Metabolism of triglyceride-rich nascent rat hepatic high density lipoproteins

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Abstract Nascent high density lipoprotein (HDL) and nascent very low density lipoprotein (VLDL) were isolated from rat livers that had been perfused with [3H]glycerol to label the triglyceride. When injected into intact rats, the labeled HDL-triglyceride disappeared as rapidly as the VLDL-triglyceride, with only 10% of the injected label remaining in the plasma after 30 min. The protein moiety of nascent HDL was labeled with [%3]methionine in a similar fashion and the labeled nascent HDL was separated into nonretained (NR) and retained (R) fractions by heparin-Sepharose affinity chromatography. When injected into rats, 55% of the injected label in nascent fraction NR and 72% of that in nascent fraction R was recovered from plasma at 30 min, compared to only 10% of the triglyceride label from unfractonated nascent HDL, indicating dissociation of triglyceride and apolipoprotein clearance. The plasma decay curves for both triglyceride and protein were biexponential. By 5 min, 15% of the 35S label remaining in plasma represented apoE and apoC that had been transferred from nascent HDL fractions NR and R to the d < 1.063 g/ml fraction of plasma. Plasma HDL was labeled in vivo with [35S]methionine, separated into fractions NR and R, and the clearance of the two plasma HDL fractions was compared with that of the corresponding nascent HDL fractions. Except for a faster rate of removal of the nascent HDL fractions during the first 5 min, the serum decay curves were very similar. We infer that the first step in the maturation of nascent hepatic HDL into plasma HDL is rapid triglyceride hydrolysis by lipoprotein lipase, and suggest that transient binding of the triglyceride-rich nascent particles to lipoprotein lipase may contribute to the faster initial clearance rate of the nascent HDL fractions as compared to the corresponding fractions from rat plasma.—Winkler, K. E., and J. B. Marsh. Metabolism of triglyceride-rich nascent rat hepatic high density lipoproteins. J. Lipid Res. 1989. 30: 989-996.

Supplementary key words apolipoprotein • VLDL • catabolism • lipoprotein lipase

METHODS

Rats

Male Sprague-Dawley rats (Ace Animals, Boyertown, PA), maintained on standard laboratory chow, were used for all experiments. The rats weighed 200–250 g for the liver perfusions and 175–225 g for the catabolism studies.

Labeling of perfusate nascent lipoproteins

Rats were anesthetized by an intraperitoneal injection of sodium pentobarbital, 60 mg/kg. Livers were perfused in a recirculating fashion for 2 h as previously described (1). The perfusate was a Krebs-Ringer bicarbonate buffer

Abbreviations: apo, apolipoprotein; EDTA, ethylenediamine tetraacetic acid; HDL, high density lipoprotein; LDL, low density lipoprotein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; VLDL, very low density lipoprotein.

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containing 0.1% glucose, 0.1% albumin, and Eagle's minimal essential amino acids (560 mg/l). In order to label newly synthesized triglyceride and protein, 0.25 mCi of $[^3]H$glycerol or $[^35]S$methionine was added to each perfusion. When $[^35]S$methionine was used Eagle's nonessential amino acids (80 mg/l) and L-glutamine (200 mg/l) were added in place of essential amino acids to avoid dilution of the methionine pool. Protease inhibitors were added to the collected perfusate as previously described (1).

**Labeling of plasma HDL**

$[^35]S$Methionine (0.25 mCi) was injected into the saphenous vein of rats. Blood was collected from the abdominal aorta after 2 h and HDL was isolated as described below.

**Lipoprotein isolation**

Nascent VLDL, as well as other lower density lipoproteins, were isolated from perfusates at d < 1.063 g/ml by sequential density flotation (3) in a Beckman L3-50 ultracentrifuge using a Beckman Ti 60 rotor. HDL was isolated as previously described (1). Lipoproteins were dialyzed against 0.15 M NaCl-2 mM EDTA. Prior to heparin-Sepharose affinity chromatography, HDL was dialyzed against 20 mM phosphate-2 mM EDTA at pH 7.4.

**Isolation of HDL subfractions**

HDL was separated into fractions NR and R as previously described (1), using a combination of heparin-Sepharose affinity chromatography and concanavalin-A affinity chromatography (4). Both fractions were dialyzed against 0.15 M NaCl-2 mM EDTA at pH 7.4.

**Lipid analysis**

Triglycerides were measured by an enzymatic kit (Boehringer Mannheim Diagnostics, Inc., No. 701882, Indianapolis, IN). In order to determine the relative amount of label in each lipid class, lipids were extracted by the method of Bligh and Dyer (5), separated by thin-layer chromatography on silica gel G plates developed in hexane-diethylether-acetic acid 90:10:1, and localized by staining with I$_2$. Radioactivity was assayed in a Beckman LS-1801 liquid scintillation counter following the addition of ScintiVerse.

**Protein analysis**

Protein was measured by the method of Lowry et al. (6) as modified by Markwell et al. (7) using 1% sodium dodecyl sulfate and a bovine serum albumin standard. Apolipoproteins were analyzed by SDS-PAGE on 5% gels (8) following delipidation by the procedure of Lux, John, and Brewer (9). After being stained with Coomassie Blue R-250, the protein bands were excised and dissolved in 200 μl of 30% H$_2$O$_2$ at 56–60°C for 18 h. Radioactivity was assayed using ScintiVerse in a Beckman LS-1801 or LS-6800 liquid scintillation counter. In some cases, Readi-Solve was used to solubilize large-volume aqueous samples.

**Catabolism of the triglyceride of nascent lipoproteins**

$^3$H-Labeled VLDL (300,000 cpm) or $^3$H-labeled HDL (150,000 cpm), isolated from recirculating rat liver perfusates, was injected into the saphenous vein of rats anesthetized by an intraperitoneal injection of sodium pentobarbital, 60 mg/kg. Blood samples (approximately 0.5 ml) were collected through the tail vein at 1, 5, 15, and 30 min following injection. The red blood cells were removed by centrifugation and aliquots of plasma were assayed for radioactivity. The radioactivity remaining in plasma at each time point was calculated by assuming the total plasma volume to be 4.5% of the body weight.

**Catabolism of the protein of nascent and plasma HDL**

Rats, anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg), were injected via the saphenous vein with $^{35}$S-labeled HDL fractions NR and R isolated from perfusate or rat plasma (50,000 to 100,000 cpm). Blood was sampled from the tail vein at 5 min, 30 min, and 1, 2, 4, and 8 h following injection. The radioactivity remaining in plasma was determined as described above.

**Statistics**

The plasma radioactivity decay curves were analyzed using Enzfitter, a nonlinear regression data analysis program for the IBM-PC that employs the advanced algorithm of Marquardt (10).

**Materials**

Translation system $[^35]S$methionine (No. 51006, 1000 Ci/mmol) and [2-$^3$H]glycerol (No. 27059, 200 mCi/mmol) were obtained from ICN, (Irvine, CA). Albumin (No. A-7638) was obtained from Sigma Chemical Co., (St. Louis, MO). Eagle's essential amino acids (No. 13-606) and L-glutamine (No. 17-605) were obtained from Whittaker Microbiological Associates (Bethesda, MD). MEM nonessential amino acids (No. 320-1140) were obtained from Gibco Laboratories (Grand Island, NY). Readi-Solve was obtained from Beckman (Fullerton, CA) and ScintiVerse from Fisher Scientific, (Pittsburgh, PA).

**RESULTS**

**Labeling of the triglyceride of nascent lipoproteins**

Nascent VLDL was isolated at d < 1.063 g/ml, rather than d < 1.006 g/ml, because it has been demonstrated
that 76% of the lipids isolated in this density class from rat liver perfusates are VLDL (11). Most of the incorporated \(^3\)H label was in triglyceride, 97% and 87% for VLDL and HDL, respectively, with the remaining label in phospholipid. No label was detected in any other lipid or in protein. The following data were corrected for residual label in phospholipid in order to measure triglyceride radioactivity. The specific activity of the triglyceride in nascent VLDL and HDL was 450 cpm/pg and 240 cpm/pg, respectively.

**Catabolism of the triglyceride of nascent lipoprotein**

Fig. 1 compares the plasma decay curve of nascent HDL-triglyceride to that of nascent VLDL-triglyceride. Both curves were biexponential, consisting of an initial rapid component followed by a second, slower component. An analysis of the rates and relative pool sizes for each component is shown in Table 1. The \(t_{1/2}\) of the initial phase was 0.5 min for VLDL-triglyceride; however, disappearance of HDL-triglyceride was so rapid that a reliable measurement could not be obtained using the analytical methods available. The \(t_{1/2}\) of the second component was 13.9 min for HDL and 8.7 min for VLDL.

**Labeling of nascent and plasma protein**

Nascent and plasma HDL fractions NR and R were labeled with \(^{35}\)S methionine. The specific activities of the labeled fractions were 830 cpm/pg for nascent fraction NR, 4970 cpm/pg for nascent fraction R, 70 cpm/pg for plasma fraction NR, and 110 cpm/pg for plasma fraction R. Table 2 shows the distribution of label among the various apolipoproteins of HDL fractions NR and R, and the relative specific activity of the individual apolipoproteins. In general, the distribution of \(^{35}\)S label corresponds to the apolipoprotein mass composition previously described for both nascent (1) and plasma HDL (2).

**Catabolism of nascent and plasma HDL protein**

The disappearance of HDL protein was biexponential for both nascent and plasma fractions NR and R. Table 3 shows a comparison of the rates of disappearance and pool sizes for the four HDL fractions. Initially, nascent HDL fraction NR \((t_{1/2} = 2.2\) min) was removed at a faster rate than plasma HDL fraction NR \((t_{1/2} = 3.9\) min). The total amount of protein removed was significantly greater for nascent HDL fraction NR at most timepoints as illustrated in Fig. 2. Nascent HDL fraction R \((t_{1/2} = 32\) min) was also removed faster than the corresponding plasma HDL fraction \((t_{1/2} = 66\) min) during the initial phase of catabolism; however, the total mass of protein removed was significantly greater for nascent fraction R only at 5 min (Fig. 3). A direct comparison of nascent fraction NR to nascent fraction R (Fig. 4) and plasma fraction NR to plasma fraction R (Fig. 5) reveals that in each case fraction NR had a faster initial \(t_{1/2}\) than fraction R. The \(t_{1/2}\) for the second phase of catabolism was similar for all four HDL fractions.

**Transfer of labeled apolipoproteins from nascent HDL to the \(d < 1.063\) g/ml fraction of plasma**

Lipoproteins were isolated from plasma at 5 min and 4 h following the injection of \(^{35}\)S-labeled nascent HDL

![Graph](image-url)
TABLE 2. Labeling of the apolipoproteins of HDL fractions NR and R

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Nascent</th>
<th>Relative Specific Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NR</td>
<td>R</td>
<td>NR</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>18.5 ± 0.3</td>
<td>6.3 ± 0.6</td>
<td>7.7 ± 1.8</td>
</tr>
<tr>
<td>ApoE</td>
<td>5.5 ± 0.7</td>
<td>71.3 ± 3.3</td>
<td>28.0 ± 1.0</td>
</tr>
<tr>
<td>ApoA-IV</td>
<td>43.0 ± 0.3</td>
<td>5.2 ± 0.6</td>
<td>24.0 ± 0.6</td>
</tr>
<tr>
<td>ApoC</td>
<td>33.0 ± 0.3</td>
<td>17.2 ± 1.1</td>
<td>41.0 ± 1.5</td>
</tr>
</tbody>
</table>

Plasma and nascent HDL, labeled with [35S]methionine, were subjected to SDS-PAGE and the protein bands were assayed for radioactivity as detailed in Methods. The relative specific activity was calculated by dividing the % of total label, shown in this table, by the % of total protein mass (1). Data are expressed as mean ± SE, n = 3.

DISCUSSION

Our preceding report (1) shows that nascent HDL, isolated from rat liver perfusates, is composed of at least two distinct fractions, NR and R, each containing a considerable amount of triglyceride (44% and 26% of total lipid, respectively). The present experiments were designed to test the hypothesis that the triglyceride moiety of nascent HDL was catabolized as rapidly, and extensively, as that of VLDL. A comparison of the disappearance from plasma of [3H]-labeled nascent HDL and VLDL confirmed this hypothesis. By 30 min, only 10% of [3H] from the [3H]-labeled triglyceride remained in the plasma for both nascent HDL and VLDL. This is in agreement with previous reports on the disappearance of serum VLDL-triglyceride (13, 14). Because the catabolism of the labeled triglyceride of nascent HDL occurred so quickly, the two nascent HDL subfractions were not examined separately, but both must have been cleared from plasma quite rapidly as indicated by the extent of total label removed by 30 min. An interesting finding from the study of triglyceride metabolism was that the specific activity of the [3H]-labeled triglyceride of nascent HDL (240 cpm/µg) was half that of nascent [3H]VLDL (450 cpm/µg). This suggests that there might be two metabolically distinct pools of triglyceride involved in the synthesis of nascent HDL and VLDL.

The rapid clearance of nascent HDL triglyceride from the plasma could have resulted from lipolysis of the triglyceride moiety or removal of the whole particle. Analysis of the plasma decay curves of [35S]-labeled nascent HDL fractions NR and R demonstrated that the protein of nascent HDL was catabolized at a rate different than that of the triglyceride (Table 2, Fig. 4). By 30 min, 55% and 72% of the protein label was still in the plasma for fractions NR and R, respectively, compare to only 10% of the triglyceride label. Therefore, it is likely that lipolysis, and not whole particle removal, was responsible for the rapid disappearance of triglyceride label. This is analogous to previous work, using rat plasma HDL labeled with [125I] and cholesteryl-linoleyl ether, which showed that a significant fraction of plasma HDL is not degraded as a whole.

TABLE 3. Kinetic parameters of the plasma decay of [35S]-labeled HDL

<table>
<thead>
<tr>
<th></th>
<th>Nascent</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NR</td>
<td>R</td>
</tr>
<tr>
<td>Fraction in pool 1</td>
<td>0.39 ± 0.01</td>
<td>0.44 ± 0.19</td>
</tr>
<tr>
<td>Clearance rate: t1/2 (min)</td>
<td>2.2 ± 0.2</td>
<td>32.0 ± 19.7</td>
</tr>
<tr>
<td>Fraction in pool 2</td>
<td>0.61 ± 0.01</td>
<td>0.52 ± 0.20</td>
</tr>
<tr>
<td>Clearance rate: t1/2 (hr)</td>
<td>4.6 ± 3.1</td>
<td>5.0 ± 3.6</td>
</tr>
</tbody>
</table>

The plasma decay curves of [35S]-labeled nascent HDL and nascent VLDL were analyzed by nonlinear regression as described in Methods. Calculated values ± SE in the four columns were derived from the data in Figs. 2, 3, 4, and 5, respectively.
The disappearance from plasma of nascent and plasma HDL fraction NR. Nascent and plasma HDL fraction NR was labeled with $[^{35}S]$methionine and injected into rats as detailed in Methods. The data points represent experimental values for radioactivity remaining in plasma over time, whereas the decay curves were computer-generated by the Enzfitter program. Data are expressed as mean ± SE, n = 4-10 rats. Labeled HDL fraction NR was obtained from two pooled samples of perfusates or plasma from four rats each; significantly different at **P < 0.01 and ***P < 0.001.

The hydrolysis of nascent HDL triglyceride was probably the result of the action of peripheral lipoprotein lipase, rather than hepatic lipase, as the nascent HDL had already been exposed to hepatic lipase during a 1-h recirculation period and had lost some of its triglyceride (1).

It is interesting to note that in familial apolipoprotein C-II deficiency, plasma HDL is enriched fourfold in its triglyceride/cholesterol ratio (17). As apoC-II is a required cofactor for lipoprotein lipase, these findings are compatible with our hypothesis. In guinea pigs, which are deficient in hepatic lipase, plasma HDL is likewise enriched fourfold in its triglyceride/cholesterol ratio (18).

Analysis of the plasma decay curves for HDL protein is complex because it involves the metabolism of more than one apolipoprotein. These apolipoproteins, particularly apoC and apoE, can transfer to other plasma lipoproteins, making interpretation of the data quite difficult. However, several interesting points can be made concerning the catabolism of protein from the various HDL fractions. The $t_{1/2}$ for the second, slower component was similar for all HDL fractions (4.6 to 6.9 h) and in agreement with the value reported by van Tol et al. (19) of 6.2 h for unfractionated rat plasma HDL. This finding was not surprising, despite the vastly different apolipoprotein compositions of each fraction (1, 5), because numerous reports in the literature document the similarity in catabolic rate among the individual apolipoproteins of unfractionated plasma HDL (20-24).

Of considerable interest is the observation that, in contrast to the slow component of the protein decay curves, the initial, rapid component varied greatly among the four HDL fractions (Table 2). The initial $t_{1/2}$ of nascent fraction NR (2.2 min) was considerably smaller than that of any other fraction. One possible explanation for this phenomenon is that nascent fraction NR was bound to lipoprotein lipase during triglyceride hydrolysis, and thus, temporarily removed from the plasma. As nascent fraction NR contained more triglyceride (44% of total lipid) than the other HDL fractions, and the initial $t_{1/2}$ for nascent fraction NR (2.2 min) was similar to that for VLDL-triglyceride (0.5 min), it seems appropriate to re-
Late triglyceride hydrolysis to the faster initial disappearance of nascent fraction NR from the plasma. Fraction R also contained a considerable amount of triglyceride (26% of total lipid); however, the rapid phase of nascent fraction R protein clearance (32 min) was two orders of magnitude slower than that of VLDL-triglyceride. Thus, it appears that while triglyceride hydrolysis did occur, it had little effect on the initial phase of fraction R protein catabolism.

We considered two additional possibilities that might have contributed to the faster initial disappearance of nascent fraction NR protein. One is that nascent fraction NR (400,000 daltons), which is considerably smaller than nascent fraction R (860,000 daltons), had a higher rate of filtration into the extravascular space. However, it has been reported (25, 26) that molecules differing as much in size as albumin (60,000 daltons) and LDL (2.3 × 10^6 daltons) are removed from the circulation at similar rates. Therefore, it appears that size is not the primary factor controlling the equilibration of a particle with the extravascular fluid. It was also conceivable that during triglyceride hydrolysis there was more transfer of labeled apolipoproteins from fraction NR to VLDL than from fraction R. As VLDL protein has a short half-life in plasma, about 7 min (27), fraction NR protein would be cleared at a faster initial rate than fraction R protein. However, the present data do not support transfer of apolipoproteins to VLDL as an explanation for the difference in initial clearance rates of nascent HDL fractions NR and R, as there was significantly more labeled apolipoprotein transferred from fraction R, which had the slower initial removal rate, to the d < 1.063 g/ml fraction of plasma, than from fraction NR (Table 4).

The finding that the initial catabolism of fraction R, both nascent and plasma, was slower than the initial catabolism of either nascent or plasma fraction NR, was somewhat unexpected. ApoE is the main apolipoprotein of fraction R, and other apoE-rich particles, such as chylomicron remnants (28, 29) and HDL (30) are known to be cleared rapidly (t_{1/2} < 10 min), by receptor-mediated
TABLE 4. Distribution of $^{35}$S label in plasma

<table>
<thead>
<tr>
<th></th>
<th>Col 1 NR</th>
<th>Col 2 NR</th>
<th>Col 3 R</th>
<th>Col 4 R</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of Total Label Remaining</td>
<td>5 min</td>
<td>4 h</td>
<td>5 min</td>
<td>4 h</td>
</tr>
<tr>
<td>VLDL</td>
<td>13.0 ± 1.0</td>
<td>6.3 ± 0.6.&quot;</td>
<td>18.3 ± 0.3.&quot;</td>
<td>15.7 ± 2.0.'</td>
</tr>
<tr>
<td>HDL</td>
<td>59.7 ± 0.6</td>
<td>58.0 ± 1.5</td>
<td>32.7 ± 2.3.&quot;</td>
<td>35.3 ± 3.3.&quot;</td>
</tr>
<tr>
<td>d &gt; 1.21</td>
<td>27.7 ± 0.3</td>
<td>35.0 ± 1.0.&quot;</td>
<td>49.0 ± 2.3.&quot;</td>
<td>49.7 ± 5.0.&quot;</td>
</tr>
</tbody>
</table>

Rats were injected with $^{35}$S-labeled nascent HDL fractions NR or R and blood was collected at 5 min or 4 hr. Radioactivity in each isolated lipoprotein fraction was assayed as detailed in Methods. Data are expressed as mean ± SE, n = 3. The following symbols represent significant differences from corresponding values: *P < 0.01 vs. col 1; †P < 0.001 vs. col 1; ‡P < 0.02 vs. col 2; ††P < 0.01 vs. col 2; †††P < 0.05 vs. col 2.

mechanisms, on the basis of their apoE content. Therefore, it appears that the apoE in both nascent and plasma HDL fraction R must be in a conformation that is not readily recognized and cleared by either the apoB,E or apoE receptor. An interesting, yet unrelated, observation regarding apoE is that the relative specific activity of this apolipoprotein was the same in both nascent HDL fractions (Table 2). This suggests that a single intracellular pool of apoE exists, from which both lipoprotein fractions are formed.

The current studies demonstrated that the triglyceride of nascent HDL fractions NR and R was rapidly hydrolyzed in the plasma, and therefore suggest a primary role for lipoprotein lipase in the metabolism of nascent HDL. Thus, we support the hypothesis that the rapid, initial removal from the circulation of the protein of nascent HDL fractions NR and R represents, at least in part, a remodelling of the nascent particles, whereas the second, slow phase depicts the catabolism of the mature plasma HDL.

The following scheme, illustrated in Fig. 6, integrates lipoprotein lipase hydrolysis of nascent HDL triglyceride into other aspects of nascent HDL and VLDL metabolism. Rat liver secretes a heterogeneous population of lipoprotein particles which differ with respect to size, density, and apolipoprotein composition (11). In the fed state, all of these lipoproteins contain triglyceride as the most abundant lipid. Nascent HDL, secreted as at least two separate subfractions (NR and R), undergoes a very rapid hydrolysis of triglyceride by peripheral lipoprotein lipase and, in the process, some of the apoE and apoC is transferred to plasma VLDL, as previously proposed (31). The nascent HDL subfractions, depleted of triglyceride, can then be acted upon by LCAT, as described by Hamilton et al. (32), with the resultant formation of cholesteryl ester. The end result is the transformation of the nascent HDL fractions into mature plasma HDL. If, however, LCAT activity is low, nascent HDL fractions can form discoidal structures.

![Fig. 6](image)

Fig. 6. The metabolism of nascent hepatic HDL in the rat. Spherical triglyceride-rich nascent HDL is secreted from rat liver. In the plasma the triglyceride is hydrolyzed by lipoprotein lipase and some apoE and apoC is transferred to VLDL. Following lipolysis, LCAT acts on the particles to esterify cholesterol. The final result is the conversion of nascent HDL into mature spherical plasma HDL. Plasma HDL is an acceptor of the apoE and apoC lost from the surface of VLDL during lipoprotein lipase hydrolysis. If LCAT activity is low, nHDL can form discoidal structures.

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REFERENCES


