Proc. Natl. Acad. Sci. USA
Vol. 78, No. 3, pp. 1396–1400, March 1981
Biochemistry

Saturation and suppression of hepatic lipoprotein receptors: A mechanism for the hypercholesterolemia of cholesterol-fed rabbits

(β very low density lipoprotein/low density lipoprotein/apoproteins B and E/regulation of receptors/clearance of plasma lipoproteins)

PETRI T. KOVANEN, MICHAEL S. BROWN, SANDIP K. BASU, DAVID W. BILHEIMER, AND JOSEPH L. GOLDSTEIN

Departments of Molecular Genetics and Internal Medicine, University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235

Contributed by Michael S. Brown, November 6, 1980

ABSTRACT **Cholesterol-fed rabbits develop a marked increase in plasma cholesterol levels. Most of the excess plasma cholesterol is contained in β-migrating very low density lipoprotein (β-VLDL), a cholesterol-rich particle that contains apoproteins B and E. When ^{125}I-labeled β-VLDL from cholesterol-fed rabbits was injected intravenously into normal rabbits, the lipoprotein was cleared rapidly from plasma, 80% of the radioactivity appearing in the liver within 4 min. *In vitro* binding assays showed that this uptake was due to the presence on liver membranes of a high-affinity, low-capacity binding site that resembles the low density lipoprotein receptor previously characterized on extrahepatic tissues. When the ^{125}I-labeled β-VLDL was injected into cholesterol-fed rabbits, hepatic uptake was reduced by more than 95% and the lipoprotein remained in the plasma. This defective uptake in cholesterol-fed rabbits was due to two factors: (*i*) saturation of the lipoprotein receptors by the high concentration of endogenous plasma β-VLDL and (*ii*) a 60% reduction in the number of hepatic receptors after cholesterol feeding. Of the two factors, saturation of receptors was quantitatively more important. We suggest that, as a result of the saturation and suppression of receptors, the hepatic removal of β-VLDL in the cholesterol-fed rabbit fails to increase commensurate with the diet-induced increase in β-VLDL synthesis and profound hypercholesterolemia ensues.**

The rabbit is unique among animal species in its extreme sensitivity to dietary cholesterol. Within days after the initiation of a high cholesterol diet, the plasma cholesterol level rises more than 10-fold. Most of the excess plasma cholesterol is contained in lipoproteins of density (ρ) <1.019 g/ml, which include very low density lipoprotein (VLDL) and intermediate density lipoproteins (1–3). In comparison with normal VLDL, in which triglycerides are the predominant lipid and apoprotein B is the major protein, the VLDL particles of cholesterol-fed rabbits contain cholesteryl ester as the major lipid and are markedly enriched in apoprotein E (also called arginine-rich protein) (1, 3). Because these particles show an abnormal β-mobility on electrophoresis, they are called β-VLDL (4–6). The β-VLDL particles are believed to be remnant lipoproteins generated by the action of lipoprotein lipase on triglyceride-rich chylomicrons and VLDL (2). In the normal rabbit, cholesterol-rich remnant particles are rapidly cleared by the liver but, in cholesterol-fed animals, hepatic uptake is markedly reduced (2, 3, 7).

A mechanism to account for the impaired removal of β-VLDL from plasma in cholesterol-fed rabbits is suggested by the finding that lipoproteins can enter cells by binding to high-affinity, saturable cell surface receptors (8). Originally found on the surface of cultured human fibroblasts, these receptors mediate the binding, uptake, and lysosomal degradation of low density lipoprotein (LDL), supplying cholesterol to the cell (8). The LDL receptors of fibroblasts have a higher affinity for lipoproteins that contain apoprotein E, such as β-VLDL, than they do for LDL, which contains only apoprotein B (9). Similar receptors have recently been identified on membranes isolated from the liver of the rat (10, 11) and the dog (12). These hepatic receptors also bind apoprotein E-containing lipoproteins with higher affinity than apoprotein B-containing lipoproteins. Like the LDL receptors of extrahepatic cells, they fail to recognize methylated LDL and HDL_3, a lipoprotein that is devoid of apoproteins B and E (10–12). In the dog, the number of hepatic receptors increases when hepatic cholesterol requirements are enhanced, suggesting that the liver receptor is regulated by cholesterol in a manner similar to the fibroblast LDL receptor (12).

If the liver of the rabbit contains high-affinity receptors that mediate the uptake of cholesterol-rich lipoproteins, then hypercholesterolemia might be accelerated when these receptors become saturated or suppressed as a result of cholesterol feeding. Our current studies were designed to test this hypothesis by measuring the binding of ^{125}I-labeled β-VLDL to liver membranes from normal and cholesterol-fed rabbits and correlating these *in vitro* binding results with studies of the hepatic uptake of intravenously injected ^{125}I-labeled β-VLDL *in vivo*. The results suggest that rabbit liver does contain saturable binding sites that recognize β-VLDL and that a combination of saturation and suppression of these sites is responsible for the defective removal of these particles from the plasma of cholesterol-fed rabbits.

METHODS

Animals and Diets. Male New Zealand White rabbits weighing 1.5–2.5 kg were kept in an animal room that had alternating 12-hr periods of light and dark. The animals had continuous access to water and the indicated diet. The 2% cholesterol diet (2% Cholesterol Diet Complete, Pelleted for Rabbits) was purchased from Nutritional Biochemicals. The 2% cholesterol–10% corn oil diet was prepared by dissolving cholesterol (U.S.P. grade, ICN Pharmaceuticals), in hot corn oil and then mixing it with Purina Rabbit Laboratory Chow (Purina). The control diet consisted of Purina Rabbit Laboratory Chow.

Lipoproteins. Human LDL (ρ, 1.019–1.063 g/ml) and HDL_3 (ρ, 1.125–1.215 g/ml) were isolated from the plasma of healthy subjects by ultracentrifugation (10). Acetylated LDL (acetyl-LDL) and methylated LDL (methyl-LDL) were prepared as described (12). The mass ratios of cholesterol to protein for human LDL and HDL_3 were 1.5 and 0.3, respectively. Blood for isolation of rabbit lipoproteins was obtained from animals that

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein that has pre-β mobility on electrophoresis and contains predominantly triglycerides; β-VLDL, very low density lipoprotein that has β-mobility on electrophoresis and contains predominantly cholesteryl esters.

had been fasted overnight. Normolipidemic rabbit VLDL (ρ, <1.006 g/ml), LDL (ρ, 1.019–1.063 g/ml), and HDL_3 (ρ, 1.125–1.215 g/ml) were isolated by ultracentrifugation from EDTA-anticoagulated plasma obtained from rabbits on the control diet (6). The mass ratios of cholesterol to protein for normal rabbit VLDL, LDL, and HDL_3 were 0.64, 0.74, and 0.16, respectively. Hyperlipidemic β-VLDL was isolated from rabbits fed the 2% cholesterol–10% corn oil diet for 2–5 weeks. Plasma cholesterol levels ranged from 1100 to 1800 mg/dl. The hyperlipidemic (ρ <1.006) fraction was isolated by centrifugation at 49,000 rpm for 14 hr at 4° and was washed by recentrifugation in 0.15 M NaCl at 49,000 rpm for 14 hr (6). More than 90% of the lipid in this fraction was contained in β-VLDL, as judged by visual inspection of overloaded agarose gel electrophoretograms (5, 6). The average mass ratio of cholesterol to protein for rabbit β-VLDL was 7.6. The concentration of each lipoprotein is given in terms of its protein content unless otherwise indicated.

^{125}I-Labeled Lipoproteins. Human LDL, rabbit β-VLDL, and rabbit HDL_3 were radioiodinated by the iodine monochloride method (13), and the iodinated products were dialyzed against buffer containing 0.15 M NaCl and 0.01% EDTA (pH 7.4) and then passed through a Millex-HA 0.45-μm filter (Millipore). For ^{125}I-labeled human LDL, rabbit β-VLDL, and rabbit HDL_3, averages of 2%, 15%, and 3%, respectively, of the radioactivity was in lipid, as determined by chloroform/methanol extraction.

Intravenous Injection of Rabbit ^{125}I-Labeled β-VLDL and Measurement of Hepatic Content of ^{125}I-Radioactivity. Solutions of rabbit ^{125}I-labeled-β-VLDL were prepared in 0.15 M NaCl containing bovine serum albumin at 20 mg/ml, passed through a Millex-HA 0.45-μm filter, and administered as a 5-ml bolus injection into an ear vein. Immediately before killing, the rabbits were anesthetized by intravenous injection of 100–200 mg of sodium pentobarbital and the abdomen was opened. Blood was obtained from the inferior vena cava, and the liver was perfused for 3 min *in situ* via the portal vein (10). The liver was then removed, and 1-g pieces were counted for ^{125}I-radioactivity. More than 90% of the total ^{125}I-radioactivity in plasma and liver could be precipitated by 10% trichloroacetic acid.

Preparation of Liver and Adrenal Membranes. Tissues were homogenized, and membranes (the fraction sedimenting at 8000–100,000 × *g*) were prepared in buffer A (0.15 M NaCl/1 mM $CaCl_2$/1 mM phenylmethylsulfonyl fluoride/20 mM Tris·HCl, pH 8) by centrifugation as described (10), except that the final pellet was subjected to an additional resuspension and sedimentation (100,000 × *g*, 60 min, 4°C). The pellets were assayed immediately or were frozen in liquid nitrogen and stored at −170°C. On the day of an experiment, the pellets were suspended in buffer B (50 mM NaCl/1 mM $CaCl_2$/20 mM Tris·HCl, pH 8) by flushing five times through a 25-gauge needle. The suspensions were then sonicated for 45 sec (10) and diluted with buffer B to a protein concentration of 5 mg/ml.

Binding of ^{125}I-Labeled Lipoproteins to Liver and Adrenal Membranes. The standard binding assay (10, 12) was conducted at pH 8 in 80 μl of buffer C (25 mM NaCl/0.5 mM $CaCl_2$/50 mM Tris·HCl/bovine serum albumin at 20 mg/ml) containing 100 μg of membrane protein and the indicated amount of ^{125}I-labeled lipoprotein in the absence or presence of excess unlabeled lipoprotein. The tubes were incubated for 60 min in an ice water bath. Membrane-bound ^{125}I-labeled lipoprotein was separated from free ^{125}I-labeled lipoprotein by centrifugation at 100,000 × *g* in a Beckman Airfuge using a 30° angle rotor (10). Samples were processed for scintillation counting as described (10). Unless otherwise indicated, all data points represent the average of duplicate assays.

Other Assays. The total cholesterol content of plasma and lipoprotein fractions was measured by the cholesterol oxidase method (Boehringer Mannheim). The total cholesterol content of homogenates of liver was determined by gas-liquid chromatography (14). Proteins were determined by the method of Lowry *et al.* (15).

RESULTS

β-VLDL was isolated from the plasma of cholesterol-fed rabbits and iodinated with ^{125}I. When the ^{125}I-labeled β-VLDL was incubated with liver membranes from normal rabbits, the lipoprotein bound with high affinity to a single class of binding sites that was susceptible to competition by an excess of unlabeled β-VLDL (Fig. 1*A*). A Scatchard plot of the specific binding data gave a line whose intercepts indicated an apparent K_d of 0.5 μg of protein/ml and an apparent maximal binding of 160 ng of protein/mg of membrane protein (Fig. 1*B*). Time course studies showed that maximal binding of labeled β-VLDL was reached after incubation for 30 min at 0°C.*

The binding of rabbit ^{125}I-labeled β-VLDL to rabbit liver membranes was inhibited competitively by excess β-VLDL as well as by excess LDL and VLDL obtained from normal rabbits (Fig. 2*A*). On the other hand, rabbit HDL_3 did not compete for binding.† The binding of rabbit labeled β-VLDL was also inhibited competitively by human LDL but not by acetylated or methylated human LDL nor by human HDL_3 (Fig. 2*B*). The high-affinity binding was also completely inhibited by 7.5 mM EDTA, and the binding site was destroyed by prior treatment of the membranes with pronase at 10 μg/ml for 20 min at 37°C.

When rabbits were fed a high cholesterol diet, the plasma

* The entire ^{125}I-labeled β-VLDL particle appeared to bind to the hepatic receptor, as indicated by the following experiment: $NaDodSO_4$/polyacrylamide gel electrophoresis of the ^{125}I-labeled β-VLDL, followed by autoradiography and scintillation counting, showed labeled bands that corresponded to apoproteins B, E, A-I, A-IV, and C in the ratio 15:6:2.5:1.5:1. When the ^{125}I-labeled β-VLDL was allowed to bind to normal liver membranes and the membranes were then isolated by centrifugation, solubilized with $NaDodSO_4$, and subjected to electrophoresis, the membrane-bound ^{125}I-radioactivity was distributed in the protein bands in similar proportions to those of the starting ^{125}I-labeled β-VLDL.

† Direct binding studies showed the presence on rabbit liver membranes of a high-affinity binding site for rabbit ^{125}I-labeled HDL_3 that was distinct from the β-VLDL binding site. The HDL_3 binding site had an apparent K_d of 63 μg of protein/ml and a maximal binding of 530 ng/mg of membrane protein, as determined by Scatchard plot analysis of the specific binding data.

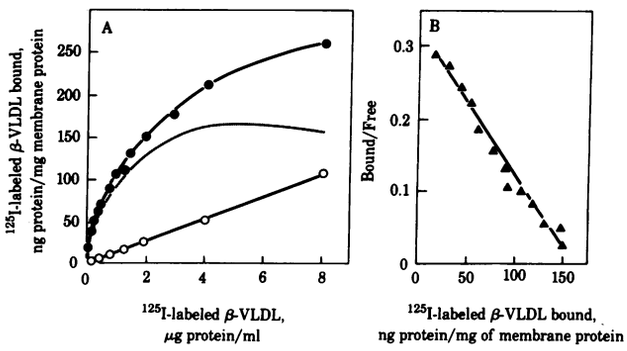

FIG. 1. Saturation curve (*A*) and Scatchard plot (*B*) of binding of rabbit ^{125}I-labeled β-VLDL to liver membranes from normal rabbits. Each assay tube contained the indicated concentration of ^{125}I-labeled β-VLDL (650 cpm/ng of protein) in the absence (●) or presence (○) of unlabeled β-VLDL at 60 μg/ml. Specific binding (———) was calculated by subtracting the amount of ^{125}I-labeled β-VLDL bound in the presence of excess unlabeled β-VLDL from that bound in its absence.

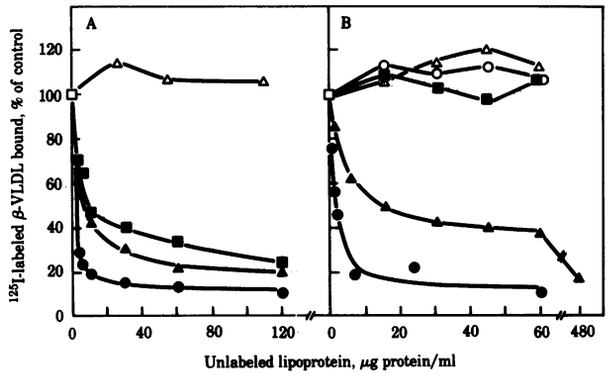

FIG. 2. Ability of unlabeled rabbit (*A*) and human (*B*) lipoproteins to compete with rabbit ^{125}I-labeled β-VLDL for binding to liver membranes from normal rabbits. Each assay tube contained rabbit ^{125}I-labeled β-VLDL (0.75 μg of protein/ml; 540 cpm/ng of protein) and the indicated concentration of one of the following unlabeled lipoproteins: (*A*) △, rabbit HDL_3; ■, rabbit VLDL; ▲, rabbit LDL; or ●, rabbit β-VLDL. (*B*) ■, human acetyl-LDL; ○, human methyl-LDL; △, human HDL_3; ▲, human LDL; ●, rabbit β-VLDL. The "100% control values" for ^{125}I-labeled β-VLDL binding in the absence of unlabeled lipoproteins (□) were 78 and 114 ng of protein bound per mg of membrane protein for *A* and *B*, respectively.

cholesterol level rose progressively over 8 days (Fig. 3*A*), as did the concentration of cholesterol in the liver (Fig. 3*B*). The number of hepatic ^{125}I-labeled β-VLDL receptors, as estimated by the amount of specific binding of labeled β-VLDL at 0.75 μg/ml, decreased slowly (Fig. 3*C*). After 8 days, the specific binding had fallen by about 60%. The nonspecific binding—i.e., binding observed in the presence of excess unlabeled β-VLDL—did not change during this period. Saturation curves for the binding of labeled β-VLDL to liver membranes showed that cholesterol feeding for 2–8 days reduced the number of binding sites without reducing the apparent affinity (data not

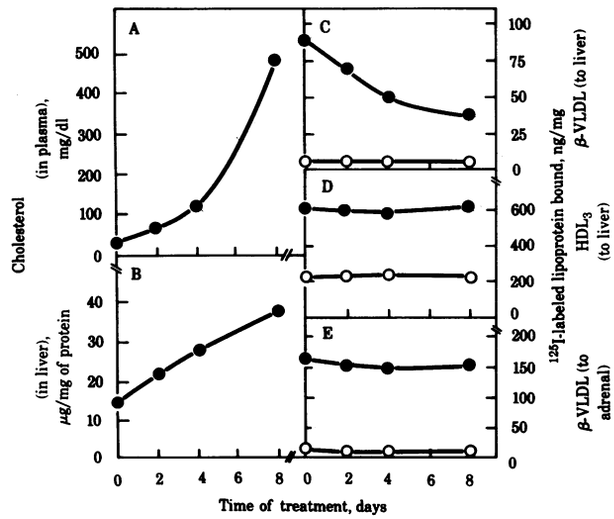

FIG. 3. (*A*) Plasma cholesterol levels. (*B*) Liver cholesterol levels. Binding of rabbit ^{125}I-labeled β-VLDL (*C*) and of rabbit ^{125}I-labeled HDL_3 (*D*) to liver membranes and binding of rabbit ^{125}I-labeled β-VLDL to adrenal membranes (*E*) during treatment of rabbits with a 2% cholesterol diet. Groups of rabbits, two per group, were fed a 2% cholesterol diet for the indicated time. For binding assays, each tube contained 0.75 μg of protein/ml of rabbit ^{125}I-labeled β-VLDL (613 cpm/ng) in the absence (●) or presence (○) of unlabeled β-VLDL at 60 μg/ml (*C* and *E*); or 30 μg of protein/ml of rabbit ^{125}I-labeled HDL_3 in the absence (●) or presence (○) of unlabeled rabbit HDL_3 at 800 μg/ml (*D*). Each point represents the average of duplicate assays of individual samples from two rabbits.

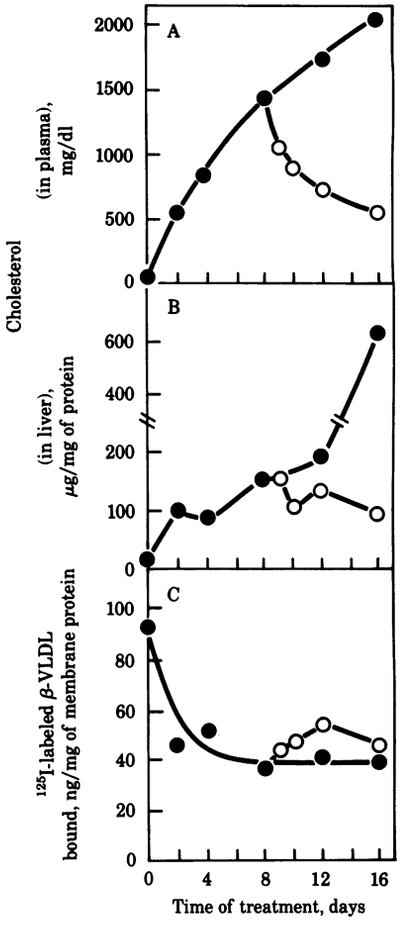

FIG. 4. (*A*) Plasma cholesterol levels, (*B*) liver cholesterol levels, and (*C*) binding of rabbit ^{125}I-labeled β-VLDL to liver membranes during treatment of rabbits with 2% cholesterol–10% corn oil diet (●) and after resumption of a control diet (○). Groups of rabbits (two per group) were treated with 2% cholesterol–10% corn oil diet for the indicated number of days (●). After 8 days, four groups of rabbits were returned to the control diet for 1, 2, 4, and 8 days as indicated (○). The onset of the diets was staggered so that all rabbits could be killed on the same day. For binding assays, each tube contained 0.75 μg protein/ml of rabbit ^{125}I-labeled β-VLDL (492 cpm/mg) in the absence or presence of 60 μg/ml of unlabeled β-VLDL. Specific binding was calculated as described in the legend to Fig. 1. Each point represents the average of duplicate assays of pooled liver membranes from two similarly treated rabbits.

shown). For comparison purposes, we also measured the specific binding of ^{125}I-labeled rabbit HDL_3, which bound to a site on hepatic membranes different from the β-VLDL binding site.[†] The binding of ^{125}I-labeled HDL_3 did not change during the cholesterol feeding period (Fig. 3*D*). Membranes from the adrenal glands of normal rabbits also bound labeled β-VLDL with high affinity and with the same specificity as the hepatic binding site, but the adrenal binding site was not suppressed by 8 days of cholesterol feeding (Fig. 3*E*).

When the rabbits were fed a diet containing cholesterol and corn oil, the plasma cholesterol level rose even more rapidly than on the cholesterol diet alone (Fig. 4). By day 8, the plasma cholesterol level reached 1500 mg/dl and hepatic ^{125}I-labeled β-VLDL binding was reduced by about 60%. A group of animals was then returned to a control diet, after which the plasma cholesterol level decreased to 500 mg/dl over the next 8 days. The hepatic cholesterol content remained elevated and did not increase further when the control diet was reinstituted. The amount of labeled β-VLDL binding increased slightly when the

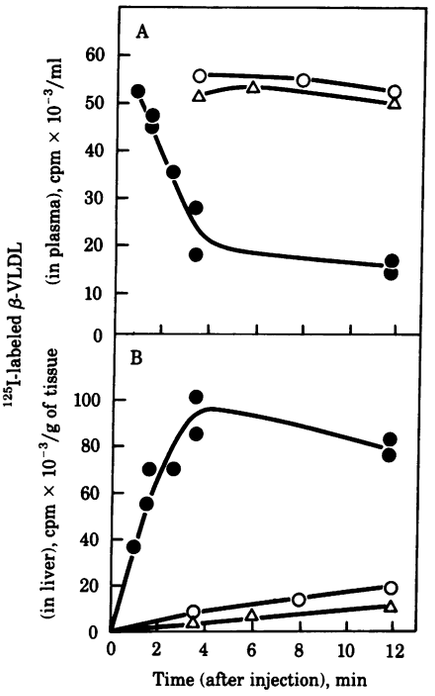

FIG. 5. Disappearance of ^{125}I-labeled β-VLDL from the plasma (*A*) and appearance in liver (*B*). Eight control rabbits (●) and three rabbits fed a 2% cholesterol–10% corn oil diet for 4 days (△) were injected intravenously with rabbit ^{125}I-labeled β-VLDL (26 μg of protein, 1 × 10^7 cpm). Three additional control rabbits received 52 mg of protein unlabeled rabbit β-VLDL together with the ^{125}I-labeled β-VLDL (○). At the indicated time after injection, each animal was killed, and the ^{125}I radioactivity in the plasma and liver was determined. Each point represents one rabbit.

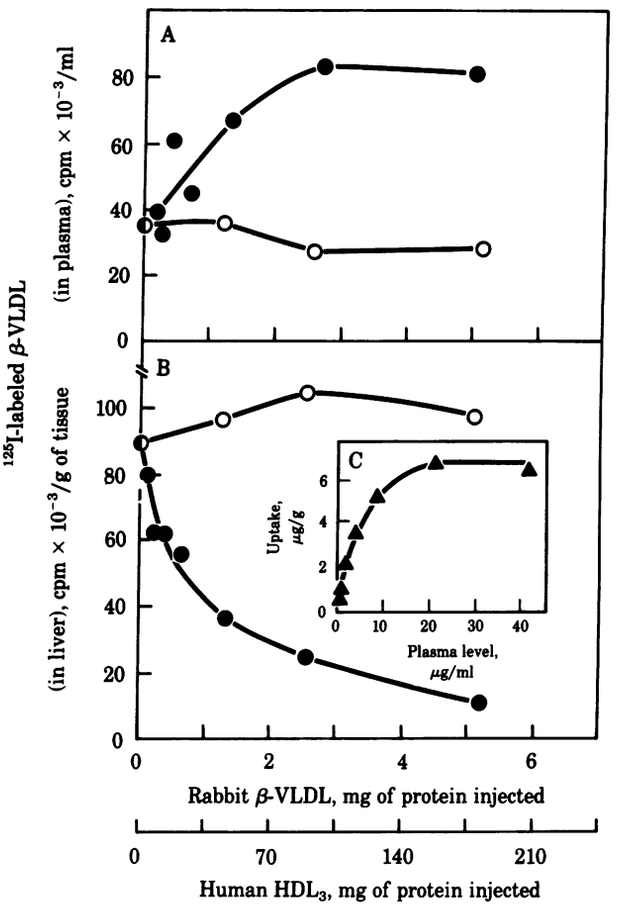

FIG. 6. Inhibition of plasma clearance (*A*) and hepatic uptake (*B*) of ^{125}I-labeled β-VLDL by increasing amounts of unlabeled rabbit β-VLDL (●) but not by human HDL$_3$ (○). Control rabbits were injected intravenously with ^{125}I-labeled β-VLDL (50 μg of protein, 1 × 10^7 cpm) either in the absence (◐) or the presence of increasing concentrations of unlabeled rabbit β-VLDL (●) or human HDL$_3$ (○). Four minutes after injection each animal was killed, and the ^{125}I radioactivity in the plasma and liver was determined. Each point represents one rabbit.

high cholesterol diet was stopped, but remained less than 50% of normal 8 days after the control diet was reinstituted, presumably because plasma and tissue cholesterol levels remained far above normal.

To determine whether a correlation exists between the hepatic β-VLDL binding site and hepatic uptake of β-VLDL, we administered ^{125}I-labeled β-VLDL intravenously to a series of rabbits and killed the animals at intervals after the injection (Fig. 5). In normal rabbits, the concentration of labeled β-VLDL in the plasma decreased rapidly between 1 and 3.5 min after injection. During this time, there was a rapid increase in the labeled β-VLDL content in the liver. By 3.5 min, 80% of the administered radioactivity was contained in the liver. When the same injection was given to cholesterol-fed animals, the plasma concentration of labeled β-VLDL remained high 12 min after the injection, and there was little uptake of the labeled β-VLDL into the livers. The block in hepatic uptake of labeled β-VLDL in cholesterol-fed animals could be reproduced in control rabbits by injection of an excess of unlabeled β-VLDL. Such injection inhibited the hepatic uptake of labeled β-VLDL by more than 90%, and the plasma radioactivity failed to decrease during the course of the experiment.

These data suggest that rabbit liver takes up ^{125}I-labeled β-VLDL by a rapid and saturable process. To estimate the affinity of this uptake mechanism *in vivo*, we injected normal rabbits with a tracer dose of labeled β-VLDL together with increasing amounts of unlabeled β-VLDL (Fig. 6). The hepatic uptake of the labeled β-VLDL was inhibited by 50% when 1 mg of unlabeled β-VLDL was injected. This amount of β-VLDL did not raise the plasma cholesterol level significantly. The same amount of unlabeled β-VLDL also blocked the disappearance of the labeled β-VLDL from the plasma. As a control for this experiment, we also injected human HDL$_3$, which does not bind to the hepatic β-VLDL receptor. Injections of as much as 180 mg of HDL$_3$ protein did not prevent hepatic uptake of β-VLDL. The total amount of β-VLDL taken up by the liver was plotted as a function of the plasma concentration of β-VLDL at the end of the experiment (see Fig. 6*C*). These values were obtained by converting the measured plasma radioactivity at the time of killing of the animals to the total plasma concentration of VLDL protein, taking into account the dilution in specific activity created by addition of the unlabeled β-VLDL. Analysis of this saturation curve by a double-reciprocal plot indicated that half-maximal β-VLDL uptake occurred at a plasma concentration of β-VLDL protein of ≈1.9 μg/ml.

DISCUSSION

Two unexpected findings emerged from this study. The first was the high rate of uptake of ^{125}I-labeled β-VLDL by the normal rabbit liver. When small amounts of ^{125}I-labeled β-VLDL were administered intravenously, fully 80% of the labeled ligand was found in the liver within 4 min. *In vitro* binding studies showed that this rapid hepatic uptake resulted from the presence on liver membranes of a high-affinity lipoprotein binding site that resembled the LDL receptors initially identified in human fibroblasts (8, 9) and subsequently found in adrenal (14, 16) and liver (10–12) tissue from several species. Like the receptors

studied earlier, the rabbit hepatic receptors had an extremely high affinity for apoprotein E-containing lipoproteins such as β-VLDL. In addition, they bound LDL, but not methyl-LDL or lipoproteins devoid of apoproteins B and E.

The second unexpected finding was the ease with which this uptake mechanism became saturated *in vivo*. In the presence of unlabeled β-VLDL, the ^{125}I-labeled β-VLDL was not taken up by the liver but remained in the plasma, apparently because the unlabeled β-VLDL competed with the labeled β-VLDL for binding to a limited number of saturable high-affinity binding sites. Half-maximal binding occurred when the plasma β-VLDL protein concentration was ≈1.9 μg/ml, which is equal to a β-VLDL cholesterol level of only 1.4 mg/dl. When the β-VLDL concentration was above this level, the removal mechanism began to be saturated and the fractional clearance rate decreased.

The concentration of β-VLDL protein that gave half-maximal hepatic uptake *in vivo* (1.9 μg/ml) was somewhat higher than the concentration (0.5 μg/ml) required for half saturation of the receptors at 0°C *in vitro*, a difference that can probably be attributed to differences in temperature and other conditions between the *in vivo* and *in vitro* measurements. Human HDL_3, which failed to compete with ^{125}I-labeled β-VLDL for binding to liver membranes, also failed to compete for the uptake of labeled β-VLDL by the liver of the intact animal, a finding that reinforces the conclusion that the high affinity binding site detectable *in vitro* was responsible for the high affinity uptake of β-VLDL *in vivo*.

Considered together with recent studies of lipoprotein uptake in perfused rat liver (11, 17, 18) and dog liver *in vivo* (19), the current data support the concept that a lipoprotein receptor system analogous to the LDL receptor pathway of fibroblasts mediates the rapid hepatic uptake of cholesterol-rich lipoprotein particles that contain apoprotein E. One likely function of this receptor system in normal animals is to mediate the hepatic uptake of cholesterol-rich remnant particles formed by the action of lipoprotein lipase on chylomicrons and VLDL (2). Although this receptor system is of extremely high affinity, it is also of low capacity.

When the rabbit consumes a high cholesterol diet, the increased production of β-VLDL overwhelms the limited capacity of the hepatic clearance mechanism and the concentration of β-VLDL in plasma increases enormously. In addition, the number of lipoprotein receptors becomes suppressed by about 60% and this suppression further reduces the hepatic uptake of plasma β-VLDL. We suggest that this combination of receptor saturation and suppression provides a mechanistic explanation for the findings of other workers who reported a decrease in the rate of clearance of cholesterol-rich lipoproteins from the plasma of cholesterol-fed rabbits (2, 3, 7).

The question arises as to why other animal species, such as rats and dogs, fail to show the same profound hypercholesterolemic response to dietary cholesterol as do rabbits. In the rat and dog, cholesterol-rich remnant particles are believed to be formed as in the rabbit (20), and the livers of these animals contain roughly the same number of high-affinity lipoprotein receptors as do rabbits (10–12). Yet, when rats and dogs are fed a high cholesterol diet, the hepatic receptors are not easily overwhelmed, and β-VLDL does not rapidly accumulate in the plasma. In one study in dogs, the clearance of ^{125}I-labeled apo $E\text{-}HDL_c$, which contains apoprotein E as its major protein, was only slightly retarded in cholesterol-fed animals, suggesting that there was not a sufficient accumulation of plasma β-VLDL to saturate the hepatic clearance mechanism (19). Two factors are likely to account for the different response in rabbits: (*i*) the rabbit absorbs more dietary cholesterol than the other species and hence may synthesize more β-VLDL (21) and (*ii*) the rabbit fails to develop high rates of bile acid production in response to dietary cholesterol (21), a defect that may lead to a secondary increase in β-VLDL production by the liver. In addition, it is possible that rats and dogs possess an additional hepatic lipoprotein removal system (i.e., a low-affinity, high-capacity receptor) that is not expressed in the rabbit and allows them to clear greater amounts of β-VLDL from plasma.

In view of its postulated role in the physiologic clearance of lipoproteins from the plasma, the receptor described in this paper is likely to exist on parenchymal cells rather than nonparenchymal cells of the liver. This conclusion is supported by the experiments of Chao *et al.*, who showed by autoradiography that ^{125}I-labeled LDL is taken into hepatic parenchymal cells of perfused rat livers by a receptor similar to the one described here (18). Although macrophages possess a receptor that binds β-VLDL with high affinity (5, 6), binding of ^{125}I-labeled β-VLDL to the macrophage receptor is not inhibited competitively by LDL or apo $E\text{-}HDL_c$, both of which inhibit binding of labeled β-VLDL to rabbit hepatic membranes.

We are grateful to Richard Gibson, John J. Jaramillo, and Michael Funk for excellent technical assistance. This work was supported by Research Grants HL-20948 and HL-15949 from the National Institutes of Health.

1. Shore, V. G., Shore, B. & Hart, R. G. (1974) *Biochemistry* **13**, 1579–1585.
2. Ross, A. C. & Zilversmit, D. B. (1977) *J. Lipid Res.* **18**, 169–181.
3. Kushwaha, R. S. & Hazzard, W. R. (1978) *Biochim. Biophys. Acta* **528**, 176–189.
4. Mahley, R. W. & Weisgraber, K. H. (1974) *Circ. Res.* **35**, 722–733.
5. Goldstein, J. L., Ho, Y. K., Brown, M. S., Innerarity, T. L. & Mahley, R. W. (1980) *J. Biol. Chem.* **255**, 1839–1848.
6. Mahley, R. W., Innerarity, T. L., Brown, M. S., Ho, Y. K. & Goldstein, J. L. (1980) *J. Lipid Res.* **21**, 970–980.
7. Redgrave, T. G., Dunne, K. B., Roberts, D. C. K. & West, C. E. (1976) *Atherosclerosis* **24**, 501–508.
8. Goldstein, J. L. & Brown, M. S. (1977) *Annu. Rev. Biochem.* **46**, 897–930.
9. Innerarity, T. L. & Mahley, R. W. (1978) *Biochemistry* **17**, 1440–1447.
10. Kovanen, P. T., Brown, M. S. & Goldstein, J. L. (1979) *J. Biol. Chem.* **254**, 11367–11373.
11. Windler, E. E. T, Kovanen, P. T., Chao, Y.-S., Brown, M. S., Havel, R. J. & Goldstein, J. L. (1980) *J. Biol. Chem.* **255**, 10464–10471.
12. Kovanen, P. T., Bilheimer, D. W., Goldstein, J. L., Jaramillo, J. J. & Brown, M. S. (1981) *Proc. Natl. Acad. Sci USA* **78**, 1198–1202.
13. Bilheimer, D. W., Eisenberg, S. & Levy, R. I. (1972) *Biochim. Biophys. Acta* **260**, 212–221.
14. Kovanen, P. T., Goldstein, J. L., Chappell, D. A. & Brown, M. S. (1980) *J. Biol. Chem.* **255**, 5591–5598.
15. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
16. Kovanen, P. T., Basu, S. K., Goldstein, J. L. & Brown, M. S. (1979) *Endocrinology* **104**, 610–616.
17. Sherrill, B. C., Innerarity, T. L. & Mahley, R. W. (1980) *J. Biol. Chem.* **255**, 1804–1807.
18. Chao, Y.-S., Jones, A. L., Hradek, G. T., Windler, E. E. T. & Havel, R. J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 597–601.
19. Mahley, R. W., Innerarity, T. L., Weisgraber, K. H. and So, S. Y. (1979) *J. Clin. Invest.* **64**, 743–750.
20. Havel, R. J., Goldstein, J. L. & Brown, M. S. (1980) in *Metabolic Control and Disease*, eds. Bondy, P. K. & Rosenberg, L. E. (Saunders, Philadelphia), pp. 393–494.
21. Dietschy, J. M. & Wilson, J. D. (1970) *N. Engl. J. Med.* **282**, 1128–1138; 1179–1183; 1241–1249.