A New In Vitro Method for the Simultaneous Evaluation of Cholesteryl Ester Exchange and Mass Transfer Between HDL and ApoB-Containing Lipoprotein Subspecies

Identification of Preferential Cholesteryl Ester Acceptors in Human Plasma

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Abstract

To date, several methods have been developed to determine the activity of plasma lipid transfer proteins. These methods have largely involved the addition of the transfer protein in question to labeled substrates, followed by prolonged incubation (4 to 18 hours) and subsequent evaluation of the radioactivity transferred to precipitated low-density lipoprotein (LDL). While adequate for determining the activity of cholesteryl ester transfer protein (CETP), these methods generally do not take into account the composition or levels of lipoproteins present within a given individual plasma because pools of high-density lipoprotein (HDL) are labeled and used for the transfer experiments. Both the direction and the extent of lipid transfer are dependent on the composition and relative abundance of both donor and acceptor particles as well as the activity of the lipid transfer protein(s). Here we describe a new method for the determination of the capacity of plasma samples to facilitate cholesteryl ester transfer from HDL to LDL and very-low-density lipoprotein (VLDL), a method that has several advantages. First, the subject’s HDL is labeled and used for transfer. Second, the labeled HDL, in a quantity equivalent to 1% of the plasma HDL mass, is added to the subject’s plasma, and therefore the relative abundance of both donor and acceptor particles is preserved at their physiological levels. Third, both cholesteryl ester mass and radioactivity are determined, allowing the net mass transfer of cholesteryl ester and cholesteryl ester exchange to be quantified separately. Fourth, the application of an ultracentrifugal density gradient for the subsequent reisolation of the lipoproteins permits estimation of the transfer of cholesteryl esters to various subfractions of LDL; such measurement is not possible when precipitation techniques are used to determine total LDL radioactivity. The method allows estimation of the total physiological capacity of a plasma sample to mediate cholesteryl ester transfer, which may represent a more relevant measurement than that of CETP activity alone. Cholesteryl ester exchange and mass transfer were determined in four normolipidemic and two moderately hypertriglyceridemic subjects by this new in vitro method. Net transfer of cholesteryl ester from HDL to VLDL was increased twofold in mildly hypertriglyceridemic subjects (triglycerides >100 and <150 mg/dL). Cholesteryl ester mass transfer predominated for the first 6 hours of incubation, after which cholesteryl ester exchange predominated. Our data indicated that on a quantitative basis VLDL and the light LDL subspecies (LDL1, d=1.019 to 1.023 g/mL) are preferential cholesteryl ester acceptors among the apolipoprotein B-containing lipoproteins. (Atherosclerosis. 1994;14:199-206.)

Key Words • reverse cholesterol transport • cholesteryl ester transfer protein • hypertriglyceridemia

The movement of cholesterol from peripheral tissues to the liver is termed reverse cholesterol transport. The first step in this process involves efflux of free cholesterol from cells to various acceptors, including nascent discoidal high-density lipoproteins (HDLs), apolipoprotein-phospholipid complexes, and free apolipoproteins. The enzyme lecithin:cholesterol acyltransferase (LCAT) assures the esterification of such free cholesterol, and its continuous action results in the formation of larger, cholesteryl ester (CE)-rich HDL particles. A major part of the CE generated by the LCAT reaction in plasma is subsequently transferred to acceptor particles, primarily the apolipoprotein (apo)B-containing lipoproteins, chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and low-density lipoprotein (LDL). The CE transfer protein (CETP) mediates both the transfer and exchange of CEs and triglycerides between donor and acceptor lipoprotein particles in plasma. The final step in reverse cholesterol transport involves the uptake of CE from both apoA-I- and apoB-containing particles by the liver.

At this time, several methods have been used to evaluate CE transfer from HDL to apoB-containing lipoproteins of lower density. Earlier methodologies have frequently involved quantification of CE transfer by the use of HDL containing radiolabeled CE. Direct determination of lipoprotein CE mass has also been performed. In these studies, either heparin-manganese precipitation or a combination of ultracentrifugation and heparin-manganese precipitation has been used to separate apoB-containing lipoproteins from...
those containing apoA-I after incubation of isolated lipoproteins or plasma. The techniques do not permit evaluation of CE transfer between particles within each major lipoprotein class, and the evaluation of CE mass transfer by radiochemical methods does not generally take into account CE exchange, particularly between HDL and LDL. To circumvent these limitations and to provide a more in-depth evaluation of CE transfer as it exists in plasma at physiological concentrations of plasma lipoproteins and CETP, we developed a new methodology that involves the simultaneous determination of CE mass and radioactivity. Our method measures both net CE mass transfer and exchange from HDL to potential apoB-containing CE acceptors, ie, VLDL, IDL, and LDL; moreover, our methodology respects physiological ratios of lipoprotein concentrations, most notably those of VLDL-HDL and LDL-HDL. Furthermore, isolation of each of the lipoprotein classes known to be potential CE acceptors allowed us to identify the major CE acceptor particles. The applicability of this method was tested in 4 normolipidemic and 2 mildly hypertriglyceridemic subjects, and the preferential CE acceptors among apoB-containing lipoprotein sub species were identified.

**Methods**

After informed consent and approval by the Human Subjects Review Committee of the hospital were obtained, mildly hypertriglyceridemic subjects with a fasting total plasma triglyceride level of >100 mg/dL and <150 mg/dL and total cholesterol level of <250 mg/dL, respectively. Blood samples (up to 40 mL) were obtained by venipuncture after an overnight fast. EDTA (final concentration, 1 mg/mL) was used as an anticoagulant. The cellular material was immediately separated by low-speed centrifugation at 4°C. EDTA (0.1 g/L), NaN3 (0.01%), and iodoacetamide were then added to the plasma, which was maintained at 4°C until use on the same day.

**Lipid Analysis**

Lipids in plasma or in isolated lipoprotein fractions were quantified enzymatically by using Bio-Merieux kits (69280, Marcy l’Etoile, France) for total cholesterol, free cholesterol, phospholipids, and triglycerides. CE mass was calculated as (total cholesterol—free cholesterol) x 1.67 and thus represents the sum of the esterified cholesterol and fatty acid moieties. Lipoprotein mass was calculated as the sum of the individual lipid components plus the associated protein as determined by the method of Lowry et al.

**Measurement of LCAT Activity**

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

**Preparation of [3H]CE-Labeled HDL**

The method of Folch et al.5 The lipids were separated by thin-layer chromatography on silica gel H plates using a solvent system of n-heptane, isopropyl ether, glacial acetic acid, and methanol (60:40:4:2, vol/vol/vol/vol). Free and esterified cholesterol were visualized with iodine vapor, and the radioactivity associated with each was quantified by liquid scintillation spectrometry using a Pharmacia RackBeta 509. After 18 hours of incubation, the HDL contained the esterified, radiolabeled CE was isolated from the d>1.063 g/mL fraction by adjusting the density to 1.21 g/mL by addition of dry solid potassium bromide (KBr) and centrifuged at 541 000g max/min at 10°C as before. The isolated HDL was dialyzed against phosphate-buffered saline, and its individual lipid components and radioactive contents were determined as described above. The chemical composition of the radio labeled HDL was not significantly different from the unlabeled HDL present in the individual patient’s plasma (data not shown).

**Determination of CE Exchange and Mass Transfer**

A known concentration of homologous radio labeled HDL equivalent to 1% of the mass of HDL CE present in the subject’s plasma was added to serial 1-mL aliquots of plasma containing 150 mmol/L iodoacetamide to inhibit plasma LCAT activity. The plasma samples were then incubated at 37°C for various times up to 18 hours. A control plasma sample was incubated at 4°C for 18 hours. CE transfer was observed during incubation of plasma at 37°C for up to 18 hours in the presence of labeled HDL CE. Total plasma CE mass was not significantly modified during incubation (data not shown). Moreover, no LCAT activity was detected in the plasma of each subject tested after incubation for 18 hours at 37°C in the presence of iodoacetamide. At the indicated times, the lipoproteins present within the plasma aliquots were isolated by density gradient ultracentrifugation by a slight modification of the method of Chapman et al as follows. The density of the plasma aliquot (1 mL) was adjusted to 1.21 g/mL by the addition of dry, solid potassium bromide, and a potassium bromide solution of density 1.24 g/mL, followed by the 3-mL NaCl-KBr solution at d=1.019 g/mL. All density solutions used were at pH 7.4 and contained 0.01% NaN3. Each sample was layered onto a discontinuous density gradient of 56×105 rpm/min at 15°C in a Beckman SW41 rotor in a Beckman XL70 ultracentrifuge. After centrifugation, the gradients were collected from the meniscus downward in 30 fractions of 0.4 mL each by using a precision pipette. The first 25 fractions were dia lyzed in Spectrapor membrane tubing at 4°C against 20 mmol/L NH4HCO3, buffer containing 0.01% EDTA, pH 7.4. Density fractions, either individual or pooled, were analyzed for their lipid and protein content and radioactivity as described above. Fractions 1 and 2 were analyzed separately; however, there was significant contamination of fraction 2 with the VLDL present in fraction 1, particularly in hypertriglyceridemic subjects. Consequently, the data for fractions 1 and 2 were summed and in this report are termed VLDL (d<1.019 g/mL).

**Results**

**Plasma Lipoprotein Distribution**

To evaluate our method, CE transfer and exchange were measured in plasma from 6 different subjects, 4 of
TABLE 1. Plasma Lipid Levels

<table>
<thead>
<tr>
<th>Subjects</th>
<th>TC</th>
<th>FC</th>
<th>CE</th>
<th>TG</th>
<th>PL</th>
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<td>Normolipidemic</td>
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<td></td>
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<td>178.9</td>
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<td>166.8</td>
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<td>150.4</td>
<td>44.4</td>
<td>176.9</td>
<td>113.9</td>
<td>160.2</td>
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</tbody>
</table>

TC indicates total cholesterol; FC, free cholesterol; CE, cholesteryl ester; TG, triglycerides; and PL, phospholipids. Values are expressed in milligrams per deciliter.

whom were normolipidemic and 2 of whom were mildly hypertriglyceridemic (Table 1). The choice of mildly hypertriglyceridemic subjects permitted us to determine the effects of varying HDL-VLDL and HDL-LDL ratios on the HDL-mediated reverse cholesterol transport system in the absence of very large differences in total plasma lipid levels. This approach provided us with an indication of the sensitivity of the technique. The plasma lipoprotein distribution patterns were determined by density gradient ultracentrifugation for each subject (Table 2). Moderately hypertriglyceridemic subjects had plasma VLDL levels that were some twofold elevated in comparison with values obtained in normolipidemic subjects. Although the mildly hypertriglyceridemic subjects’ lipoprotein profiles were similar, plasma VLDL levels were higher for subject 5 than for subject 6. Plasma lipoprotein profiles were similar among normolipidemic subjects. Analysis of the HDL from each individual showed the predicted enrichment in triglycerides for the HDL from mildly hypertriglyceridemic individuals. The LDL subfraction profile was distinct in each subject studied (Table 3). The most abundant LDL subfraction was either LDL2, LDL3, or LDL4. The light LDL subfraction (LDL1) and dense LDL subfraction (LDL5) consistently represented minor components in the total LDL profile, with the exception of LDL5 in subject 6, in whom the proportion of this subfraction attained 19%. Under these conditions, each subject tested presented distinct mass ratios of VLDL-HDL and of LDL subfractions-HDL.

Production and Characterization of [3H]CE-Labeled HDL

As the objective of this study was to develop a method to measure the ability of plasma from a variety of normal and dyslipoproteinemic subjects to facilitate HDL-mediated reverse transport and exchange of CE, we considered it desirable to radiolabel the individual’s own HDL and thus approximate as closely as possible the in vivo situation rather than to employ an unrelated HDL pool. In hyperlipidemic subjects both the lipid composition and relative proportions of the HDL subfractions varied significantly from normolipidemic individuals (Table 2 and Fig 1). As a consequence, the ability of radiolabeled CE present within the HDL to undergo exchange with the HDL CE pool and other lipoproteins when added back to plasma may vary from that observed when radiolabeled HDL from normolipidemic subjects is added to dyslipoproteinemic plasma. Similar considerations also led us to produce radiolabeled CE from radiolabeled cholesterol within the HDL particle population by exploitation of the physiological esterification reaction present in plasma and mediated by LCAT. As LCAT in plasma is labile on extended

TABLE 2. Plasma Lipoprotein Concentrations and HDL Lipid Composition

<table>
<thead>
<tr>
<th>Subjects</th>
<th>VLDL, mg/dL</th>
<th>LDL, mg/dL</th>
<th>HDL, mg/dL</th>
<th>TC, mg/dL</th>
<th>FC, mg/dL</th>
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VLDL indicates very-low-density lipoprotein (d<1.019 g/mL); LDL, low-density lipoprotein (d=1.019 to 1.063 g/mL); HDL, high-density lipoprotein (d=1.063 to 1.145 g/mL); TC, total cholesterol; FC, free cholesterol; CE, cholesteryl ester; and TG, triglycerides. Density limits for the major lipoprotein classes were as determined before. Concentrations were determined by chemical analysis (see “Methods”). The total mass of lipoprotein represents the sum of all lipid components plus protein.
Table 3. Plasma LDL Subfraction Levels

<table>
<thead>
<tr>
<th>Subjects</th>
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<th>%</th>
<th>LDL₂</th>
<th>%</th>
<th>LDL₃</th>
<th>%</th>
<th>LDL₄</th>
<th>%</th>
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LDL indicates low-density lipoprotein; LDL₁, \(d=1.019\) to \(1.023\) g/mL; LDL₂, \(d=1.023\) to \(1.029\) g/mL; LDL₃, \(d=1.029\) to \(1.039\) g/mL; LDL₄, \(d=1.039\) to \(1.050\) g/mL; LDL₅, \(d=1.050\) to \(1.063\) g/mL. LDL subfractions were isolated by density gradient ultracentrifugation and their chemical compositions analyzed (see "Methods"). The values indicated represent the sum of all lipids and the protein component.

Storage, it was important to maintain plasma at \(4°C\) after venipuncture and to undertake radiolabeling of the plasma HDL rapidly thereafter. All subjects in this study had an endogenous plasma cholesterol esterification rate within the normal range, i.e., \(64.7\pm9.3\) nmol CE formed per hour per milliliter for the normolipidemic subjects and \(77\) to \(90\) nmol CE formed per hour per milliliter for the two mildly hypertriglyceridemic subjects. Upon centrifugation, LCAT is located in the \(d>1.063\) g/mL fraction and is available for esterification.

![Graph A: Hypertriglyceridemic](image)

![Graph B: Normolipidemic](image)

Fig 1. Line graphs showing distribution of \([3H]\)cholesteryl ester between lipoprotein species in plasma as a function of the time of incubation in a mildly hypertriglyceridemic (A) and a normolipidemic (B) subject. Shown is the distribution of radioactivity contained in the different ultracentrifugal fractions after an incubation of plasma for 18 hours at \(4°C\) (-○-), 4 hours at \(37°C\) (-▪-), and 18 hours at \(37°C\) (-×-). An increase of radioactivity in very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) with a diminution of that in high-density lipoprotein (HDL) was found in each case. Density limits for the distribution of the major lipoprotein classes were as determined before. IDL indicates intermediate-density lipoprotein.
of the added \([1^3]H\)cholesterol. After an 18-hour incubation of the \(d>1.063 \, \text{g/mL}\) fraction, only 1.5% of the added radioactivity remained associated with free cholesterol; the majority of the radioactivity was present as CE and thus indicative of functional LCAT. Specific activity values for HDL obtained by this method for each subject studied ranged from 3700 to 7970 cpm/nmol HDL CE. This variation was explained by the different mass of HDL present in the \(d>1.063 \, \text{g/mL}\) fraction of plasma from each individual (Table 2). We next sought to verify that our in vitro method for labeling HDL resulted in a distribution of radiolabeled CE throughout the entire HDL density range. After its isolation from the \(d>1.063 \, \text{g/mL}\) fraction of normolipidemic plasma, labeled HDL was applied to a density gradient; after ultracentrifugal fractionation, the radioactivity was quantified in each HDL subfraction. The peak HDL radioactivity was in fraction 15, which corresponded exactly with the peak in HDL protein in normolipidemic plasma. Furthermore, the radioactivity was distributed in a normal fashion around fraction 15 and was only found in association with those fractions normally containing HDL (gradient fractions 12 through 22, \(d=1.063\) to 1.167 g/mL). The presence of HDL in these fractions was confirmed by detection of apoA-I as the major apolipoprotein after sodium dodecyl sulfate (5% to 19%)-polyacrylamide gradient gel electrophoresis. Neither lipids nor radioactivity was detectable in gradient fractions 23 through 30 of \(d>1.17 \, \text{g/mL}\) (Fig 1B). HDL particles were absent from these latter fractions, and apoA-I present at the end of the gradient \((d>1.17 \, \text{g/mL})\) was detected as free apoA-I. HDL from hypertriglyceridemic subjects showed a peak in gradient fraction 18 \((d=1.12 \, \text{g/mL})\), and after radiolabeling, the radioactive CE was normally distributed throughout the HDL density range in these subjects (Fig 1A). During incubation of plasma containing the radiolabeled HDL at \(37^\circ\text{C}\), a progressive, time-dependent transfer of the radioactive HDL CE from the HDL density range into LDL and VLDL occurred. Transfer did not occur to any appreciable extent (less than 10% of total radioactivity) when the plasma was incubated at \(4^\circ\text{C}\).

**CE Transfer From HDL to ApoB-Containing Lipoproteins**

Fig 2 shows the effects of incubation on the CE-specific radioactivities in HDL and the apoB-containing lipoproteins. During the first 6 hours of incubation, the CE-specific radioactivity in VLDL and LDL increased rapidly, consistent with an initial mass transfer of CE to these lipoproteins. Thereafter the specific radioactivity increased slowly and, as expected, became asymptotic with the declining HDL CE-specific radioactivity. HDL CE-specific radioactivity declined at a similar rate throughout the time course of the incubation, indicating a continual exchange of CE between HDL and the apoB-containing lipoproteins. The observation of declining HDL-specific radioactivity also emphasized the need to use the actual HDL CE-specific radioactivity at each individual time point when assessing CE mass transfer from CE radioactivity. Fig 3 shows the mass transfer of CE into VLDL and LDL with time as determined directly by monitoring the increase in CE mass and by calculation from the known specific radioactivity of HDL CE. During the first 6 hours of incubation from HDL CE, a continual exchange of CE between HDL and the apoB-containing lipoproteins occurred. Transfer did not occur to any appreciable extent (less than 10% of total radioactivity) when the plasma was incubated at \(4^\circ\text{C}\).
Fig 4. Bar graphs showing total mass of high-density lipoprotein cholesteryl ester transferred to very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL). During the first 6 hours of incubation at 37°C, the majority of cholesteryl ester transferred resulted from a net mass transfer. After 6 hours of incubation, an exchange of cholesteryl ester between lipoproteins occurred. Density limits of lipoprotein fractions are shown in Fig 1. Solid part of bars indicates exchange of cholesteryl ester; hatched part of bars, net mass transfer of cholesteryl ester.

calculation of mass transfer based solely on radioactivity would be progressively inaccurate at incubation times longer than 6 hours. The situation with LDL (Fig 3B) was generally similar to VLDL; however, the two methods were only equivalent for the first 3 to 4 hours of incubation, after which mass transfer slowed, ceasing at 6 hours, while radioactive transfer continued at the same rate. Thus, for the first 6 hours of incubation, the exchange component was somewhat higher for LDL than for VLDL. The relative extents to which net mass transfer and exchange of CE occurred during the first 6 hours and after 18 hours of incubation in plasma from normolipidemic and mildly hypertriglyceridemic subjects are shown in Fig 4. Several points emerged from these data. First, for VLDL, the exchange component was small and insignificant during the first 6 hours of incubation. Second, more CE mass was transferred to VLDL in hypertriglyceridemic subjects, which is consistent with the presence of higher concentrations of VLDL in these subjects. Third, LDL was the major CE acceptor in normolipidemic subjects, and the exchange component was significant during the first 6 hours of incubation. In hypertriglyceridemic subjects, LDL was a relatively minor CE acceptor, and during the first 6 hours of incubation the exchange component exceeded net mass transfer. Fourth, the CE exchange component was very significant after 18 hours of incubation in all subjects tested and was particularly pronounced for the LDL fraction. Clearly, as LDL is a major lipoprotein class in all human plasma, CE exchange represents a significant component of reverse cholesterol transport, and caution must be used when interpreting data based on CE radioactivity, particularly in subjects in whom LDL levels are high.

Table 4 shows the rates of CE mass transfer to VLDL and LDL in plasma from normal and mildly hypertriglyceridemic subjects. These data were calculated from the enzymatic determination of changes in the VLDL and LDL CE mass during the first 6 hours of incubation. The rate of transfer to VLDL in hypertriglyceridemic subjects was clearly higher than the rate of transfer to VLDL in normolipidemic individuals, and the increase in CE mass in the hypertriglyceridemic subjects was twofold greater than in the normolipidemic individuals (138±10.7 and 62.9±9.0 μg CE, respectively). The rate of transfer to LDL in hypertriglyceridemic patients was, in contrast, lower than the rate of transfer to LDL in normal subjects, and after 6 hours of incubation the CE content of LDL from normolipidemic subjects had increased more than that from hypertriglyceridemic individuals (165.7±46.0 and 62.5±14.6 μg CE, respectively). At the same time, a marked reduction (21.4±7.6%) of CE mass occurred in HDL. These rate calculations, while reflecting the time-dependent net flux of CE, did not indicate whether the observed overall rate was a function of lipoprotein acceptor quantity or chemical composition. Some insight can be gained by expressing the net mass transfer to apoB-containing lipoproteins after 6 hours of incubation as a function of the plasma concentration (Fig 5). When expressed in this fashion it becomes apparent that despite the fact that the VLDL fraction from hypertriglyceridemic subjects accepted approximately twice the quantity of CE as normolipidemic subjects, transfer was a function of the increased VLDL levels and, on a per unit mass basis, there was little difference in the ability of VLDL from normal and mildly hypertriglyceridemic individuals to accept CE. In both subject groups, VLDL represented the most active CE acceptor per unit mass of lipoprotein. This was not the case with the individual LDL subfractions, in which, qualitatively, the LDL of normolipidemic subjects was a more active CE acceptor than that of mildly hypertriglyceridemic individuals. For each normolipidemic or moderately hypertriglyceridemic subject tested, the lightest LDL subfraction (LDL₁, d=1.019 to 1.023 g/mL) exhibited the greatest capacity to accept CE per milligram lipoprotein mass compared with the other LDL subfractions (LDL₄).

Table 4. Rate of Cholesteryl Ester Mass Transfer From HDL to Apolipoprotein B–Containing Lipoproteins

<table>
<thead>
<tr>
<th>Lipoprotein Fraction</th>
<th>Normolipidemic</th>
<th>Hypertriglyceridemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>12.3±2.8</td>
<td>29.2 (28-30)</td>
</tr>
<tr>
<td>LDL</td>
<td>33.5±12.1</td>
<td>10.8 (8.4-13.1)</td>
</tr>
</tbody>
</table>
Our present study detailed a new methodology for the measurement of CE transfer from HDL to apoB-containing lipoproteins, primarily VLDL and LDL, in individual human plasma under physiological conditions. The more commonly used method is based on a determination of the change in CE mass after plasma incubation. Typically, the mass transfer of CE is measured by incubation of plasma over a 6-hour period at 37°C in the presence of 1.5 mmol/L 5,5'-dithiobis-2-nitrobenzoic acid, an inhibitor of LCAT activity. Subsequently, an aliquot of plasma is removed and VLDL and LDL are isolated by precipitation with heparin/MnCl₂. The transfer of CE from HDL to apoB-containing lipoproteins is determined from the measurement of the decrease in the mass of CE present in the supernatant containing HDL. Under these conditions, there is no estimation of the increment of CE content in VLDL or in LDL, and it is impossible to determine which CE acceptor particles are the most efficient. Other methods, which are based on the determination of CETP activity, have also been used. In one, the activity of CETP is followed by incubation at 37°C of small aliquots of diluted plasma as a CETP source in the presence of isolated LDL as acceptor in the absence of VLDL. The transfer of radioactive CE is related to the amount of supernatant radioactivity remaining after precipitation of the LDL. The main drawback of this method is the use of nonphysiological lipoprotein mass ratios. A third generally used method is a CE assay in which labeled HDL is added to isolated LDL in the presence of purified CETP. LDL and HDL are then separated, and the radioactive content of each fraction is determined. This method requires large quantities of plasma for the isolation of CETP. In these latter two methods, CE transfer is evaluated in isolated lipoproteins; moreover, the authors measured only CE exchange between HDL and the apoB-containing lipoproteins rather than a real transfer of CE, as no modification in the CE mass was determined.

The method detailed here is particularly useful for several reasons. First, the purified, labeled HDL can be obtained at high specific activity. Under these conditions, addition of a small quantity of labeled HDL (1% of total HDL) to plasma was sufficient to trace CE exchange but did not alter the physiological ratio of lipoprotein mass between donor and acceptor particles. As the labeled HDL was obtained from the same individual, a homogeneous pool was obtained upon addition of the labeled HDL to plasma. Second, CE transfer and exchange are evaluated on total plasma and not on isolated lipoproteins. The experimental conditions were thus as close as possible to the in vivo conditions. However, there was no continuous input of newly synthesized lipoproteins to the system, nor were the metabolized lipoproteins continuously removed. In this respect, our in vitro system was static in comparison to the dynamic in vivo situation. Third, isolation of lipoproteins by density gradient ultracentrifugation allows separation of all lipoprotein classes and subclasses for analysis. Finally, our method permits a simultaneous evaluation of both net mass CE transfer and exchange. It is evident from Fig 3 that the use of the HDL-specific radioactivity to calculate mass transfer of CE to the apoB-containing lipoproteins is inappropriate if incubations of plasma exceed 4 to 6 hours, because there is a significant CE exchange component during the first 6 hours of incubation, particularly with the LDL.

The rate and the magnitude of the net mass transfer of CE from HDL to VLDL during in vivo incubations of human plasma are a function of the VLDL concentration. The rate of exchange of CE between HDL and LDL increases with increasing concentrations of LDL. Other studies indicate an increase in CE transfer in dyslipidemic subjects, hypercholesterolemic subjects, and patients with insulin-dependent diabetes mellitus. Taken together, these results indicate that CE transfer between either VLDL or LDL and HDL is influenced by both the relative and absolute concentrations of the various lipoprotein donor and acceptor particles. Recent data from our laboratory have also shown that in subjects with heterozygous familial hypercholesterolemia resulting in high plasma LDL levels, the CE exchange component is even more pronounced than noted here for normolipidemic and mildly hypertriglyceridemic individuals. Thus the extent to which the exchange component contributes to the net mass transfer of CE calculated from the HDL-specific radioactivity depends on the relative mass ratios of VLDL to LDL and is influenced by the triglyceride content of the latter. These considerations underline the requirement for performing time-course experiments for individual subjects when estimating CE transfer to apoB-containing lipoproteins by radioactive methods. In estimating net CE mass transfer to the apoB-containing lipoproteins, a direct chemical determination of the increase in CE mass within these lipoprotein species appears to provide the most accurate data. The advantage of the method described here is that the nature and physiological ratios of each subject’s lipoproteins are pre-
The net mass transferred of HDL CE to VLDL was twofold higher in mildly hypertriglyceridemic than in normolipidemic subjects, in agreement with an earlier study. However, if lipoprotein mass is considered, then the VLDL of both the normolipidemic and mildly hypertriglyceridemic subjects exhibited the same ability to accept CE from HDL. Conversely, the LDL of moderately hypertriglyceridemic subjects was a poor CE acceptor compared with its counterparts in normolipidemic subjects. Moreover, for the first time, this study suggested heterogeneity in CE transfer from HDL to LDL particle subfractions. Indeed, when CE mass transfer was expressed relative to the mass of the lipoprotein acceptor fraction, then light LDL subfractions appeared to be superior acceptors of CE from HDL compared with subfractions of higher density (LDL1 through LDL2).

Qualitatively, the proportion of triacylglycerol and CE contained in each lipoprotein fraction appeared to be an essential determinant of the capacity of these particles to accept CE. VLDL and LDL1, which are triglyceride-rich and CE-poor particles, were the most efficient acceptors of CE from HDL. Elevated VLDL levels in mildly hypertriglyceridemic subjects altered the ratio of the concentrations of the plasma lipoproteins, such that VLDL became the preferential CE acceptor. In normolipidemic subjects, however, we observed that on a quantitative basis, the major acceptor of CE from HDL was LDL, the major lipoprotein class. Moreover, among the LDL particles, it was the abundant subfractions of intermediate density (LDL2 through LDL4) that on a quantitative basis accepted the most CE from HDL. CETP thus promoted the redistribution of the CE pool from HDL to LDL and preferentially to LDL subfractions with intermediate density, whose elevated affinity for the cellular LDL receptor facilitates their rapid removal from plasma. For this reason, CETP may have an antiatherogenic role in normolipidemic subjects by promoting CE removal from the plasma via the LDL receptor pathway.

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