Preferential Cholesteryl Ester Acceptors Among the LDL Subspecies of Subjects With Familial Hypercholesterolemia

Maryse Guérin, Peter J. Dolphin, M. John Chapman

Abstract Elevated cholesteryl ester transfer protein–mediated transfer of cholesteryl ester (CE) from high-density lipoprotein (HDL) to low-density lipoprotein (LDL) may contribute to the atherogenicity of LDL in subjects with familial hypercholesterolemia (FH). To identify the major CE acceptors among LDL subspecies, we investigated the qualitative and quantitative features of CE transfer and exchange to LDL on incubation of plasma under physiological conditions. LDL subfractions were fractionated by density-gradient ultracentrifugation. Both mass transfer and exchange of HDL CE to and with very-low-density lipoprotein plus intermediate-density lipoprotein and LDL were linear for the first 6 hours of incubation. Thereafter mass transfer ceased, but exchange continued at a comparable rate. The rate of CE mass transfer to apolipoprotein B–containing lipoproteins was significantly enhanced in heterozygous FH subjects compared with normolipidemic individuals (91.6±28.2 versus 52.9±19.6 CE/h per milliliter plasma, FH versus normal subjects, P<.02). In FH subjects the predominant LDL subspecies (LDL 3 and 4, d=1.029 to 1.050 g/mL) accounted for 59.7±9.2% of the total CE transferred to LDL from HDL. By contrast, expression of mass transfer relative to the mass of each lipoprotein acceptor showed the triglyceride (TG)-rich (10.7% to 17.3%), light LDL subspecies (LDL 1 and 2, d=1.019 to 1.029 g/mL) to represent the preferential CE acceptors (LDL 1 and 2, 95.8 to 136.5 μg CE/mg LDL mass; LDL 3 through 5 [d=1.029 to 1.063 g/mL], 47.1 to 64.1 μg CE/mg LDL mass). In comparison, TG-rich (11.4%) LDL-1 in normolipidemic subjects was the most active acceptor of CE from HDL (LDL-1, 99.4±44.3 and LDL 2 through 5, 52.7 to 69.2 μg CE/mg LDL mass). The capacity of LDL subspecies to accept CE from HDL was correlated with the relative content of TG in the LDL (r=.5, P<.0001); the TG-rich particles were the preferred CE acceptors. The quantitative features of CE transfer to LDL subspecies are determined principally by the concentration of acceptor particles (r=.75, P<.0001), while the TG content of LDL appears to be the major qualitative determinant of CE transfer.

Key Words • cholesteryl ester exchange • cholesteryl ester mass transfer • cholesteryl ester transfer protein • familial hypercholesterolemia • LDL particle subspecies • cholesteryl ester acceptors

Cholesteryl ester transfer protein (CETP) plays a key role in the reverse cholesterol transport system by mediating the transfer of cholesteryl ester (CE) from high-density lipoprotein (HDL) to the apolipoprotein (apo) B–containing lipoproteins, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and low-density lipoprotein (LDL). CETP transfer is modulated by the composition and concentration of plasma lipoproteins. For example, lipid transfer mediated by CETP is influenced by the concentration of unesterified cholesterol in the donor and acceptor lipoproteins, and the rate of the net mass transfer of CE from HDL to VLDL or LDL is correlated with elevated concentrations of VLDL and LDL, respectively. CETP transfer is increased in the plasma of hypercholesterolemic patients. This augmentation of plasma CETP activity is correlated with increased plasma concentrations of CETP.

In plasma, LDL is distributed as a continuum of particles over the density range between d=1.019 and d=1.063 g/mL. In this density interval, multiple LDL subspecies possessing different physical, chemical, hydrodynamic, immunological, and structural properties have been described in the plasma of normolipidemic and hypercholesterolemic subjects. In this study, we determined both exchange and net mass transfer of CE from HDL to LDL fractions by a methodology involving the simultaneous determination of CE radioactivity and mass in both normolipidemic and hypercholesterolemic subjects. We investigated possible relations between CE transfer and the establishment of the LDL profile in human plasma. Moreover, we identified which LDL subspecies may represent the preferential CE acceptors in patients with familial hypercholesterolemia (FH) compared with those in normolipidemic subjects. Elevated CE transfer to preferred LDL subspecies may represent a determinant factor in promoting the atherogenic LDL profile generally observed in FH patients.

Methods

Subjects and Blood Samples

Approval by the Human Subjects Review Committee of the hospital and informed consent from all participants was obtained. Six heterozygous FH subjects (4 men and 2 women)
with a total plasma cholesterol of >300 mg/dL were selected and were taken off all lipid-modifying drugs for 6 weeks before blood sampling. Two of the male subjects were aged 19 and 24 years with a body mass index (BMI) of 21.4 and 21.4, respectively. The remaining 4 patients were between 53 and 65 years of age with BMIs between 21.4 and 26.2. The mean BMI for all the heterozygous FH subjects was 23.0±2.2. Five normolipidemic subjects (4 men and 1 woman) displayed cholesterol levels of <250 mg/dL and ranged in age from 26 to 50 years old. Their mean BMI was 21.4±1.4. Blood samples (up to 40 mL) were obtained by venipuncture after an overnight fast and placed in EDTA-containing tubes (final concentration, 1 mg/mL). Plasma was separated by low-speed centrifugation at 4°C. EDTA (0.1 g/L), NaN₃ (0.01%), and gentamicin (0.05%) were added to the plasma, which was maintained at 4°C. All subjects in this study had low levels of lipoprotein(a) (<12 mg/dL) and were nonsmokers.

Measurement of Exchange and Net Mass Transfer of CE

CE exchange and net mass transfer were determined in whole plasma from each normolipidemic and hypercholesterolemic subject. Briefly, radiolabeled HDL was obtained from the d>1.063 g/mL fraction of 1 mL plasma isolated by ultracentrifugation at 541 000 g/min at 10°C by using a Beckman TL100 centrifuge as follows. The d>1.063 g/mL fraction was incubated for a total of 18 hours at 37°C in the presence of 4 μL [l,2,6,7-3H]cholesterol (specific activity, 71 Ci/mmol). This quantity corresponds to 20.8 ng of free cholesterol added as labeled CE. Moreover, the chemical composition of the radiolabeled HDL was not significantly different from the unlabeled HDL present in the individual patient’s plasma (data not shown). Several 1-mL aliquots of each subject’s plasma containing radiolabeled HDL (equivalent to 1% of the total HDL CE mass present in 1 mL of the subject’s plasma) were incubated at 37°C for up to 18 hours in the presence of 150 mmol/L sodium bromide and centrifuging at 541 000 g/min at 10°C. The radiolabeled HDL was dialyzed against phosphate-buffered saline buffer. A typical radiolabeled HDL preparation had a specific radioactivity of approximately 5000 cpm/nmol (free cholesterol). This activity was adjusted to 1.21 g/mL by adding dry solid potassium bromide and centrifuging at 541 000 g/min at 10°C. The radioactive content and lipid density interval of each subject’s plasma containing radiolabeled HDL (equivalent to that of the total HDL CE mass present in 1 mL of the subject’s plasma) was incubated at 37°C for up to 18 hours in the presence of 150 mmol/L sodium bromide and centrifuging at 541 000 g/min at 10°C. For each incubation time, lipoproteins present within the VLDL fraction from the gradient, the first two gradient fractions were pooled and represent a relative proportion of HDL was significantly decreased in FH patients compared with normolipidemic subjects (28.5±5.2% and 42.8±7.8% respectively; P=.0006). Under these conditions, each group had distinct mass ratios of LDL/HDL; 1.2 in normolipidemic and 2.3 in FH subjects. The mass distribution profiles of LDL subfractions differ these conditions, each group had distinct mass ratios of LDL/HDL; 1.2 in normolipidemic and 2.3 in FH subjects. The mass distribution profiles of LDL subfractions.

<table>
<thead>
<tr>
<th>TABLE 1. Plasma Lipid and Apolipoprotein Levels</th>
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<tbody>
<tr>
<td>NL, mg/dL</td>
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<tr>
<td>Total cholesterol</td>
</tr>
<tr>
<td>Free cholesterol</td>
</tr>
<tr>
<td>Cholesterol esters</td>
</tr>
<tr>
<td>Triglycerides</td>
</tr>
<tr>
<td>Phospholipids</td>
</tr>
<tr>
<td>VLDL+IDL cholesterol</td>
</tr>
<tr>
<td>LDL cholesterol</td>
</tr>
<tr>
<td>HDL cholesterol</td>
</tr>
<tr>
<td>ApoB</td>
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<tr>
<td>ApoA-I</td>
</tr>
</tbody>
</table>

NL indicates normolipidemic subjects (n=5); FH, heterozygous familial hypercholesterolemic patients (n=6); VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Apo, apolipoprotein. Values are mean±SD. VLDL+IDL cholesterol, LDL cholesterol, and HDL cholesterol were determined by analysis of VLDL+IDL (d<1.019 g/mL), LDL (d=1.019-1.063 g/mL), and HDL (d=1.063-1.145 g/mL), respectively, following isolation by density-gradient ultracentrifugation (see "Methods"). *P<.005, **P<.05, vs normolipidemic subjects.

Lipid and Protein Analyses

Lipids in plasma or in isolated lipoprotein fractions were quantified enzymatically by using Bio-Merieux kits (model 69280) for total cholesterol, free cholesterol, triglyceride (TG), and phospholipid levels. CE mass was calculated as 1.67x(free cholesterol mass). Protein was measured by the method of Lowry et al using bovine serum albumin as the standard. Lipoprotein mass corresponded to the sum of the mass of the individual components (free cholesterol, CE, TG, phospholipids, and protein) for each lipoprotein fraction.

Measurement of LCAT Activity

LCAT activity was measured by nonradioactive endogenous cholesterol esterification methods in which the decrease in plasma free cholesterol content with time upon incubation at 37°C was determined.

Statistical Analysis

Nonparametric analysis was performed to evaluate the significance of differences between sample groups according to the method of Mann and Whitney by using the STATVIEW program for Apple Macintosh. Data in Table 4 and in Fig 1 were transformed into natural logarithms before nonparametric analysis.

Results

Plasma Lipoprotein Profile and Chemical Composition

Plasma lipid and apolipoprotein levels in both normolipidemic and FH subjects are shown in Table 1. No significant difference was observed in plasma VLDL+IDL levels between each group of subjects. By contrast, the mean total LDL cholesterol concentration was twofold higher in FH patients compared with normolipidemic subjects (240.4±68.3 and 129.2±44.1 mg/dL, respectively; P<.0005). Moreover, despite the observation that plasma HDL concentrations were similar in each group, the relative proportion of HDL was significantly decreased in FH patients compared with normolipidemic subjects (28.5±5.2% and 42.8±7.8% respectively; P=.0006). Under these conditions, each group had distinct mass ratios of LDL/HDL; 1.2 in normolipidemic and 2.3 in FH subjects. The mass distribution profiles of LDL subfractions.
tions observed in both groups were quite distinct (Table 2). The most abundant LDL subfractions in normolipidemic subjects were LDL-2 and LDL-3 (d=1.023 to 1.039 g/mL), which corresponded to 64±4% of the total LDL mass. By contrast, the LDL profile in FH patients displayed significant increases in the masses of the LDL-3, LDL-4 (d=1.039 to 1.050 g/mL), and LDL-5 (d=1.050 to 1.063 g/mL) subfractions (P<.005).

Moreover, the LDL-3 and LDL-4 subfractions from FH patients contained a slightly elevated proportion of CE (40.0±3.2% and 39.2±2.8%, respectively) compared with their normolipidemic counterparts (37.3±2.7%, P<.05 and 35.2±3.3%, P<.005, respectively; data not shown). These results were consistent with those of earlier studies.17,21,22

**Measurement of CE Exchange and Mass Transfer in Whole Plasma**

CE exchange was evaluated in each subject by measuring the rate of radiolabeled CE transfer from HDL to apoB-containing lipoproteins during incubation of whole plasma at 37°C for up to 18 hours. The Figure shows a progressive increase of radiolabeled CE content in the apoB-containing lipoproteins (VLDL, IDL+LDL) as a function of the period of incubation in both normolipidemic and heterozygous FH subjects. CE radioactivity increased linearly during the first 4 through 6 hours of the incubation and in normal subjects paralleled the increase in CE mass. Thus, in these individuals, calculation of mass transfer based on the specific radioactivity of HDL CE reflects, with a fair degree of accuracy, the actual mass transfer for the first 6 hours of incubation. In the heterozygous FH subjects, in contrast, the mass transfer calculated from the HDL CE specific radioactivity was somewhat greater than the chemically determined mass transfer over the first 6 hours of incubation (Figure). This indicates the presence of a significant exchange component in addition to net mass transfer over this period.

After 6 hours of incubation, net mass transfer of CE to the apoB-containing lipoproteins in both subject groups ceased; however, radioactive CE continued to increase in the VLDL, IDL+LDL fraction, indicating a continuing exchange of lipid between the lipoprotein particles. For normolipidemic and mildly hypertriglyceridemic subjects,20 the latter phase of incubation (from 6 hours through 18 hours) reflected only CE exchange between the donor and acceptor particles. The observed linear increment in both net mass transfer and radioactivity over the first 6 hours of incubation permitted calculation of both the rate of CE mass transfer from HDL to the apoB-containing lipoproteins (VLDL, IDL+LDL) and the rate of CE exchange between these particles (Table 3). The increase in CE mass calculated from the specific radioactivity of the HDL CE reflects both mass transfer and exchange. The difference between these values and that determined by enzymatic analysis represents the differences observed in both groups were quite distinct (Table 2). The most abundant LDL subfractions in normolipidemic subjects were LDL-2 and LDL-3 (d=1.023 to 1.039 g/mL), which corresponded to 64±4% of the total LDL mass. By contrast, the LDL profile in FH patients displayed significant increases in the masses of the LDL-3, LDL-4 (d=1.039 to 1.050 g/mL), and LDL-5 (d=1.050 to 1.063 g/mL) subfractions (P<.005).

## Table 2. Concentrations of Plasma LDL Subfractions

<table>
<thead>
<tr>
<th>LDL Subfractions</th>
<th>Density, g/mL</th>
<th>NL, mg/dL</th>
<th>FH, mg/dL</th>
<th>NL, %</th>
<th>FH, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-1</td>
<td>1.019-1.023</td>
<td>44±16</td>
<td>44±25</td>
<td>13±5</td>
<td>6±3†</td>
</tr>
<tr>
<td>LDL-2</td>
<td>1.023-1.029</td>
<td>114±34</td>
<td>100±68</td>
<td>32±8</td>
<td>13±8†</td>
</tr>
<tr>
<td>LDL-3</td>
<td>1.029-1.039</td>
<td>112±33</td>
<td>315±114*</td>
<td>32±8</td>
<td>41±6*</td>
</tr>
<tr>
<td>LDL-4</td>
<td>1.039-1.050</td>
<td>52±18</td>
<td>227±97†</td>
<td>15±5</td>
<td>30±10†</td>
</tr>
<tr>
<td>LDL-5</td>
<td>1.050-1.063</td>
<td>27±6</td>
<td>64±23*</td>
<td>8±1</td>
<td>9±3</td>
</tr>
</tbody>
</table>

**Normal Subjects**

![Line graph showing time course of cholesterol ester (CE) transfer from high-density lipoprotein (HDL) to the apolipoprotein B-containing lipoproteins in normal subjects (dotted line) and heterozygous FH subjects (solid line). The incubation time-dependent increase in CE mass in VLDL and LDL calculated as a function of the HDL CE specific radioactivity at each time point (---). The incubation time-dependent increase in CE mass in VLDL and LDL was determined as described in "Methods."](http://atvb.ahajournals.org/)

**Heterozygous FH Subjects**

![Line graph showing time course of cholesterol ester (CE) transfer from high-density lipoprotein (HDL) to the apolipoprotein B-containing lipoproteins in heterozygous FH subjects. The incubation time-dependent increase in CE mass in VLDL and LDL calculated as a function of the HDL CE specific radioactivity at each time point (---). The incubation time-dependent increase in CE mass in VLDL and LDL was determined as described in "Methods."](http://atvb.ahajournals.org/)

*P<.05, **P<.002 vs normolipidemic subjects.
exchange component. CE mass transfer to VLDL, IDL+LDL in terms of the overall quantity (Figure) and the rate of transfer (Table 3) was significantly higher in the plasma of heterozygous FH patients than in normolipidemic subjects. Similarly, the rate of CE exchange between HDL and the apoB-containing lipoproteins over the first 6 hours of incubation was also significantly higher in the heterozygous FH subjects than in the normal subjects. These observations could not be attributed to any differences in plasma LCAT activity between the two subject groups (92.5 ±44.3 for normal subjects and 83.6 ±31.6 nmol CE formed/h per milliliter for FH subjects). The plasma VLDL+IDL concentrations were also similar in both groups (Table 1) and accounted for 26.0 ±6.8% of the total mass transfer to apoB-containing lipoproteins in normal subjects and 13.5 ±7.3% in the heterozygous FH patients. Table 3 also shows that the LDL fraction in both normal and heterozygous FH subjects accounts for the majority of the net mass transfer of CE from HDL in plasma over the first 6 hours of incubation. This lipoprotein fraction is also responsible for the CE mass transfer to the LDL-3 and LDL-4 subfractions. The quantitative features of CE transfer from HDL to LDL sub species appear to be determined principally by the concentration of acceptor particles.

### Table 3. Rates of CE Exchange and Transfer Between HDL and Apolipoprotein B–Containing Lipoproteins

<table>
<thead>
<tr>
<th>LDL Subfractions</th>
<th>Normal</th>
<th>Heterozygous FH</th>
<th>Normal</th>
<th>Heterozygous FH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass transfer</td>
<td>53±20</td>
<td>92±26*</td>
<td>40±18</td>
<td>72±26*</td>
</tr>
<tr>
<td>Transfer+exchange</td>
<td>65±25</td>
<td>130±46†</td>
<td>64±39</td>
<td>113±49</td>
</tr>
</tbody>
</table>

*CE indicates cholesteryl ester; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; and FH, familial hypercholesterolemia. Rates of exchange and transfer were estimated over the first 6 hours of incubation using the gradients of the slopes of the lines obtained when data for each subject were plotted as shown in the Figure. Each value represents mean±SD for five subjects. The interassay coefficients of variation for the transfer assay were <5%. Values are micrograms CE per hour per milliliter plasma.

**P<.05, †P<.02 vs normal subjects after logarithmic transformation of data.**

Net Mass Transfer of CE From HDL to LDL Subfractions

The CE mass content of each LDL subfraction was determined as a function of the period of incubation after fractionation of the plasmas by density-gradient ultracentrifugation. The total increase in CE mass determined in each LDL subfraction in both normolipidemic and FH subjects is shown in Table 4. On a quantitative basis, no significant difference of CE mass increase in the light LDL subfractions, LDL-1 (d=1.019 to 1.023 g/mL) and LDL-2 (d=1.023 to 1.029 g/mL), was observed between normolipidemic and FH subjects. Net mass transfer of CE in the denser LDL subfractions, LDL-3 (d=1.029 to 1.039 g/mL), LDL-4 (d=1.039 to 1.050 g/mL), and LDL-5 (d=1.050 to 1.063 g/mL), of FH patients was significantly greater than in their normolipidemic counterparts (P<.02). In both subject groups, the predominant LDL subfractions of intermediate density, LDL-2, LDL-3, and LDL-4, accounted for 75.1% to 78.4% of the total CE transferred from HDL to LDL. However, in FH patients 61.5% of the CE was transferred to the LDL-3 and LDL-4 subfractions. The quantitative features of CE transfer from HDL to LDL subspecies appear to be determined principally by the concentration of acceptor particles (r= .75, P<.0001).

### Table 4. Net Mass Transfer of Cholesteryl Ester From HDL to LDL Subspecies

<table>
<thead>
<tr>
<th>LDL Subfractions</th>
<th>Density, g/mL</th>
<th>Total CE Transferred, µg/mL plasma</th>
<th>CE Transferred, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NL</td>
<td>FH</td>
</tr>
<tr>
<td>LDL-1</td>
<td>1.019-1.023</td>
<td>34±27</td>
<td>41±15</td>
</tr>
<tr>
<td>LDL-2</td>
<td>1.023-1.029</td>
<td>58±42</td>
<td>70±30</td>
</tr>
<tr>
<td>LDL-3</td>
<td>1.029-1.039</td>
<td>53±19</td>
<td>142±76*</td>
</tr>
<tr>
<td>LDL-4</td>
<td>1.039-1.050</td>
<td>41±22</td>
<td>111±42†</td>
</tr>
<tr>
<td>LDL-5</td>
<td>1.050-1.063</td>
<td>16±5</td>
<td>47±23†</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; CE, cholesteryl ester; NL, normolipidemic subjects (n=5); and FH, heterozygous familial hypercholesterolemia patients (n=6). LDL subfractions were isolated by density-gradient ultracentrifugation. Net mass transfer of CE from HDL to LDL subfractions was determined following incubation of whole plasma (see "Methods"). Percent CE transferred represents the proportion (as percent) of total CE transferred from HDL to each LDL subfraction.

*P<.03, †P<.01 vs normolipidemic subjects.
LDL-4 from FH patients accepted up to 59.7% of total major CE acceptors in both subject groups. LDL-3 and LDL-1 in FH patients represented the LDL subfractions of higher density (LDL fractions 3 through 5, 47.1 ± 64.1 versus LDL-1, 136.5 ± 40.8 μg CE/mg LDL mass, P < .0005, and LDL-2, 94.8 ± 66.8 μg CE/mg LDL mass, P < .05). Comparison of each LDL subfraction in both groups for CE transfer was determined by measuring the increase in μg CE/mg LDL mass, P < .05). In FH subjects, both the LDL-1 and LDL-2 subfractions were active CE acceptors compared with the LDL subfractions of higher density (LDL fractions 3 through 5, 47.1 ± 64.1 versus LDL-1, 136.5 ± 40.8 μg CE/mg LDL mass, P < .0005, and LDL-2, 94.8 ± 66.8 μg CE/mg LDL mass, P < .05). Comparison of each LDL subfraction in both groups for their ability to accept CE from HDL showed that the LDL-1 fraction in normolipidemic subjects and the LDL-2 in FH patients displayed a similar affinity for CE. The ability of LDL subtypes to accept CE from HDL was positively correlated with their relative TG content (r = 5, P < .0001, n = 50).

**Discussion**

We determined the quantitative and qualitative features of CE exchange and transfer from HDL to the apoB-containing lipoproteins in both normolipidemic and heterozygous FH subjects by using a new methodology. We also identified and compared the major CE acceptors among the LDL subspecies in these individuals. Most significantly, LDL-1 in normolipidemic subjects and LDL-1 and LDL-2 in FH patients represented the preferential CE acceptors on a qualitative basis. In addition, a significant positive correlation was observed between net mass transfer of CE from HDL to LDL subspecies and the mass of each lipoprotein subfraction in both subject groups. Furthermore, the capacity of LDL subtypes to accept CE from HDL was correlated with the relative TG content of the lipoprotein acceptors. Finally, plasma CE mass transfer and exchange was significantly increased in FH patients compared with normolipidemic subjects.10,11,24

CE transfer was determined by measuring the increase of CE mass in each LDL subfraction isolated by density-gradient ultracentrifugation after plasma incubation at 37°C. Total net CE mass transfer of CE from HDL to LDL particles was twofold higher in FH patients than in normolipidemic subjects (453.5 ± 119.5 versus 203.9 ± 94.5 μg CE transferred, respectively). LDLs of intermediate density (LDL-2 through LDL-4) were quantitatively the major CE acceptors in both subject groups. LDL-3 and LDL-4 from FH patients accepted up to 59.7% of total CE transferred from HDL to LDL. The normolipidemic group exhibited an LDL/HDL ratio significantly lower than the FH group (1.4 ± 0.4 versus 2.4 ± 0.7, respectively, P < .0005). However, no differences were observed in the VLDL/HDL ratios in these two subject groups (0.15 ± 0.07 versus 0.14 ± 0.05 in normolipidemic subjects and FH patients, respectively). In mildly hypertriglyceridemic subjects with elevated VLDL/HDL ratios, the net mass transfer of CE from HDL to VLDL is twofold higher than normolipidemic subjects.20 In this case, the ability of VLDL to accept CE from HDL was the same in both groups of subjects when expressed on a per particle basis. These results suggested that the relative proportions of donor and acceptor particles present in plasma are significant determining factors of CE transfer activity.

Unesterified cholesterol has a potent role in regulating lipid transfer mediated by CETP.26 Dullaart et al27 studied the effect of the composition of different apoB-containing lipoproteins on their ability to accept CE from HDL and showed that the unesterified cholesterol/phospholipid molar ratio affects the CE transfer rate to VLDL. These studies indicate a role of the surface of VLDL particles in the CE transfer process. Our chemical compositional analyses of native LDL subfractions showed that LDL-3 and LDL-4 from heterozygous FH patients displayed a slight elevation of their CE content compared with LDL subfractions from normolipidemic subjects. However, no significant difference in the unesterified cholesterol/phospholipid ratio was noted between LDL fractions isolated from either group of individuals in this study. In contrast, a correlation did exist between the relative TG content of the LDL subtypes and their qualitative ability to accept HDL CE. Thus, we conclude that the quantitative difference in CE mass transferred to each LDL subfraction is most probably strongly related to its relative concentrations, whereas qualitative difference in the acceptor capacity between these particles is related to their relative TG contents.

McPherson et al11 show that CETP activity is positively correlated with plasma CETP mass, but there is no direct evidence indicating that plasma CETP concentration is the major determinant of CETP activity in vivo. Several other plasma factors could influence CE transfer activity. Among them, the structure and composition of both donor and acceptor lipoproteins appear to play an important role.25-27 LCAT and lipoprotein lipase increase CETP activity by increasing the binding of CETP to lipoprotein particles.28,29 Moreover, nonesterified fatty acids modulate the CE transfer activity of CETP.20 Recent studies have demonstrated the role of CETP activity on the size redistribution of LDL and HDL profiles in normolipidemic subjects.31 These data suggest that lipid exchange between HDL and the apoB-containing lipoproteins mediated by CETP could actively modulate the establishment of the LDL profile.

Marzetta et al32 examined the transfer of HDL-derived CE to apoB-containing lipoprotein subfractions in normolipidemic subjects. Our data for normolipidemic individuals are similar to theirs in that the lighter LDL subfractions appear to be the preferential acceptors of HDL CE. Marzetta et al, who used a similar approach, calculated CE mass transfer on the basis of radioactivity, and there appeared to be no direct chemical determination of mass transfer. Thus, net mass transfer and exchange were not simultaneously deter-
mined in their study. In addition, we reported data for five LDL subfractions in both normolipidemic and heterozygous FH subjects compared with Marzetta et al., who divided the LDL fraction from normolipidemic subjects into light (LDL-1, d=1.026 to 1.039 g/mL) and heavy (LDL-2, d=1.037 to 1.054 g/mL) subfractions. These fractions are equivalent to our LDL-2 and LDL-3 (d=1.023 to 1.039 g/mL) and LDL-4 (d=1.039 to 1.050 g/mL), respectively, and do not include the lightest (LDL-1, d=1.019 to 1.023 g/mL) and heaviest (LDL-5, d=1.050 to 1.063 g/mL) fractions. Furthermore, we also presented data indicating which lipoprotein subspecies are superior CE acceptors on a per unit mass basis. When expressed in this fashion, our LDL-1 in normolipidemic subjects and both our LDL-1 and LDL-2 in FH subjects are qualitatively the best acceptors of HDL CE. Our report confirms the findings of Marzetta et al.22 and extends their study to FH subjects for whom both net CE mass transfer and exchange were simultaneously measured in vitro over an extended time period.

In FH subjects the normal LDL profile was modified such that there was an increase in the concentrations and relative proportions of the denser LDL 3 through 5 subfractions. LDL-3 and LDL-4 contained elevated proportions of CE compared with normolipidemic subjects (data not shown). Furthermore, 73% of the total mass of CE from HDL was transferred to these dense LDL subfractions in FH subjects, as opposed to 54% in normal subjects. These results suggest that CETP, which is elevated in hypercholesterolemic subjects, plays an important role in the establishment of the LDL profile and may contribute to the formation of the denser LDL subfractions. Dense LDL subspecies display a low binding affinity for the LDL receptor in normolipidemic subjects,33 suggesting that their removal from the circulation may be delayed. Indeed, it is relevant that such subspecies are present at elevated concentrations in FH (Table 2). Furthermore, dense, small LDL subspecies in both normolipidemic and combined hyperlipidemic subjects possess a diminished resistance to oxidation.23,34 The preferential mass transfer of CE to dense LDL subspecies clearly suggests that CETP enhances the atherogenic potential of LDL particles in hypercholesterolemia.

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Preferential cholesteryl ester acceptors among the LDL subspecies of subjects with familial hypercholesterolemia.

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