Regulation of the hepatic removal of chylomicron remnants and β-very low density lipoproteins in the rat

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Abstract The contribution of the low density lipoprotein (LDL) receptor to the removal of chylomicron remnants was determined in vitro and in vivo by using interventions that up- or down-regulate the LDL receptor but not the LDL receptor-related protein (LRP). In vitro, chylomicron remnants and β-very low density lipoprotein (VLDL) bind to the LDL receptor on endosomal membranes; their binding can be competed by LDL and β-VLDL and the binding capacity is greatly augmented in membranes from estradiol-treated rats. Likewise, estradiol treatment almost doubled the removal of chylomicron remnants during a single pass through perfused rat livers. However, in vivo the removal of chylomicron remnants and β-VLDL was very rapid even in untreated rats so that the effect of the stimulation by estradiol was barely detectable when trace amounts of lipoproteins were injected. Yet, when saturating doses of either lipoprotein were injected, the effect of estradiol treatment on the removal of chylomicron remnants and β-VLDL was readily disclosed. In rats fed a diet containing lard, cholesterol, and bile acids, removal of chylomicron remnants or β-VLDL was significantly retarded. Likewise, perfused livers from diet-fed rats removed only a mean of 16% of chylomicron remnants during a single passage as compared to 29% in livers from control animals. Also, when large doses of β-VLDL had been infused into rats for 4 h, in subsequent perfusions of the livers the removal of chylomicron remnants was decreased to 11%. From these results it is concluded that the LDL receptor mediates the hepatic removal of a major fraction of chylomicron remnants and β-VLDL.—**Jäckle, S., F. Rinninger, J. Greeve, H. Greten, and E. Windler.** Regulation of the hepatic removal of chylomicron remnants and β-very low density lipoproteins in the rat. *J. Lipid Res.* 1992. **33:** 419–429.

Supplementary key words chylomicron remnants • β-VLDL • LDL receptor • receptor-mediated endocytosis

The terminal catabolism of most plasma lipoproteins occurs chiefly in the liver by means of receptor-mediated endocytosis (1, 2). The low density lipoprotein (LDL) receptor mediates the removal of most apolipoprotein B-100-containing lipoproteins such as LDL and remnants of very low density lipoproteins (VLDL) from the blood (2, 3). It has been suggested that chylomicron remnants may be removed by a mechanism distinct from the LDL receptor. This is based on the following observations (2–4). First, the hepatic uptake of chylomicron remnants is apparently normal in patients with homozygous familial hypercholesterolemia and in Watanabe heritable hyperlipidemic (WHHL) rabbits, which express no LDL receptors or have a severe deficiency of LDL receptors (2, 5); second, interventions that affect the number of LDL receptors seem to have little effect on chylomicron remnant removal by the liver (6–11); third, antibodies against the hepatic LDL receptor have failed to inhibit binding of chylomicron remnants to liver membranes (12, 13).

However, a recent investigation demonstrated clear differences in the removal of chylomicron remnants between normal rabbits and heterozygous and homozygous WHHL rabbits (14). This is in line with many observations in vitro showing that chylomicron remnants and β-very low density lipoproteins (β-VLDL) bind to the hepatocytic LDL receptor (5, 15–17) and their uptake is mediated by the LDL receptor in mouse peritoneal macrophages (18, 19), human monocyte-derived macrophages (20), human skin fibroblasts (21), rat Fu5AH hepatoma cells (22), and cultured rat hepatocytes (13). Chylomicron remnant removal is regulated in cultured hepatocytes by interventions that change the number of LDL receptors, as HMG-CoA reductase inhibitors, mevalonic acid, estrogen, insulin, and lipoprotein-deficient serum (15, 23–27) and excess LDL compete for the uptake of chylomicron remnants in liver perfusions (16).

Abbreviations: β-VLDL, β-very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; WHHL rabbits, Watanabe heritable hyperlipidemic rabbits; LRP, LDL receptor-related protein.

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Thus, the contribution of the LDL receptor for the removal of chylomicron remnants is still controversial. In this study we demonstrate that interventions known to alter the number of LDL receptors affect the removal of chylomicron remnants in vivo. The rate of removal of chylomicron remnants and β-VLDL can be up-regulated, but also be down-regulated below physiological levels.

MATERIALS AND METHODS

Reagents

Asialofetuin and 17α-ethinyl estradiol were from Sigma (St. Louis, MO). [^{125}I]iodide (carrier-free) and [1,2-^{3}H]cholesterol were from Amersham-Buchler (Braunschweig, FRG). Chymotrypsin-activated ^{125}I-labeled α_2-macroglobulin was a generous gift from Dr. J. Gliemann, Århus, Denmark.

Animals

Male Sprague-Dawley rats (250–300 g) were treated with 17α-ethinyl estradiol (1 mg/ml propylene glycol) as described (27). In some experiments the animals received a chow diet supplemented with 2% cholesterol, 0.3% taurocholate, and 5% lard (w/w) (28) for 8 days. Male New Zealand White rabbits were maintained for 3–4 weeks on laboratory chow containing 1.5% cholesterol (w/w).

Lipoproteins

Human LDL ($1.025 < d < 1.050$ g/ml) were isolated from blood of normolipidemic adult humans by sequential ultracentrifugation (29) and purified by recentrifugation at the upper density limit. β-VLDL ($d < 1.006$ g/ml) were isolated from the blood of cholesterol-fed rabbits (30). Small chylomicron remnants were produced by a standard procedure (16). Lymph was collected from rats with an intestinal lymph fistula while 50 ml of 0.15 M NaCl containing 5 g glucose was infused into the duodenum for 25 h (31). Chylomicrons were isolated by centrifugal flotation at $3 \times 10^7 g_{av}$·min (32). Chylomicron remnants were produced in vitro by incubation of lymphatic chylomicrons with the $d > 1.019$ g/ml plasma fraction obtained after injection of heparin into rats (16). The protein and lipid composition of lymph chylomicrons and chylomicron remnants was similar to that reported previously (16). Serum of control and diet-fed rats was applied to density gradient centrifugation as described (33). Different plasma lipoproteins were separated by centrifugation of a KBr density gradient containing 1 ml H_2O, 3 ml KBr solution (d 1.019 g/ml), 3 ml KBr solution (d 1.063 g/ml) and 3 ml serum, adjusted to a density of 1.21 g/ml, for 24 h in a SW 40 Ti swinging bucket rotor.

Radiolabeling of ligands

LDL, β-VLDL, and asialofetuin were labeled with ^{125}I by the iodine monochloride method of McFarlane (34) to a specific activity of 200–300 cpm/ng protein of LDL and β-VLDL and 200–400 cpm/ng asialofetuin as described (35). More than 97% of radioactivity was precipitable in 10% trichloroacetic acid. Chylomicrons were labeled with [^{3}H]cholesterol as described (32). [1,2,-^{3}H]cholesterol (Amersham-Buchler) complexed to albumin was added to the intraduodenal infusion, resulting in a specific activity of 2–4 cpm/ng of cholesteryl esters in chylomicrons or their remnants.

Isolation of organelles

Endosomes were isolated from livers of estradiol-treated or normal rats exactly as described previously (36, 37). The purity of endosomal fractions was routinely checked by SDS-gel electrophoresis. Plasma membranes were prepared from liver homogenate of untreated rats (38).

Kinetics of lipoproteins and asialofetuin in blood plasma

To study the plasma removal of lipoproteins or asialofetuin in vivo, rats were anesthetized with diethyl ether and the radiolabeled ligands were injected into a femoral vein; blood samples were taken from the tail vein. In competition experiments unlabeled ligands were injected into a femoral vein 5–10 min before the radiolabeled ligand was given. The injected dose of tracer per estimated plasma volume was taken as 100%. The calculation was based on a volume of 4.5% of body weight.

Liver perfusion experiments

Removal of ^{3}H-labeled chylomicron remnants and activated α_2-macroglobulin by perfused livers was measured by a modified single pass technique (16, 32). Rats were anesthetized with ether and the abdomen was opened; ligations were put around the arteria hepatica and arteria mesenterica superior and around the vena cava inferior. The vena portae was cannulated and the liver was flushed with Krebs-Henseleit-buffer, pH 7.4, with a flow rate of 12 ml/min at 37°C (32). The ligations were closed and the vena cava superior was cannulated. After 5 min radiolabeled lipoproteins in 1 ml of buffer were injected into the cannula of the hepatic vein over 1 min and the effluent perfusate was collected from the vena cava superior for 5 min. Four perfusions with ^{125}I-labeled chylomicron remnants showed that the fraction of the

injected dose that was not taken up by the liver was collected in a single peak (7% of the eluted ^{125}I was collected within 1 min after injection, 54% within 2 min, 25% within 3 min, 7% within 4 min, 4% within 5 min, and 2% within 6 min). In some experiments 5 ml β-VLDL (3.5 mg protein/ml) was continuously infused into a femoral vein for 4 h before the liver perfusion was started.

Binding assays of lipoproteins

Binding of radiolabeled lipoproteins to rat liver endosomal membranes was quantified in a direct binding assay using 2–3 μg (estradiol-treated rats) or 10 μg (normal rats) endosomal membrane protein in a volume of 50 μl as described (17, 39). To study the effects of different lipoproteins on ligand binding, the membranes were preincubated with the unlabeled competitor for 30 min on ice before adding the radiolabeled ligand.

Statistical analysis

For the calculation of levels of significance for differences between experimental groups, a two-group, unpaired, one-tailed *t*-test was performed.

RESULTS

Competition of chylomicron remnants and various ligands in vivo

In vivo, chylomicron remnants are rapidly taken up by rat livers. Two min after intravenous injection of [^{3}H]cholesterol-labeled rat chylomicron remnants (30 μg protein) more than 50% was cleared from the circulation (**Fig. 1A**). Chylomicrons are poorly taken up into perfused rat livers (2), yet in vivo conversion of chylomicrons to chylomicron remnants occurs rapidly, resulting in a similar removal from plasma of chylomicrons as compared to their remnants (Fig. 1A).

To identify the mechanism involved in the removal of chylomicron remnants, competition experiments with various lipoproteins were performed in vivo. Intravenous injection of human LDL (22 mg protein) raised the plasma concentration of cholesterol about 6-fold to ca. 400 mg/dl. However, the rate of removal of ^{3}H-labeled chylomicron remnants injected 10 min later remained unchanged (Fig. 1B). Therefore, β-VLDL, isolated from cholesterol-fed rabbits, as an alternative lipoprotein containing apolipoprotein B-100 was tested, since β-VLDL has been shown to bind with much higher affinity to the LDL-receptor than LDL itself (17). Injection of β-VLDL (1.9 mg protein equivalent to 30 mg cholesteryl esters) raised the plasma cholesterol concentration 2.5-fold to ca. 200 mg/dl. Under these conditions the removal of ^{3}H-labeled chylomicron remnants from plasma was substantially retarded (Fig. 1C). After 30 min, 64% of ^{3}H-labeled chylomicron remnants still remained in plasma as compared to only 13% in control rats.

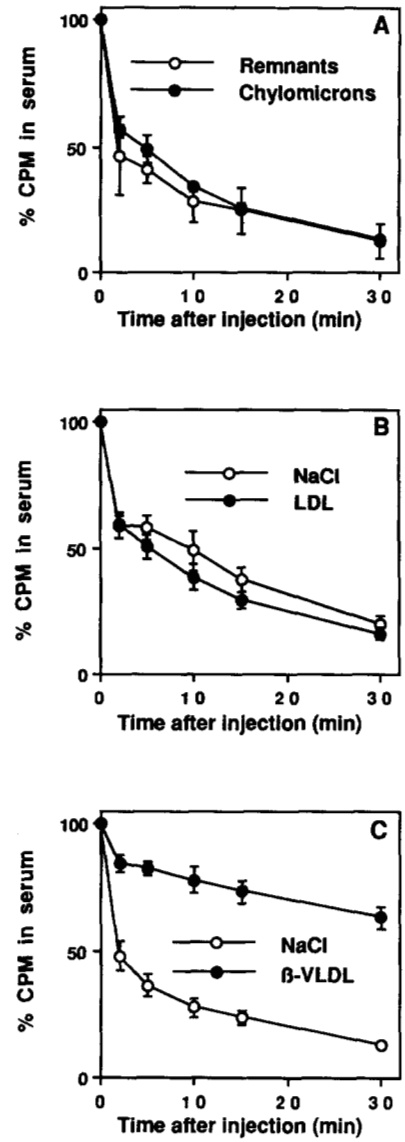

Fig. 1. In vivo competition of chylomicron remnants, β-VLDL, and LDL in rats. (A) Blood samples were drawn from the tail vein after intrafemoral injection of ^{3}H-labeled rat chylomicron remnants (30 μg protein) or ^{3}H-labeled rat chylomicrons (70 μg protein, which is a comparable dose of cholestryl esters/rat). (B) Human LDL (22 mg protein) or the same volume of 0.15 M NaCl were injected intrafemorally followed 10 min later by ^{3}H-labeled rat chylomicron remnants (140 μg protein), and blood samples were drawn from the tail vein. (C) Rabbit β-VLDL (1.92 mg protein) or the same volume of 0.15 M NaCl was injected intrafemorally followed 10 min later by ^{3}H-labeled rat chylomicron remnants (140 μg protein), and blood samples were drawn from the tail vein. Values are given as percent of the injected ^{3}H remaining in blood plasma. Each point represents the mean of three experiments; bars indicate 1 SD.

Evidence from findings in HepG2 cells and human liver membranes suggests a contribution of the asialoglycoprotein receptor for chylomicron remnant

removal in man (26). Therefore, competition experiments with chylomicron remnants, β-VLDL, and asialofetuin, a ligand of the asialoglycoprotein receptor, were performed in the rat. Asialofetuin was rapidly cleared from the circulation by a saturable mechanism (**Fig. 2A**) and accumulated mostly in the liver. Two min after intrafemoral injection of 40 μg ^{125}I-labeled asialofetuin, 57.4 ± 6.4% (n = 3) of the radioactivity was found in the liver, 0.5 ± 0.1% in the spleen, 1.6 ± 0.1% in the kidney, 0.1 ± 0.1% in the heart, and 0.4 ± 0.1% in the lungs. Saturating dosages of β-VLDL (1.9 mg protein/rat), injected 10 min before ^{125}I-labeled asialofetuin was administered, did not inhibit the removal of asialofetuin (Fig. 2B), and saturating dosages of unlabeled asialofetuin (150 mg/rat) did not compete for the uptake of ^{3}H-labeled chylomicron remnants (Fig. 2C).

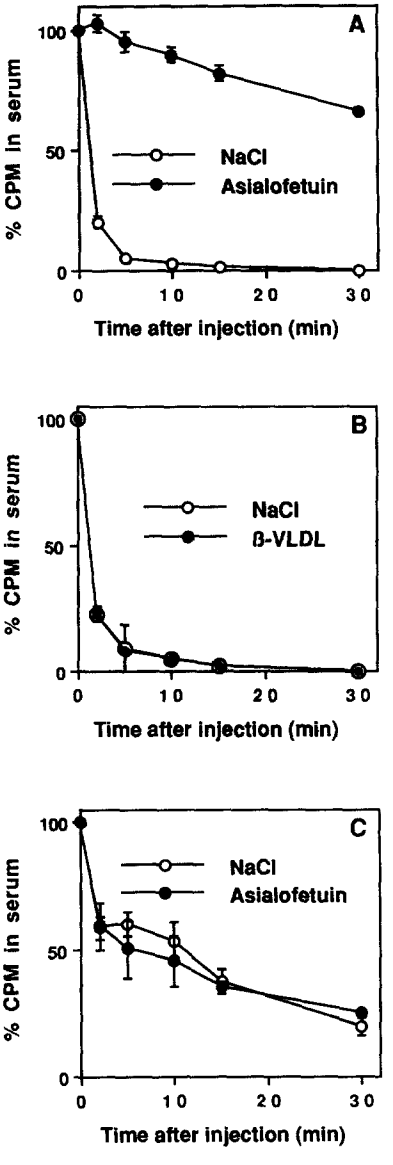

Fig. 2. In vivo competition of chylomicron remnants, β-VLDL, and asialofetuin in rats. (A) 150 mg unlabeled asialofetuin was intrafemorally injected into normal rats followed by 20 μg ^{125}I-labeled asialofetuin and blood samples were drawn from the tail vein. (B) β-VLDL (1.9 mg protein) was injected followed by 20 μg ^{125}I-labeled asialofetuin. (C) Injection of 150 mg unlabeled asialofetuin was followed by ^{3}H-labeled chylomicron remnants (144 μg protein). In control rats a volume of 0.15 M NaCl equivalent to that of the unlabeled ligand was injected. Values are given as percent of the injected ^{3}H or ^{125}I remaining in blood plasma. Each point represents the mean of three experiments; bars indicate 1 SD.

Up-regulation of the rate of removal of chylomicron remnants and β-VLDL by estradiol

To examine the LDL receptor as a removal mechanism for chylomicron remnants, the effect of interventions that change the number of hepatic LDL receptors was investigated in rats. Three days of treatment with 17α-ethinyl-estradiol increased the rate of removal of LDL from plasma (**Fig. 3A**), as demonstrated previously (27). Small quantities of β-VLDL (20 μg protein) were cleared from the circulation of untreated rats very rapidly, and estradiol treatment failed to further stimulate the removal (Fig. 3B). However, after injection of high dosages of β-VLDL (1.7 mg protein), the removal of β-VLDL in normal rats was saturated; under these conditions previous estradiol treatment enhanced the removal of β-VLDL substantially (Fig. 3C). The removal of ^{3}H-labeled chylomicron remnants (250 μg protein) was slower as compared to β-VLDL (20 μg protein) and a slight effect of estradiol treatment was detectable (Fig. 3D). Yet, the effect of estradiol became even more apparent after saturating the removal of chylomicron remnants by simultaneous injection of high amounts of unlabeled β-VLDL (1 mg protein) (Fig. 3E).

The enhanced hepatic uptake of remnants could be demonstrated directly using perfused livers. During a single pass perfusion the removal of ^{3}H-labeled chylomicron remnants by livers from estradiol-treated rats was almost doubled as compared to livers from control rats (see Fig. 7). To exclude a nonspecific stimulation of receptor-mediated endocytosis in the liver by estradiol, the plasma decay of low and high doses of asialofetuin, a ligand of the asialoglycoprotein receptor, was studied. The plasma turnover of both, 13 μg and 150 mg asialofetuin, was unchanged in estradiol-treated rats (data not shown).

The binding capacity for LDL, β-VLDL, and chylomicron remnants was greatly increased on hepatic membranes from estradiol-treated rats (**Fig. 4A–C**) (17, 28, 40). Evidently, the number of binding sites for all three ligands is stimulated by estradiol to a similar extent[2]. Binding of chylomicron remnants to endosomal membranes from normal rats can be inhibited efficiently by increasing concentrations of un-

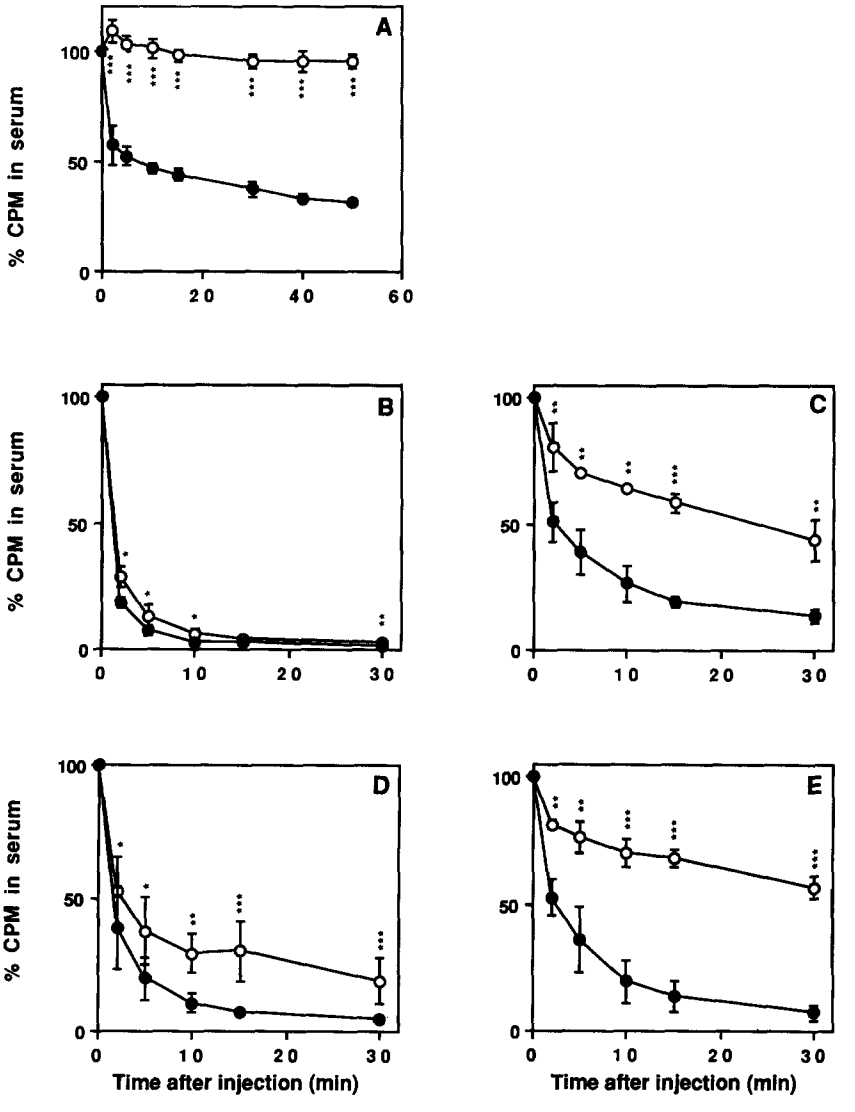

Fig. 3. Regulation of the removal of LDL, chylomicron remnants, and β-VLDL in rats by estradiol. Rats were treated with 17-α-ethinyl estradiol (5 mg/kg body weight) for 3 days. Blood was drawn from the tail veins of untreated (○) or estradiol-treated (●) rats after intrafemoral injection of (A) human ^{125}I-labeled LDL (20 μg protein), (B) rabbit ^{125}I-labeled β-VLDL (20 μg protein), (C) rabbit ^{125}I-labeled β-VLDL (1.65 mg protein), (D) rat ^{3}H-labeled chylomicron remnants (250 μg protein), and (E) rat ^{3}H-labeled chylomicron remnants (114 μg protein) plus rabbit β-VLDL (1 mg protein). Values are given as percent of the injected tracer remaining in plasma. Each point represents the mean of three (A, B, C, E), or six (D) experiments; bars indicate 1 SD. Levels of significance: $*P < 0.1$; $**P < 0.01$; $***P < 0.001$.

labeled β-VLDL or human LDL (**Fig. 5A and B**). However, β-VLDL competes much more efficiently for the binding of chylomicron remnants than LDL (Fig. 5A and B). Similar observations were made using isolated plasma membranes (data not shown).

[2]Binding assays with β-VLDL and chylomicron remnants on endosomal membranes of estradiol-treated rats show a certain kind of curvilinearity in Scatchard plots (17); curvilinearity in Scatchard plots can be caused by ligand heterogeneity, and it is more likely to be detected when the concentration of receptor is high relative to the K_d of the higher affinity ligand (62). The number of receptors cannot be calculated from this kind of curvilinear Scatchard plot.

Down-regulation of the rate of removal of chylomicron remnants and β-VLDL by dietary intervention

The hepatic LDL receptor can be down-regulated by feeding rats a diet rich in saturated fatty acids, cholesterol, and taurocholate for 8 days (28). The concentration of cholesteryl esters in the liver and the concentration of plasma cholesterol was substantially increased in diet-fed rats (**Table 1**), and the removal of chylomicron remnants from plasma was significantly delayed (**Fig. 6A**).

The increased hepatocytic cholesterol content may down-regulate the number of LDL receptors and addi-

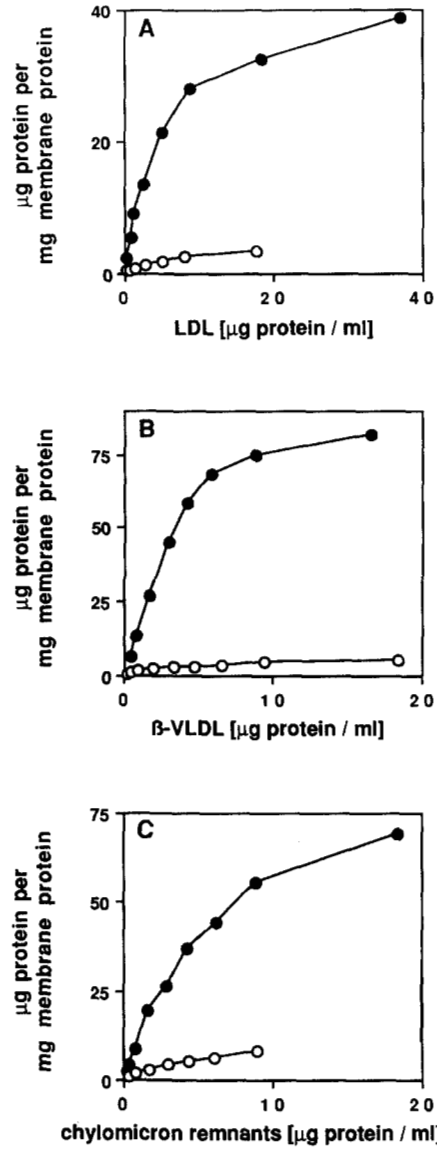

Fig. 4. Binding of lipoproteins to endosomal membranes from livers of normal and estradiol-treated rats. Specific binding is given as the difference of total binding and binding in the presence of 50-fold excess unlabeled ligand. Each tube (50 μl) contained 3 μg of endosomal membrane protein for estradiol-treated rats (●), or 10 μg for untreated rats (○), and the indicated concentrations of ^{125}I-labeled LDL (A), ^{125}I-labeled β-VLDL (B), or ^{125}I-labeled chylomicron remnants (C). Each point represents the mean of duplicate assays.

tionally stimulate the synthesis of triglyceride-rich lipoproteins. In accordance with this, plasma of diet-fed rats contained substantially higher concentrations of cholesterol and triglycerides (Table 1), by an increase of lipoproteins of $d < 1.019$ g/ml (data not shown). These lipoproteins may compete with the injected radiolabeled chylomicron remnants. Both mechanisms could have contributed to the delay of the chylomicron remnant removal. Therefore, lipoproteins ($d <$ 1.20 g/ml) isolated from two diet-fed rats were injected into normal rats followed by ^{3}H-labeled chylomicron remnants 5 min later. Plasma cholesterol and triglycerides increased to the same level as in diet-fed rats (Table 1). Removal of ^{3}H-labeled chylomicron remnants was somewhat retarded in these rats as compared to control rats, into which lipoproteins of rats fed a regular diet had been injected; however, the delay was less pronounced than that in diet-fed rats themselves (Fig. 6A and B). Analogous to chylomicron remnants, the plasma decay of β-VLDL was delayed in diet-fed rats (Fig. 6C).

To examine the contribution of down-regulation of the LDL receptor in diet fed rats independent of elevated concentrations of endogenous lipoproteins, chylomicron remnant removal was studied in single-pass liver perfusions. In livers of diet-fed rats uptake of chylomicron remnants was reduced to 54% of that found in rats fed a regular diet (**Fig. 7**). Similarly, a marked decrease to 37% in the removal of chylomicron remnants by perfused livers was achieved when, for 4 h, a high dose of unlabeled β-VLDL (14 mg protein) had been intravenously infused into rats before their livers were perfused (Fig. 7).

To rule out a contribution of LRP to the variation of the removal of chylomicron remnants in estradiol-treated or diet fed rats, the hepatic uptake of activated α_2-macroglobulin was measured. Treatment of rats with estradiol had no effect on the removal of activated α_2-macroglobulin by perfused livers, and feeding the diet rich in lard, cholesterol, and taurocholate even led to a somewhat increased uptake (**Fig. 8**). Thus, the hepatic removal of chylomicron remnants appears to be influenced by the activity of the LDL receptor, but not of LRP.

DISCUSSION

This investigation clearly demonstrates that in the rat the removal of chylomicron remnants can be up- and down-regulated in vivo by interventions that regulate the LDL receptors but not LRP or the asialoglycoprotein receptor. This has been questioned, since previous studies in experimental animals failed to show such an effect. Observations in patients and animals deficient in the LDL receptor led to the conclusion that the removal of LDL and VLDL is impaired, while that of chylomicron remnants may be independent of the LDL receptor activity. The results of this paper, however, contradict this view. For the first time these data provide strong evidence that the LDL receptor mediates the hepatic removal of at least a major fraction of chylomicron remnants in vivo under physiological circumstances.

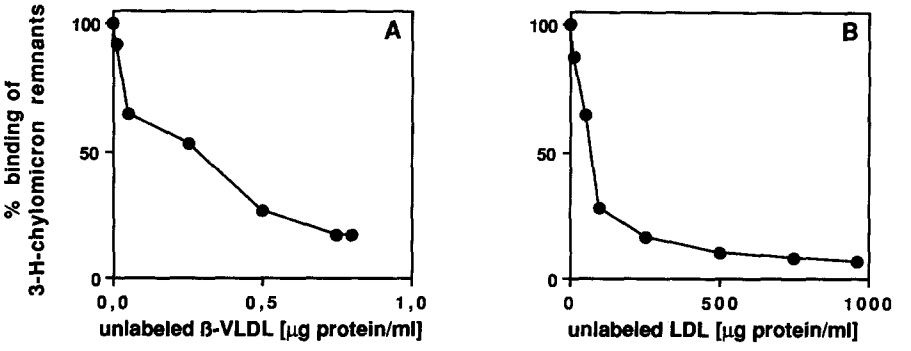

Fig. 5. Competition of chylomicron remnants with β-VLDL or LDL for the binding to rat hepatic endosomal membranes. Each tube (50 µl) contained ^{3}H-labeled chylomicron remnants (20 µg protein/ml), 10 µg of endosomal membrane protein, and the indicated concentrations of unlabeled β-VLDL (A) or unlabeled LDL (B). Each point represents the mean of duplicate assays.

Small chylomicron remnants, produced from lymphatic rat chylomicrons, are composed chiefly of triglycerides (about 73% of the particle mass) (31); the apoB of these particles is exclusively B-48 (41). In contrast, the core of β-VLDL, isolated from blood of cholesterol-fed rabbits, is composed mainly of cholesteryl esters (about 65% of particle mass) (42), and the apoB is mainly B-100 with little B-48 (1). Beyond these differences both lipoproteins resemble one another in their high content of apoE, which has been shown to mediate their rapid uptake by the liver (2, 4, 43, 44). Despite the structural differences, the competition experiments in vivo and in vitro of this investigation indicate that both lipoproteins are taken up into rat livers by the same receptor.

Several receptors have been proposed to interact with remnants. The LDL receptor in hepatocytes is regulated by the status of cellular cholesterol (2, 3). Diet rich in cholesterol and saturated fats tend to reduce the number of LDL receptors (45), while pharmacological doses of 17α-ethinyl estradiol increase the number of LDL receptors (27, 39, 40, 46, 47). The LDL receptor-related protein (LRP), which was shown to have sequence identity with the α_2-macroglobulin receptor (48, 49), was proposed to mediate the uptake of apoE-containing lipoproteins (50–52). Yet, unlike the LDL receptor, the amount of LRP is not regulated by estradiol treatment or sterols. The concentration of LRP was unchanged after treatment with estradiol as demonstrated in ligand blots on endosomal membranes of normal and estradiol-treated rats using chylomicron remnants, β-VLDL, LDL, and ^{45}Ca (53). In fibroblasts from normal subjects and patients with familial hypercholesterolemia, the synthesis of LRP was unimpaired by treatment with high concentrations of cholesterol and 25-hydroxycholesterol (51). In line with these results, the removal of activated α_2-macroglobulin by perfused rat livers was unimpaired by treatment of rats with estradiol or a diet containing cholesterol, lard, and taurocholate. The number of asialoglycoprotein receptors, which may also contribute to chylomicron remnant removal (26), was also unchanged by estradiol treatment.

To examine the role of the LDL receptor in the hepatic removal of chylomicron remnants and β-VLDL in vivo, turnover studies were performed in rats after interventions that changed the number of hepatocytic LDL receptors, but not of LRP or the asialoglyco-

TABLE 1. Cholesterol and triglyceride concentrations in plasma and liver

	Control (n = 6)	Estradiol (n = 3)	Diet (n = 9)	Lipoproteins (n = 3)
Plasma				
Cholesterol (mg/dl)	65.90 ± 12.90	18.60 ± 2.20	92.30 ± 10.60	119.80 ± 8.00
Triglycerides (mg/dl)	54.70 ± 19.10	43.20 ± 30.30	129.30 ± 77.60	127.20 ± 28.30
Liver				
Cholesterol (mg/g liver ww)	3.04 ± 0.78	5.75 ± 0.49	10.59 ± 3.80	
Triglycerides (mg/g liver ww)	11.97 ± 0.60	10.96 ± 0.55	32.74 ± 7.90	

Blood was drawn and livers were removed from normal rats (Control), rats treated for 3 days with estradiol (Estradiol), rats fed a diet containing cholesterol, lard, and bile acids for 8 days (Diet), and from normal rats 10 min after intravenous injection of the lipoprotein fraction (d < 1.20 g/ml) of two diet-fed rats (Lipoproteins); ww, wet weight.

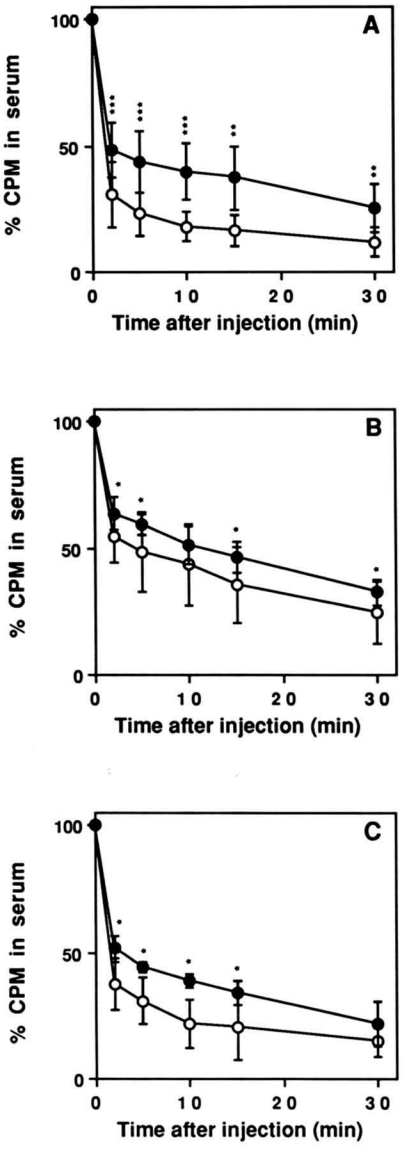

Fig. 6. Regulation of the removal of chylomicron remnants and β-VLDL by dietary intervention in normal rats. Rats were fed a diet containing 2% cholesterol, 0.3% taurocholate, and 5% lard for 8 days; control rats received regular laboratory chow. (A) Blood samples were taken from the tail vein after intrafemoral injection of rat ^{3}H-labeled chylomicron remnants (250 μg protein) into control (○) or diet-fed (●) rats. (B) Lipoproteins (d < 1.20 g/ml) were isolated from two diet-fed rats and intrafemorally injected into a normal rat followed 10 min later by ^{3}H-labeled chylomicron remnants (250 μg protein) (●); In control rats a similar volume of serum from untreated rats was injected (○). (C) Plasma removal of ^{125}I-labeled β-VLDL (10 μg protein) in control (○) and diet-fed (●) rats. Values are given as percent of the injected ^{3}H or ^{125}I remaining in plasma. Each point represents the mean of nine (A), six (B), or three (C) experiments; bars indicate 1 SD. Levels of significance: *P < 0.1; **P < 0.01; ***P < 0.001.

protein receptor. When only trace amounts of β-VLDL or chylomicron remnants are injected, receptor affinity and hemodynamic parameters are the rate-limiting factors for the removal, but not receptor number. However, the number of receptors becomes rate-limiting when higher concentrations of chylomicron remnants or β-VLDL are used, which saturate the binding capacity. Under these conditions a substantial increase in removal could be demonstrated as a result of the estradiol treatment. The removal of chylomicron remnants is also highly stimulated in isolated perfused livers of estradiol-treated rats. For the first time these data demonstrate unambiguously that the hepatic removal of chylomicron remnants can be augmented in vivo by estradiol treatment. Conflicting data concerning the regulation of the removal of chylomicron remnants, β-VLDL, or apoE-containing HDL in vivo may be due to differences in species, in ligands or, most likely, in ligand concentrations (4–7, 10, 11, 27, 54–57).

Binding of chylomicron remnants and β-VLDL to plasma or endosomal membranes can be stimulated by estradiol treatment and can be efficiently competed by excess human LDL or β-VLDL. These results agree with our experiments using HepG2 cells (26) and with observations of several investigators, who found binding of chylomicron remnants to the LDL receptor in vitro (5, 12, 13, 15–17). The in vitro findings support the interpretation of the results obtained in vivo and with perfused livers.

In vivo the removal of chylomicron remnants was substantially delayed in rats fed a diet rich in cholester-

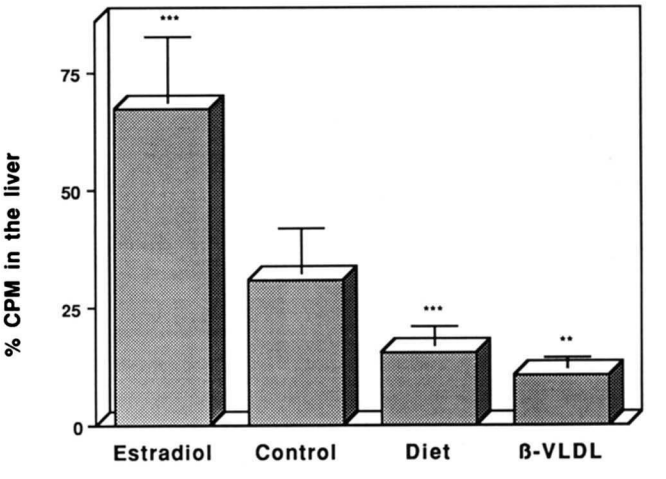

Fig. 7. Removal of chylomicron remnants by perfused livers from normal, estradiol-treated, diet-fed, and β-VLDL-infused rats. Rats were either treated with ethinyl-estradiol for 3 days (Estradiol), fed a diet containing 2% cholesterol, 0.3% taurocholate, and 5% lard for 8 days (Diet), or were continuously infused with 5 ml β-VLDL (3.5 mg protein/ml) into a femoral vein for 4 h before the liver perfusion was started (β-VLDL). Rat ^{3}H-labeled chylomicron remnants (136–275 μg protein) in 1 ml 0.15 M NaCl were injected into the perfusion buffer over 1 min. After 4 additional min of single pass perfusion, livers were homogenized and the removed radiolabel was determined. Each column represents the mean of three (Estradiol), twelve (Control), nine (Diet), or two (β-VLDL) experiments; bars indicate 1 SD. Levels of significance: *P < 0.1; **P < 0.01; ***P < 0.001.

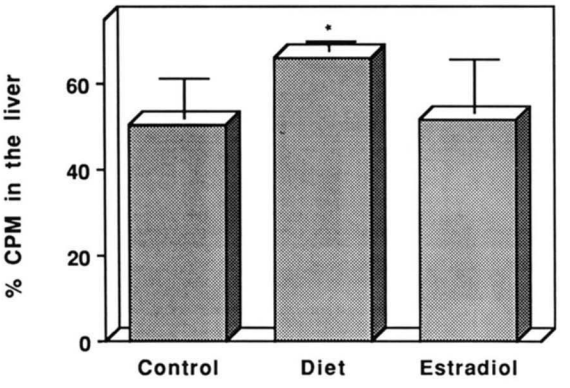

Fig. 8. Removal of α_2-macroglobulin by perfused livers from normal, estradiol-treated, and diet-fed rats. Rats were either treated with ethinyl-estradiol for 3 days (Estradiol) or fed a diet containing 2% cholesterol, 0.3% taurocholate, and 5% lard for 8 days (Diet). Activated ^{125}I-labeled α_2-macroglobulin (10 μg protein) in 1 ml 0.15 M NaCl were injected into the perfusion buffer over 1 min. After 4 additional min of single pass perfusion, livers were homogenized and the removed radiolabel was determined. Each column represents the mean of three experiments; bars indicate 1 SD. Levels of significance versus control: $*P < 0.1$; $**P < 0.01$; $***P < 0.001$.

ol, saturated fats, and bile acids. This diet was previously shown to down-regulate the LDL receptor of estradiol-treated rats (28). Unlike humans and male hamsters, rats respond to dietary cholesterol or saturated fats primarily with down-regulation of sterol synthesis, but not of LDL receptors (58, 59). Thus, the cholesterol content of the liver increased dramatically only after supplementation of the diet with 0.3% taurocholate, and chylomicron remnant removal was greatly retarded in vivo. Moreover, the removal of chylomicron remnants was also greatly decreased in perfused livers supporting the interpretation that down-regulation of LDL receptors is primarily responsible for the retarded remnant catabolism, rather than increased production of competing lipoproteins. Not only these dietary interventions but also previous infusion of large amounts of β-VLDL into rats led to a substantial reduction of chylomicron remnant removal by perfused livers. In normal rats the LDL receptor evidently largely contributes to the removal of both apoB-100-containing LDL and β-VLDL, and B-48-containing chylomicron remnants.

Chylomicron remnant uptake in perfused livers was reduced by the diet to 54% and by previous β-VLDL infusion to 37%. These data may reflect a minimal estimate of the contribution of the LDL receptor, though other mechanisms may be operative in the removal of the residual fraction. Distribution into the space of Disse, but also attachment to components of the sinusoidal surface as heparan sulfate or hepatic lipase, may contribute. LRP or the asialoglycoprotein receptor may also be involved in this removal and may become more important in situations in which the LDL receptor is down-regulated or missing. Cross-competition experiments with α_2-macroglobulin and remnants have demonstrated incomplete (60) or almost no competition (S. Jäckle, S. Moestrup, J. Gliemann, and U. Beidiegel, unpublished data, and T. J. van Berkel, personal communication). There was also no cross-competition of remnants and asialofetuin in the rat in vivo and in liver perfusions (61). Yet, the negative cross-competition experiments do not exclude binding to one of these receptors, since these ligands may bind to different binding sites on the same receptor molecule. Further in vivo investigations are required using antibodies that block specifically the lipoprotein-binding sites of the LDL receptor, LRP, or the asialoglycoprotein receptor.

In conclusion, up-regulation of the LDL receptor by estradiol treatment demonstrates that the LDL receptor has the ability to mediate remnant removal. Thus, pharmacological stimulation of the LDL receptor may influence postprandial hyperlipidemia. However, beyond this, the effect of down-regulation of LDL receptors in normal rats clearly underlines the predominant role of the LDL receptor in the removal of chylomicron remnants under physiological conditions.

We thank Birgit Biermann, Ulrike Christ, and Andrea Krah for excellent technical assistance. We are grateful to Dr. J. Gliemann, Århus, for providing us with chymotrypsin-activated ^{125}I-labeled α_2-macroglobulin. This work was supported by the Deutsche Forschungsgemeinschaft (grants Ja-421/2-1, R-436/4-1, and Sonderforschungsbereich 232, Project C2).

Manuscript received 25 September 1991 and in revised form 26 November 1991.

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