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Hepatic uptake of chylomicron remnants in WHHL rabbits: A mechanism genetically distinct from the low density lipoprotein receptor

(familial hypercholesterolemia/cholesteryl ester metabolism)

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ABSTRACT **Homozygous Watanabe hereditary hyperlipidemic (WHHL) rabbits have a near-complete deficiency of low density lipoprotein (LDL) receptors in liver and other tissues. As a result, these rabbits clear LDL from plasma at an abnormally slow rate. In the current studies we show that WHHL rabbits clear chylomicrons from plasma at a normal rate. Chylomicrons are cleared by a two-step process: (*i*) hydrolysis of triglycerides in extrahepatic tissues to yield cholesteryl ester-rich remnant particles and (*ii*) rapid uptake of the remnants by the liver. Normal and WHHL rabbits were given intravenous injections of rat chylomicrons labeled either in the lipid portion with [^{3}H]cholesterol and [^{14}C]palmitate or in the protein portion with [^{125}I]iodine. All radiolabeled components were removed from plasma at comparable rates in normal and WHHL rabbits. Comparable amounts of radioactivity accumulated in livers of animals from both genotypes. *In vitro* assays showed that liver membranes from WHHL rabbits were markedly deficient in the binding of ^{125}I-labeled chylomicron remnants as well as ^{125}I-labeled LDL, implying that chylomicron remnants can bind to the hepatic LDL receptor. We conclude that the rabbit liver normally has at least two genetically distinct lipoprotein uptake mechanisms, both of which recognize chylomicron remnants: (*i*) the LDL receptor and (*ii*) a specific chylomicron remnant uptake mechanism that is not measured adequately by current *in vitro* membrane binding assays. WHHL rabbits possess a normal chylomicron remnant uptake mechanism that allows them to clear chylomicrons from plasma at a rapid rate despite their genetic deficiency of LDL receptors.**

Receptors in the liver play a major role in the removal of lipoproteins from blood. Livers of rabbits, dogs, pigs, and rats express receptors that are similar to the low density lipoprotein (LDL) receptors originally described in extrahepatic cells (for review, see ref. 1). These receptors bind lipoproteins containing apoprotein B (apo-B) or apoprotein E (apo-E), including LDL, β-very low density lipoprotein (β-VLDL), and a cholesterol-induced high density lipoprotein containing apo-E as its predominant protein (apo-E-HDL$_c$). These hepatic LDL receptors are subject to metabolic regulation and are expressed in variable amounts under differing conditions. For example, they are suppressed by cholesterol feeding (2, 3) and enhanced when animals are given bile acid binding resins (3–5), cholesterol synthesis inhibitors (4), or 17α-ethinyl estradiol (6).

In addition to the lipoproteins listed above, the hepatic LDL receptor is able to bind chylomicron remnants that are formed during the two-step pathway for chylomicron catabolism (1, 7). This pathway begins when dietary cholesterol and triglyceride are incorporated into chylomicrons. Within extrahepatic capillaries, the chylomicrons are digested by lipoprotein lipase which liberates a large fraction of the triglycerides, reducing the size of the chylomicron and forming a remnant particle. The remnant—which retains its cholesteryl ester—dissociates from the endothelium and reenters the circulation whereupon it is rapidly cleared by the liver, apparently by binding to a receptor.

Although chylomicron remnants can bind to the LDL receptor, clinical observations suggest that remnants can also enter the liver by another pathway distinct from the LDL receptor (8). This conclusion is based on findings in patients with homozygous familial hypercholesterolemia who have a genetic deficiency of LDL receptors. As a result of this deficiency, LDL is degraded slowly and it accumulates to massive levels in plasma. Yet chylomicron remnants do not accumulate in plasma of these individuals, suggesting that remnants can be removed normally from the circulation despite a deficiency of LDL receptors (7, 8).

The uptake of chylomicron remnants by liver is likely to be mediated by a binding site that recognizes one or more of the protein components of the remnant, either apo-E (9–11) or apo-B-48 (11), a unique form of apo-B that is present in chylomicrons but not in LDL (12, 13). Using *in vitro* membrane binding assays, Mahley and co-workers have shown that the liver of dog, swine, and man contains a binding site that recognizes apo-E but not apo-B, in addition to the LDL receptor, which recognizes both apo-B and apo-E (3, 5). Like the LDL receptor, this apo-E binding site is inhibited by EDTA. In rats, Cooper *et al.* have described an EDTA-resistant binding site that also seems specific for apo-E (14). It has not yet been directly shown that the apo-E binding site as measured by these *in vitro* assays is responsible for the *in vivo* uptake of chylomicron remnants.

A new way to study the chylomicron remnant receptor has become available as a result of the discovery of the Watanabe heritable hyperlipidemic (WHHL) rabbit (15). Homozygous WHHL rabbits have a deficiency of LDL receptors, analogous to that of human homozygous familial hypercholesterolemia (16, 17). As in the human disease, LDL is cleared slowly from the circulation of WHHL rabbits and accumulates to massive levels in plasma (18). These rabbits have a near-complete deficiency of LDL receptors in liver as revealed by membrane

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Abbreviations: apo-B, apoprotein B; apo-E, apoprotein E; apo-E-HDL$_c$, a cholesterol-induced lipoprotein containing apo-E as its predominant protein; LDL, low density lipoprotein; HDL, high density lipoprotein; β-VLDL, β-migrating very low density lipoprotein; WHHL, Watanabe heritable hyperlipidemic.

binding studies (17) or by studies of LDL uptake in isolated hepatocytes (19). The current studies were designed to determine whether the WHHL rabbit is able to clear chylomicron remnants rapidly from the circulation.

METHODS

Animals. Male New Zealand White rabbits were purchased from Nitabell (Hayward, CA) or from Sunny Acres Rabbitry (Tyler, TX). Male homozygous WHHL rabbits were raised in Dallas from a mating pair of homozygous WHHL rabbits (17). Animals were maintained on Purina Lab Rabbit Chow and used at 3–4 months of age. In WHHL and normal rabbits, plasma cholesterol levels averaged 640 and 50 mg/dl, respectively, and plasma triglyceride levels averaged 410 and 110 mg/dl, respectively.

Lipoproteins. Endogenously labeled small chylomicrons were prepared by centrifugation of intestinal lymph obtained from rats fed a glucose diet supplemented with [^{3}H]cholesterol and [^{14}C]palmitate (20). Of the ^{3}H radioactivity in the chylomicrons, 15% was in free [^{3}H]cholesterol and 85% was in [^{3}H]cholesteryl esters; of the ^{14}C radioactivity, >95% was in triglycerides, 3–4% was in phospholipids, and <1% was in cholesteryl esters. Double-labeled rabbit chylomicrons were obtained by a similar method from intestinal lymph of a New Zealand White rabbit fed [^{3}H]cholesterol and [^{14}C]palmitate.

To prepare ^{125}I-labeled chylomicron (^{125}I-chylomicron) remnants, we radiolabeled rat chylomicrons (21) and injected them into the femoral veins of functionally eviscerated rats (20). The rats were bled 30 min later. The serum was adjusted to a density (ρ) of 1.019 g/ml by addition of D_2O in 0.15 M NaCl, layered beneath Krebs–Henseleit buffer, and subjected to ultracentrifugation for $10^8 \times g_{av}$·min. The ^{125}I-chylomicron remnants (top fraction) were obtained by tube-slicing. Of the ^{125}I radioactivity in the remnants, 98% was precipitated by trichloroacetic acid and 13% was extracted into chloroform/methanol. ^{125}I-Labeled canine apo-E-HDL$_c$ (3) was kindly provided by T. L. Innerarity and R. W. Mahley. Rabbit β-VLDL, LDL, and HDL$_3$ were prepared as described (2).

***In Vivo* Injection of Double-Labeled Chylomicrons.** Nonfasted rabbits were tranquilized with xylazine at 7 mg/kg (Cutter) and anesthetized with ketamine at 80 mg/kg (Bristol), after which a femoral artery and vein were cannulated. A small epigastric incision was made to permit samples of liver to be obtained, and the incision was closed with surgical clips. One milliliter of the [^{3}H]cholesterol/[^{14}C]palmitate-labeled chylomicrons (containing 16 mg of triglycerides, 0.91 mg of cholesteryl ester, and 0.35 mg of free cholesterol) was injected into the venous catheter, followed by 1 ml of ^{125}I-labeled human serum albumin (2.4 μCi; 1 Ci = 3.7×10^{10} becquerels). Samples of blood (3 ml) were obtained from the femoral arterial cannula as indicated. The blood was mixed with 0.3 mg of disodium EDTA and immediately placed on ice. One-milliliter samples of blood plasma, obtained by centrifugation at 7,000 $\times g_{av}$·min, were layered beneath Krebs–Henseleit buffer and subjected to ultracentrifugation at plasma density for $10^8 \times g_{av}$·min at 12°C. Supernatant and infranatant fractions were recovered after tube-slicing, extracted, and subjected to TLC (22). The labeled spots containing [^{14}C]triglycerides, [^{3}H]cholesteryl esters, and [^{3}H]cholesterol were isolated and subjected to scintillation analysis. Immediately after the 15- and 30-min blood samples were obtained, pieces (≈200 mg) were cut from the edge of a superficial lobe of the liver and weighed, and the lipids were extracted into ethanol/acetone (1:1). Bleeding from the cut liver edge was arrested with Gelfoam (Upjohn). After the 60-min sample was taken, the portal vein was cannulated, the vena cava was incised, and the liver was perfused with 100 ml of ice-cold 0.15 M NaCl. The liver was weighed, and lipids were extracted from pieces taken from each lobe, as above. Lipids were also extracted from other tissues, separated, and quantified as above.

Membrane Binding of ^{125}I-Labeled Lipoproteins (^{125}I-Lipoproteins). Rabbit liver membranes (fraction sedimenting between 8,000 and 100,000 × *g*) were prepared as described (17). The standard binding assay (17) was conducted at pH 8 in 80 μl of buffer (20 mM NaCl/0.63 mM $CaCl_2$/50 mM Tris·HCl/bovine serum albumin, 20 mg/ml) containing 100 μg of membrane protein and the indicated amount of ^{125}I-lipoprotein in absence and presence of EDTA or excess unlabeled lipoprotein. Tubes were incubated for 100 min at 0°C, and membrane-bound ^{125}I-lipoprotein was measured after centrifugation at 100,000 × *g* (17). EDTA-sensitive binding was calculated by subtracting the amount of ^{125}I-lipoprotein bound in the presence of EDTA from that bound in the absence of EDTA.

Assays. Protein was determined by a modified Lowry procedure (23). Triglycerides were estimated by an automated procedure.

RESULTS

Normal and WHHL rabbits were injected with double-labeled rat chylomicrons containing [^{3}H]cholesterol (largely in the form of cholesteryl esters) and [^{14}C]palmitate (largely in the form of triglycerides). At various times after injection, blood samples were obtained, the plasma fraction of ρ <1.006 g/ml was isolated, and its content of labeled lipids was determined by extraction and TLC. In normal rabbits, the [^{3}H]cholesteryl esters and [^{14}C]triglycerides disappeared from the plasma fraction of ρ <1.006 g/ml rapidly and at comparable rates with an initial half-time of 15 min (Fig. 1*A*). In WHHL rabbits, the rates of removal of the two labeled lipids were also comparable but more rapid than in the normals, with an initial half-time of 8 min (Fig. 1*B*). In normal and WHHL rabbits, a portion of injected [^{3}H]cholesteryl esters was found in the plasma fraction of ρ >1.006 g/ml in the earliest sample taken (2 min after injection), amounting in normal rabbits to about 15% and in WHHL rabbits to 24% of that injected. Thereafter, in normal animals the amount of [^{3}H]cholesteryl esters in this fraction tended to re-

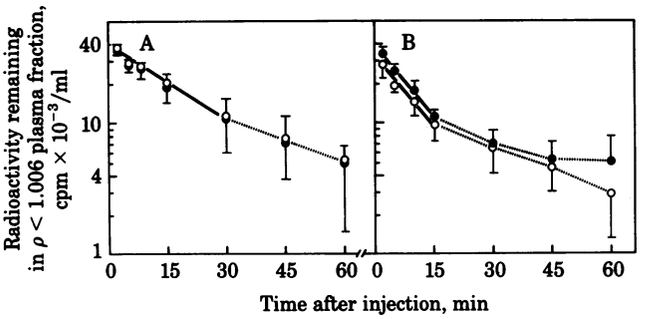

FIG. 1. Disappearance of [^{14}C]triglycerides (●) and [^{3}H]cholesteryl esters (○) from the plasma fraction of ρ <1.006 g/ml of four normal rabbits (*A*) and four WHHL rabbits (*B*) after intravenous injection of double-labeled rat lymph chylomicrons. Animals were injected as described in *Methods* and blood samples were obtained at the indicated times. The values (mean ± SD) have been normalized to those calculated for animals with a body weight of 2 kg and an injected dose of 4.1×10^6 cpm of each labeled lipid. This was done according to the following formula: normalized cpm/ml = observed cpm/ml × [body weight (kg)/2] × (4.1×10^6 cpm/cpm injected). The body weights (mean ± SD) for the normal and WHHL rabbits were 1.76 ± 0.28 and 1.79 ± 0.22 kg, respectively. The injected dose of [^{3}H]cholesteryl esters and [^{14}C]triglycerides was 4.1×10^6 and 5.5×10^6 cpm, respectively.

Table 1. Hepatic uptake of chylomicron [^{3}H]cholesterol in normal and WHHL rabbits

Component analyzed	Minutes after injection	Normal rabbits: cpm in total liver $\times 10^{-6}$	Normal rabbits: cpm cleared from plasma $\times 10^{-6}$	Normal rabbits: % hepatic uptake	WHHL rabbits: cpm in total liver $\times 10^{-6}$	WHHL rabbits: cpm cleared from plasma $\times 10^{-6}$	WHHL rabbits: % hepatic uptake
[^{3}H]Cholesteryl esters	15	0.79 ± 0.23	1.51 ± 0.21	52	1.36 ± 0.52	2.31 ± 0.55	59
	30	1.10 ± 0.18	2.26 ± 0.53	48	1.19 ± 0.19	2.45 ± 0.94	49
	60	1.42 ± 0.39	2.90 ± 0.21	49	1.32 ± 0.08	2.19 ± 0.08	60
Total [^{3}H]cholesterol	60	3.27 ± 0.49	3.58 ± 0.21	91	2.68 ± 0.37	2.62 ± 0.12	102

Animals were injected intravenously with double-labeled rat chylomicrons as described in *Methods* and in the legend to Fig. 1. At 15 and 30 min, liver biopsy specimens were obtained, and at 60 min the animals were killed and the livers were excised. The content of [^{3}H]cholesteryl esters and total [^{3}H]cholesterol was measured as described. All values were corrected for plasma ^{3}H radioactivity that was trapped within the liver as deduced from simultaneous measurements of ^{125}I-labeled albumin in plasma and liver. Values are means ± SD for four animals. Data are expressed as cpm in total liver and as percent hepatic uptake. The latter represents the hepatic content as a percentage of the total amount of labeled lipid that disappeared from unfractionated blood plasma during that interval. The liver weights (mean ± SD) for the normal and WHHL rabbits were 109 ± 40 and 66 ± 8 g, respectively.

main constant but in the WHHL rabbits it increased, and by 60 min it amounted to about 40% of the injected [^{3}H]cholesteryl esters. A smaller amount of the injected [^{14}C]triglycerides was found in the fraction of $\rho > 1.006$ g/ml 2 min after injection, amounting in normal and WHHL rabbits to about 7% of that injected. Thereafter, the amount decreased gradually.

Table 1 shows the hepatic content of [^{3}H]cholesteryl esters at 15, 30, and 60 min after injection and the total content of [^{3}H]cholesterol (free plus esterified) at 60 min. At 15 min, the [^{3}H]cholesteryl ester content of WHHL livers was higher than that of normals. In both strains, the [^{3}H]cholesteryl ester content of liver accounted for 52–59% of the [^{3}H]cholesteryl ester that had disappeared from blood. Thereafter, the content of [^{3}H]cholesteryl ester rose slowly in normal rabbits but remained essentially constant in WHHL rabbits. At 60 min, the liver contained comparable amounts of total [^{3}H]cholesterol in the normal and WHHL rabbits. In the normal rabbits, the hepatic content of total [^{3}H]cholesterol accounted for 91% of the total [^{3}H]cholesterol that had disappeared from plasma. In the WHHL rabbits, the corresponding value was 102%.

At 60 min after injection of the double-labeled chylomicrons, only trace amounts of [^{3}H]cholesteryl esters and free [^{3}H]-cholesterol were recovered in other viscera (kidney, spleen, lung, heart, and adipose tissue). The amount of [^{3}H]cholesteryl esters recovered in the adrenal gland was about 8-fold higher in normal than in WHHL rabbits, but even in normal animals it amounted to only about 0.01% of that injected. Recovery of injected [^{14}C]triglycerides in hepatic triglycerides was considerably less than that of [^{3}H]cholesteryl esters, amounting—in the samples taken 15 and 30 min after injection—to about 25% of the [^{14}C]triglycerides removed from the plasma (data not shown).

Normal and WHHL rabbits cleared rat ^{125}I-chylomicrons from the plasma at comparable rates (Fig. 2). The initial half-time for the disappearance of ^{125}I radioactivity from whole plasma was 20–25 min in both strains. To compare the hepatic uptake of ^{125}I-chylomicrons, the animals were killed after 15 min, the livers were perfused to remove excess blood, and samples of tissue were taken for measurement of ^{125}I radioactivity (2). In two WHHL rabbits, values of 15% and 21% of injected radioactivity were found in the liver. In two normal rabbits, the corresponding values were 13% and 17%. During this interval about 30% of the injected ^{125}I radioactivity had disappeared from plasma in both species (Fig. 2). Thus, about 50% of the ^{125}I radioactivity that disappeared from the plasma was found in the liver.

Fig. 3*A* shows that membranes from the livers of normal rabbits had high affinity binding of rat ^{125}I-chylomicron remnants when assayed under standard conditions previously shown to optimize binding of lipoproteins to hepatic LDL receptors (17). Binding was competitively reduced by excess unlabeled β-VLDL obtained from plasma of cholesterol-fed rabbits. Binding was also inhibited by EDTA. Both of these agents prevent ^{125}I-labeled LDL binding to the hepatic LDL receptor (1, 17). WHHL liver membranes, which lack LDL receptors (17), showed a marked reduction in high affinity binding of ^{125}I-chylomicron remnants (Fig. 3*B*). The small amount of residual binding was not inhibited by EDTA, but it was inhibited by β-VLDL. Fig. 3*C* compares the EDTA-sensitive binding in normal and WHHL rabbits and shows the marked difference between the two. We found that the EDTA-sensitive binding of rat ^{125}I-chylomicron remnants in normal liver was competitively inhibited by rabbit LDL but not by rabbit HDL_3 (experiments not shown). We observed no EDTA-sensitive binding of rat ^{125}I-chylomicrons to liver membranes from normal or WHHL rabbits.

Chylomicron remnants are believed to bind to hepatic LDL receptors on the basis of their content of apo-E (1, 2). ^{125}I-Labeled apo-E-HDL_c (^{125}I-apo-E-HDL_c), which contains no proteins except apo-E, bound with high affinity to liver membranes

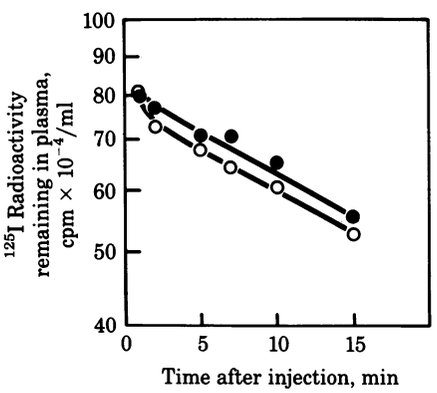

FIG. 2. Disappearance of rat ^{125}I-chylomicrons from the unfractionated plasma of two normal rabbits (○) and two WHHL rabbits (●). Rat ^{125}I-chylomicrons (544 μg of protein; 7×10^7 cpm) were injected into a marginal ear vein of each unanesthetized rabbit. At the indicated time after injection, blood was obtained from a cannula in the internal jugular vein. The values (average of two animals) have been normalized to those calculated for animals with a body weight of 2 kg. The average body weights for normal and WHHL rabbits were 1.73 and 2.14 kg, respectively.

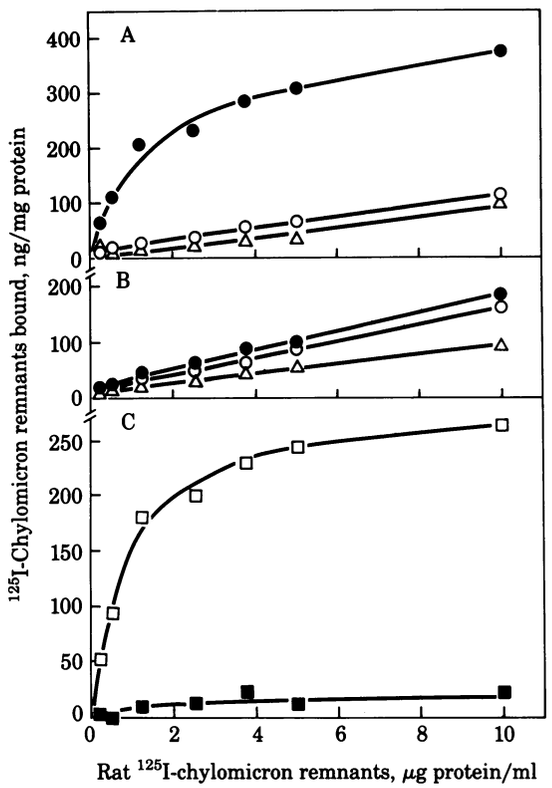

FIG. 3. Saturation curves for binding of rat ^{125}I-chylomicron remnants to liver membranes from normal (*A*) and WHHL (*B*) rabbits. Each tube contained the indicated concentration of rat ^{125}I-chylomicron remnants (74 cpm/ng of protein) in the absence (●) or presence of either 10 mM EDTA (○) or unlabeled rabbit β-VLDL at 300 μg of protein per ml (△). Values for the EDTA-sensitive binding site in normal (□) and WHHL (■) rabbits are shown in *C*.

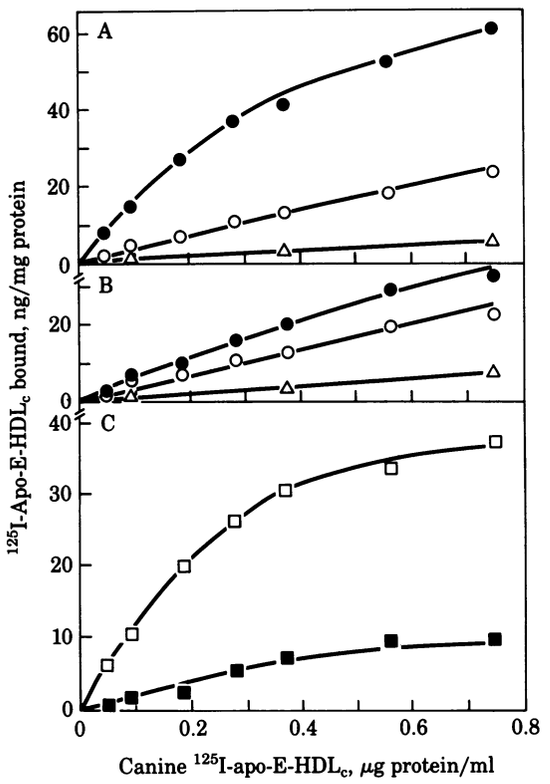

FIG. 4. Saturation curves for binding of canine ^{125}I-apo-E-HDL$_c$ to liver membranes from control (*A*) and WHHL (*B*) rabbits. Each tube contained the indicated concentration of ^{125}I-apo-E-HDL$_c$ (512 cpm/ng of protein) in the absence (●) or presence of either 7 mM EDTA (○) or unlabeled apo-E-HDL$_c$ at 233 μg of protein per ml (△). Values for EDTA-sensitive binding in normal (□) and WHHL (■) rabbits are shown in *C*.

from normal rabbits (Fig. 4*A*). Binding was inhibited by EDTA. Unlabeled apo-E-HDL$_c$ reduced the binding to a greater extent than EDTA, suggesting that the ^{125}I-apo-E-HDL$_c$ binds both to EDTA-sensitive and to EDTA-resistant sites. WHHL rabbits had some ability to bind the apo-E-HDL$_c$, and there was definite inhibition by EDTA (Fig. 4*B*). However, the amount of EDTA-sensitive binding was reduced by 80% when compared with the normal (Fig. 4*C*).§ The amount of EDTA-resistant binding was similar in the two strains.

DISCUSSION

The current results demonstrate that the livers of WHHL rabbits take up chylomicron remnants at a normal rate despite a marked deficiency of hepatic LDL receptors. Rapid hepatic uptake of chylomicrons was apparent when the animals were given chylomicrons that were labeled in the lipid portion with [^{3}H]cholesterol and [^{14}C]palmitate or in the protein portion with [^{125}I]iodine. The normal disappearance of chylomicrons from the plasma of WHHL rabbits stands in striking contrast to the markedly decreased rate of removal of LDL from the plasma of these mutant animals (18). Using a membrane binding assay previously shown to maximize lipoprotein binding to hepatic LDL receptors, we found that chylomicron remnant binding to liver membranes is markedly reduced in WHHL rabbits as compared with the normals. This finding suggests that the LDL receptor is responsible for most of the chylomicron remnant binding observed *in vitro*. This observation is consistent with earlier studies showing that chylomicron remnants bind to the LDL receptors of cultured human fibroblasts (24). Despite their lack of LDL receptors and the resultant decreased binding of chylomicron remnants *in vitro*, the livers of WHHL rabbits were nonetheless able to take up chylomicron remnants at a rapid rate *in vivo*.

The simplest explanation for the current data is that the rabbit liver normally has at least two genetically distinct lipoprotein uptake mechanisms. One of these is mediated by the LDL receptor, which binds chylomicron remnants and VLDL remnants (β-VLDL) as well as LDL. The other mechanism is specific for chylomicron remnants. Whereas the activity of the LDL receptor is maximized by the current *in vitro* binding assay, the activity of the putative chylomicron remnant receptor is not. In the WHHL rabbit the LDL receptor is deficient (17), accounting for the decreased binding of chylomicron remnants to liver membranes *in vitro*. However, the normal hepatic uptake of chylomicron remnants by WHHL rabbits *in vivo* indicates that these rabbits must have a normal chylomicron remnant uptake mechanism. It is likely that this uptake mechanism involves receptor-mediated endocytosis—like the LDL receptor-mediated mechanism—because the WHHL livers took up the ^{125}I-labeled protein component of the remnants as well as the lipid components.

Although rat chylomicrons were used in the current study, we believe that the findings are valid for rabbit chylomicrons. In a separate study we found that double-labeled chylomicrons

§ Similar observations have been made by D. Hui, T. L. Innerarity, and R. W. Mahley, who have found that liver membranes from WHHL rabbits have a near-complete deficiency in EDTA-sensitive, high affinity binding of rabbit ^{125}I-apo-E-HDL$_c$ at 0°C (personal communication).

from rabbit intestinal lymph, injected into two normal rabbits, were removed from the blood at rates similar to those observed with double-labeled rat lymph chylomicrons. In samples of normal liver taken 30 min after injection, 67% and 70% of the [^{3}H]cholesteryl esters removed from plasma were found in the liver (data not shown).

In WHHL rabbits injected with double-labeled rat chylomicrons, a substantial fraction (40%) of the [^{3}H]cholesteryl esters was found in the plasma fraction of $\rho > 1.006$ g/ml after 60 min, as compared to 15% in normal rabbits. This may be attributable to the high activity of cholesteryl ester transfer protein present in rabbit plasma (25) and the large pool of cholesteryl ester in the LDL fraction of the WHHL rabbit (18).

It is noteworthy that liver membranes from WHHL rabbits bound small amounts of chylomicron remnants to a site that was susceptible to competition by LDL and β-VLDL but was not inhibited by EDTA, which blocks binding to the LDL receptor. This EDTA-resistant binding (17) is difficult to evaluate because it was apparently of much lower affinity than binding to the LDL receptor—i.e., it did not show saturation within the concentration range of chylomicron remnants used in the current studies. In the rat, Cooper *et al.* have shown that hepatic binding of chylomicron remnants to an EDTA-resistant site is much higher at 37°C than at 4°C (14). We have not evaluated the binding of chylomicron remnants to rabbit liver membranes at 37°C. In contrast to the EDTA-sensitive site that is specific for lipoproteins containing apo-B and apo-E, binding of ^{125}I-lipoproteins to the EDTA-resistant site at 0°C was competitively inhibited by all lipoproteins tested, including HDL_3 and methylated LDL. The 8,000–100,000 × *g*-fraction of liver membranes was used in the current studies, but similar results were obtained when the whole homogenate or the 8,000 × *g*-pellet was used at 0°C (data not shown). Because there are no specific inhibitors of this EDTA-resistant binding and because there are no mutants in which this site is absent, it is not yet possible to determine whether this EDTA-resistant binding site functions as a remnant receptor *in vivo*.

It has been suggested that apo-E is the protein component of chylomicron remnants that is recognized by the remnant receptor (9–11). In normal rabbits this point is difficult to study because chylomicron remnants bind to LDL receptors as well as to remnant receptors. Binding of remnants to LDL receptors is mediated by the content of apo-E, which binds with high affinity to LDL receptors (1, 3). Whether apo-E also mediates binding to the rabbit chylomicron remnant receptor is not known. In the current studies, we found that the binding of ^{125}I-labeled canine apo-E-HDL_c to WHHL liver membranes was markedly decreased, consistent with the reduction in LDL receptors.§ However, both normal and WHHL membranes exhibited similar—though small—amounts of EDTA-resistant binding of apo-E-HDL_c. If the EDTA-resistant binding represents the chylomicron remnant receptor, these data would indicate that the remnant receptor recognizes apo-E. It should also be noted that the apo-B of chylomicrons differs from that of VLDL and LDL not only in rats (13) and humans (12) but also in rabbits (unpublished observations). It is possible that the chylomicron apo-B (so-called B-48) mediates binding to the remnant receptor.

Mahley and co-workers have shown that canine and human liver contain a specific apo-E binding site that is distinct from the LDL receptor (which binds both apo-B and apo-E) (3, 5). In their studies, the apo-E specific site was inhibited by EDTA—i.e., it was EDTA-sensitive. In the current studies, we found that the WHHL rabbit has a marked deficiency of EDTA-sensitive apo-E binding sites, suggesting that rabbits may not produce an EDTA-sensitive receptor other than the LDL receptor.§ As discussed above, if there is a separate apo-E receptor in these animals, it is possibly EDTA-resistant.

The current findings in WHHL rabbits have relevance to homozygous familial hypercholesterolemia, a human disease in which the LDL receptor is also genetically absent. Despite the absence of LDL receptors and the resultant massive accumulation of plasma LDL, these individuals do not appear to accumulate chylomicron remnants (7, 8). Thus, it seems that humans—like rabbits—must have a chylomicron remnant uptake mechanism that differs genetically from the LDL receptor and remains intact when the LDL receptor is genetically absent.

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1. Brown, M. S., Kovanen, P. T. & Goldstein, J. L. (1981) *Science* **212**, 628–635.
2. Kovanen, P. T., Brown, M. S., Basu, S. K., Bilheimer, D. W. & Goldstein, J. L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1396–1400.
3. Hui, D. Y., Innerarity, T. L. & Mahley, R. W. (1981) *J. Biol. Chem.* **256**, 5646–5655.
4. Kovanen, P. T., Bilheimer, D. W., Goldstein, J. L., Jaramillo, J. J. & Brown, M. S. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1194–1198.
5. Mahley, R. W., Hui, D. Y., Innerarity, T. L. & Weisgraber, K. H. (1981) *J. Clin. Invest.* **68**, 1197–1206.
6. 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.
7. Havel, R. J., Goldstein, J. L. & Brown, M. S. (1980) in *Metabolic Control and Disease*, eds. Bondy, P. K. & Rosenberg, L. E. (W. B. Saunders, Philadelphia), 8th Ed., pp. 393–494.
8. Brown, M. S., Luskey, K., Bohmfalk, H. A., Helgeson, J. & Goldstein, J. L. (1976) in *Lipoprotein Metabolism*, ed. Greten, H. (Springer, Berlin), pp. 82–89.
9. Havel, R. J., Chao, Y.-S., Windler, E. E., Kotite, L. & Guo, L. S. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4349–4353.
10. Sherrill, B. C., Innerarity, T. L. & Mahley, R. W. (1980) *J. Biol. Chem.* **255**, 1804–1807.
11. Windler, E., Chao, Y.-S. & Havel, R. J. (1980) *J. Biol. Chem.* **255**, 8303–8307.
12. Kane, J. P., Hardman, D. A. & Paulus, H. E. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2465–2469.
13. Krishnaiah, K. V., Walker, L. F., Borensztajn, J., Schonfeld, G. & Getz, G. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3806–3810.
14. Cooper, A. D., Erickson, S. K., Nutik, R. & Shrewsbury, M. A. (1982) *J. Lipid Res.* **23**, 42–52.
15. Watanabe, Y. (1980) *Atherosclerosis* **36**, 261–268.
16. Tanzawa, K., Shimada, Y., Kuroda, M., Tsujita, Y., Arai, M. & Watanabe, Y. (1980) *FEBS Lett.* **118**, 81–84.
17. Kita, T., Brown, M. S., Watanabe, Y. & Goldstein, J. L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2268–2272.
18. Bilheimer, D. W., Watanabe, Y. & Kita, T. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3305–3309.
19. Attie, A. D., Pittman, R. C., Watanabe, Y. & Steinberg, D. (1981) *J. Biol. Chem.* **256**, 9789–9792.
20. Windler, E., Chao, Y.-S. & Havel, R. J. (1980) *J. Biol. Chem.* **255**, 5475–5480.
21. Sigurdsson, G., Noel, S.-P. & Havel, R. J. (1978) *J. Lipid Res.* **19**, 638–644.
22. Fielding, C. J. (1979) *Biochim. Biophys. Acta* **573**, 255–265.
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
24. Florén, C.-H., Albers, J. J., Kudchodkar, B. J. & Bierman, E. L. (1981) *J. Biol. Chem.* **256**, 425–433.
25. Barter, P. J. & Lally, J. I. (1978) *Atherosclerosis* **31**, 355–364.