Metabolism of Canine β-Very Low Density Lipoproteins in Normal and Cholesterol-Fed Dogs

Menahem Fainaru, Harald Funke, Janet K. Boyses, Erwin H. Ludwig, Thomas L. Innerarity, and Robert W. Mahley

Cholesteryl ester-rich β-very low density lipoproteins (β-VLDL) are β-migrating lipoproteins that accumulate in the plasma of cholesterol-fed animals and of patients with type III hyperlipoproteinemia. There are two distinct fractions: fraction I β-VLDL are chylomicron remnants of intestinal origin, and fraction II β-VLDL are cholesteryl-rich VLDL of hepatic origin. The liver rapidly clears fraction I β-VLDL from the plasma of both normal and cholesterol-fed dogs. The liver also clears fraction II β-VLDL rapidly and efficiently from the plasma of normal dogs by receptor-mediated uptake. In cholesterol-fed dogs the clearance is biphasic: an initial rapid drop-away of about 30% to 40% of the injected dose within 5 minutes, followed by a slow clearance of plasma radioactivity (a half-life of more than 20 hours). The rapid, initial phase of fraction II β-VLDL clearance appears to be related to sequestration of the lipoproteins presumably on endothelial cells and is apparently associated with lipolytic processing. Treatment of the fraction II β-VLDL with lipoprotein lipase abolishes this rapid phase. In the cholesterol-fed dog, the slow, late phase of clearance corresponds to the conversion of fraction II β-VLDL to the smaller, denser intermediate and low density lipoproteins (IDL and LDL), which are slowly cleared from the plasma. It is concluded that fraction II β-VLDL are catabolized in the normal dog by rapid uptake mediated at least in part by the apo B,E(LDL) receptor of hepatic parenchymal cells. In cholesterol-fed dogs, in which these receptors are markedly down-regulated, fraction II β-VLDL are apparently initially bound to endothelial cells and converted to IDL and LDL by lipolytic processing. (Atherosclerosis 8:130–139, March/April 1988)

Cholesterol feeding of experimental animals results in marked changes in plasma lipoproteins. Unlike normal very low density lipoproteins (VLDL) and chylomicrons, the d < 1.006 g/ml lipoproteins that accumulate in these animals are rich in cholesteryl esters, possess primarily apolipoproteins (apo) B and E, and display β-electrophoretic mobility. These lipoproteins are referred to as β-VLDL and appear to be atherogenic. Their apparent atherogenicity seems to be related to their ability to cause the deposition of cholesteryl esters in macrophages both in vivo and in vitro.

It has been shown that β-VLDL obtained from cholesterol-fed dogs and from patients with type III hyperlipoproteinemia are heterogeneous: they have at least two subpopulations of lipoproteins, which differ in size, chemical composition, apo B isoforms, and sites of synthesis. One fraction (fraction I β-VLDL) resembles chylomicron remnants in size, electrophoretic mobility, and lipid and apolipoprotein composition; another similarity is that a large proportion of its apo B is in the low molecular weight form (B-48), which is characteristic of lipoproteins of intestinal origin. The second subclass (fraction II β-VLDL) is morphologically and biochemically similar to VLDL remnants or cholesterol-enriched VLDL of hepatic origin in cholesterol-fed animals. Fraction II β-VLDL possess primarily the high molecular weight form of apo B (B-100). One additional difference is that fraction I β-VLDL promote cholesterol esterification and accumulation in mouse peritoneal macrophages somewhat more actively than fraction II β-VLDL.

It is known that chylomicron and VLDL remnants are removed from the plasma by the liver (for review, see reference 11). There are two lipoprotein receptors in the liver: 1) the apo B,E(LDL) receptor, which is identical to the apo B,E(LDL) receptor in extrahepatic tissues, and 2) the chylomicron remnant receptor (for review, see references 12 and 13). Whereas VLDL remnants are cleared from the circulation by the hepatic apo B,E(LDL) receptor, chylomicron remnants are cleared mainly by the chylomicron remnant receptor. Since fraction I and fraction II β-VLDL are similar to chylomicron and VLDL remnants, respectively, the present study was undertaken to investigate in vivo the metabolism of the two fractions of canine β-VLDL in normal and cholesterol-fed dogs. Radiolabelled β-VLDL were injected intravenously, and their clearance from the plasma was compared under these different conditions.
Methods

Animals

Pure-bred male adult (20 to 30 months of age) foxhounds (Brink Farm, Paola, Kansas) weighing 25 to 30 kg were fed a semisynthetic diet prepared by Teklad Mills (Madison, Wisconsin). The diet consisted (by weight) of a mixture of 30% sucrose, 20% casein, 19.3% cellulose, 16% hydrogenated coconut oil, 9% salt mixture, 5% cholesterol, and 0.7% vitamin mixture.14 Fed ad libitum, the dogs were maintained on this diet for over a year and had plasma cholesterol levels above 700 mg/dl during the study. Additional male foxhounds of similar breed, age, and weight were maintained on normal dog chow (Purina dog meal). Male Sprague-Dawley rats weighing 250 to 300 g were obtained from Simonsen Labs (Gilroy, California) and maintained on standard rat chow. All animal studies were performed in accordance with institutional policies.

Materials

Agarose A-15 m was purchased from Bio-Rad (Richmond, California). Sodium125I and Na131I were purchased from Amersham/Searte (Arlington Heights, Illinois).

The unfractionated β-VLDL and the β-VLDL fractions were labelled with either 125I or 131I by the iodine monochloride method.17 The free iodine was removed from radiolabelled lipoproteins by dialysis in 0.15 M NaCl containing 0.01% disodium EDTA. The labelled lipoproteins were similar to unlabelled lipoproteins in electrophoretic mobility on Pevikon block and in elution volume by gel filtration on an agarose A-15 m column. The lipoproteins were characterized by their lipid- and apolipoprotein-associated label (Table 1). Apart from the lower specific activity achieved with 131I, no appreciable differences were observed among the fractions and isolates. Mixing experiments, in which each fraction was labelled alternately by the isotopes and mixed with the other fraction, followed by incubations with normal or cholesterol-fed dog plasma for up to 6 hours at 37°C, did not result in appreciable exchange of the label among the lipoproteins as determined by both gel chromatography on agarose A-15 m column and density gradient ultracentrifugation (data not shown).

Arginine residues of the apolipoproteins of canine β-VLDL were modified by treatment with 1,2-cyclohexane-dione as previously described.18

Table 1. Characterization of Radiolabelled Canine B-VLDL Fractions

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Fraction I</th>
<th>Fraction II</th>
</tr>
</thead>
<tbody>
<tr>
<td>125I</td>
<td>131I</td>
<td>125I</td>
</tr>
<tr>
<td>Specific activity (cpm/ng protein)</td>
<td>649</td>
<td>100</td>
</tr>
<tr>
<td>Lipid-associated label (%)*</td>
<td>8.4</td>
<td>6.2</td>
</tr>
<tr>
<td>Protein-associated label (%)†</td>
<td>94.1</td>
<td>92.3</td>
</tr>
<tr>
<td>Apolipoprotein-associated label (%)‡</td>
<td>Apo B</td>
<td>77.0</td>
</tr>
<tr>
<td></td>
<td>B-100§</td>
<td>52.0</td>
</tr>
<tr>
<td></td>
<td>B-48§</td>
<td>48.0</td>
</tr>
<tr>
<td></td>
<td>Apo E</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Apo C</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Canine B-VLDL fractions (I and II) were labelled independently with either 125I or 131I by using the modified McFarlane method. After overnight dialysis in which there were three to four changes (3000 vol) of 0.15 M NaCl, 0.01% EDTA, the radiolabelled lipoproteins were used in metabolic studies and characterized. Detailed are the average results of three experiments (the standard deviation was within 10% of the mean).

*The lipid-associated label was determined by extraction in chloroform/methanol in a 2:1 ratio.
†The protein-associated label was determined by precipitation in 10% trichloroacetic acid solution at 4°C.
‡The distribution of the label among the apolipoproteins was determined by electrophoresing the delipidated lipoproteins on (0.1%) sodium dodecyl sulfate-polyacrylamide gels (11%). The gels were stained in 0.2% Coomassie brilliant blue, and the bands were identified and counted. Lipoprotein protein (20 μg to 60 μg) containing about 2 × 106 cpm was applied to the gel. The recovery of the various bands was expressed as the percent of the amount applied to the gels. The results are the average of two lipid-preparations (the range is within 10% of the mean).
**In Vivo Studies**

The in vivo studies were performed on animals that had fasted overnight (15 to 18 hours). The radiolabeled (125I or 131I) lipoproteins (0.1 to 0.5 mg of protein) were injected into the cephalic vein of conscious normal or cholesterol-fed foxhounds. At designated times, blood samples were drawn from the jugular vein into tubes containing disodium EDTA (0.1% wt/vol, final concentration; pH 7.4), and the plasma was separated promptly (3000 rpm for 20 minutes at 4°C). Aliquots of plasma (0.5 to 1.0 ml) were counted for 10 minutes in a gamma counter (Gamma 4000, Beckman Instruments). For determination of the percentage of the radiolabeled in the protein, an equal volume of trichloroacetic acid (20% wt/vol) was added to the plasma aliquots (10% final concentration), vortexed, kept on ice for 20 minutes, and centrifuged (3000 rpm for 20 minutes, 45°C). An aliquot of the supernatant was counted to determine the amount of label not associated with protein.

Calculations of the percentage of injected dose remaining in the plasma at various time intervals were based on plasma volumes of 4.5% of body weight (vol/wt) for dogs and rats. In some experiments, liver biopsies (slices of <200 mg) were obtained from anesthetized dogs through an abdominal incision from two different sites at designated times. An intravenous injection of thiopental sodium, 25 mg/kg of body weight, was used for induction of anesthesia, followed by inhalation of methoxyflurane 1% for 25 mg/kg of body weight, was used for induction of anesthesia, followed by inhalation of methoxyflurane 1% for maintenance of anesthesia. Bleeding was stopped using Gelfoam (Upjohn, Kalamazoo, Michigan). The liver slices (1 to 2 mm in thickness) were washed in saline to remove blood and were blotted, weighed, and digested in Protosol, and their radioactivity was counted. The total weight of the liver was determined by weighing the liver of the dog at the end of the study, after the excess of blood had been removed.

Studies in rats were performed after the animals were anesthetized with sodium pentobarbital (65 mg/kg of body weight). The radiolabeled B-VLDL fractions (65 mg/kg of body weight) were injected into an exposed femoral vein, and 100-μl samples of blood were obtained from the opposite femoral vein. Liver samples obtained from various lobes were processed for determination of radioactivity. For autoradiography, samples of liver were taken after perfusion of the liver with 120 to 180 ml of modified Eagle’s medium (GIBCO, Grand Island, New York), followed by perfusion of the fixative (freshly prepared 2% formaldehyde and 0.5% glutaraldehyde in 0.08 M sodium cacodylate, pH 7.3). The tissue was processed, as described, to allow identification of Kupffer cells by localization of endogenous peroxidase activity, and then was embedded in JB-4 (Polysciences, Warrington, Pennsylvania). Sections (2-μm) were mounted on slides and dipped in Ilford L-4 emulsion (Polysciences). Slides were developed and stained or observed by phase-contrast microscopy.

**Density Gradient Ultracentrifugation**

One milliliter of plasma or lipoprotein sample was overlaid on a linear density gradient of NaCl-KBr (1.006 to 1.03 or 1.006 to 1.21 g/ml) in an SW-41 rotor using a gradient mixer (Beckman). The gradient was ultracentrifuged at 40,000 rpm for 48 hours at 4°C. Fractions (0.5 to 1.0 ml) were collected by piercing the tube bottom, and aliquots were measured to determine the radioactivity. The density of each fraction was determined from the refractive index.

**Analytical Methods**

Paper electrophoresis of lipoproteins was performed as described. Polycrylamide gel electrophoresis (11% or 4%) was performed with sodium dodecyl sulfate (0.1%) as previously described. Lipid and protein analyses were determined as described.

**Results**

The radiolabeled canine B-VLDL fractions I and II injected into normal chow-fed dogs were cleared from the plasma in nearly identical times: approximately 50% of the injected dose was removed within 10 minutes, and about 90% by 1 hour (Figure 1A). The liver was responsible for the clearance of both fractions. Ten minutes after injection, when about 50% of the fraction II B-VLDL had been removed from the plasma, about 45% of the radiolabel was detected in the liver of the normal dog (Figure 1B). Essentially identical results were obtained for the radiolabeled fraction I B-VLDL (data not shown), emphasizing the importance of the liver in clearing these fractions from the plasma.

In contrast, radiolabeled fractions I and II B-VLDL injected into cholesterol-fed dogs were cleared from the plasma in patterns distinctly different from each other. Whereas fraction I B-VLDL were rapidly cleared from the plasma (about 50% in 10 minutes, similar to the clearance of this fraction from the plasma of normal dogs), fraction II B-VLDL clearance was retarded (Figure 2A). After an initial, rapid disappearance (5 minutes or less) of about 35% of fraction II B-VLDL from the plasma compartment (ranging between 30% and 40% in five different dogs), the remainder of the injected dose was cleared more slowly. The latter component had a half-life of 26.5 ± 1.8 hours (mean ± SD, n = 5), which is similar to the clearance of canine low density lipoproteins (LDL) from plasma. Approximately half of the radiolabel of fraction II B-VLDL cleared from the plasma was detected in the liver (Figure 2B), whereas virtually all of the fraction I B-VLDL cleared could be accounted for within the liver (data not shown). Very similar results have been observed in both normal and cholesterol-fed dogs by using 10 different preparations of canine B-VLDL fractions I and II that were labelled with either 125I or 131I and injected simultaneously into 12 different rodents. No appreciable exchange of radiolabel among the lipoproteins was observed during in vivo metabolism, as measured by gel chromatography (see Figure 8 below).

Consideration was given to the possibility that the altered clearance of fraction II B-VLDL (the hepatic VLDL remnant lipoproteins) in cholesterol-fed dogs was secondary to diet-induced down-regulation of the hepatic apo B,E(LDL) receptors, whereas the unaltered clearance of fraction I B-VLDL (the intestinal chylomicron remnant fraction) reflected their clearance by other mechanisms that are not down-regulated by cholesterol feeding.
Figure 1. A. Plasma clearance of $^{131}$I-labelled fraction I (Fx I) $\beta$-very low density lipoproteins (B-VLDL) (•) and $^{125}$I-labelled fraction II (Fx II) B-VLDL (○) in a normal dog. Approximately 0.5 mg of lipoprotein protein of each B-VLDL fraction was injected into the cephalic vein of a normolipidemic foxhound (plasma cholesterol = 150 mg/dl). Blood samples were obtained from the jugular vein. The results are expressed as trichloroacetic acid-precipitable counts remaining in the plasma at each time interval. B. Liver uptake of $^{125}$I-labelled fraction II B-VLDL (○) in a normal dog. The foxhound was anesthetized with sodium pentobarbital and methoxyflurane, and blood samples were drawn at the designated times (•, plasma die-away). At the designated time intervals, liver biopsies (about 100 to 200 mg wet weight) were obtained from two different sites. They were washed in saline, blotted, and weighed. This tissue was digested in Protosol and counted for radioactivity. The total radioactivity in the liver was calculated by weighing the liver after the animal was exsanguinated at the end of the study.

Figure 2. A. Plasma clearance of $^{131}$I-labelled fraction I $\beta$-VLDL (•) and $^{125}$I-labelled fraction II $\beta$-VLDL (○) in a cholesterol-fed dog. Approximately 0.5 mg of lipoprotein protein of each $\beta$-VLDL fraction was injected into the cephalic vein of a cholesterol-fed foxhound (plasma cholesterol = 370 mg/dl). The results are expressed as protein-associated counts remaining in the plasma at each time interval. B. Liver uptake of $^{125}$I-labelled fraction II $\beta$-VLDL (○) in a cholesterol-fed dog (plasma cholesterol = 370 mg/dl). Liver biopsies were obtained and their radioactivity determined as described in the legend of Figure 1. The plasma clearance (•) of the $\beta$-VLDL is shown.

became concerned about the possible denaturation (even under the most careful conditions, including in vivo screening) of fraction I $\beta$-VLDL, as implied by their partial uptake by Kupffer cells and other macrophages of the reticuloendothelial system (data not shown). Because the question of possible denaturation remains unresolved, we chose to focus on the metabolism of fraction II $\beta$-VLDL to determine whether their clearance was receptor-mediated in both normal and cholesterol-fed dogs.

As shown in Figure 3A, cyclohexanedione modification of arginine residues of apolipoproteins of fraction II...
B-VLDL substantially inhibited the rapid phase of clearance of the radio labelled lipoproteins from the plasma of a normal dog. For example, at 1 hour after injection, only 7% of the unmodified (native) fraction II B-VLDL remained in the plasma, as compared with about 50% of the modified fraction II B-VLDL. Selective chemical modification of arginine residues of various apolipoproteins by cyclohexanedione treatment has previously been shown to prevent lipoprotein binding to apo B,E(LDL) receptors.18

However, cyclohexanedione-modified fraction II B-VLDL injected into cholesterol-fed dogs had little effect on the plasma clearance of these lipoproteins (Figure 3B). The initial, rapid phase of clearance did not appear to depend on recognition of the modified fraction II B-VLDL by hepatic apo B,E(LDL) receptors. The late, slow phase of clearance was only slightly affected by the chemical modification at the early times examined (up to 6 hours). Therefore, whereas fraction II B-VLDL clearance in normal dogs appeared to be mainly receptor-mediated, their clearance in cholesterol-fed dogs did not.

The role of receptor-mediated clearance of fraction II B-VLDL in normal dogs was further evaluated by the intravenous infusion of the bile acid taurocholate. Taurocholate infusion has been previously shown to down-regulate the expression of hepatic apo B,E(LDL) receptors.20 As shown in Figure 4, taurocholate infusion at two concentrations for 4 and 5 hours, respectively, prior to injection of the 125I-labelled fraction II B-VLDL in the normal dog. At 40 minutes, 92% of the injected dose had been cleared from the plasma of the control dog, whereas respectively about 75% or 60% had been cleared from the plasma of dogs infused with 2.5 and 4 µg/kg/min of taurocholate, respectively.

The hepatic cell type responsible for the uptake of canine fraction II B-VLDL was determined by electron microscopic autoradiography. It was necessary to conduct these studies in rats because their livers (smaller than that of a dog) allowed a sufficient concentration of 125I-labelled uptake for accurate evaluation of the autoradiographs. The kinetics of the clearance of canine fraction II B-VLDL from the plasma of rats was similar to that observed in dogs. Whereas about 65% of the fraction II B-VLDL dose injected into normally fed rats was cleared from the plasma within 20 minutes, the same dose injected into cholesterol-fed rats was cleared slowly, a result similar to that observed in cholestrol-fed dogs. Furthermore, cyclohexanedione modification of fraction II B-VLDL blocked their plasma clearance in normally fed rats but not in cholesterol-fed rats, a result also similar to that observed in dogs (data not shown). As shown in Figure 5, the parenchymal cells were primarily responsible for the uptake of fraction II B-VLDL. Furthermore, it was possible to abolish the parenchymal cell uptake by modifying these lipoproteins with cyclohexanedione (Figure 6). The only significant hepatic uptake of the modified fraction II B-VLDL was by kupffer cells. This uptake by Kupffer cells, which is not seen with the unmodified lipoproteins, can partially explain the continued clearance of some modified particles by the liver. These morphologic studies further confirm that parenchymal cells clear fraction II B-VLDL by receptor-mediated uptake. Consideration was given to the mechanisms responsible for the biphasic character of the plasma clearance of the fraction II B-VLDL in cholesterol-fed dogs. The rapid, initial phase was not receptor-mediated, as indicated by the fact that cyclohexanedione modification of the particle

Figure 3. Effect of cyclohexanedione (CHD) modification of arginine residues on fraction II B-VLDL clearance from the plasma of normal (A) and cholesterol-fed (B) dogs. The 125I-CHD-fraction II B-VLDL (0.3 mg of protein) were injected into normal (•) and cholesterol-fed (A) dogs. Approximately 80% of the arginine residues of the B-VLDL protein were modified by the CHD treatment. The results are expressed as protein-associated label (see legend to Figure 1).

![Figure 3](image-url)

Figure 4. Effect of taurocholate infusion on fraction II B-VLDL clearance from the plasma of a normolipidemic dog. Two normal dogs were anesthetized with sodium pentobarbital and infused with either 2.54 or 40.1 µmol taurocholic acid/kg body weight/min for 4 and 5 hours, respectively, prior to injection of the 125I-labelled fraction II B-VLDL. The experiment was continued for an additional 2.5 hours. The plasma clearance of the 125I-labelled fraction II B-VLDL in the dog receiving 2.54 (•) or 40.1 (A) µmol/kg/min is compared with the clearance of 125I-labelled fraction II B-VLDL (•) in a saline-infused normal dog. The results are expressed as protein-associated label (see legend to Figure 1).

![Figure 4](image-url)
did not alter the clearance profile (Figure 3B). Consideration was therefore given to the possibility that the initial phase might represent endothelial binding and lipolytic processing. To evaluate this, fraction II B-VLDL were incubated for 16 hours at 37°C with post-heparin plasma, which possesses lipolytic activity (similar results were obtained using purified lipoprotein lipase). After lipolytic treatment, fraction II B-VLDL that floated at d < 1.03 g/ml were injected into a cholesterol-fed dog. As shown in Figure 7A, lipolytic treatment of the fraction II B-VLDL abolished the initial, rapid phase of clearance. Similarly, as shown in Figure 7B, canine intermediate density lipoproteins (IDL, d = 1.006 to 1.03 g/ml) isolated from the plasma of a cholesterol-fed dog displayed only a slow phase of clearance, paralleling the late, slow phase of fraction II B-VLDL clearance. It is of interest that the clearance of canine IDL from the plasma of a normal dog was similar to the clearance of fraction II B-VLDL in normal dogs (Figure 7B). These data suggested that the late, slow phase of clearance might reflect the conversion of fraction II B-VLDL to smaller, lower density IDL or LDL, which are cleared more slowly from the plasma of the cholesterol-fed dog.

To determine whether the fraction II B-VLDL were converted to IDL or LDL, plasma was subjected to agarose column chromatography at various time intervals after injection of radiolabelled fraction II B-VLDL (Figure 8). Fraction II B-VLDL injected into a cholesterol-fed dog were converted to smaller particles over time. In Figure 8B, compare the elution profile at 15 minutes and at 4 hours. By contrast, fraction II B-VLDL injected into a normal dog appeared to be cleared from the plasma without conversion to smaller particles (Figure 8A).

The apparent conversion of the fraction II B-VLDL to IDL and LDL in the cholesterol-fed dog was demonstrated by using density gradient ultracentrifugation (Figure 9). By 5 hours, and even as late as 24 hours, the radiolabelled lipoproteins that remained in the plasma had the density of IDL and LDL. Therefore, the late, slow phase of fraction II B-VLDL clearance in cholesterol-fed dogs appears to reflect the clearance of IDL and LDL.

Discussion

The B-VLDL in cholesterol-fed dogs and in type III hyperlipoproteinemia in humans are heterogeneous, consisting of two subpopulations: fraction I, originating in the intestine and composed of chylomicron remnants, and fraction II, originating in the liver and composed of cholesteryl esterrich VLDL remnants. In the present study, the metabolism of these two B-VLDL subfractions in normal and cholesterol-fed dogs was investigated. In the normal dog, both fractions disappeared rapidly from the circulation (> 90% with-
in 30 to 60 minutes). In the cholesterol-fed dog, fraction I B-VLDL (chylomicron remnants) were cleared with similar rapidity. It has previously been shown that parenchymal cells of the rat liver take up chylomicron remnants by a receptor-mediated process. In contrast to fraction I, most fraction II B-VLDL in the cholesterol-fed dog were retained in the plasma for much longer. After an initial, rapid phase of clearance, resulting in approximately a 30% to 40% decrease in plasma radioactivity, fraction II B-VLDL entered a slow phase of clearance (a half-life of >20 hours).

In the normal (chow-fed) dog, most fraction II B-VLDL disappeared rapidly from the plasma without any appreciable change in size or density and were recovered almost quantitatively in the liver. Furthermore, cyclohexanone modification of the arginine residues of the B-VLDL apolipoproteins resulted in a less rapid plasma clearance and a blocking of their uptake by rat liver parenchymal cells, thus suggesting that their uptake is mediated mainly by an apo B,E(LDL) receptor-specific mechanism on parenchymal cells.

Current evidence indicates that the liver contains two lipoprotein receptors, the apo B,E(LDL) and the chylomicron remnant receptor (for review, see references 12 and 13). To investigate further the role of receptor-mediated clearance of B-VLDL in normolipidemic dogs, we took advantage of the observation that apo B,E(LDL) receptors can be down-regulated by the infusion of bile acids. In the present study, the uptake of fraction II B-VLDL was delayed in normal dogs by an infusion of taurocholate bile acid, further suggesting that the apo B,E(LDL) receptor is a major pathway for fraction II B-VLDL clearance in normal dogs. Furthermore, autoradiography performed in rats has shown that the hepatocyte is the major site of fraction II B-VLDL uptake in vivo. It can be concluded from these results that, in the normolipidemic dog, fraction II B-VLDL are taken up rapidly by the liver and that the uptake appears to be mediated by the apo B,E(LDL) receptors on the hepatic parenchymal cell.

In the cholesterol-fed dog, fraction II B-VLDL metabolism is different. The apo B,E(LDL) receptors in the choles-
terol-fed dog are known to be down-regulated, as they are in cholesterol-fed rabbits and rats. The impaired clearance of these lipoproteins in cholesterol-fed dogs is therefore consistent with our findings that demonstrate the importance of the apo B:E(LDL) receptors in the rapid hepatic clearance of fraction II B-VLDL in normal dogs. In the absence of expression of apo B:E(LDL) receptors, it appears that fraction II B-VLDL are processed through lipolytic conversion to IDL and LDL, which are cleared slowly from the plasma. Similarly, in Watanabe Heritable Hyperlipidemic (WHHL) rabbits, which lack normal apo B:E(LDL) receptors, clearance of VLDL and VLDL remnants decreases and production of LDL from the VLDL increases.

As with fraction I B-VLDL, the clearance of chylomicron remnants does not appear to be mediated solely by apo B:E(LDL) receptors. Chylomicron remnant catabolism is impaired only slightly, if at all, in cholesterol-fed animals, WHHL rabbits, and humans with familial hypercholesterolemia. These data suggest the existence of a second hepatic lipoprotein receptor, the chylomicron remnant receptor. Although we originally postulated that the apo E receptor is the chylomicron remnant receptor, we now know that binding data obtained with liver membranes in vitro may not reflect in vivo internalization. We have reason to believe that the so-called apo E receptor represents more than one protein, and that they are not necessarily involved in chylomicron remnant metabolism (Beischer, U., et al., unpublished data). Further studies are clearly indicated to resolve these questions.

The present study demonstrates that both fraction I and fraction II B-VLDL appear to be cleared from the plasma by the liver, but through different mechanisms that presumably are different lipoprotein receptors. While fraction II B-VLDL are cleared by the hepatic apo B:E(LDL) receptor, fraction I B-VLDL appear to be cleared by another mechanism, presumably the chylomicron remnant receptor. In addition, the apo B:E(LDL) receptor may also participate in the metabolism of fraction I B-VLDL since it can bind to the latter in vitro. The structural features that differentially target these two types of lipoprotein particles are not known. Several differences distinguish fraction I from fraction II B-VLDL, including the presence of apo B-48 in fraction I B-VLDL, which possess apo B-100. However, in vitro binding of both fraction I and fraction II B-VLDL and the receptor clearance in vivo appear to be mediated by apo E, not by B-48. In fact, studies in an apo E-deficient patient and an apo E2 type III hyperlipoproteinemic patient revealed that large amounts of apo B-48-containing B-VLDL...
accumulate in the fasting plasma. Despite the lack of apo B-48 binding to the receptors, it is reasonable to postulate that the type of apo B on a particle may modulate the expression of apo E and affect its recognition. Apolipoproteins B would not necessarily have to mediate binding directly to have these effects; it could alter the conformation of apo E or its availability for binding and thereby account for the separate metabolic fates of both B-VLDL fractions. Another factor that may affect the metabolic fates of these lipoprotein particles, but is not addressed directly in our study, is the content of C apolipoproteins, which has been shown to affect hepatic clearance of triglyceride-rich lipoproteins.  

Figure 9. Density gradient ultracentrifugation of plasma from a cholesterol-fed dog injected with $^{125}$I-labelled fraction II B-VLDL. The plasma (1 ml) was subjected to ultracentrifugation on a NaCl-KBr gradient of d = 1.006 to 1.09 g/ml (SW41 rotor, 40 000 rpm for 48 hours at 4°C). The distribution on the gradients was determined by addition of an aliquot to plasma at 0 minutes ( ), 5 hours ( ), and 24 hours ( ) after intravenous injection of 0.5 mg of $^{125}$I-labelled fraction II B-VLDL. The 0 time represents an aliquot of the injected $^{125}$I-labelled fraction II B-VLDL added to control plasma.

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