Lipid Transfer Protein-Mediated Distribution of HDL-Derived Cholesteryl Esters Among Plasma Apo B-Containing Lipoprotein Subpopulations

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The substrate specificity of lipid transfer protein has been examined in whole plasma in vitro by following the redistribution of high density lipoprotein (HDL)-derived \([^{3}H]\) cholesteryl ester (CE) into apolipoprotein (apo) B-containing lipoproteins using density gradient ultracentrifugation. HDL-derived \([^{3}H]\) CEs were incubated with plasma or isolated lipoprotein classes (very low density lipoprotein, intermediate density lipoprotein, and low density lipoprotein [LDL] subpopulations from the HDL donor) with and without lipoprotein lipase for 0.5–6 hours at 37°C. After incubation, lipoproteins were separated into 38 fractions after density gradient ultracentrifugation, and radioactivity, protein, and cholesterol were monitored across the profiles. These studies indicate that 1) lipid transfer protein activity varied among the individuals as well as within an individual; 2) the majority of the \([^{3}H]\) CE was associated with LDL; 3) in most individuals (71%), more HDL-derived \([^{3}H]\) CE distributed within the buoyant LDL density region; and 4) the distribution of HDL-derived \([^{3}H]\) CE was similar to the distribution of lipoprotein lipase–derived "remnant" particles within buoyant LDL. These in vitro studies support the hypothesis that HDL-derived \([^{3}H]\) CEs vary in their distribution among apo B–containing particles and that more HDL-derived \([^{3}H]\) CEs are transferred to lipoproteins within the buoyant LDL density range. Additional studies suggest that lipoprotein heterogeneity within this density range, such as the presence of remnant-like lipoproteins, may contribute to the selective distribution of HDL-derived \([^{3}H]\) CE into buoyant LDL.

(In Arteriosclerosis and Thrombosis 1993;13:834–841)

KEY WORDS • cholesteryl ester transfer protein • LDL • HDL • VLDL

However, the transfer of CE to VLDL and LDL may contribute to the formation of CE-enriched apo B–containing particles, which have been associated with an increased risk for developing atherosclerosis.

Little is known about the distribution of LTP-mediated HDL-derived CE among the various apo B–containing lipoproteins. What determines the ability of a lipoprotein to accept an HDL-derived CE is likewise not well understood. Both surface and core lipids are thought to influence LTP activity. The phospholipid moiety, the presence of core lipids, the ratio of triglyceride (TG) to CE within the core, the species of the CEs, the free cholesterol–to-phospholipid ratio, the free cholesterol concentration, and the presence of fatty acids in lipoproteins have all been shown to influence the activity of LTP. These studies have been done primarily with isolated lipoproteins and semipurified LTP.

The present studies were designed to examine in detail the distribution of HDL-derived CE among all apo B–containing lipoproteins in whole plasma by use of density gradient ultracentrifugation (DGUC). In addition, comparisons were made between the in vitro formation of VLDL "remnants" and the distribution of HDL-derived \([^{3}H] \) CE among apo B–containing lipoproteins in whole plasma.

Methods

Materials

\([\text{Cholesteryl-1,2,6,7-}^{3}H(n)]\) cholesteryl oleate (75.3 Ci/mmol; Dupont, Boston) or \([\text{cholesteryl-4,14C}]\) choleseeryl ester activity was first recognized by Nichols and his colleagues in the mid 1960s and has stimulated much interest and speculation on its physiological role. It has been suggested that this lipid transfer protein (LTP) activity is involved in a sequence of events in which free cholesterol from peripheral tissue is taken up by high density lipoprotein (HDL) esterified by lecithin :cholesterol-acyl transferase (LCAT), and then transferred to very low density lipoprotein (VLDL) or low density lipoprotein (LDL) in a process called reverse cholesterol transport. Although reverse cholesterol transport is thought to be an antiatherogenic event by which cholesterol is removed from peripheral tissue to be transported and cleared from the circulation by the liver, it is unclear whether the transfer of CE from HDL to apolipoprotein (apo) B–containing lipoproteins is atherogenic or antiatherogenic. If the ultimate outcome of such a process results in the net movement of cholesterol from peripheral tissue to the liver, then LTP activity would appear to be beneficial.
lesterly oleate (52.5 mCi/mmol; New England Nuclear, Boston), glycerol-tri-[1-14C]oleate (Amersham, Arlington Heights, Ill.), and [125I]NaI (New England Nuclear) were used. Purified bovine milk lipoprotein lipase (LpL) was isolated as described previously. The paraoxon was a gift from Dr. Gerhard Schrader.

Isolation and Radiolabeling Lipoproteins

All subjects used in these studies were volunteers. The subjects were normolipidemic except for subject 6, who was mildly hypertriglyceridemic (195 mg/dL), and subjects 10 and 13, who had familial combined hyperlipidemia according to the criteria described previously. Subject 10 had been taking 1 g/day of niacin for approximately 6 months before the study. No other subject was taking medications known to alter lipid or lipoprotein metabolism. The study protocol was approved by the Human Subjects Review Committee of the University of Washington, and informed consent was obtained from each subject.

HDL-derived [3H]CE. The density of 50 mL of whole plasma was raised to 1.080 g/mL with solid potassium bromide. The 1.080-g/mL whole plasma was divided into two 39-mL quick-seal tubes (Beckman, Palo Alto, Calif.), then overlaid with a 1.080-g/mL solution and subjected to ultracentrifugation in a 50-Ti rotor for 18 hours at 50,000 rpm at 4°C. The d > 1.080 g/mL lipoproteins were dialyzed to 1.006 g/mL in 0.9% NaCl containing 0.01% EDTA and 0.01% NaN3. Dithiobisnitrobenzoic acid (DTNB; final concentration of 1.4 mmol/L) was added to 12 mL of the d > 1.080 g/mL lipoproteins and then incubated at 37°C in a shaking water bath for 6–9 hours in a 15-mL glass conical tube that had a thin layer of [cholesteryl-l,2,6,7-3H(2)]cholesteryl oleate (8–500×10^6 cpm) dried to the wall. After incubation, the density of the d > 1.080 g/mL lipoproteins was raised to 1.21 g/mL with potassium bromide, poured into two ultracentrifugation tubes, overlaid with a 1.21-g/mL solution, and subjected to ultracentrifugation in an SW-41 rotor for 40 hours at 10°C and 41,000 rpm. The concentrated HDL was obtained by tube slicing, and an average of 24.5±14% (mean±SD, n=8) of the [3H]CE was incorporated into HDL.

The d > 1.080 plasma fraction was used for the HDL radiolabeling methods because this fraction includes active LTP and HDL that had been subjected to minimal ultracentrifugation. The HDL CE radiolabeling method was done in the presence of DTNB to inhibit LCAT and minimize compositional changes of HDL during the 6–9-hour incubations. Except in studies requiring substantial amounts of radiolabeled HDL (e.g., multiple-subject screening studies), fresh plasma was obtained on the day of the incubation study from the same subject who served as the donor for the HDL-derived [3H]CE.

VLDL [14C]TG. VLDL was isolated from plasma at 1.006 g/mL after ultracentrifugation in an SW-41 rotor for 16 hours at 10°C and 41,000 rpm. The isolated VLDL was centrifuged further in a dialysis bag using carboxymethyl cellulose (Sigma, St. Louis, Mo.). The concentrated VLDL was then added to 10 mL plasma (from the same VLDL donor) in the presence of DTNB (final concentration, 1.4 mmol/L) and incubated in tubes that had a thin layer of glycerol-tri-[1-14C]oleate (4–10×10^6 cpm) dried to the wall as described for the HDL-derived [3H]CE. The radiolabeled VLDL was isolated after incubation by ultracentrifugation as described earlier. Approximately 6.1±4% (mean±SD, n=4) of the [14C]TG was incorporated into VLDL.

VLDL 125I-protein. VLDL was isolated from plasma and concentrated as outlined above. The concentrated VLDL was then radioiodinated with 125I (New England Nuclear) by the method of McFarlane as modified by Bilheimer et al.

Incubation Procedures

Lipid transfer activity assay. Radiolabeled HDL-derived [3H]CE (70,000–580,000 cpm) was added to an aliquot of freshly isolated plasma and incubated for 30 minutes to 6 hours at 37°C in a shaking water bath. The assays were stopped by placing the samples on ice or ice and paraoxon when LpL had been added to the incubation. The postincubation samples were then separated and characterized by DGUC. The density of 2 mL of each sample was raised to 1.21 g/mL and underlayered into a discontinuous salt gradient consisting of 3.7 mL of a 1.006 g/mL solution, 6.5 mL of a 1.063 g/mL solution, and 2.3 mL of the 1.21 g/mL whole plasma postincubation sample. The samples were then subjected to ultracentrifugation in an SW-41 rotor at 41,000 rpm for 24 hours at 15°C. After ultracentrifugation, the samples were drained through a flow-cell UV monitor into a fraction collector as described previously. The distribution of HDL-derived [3H]CE among the apo B-containing lipoproteins was determined by counting an aliquot of each fraction and correcting for background radioactivity and quench. Recovery of radioactivity after DGUC averaged 77.3±10% (mean±SD of five representative experiments).

When the movement of HDL-derived [3H]CE to apo B-containing particles was examined, in most studies the incubation conditions were achieved by use of freshly isolated plasma in which LTP and LCAT were active to mimic in vivo conditions as closely as possible.

In some experiments, total cholesterol per fraction after separation by DGUC was measured enzymatically with a Total Cholesterol Kit (Boehringer Mannheim, Indianapolis, Ind.) or by the method of Allain et al.

Lipid transfer activity assay in the presence of LpL. Whole plasma, HDL-derived [3H]CE, 1,000 units of LpL (nanomoles of free fatty acids released per minute per milliliter at 37°C), and 150 μg human serum albumin (Sigma) were incubated at 37°C in a shaking water bath for 30–90 minutes. In some studies, [125I]-VLDL or [14C]TG were added to the whole plasma. The incubations were stopped by placing the samples on ice in tubes containing paraoxon (final concentration, 2 mmol/L). An aliquot of each sample was taken for free fatty acid determinations. Lipoproteins were then separated and characterized by DGUC as described above. Aliquots were taken for radioactivity and corrected for background, quench, and [125I] radioactivity in the tritium window.

Studies using isolated lipoprotein subpopulations. The density of freshly isolated whole plasma was raised to
were then underlayered into a discontinuous salt gradient consisting of 8 mL of a 1.006 g/mL solution, 20 mL of the lipoprotein sample at 1.030 g/mL, and 11 mL of a 1.063 g/mL solution. The samples were then subjected to 5.5 hours of ultracentrifugation at 50,000 rpm and 20°C and fractionated as described previously. Material within specific fractions was pooled and dialyzed to 1.006 g/mL, and aliquots were taken for cholesterol determinations. Three milliliters of the d>1.080 lipoproteins was raised to 1.21 g/mL, and a 1.063 g/mL solution. The samples were then subjected to 5.5 hours of ultracentrifugation at 50,000 rpm and 20°C and fractionated as described above. The density of the [3H]CE d>1.080 lipoproteins was raised to 1.21 g/mL, and radiolabeled HDL was isolated by ultracentrifugation in a 40.3-Ti rotor at 36,000 rpm for 24 hours at 10°C. Incubation conditions consisted of 1) 2 mL of freshly isolated whole plasma and HDL-derived [3H]CE (207,500 cpm) for the whole-plasma control; 2) 378 µg VLDL cholesterol, 980 µg LDL cholesterol, 208.5 µg of d>1.080 unlabeled lipoproteins (HDL, LTP, LCAT, and plasma proteins), and 207,500 cpm of HDL-derived [3H]CE for the LDL1 sample; 3) 378 µg VLDL cholesterol, 1,522 µg LDL2 cholesterol, 208.5 µg of d>1.080 unlabeled lipoproteins, and 207,500 cpm of HDL-derived [3H]CE for the LDL2 sample; and 4) 378 µg VLDL cholesterol, 980 µg LDL cholesterol, 1,522 µg LDL cholesterol, 208.5 µg of d>1.080 unlabeled lipoproteins, and 207,500 cpm of HDL-derived [3H]CE for the LDLi and LDLj samples. Total volumes were made equivalent (3.9 mL) with saline. All samples were incubated for 2 hours at 37°C in a shaking water bath. Incubations were stopped by placing the samples on ice, and then each sample was subjected to DGUC in the following discontinuous salt gradient: 3.5 mL of 1.006 g/mL, 5.7 mL of 1.030 g/mL, and 2.4 mL of the incubation sample at 1.21 g/mL. The samples were ultracentrifuged and fractionated as described earlier.

Results

An example of the distribution of HDL-derived [3H]CE among apo B-containing lipoproteins separated and characterized by DGUC before and after incubation is shown in Figure 1A. The percentages of HDL-derived [3H]CE isolated within VLDL+intermediate density lipoprotein (IDL) and LDL and HDL were determined by summing the radioactivity within VLDL+IDL, LDL, and HDL based on the DGUC profile. After 6 hours of incubation at 37°C, the distribution of the HDL-derived [3H]CE was found primarily associated with LDL (42%) and was essentially superimposable with the distribution of LDL cholesterol, whereas <10% of the [3H]CE was isolated within the VLDL+IDL fraction (Figure 1B).

The distribution of HDL-derived [3H]CE among LDL was not usually superimposable with the plasma LDL mass. Shown in Figure 2 are three examples of DGUC profiles in which the HDL-derived [3H]CE was distributed primarily within the buoyant LDL density range after incubation. As shown in these examples, the peak of the [3H]CE radioactivity occurred at a lower density compared with the peak of the LDL protein mass (1.037 versus 1.039 g/mL, 1.033 versus 1.036 g/mL, and 1.038 versus 1.040 g/mL for panels A, B, and C, respectively). Among the studies in which HDL-derived [3H]CEs were incubated with freshly isolated plasma, the distribution of the [3H]CE was primarily in the buoyant region of LDL in 10 of 14 studies (71.4%), compared with three of 14 studies (21.4%) in which the radiolabeled CE was superimposable with the LDL protein or cholesterol mass. In one subject (subject 10), the [3H]CE distributed preferentially into a more dense region of the LDL.

On the basis of each subject’s unique DGUC LDL protein peak, LDL was divided equally into buoyant (LDLd) and dense (LDLs) subpopulations, and the percentage of [3H]CE radioactivity isolated within each lipoprotein subpopulation was determined (Table 1). On average, 17.9±6% of the total [3H]CE radioactivity
LDL₂ was defined as fractions 14-18 (1.037<d<1.051). LDL₁, was defined as fractions 9-13 (1.026<d<1.037), and LDL₂ was defined as fractions 10-14 (1.031<d<1.039), and LDL₂ was isolated within the LDL density range and 11.8±4% of the [³H]CE radioactivity within the VLDL+IDL region after 1.5-2 hours of incubation at 37°C (p<0.005, unpaired t test). In addition, significantly more [³H]CE radioactivity was isolated within the buoyant LDL₁ subpopulation compared with the more dense LDL₂ subpopulation (9.5±3% versus 8.4±3%, respectively; p<0.005, unpaired t test).

The rate of change of radioactivity into LDL₁, LDL₂ for subject 1 (Figure 3B) were 0.233 and 0.137, respectively. That is, the rate of movement of HDL-derived [³H]CE into LDL₁ was approximately 2.5 times greater than VLDL, and LDL₂ for subject 13 (Figure 3A) were 0.233 and 0.137, respectively. The rates of change of radioactivity into LDL₁ and LDL₂ for subject 1 (Figure 3B) were 0.083 and 0.045, respectively. That is, the rate of movement of HDL-derived [³H]CE into LDL₁, was approximately 2.5 times greater than VLDL, and LDL₂ for subject 13 (Figure 3A) were 0.233 and 0.137, respectively. That is, the rate of movement of HDL-derived [³H]CE into LDL₁, was approximately 2.5 times greater than VLDL, 4.4 times greater than LDL₁, and 2.6 times greater than LDL₂ in both subjects.

Studies were done in which LDL subpopulations (buoyant and dense) were first isolated and then incubated separately or together with radiolabeled HDL to test the hypothesis that HDL-derived CE would transfer preferentially into the buoyant LDL. Plasma LDL was first subfractionated by DGUC in a vertical rotor and divided into VLDL+IDL, LDL₁, and LDL₂ on the basis of the protein profile. An aliquot of HDL (d>1.080) was radiolabeled with [³H]CE and incubated at the donor's physiological concentrations of VLDL cholesterol (d<1.066 fraction; 9.7 mg/dL); LDL₁ cholesterol (25.2 mg/dL), LDL₂ cholesterol (39.1 mg/dL), or both; unlabeled HDL cholesterol (d>1.080; 53.8...
FIGURE 3. Graphs showing changes in the high density lipoprotein (HDL)-derived [3H]cholesteryl ester (CE) radioactivity with time among the various lipoprotein subpopulations in subject 13 (panel A) and subject 1 (panel B). Plasma from each subject was incubated with HDL-derived [3H]CE for 30, 60, and 90 minutes in the presence of lipoprotein lipase (LpL). After incubation, each sample was separated by density gradient ultracentrifugation, and radioactivity within each lipoprotein subpopulation was measured. The data at 0 minutes of incubation represent the radioactivity in each lipoprotein subpopulation of the 4°C control (without LpL). LDL, low density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein.

FIGURE 4. Density gradient ultracentrifugation profiles of the percentage distribution of very low density lipoprotein-derived [125I]-protein (A), high density lipoprotein-derived [3H]cholesterol ester (CE) (●), and relative protein (solid line; optical density at 280 nm) of whole plasma incubated at either 4°C (upper panel) or after 1 hour of incubation at 37°C in the presence of lipoprotein lipase (LpL) (lower panel).

mg/dL); and radiolabeled HDL-derived [3H]CE (total cholesterol, 8.8 mg/dL) for 2 hours at 37°C and characterized by DGUC as described earlier. Table 2 gives the calculated specific activities for each LDL subpopulation. Under all conditions, the buoyant LDL1 fraction had a higher specific activity than did the more dense LDL2 fraction (25.4±8 and 15.3±5, respectively, mean±SD; n=3 each).

Additional studies were done to test whether the distribution of HDL-derived [3H]CE into buoyant LDL could be due, at least in part, to the presence of

Table 2. Specific Activities for LDL1 and LDL2 After Incubation at 37°C for 2 Hours

<table>
<thead>
<tr>
<th>CE acceptor</th>
<th>Lipoprotein fraction</th>
<th>SA* (cpm/CE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>LDL1</td>
<td>25.3</td>
</tr>
<tr>
<td></td>
<td>LDL2</td>
<td>13.3</td>
</tr>
<tr>
<td>LDL1</td>
<td>LDL1</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>LDL2</td>
<td>21.5</td>
</tr>
<tr>
<td>LDL1+LDL2</td>
<td>LDL1</td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td>LDL2</td>
<td>11.2</td>
</tr>
</tbody>
</table>

LDL, low density lipoprotein; CE, cholesteryl ester.

*SA, specific activity; cholesteryl ester (μg per subpopulation) calculated as 0.75×total cholesterol of each fraction.
FIGURE 5. Density gradient ultracentrifugation profiles of the percentage distribution of very low density lipoprotein-derived \([14C]\) triglycerides (TG) (A), high density lipoprotein-derived \([14C]\) cholesteryl ester (CE) (○), and relative protein (solid line; optical density at 280 nm) in plasma incubated at \(4^\circ\) C (upper panel) or after 90 minutes at \(37^\circ\) C in the presence of lipoprotein lipase (LpL) (lower panel). The \(14\) C radioactivity represents the percentage distribution through fraction 32 only, which was 32.4% of the total \(\text{\textsuperscript{14}C}\) radioactivity. The remaining 67.6% was associated with albumin (as free fatty acids) at the bottom of the gradient (fractions 33–37).

"remnant-like" particles. First, iodinated VLDL and HDL-derived \([\text{\textsuperscript{3}H}]\text{CE}\) were added to plasma in the presence of LpL to generate remnant-like particles, and the distribution of \([\text{\textsuperscript{3}P}]\text{-protein and [\text{\textsuperscript{3}H}]CE}\) was examined simultaneously. As shown in Figure 4, after 1 hour of incubation at \(37^\circ\) C in the presence of LpL, the distribution of the \([\text{\textsuperscript{3}P}]\text{-protein was very similar to the distribution of the [\text{\textsuperscript{3}H}]CE within the apo B-containing particles. Consistent with previous observations, the radioactivity distributed primarily in the buoyant region of the LDL.

Second, experiments were done by incubating VLDL \([\text{\textsuperscript{14}C}]\text{TG}\) and HDL-derived \([\text{\textsuperscript{3}H}]\text{CE}\) into plasma, again in the presence of LpL. Again, after 90 minutes of incubation at \(37^\circ\) C, both isotopes distributed similarly within the buoyant region of LDL (Figure 5). Although the percentage of \(\text{\textsuperscript{14}C}\) radioactivity that was associated with free fatty acids could not be determined for each fraction within LDL, approximately 50% of the total radioactivity isolated within the fractions pooled within LDL (fractions 11–19) was found to be associated with the TG moiety.

Discussion
The movement of HDL-derived CE into VLDL and LDL has been described previously; however, those studies have been done with isolated lipoprotein classes in a buffer system.\(^{25–28}\) The studies presented here describe the transfer of HDL-derived \([\text{\textsuperscript{3}H}]\text{CE}\) into various apo B–containing lipoproteins in whole plasma under in vitro conditions, which more closely mimic the physiological state (in plasma with LCAT and LTP active). After incubation, the lipoproteins were separated and characterized by DGUC and/or gel filtration so that the distribution of the HDL-derived radiolabeled CE into all apo B–containing lipoproteins could be defined in more detail. A number of informative observations have been made from these studies. First, at physiological concentrations of plasma lipoproteins, significantly more HDL-derived \([\text{\textsuperscript{3}H}]\text{CE}\) was transferred to LDL than to VLDL. Second, in most subjects, more HDL-derived \([\text{\textsuperscript{3}H}]\text{CE}\) distributed into the buoyant LDL subpopulation compared with the dense LDL subpopulation. And finally, studies in which remnant-like particles were produced in vitro suggested that these lipoprotein particles are avid acceptors for HDL-derived \([\text{\textsuperscript{3}H}]\text{CE}\) and may contribute to the skewed distribution of \([\text{\textsuperscript{3}H}]\text{CE}\) among buoyant LDL.

Studies characterizing the distribution of HDL CE among apo B–containing lipoproteins have been inconsistent. Contrary to the observations reported here, Barter et al.\(^{28}\) and Eisenberg\(^{26}\) suggested that plasma LDL are inefficient HDL CE acceptors in in vitro incubation systems. In Eisenberg’s studies, however, isolated LDL and HDL were incubated at equivalent CE concentrations in artificial buffer systems. Since normolipidemic subjects generally have between five and nine times more CE mass in LDL than in VLDL,\(^{17}\) it is difficult to extrapolate the results of these studies back to the physiological state. Barter et al.\(^{28}\) examined the movement of HDL-derived \([\text{\textsuperscript{3}H}]\text{CE}\) into VLDL and LDL after incubation in whole plasma and noted that the specific activity of the LDL was approximately four times lower than the plasma VLDL. In other studies using whole plasma\(^{29–30}\) or whole sera,\(^{1,2,30}\) however, the amount of radiolabeled CE or CE mass transferred into LDL was at least equivalent to, if not greater than, into VLDL.

In the present studies, the transfer of HDL-derived \([\text{\textsuperscript{3}H}]\text{CE}\) into apo B–containing lipoproteins was examined in whole plasma. At each subject’s physiological concentrations of lipoproteins, significantly more HDL-derived \([\text{\textsuperscript{3}H}]\text{CEs were transferred to LDL than to VLDL+IDL. The inconsistent results among the various published reports may reflect differences in the incubation conditions (concentrations of donors and acceptors, isolated lipoproteins versus plasma or sera, enzyme inhibitors used, etc.), length of time of the incubations, and/or differences in study subjects.

Although we assumed that the amount of the HDL-derived \([\text{\textsuperscript{3}H}]\text{CE associated with apo B–containing lipoproteins would be directly related to the lipoprotein mass,\(^{31}\) in most of the subjects studied, the HDL-derived \([\text{\textsuperscript{3}H}]\text{CE distributed preferentially within the buoyant region of the LDL. This preference for buoyant LDL (fractions 11–19) was found to be associated with the TG moiety.
LDL as the CE acceptor was supported by additional studies in which the rate of transfer of LDL-derived [$^3$H]CE was greater into LDL than LDL$_2$, and the specific activities of isolated LDL$_2$ were greater than isolated LDL$_1$. Recently, Phair et al$^{32}$ have tested various kinetic models describing "reverse cholesterol transport." In their theoretical models, which include the transfer of HDL cholesterol to apo B-containing lipoproteins, particles between 2.5 and 3.5 $\times 10^6$ g/mol were the predicted preferred acceptors for HDL-derived CE. That is, our observation suggesting large, buoyant LDL as preferred acceptors for HDL-derived CE is consistent with their model predictions. In addition, recent work by Gambert et al$^{33}$ has also been consistent with the present observations. In a series of studies, they demonstrated that when LDL and HDL were incubated together in the presence of LTP, the buoyant LDL as preferred acceptors for HDL-derived lipoproteins, particles between 2.5 and 3.5 $\times 10^6$ g/mol were the predicted preferred acceptors for HDL-derived CE. That is, our observation suggesting large, buoyant LDL as preferred acceptors for HDL-derived CE is consistent with their model predictions. In addition, recent work by Gambert et al$^{33}$ has also been consistent with the present observations. In a series of studies, they demonstrated that when LDL and HDL were incubated together in the presence of LTP, the distribution of esterified cholesterol within LDL was reisolated in larger particles.

The studies presented here offer insight into several forms of lipoprotein heterogeneity. As expected, LTP activity varies among individuals. However, these data also suggest that variability in the transfer of HDL-derived [$^3$H]CE among the apo B-containing particles exists within an individual. Finally, various subpopulations of lipoproteins within the IDL–LDL density range, such as remnant-like particles, may also contribute to the distribution of HDL-derived [$^3$H]CE among the apo B-containing lipoproteins. This last observation is consistent with studies by Sammett and Tall$^{15}$ showing that during LpL-mediated TG hydrolysis, HDL-derived [$^3$H]CE are transferred to the large LDL remnant-like particles produced in vitro. Although the notion of metabolic heterogeneity within the LDL density range is certainly not new,$^{18-34,39}$ the factors that influence this phenomenon are still poorly understood.

In conclusion, the present studies suggest heterogeneity among apo B-containing lipoproteins as acceptors of HDL-derived CE. In most subjects studied, more of the HDL-derived [$^3$H]CE distributed within LDL than VLDL. In addition, in most subjects, more HDL-derived [$^3$H]CE distributed within buoyant LDL than dense LDL. This distribution of HDL-derived [$^3$H]CE into buoyant LDL may be related, in part, to the presence of remnant-like particles that may reside within this density region.

Acknowledgments
The authors thank Martha Kimura and Steve Hashimoto for their excellent technical expertise and Dr. John Brunzell for the purified LpL and for his support throughout these studies.

References


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Arterioscler Thromb Vasc Biol. 1993;13:834-841
doi: 10.1161/01.ATV.13.6.834
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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