Metabolism of Apolipoprotein E in Plasma High Density Lipoproteins from Normal and Cholesterol-fed Rats*

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High density lipoproteins of rat blood plasma were labeled in vitro with radiiodinated apolipoprotein E and biologically with [3H]cholesteryl esters. These two components, present in high density lipoproteins separated from serum of normal or cholesterol-fed rats by molecular sieve chromatography, were removed slowly from perfused livers and the labeled apolipoprotein E was also removed slowly from the blood of intact rats. However, when labeled serum was subjected to ultracentrifugation at a density of 1.21 g/ml before the floating apolipoprotein E-labeled high density lipoproteins were separated by chromatography, the labeled protein was rapidly removed from the blood of intact rats by uptake into the liver. About one-half of the labeled apolipoprotein E associated with high density lipoproteins was dissociated during ultracentrifugation, but most of it reassociated with these lipoproteins when the floating lipoproteins were remixed with the sedimented serum proteins. The apolipoprotein E in such reassociated high density lipoproteins was removed from the blood of intact rats at the slow rate observed when the high density lipoproteins were separated chromatographically from whole serum. About 90% of the labeled apolipoprotein E in uncentrifuged or centrifuged high density lipoproteins was shown by affinity chromatography to be associated with particles containing apolipoprotein A-I. Rapid hepatic uptake of apolipoprotein E in centrifuged high density lipoproteins may result from an altered conformation of the apolipoprotein E on the particle surface.

Apolipoprotein E appears to be critically involved in the hepatic uptake and metabolism of partially degraded (remnant) lipoproteins of intestinal and hepatic origin (1-4). The concentration of apo-E in rat plasma is the highest reported among mammals fed cholesterol-poor diets. This apo-E is found mainly in high density lipoproteins (5, 6). We have developed a method to study the metabolism of apo-E in plasma lipoproteins of rats by a specific labeling technique and have shown that this protein is slowly removed from blood plasma when injected in either very low density lipoproteins or HDL (7). However, apo-E in VLDL was found to be removed rapidly, together with cholesteryl esters, from perfusates of isolated rat livers. The metabolism of apo-E in vivo appeared to result from rapid equilibration of apo-E between VLDL and HDL, in which it was rapidly metabolized, and HDL, in which it was metabolized slowly.

VLDL and HDL are the major circulating lipoproteins containing apo-E and they are the major hepatic uptake lipoproteins (5, 6, 12). Rapid hepatic uptake of apo-E was slow, as was the uptake of component cholesteryl esters of this lipoprotein.

EXPERIMENTAL PROCEDURES

Treatment of Rates—Male Sprague-Dawley rats weighing 300-350 g were maintained on standard Purina Rat Chow (Ralston Purina Co., St. Louis, MO) or fed a cholesterol-rich chow containing 5% lard, 1% cholesterol, 0.1% propylthiouracil, and 0.3% taurocholic acid for 2-3 weeks (6). Blood was collected from the animals as described earlier (7).

Labeling Procedure and Separation of Lipoproteins—Isolation and radioiodination of apo-E, incorporation of 125I-apo-E into lipoproteins of whole serum by incubation at 6 °C, and separation of labeled VLDL and HDL on columns of 8% agarose gel at 6 °C were performed as described (7). The separated lipoproteins were dialyzed for 2 h against 100 volumes of Krebs-Henseleit buffer (13) at 6 °C and were used in metabolic experiments within 3 h. In some experiments, apo-E-labeled plasma was brought to a non-protein solvent density of 1.21 g/ml with solid KBr and subjected to ultracentrifugation at 12 °C for 48 h at 35,000 rpm (294 × 10^6 g force) in the 40.3 rotor of a Beckman preparative ultracentrifuge (14). The floating lipoproteins were obtained by tube slicing, brought to a volume of 10 ml with phosphate-buffered saline (7), and subjected to chromatography on 6% agarose gel to obtain the labeled HDL. Alternatively, the 40.3 rotor tubes, after centrifugation, were gently mixed at room temperature for 15 min and the entire contents of the tube were brought to a volume of 10 ml as above and subjected to chromatography. To label cholesteryl esters of VLDL and HDL biologically, 250 μCi of [3H]cholesteryl, stabilized by bovine serum albumin (15), was injected into donor rats 8 h before they were bled, under diethyl ether anesthesia, from the abdominal aorta.
Metabolic Studies—Labeled lipoproteins containing an amount of cholesterol equivalent to less than 5% of that in the corresponding lipoprotein in the blood of the recipient rat were injected through the femoral vein of animals anesthetized with diethyl ether and blood samples were collected from a tail vein (7). In some experiments, samples of liver were obtained at the end of the experiment for analysis of labeled cholesterol (7). Isolated livers were perfused in a recirculating system with Krebs-Henseleit/glucose buffer containing 18% rat erythrocytes (7). For experiments with labeled HDL, the perfusate volume was 60 ml and samples of 2 ml were taken for analysis at the indicated intervals. For experiments with labeled VLDL, the perfusate volume was 35 ml and sample volumes were 1 ml. At the end of perfusions, livers were flushed with 60 ml of fresh perfusate and several samples were taken for analysis. Samples of blood, perfusate, and liver were subjected to analysis of trichloroacetic acid-soluble and insoluble [3H]- or [125]I-cholesteryl esters exactly as described (7).

Chemical Analyses—Protein was determined by a modified procedure of Lowry with bovine serum albumin as standard (16). Apo-protein E was measured by specific radioimmunoassay (5). Cholesterol in serum and in lipoprotein fractions separated by sequential preparative ultracentrifugation (14), or molecular sieve chromatography, was measured by a modification of a fluorometric enzymatic method (17).

Affinity Chromatography of HDL—An amount of chromatographically separated HDL, equivalent to that contained in 1 ml of plasma, was dialyzed against 0.15 M NaCl, 0.02% sodium azide, and 0.04% sodium deoxycholate, pH 7.4, and applied to an affinity column (18) (1 x 10 cm) prepared with monospecific rat anti-apo-A-1. The column was maintained at 6°C. Fractions containing unbound lipoprotein were collected for analysis of apo-E. The capacity of this column for apo-A-1 was ~3 mg. When 1 mg of isolated apo-E was applied to the column, no detectable protein was retained.

RESULTS

Uptake and Catabolism of Chromatographically Separated [125]I-Apo-E HDL by the Perfused Liver—HDL from chow-fed rats, labeled biologically with [3H]cholesterol and in vitro with [125]I-apo-E, were separated from serum on 6% agarose columns and dialyzed against Krebs-Henseleit buffer (Fig. 1). In four experiments, about one-fourth of the [125]I cholesterol esters was removed from the perfusate in 4 h. After a lag of 30 min, trichloroacetic acid-soluble [125]I increased linearly in the perfusate and, after 4 h, the amount of [125]I-apo-E catabolized approximately equaled the amount of the labeled protein taken up. At this time, 14.7 ± 3.5% of the [3H] cholesteryl esters and 12.5 ± 2.7% of the [125]I-apo-E in the perfusate was associated with VLDL, separated by chromatography. Thus, under the conditions of this experiment, the bulk of the labeled cholesteryl esters and apo-E remained associated with HDL. The total amount of [125]I and [3H] recovered in perfusate and liver at the end of the perfusion was 95.6 ± 2.1% and 97.3 ± 4.1%, respectively.

In these experiments, the rate of accumulation of trichloroacetic acid-soluble [125]I in the perfusates (38% after 4 h) exceeded the rate of removal of [3H]cholesteryl esters (23%) (P < 0.05). This observation raises the possibility that a subfraction of HDL, rich in apo-E, is rapidly degraded by the liver. This was investigated further in experiments with apo-E-rich and apo-E-poor subfractions of HDL, taking advantage of the fact that apo-E is contained mainly in larger particles than those in which apo-A-1 is the major protein component (7, 12). Such subfractions of HDL were obtained from the leading and trailing portions of the HDL elution profile from 6% agarose columns, each of which contained approximately equal amounts of cholesterol. As shown in Fig. 2, the rates of removal of [3H]cholesteryl esters from apo-E-rich and apo-E-poor HDL subfractions by the perfused liver were virtually identical.

Effect of Ultracentrifugation on the Composition and Metabolic Properties of [125]I-Apo-E HDL—Rat serum, incubated with [125]I-apo-E, was subjected to ultracentrifugation at a nonprotein solvent density of 1.21 g/ml. The supernatant fraction was subjected to chromatography on 6% agarose gel. The infranatant fraction contained 47.7 ± 2.3% of the [125]I-apo-E (n = 4), which represented about one-half of the recovered [125]I (total recovery was 94.1 ± 1.7%). When the original serum was subjected to chromatography on 6% agarose gel, only 13.6 ± 1.6% of the [125]I-apo-E was recovered in the region expected for unbound apo-E, indicating that about 35% of the [125]I-apo-E was dissociated from lipoproteins during ultracentrifugation. In Fig. 3A, elution profiles of [125]I-apo-E-labeled serum and the labeled lipoproteins separated by ultracentrifugation at 1.21 g/ml are compared. It is evident that most of the [125]I-
The extent to which ultracentrifugation affected the association of apo-E and apo-A-I, the other major protein component of rat HDL, on HDL particles was evaluated by chromatography on 6% agarose columns and then passed through an anti-A-I affinity column. The fraction of apo-E that was not adsorbed to the column was taken as an estimate of the unassociated apo-E. As shown in Table I, only about 10% of the apo-E appeared to be in HDL particles that did not contain apo-A-I, both in native serum and after ultracentrifugation.

$^{125}$I-apo-E HDL, isolated by gel chromatography of either whole serum or the $d < 1.21$ g/ml fraction obtained by ultracentrifugation, was injected into intact rats. As shown in Fig. 4A, ultracentrifugation caused a dramatic increase in the rate of removal of the $^{125}$I-apo-E from the blood. In two experiments, 51.2% and 58.7% of the injected $^{125}$I-apo-E in ultracentrifuged HDL was found in the liver 15 min after injection of the ultracentrifuged HDL. In the experiments shown in Fig. 4A, the HDL was injected into rats approximately 3 days after the serum was labeled with apo-E. Comparable results were obtained when HDL prepared from uncentrifuged serum was injected immediately after elution from agarose columns.

### Table I

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<td>107 ± 12</td>
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 apo-E dissociated by ultracentrifugation was derived from the HDL. In four such experiments, 10.2 ± 4.5% of the $^{125}$I-apo-E added to the serum was lost from VLDL, whereas 48.1 ± 9.1% was lost from HDL. In spite of this large loss of $^{125}$I-apo-E from HDL, the elution volume of the HDL-associated apo-E was unaltered by ultracentrifugation. In one experiment, in which unlabeled serum and its 1.21 g/ml supernatant fraction were compared, elution of apo-E from the 6% agarose columns was measured by radioimmunounoassay. Losses of apo-E mass from VLDL and HDL during ultracentrifugation were comparable to those observed with $^{125}$I-apo-E (not shown).

The extent to which ultracentrifugation affected the association of apo-E and apo-A-I, the other major protein component of rat HDL, on HDL particles was evaluated by immunoadsorption. The HDL fraction of samples of rat serum and of the 1.21 g/ml supernatant fraction of serum were obtained by chromatography on 6% agarose columns and then passed through an anti-A-I affinity column. The fraction of apo-E that was not adsorbed to the column was taken as an estimate of the unassociated apo-E. As shown in Table I, only about 10% of the apo-E appeared to be in HDL particles that did not contain apo-A-I, both in native serum and after ultracentrifugation.

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As shown in Fig. 3B, the $^{125}$I-apo-E dissociated from HDL during ultracentrifugation at a density of 1.21 g/ml was found to reassociate with the lipoprotein when the supernatant and infranatant fractions were remixed. After such recombination of $^{125}$I-apo-E, only 20.2 ± 2.1% of the labeled protein was unassociated with VLDL and HDL separated on 6% agarose columns, as compared with 12.7 ± 3.7% when the same samples of uncentrifuged serum were applied to the columns. Comparable results were obtained for reassociation of apo-E mass, as determined by radioimmunooassay in other experiments.

**TABLE II**

<table>
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<tr>
<th>Fraction (density in g/ml)</th>
<th>Total cholesterol mg/dl</th>
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<tr>
<td>&lt;1.006</td>
<td>130.6 ± 10.8*</td>
</tr>
<tr>
<td>1.006-1.019</td>
<td>76.0 ± 11.8</td>
</tr>
<tr>
<td>1.019-1.063</td>
<td>45.1 ± 6.3</td>
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<tr>
<td>&gt;1.063</td>
<td>15.5 ± 4.6</td>
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* Mean ± S.D. (total cholesterol and apo-E levels in unfractionated serum were 295.7 ± 6.9 and 41.9 ± 5.1 mg/dl, respectively).

**FIG. 5.** Distribution of cholesterol and apo-E in lipoproteins of serum from rats fed standard or cholesterol-rich diets. **A**, separation on a column of 6% agarose gel of total cholesterol (C—C) and apo-E (A—A) in 10 ml of serum from cholesterol-fed rats fasted for 24 h. The two peaks represent (from left) VLDL and HDL. The absorbance of serum lipoproteins and proteins is also shown (O—O). **B**, separation on a column of 6% agarose gel of cholesterol and apo-E in 10 ml of serum from a rat fed regular chow and fasted for 24 h. Symbols are as in **A**.

**FIG. 6.** Uptake and metabolism of doubly labeled HDL by isolated, perfused rat livers. The serum lipoproteins of cholesterol-fed rats were labeled biologically with [3 H]cholesterol and $^{125}$I-apo-E (7) and the HDL, separated on columns of 6% agarose gel, were dialyzed for 1-2 h against Krebs-Henseleit buffer and then added to perfusates of isolated rat livers. Concentration of $^3$H in cholesteryl esters and trichloroacetic acid (TCA)-soluble $^{125}$I in perfusates was measured in samples taken at the times indicated. The mass of total cholesterol in the HDL added to the perfusate was 0.76 ± 0.09 mg. Results are mean values ± S.D. for three experiments.

**FIG. 7.** Uptake of [3 H]cholesteryl esters and $^{125}$I-apo-E during perfusion of isolated rat livers from chow-fed rats with plasma VLDL from hypercholesterolemic rats, separated from serum on columns of 6% agarose gel. The mass of total cholesterol in the VLDL added to the perfusates was 1.27 ± 0.13 mg. Results are mean values ± S.D. for three experiments.

Metabolism of $^{125}$I-Apo-E in Lipoproteins from Hypercholesterolemic Rats—As shown in Table II, the cholesterol content of VLDL, HDL, and LDL and of apo-E in serum was substantially increased in rats fed the cholesterol-rich diet for 2 weeks. In confirmation of previous reports, the VLDL had predominantly $\beta$-mobility upon electrophoresis in agarose gel. The level of apo-E was about double that of chow-fed rats (12) and, in contrast to the situation in chow-fed rats, most of the apo-E was associated with VLDL rather than HDL (Fig. 5A). However, the apo-E in HDL was associated with larger particles in serum of cholesterol-fed rats (compare Fig. 5A in which the elution peak for HDL is at tube 51 with Fig. 5B in which the elution peak is at tube 58). The distribution of $^{125}$I-
apo-E, added to serum from hypercholesterolemic rats, was comparable to that of apo-E mass; 15.7 ± 2.4% of the 125I-apo-E remained unassociated with VLDL or HDL (n = 4). As in normal rats (7), the 125I-apo-E was stable as determined by rechromatography of the isolated fractions, and the labeled apo-E readily exchanged between VLDL and HDL, both in vitro and after injection into intact rats (not shown). The rate of removal of [3H]cholesteryl esters in HDL from hypercholesterolemic rats, during perfusion through livers of chow-fed rats (Fig. 6), was similar to that observed with HDL from chow-fed rats (Fig. 1), as was the rate of production of trichloroacetic acid-soluble 125I-apo-E. In these experiments with HDL from cholesterol-fed rats, 17.2 ± 5.7% of the [3H]cholesteryl esters and 12.4 ± 3.5% of the 125I-apo-E remaining in the perfusate after 4 h was associated with VLDL. Recovery of 125I and 3H in liver and perfusate at the end of the experiments was 96.3 ± 2.5 and 92.7 ± 5.1%, respectively.

Similar experiments were performed with doubly labeled VLDL from hypercholesterolemic rats (Fig. 7). The rates of removal of 125I-apo-E and [3H]cholesteryl esters from the perfusate were indistinguishable, but somewhat more rapid than observed with VLDL from chow-fed rats (7). At the end of the 21-min perfusion period, 11.2 ± 2.0% of the [3H]cholesteryl esters and 17.9 ± 7.2% of the 125I-apo-E remaining in the perfusate were associated with HDL. Recovery of [3H]cholesteryl esters and 125I-apo-E from liver and perfusate at the end of the experiments was 96.1 ± 2.1% and 93.7 ± 4.1%, respectively.

The effect of ultracentrifugation upon the composition and metabolic properties of labeled HDL was studied, as described for HDL from chow-fed rats. Less 125I was dissociated from lipoproteins during ultracentrifugation of serum of hypercholesterolemic rats at a density of 1.21 g/ml (17.4 ± 3.4%) than in the case of chow-fed rats. Most of the apo-E was lost from HDL, such that only 64.7 ± 8.9% of that originally present remained after ultracentrifugation. As with chow-fed rats, 125I-apo-E in ultracentrifuged HDL from cholesterol-fed rats was removed much more rapidly from the blood than uncentrifuged HDL (Fig. 8).

**Discussion**

In confirmation of our earlier research in which 125I-apo-E HDL from chow-fed rats was injected into intact rats (7), we found that the labeled protein was removed slowly from periwormes of isolated rat livers and then catabolized completely. [3H]Cholesteryl esters of these chromatographically separated HDL were taken up from the perfusate at a lower rate than 125I-apo-E, but labeled cholesteryl esters in the larger, apo-E-rich HDL particles were not removed rapidly than those in the smaller, apo-E-poor ones. It seems likely, therefore, that the two types of particles are removed by the liver at a similar rate.

The fractional rates of removal of cholesteryl esters and apo-E of chromatographically separated HDL by perfused livers were more rapid than those observed earlier with HDL isolated from rat blood plasma between densities of 1.085 and 1.21 g/ml, which were labeled endogenously in the cholesteryl ester moiety with 3H or exogenously in the protein moiety with 125I (19). In those experiments, the major labeled protein was apoprotein A-I and much higher concentrations of HDL were routinely added to the perfusates. However, even when amounts of HDL comparable to those used in the present experiments were added, the fractional rates of removal of 125I did not approach those observed in the present studies. The reasons for the more rapid removal of the chromatographically separated HDL by the perfused liver are unclear, but it is possible that 125I-apo-E exchanged with unlabeled apo-E secreted from the liver in VLDL and was then taken up by the liver during endocytosis of these VLDL (1). In our previous studies in which 125I-apo-E and 125I-apo-A-I HDL were injected into intact rats, removal of 125I-apo-E slightly exceeded that of 125I-apo-A-I (7), but the rates for both were similar to those of ultracentrifugated separated, radioiodinated HDL (19).

As expected from earlier work (6), apo-E-containing HDL from cholesterol-fed rats (HDLc) were larger than those from rats fed regular chow. Removal of [3H]cholesteryl esters and 125I-apo-E from these HDL by perfused livers did not exceed that of such HDL obtained from rats fed regular chow. Others have obtained variable results in their studies of the metabolism of HDL containing apo-E. The apo-E of the HDLc, that has been obtained from blood plasma of cholesterol-fed dogs by a combination of ultracentrifugation and preparative electrophoresis is rapidly removed from the blood by the liver (9, 10). Apo-E-containing HDL from chow-fed rats, obtained from ultracentrifuged HDL by chromatography on heparin-Sepharose (11), is also rapidly taken up from the blood by the liver.
do not constitute a unique vehicle in the rat for transport to the liver of cholesterol esters synthesized by lecithin-cholesterol acyltransferase, they do not exclude an important role for such HDL in cholesteryl ester transport. As suggested earlier (19), arachidonate-rich cholesteryl esters of HDL may participate in the transport of cholesteryl esters to extrahepatic tissues. In view of the low concentration of LDL and the very limited cholesteryl ester transfer activity in rat plasma (26), those HDL that contain apo-E may be a major vehicle for the transport of cholesterol to cells containing active “LDL receptors.” The amount of such HDL in the rat may be sufficient to provide an effective substitute for LDL in the delivery of cholesterol to the liver as well as extrahepatic tissues.

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REFERENCES

Metabolism of apolipoprotein E in plasma high density lipoproteins from normal and cholesterol-fed rats.
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