High density lipoprotein subfractions isolated by heparin-Sepharose affinity chromatography and their role in cholesteryl ester transfer to very low density lipoproteins

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Abstract Normal human plasma HDL was applied to a column of heparin-Sepharose in the presence of MnCl₂ and three fractions were obtained by stepwise elution with increasing NaCl concentrations: a non-retained fraction (NR, 78% of protein) and two retained fractions (R₁ and R₂, 18 and 2.5% of protein, respectively). Both unesterified and esterified cholesterol increased from NR to R₁ to R₂ but the increment was more pronounced for unesterified cholesterol. ApoA-II to apoA-I ratio was lower in R₁ compared to NR but R₂ contained more apoC than NR. ApoE increased from NR to R₁ to R₂ (0.07, 0.4, and 14% of protein in each fraction, respectively) while apoB was found only in R₂. Agarose gel electrophoresis and immunoadsorbents for apoB and apoE showed that R₁ consisted of two major lipoprotein populations, one containing apoB and some apoE and the other containing apoE and no apoB. Cholesteryl ester transfer between each HDL subfraction and VLDL in the presence of partially purified cholesterol ester transfer protein was studied. NR and R₁ gave the highest initial rates of transfer for labeled cholesteryl ester which were corroborated by significant mass transfer of cholesteryl esters. From these results, we concluded that there is no connection between cholesteryl ester transfer and apoE. On the other hand, transfer from R₂ to VLDL followed different kinetics with a high zero hour transfer but with subsequently lower rates when compared to NR and R₁. The cholesteryl ester transfer activity in R₂ was mainly due to the presence of apoE-containing lipoproteins whereas those containing apoB had minimal transfer activity. However, because this transfer of label was not translated into significant mass transfer of cholesteryl ester to VLDL, the apoE-containing lipoproteins appear involved mainly in the equilibration of cholesteryl esters. — Marcel, Y. L., C. Vezina, D. Emond, R. B. Verdery, and R. W. Milne. High density lipoprotein subfractions isolated by heparin-Sepharose affinity chromatography and their role in cholesteryl ester transfer to very low density lipoproteins. J. Lipid Res. 1981. 22: 1198–1205.

Supplementary key words apoE · apoB · apoC · LCAT

Protein-mediated transfer of cholesteryl esters between lipoproteins (1) has recently been shown to be closely related to cholesteryl ester formation by LCAT (2, 3) and its transport by different lipoproteins. However, conflicting evidence has been presented on the characteristics of the cholesteryl ester transfer proteins (2, 4). In contrast to the equilibrium existing between HDL and LDL (5), we have shown net transfer of cholesteryl esters from HDL to triglyceride-rich lipoproteins at a rate comparable to the reaction rate of LCAT (6). We therefore proposed that this process supplies most of the cholesteryl esters found in plasma lipoproteins and that LDL cholesteryl esters are derived from those originally transferred to VLDL.

Because of the heterogeneity of HDL, we have studied the ability of HDL subfractions to serve as cholesteryl ester donors in the transfer process to VLDL. To this end, HDL subclasses have been isolated as a function of their apoE content by heparin-affinity chromatography according to methods previously described (7, 8). Using these subfractions, we investigated whether apoE-containing HDL played a special role in cholesteryl ester transfer, as previous studies had indirectly suggested (9, 10).

MATERIALS AND METHODS

Preparation of lipoproteins

Blood from normal volunteers was collected in blood packs containing citrate-phosphate-dextrose (Fenwall Laboratories, Deerfield, IL). Upon separation of the plasma, sodium azide was added (1 mg/ml) to all samples and this concentration was maintained throughout dialysis and handling.

Abbreviations: CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase.
Plasma lipoproteins were obtained by sequential preparative ultracentrifugation at 5°C in a Beckman 50.2 Ti rotor (11, 12). Chylomicrons were removed from the plasma of postprandial donors by flotation (1.1 \times 10^3 \text{ g-min}) through a layer of saline (0.15 M NaCl). Density for all other separations was adjusted by addition of solid KBr. VLDL was isolated at d > 1.25 g/ml. The fraction retained by phenyl-Sepharose was used as the source of cholesteryl ester transfer protein in most experiments and in some experiments this was further purified by chromatography on Con A-Sepharose and CM-cellulose (4).

HDL or HDL subfractions labeled with radiocholesterol (15 \mu g of cholesteryl ester) were incubated with unlabeled VLDL (20 \mu g of cholesteryl ester, unless otherwise stated) in the presence of CETP (140 \mu g of protein) with or without albumin (3 mg/ml, human albumin, Pentex, Montreal, Quebec). LCAT activity was inhibited by the addition of DTNB (1.4 mM). Final volume was adjusted to 1 ml with 10 mM Tris, 0.15 M NaCl, 1 mM EDTA (pH 7.4) and the mixtures were incubated at 37°C for various times. In some control experiments, the VLDL was omitted from the incubations. In time course experiments, the zero hour value represents the mixing of incubation components at 4°C and their further processing for centrifugation at the same temperature. Immediately after incubation, the mixture was chilled to 4°C, overlaid with cold saline solution (0.9%), and centrifuged in a Beckman 40.3 rotor with 2-ml adaptors to float VLDL. The fractions d < 1.006 g/ml and d > 1.006 g/ml were quantitatively recovered and analyzed for cholesterol concentration and radioactivity. In some experiments, each centrifugal fraction was analyzed both for cholesterol and for distribution of apoprotein radioactivity. Separation and counting procedures for cholesterol and cholesteryl ester were as described previously (6). The amount of cholesteryl ester transferred to VLDL was calculated from the ratio of radioactivity in VLDL cholesteryl ester to the total radioactivity recovered in VLDL and HDL cholesteryl ester, and from the initial cholesteryl ester concentration in HDL.

In selected experiments, VLDL was linked to CNBr-activated Sepharose (13) and the appropriate weight of VLDL-Sepharose to supply the desired amount of VLDL cholesteryl esters was included in the incubation mixture instead of VLDL. After incubation, the supernatant was collected, the VLDL-Sepharose was washed 3 times with 10 ml of Tris-buffered saline containing 1% (w/v) bovine serum albumin and analyzed for cholesterol ester as described above.

**Analyses**

Protein levels were measured by the method of Lowry et al. (16) using bovine serum albumin as a

**Assay system for the study of cholesteryl ester transfer between lipoproteins**

Cholesteryl ester transfer protein was partially purified as described by others (4) from the plasma fraction of d > 1.25 g/ml. The fraction retained by phenyl-Sepharose was used as the source of cholesteryl ester transfer protein in most experiments and in some experiments this was further purified by chromatography on Con A-Sepharose and CM-cellulose (4).

Heparin-Sepharose was prepared by covalent linkage of 120 mg of pig mucosal heparin (Upjohn, Don Mills, Ontario) per 50 ml of CNBr-activated Sepharose-4B (Pharmacia) (13). Chromatography was done according to the method of Weisgraber and Mahley (8). Typically, HDL (60–120 mg of protein) was dialyzed against 5 mM Tris Buffer, pH 7.5, 50 mM NaCl, and made up to 25 mM MnCl₂ prior to application. The sample was applied to the column (1.6 \times 25 \text{ cm}) conditioned with the same buffer and the HDL was allowed to equilibrate with the column overnight. The non-retained fraction (NR) was eluted with the starting buffer, and two other fractions (R₁ and R₂) were eluted with the same buffer without MnCl₂, but with 70 mM and 300 mM NaCl, respectively.

**Iodination of cholesteryl ester-labeled HDL subfractions**

Cholesterol-labeled HDL was fractionated by heparin-Sepharose chromatography as described above and the HDL subfractions (NR, R₁ and R₂; 1 mg protein/ml) were dialyzed against 0.1 M borate buffer, pH 8.5. After iodination with ¹²⁵I by the Bolton-Hunter procedure (14), the doubly labeled lipoproteins were exhaustively dialyzed against 10 mM Tris, 1 mM EDTA, pH 7.4, and filtered before use. Distribution of ¹²⁵I among apoproteins of each HDL subfraction was determined by SDS polyacrylamide gel electrophoresis (15). After staining and identification of the various apoproteins by molecular weight, the gel was cut into 2-mm slices with a multiple blade gel slicer (BioRad, Richmond, CA) and the ¹²⁵I was measured.
standard. Total cholesterol and triglycerides were measured enzymatically (17, 18) with an auto-analyzer (ABA-100 bichromatic analyzer, Abbott Laboratories). Unesterified cholesterol was determined by gas–liquid chromatography (19) and esterified cholesterol was calculated from the levels of total and unesterified cholesterol. Total phospholipids were measured as described previously (20).

Apolipoproteins were analyzed by SDS polyacrylamide gel electrophoresis (15) and by analytical isoelectric focusing on polyacrylamide gel (21). Lipoproteins were electrophoresed on agarose gel (Agarose C, Pharmacia) by the method of Noble (22). ApoE and apoB concentrations were measured by radioimmunoassays using monoclonal mouse anti-apoE antibody and rabbit anti-apoB IgG, respectively (23).

**RESULTS**

**Separation of HDL subfractions by heparin-Sepharose affinity chromatography**

With the stepwise elution system (10), a very reproducible series of HDL subfractions was obtained both within and between HDL samples. The non-retained fraction (NR) represented 78 ± 0.4% of HDL protein; the retained fraction eluted with 70 mM NaCl (R₁) represented 18 ± 0.2% and the retained fraction eluted with 0.3 M NaCl (R₂) represented 2.5 ± 0.3%. The recovery of protein was always greater than 95%.

Relative composition of these HDL subfractions was determined in four different HDL preparations (Table 1). Retained fractions, R₁ and R₂, were characterized by lower protein content compared to NR. This difference translated into significantly higher total cholesterol in R₁ and R₂. Both unesterified and esterified cholesterol increased from NR to R₁ to R₂ but the increment was more pronounced for unesterified cholesterol (12.5, 17.6, and 21.3% of total cholesterol in NR, R₁, and R₂, respectively).

**TABLE 1. Relative composition of HDL subfractions isolated by heparin-Sepharose chromatography**

<table>
<thead>
<tr>
<th>Subfraction</th>
<th>Protein (%)</th>
<th>Total Cholesterol (%)</th>
<th>Triglycerides (%)</th>
<th>Phospholipids (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR</td>
<td>57.5 ± 5.4</td>
<td>13.3 ± 0.6</td>
<td>3.7 ± 1.0</td>
<td>25.1 ± 4.5</td>
</tr>
<tr>
<td>R₁</td>
<td>52.3 ± 3.3</td>
<td>15.9 ± 2.3</td>
<td>3.5 ± 0.6</td>
<td>28.1 ± 2.4</td>
</tr>
<tr>
<td>R₂</td>
<td>51.6 ± 3.7</td>
<td>24.2 ± 3.2</td>
<td>3.3 ± 2.3</td>
<td>20.8 ± 1.5</td>
</tr>
</tbody>
</table>

* Mean and S.D. (n = 4).

Table 1 shows that NR and R₁ had different ratios of apoA-I to apoA-II and R₁ contained more apoC than NR (Fig. 1). Radioimmunoassays of apoB and apoE indicated that all apoB was present within the R₂ fraction while apoE concentration increased progressively from NR to R₁ to R₂ (Table 2). Radiolabeled apoproteins of each HDL subfraction were separated by SDS gel electrophoresis and the radioactivities of the various molecular weight zones were counted. The apoproteins of the NR and R₁ fractions had a similar distribution of radioactivity, with 50% present within the molecular weight range of apoA-I and 20% present within the molecular weight range of apoA-II and apoC (Table 3). The apoproteins of the R₂ fraction were clearly different, with 50% of the radioactivity present in the apoB region, 13% in the apoA-I region, and only 9% in the apoA-II-apoC region. In contrast, the distribution of radioactivity in the molecular weight range of apoE did not differ significantly between HDL subfractions.

Agarose-gel electrophoresis of the HDL subfractions clearly demonstrated the heterogeneity of the R₂ fraction which was resolved into two major components (Fig. 2): the slower component comigrated...
TABLE 2. Concentration of apoE and apoB in HDL subfractions

<table>
<thead>
<tr>
<th>HDL Subfractions</th>
<th>apoE</th>
<th>apoB</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR</td>
<td>0.7 ± 0.7*</td>
<td>0*</td>
</tr>
<tr>
<td>R1</td>
<td>4.0 ± 5.7</td>
<td>0</td>
</tr>
<tr>
<td>R2</td>
<td>140 ± 140</td>
<td>40 ± 30</td>
</tr>
</tbody>
</table>

* Values are expressed as the ratio of apoE or apoB protein to total protein × 10^6 (mean ± S.D., n = 11).

When the R2 fraction was passed on an anti-apoE immunoadsorber column, cholesteryl ester transfer was significantly decreased, whereas there was no change caused by passage on an immunoadsorber column against normal mouse serum (Fig. 4). On the contrary, passage of R2 over an anti-apoB immunoadsorber column, which removed most of the apoB but left most of the immuno-reactive apoE un-retained, significantly increased the transfer rate to VLDL (Fig. 4). The simultaneous assay of the different HDL subfractions allowed us to compare them for cholesteryl ester transfer to VLDL: NR = R1 > R2 un-retained on anti B > R2. Similar results were obtained with each HDL subfraction when VLDL-Sepharose was substituted for VLDL as described under Methods (not shown).

Chemical determination of cholesteryl ester concentrations in VLDL before and after incubation with each HDL subfraction (Table 5) demonstrated that NR and R1 could give significant net transfer of cholesteryl ester to VLDL whereas that measured with R2 did not reach significance. In addition, the cholesteryl ester concentration in VLDL at 0 hr of the incubation with R2 (34.0 ± 1.0 µg) was not significantly different from that of VLDL incubated without R2 (33.7 ± 1.4 µg) and thus does not corroborate the high initial transfer of labeled cholesteryl ester found with R2.

Role of HDL subfractions in cholesteryl ester transfer to VLDL

Normal plasma HDL was labeled in the cholesterol and cholesteryl ester moieties as described under Methods, applied to the heparin-Sepharose column, and eluted. The resulting NR, R1, and R2 subfractions all contained labeled cholesteryl esters with specific activities of 19, 18, and 12 × 10^6 cpm/µg cholesterol, respectively. Cholesterol-labeled HDL subfractions were used to study cholesteryl ester transfer to VLDL. The NR and R1 subfractions were equally effective cholesteryl ester donors to VLDL in the presence of CETP (Fig. 3), with an identical initial transfer velocity. The R2 fraction exhibited very different properties (Fig. 3): it was characterized by a high initial transfer to VLDL, that is, a zero time value for cholesteryl ester transfer which varied from 5 to 30% of the R2 cholesteryl ester and which may have been related to the unstability of the R2 fraction causing its adsorption to VLDL. Subsequently, the transfer was a function of time and was linear for 2 hr. When VLDL was omitted from the incubation, the amount of cholesteryl ester from the R2 fraction that floated at d 1.006 g/ml was markedly reduced and further decreased in the presence of albumin (Table 4). Therefore the R2 fraction appeared to be stabilized by albumin and flotation of its labeled cholesteryl esters at 1.006 g/ml required the presence of VLDL. In addition, the R2 fraction possessed some endogenous transfer activity whether alone or in the presence of albumin, but the transfer was significantly enhanced by addition of CETP (Table 4).

<table>
<thead>
<tr>
<th>Fraction Analyzed</th>
<th>Percent Radioactivity in Various Molecular Weight Regions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;80^a</td>
</tr>
<tr>
<td>NR</td>
<td>2.0^a</td>
</tr>
<tr>
<td>R1</td>
<td>4.4</td>
</tr>
<tr>
<td>R2</td>
<td>51.3</td>
</tr>
</tbody>
</table>

* Expressed as k daltons.

With NR, R1, and R2.

TABLE 3. Relative composition of apoproteins in [125I]-labeled HDL subfractions analyzed by SDS polyacrylamide gel electrophoresis

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there was a significant transfer of label within the molecular weight range of apoA-I; but only with R₂ was there a transfer of radioactivity in the molecular weight region of apoE and in the region of 22 to 18,000 daltons. When apoE levels were measured by radioimmunoassay in VLDL before and after incubation with HDL subfractions, there was no significant change although the variation in the assay (6%) may have been too important to measure an hypothetical transfer of apoE in the range of 10% of VLDL levels.

DISCUSSION

Application of the HDL to heparin-Sepharose in the presence of Mn²⁺ and stepwise elution as described by Weisgraber and Mahley (10) yielded three fractions which were different from those previously described. However, HDL subfractions retained by heparin always share common characteristics: as in previous works (9, 10), fractions retained on heparin-Sepharose were richer in total cholesterol compared to nonretained fractions. Also significant was the progressive increase in relative concentration of unesterified cholesterol with increasing heparin affinity of the fractions (R₂ > R₁ > NR). The relative concentration of apoE in the R₁ fractions, 0.4% of total protein, was far too low to explain the affinity of this fraction for heparin on the basis of one apoE molecule per particle. Both NR and R₁ fractions contained apoA-I and apoA-II as their major apoproteins, but the densitometric scanning of the isoelectric focusing gel electrophoretograms of their apoproteins indicated that the ratio of apoA-II to apoA-I was significantly lower in R₁ compared to NR, but that this decrease in apoA-II in R₁ was accompanied by an increase in apoC-I, apoC-II, and apoC-III in this fraction (Fig. 1). Since it could not be explained on the basis of its apoE content, the retention of R₁ on heparin-Sepharose might be related to a secondary affinity process: the original ligand, heparin, binds apoE and/or apoE-containing lipoproteins, which become a secondary ligand; these bound apoE molecules may have an affinity for certain HDL particles such as R₁ and thus cause their retention on the column in the presence of Mn²⁺. Immunodiffusions with antisem against apoD have shown the presence of apoD in all fractions from NR to R₁ to R₂, indicating that this apoprotein, which has been proposed as cholesteryl ester transfer protein (2), does not interact with heparin.

The R₂ subfraction was heterogeneous as evidenced by agarose gel electrophoresis (Fig. 2). The relative concentration of apoB and apoE varied greatly from preparation to preparation inasmuch as apoB levels in certain HDL were found to be very low. When R₂ was passed through an anti-apoB immunoadsorber column, the unretained fraction contained no apoB and half of the initial apoE concentration. When R₂ was passed through an anti-apoE immunoadsorber column, the unretained fraction contained no apoE and most of the initial apoB concentration. From these experiments, it can be concluded that the R₂ fraction is composed of at least two populations of lipoproteins, one containing both apoB and apoE and one containing apoE and no apoB.

When cholesteryl ester-labeled HDL was fraction-
ester-labeled, but the specific activity of the lower reaction rates of brate at the same rates in NR and R, but not in R,. containing HDL (9), although another interpreta-
These findings corroborate our earlier observations in NR and R, and this difference persisted in the R, fraction unretained on anti-B-Sepharose. These results indicate that cholesteryl esters are formed or equili-
rate on heparin-Sepharose, each subfraction was calculated for the R2 fraction was always lower than
cholesteryl ester (24 pg cholesteryl ester) as described under Methods.

TABLE 4. Flotation of cholesteryl esters at d 1.006 g/ml upon incubation of the R2 fraction with or without VLDL and under various conditions

<table>
<thead>
<tr>
<th>Additions to Incubation Mixture</th>
<th>Percent of labeled CE in d &lt; 1.006 g/ml at 0 hr</th>
<th>Percent of labeled CE in d &lt; 1.006 g/ml at 4 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>Protein</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>7.1</td>
</tr>
<tr>
<td>0</td>
<td>CETP* (140 μg)</td>
<td>5.0</td>
</tr>
<tr>
<td>0</td>
<td>Albumin (3 mg)</td>
<td>1.9</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>32.4</td>
</tr>
<tr>
<td>+</td>
<td>CETP* (140 μg)</td>
<td>26.6</td>
</tr>
<tr>
<td>+</td>
<td>Albumin (3 mg)</td>
<td>18.1</td>
</tr>
</tbody>
</table>

* Cholesteryl ester transfer protein.

The R2 fraction (14 pg cholesteryl ester) was incubated with or without VLDL (24 pg cholesteryl ester) as described under Methods.

ated on heparin-Sepharose, each subfraction was cholesteryl ester-labeled, but the specific activity calculated for the R2 fraction was always lower than in NR and R, and this difference persisted in the R2 fraction unretained on anti-B-Sepharose. These results indicate that cholesteryl esters are formed or equili-
brate at the same rates in NR and R, but not in R2. These findings corroborate our earlier observations on the lower reaction rates of LCAT with apoE-containing HDL (9), although another interpreta-
might be that newly formed labeled cholesteryl esters are transferred from R2 to NR and R, during the incubation of d > 1.065 g/ml.

NR and R, fractions gave the highest rates of cholesteryl ester transfer to VLDL and there was no difference between these two fractions. Cholesteryl ester transfer from NR and R, to VLDL was dependent upon the presence of cholesteryl ester transfer protein and was a function of time. As the NR fraction has negligible apoE, it can be concluded that cholesteryl ester transfer between HDL and VLDL can take place independently of apoE. Experiments with HDL subfractions labeled in both cholesteryl ester and apoproteins showed that there were small but significant transfers or equilibration of labeled apoproteins concomitant with cholesteryl ester transfer. With NR and R,, the transfer to VLDL of apoprotein label was mainly within the molecular weight range of apoA-I in addition to the known transfer and equili-
ration of apoC. Since VLDL does not contain any significant level of apoA-I, the process of cholesteryl ester transfer from the NR and R, subfractions of HDL to VLDL may involve a temporary fusion of the particles which results in a net increase in VLDL cholesteryl esters and in a probable transient transfer of some HDL apoproteins to VLDL. However the definitive demonstration of such a process would require the demonstration by immunoassays that apoA-I increases in VLDL.

The kinetics of cholesteryl ester transfer from R2 to VLDL were quite different from those of NR and R, and were characterized by a high initial transfer at 0 h to VLDL. Although this initial transfer could be decreased in the presence of physiological concentrations of albumin, it could not be eliminated altogether. Experiments have shown that this transfer is not due to the flotation of R2 fraction at d 1.006 g/ml and that it does require the presence of VLDL (Table 4). R2 also contains some endogenous cholesteryl ester transfer activity but the process is further enhanced by addition of a partially purified cholesteryl ester transfer protein.

![Fig. 4. Time course of cholesteryl ester transfer from cholesteryl ester-labeled R2 fraction to VLDL: O——O, original R2 fraction; ■——■, R2 unretained on anti-normal mouse serum-Sepharose; □——□, R2 unretained on anti-apoE-Sepharose; △——△, R2 unretained on anti-apoB-Sepharose. The various R2 fractions (15 μg cholesteryl ester each) were incubated with VLDL (20 μg cholesteryl ester) as described under Methods.](https://www.jlr.org)

**TABLE 5. Chemical determination of cholesteryl ester transfer from individual HDL subfractions to VLDL**

<table>
<thead>
<tr>
<th>HDL Subfraction Added</th>
<th>Cholesteryl Esters (μg) Recovered in VLDL at 0 hr</th>
<th>Cholesteryl Esters (μg) Recovered in VLDL at 4 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR</td>
<td>34.2 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.0 ± 1.5 &lt;i&gt;P &lt; 0.02&lt;/i&gt;</td>
</tr>
<tr>
<td>R1</td>
<td>35.7 ± 2.6</td>
<td>38.9 ± 1.9 &lt;i&gt;P &lt; 0.05&lt;/i&gt;</td>
</tr>
<tr>
<td>R2</td>
<td>34.0 ± 1.9</td>
<td>35.0 ± 2.9 &lt;i&gt;NS&lt;/i&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.D., n = 6.

<sup>b</sup> Significantly higher than 0-hr value or not significantly higher (NS).

VLDL and each HDL subfraction were incubated separately in the presence of the cholesteryl ester transfer protein fraction for 0 and 4 hr and the VLDL was isolated by centrifugation at d 1.006 g/ml for subsequent determination of cholesteryl ester concentrations.
transfer protein fraction. Most importantly, we have shown that within the R₂ fraction, the labeled cholesteryl esters of apoE-containing lipoproteins could transfer to or equilibrate with VLDL whereas those of apoB-containing lipoproteins could not. It should be emphasized that the apoE-containing lipoproteins (R₂ fraction unretained on anti-B-Sepharose) did not react with antisera against apoB and therefore were not contaminated with LDL or Lp(a).

When VLDL-Sepharose was used as a cholesteryl ester acceptor, the transfers of labeled cholesteryl ester from each HDL subfraction were similar to those noted with VLDL as an acceptor, confirming that the transfers do not result from a centrifugal artifact. Determinations of VLDL cholesteryl ester concentrations before and after incubation with various HDL subfractions and after reisolation by centrifugation demonstrated that NR and R₁ could give significant net transfer of cholesteryl ester to VLDL during a 4-hr incubation (Table 5) and corroborated the results obtained with labeled cholesteryl esters. However, when cholesteryl ester levels in VLDL incubated with cholesteryl ester transfer protein and albumin with or without R₂ at 4°C were compared, these levels were the same and therefore do not corroborate the high initial transfer of labeled cholesteryl esters that was found with R₂ (Table 4). This discrepancy may be only apparent and related to an active equilibration of cholesteryl esters between R₂ and VLDL in the absence of mass transfer, but it could also result from artifacts such as intrinsic instability of R₂ caused perhaps by the incubation of d 1.065 g/ml infranatant or to a loss of VLDL cholesteryl esters upon centrifugation. It should be recognized that the ultracentrifugation used to separate VLDL after incubation was a self-defeating, but necessary, step which may have obscured certain transfer taking place physiologically. Precipitation methods were not practical because they precipitate apoE-containing lipoproteins while the variations observed with VLDL-Sepharose were too important to allow the study of cholesteryl ester mass transfer.

Earlier experiments had shown that incubation of an LCAT-deficient serum with a partially purified LCAT caused a shift of apoE from HDL to VLDL and IDL which occurred together with the increase in cholesteryl esters in these lipoproteins (9). These results have recently been confirmed by the same group (24) but Utermann et al. (25), who used a highly purified LCAT and an electroimmunoassay for apoE, did not corroborate these findings. Our experiments clearly demonstrate that cholesteryl ester transfer between HDL and VLDL isolated from normal plasma can take place independently of apoE. However, R₂ nonretained on anti apoB-Sepharose, i.e., the apoE-containing HDL, can also transfer cholesteryl ester to VLDL, although through a mechanism which involves equilibration rather than net transfer.

Further experiments with VLDL isolated from LCAT-deficient plasma or with nascent VLDL isolated from perfused animal liver will be necessary to define the role of apoE-containing HDL in cholesteryl ester transfer to triglyceride-rich lipoproteins.

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