Concentration and Composition of Lipoproteins in Blood Plasma of the WHHL Rabbit

An Animal Model of Human Familial Hypercholesterolemia

Richard J. Havel, Toru Kita, Leila Kotite, John P. Kane, Robert L. Hamilton, Joseph L. Goldstein, and Michael S. Brown

Lipoproteins in blood plasma have been quantified and characterized in homozygous Watanabe-heritable hyperlipidemic (WHHL) rabbits, an animal model of human familial hypercholesterolemia. Like homozygous human hypercholesteroleemics, WHHL rabbits have a severe deficiency of low density lipoprotein (LDL) receptors, a prolonged residence time for LDL, and an increased absolute rate of LDL catabolism. Although lipoproteins containing apolipoprotein B in WHHL rabbits are enriched in cholesteryl esters, their LDL as well as intermediate density lipoproteins (IDL) and very low density lipoproteins (VLDL) also contain a substantial amount of triglycerides and they consistently exhibit hypertriglyceridemia as well as hypercholesterolemia. The cholesteryl esters accumulating in lipoproteins of WHHL rabbits are rich in cholesteryl linoleate and appear to be produced almost exclusively by lecithin-cholesterol acyltransferase. Levels of apolipoprotein B-100 are elevated in VLDL and IDL as well as in LDL of WHHL rabbits and only trace amounts of apolipoprotein B-48 are present. Plasma levels of apolipoprotein E are also substantially increased, and VLDL and IDL are enriched in this protein. The accumulation of lipoproteins with the expected characteristics of remnants of hepatogenous triglyceride-rich lipoproteins contrasts with the efficient hepatic clearance of chylomicron remnants in WHHL rabbits.

The Watanabe-heritable hyperlipidemic (WHHL) rabbit is a strain of Japanese rabbit discovered by Watanabe which is characterized by grossly elevated levels of serum cholesterol, phospholipids, and triglycerides. Homozygous WHHL rabbits have a nearly complete deficiency of low density lipoprotein (LDL) receptors in liver and other tissues and, consequently, clear LDL from plasma with reduced efficiency. WHHL rabbits develop atherosclerosis spontaneously by 3 to 5 months of age and tendinous xanthomas by 15 months of age. These animals thus appear to represent an animal model of human familial hypercholesterolemia.

As part of a systematic study of the disordered lipoprotein metabolism in WHHL rabbits, we have determined the concentration and composition of major lipid and protein components of their plasma lipoproteins. In addition to increased levels of LDL, we have found these animals to have increased concentrations of intermediate density lipoprotein (IDL) and very low density lipoproteins (VLDL), both of which are enriched in cholesterol and apolipoprotein B-100. WHHL rabbits are also characterized by low concentrations of high density lipoproteins (HDL) and increased concentrations of plasma apolipoprotein E.
Methods

Homozogous WHHL rabbits were raised in Dallas from a mating pair of homozogous WHHL rabbits. Male Japanese White rabbits were obtained from Chubu Kagaku Shizai (Tomatsu, Japan) and male New Zealand White rabbits from Nitabell, Incorporated (Hayward, California). Animals were maintained on Purina Rabbit Laboratory Chow and used at 3–5 months of age.

Lipoproteins

Blood was collected from the ear veins of unanesthetized rabbits or from the abdominal aorta of rabbits anesthetized with xylazine and ketamine, mixed with 0.1 mg disodium EDTA per milliliter, and placed on ice. Lipoproteins were separated from plasma by sequential ultracentrifugation. For analysis of lipoprotein composition and for separation of protein components, samples were recentrifuged at their upper density limits.

Analyses

Total cholesterol and triglycerides were estimated in plasma and lipoprotein fractions by an automated technique. For measurements of lipoprotein composition, we also estimated free and esterified cholesterol, phospholipids, and protein. The fatty acid composition of cholesteryl esters separated from other lipids on columns of silicic acid was determined by a gas-liquid chromatographic technique, using a column of 5% DEGS-PS on 100/200 Supelcoport (Supelco, Inc., Bellefonte, Pennsylvania) and a flame ionization detector. Total protein of lipoprotein fractions and protein soluble in 1,1,3,3-tetramethyl urea were determined as described, and apoprotein B was estimated by difference. Less than 4% of the protein precipitated from VLDL of normal rabbits by tetramethyl urea could be extracted into 2 M urea-Tris buffer, pH 8.2, indicating that little or no protein other than apo B was precipitated under these conditions. Molecular species of apo B were visualized by staining with Coomassie blue after electrophoretic separation in 3% polyacrylamide gels containing 0.1% sodium dodecyl sulfate (SDS), and other species of apolipoproteins were separated in 10% polyacrylamide gels containing SDS or by analytical isoelectric focussing polyacrylamide gel electrophoresis, pH 3.5–7.0. Apo E in plasma delipidated with ethanol-ether, 3:1 vol/vol, and dissolved in 0.1 M sodium decyl sulfate was estimated by radial immunodiffusion or radioimmunoassay. For these assays, rabbit apo E was prepared as described for the rat protein and an antiserum against rabbit apo E was raised in a sheep. Agarose gel electrophoresis of lipoproteins in plasma and lipoprotein fractions was performed as described. The distribution of particle diameters of negatively stained lipoproteins was estimated from photographic prints at a magnification of 180,000 by a semiautomated method, utilizing a magnetic digitizer. Three points were marked on the circumference of each of 100 particles and the diameters were calculated by computer, programmed for a three-point geometric algorithm.

### Table 1. Concentration of Total Cholesterol and Triglycerides in Plasma and Lipoprotein Fractions of Normal and WHHL Rabbits (mg/dl)

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>VLDL</th>
<th>IDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cholesterol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Japanese males*</td>
<td>38.3 ± 12.6</td>
<td>9.0 ± 7.8</td>
<td>6.7 ± 3.5</td>
<td>12.0 ± 3.5</td>
<td>7.0 ± 2.7†</td>
</tr>
<tr>
<td>WHHL males*</td>
<td>731.3 ± 53.2</td>
<td>91.7 ± 10.1</td>
<td>46.7 ± 20.0</td>
<td>451.3 ± 16.5</td>
<td>6.0 ± 2.7†</td>
</tr>
<tr>
<td>Control NZW males</td>
<td>58.5 ± 1.9</td>
<td>17.0 ± 7.2</td>
<td>7.3 ± 3.5</td>
<td>11.5 ± 3.3</td>
<td>22.3 ± 4.4‡</td>
</tr>
<tr>
<td>WHHL males</td>
<td>487.5 ± 75.8</td>
<td>110.3 ± 41.5</td>
<td>116.3 ± 26.4</td>
<td>218.0 ± 68.1</td>
<td>8.3 ± 2.9‡</td>
</tr>
<tr>
<td>Control NZW females</td>
<td>46.8 ± 4.0</td>
<td>15.5 ± 4.4</td>
<td>8.5 ± 1.7</td>
<td>11.8 ± 1.3</td>
<td>18.0 ± 2.5†</td>
</tr>
<tr>
<td>WHHL females*</td>
<td>582.0 ± 143.3</td>
<td>106.0 ± 23.8</td>
<td>110.7 ± 21.1</td>
<td>338.0 ± 100.5</td>
<td>12.3 ± 2.5†</td>
</tr>
<tr>
<td><strong>Triglycerides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Japanese males*</td>
<td>64.3 ± 37.8</td>
<td>37.3 ± 30.4</td>
<td>7.0 ± 3.0</td>
<td>12.7 ± 4.0</td>
<td>5.0 ± 2.7</td>
</tr>
<tr>
<td>WHHL males*</td>
<td>288.3 ± 106.1</td>
<td>57.0 ± 18.5</td>
<td>23.3 ± 12.9</td>
<td>162.0 ± 39.9</td>
<td>1.7 ± 1.1</td>
</tr>
<tr>
<td>Control NZW males</td>
<td>213.3 ± 27.4</td>
<td>128.5 ± 37.0</td>
<td>14.0 ± 5.8</td>
<td>15.5 ± 3.9</td>
<td>24.8 ± 4.9</td>
</tr>
<tr>
<td>WHHL males</td>
<td>435.0 ± 94.7</td>
<td>153.5 ± 48.0</td>
<td>78.3 ± 26.0</td>
<td>146.0 ± 32.8</td>
<td>4.8 ± 2.8</td>
</tr>
<tr>
<td>Control NZW females</td>
<td>108.0 ± 43.5</td>
<td>62.5 ± 32.5</td>
<td>11.8 ± 1.9</td>
<td>11.8 ± 1.7</td>
<td>24.7 ± 6.5</td>
</tr>
<tr>
<td>WHHL females*</td>
<td>308.7 ± 128.8</td>
<td>124.0 ± 80.7</td>
<td>47.0 ± 21.7</td>
<td>129.7 ± 19.5</td>
<td>8.7 ± 1.5</td>
</tr>
</tbody>
</table>

Values are means ± so. The second group of NZW and WHHL males was nonfasting; other groups fasted overnight. WHHL = Watanabe-heritable hyperlipidemic; NZW = New Zealand White rabbit.

* n = 3; other groups, n = 4.
†Values from d > 1.063 g/ml fraction.
‡Values from 1.063 < d < 1.21 g/ml fraction.
Table 2. Composition of Plasma Lipoproteins of Normal and WHHL Male Rabbits

<table>
<thead>
<tr>
<th>Component</th>
<th>% Mass</th>
<th>% Volume</th>
<th>Calculated diameter (Å)</th>
<th>Cholesterol (unesterified) (%) of total cholesterol</th>
<th>Apoprotein B (% of total protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control NZW</td>
<td>WHHL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesteryl esters</td>
<td>6.6 ± 1.1</td>
<td>21.9 ± 7.4*</td>
<td>73.4 ± 3.3</td>
<td>49 ± 3</td>
<td>40.9 ± 6.1</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>62.2 ± 0.4</td>
<td>41.9 ± 10.5*</td>
<td>68.5 ± 3.2</td>
<td>32 ± 2*</td>
<td>55.1 ± 7.4*</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>3.7 ± 0.4</td>
<td>6.1 ± 1.5*</td>
<td>6.1 ± 1.6</td>
<td>6.1 ± 0.3*</td>
<td>6.9 ± 0.3*</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>15.6 ± 2.1</td>
<td>17.6 ± 1.6</td>
<td>19.7 ± 1.4</td>
<td>19.7 ± 1.4</td>
<td>20.0 ± 2.7</td>
</tr>
<tr>
<td>Total protein</td>
<td>11.9 ± 1.3</td>
<td>12.2 ± 1.3</td>
<td>19.9 ± 1.3</td>
<td>21.6 ± 0.9</td>
<td>29.3 ± 2.8</td>
</tr>
</tbody>
</table>

% Volume

| Cholesteryl esters + triglycerides | 73.4 ± 3.3 | 68.5 ± 3.2 | 56.6 | 56.8 ± 1.1 | 52.3 ± 0.9 | 55.8 ± 0.9* |

Calculated diameter (Å)

| 440 | 355 | 249 | 251 | 222 | 244 |

Cholesterol (unesterified)

| 49 ± 3 | 32 ± 2* | 36 | 31 ± 2 | 31 ± 2 | 28 ± 2 |

Apoprotein B

| 40.9 ± 6.1 | 55.1 ± 7.4* | 72.7 | 81.7 ± 5.5 | 91.3 ± 1.1 | 90.8 ± 1.2 |

Values are mean ± SD (n = 4) except for control IDL (n = 2).

*Significantly different from control NZW (p < 0.05; Student's t test, unpaired).

NZW = New Zealand White; WHHL = Watanabe-heritable hyperlipidemic rabbits.

Results

Lipoprotein-Lipid Concentrations and Lipoprotein Composition

Lipoprotein total cholesterol and triglyceride concentrations were measured in three groups of WHHL rabbits, 4 to 5 months of age (table 1). All groups had grossly elevated levels of serum cholesterol, owing to elevated levels of cholesterol in VLDL and IDL, as well as LDL. All were also hypertriglyceridemic, owing to increased levels of triglycerides in IDL and LDL as well as VLDL. Levels of HDL-cholesterol and triglycerides were reduced in male and female WHHL rabbits, as compared with New Zealand White rabbits of comparable age. However, HDL-cholesterol levels were as low in unaffected Japanese White male rabbits as in WHHL males. As compared with control rabbits, the percentage mass of cholesteryl esters was higher and that of triglycerides was lower in all lipoprotein fractions of WHHL rabbits (table 2). The fatty acid composition of the cholesteryl esters was the same in VLDL, IDL, and LDL of WHHL rabbits; cholesteryl linoleate comprised about 50% of these esters (table 3). The fraction of total cholesterol esterified in each of these lipoprotein fractions was also quite similar (table 2). The same distribution of fatty acids was found in cholesteryl esters of HDL and LDL in New Zealand White rabbits and the fraction of esterified cholesterol in LDL was comparable. However, VLDL of New Zealand White rabbits contained less cholesteryl linoleate and more cholesteryl oleate and a smaller

Table 3. Composition of Cholesteryl Esters of Plasma Lipoproteins of Normal and WHHL Male Rabbits (weight %)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>VLDL Control NZW</th>
<th>WHHL</th>
<th>IDL Control NZW</th>
<th>WHHL</th>
<th>LDL Control NZW</th>
<th>WHHL</th>
<th>HDL Control NZW</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>13.7</td>
<td>13.6</td>
<td>15.5</td>
<td>14.4</td>
<td>15.3</td>
<td>14.3</td>
<td>12.4</td>
</tr>
<tr>
<td>16:1</td>
<td>4.8</td>
<td>2.1</td>
<td>3.7</td>
<td>5.2</td>
<td>2.4</td>
<td>6.7</td>
<td>4.4</td>
</tr>
<tr>
<td>18:0</td>
<td>3.6</td>
<td>3.0</td>
<td>2.3</td>
<td>2.4</td>
<td>2.5</td>
<td>1.8</td>
<td>3.6</td>
</tr>
<tr>
<td>18:1</td>
<td>34.0</td>
<td>24.8</td>
<td>28.3</td>
<td>22.3</td>
<td>23.3</td>
<td>22.3</td>
<td>22.0</td>
</tr>
<tr>
<td>18:2</td>
<td>40.4</td>
<td>52.4</td>
<td>45.5</td>
<td>51.9</td>
<td>51.1</td>
<td>50.9</td>
<td>51.7</td>
</tr>
<tr>
<td>18:3</td>
<td>2.2</td>
<td>2.9</td>
<td>2.4</td>
<td>2.8</td>
<td>2.4</td>
<td>2.6</td>
<td>3.1</td>
</tr>
<tr>
<td>20:4</td>
<td>1.4</td>
<td>1.4</td>
<td>2.4</td>
<td>1.3</td>
<td>2.9</td>
<td>1.4</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Cholesteryl esters from pooled lipoprotein fractions from four control and four WHHL rabbits were analyzed.

VLDL = very low density lipoproteins; IDL = intermediate density lipoproteins; HDL = high density lipoproteins; NZW = New Zealand White; WHHL = Watanabe-heritable hyperlipidemic.
fraction of the cholesterol was esterified. The same trends were observed for the cholesteryl esters of IDL, but the differences between New Zealand White and WHHL rabbits were considerably less pronounced.

**Lipoprotein Size and Electrophoretic Mobility**

The size of VLDL, IDL, and LDL was estimated from the volume percentage of the major nonpolar lipid components, cholesteryl esters and triglycerides. On this basis, VLDL of WHHL rabbits were somewhat smaller and LDL somewhat larger than those of control rabbits, whereas IDL did not differ (table 2). Two samples of each of these lipoprotein fractions from a different set of control and WHHL rabbits were examined by electron microscopy. The distribution of particle diameters (figure 1) also indicated that VLDL from WHHL rabbits were somewhat smaller and IDL were of similar size to those of controls, but no difference in size of LDL was evident. In all cases, particles in all classes had a typically round appearance. On agarose electrophoresis (not shown), the mobility of VLDL from WHHL rabbits was in the prebeta region, but was slightly less than that of controls. IDL and LDL from both groups had beta mobility.

![Figure 1](http://atvb.ahajournals.org/)

**Figure 1.** Distribution of particle diameters measured on photographic prints of negatively stained lipoproteins from a male New Zealand White rabbit control (C) and a Watanabe-heritable hyperlipidemic rabbit (W). Comparable results were obtained in a second pair of animals.

**Apolipoprotein Concentration and Composition**

Apolipoprotein B comprised a larger fraction of VLDL-protein and a slightly larger fraction of IDL protein in WHHL rabbits than in controls (table 2). In LDL, apolipoprotein B comprised about 90% of total protein in both groups. The levels of apolipoprotein B were calculated for one set of animals in which both lipoprotein composition and lipoprotein-lipid concentrations had been determined. Apo B levels were about 2.5-fold higher in VLDL of WHHL rabbits than in controls (24.7 vs 10.0 mg/dl), but 10-fold higher in LDL (68.7 vs 6.6 mg/dl) and LDL (167.5 vs 15.7 mg/dl). In all fractions, apo B-100 comprised all or the great majority of stainable apo B in 3% SDS polyacrylamide gel electrophoretograms (figure 2). Minor components corresponding to apo B-95 were seen in WHHL and control IDL and LDL, and minor components with the mobility of apo B-48 were evident in WHHL IDL and LDL. In 10% SDS gel electrophoretograms, a component with the mobility of apo E was more prominent in the VLDL-protein of WHHL rabbits than in controls (figure 3). This was also evident for both VLDL and IDL in isoelectric focussing gel electrophoretograms (figure 4). Components presumably corresponding to anionic C apoproteins were correspondingly less prominent in VLDL and IDL of WHHL rabbits than in controls. The non-apo B components of LDL of both WHHL and normal rabbits consisted mainly of these anionic species. Notably, the number of these and other focussing species was reduced by treatment of the apoproteins of LDL with beta-mercaptoethanol. A protein with molecular weight corresponding to that of human apo A-I was the major species in HDL of both WHHL and control rabbits (figure 3).

The concentration of apo E was four- to fivefold higher in the serum of WHHL than in control rabbits (table 4). In WHHL and controls alike, concentrations of apo E increased significantly after the animals were fasted overnight. The levels of apo E increased much more in WHHL rabbits fed a diet containing 0.1% cholesterol for 3 months than in controls (table 4), as did levels of total cholesterol and triglycerides.

**Discussion**

The alterations demonstrated here in the plasma lipoproteins of homozygous WHHL rabbits are in some, but not all, respects those expected from observations in humans with homozygous familial hypercholesterolemia. As reported by Tanzawa and associates, plasma and LDL cholesterol levels are grossly elevated in WHHL rabbits. They found significant but more modest elevations of cholesterol in VLDL and IDL as well. Plasma total cholesterol levels were similarly elevated in our WHHL homozygotes (in the range of 500–700 mg/dl), but in many cases no more than one-half was found in LDL, with the remainder in IDL and VLDL. Unlike human homozygotes, WHHL rabbits are also hypertriglyceri-
Figure 2. SDS polyacrylamide gel (3%) electrophoreto-
grams of apoproteins of VLDL, IDL and LDL from control
Japanese White (left member of each pair) and Watanabe-
heritable hyperlipidemic (WHHL) rabbits (right member of
each pair). The mass of tetramethyl urea-insoluble protein
applied was, from left: 20, 10, 30, 40, 20 and 45 μg, re-
spectively. In each gel, the major protein band has the
mobility of apoprotein B-100. Minor components with the
mobility of apoprotein B-95 are visible in the gel containing
apoproteins of LDL from a normal rabbit and IDL and LDL
from a WHHL rabbit. A minor component with the mobility
of B-48 is visible in the gel containing WHHL IDL and LDL;
WHHL LDL also contains a component of intermediate
mobility.

Table 4. Concentration of Apolipoprotein E in Plas-
ma of Normal and WHHL Male Rabbits (mg/dl)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NZW</th>
<th>WHHL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonfasting</td>
<td>1.7 ± 0.2*</td>
<td>7.2 ± 2.1†</td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>2.6 ± 1.0†</td>
<td>10.3 ± 1.7†</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonfasting</td>
<td>1.8 ± 0.4*</td>
<td>9.9 ± 2.3†</td>
<td></td>
</tr>
<tr>
<td>Nonfasting, cholesterol-fed</td>
<td>3.8 ± 2.0†</td>
<td>33.2 ± 23.8†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± sd. All values shown were ob-
tained by radial immunodiffusion. During this study, 18
samples were analyzed by both radial immunodiffusion
(mean = 8.95 mg/dl) and by radiolimunonassay (mean = 8.77 mg/dl). The mean difference between the two values
was 1.00 mg/dl.

* n = 4.
† n = 5.
‡ n = 3. These animals had been fed a diet containing
0.1% cholesterol for 3 months; mean values for plasma
total cholesterol were 261 ± 77 and 1187 ± 33 mg/dl and
for plasma triglycerides were 150 ± 77 and 767 ± 251
mg/dl in NZW and WHHL rabbits, respectively.

NZW = New Zealand White; WHHL = Watanabe-heri-
table hyperlipidemic.

demic, and LDL as well as IDL and VLDL contain a
substantial amount of triglycerides. As compared
with normal rabbits, however, all these lipoprotein
classes are enriched in cholesteryl esters and con-
tain less triglyceride. This has also been observed in
human homozygotes. With the possible exception
of LDL, these cholesterol-enriched lipoproteins are
not larger than the corresponding classes of normal
rabbits, because the increased volume of the core of
the particles occupied by cholesteryl esters is ac-
panied by a corresponding reduction in the vol-
ume of triglycerides. These cholesteryl esters are
mainly cholesteryl linoleate. The mixture of chole-
sterol esters and triglycerides present in the LDL and
IDL, as well as in VLDL, is such that the particle core
should be liquid and disordered at body temperature.
Thus, the putative atherogenicity of these lipopro-
etins evidently does not depend upon an ordered
particle core. In humans with homozygous familial
hypercholesterolemia, the triglyceride content of
LDL is very low, but the molecular weight is not
increased.

The major B apoprotein of VLDL, IDL, and LDL of
WHHL rabbits is B-100. This finding is in accord
with the normal rate of chylomicron lipid and protein me-
Figure 4. A. Isoelectric focussing gel electrophoretograms of apoproteins of human VLDL (left pair of gels) and control rabbit VLDL, IDL, and LDL. The protein applied to the right member of each pair of gels was incubated with beta-mercaptoethanol. The mass of tetramethyl urea-soluble protein applied to each pair of gels was 20, 30, 20 and 20 μg, respectively. Left pair of gels, reference apo VLDL from an individual with apo E phenotype E4/3. Note that components of rabbit apo E are visible only in VLDL and IDL and that no effect of reduction of disulfide bonds is evident. Contaminating albumin is visible only in gels containing unreduced proteins (albumin aggregates upon treatment with beta-mercaptoethanol). The proteins with the lowest isoelectric points (brackets) are of low molecular weight as determined by gel chromatography and presumably are homologous to human C apoproteins. B. Isoelectric focussing gel electrophoretograms of apoproteins of human VLDL (same gels as in A) and Watanabe-heritable hyperlipidemic (WHHL) rabbit VLDL, IDL, and LDL. The conditions are the same as in A except that rabbit VLDL (30 μg), IDL (20 μg), and LDL (60 μg) are from a WHHL rabbit. Apo E is a prominent component of the VLDL and IDL, but not of LDL, which contains more C apoproteins. Note the reduction in the number of C apoprotein components of WHHL LDL after treatment with beta-mercaptoethanol.
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tabolism in WHHL rabbits and the rapid uptake of chylomicron remnants. However, we did observe a small amount of protein with the electrophoretic mobility of apo B-48 in IDL and LDL. Perfused livers of normal rabbits secrete mainly apo B-100, but small amounts of a protein resembling apo B-48 have been found in perfusate VLDL; chylomicrons from rabbit intestinal lymph contain mainly apo B-48, with only trace amounts of apo B-100. (Hornick, Havel and Kane, unpublished observations, University of California School of Medicine, San Francisco, 1981). It is therefore possible that apo B-48 in IDL and LDL is derived from hepatogenous VLDL. It seems clear, however, that virtually none of the accumulated lipoproteins in WHHL rabbits is derived from the intestine.

Although we believe that almost all the apo B-containing lipoproteins in blood plasma of WHHL rabbits are hepatogenous, it is likely that postsecretory modifications related to lecithin-cholesterol acyltransferase (LCAT) and cholesteryl ester transfer activity influence their composition. The transfer activity in rabbit plasma is the highest observed in any species, yet the cholesteryl esters of VLDL and, to a lesser extent of IDL, in normal rabbits appear to comprise a mixture of hepatogenous (oleate-rich) cholesteryl esters, synthesized by acyl CoA acyltransferase, and linoleate-rich esters synthesized by LCAT (table 3). Presumably, this reflects the rapid turnover of VLDL known to occur in normal rabbits. By contrast, cholesteryl linolate comprises about one-half of cholesteryl esters of VLDL and IDL as well as LDL in WHHL rabbits, a value comparable to that observed in HDL and LDL of normal rabbits. This suggests that the increased concentration of cholesteryl esters in VLDL and IDL of WHHL rabbits is accompanied by a reduced fractional catabolic rate. Such a reduced rate of catabolism would provide ample time for core components of all lipoprotein classes to reach an equilibrium state in WHHL rabbits, accounting for the enrichment of cholesteryl esters in VLDL and IDL. These lipoproteins, as well as LDL, may therefore reflect "end-product" lipoproteins, which have received as much as the cholesteryl esters product of the LCAT reaction as they can. Increased cholesteryl ester content of VLDL is in several situations accompanied by an increase in apo E as observed here in VLDL and IDL of WHHL rabbits. A decreased rate of apo E catabolism would be expected in the absence of the LDL receptor, which binds apo E-containing lipoproteins as well as those containing apo B. This may account for the increased concentration of apo E in plasma, but an increased rate of hepatic secretion of apo E cannot be excluded. Plasma levels of apo E are modestly increased in humans with heterozygous familial hypercholesterolemia and may be increased severalfold in human homozygotes (Havel, Bilheimer, Kane, Kotite, and Hamilton, unpublished observations, University of California School of Medicine, San Francisco, California, 1982).

The VLDL and IDL of WHHL rabbits resemble VLDL remnants in several characteristics (enrichment in cholesteryl esters and apo E, and reduced electrophoretic mobility). The concentration and rate of production of apo B of VLDL are reported normal in humans with homozygous familial hypercholesterolemia, and chylomicron triglyceride catabolism and hepatic uptake of chylomicron cholesteryl esters appear to be unimpaired in WHHL rabbits. However, the concentration of apo B in VLDL and IDL is substantially increased in WHHL rabbits and it is possible that these animals also have a defect in the catabolism of these lipoproteins, perhaps at the level of VLDL remnants. Alternatively, these lipoproteins may represent abnormal hepatic secretory products. As in human homozygotes, the rate of production of apo B in LDL of WHHL rabbits is severalfold higher than in normal rabbits. The rate of hepatic secretion of VLDL, IDL, or both may be increased as well.

The concentration of HDL cholesterol was uniformly low in WHHL rabbits. The significance of this observation is unclear, because we found comparably low levels in Japanese White rabbits of the strain in which the WHHL mutation occurred. However, in an earlier study, HDL cholesterol levels were found to be lower in WHHL rabbits than in normal Japanese rabbits. HDL levels in humans with homozygous familial hypercholesterolemia may be normal or reduced (Havel et al. unpublished observations, 1982). The relative contribution to atherogenesis of the several abnormalities of plasma lipoproteins in WHHL rabbits (increased concentration of remnant-like VLDL and IDL, increased concentration of LDL, and reduced concentration of HDL) remains to be determined.

Although some of the lipoprotein abnormalities in WHHL rabbits differ from those of humans homozygous for LDL receptor deficiency, these are likely to reflect a difference in lipoprotein metabolism in the two species rather than a fundamental difference in the LDL receptor defect. The WHHL rabbit provides a unique opportunity to determine the consequences of LDL receptor deficiency for many aspects of lipoprotein metabolism, including hepatic lipoprotein synthesis, secretion, and interconversions, as well as cellular catabolism.

References


Index terms: lipoproteins • cholesterol • triglycerides • rabbits • apolipoprotein B • apolipoprotein E • familial hypercholesterolemia
Concentration and composition of lipoproteins in blood plasma of the WHHL rabbit. An animal model of human familial hypercholesterolemia.

R J Havel, T Kita, L Kotite, J P Kane, R L Hamilton, J L Goldstein and M S Brown

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