with that of CM and LDL (VLDLs, IDL/CM ratio 4:1; P<.0005 and VLDLs, IDL/LDL ratio 2:1; P<.001, respectively). It is relevant that VLDL1 displayed an affinity for CE that was intermediate between those of CM and VLDL2. In addition, the affinity of each apoB-containing lipoprotein particle for CE was not modified by alimentary lipemia. Finally, the capacity of total apoB-containing lipoproteins to accept CE from HDL in postprandial plasma was not correlated with their relative TG content (r=−.101; P=.6047). Moreover, the affinities of both CMs and VLDL1 for CE were not correlated with their relative TG content (r=.102; P=.885, and r=.684;
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explained by differences in the composition of the oral meal. The apparently minor effect of intestinal absorption on the chemical composition of the postprandial lipoproteins reported here may be explained by differences in the composition of the oral meal. Indeed, in the present study, data were obtained after ingestion of a typical Western meal containing less than 0.5 g of fat per kilogram body weight in comparison with the more than 1 g of fat per kilogram body weight, which was consumed by normolipidemic subjects in earlier studies. 29

Physiological CE mass transfer was determined by measuring the increase in CE content in each apoB-containing lipoprotein subfraction after incubation of fresh plasma at 37°C. Total net mass transfer of CE from HDL to TRL and LDL was not modified during the postprandial phase, thereby suggesting that plasma CETP activity remained quasiconstant after consumption of a typical Western meal. Similar results have been recently reported by Lottenberg et al. 26 Contradictory findings involving a twofold to threefold increase in postprandial plasma CETP activity have been reported previously by others. 23,24 These apparently conflicting data probably reflect differences in the type of meal consumed, as well as in the amount of fat and/or of cholesterol ingested. Indeed, Tall et al 23 administered a pint of heavy cream containing 135 g fat in comparison with the solid meal composed of 66 g fat in the present study. Similarly, Castro and Fielding 23 demonstrated a 1.7-fold increase in plasma CETP activity during postprandial lipemia induced by an oral fat load containing 67 g fat and 600 mg cholesterol, which corresponds to a fourfold higher cholesterol intake compared with the current report (142 mg cholesterol). Using the indirect assay for CETP mass, 29 we did not observe any significant modification in plasma CETP levels after absorption of the test meal. Similar observations were made by Lottenberg et al 26 in both normolipidemic and diabetic subjects after a 50-g fat load. By contrast, Marcel et al 27 reported a minor increase (10% to 12%) in CETP mass in response to intake of a fatty meal (80 g fat) in five normolipidemic subjects. In addition, Dullaart et al 41 demonstrated a minor increase (5%) in plasma CE transfer activity using an exogenous CE donor and acceptor particle system after a 140 g fat load. It is of interest that increase in plasma CETP activity 23,24 as well as in plasma CETP mass 27 during postprandial lipemia in normolipidemic subjects occurred after a high cholesterol meal in which the cholesterol content was approximately 600 mg. As shown by studies in human-CETP transgenic mice, a high cholesterol diet induces increase of transcription of the CETP gene. 45 Such upregulation of CETP gene expression in response to cholesterol requires the presence of natural flanking regions of the human CETP gene. 45 Moreover, it has been proposed that the elevated plasma CETP levels observed in endogenous hyperlipidemia result in increased CETP gene expression by a mechanism similar to that involving dietary cholesterol. 46 These observations strongly suggest that elevation in CETP mass or activity during postprandial lipemia, as reported by others, 27,41 is above all the consequence of the amount of exogenous cholesterol consumed. In addition, the increase in plasma CETP levels observed by others 27,41 ranged from 5% to 12%, thereby suggesting heterogeneity in postprandial response in terms of increase in plasma CETP levels within normolipidemic subjects. Similar conclusions may be reached from analysis of data with respect to changes in plasma CETP activity during postprandial lipemia. Indeed, Castro and Fielding 23 reported a}

Discussion

For the first time, we describe the effects of a typical Western meal (1200 kcal, 14% protein, 38% carbohydrate, and 48% fat, monounsaturated/polyunsaturated ratio 4:1) on the quantitative and qualitative features of CE transfer from HDL to TRL and LDL in normolipidemic young men; this meal was distinguished by its content of typically consumed solid foodstuffs rather than a liquid formula. On a quantitative basis, VLDL1 (Sf 60 to 400) particles, but not CMs (Sf>400), represented the major CE acceptors among TRL during alimentary lipemia in these subjects. On a qualitative basis, however, VLDL2 (Sf 20 to 60) and IDL (Sf 12 to 20) displayed the highest relative capacity to accept CE. By contrast, we have established that CM particles are minor CE acceptors during postprandial lipemia.

Data presented here show that postprandial lipemia induced by a solid mixed meal is characterized by a twofold increase in plasma TG levels at 2 hours. The magnitude of the postprandial lipemia is positively correlated with their relative TG content (r=0.88; P<0.0001).

CETP-Dependent CE Transfer During Postprandial Lipemia

CETP activity was assessed before meal intake and at 2 hours and 8 hours thereafter by use of a system containing exogenous HDL donors and excess exogenous VLDL/LDL acceptor particles isolated from a control plasma (see “Methods”). The mean transfer of CE from HDL donor particles in normolipidemic plasmas was 15±4% before meal intake and 13±3% and 16±6% at 2 hours and 8 hours thereafter, respectively. Thus, postprandial lipemia did not induce any significant variation in CETP-dependent transfer activity, indicating that no modification in plasma CETP mass levels occurred during alimentary lipemia.
mean twofold increase in plasma CETP activity after feeding of an elevated amount of cholesterol (600 mg) to 10 normolipidemic subjects who could be divided into two distinct subpopulations. In five subjects, postprandial lipemia was associated with a minor (1.4-fold) increase in plasma CETP activity, whereas the five remaining subjects displayed a large (3.3-fold) elevation. The observed differences in the magnitude of the increment in plasma CETP activity during postprandial lipemia might involve variability in the upregulation of CETP gene expression induced by cholesterol in the normolipidemic population.

Postprandial lipemia did not induce any significant modification in the typical redistribution of CE in fasting normolipidemic subjects characterized by preferential CE transfer from HDL to LDL, the latter particle accepting threefold to fourfold more CE than TRL over the postprandial phase. The present rates of physiological CE mass transfer from HDL to apoB-containing lipoproteins are consistent with those previously reported by us.\(^{21,25}\) Surprisingly, the postprandial period was not characterized by an accelerated total CE transfer to TRL. Indeed, elevated total plasma TRL levels have been shown to increase CE mass transfer from HDL to apoB-containing lipoproteins.\(^{21,25}\) Moreover, according to the observation of Mann et al.\(^{21}\) who reported elevated CETP activity in hypertriglyceridemic subjects due to a marked (fourfold) increase in VLDL levels, postprandial lipemia would be expected to be associated with an enhanced CE transfer from HDL to TRL. Such observations suggest that triglyceridemia observed during the postprandial period is distinct from that observed in hypertriglyceridemia associated with dyslipoproteinemia. The structure and composition of plasma TRL particles, after their delivery in plasma, appear therefore to be dramatically different in these distinct metabolic states, and thus TRL probably does not possess identical capacity to interact with CETP to mediate CE transfer.

Further analysis of CE transfer rates from HDL to each TRL subspecies revealed that discrete but significant variation in CE redistribution occurred during postprandial lipemia. Quantitatively, VLDL1 was the major CE particle acceptor among TRL subspecies throughout the postprandial phase and even in the presence of elevated concentrations (77 mg/dL) of CMs. The most remarkable finding in the present study concerns the observation that CMs do not represent the preferential CE acceptor during postprandial lipemia, at least not under the present nutritional conditions. Several mechanisms may explain the minor participation of CM particles in the CE transfer reaction mediated by CETP during postprandial lipemia. First, CE transfer between plasma lipoproteins mediated by CETP may represent a rapid and dynamic phenomenon in vivo. Thus, immediately after CM secretion by the intestine and their delivery to the circulation via the lymphatic system, they may rapidly interact with CETP to accept CE from HDL. Moreover, it has been shown that HDL particles pass from the circulation to the intestinal lymphatics and that most of the plasma HDL-CE is subsequently transferred to the lymph.\(^ {47}\) Lipid transfer proteins may infiltrate the intestinal lymphatics directly from the circulation as a result of their low molecular weight or in association with HDL.\(^ {47}\) Consequently, plasma obtained from normolipidemic subjects during the postprandial period may contain CM particles that have in part already been involved in in vivo CE transfer reactions. Second, it has been shown that the formation of CETP-lipoprotein complexes is the direct result of an intricate balance in the electrostatic attraction or repulsion between donor or acceptor lipoprotein particles and CETP.\(^{48}\) As CMs display a significantly lower electronegative charge than VLDL, CETP-CM interactions might be insufficient, resulting in the low CE transfer from HDL to CMs that we observed.

The apparently low CE transfer rates from HDL to CMs seen in the present study may result from a higher relative capacity of VLDL particles to interact with CETP. Nonesterified fatty acids bound to the surface of lipolyzed VLDL have been shown to stimulate CETP-mediated CE transfer from HDL to VLDL and probably result from an increased electrostatic interaction between CETP and the negative charges of nonesterified fatty acids.\(^ {48}\) In addition, high concentrations of nonesterified fatty acids may induce inhibition of CETP activity as a result of an excessive affinity of CETP for lipoprotein substrates, thereby resulting in a reduced mobility of CETP between plasma lipoproteins.\(^ {48}\) Such a mechanism might explain the low affinity of CM for CE, as well as the relative lower affinity for CE observed in VLDL1 in comparison with VLDL2. Moreover, in accordance with the results obtained in the present study, Bagdade et al.\(^ {20}\) have previously described an accelerated CE transfer in plasma from hypercholesterolemic subjects due to a dysfunction of the VLDL1 subfraction rather than an elevation of plasma CETP levels. The latter authors identified the VLDL1 subfraction isolated from hypercholesterolemic patients as an active CE acceptor in comparison with the other VLDL subfractions. This increased capacity of VLDL1 to stimulate CE transfer was demonstrated to be related to the abnormal chemical composition of these particles, ie, an increased CE/TG ratio or a possible enrichment of VLDL1 with lipolytic products or free fatty acids.

It is of interest to note that VLDL2 particles were less active in reactions of CE transfer than VLDL1, as shown by the significantly lower CE mass transfer rate from HDL to VLDL2 compared with that from HDL to VLDL1 (Table 4). Among the multiple factors known to influence CE transfer, the relative proportion of acceptor lipoprotein particles has been shown to play a determinant role.\(^ {15,26,38,49}\) Thus, the reduced CE mass transfer rate from HDL to VLDL2 that we observed may result from the low plasma levels of VLDL2 (<3 mg/dL) detected during the postprandial state despite the high affinity of these particles for CE.

In conclusion, elevated TRL levels observed during postprandial lipemia in normolipidemic subjects are not associated with an enhanced CE transfer mediated by CETP, as predicted from the described mechanism of plasma CETP transfer in hypertriglyceridemia.\(^ {21}\) Quantitatively, VLDL1, but not CMs, represents the major CE acceptor among TRL during alimentary lipemia in normal plasma, whereas, qualitatively, VLDL2 and IDL exhibited the highest particle affinity for CE. By contrast with fasting VLDL from normolipidemic subjects,\(^ {15,36}\) the affinities of postprandial CM and VLDL1 particles are not positively correlated with their relative TG content. These results suggest that an optimal size, surface structure, and core content of TG (48%), similar to that observed in VLDL2, may be required in CE acceptor particles for a lipoprotein-CETP interaction.
interaction of high affinity. Furthermore, CETP promotes a massive redistribution of CE from HDL to LDL (75%) even in the postprandial phase. Thus, we hypothesize that plasma CETP activity assures an antiatherogenic role in normolipidemia by promoting active CE removal from the circulation, in the form of the apoB-containing lipoproteins via the LDL receptor pathway.

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Preferential Cholesteryl Ester Acceptors Among Triglyceride-Rich Lipoproteins During Alimentary Lipemia in Normolipidemic Subjects
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