review

Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E

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Abstract **The plasma clearance of intestinally derived remnant lipoproteins by the liver is a process that likely involves three steps. Our model suggests that the initial rapid clearance by the liver begins with sequestration of the remnants within the space of Disse, where apolipoprotein E secreted by hepatocytes enhances remnant binding and uptake. Heparan sulfate proteoglycans (HSPG), which are also abundant in the space of Disse, mediate this enhanced binding. Next, the remnants undergo further processing in the space of Disse by hepatic and lipoprotein lipases, which may also serve as ligands mediating remnant uptake. The final step, endocytosis by hepatocytes, appears to be mediated, at least in part, by the low density lipoprotein (LDL) receptor and by the LDL receptor-related protein (LRP). Cell-surface HSPG play a critical role in remnant uptake, not only in the important initial sequestration or capture step in the space of Disse, but also as an essential or integral component of the HSPG-LRP pathway. In addition, HSPG appear to function alone as a receptor and display unique handling properties for specific isoforms of apolipoprotein E.—**Mahley, R. W., and Z-S. Ji. **Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E.** *J. Lipid Res.* **1999.** 40: **1–16.**

Supplementary key words remnants • apolipoprotein E • hepatic lipase • heparan sulfate proteoglycans • lipoprotein lipase

Remnant lipoproteins are cholesterol-enriched particles derived from the lipolytic processing of intestinal chylomicrons and hepatic very low density lipoproteins (VLDL). Chylomicrons (d $<$ 0.95 g/ml) are synthesized by the small intestine to transport dietary triglyceride and cholesterol from the site of absorption by the intestinal epithelium to various cells of the body. In the circulation, the triglycerides in these particles are hydrolyzed by lipoprotein lipase (LPL), an enzyme found on the endothelial surfaces of capillaries, especially in adipose tissue, the heart, and skeletal muscle, resulting in the formation of chylomicron remnants. Relatively enriched in cholesterol by the loss of triglyceride, these remnants are normally cleared rapidly and efficiently from the plasma by the liver, through a process mediated primarily by apolipoprotein (apo) E. However, in animals whose diets are high in fat and cholesterol and in patients with type III hyperlipoproteinemia, chylomicron remnants accumulate in the plasma and have been linked to the development of accelerated atherosclerosis (for review, see refs. 1–3).

Very low density lipoproteins (d $<$ 1.006 g/ml) are synthesized in the liver and, like chylomicrons, fulfill a lipid transport function. The triglycerides in these particles are hydrolyzed by LPL in the plasma and in the liver, generating small, cholesterol-enriched lipoproteins known as intermediate density lipoproteins (IDL; d 1.006–1.019 g/ml) and low density lipoproteins (LDL; d 1.019–1.063 g/ml) (**Fig. 1**). Smaller VLDL and IDL are referred to as VLDL remnants and are atherogenic lipoproteins (1–3). VLDL remnants and chylomicron remnants are collectively called β-VLDL. About half of the VLDL remnants are cleared directly by the liver through an apoE-mediated process. The remainder are converted to LDL containing only apoB-100, the end product of VLDL catabolism and the major cholesterol-transporting lipoprotein in the plasma.

By the early 1980s, Goldstein and Brown's classic studies of the LDL receptor pathway had shown that LDL bound via apoB-100 and were internalized and degraded primarily by the liver (4). Remnant lipoproteins also bound to the LDL receptor via apoE, the other ligand for the LDL receptor (Fig. 1) (5). However, it became obvious that remnant metabolism differed from LDL metabolism. In

Abbreviations: apo, apolipoprotein; CHO, Chinese hamster ovary; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine; HL, hepatic lipase; HL-CAT$^-$, catalytically inactive HL; HL-LRP$^-$, LRP binding-defective HL; HSPG, heparan sulfate proteoglycans; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; LPL, lipoprotein lipase; LRP, LDL receptor-related protein; VLDL, very low density lipoproteins; RAP, receptor-associated protein; FGF, basic fibroblast growth factor.

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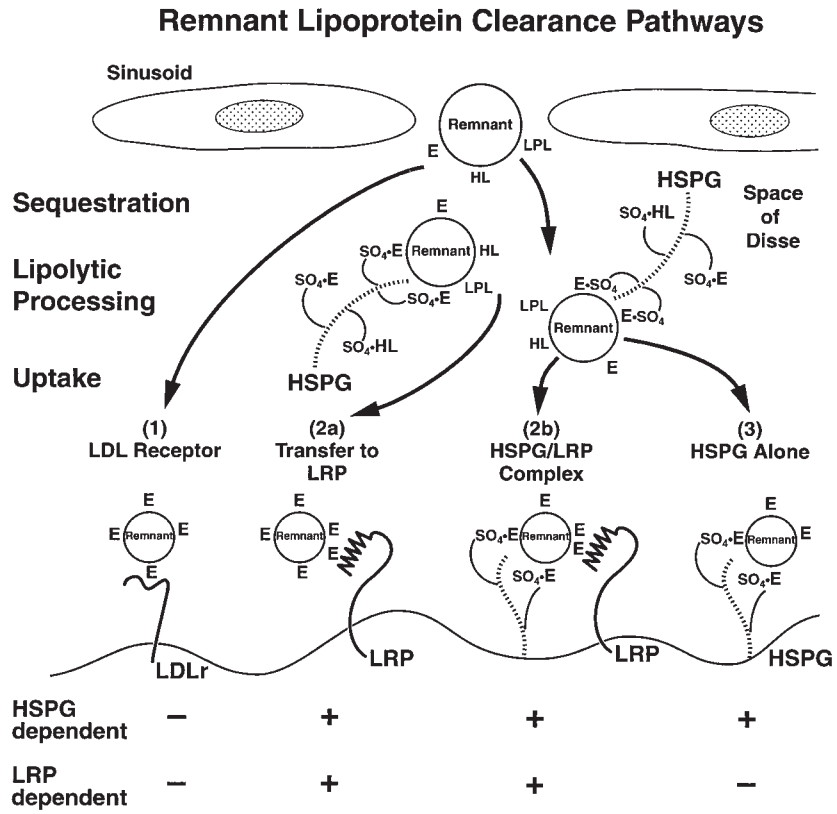

Fig. 1. Summary of remnant lipoprotein uptake by the LDL receptor, HSPG-LRP pathway, and HSPG alone. HSPG are abundant in the space of Disse and on the surface of hepatocytes. Apolipoprotein E (E) and HL, secreted by the hepatocytes, appear to bind to the HSPG and be available to enrich the remnant lipoproteins. The HSPG/apoE appear to fulfill a critical role in the sequestration or capture of the remnants. Three major pathways for internalization are illustrated: *1*) direct uptake by LDL receptor; *2*) HSPG-LRP pathway: (a) remnants transferred to LRP for uptake or (b) HSPG-LRP complex internalized; and *3*) HSPG alone mediating direct uptake.

patients with familial hypercholesterolemia, LDL were not cleared normally and accumulated in the plasma as a result of defective or absent LDL receptors. Nevertheless, remnants were cleared rather normally. Therefore, an alternate pathway appeared to be involved. Hui and colleagues (6, 7) were among the first to undertake studies to define this alternate pathway and to highlight the role for a putative apoE receptor, independent of the LDL receptor. For many years, this pathway remained elusive. In 1988, Herz et al. (8) identified a candidate molecule, the LDL receptor-related protein (LRP), which recognized only apoE and not apoB-100. Subsequently, the LRP was shown to be identical to the α_2-macroglobulin receptor (9, 10) and was demonstrated to be a multifunctional receptor capable of mediating the binding and uptake of several ligands (for review, see refs. 11, 12). In addition to the LRP, several other members of the LDL receptor family have been identified (for review, see refs. 12–14), and although these receptors can bind apoE-containing lipoproteins in vitro, they have not been shown to be expressed in the liver or to be involved in remnant lipoprotein catabolism. Other receptors, such as the asialoglycoprotein receptor (15), the lipolysis-stimulated receptor (16–19), a lactoferrin-inhibited receptor (20, 21), and a triglyceride-rich lipoprotein receptor (22, 23), have been postulated to be involved in remnant lipoprotein metabolism. However, their role in this process has not been established.

We have hypothesized that remnant lipoprotein metabolism involves three steps. First, remnants are cleared from the plasma by a process involving rapid sequestration of the particles within the space of Disse; heparan sulfate proteoglycans (HSPG) may play a major role in this "capture" step, with apoE and hepatic lipase (HL) serving as important ligands (for review, see refs. 24–28). Second, remnants may undergo further processing in the space of Disse. We (29) and others (30) have demonstrated the importance of HL in this step; other investigators, including Beisiegel, Weber, and Bengtsson-Olivecrona (31) and Mulder and associates (32) have implicated LPL. In vitro studies have shown that the lipases serve as ligands for the HSPG-LRP pathway. Third, uptake of remnants by hepatocytes may be mediated, at least in part, by LDL receptors (33) and, particularly as shown by Herz (11), Krieger and Herz (12), and Willnow (34), by the LRP. Our studies have shown that HSPG participate not only in the initial sequestration step, but also in the uptake step, either in association with the LRP or acting alone as a receptor (35–37) (Fig. 1).

Recently, Cooper (33) published an extensive review on the hepatic metabolism of chylomicron remnants, summarizing much of the early background and the importance of various ligands. Hussain et al. (38) and Willnow (34) have also reviewed this topic recently. Here we focus on the mechanisms responsible for remnant clearance from the plasma and uptake by hepatocytes, especially the importance of cell-surface HSPG.

Chylomicron remnant clearance into the space of Disse

Chylomicron remnants enter the space of Disse through the fenestrated sinusoidal endothelium, which acts as a dynamic biofilter that restricts the entry of large chylomicrons while allowing the smaller remnants to enter (39). The space of Disse is rich in HSPG and contains an abundance of apoE (40, 41) and HL (42), which are synthesized and secreted by hepatocytes, and LPL, which is presumably carried into the space of Disse on the remnants (43). Apolipoprotein E is critically important and can mediate the sequestration or trapping of the remnants by binding to HSPG (24, 25). The HSPG may thus serve as a reservoir for apoE to enrich the remnant particles. Hepatic lipase may also be bound to the matrix and cell-surface HSPG. The remnant lipoproteins are further processed and then taken up by endocytosis.

As illustrated in Fig. 1, we envision three major pathways that are important in remnant lipoprotein uptake by hepatocytes. First, LDL receptors can mediate the direct uptake of remnant lipoproteins, as shown by Choi et al. (44) and Choi and Cooper (45) and more recently by Ishibashi et al. (46), Herz et al. (47), Rohlman et al. (48), and Mortimer et al. (49). Second, the HSPG-LRP pathway can mediate uptake either by transfer of the remnants from the HSPG to the LRP for internalization or by binding of the remnant lipoproteins to HSPG forming a tertiary complex with the LRP that is then internalized. The HSPG are critical for the HSPG-LRP pathway because in their absence the remnant do not bind and are not taken up to a major extent by the LRP on the hepatocytes (25, 28, 36). Third, HSPG alone can mediate remnant lipoprotein uptake and function as a receptor. Each of these pathways will be discussed.

Apolipoprotein E: critical ligand for remnant clearance

Several approaches have shown that apoE is a critical ligand for the clearance of remnant lipoproteins. Years ago, we demonstrated that the intravenous infusion of apoE reduces remnant lipoproteins in cholesterol-fed rabbits, a model characterized by substantial accumulation of these particles in the plasma (50). Within 2–3 h after intravenous infusion of 6–12 mg of apoE, the plasma cholesterol levels decreased by 35–40%. The apoE drove the remnants into the liver. In transgenic mice expressing rat apoE, the apoE was localized to the sinusoids, presumably reflecting distribution in the space of Disse; after infusion of remnant lipoproteins, the apoE appeared to be distributed over the hepatic parenchymal cells and decreased in the sinusoids (51). More recently, Fan and colleagues (52) demonstrated that overexpression of human apoE in transgenic rabbits prevented diet-induced remnant accumulation and also accelerated remnant lipoprotein clearance from the plasma (**Fig. 2**). The accelerated clearance was accounted for by the uptake of remnants within the liver.

Studies of patients with type III hyperlipoproteinemia also implicate apoE as a critical ligand for remnant metabolism (for review, see refs. 3, 24, 25). This genetic disease is characterized by impaired remnant lipoprotein clearance as a result of various mutant forms of apoE that are defective in binding to lipoprotein receptors. All of these variants are associated with the development of the hyperlipidemia, and the mutant apoE correlates with defective clearance of remnant lipoproteins. The mutations of apoE that cause defective binding occur primarily in or near the receptor-binding domain, the region encompassing amino acids 136–150 (53).

One interesting variant we have studied is apoE (Arg_{142}→Cys), in which the arginine at residue 142 is replaced with a cysteine (54, 55). This variant is associated with the dominant transmission of the disease, causing hyperlipidemia at birth, marked remnant accumulation, and premature atherosclerosis. The apoE(Arg_{142}→Cys) variant exhibits 20% of normal binding to the LDL receptor and less than 5% of normal binding to the LRP and to HSPG (55). Expression of apoE(Arg_{142}→Cys) in transgenic mice demonstrated that this mutant protein was responsible for the impaired remnant metabolism. In these mice, Fazio et al. (53) showed that overexpression of the 142 variant resulted in a hyperlipidemia characterized by β-VLDL remnants. The mice had a hypercholesterolemia of 307 mg/dl and a hypertriglyceridemia of 378 mg/dl. These lipid levels are similar to those in patients with the apoE(Arg_{142}→Cys) variant. The apoE(Arg_{142}→Cys) transgenic mice had an increase in the VLDL fraction, specifically in the remnant lipoproteins. By comparison, the nontransgenic control mice had virtually undetectable levels of remnants in their plasma. Clearly, the binding-defective apoE results in

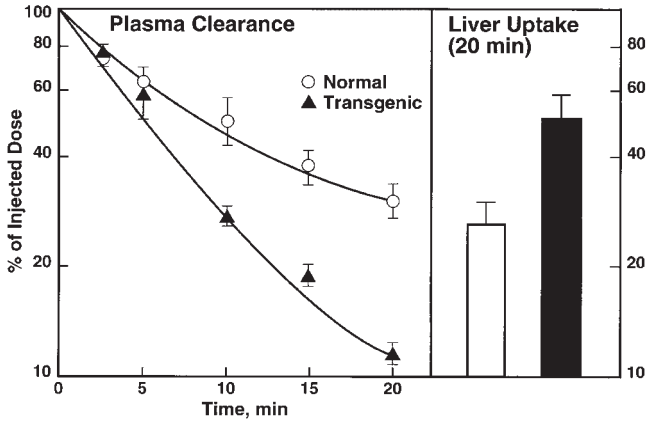

Fig. 2. Radiolabeled canine chylomicrons are rapidly cleared from the plasma of transgenic rabbits expressing human apoE3. The hepatic uptake of the chylomicrons is markedly enhanced in the apoE3-expressing transgenic rabbits (open bar, nontransgenic; black bar, apoE3 transgenic). (Reproduced with permission from *J. Clin. Invest.* 1998. **101:** 2151–2164.)

impaired remnant clearance (the mechanism will be discussed in detail later).

Plump et al. (26) and Zhang et al. (27) created apoE knockout mice that display very severe hypercholesterolemia characterized by marked accumulation of remnants, again indicating a critical role of apoE in remnant clearance. However, whereas receptor binding-defective apoE induced the accumulation of remnants enriched in both cholesterol and triglycerides, the absence of apoE induced the accumulation of highly cholesterol-enriched, triglyceride-poor remnants. This suggests that mutant apoE may affect triglyceride catabolism independently of its defective receptor binding. Early studies have suggested that some defective apoE impairs lipolytic processing of remnant lipoproteins (56–58). Recently, this concept has been confirmed and expanded in human apoE2(Arg_{158}→Cys) transgenic mice lacking mouse apoE (59). In these mice, the presence of apoE2(Arg_{158}→Cys) in VLDL or IDL impairs LPL-mediated lipolysis by ~80% and ~70%, respectively (60). Thus, defective apoE can impair remnant clearance and alter normal lipolytic processing as well.

Role of HL in remnant metabolism

As mentioned above, HL appears to serve as a ligand for remnant metabolism and may also be involved in lipolytic processing. One important question concerning the role of HL in remnant metabolism is its precise location in the liver. If HL participates in the sequestration and processing of remnants, as suggested by our model, it should be present within the space of Disse. Previous studies had suggested that HL was localized on the luminal surfaces of sinusoidal endothelial cells (61, 62) or on hepatocytes (63). To address this issue, Sanan et al. (42) created transgenic rabbits that synthesize and secrete human HL solely in the liver and used immunochemical light microscopy and gold-labeling immunoelectron microscopy to pinpoint the ultrastructural location of HL. The HL was present on the surface of luminal sinusoidal endothelial cells throughout all liver sections examined; however, substantial amounts of HL were also present on the surfaces of hepatocyte microvilli in the space of Disse and on the abluminal surfaces of endothelial cells, and additional HL was found in the interhepatocyte spaces (**Fig. 3**). Kounnas et al. (64) have demonstrated that HL binds to HSPG and the LRP. This distribution of HL is consistent with the distribution of the extracellular matrix and cell-surface HSPG that are abundant in the space of Disse and supports our hypothesis that HL participates in the sequestration and processing of remnants in the space of Disse.

We know from a number of studies that HL is involved in remnant metabolism (29, 30, 65–70). Studies in animals have shown that the intravenous injection of antibodies to HL impairs remnant lipoprotein clearance from the plasma (71–73). Conversely, treatment of chylomicron remnant lipoproteins with HL facilitates their uptake by the liver (69, 70). Thus, another important question regarding HL is whether it functions as a ligand or as an enzyme in remnant metabolism.

Rabbits, which normally have very low levels of HL, provide useful insights into the function of this enzyme in remnant metabolism. They are highly susceptible to diet-induced hyperlipidemia and are especially prone to remnant lipoprotein accumulation. This unique sensitivity to dietary cholesterol may be partly explained by low HL levels. In the studies of Fan and associates (74), overexpression of human HL in transgenic rabbits reduced triglycerides by 58% and cholesterol by 42% compared with the levels in nontransgenic littermates, and altered VLDL, IDL, and high density lipoprotein levels as well. Overexpression of HL also accelerated the clearance of radiola-

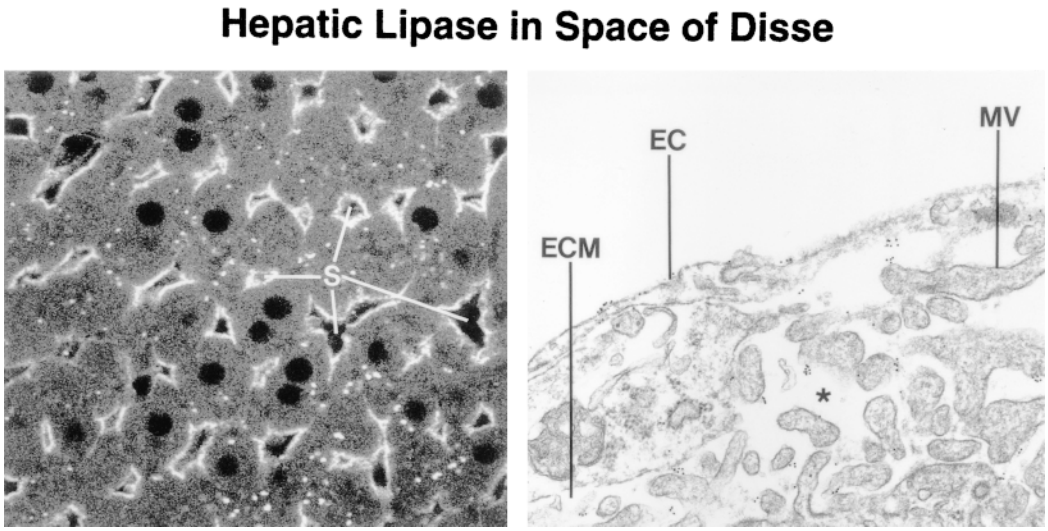

Fig. 3. Immunogold localization of HL in the liver sinusoids (S) and within the space of Disse (*) of transgenic rabbits overexpressing human HL. The gold particles labeling the HL are clearly associated with the extracellular matrix (ECM) and with the microvilli (MV) of the hepatocytes. Hepatic lipase is also present on the luminal surface of the endothelial cells (EC). (Reproduced with permission from *J. Lipid Res.* 1997. **38:** 1002–1013.)

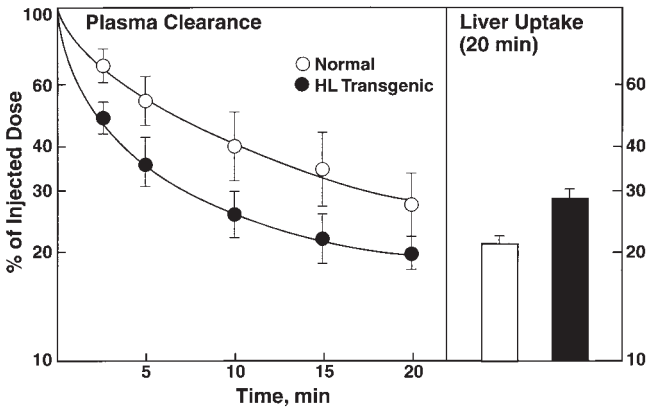

Fig. 4. Radiolabeled canine chylomicrons display enhanced plasma clearance when injected intravenously into transgenic rabbits overexpressing human HL. The hepatic uptake of chylomicrons is also significantly enhanced in the HL transgenic rabbits (open bar, nontransgenic; black bar, HL transgenic).

beled chylomicrons from the plasma and significantly enhanced their uptake by the liver (**Fig. 4**). Thus, increased levels of HL facilitate plasma clearance of the remnants. As will be discussed below, our data indicate that the facilitated uptake reflects both increased lipolytic processing and ligand-binding activity.

Role of LPL in remnant metabolism

Felts, Itakura, and Crane (75) suggested many years ago that LPL might circulate with remnant lipoproteins and participate in the delivery of the particles to the liver. Lipoprotein lipase binds to the LRP and to HSPG (31, 32, 76–80) and enhances the uptake of remnants (31, 32). Nevertheless, the relative importance of LPL in normal remnant metabolism is problematic (for review, see ref. 81). In humans with a genetic apoE deficiency and in apoE-null mice (26, 27, 82, 83), the defective remnant lipoprotein clearance persists even in the presence of normal LPL.

Huff et al. (68) reported that, at physiological concentrations, LPL did not play a major role as a ligand to promote uptake of triglyceride-rich VLDL. However, transgenic mice expressing LPL display a significantly reduced plasma VLDL (84). In a recent study, Zsigmond et al. (85) intravenously injected an adenovirus-LPL gene construct into apoE-deficient and LDL receptor knockout mice and found that overexpression of LPL reduced total plasma cholesterol and triglyceride by 40–60%. In addition, the VLDL/chylomicron remnant cholesterol and triglyceride levels were decreased by more than 83%. These findings indicate that the excess LPL may accelerate the clearance of remnant lipoproteins.

Undoubtedly, LPL plays a central role in chylomicron metabolism by lipolytic conversion of chylomicrons to somewhat triglyceride-depleted chylomicron remnants (for review, see ref. 86). During this pre-sequestration step, chylomicrons are changed in size and lipid and apolipoprotein composition in preparation for entry into the space of Disse. However, the quantity of LPL transported on remnants and the relative abundance of LPL in the space of Disse under physiologic conditions need to be determined to establish an in vivo role for LPL as a ligand responsible for remnant lipoprotein metabolism.

Sequestration and uptake of remnants: importance of HSPG

As part of the sequestration step and the LRP pathway, cell-surface HSPG mediate important interactions of apoE-enriched lipoproteins in a variety of cells (for review, see refs. 25, 28). All of the major proteins involved in chylomicron catabolism, including apoE, LPL, and HL, bind to HSPG (25, 28). Thus, cell-surface HSPG bring all the participants in the chylomicron remnant pathway together in the space of Disse. A significant role for HSPG in lipoprotein metabolism has been supported by important contributions by Eisenberg et al. (76), Williams and Fuki (87), Fuki et al. (88), and Fernández-Borja et al. (89).

In vitro studies in our laboratory have shown that HSPG participate in the binding and uptake of apoE-enriched β-VLDL (36). Treatment of a variety of cells with heparinase, which removes the sulfated glycosaminoglycan side chains from the proteoglycans, significantly inhibits the binding and uptake of apoE-enriched remnant lipoproteins. Heparinase decreased remnant binding by 80% in normal human fibroblasts, by 90% in FH fibroblasts lacking LDL receptors, and by 80% in HepG2 hepatocytes.

We have extended these studies to explore the in vivo effects of heparinase in mice. In initial control studies, we demonstrated an effect of intravenous heparinase on the release of ^{35}S-radiolabeled HSPG from the mouse liver (37). In saline-infused mice, a large fraction of the radiolabeled HSPG could be isolated from the liver, whereas in the heparinase-treated mice, the amount of labeled HSPG was reduced by about 80%. Thus, the intravenous heparinase released the sulfated glycosaminoglycan side chains from the hepatic HSPG in vivo.

Next, we examined the effect of portal vein injection of heparinase on plasma clearance and liver uptake of apoE-enriched ^{125}I-labeled β-VLDL (37). In these studies, heparinase (30 units) was slowly infused into the portal vein, followed 5 min later by infusion of the radiolabeled remnants. Plasma clearance and liver uptake of the remnants were measured 15 min later. Only 41% of the injected β-VLDL plus apoE remained in the plasma of the saline-injected controls, whereas 70% of the injected dose remained in the plasma of the heparinase-infused mice (**Fig. 5**). Thus, heparinase inhibited the clearance of β-VLDL from the plasma. This inhibition was reflected in the liver uptake of the remnants: 50% of the injected dose accumulated in the liver of the saline controls, but only about 15% of the remnants appeared in the liver after heparinase injection, a very large effect (Fig. 5). Similar studies with chylomicrons and chylomicron remnants yielded virtually identical results.

In this same study, we also looked at the uptake of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI)-labeled β-VLDL plus apoE by mouse liver in vivo (37). Hepatic uptake was marked in the saline-injected controls,

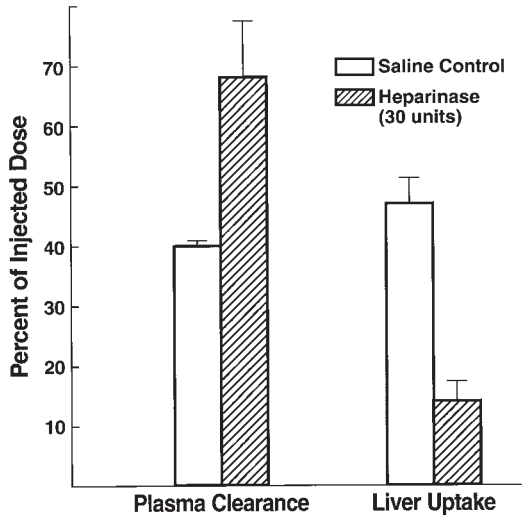

Fig. 5. Plasma clearance of ^{125}I-labeled β-VLDL enriched in apoE is markedly impaired after intravenous injection of 30 units of heparinase into the portal vein of wild-type mice. Likewise, heparinase infusion very significantly inhibits the hepatic uptake of the radiolabeled β-VLDL + human apoE3.

but decreased very significantly in the heparinase-injected mice (**Fig. 6**). In control experiments, intravenous heparinase had no effect on the clearance and uptake of activated α_2-macroglobulin by the LRP or of LDL by the LDL receptors in mouse liver (**Table 1**), indicating that heparinase did not inhibit the activity of these receptors for these ligands. The in vivo effects of heparinase on lipoprotein clearance have been confirmed by others (49, 90).

These studies clearly show that heparinase inhibits the plasma clearance and liver uptake of remnant lipoproteins. The consistency among the results of in vitro and in vivo studies demonstrates an important role for HSPG in remnant catabolism. The effect of heparinase appears to be caused by the release of heparan sulfate oligosaccharides from HSPG in the liver, which may deplete the apoE in the space of Disse or interfere with LPL or HL interactions in the liver. Thus, although the precise mechanism remains to be determined, HSPG clearly have a significant role in remnant clearance.

Uptake and endocytosis of remnants by hepatocytes: importance of LDL receptors, LRP, and HSPG

Although the LDL receptor is a critical participant in the uptake and endocytosis of remnants by hepatocytes, other molecules are also involved, including the LRP and cell-surface HSPG. It has been known for some time that apoE-containing remnants can interact with and be taken up by the LDL receptor (5). As mentioned above, however, an alternate pathway also exists, in which the remnants interact with HSPG and are either transferred to the

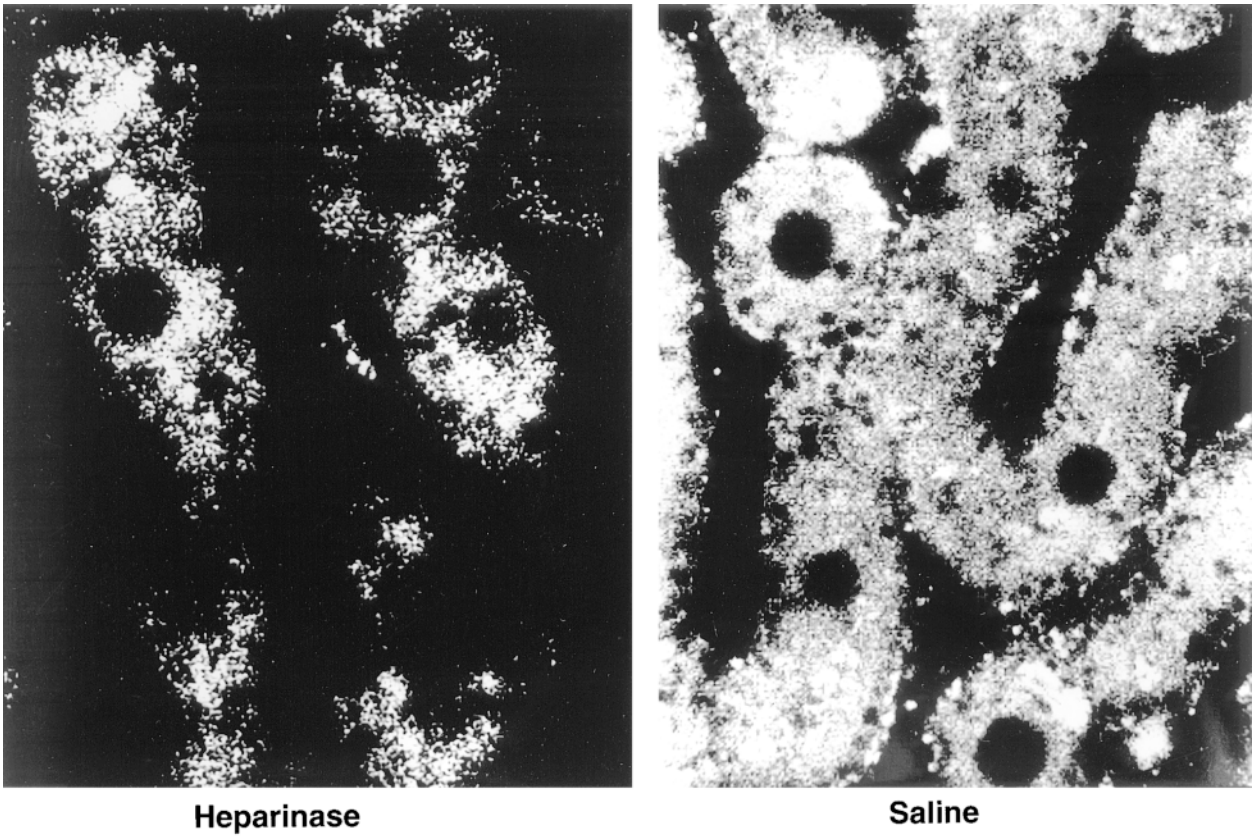

Fig. 6. By comparison with saline injection, heparinase infusion into wild-type mice markedly decreases hepatocyte uptake of DiI-labeled β-VLDL enriched in apoE. In the saline-infused controls, the β-VLDL are associated with the sinusoidal surface of the hepatocytes, suggesting that the lipoproteins are sequestered in the space of Disse. (Reproduced with permission from *J. Lipid Res.* 1995. **36:** 583–592.)

TABLE 1. Effect of intravenous heparinase on activated α_2-macroglobulin and LDL plasma clearance and liver uptake in mice

	n	Saline Control		Heparinase	
		Plasma Clearance	Liver Uptake	Plasma Clearance	Liver Uptake
		% of injected dose of labeled molecules			
α_2-Macroglobulin	20	22 ± 6	60 ± 12	21 ± 7	64 ± 11
LDL	3	74 ± 3	18 ± 7	77 ± 6	15 ± 4
β-VLDL + apoE	12	38 ± 9	48 ± 7	66 ± 12	22 ± 7

Saline or heparinase (30 units) was allowed to circulate for 5 min before injection of the radiolabeled activated α_2-macroglobulin, LDL, or β-VLDL + apoE. The plasma clearance and liver uptake of the activated α_2-macroglobulin, LDL, and β-VLDL + apoE were determined 5 min, 15 min, and 10 min later, respectively. There was no significant difference between plasma clearance and liver uptake of saline controls versus heparinase-injected mice for α_2-macroglobulin and LDL.

LRP for internalization or are taken up directly with the HSPG-LRP complex (35–37). The HSPG may serve as a reservoir for apoE, allowing the particles to be enriched in apoE, which facilitates their interaction with the LRP. Furthermore, HSPG alone serve as receptors and as an alternate pathway (to be discussed below). These apoE-mediated interactions are illustrated in Fig. 1. Before discussing each of these pathways, we will describe the LRP molecule and a useful inhibitor of its binding activity.

The LRP is a very large molecule (M_r = 600,000) that is prominently expressed on hepatocytes and has multiple ligands, including apoE, HL, LPL, activated α_2-macroglobulin, and several others (for review, see refs. 11–13). As the LRP is synthesized in the cell and transported to the cell surface, it is associated with a protein called the receptor-associated protein (RAP, or 39-kDa protein), which apparently serves as a chaperone to usher the LRP to the cell surface (91–93). Interestingly, when RAP is isolated and injected intravenously, it blocks the binding of all ligands to the LRP (94) and can also bind to cell-surface HSPG (28, 95). Heparinase treatment of Chinese hamster ovary (CHO) cells reduced the direct binding of the ^{125}I-labeled 39-kDa protein by ~40%. On the other hand, RAP, which is known to be a heparin-binding protein, does not bind to HSPG on fibroblasts (96). However, the structural diversity of HSPG is enormous for different cell types and different organs (97, 98). Hepatic HSPG are especially diverse, are highly sulfated, and are rich in heparin-like domains. Therefore, it is extremely likely that RAP binds to hepatic HSPG in vivo and that RAP can inhibit remnant lipoprotein binding to the LRP and HSPG, as well as the LDL receptor. Nevertheless, the inhibitory effects of RAP are concentration-dependent, and thus the local concentration and affinity of RAP for the various pathways become critical, yet difficult, to ascertain in vivo, and care should be taken in ascribing the inhibitory activity of RAP to only one pathway.

Several in vitro studies have established that the LRP is involved in the binding and uptake of apoE-enriched remnant lipoproteins (for review, see refs. 11–13, 25, 28). The first evidence that the LRP was involved in remnant clearance in vivo came from studies using native versus activated α_2-macroglobulin to determine whether the activated α_2-macroglobulin competes for LRP binding and uptake. Native α_2-macroglobulin does not bind to the LRP, whereas activated α_2-macroglobulin does. Our in vitro data indicated that activated α_2-macroglobulin can, to a limited extent, compete with β-VLDL for LRP binding (99). In these in vivo studies in mice, inactive α_2-macroglobulin or saline was injected into a tail vein, followed 1 min later by injection of radiolabeled chylomicron remnants into a contralateral vein. Within 15 min, about 79% of the injected chylomicrons appeared in the liver. However, after injection of activated α_2-macroglobulin and labeled chylomicrons, only about 60% of the remnants appeared in the liver. This small but significant decrease indicated that α_2-macroglobulin can compete for remnant clearance and that the LRP is involved, to some extent, in remnant clearance. These results were confirmed by Choi and Cooper (45) but not by others (100). More recently, de Faria and associates (73) attempted to make minimal estimates of the relative importance of the LDL receptor and the LRP, and the data of Jong et al. (101) established the importance of these two receptors in remnant removal in the mouse. The data from various studies consistently suggested that at least a portion of remnant clearance in mouse liver is via the LRP.

The use of transgenic and knockout mice has been informative in highlighting the role of these alternate pathways. For example, Willnow et al. (102) demonstrated that LDL receptor-null mice have an increase in total cholesterol, due mostly to an increase in LDL; remnants do not accumulate significantly. When RAP was overexpressed with an adenoviral RAP expression construct and secreted by the mouse liver, there were small increases in cholesterol, triglycerides, and remnants. However, when RAP was overexpressed to impair the HSPG-LRP pathway in the LDL receptor-null mice, cholesterol and triglycerides increased more than 10-fold, accompanied by a large increase in remnant lipoproteins in the plasma. Furthermore, in RAP knockout mice, in which LRP expression in the liver was reduced by 75%, there was little change in plasma cholesterol or triglyceride levels (92). However, in double-knockout mice lacking both RAP and LDL receptors, remnants accumulated significantly in the plasma, as reflected by an increase in both cholesterol and triglycerides. These findings are summarized in **Table 2**. Thus, single interven-

TABLE 2. Plasma cholesterol and triglyceride levels in transgenic and knockout mice

Genotype	Cholesterol	Triglyceride	Ref.
	mg/dl		
Normal (wild-type) mice	92 ± 9	94 ± 10	92, 102
$LDLr^{-/-}$	237 ± 22	155 ± 22	102
↑RAP(AdCMV-RAP)	150 ± 17	254 ± 27	102
↑RAP,$LDLr^{-/-}$	1057 ± 61	1203 ± 168	102
$RAP^{-/-}$	103 ± 3	115 ± 8	92
$RAP^{-/-}$,$LDLr^{-/-}$	487 ± 12	279 ± 25	92
$LRP^{flox/flox}$,$LDLr^{-/-}$ (AdCrel injected)	503 ± 110	185 ± 47	48

tions, such as the absence of LDL receptors or impairment of the HSPG-LRP or LRP pathways by RAP, do not impair remnant clearance to a large extent. However, blocking both pathways significantly impairs remnant clearance, indicating that both can be involved in remnant metabolism. Especially noteworthy, overexpression of RAP, which could block both the HSPG and LRP components of the HSPG-LRP pathway in the absence of LDL receptor expression, was associated with the most marked increase in remnants (Table 2), suggesting that the most dramatic impact on remnant clearance occurs when all component pathways are blocked: the LDL receptor, the LRP, and HSPG.

Further insight into the importance of these pathways comes from a study by Mortimer and associates (49), in which fluorescently labeled chylomicron-like particles were injected into LDL receptor-null or apoE-null mice and normal controls. In the control mice, remnants were associated with the liver cells at 5 min and were evenly distributed throughout the hepatocytes at 20 min. Most of the labeled remnants were catabolized within 3 h. In the LDL receptor-null mice, however, the fluorescence was localized to the sinusoidal spaces of the liver (i.e., the labeled remnants were sequestered but not endocytosed) and at 3 h was evenly distributed over the hepatocytes. This finding highlights the fact that plasma clearance of remnants is very similar in control and LDL receptor-null mice, but the distribution in the liver (rate of uptake by hepatocytes) is very different. In the absence of LDL receptors, the sequestration step can occur, followed by slow endocytosis, presumably via the HSPG or HSPG-LRP pathway. In the apoE-null mice, however, very little fluorescence was visible at any time point. Thus, in the absence of apoE, sequestration and endocytosis of remnants are extremely limited. Interestingly and importantly, Linton et al. (103) have demonstrated that in apoE-null mice the introduction of macrophage apoE production, without hepatic apoE production, accelerates remnant clearance only when LDL receptors are present. In the absence of LDL receptors, however, peripheral production of apoE had no effect, suggesting that hepatic apoE synthesis and secretion are required for remnant clearance when alternate pathways to the LDL receptor are used (i.e., for HSPG sequestration in the space of Disse and HSPG-LRP-mediated uptake). Recently, Zeng et al. (104) further established the importance of hepatic HSPG in remnant-like lipoprotein binding by suppressing syndecan expression in vitro. Remnant lipoprotein metabolism by the liver in normal, LDL receptor-null, and apoE-null mice is summarized in **Table 3**.

However, Rohlmann and associates (48) have recently conclusively established the role of the LRP in remnant lipoprotein metabolism. Because the LRP is required for normal embryonic development in mice (105), it is not possible to generate viable LRP-null mice to test directly the role of this receptor in the liver. They have now generated a mouse strain in which *loxP* sites have been inserted into the LRP gene, allowing for conditional inactivation (knockout) of the LRP after birth (48). Injection of an adenovirus containing the Cre recombinase gene resulted in selective inactivation of the *loxP*-flanked LRP in the liver. In mice with normal LDL receptors, LRP inactivation had little effect on plasma lipoproteins. In LRP-deficient livers, up-regulation of the LDL receptors fully compensated for the absence of the LRP. However, in the LDL receptor-null mice, inactivation of the LRP resulted in a marked accumulation of apoB-48-containing lipoproteins in the plasma and an increase in plasma cholesterol and triglycerides (Table 2). Therefore, under normal physiological conditions, the LDL receptor and the LRP can participate in the endocytosis of remnant lipoproteins in the liver. On the other hand, if these were the only pathways involved in remnant lipoprotein clearance, one might have expected that a more marked hyperlipidemia would have resulted after the LRP inactivation in the LDL receptor-null mice. Thus, consistent with results obtained by Jeong et al. (106) in apoE-null mice, there is in vivo evidence for a non-LDL receptor, non-LRP pathway. A likely candidate is direct HSPG-mediated uptake. However, it is also clear that at the present time we cannot quantitatively determine the relative importance of these various pathways in vivo.

TABLE 3. Remnant lipoprotein metabolism by mouse liver

	Normal	LDLr$^{-/-}$	ApoE$^{-/-}$
Plasma clearance	normal	normal	blocked
Sequestration	rapid ApoE·HSPG	rapid ApoE·HSPG	minimal HL-HSPG (?) LPL·HSPG (?)
Endocytosis	rapid LDL receptor	slow HSPG-LRP pathway	minimal

Metabolism of apoB-48- versus apoB-100-containing lipoproteins

It has been established that apoB-100-containing lipoproteins can be removed from the plasma by LDL receptors and that apoE-containing apoB-48 particles can interact with LDL receptors. In addition, as described above, recent studies have demonstrated that the LRP plays a primary role in the clearance of apoB-48-containing lipoproteins (48). However, there are three important questions that need to be answered by in vivo studies. Does the LDL receptor participate in the clearance of apoB-48-containing lipoproteins? Does the LRP pathway participate in the clearance of apoB-100-containing lipoproteins? Is the HSPG-LRP pathway only a secondary or alternative pathway or does it have a major role under normal physiological conditions? Studies by Véniant et al. (107) have provided in vivo data to answer these questions.

These investigators have demonstrated that "apoB-48-only" mice on the LDL receptor-null background have higher plasma cholesterol and LDL cholesterol levels and 2- to 3-fold higher apoB-48 levels than "apoB-48-only" mice on a wild-type background. Therefore, the LDL receptor does play a role in the clearance of apoB-48-containing lipoproteins. Furthermore, when adenovirus-

mediated expression of RAP was used to block the HSPG-LRP pathway, plasma apoB-48 levels increased markedly in the "apoB-48-only" mice regardless of whether LDL receptors were present, but plasma apoB-100 levels did not increase in LDL receptor-null, "apoB-100-only" mice. Therefore, the LRP pathway plays a major role in the clearance of apoB-48-containing lipoproteins but does not play a significant role in the clearance of apoB-100 particles (107). It must be remembered that these conclusions relate specifically to *mouse* apoB-48- and apoB-100-containing lipoproteins. Certain *human* apoB-100-containing lipoproteins might be capable of being enriched in apoE and be targeted to the HSPG-LRP pathway for uptake. Nevertheless, these data strongly suggest that the LRP pathway may be much more important in the metabolism of apoB-48-containing than of apoB-100-containing lipoproteins.

A primary role for the LRP pathway in apoB-48-containing lipoprotein clearance demonstrated by Rohlmann et al. (48) is further supported by Véniant et al. (107). They demonstrated that there was a much more marked accumulation of apoB-48-containing lipoproteins in "apoB-48-only" mice that were deficient in apoE compared to "apoB-48-only" mice lacking LDL receptor expression. These results suggest that a second, apoE-mediated pathway is involved in apoB-48 metabolism (i.e., the HSPG-LRP pathway). Likewise, RAP overexpression in wild-type mice produced a much higher accumulation of apoB-48 than of apoB-100. Thus, the LRP or HSPG-LRP pathway appears to have a major role in the uptake of apoB-48-containing lipoproteins and may be the primary pathway for apoE-mediated clearance of these particles even in the presence of LDL receptor expression. However, at the present time we cannot be sure what role the direct HSPG pathway alone may play in the uptake of specific apoB-containing lipoproteins.

HSPG-LRP pathway in remnant lipoprotein metabolism and the critical role for HSPG

To define further the role of the HSPG-LRP pathway in remnant lipoprotein metabolism, a number of in vitro studies have been performed in cultured cells, including the hepatoma cell lines McA-RH7777 and HepG2, and the results were compared with those from normal fibroblasts, fibroblasts lacking LDL receptors, fibroblasts lacking the LRP, and CHO cells lacking cell-surface HSPG (36). Heparinase was used to remove the sulfated glycosaminoglycan chains from HSPG, and the remnants were enriched with apoE to mimic the conditions that appear to prevail in the space of Disse.

The β-VLDL enriched in apoE3 displayed a 12-fold enhancement of binding to HepG2 hepatocytes compared with β-VLDL without added apoE or with LDL (**Fig. 7A**). Heparinase treatment of the cells prevented nearly all of this enhanced binding. The binding activity that remained was essentially identical to that of β-VLDL without supplementary apoE and was not affected by heparinase. Similarly, most of the binding of β-VLDL without added apoE could also be prevented by an inhibitory LDL receptor antibody; however, this antibody blocked only about

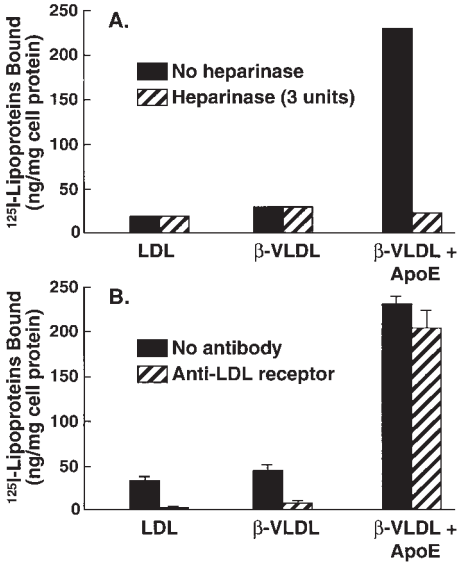

Fig. 7. A: The binding of β-VLDL, β-VLDL + human apoE3, and LDL to HepG2 hepatocytes was determined with or without pretreatment of cells with heparinase. The enhanced binding of β-VLDL enriched in apoE was abolished by heparinase treatment. B: The binding of β-VLDL, β-VLDL + human apoE3, and LDL to HepG2 cells was determined in the presence or absence of added anti-LDL receptor antibody that inhibits receptor-ligand interaction. Only a small fraction of the enhanced binding of the β-VLDL + apoE3 was mediated by the LDL receptor. (Reproduced with permission from *J. Biol. Chem.* 1993. **268:** 10160–10167.)

10% of the enhanced binding displayed by the apoE-enriched β-VLDL (Fig. 7B). Thus, the basal binding activity of β-VLDL is mediated by the LDL receptor, and most of the enhanced binding is mediated by HSPG (36).

The binding and uptake of β-VLDL by CHO mutants defective in cell-surface proteoglycan synthesis, by wild-type CHO cells, and by normal fibroblasts have also been examined (36). Mutant *pgs*D-677 CHO cells (deficient in *N*-acetylglucosamine transferase and in glucuronic acid transferase) lack cell-surface HSPG but do have chondroitin sulfate. Mutant *pgs*A-745 CHO cells (deficient in xylose transferase) completely lack glycosaminoglycan synthesis. The results of these studies are summarized in **Table 4**. Binding of β-VLDL without added apoE was essentially identical in normal fibroblasts, wild-type CHO cells, and mutant CHO cells. Heparinase had no significant effect on binding, indicating that it does not decrease basal binding activity, most of which is mediated by

TABLE 4. Effect of heparinase treatment on cell binding

		β-VLDL[a] (0.8 μg)		β-VLDL + ApoE[a] (0.8 ± 1.2 μg)	
	n	Control	Heparinase	Control	Heparinase
		ng/mg cell protein			
Fibroblasts	4	47 ± 12	53 ± 15	358 ± 104	62 ± 25
Wild-type CHO	2	49	57	209	47
Mutant CHO-677	9	47 ± 12	49 ± 16	4 ± 11	46 ± 11
Mutant CHO-745	9	56 ± 12	55 ± 13	41 ± 10	42 ± 9

[a]Amount of lipoprotein binding at 4°C for 3 h (mean ± SD).

the LDL receptor. By comparison, binding of apoE-enriched β-VLDL by normal fibroblasts and wild-type CHO cells increased 4- to 7-fold. This enhancement was eliminated by heparinase. However, binding of apoE-enriched β-VLDL by the mutant CHO cells (*pgs*D-677 or *pgs*A-745) lacking heparan sulfate was not enhanced, only the basal LDL receptor-mediated binding was displayed, even though the LRP is present in these cells. The CHO cells possessed the ability to bind and internalize activated α_2-macroglobulin, and this ability was not diminished by heparinase treatment. These results helped to establish the importance of HSPG in the binding of apoE-enriched remnants by demonstrating that LRP expression alone is insufficient to mediate the enhanced binding to the cell surface in the absence of HSPG.

As mentioned above, heparinase cleaves the heparan sulfate oligosaccharides from cell-surface heparan sulfate. We have demonstrated parallel losses of cell-surface binding of remnants and of [^{35}S]sulfate-labeled proteoglycans from the cell surface after treatment with heparinase. In addition, we have seen no effect of heparinase on the structure or function of the LRP on ligand blots or on cultured cells or of the LDL receptor (36).

In search of additional insight into the involvement of HSPG and the LRP in remnant catabolism, we examined hemizygous and homozygous LRP-null fibroblasts from fetal mice (108). The binding and uptake of apoE-enriched remnants was about 6-fold higher than the binding and uptake of remnants without added apoE (**Fig. 8**). About 10% of the enhanced binding was blocked by an anti-LDL receptor antibody and about 90% was blocked by heparinase. These results are similar to those in normal human fibroblasts. In LRP-null fibroblasts, binding was also enhanced, albeit to a lesser extent than in the hemizygous cells (Fig. 8). Thus, a fraction of the enhanced binding occurs via the LRP. Heparinase treatment eliminated most of the remaining activity. From these findings it appears that, in vitro, HSPG alone can mediate a significant fraction of remnant binding and uptake in the absence of the LRP. Therefore, although the LRP, in association with HSPG, plays a significant role, HSPG alone clearly make a major independent contribution to the metabolism of apoE-containing lipoproteins.

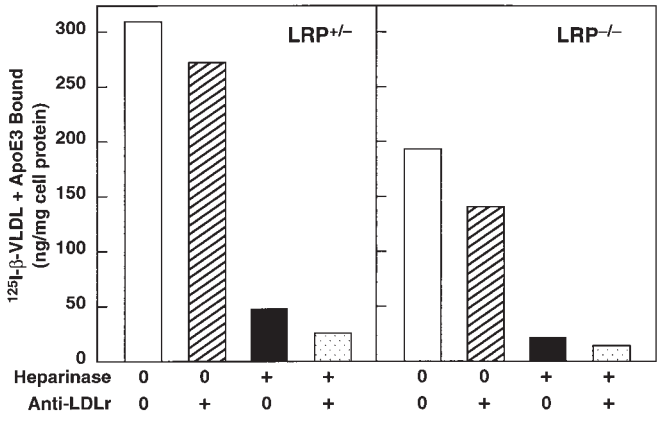

Fig. 8. Binding of ^{125}I-labeled β-VLDL enriched in human apoE3 to fibroblasts from heterozygous (LRP$^{+/-}$) or homozygous (LRP$^{-/-}$) fetal LRP knockout mice. In some cases, the cells were pretreated (+) with heparinase or with an anti-LDL receptor antibody (anti-LDLr).

How do the HSPG and the LRP function together in the HSPG-LRP pathway? That HSPG are required for the LRP to mediate the uptake of apoE-enriched remnants suggests that the remnants interact first with the HSPG and then are transferred to the LRP for internalization as illustrated in Fig. 1. Alternatively, the HSPG and the LRP may form a complex and the complex may mediate the uptake. In vivo, the binding conformation of the LRP may require the interaction of HSPG and the LRP (i.e., with HSPG serving as a scaffolding to support an extended LRP molecule capable of interacting with apoE-containing lipoproteins). On the other hand, in the absence of cell-surface HSPG, the very large chylomicron remnant particles simply may not be able to enter the cell-surface environment surrounding the LRP. There are many similarities between the apoE remnant·HSPG-LRP pathway and the basic fibroblast growth factor (FGF)·cell-surface proteoglycan-FGF receptor pathway (109–111), and evidence from other fields demonstrates that HSPG can mediate the uptake of ligands directly (112, 113).

Insights into the importance of HSPG from type III hyperlipoproteinemia

Type III hyperlipoproteinemia associated with apoE2 displays a recessive mode of inheritance in which apoE2 homozygosity is essential, but not sufficient, for the development of an overt increase in the accumulation of remnant lipoproteins in the plasma (for review, see refs. 24, 25). The vast majority of apoE2 homozygotes are not hyperlipidemic and do not have significant accumulation of remnant lipoproteins. Clearly, apoE2 acts as a susceptibility gene requiring secondary genetic or environmental factors to precipitate the hyperlipidemia. Yet apoE2 is highly defective in LDL receptor-binding activity (less than 2% of normal apoE3 activity) (5, 24). As stated, the majority of apoE2 homozygotes have efficient clearance of remnants despite possessing apoE that binds defectively to the LDL receptor. Thus, while the LDL receptor is an important pathway for remnant clearance, it is not the only pathway. Apolipoprotein E2 has significant HSPG-binding activity (50–90% of normal apoE3 activity), and therefore the HSPG pathway may serve as an efficient backup pathway for remnant lipoprotein clearance (114). Remnants accumulate when secondary factors, such as increased production or decreased clearance of VLDL or chylomicrons, overwhelm the HSPG pathway.

Further support for the importance of the HSPG pathway in remnant lipoprotein metabolism comes from studies of the dominant variants of apoE that cause type III hyperlipoproteinemia without the involvement of secondary factors (for review, see refs. 24, 25). Heterozygosity for defective apoE variants involving single amino acid substitutions at amino acid residues 142, 145, or 146 and the insertional variant called apoE-Leiden are invariably associated with type III hyperlipoproteinemia (24). These variants

are markedly defective in HSPG binding (<5% of apoE3's activity), despite possessing 25–45% of the LDL receptor–binding activity of apoE3 (25, 114). The impaired HSPG binding is the only defect consistently associated with these dominant variants of apoE and type III hyperlipoproteinemia, which strongly suggests that the HSPG interaction is critically important in remnant lipoprotein clearance. The inability of remnants to bind to HSPG would block sequestration in the space of Disse and impair access to or interaction with the LRP, leading to the accumulation of these particles in the plasma. Clearly, the retention of 25–45% of normal LDL receptor binding, in the presence of defective HSPG-binding activity, is not sufficient to maintain normal clearance of the remnant lipoproteins.

HSPG, apoE, and Alzheimer's disease

As added evidence for the role of HSPG in the metabolism of apoE-containing lipoproteins, we have now shown that cell-surface HSPG, at least in vitro, can play a unique role in lipoprotein metabolism independently of the LDL receptor or the LRP. In addition to its well-known functions in plasma lipoprotein metabolism and transport, apoE also serves to transport lipids in the nervous system (5, 115, 116). Apolipoprotein E4 is a risk factor for Alzheimer's disease, whereas apoE3, the most common allelic form in humans, is not (117, 118). These two isoforms differ at one residue, apoE4 has arginine at residue 112, where apoE3 has cysteine (5). Although the mechanism by which apoE affects the biology of the central nervous system is unknown, we have demonstrated, in cultured neurons, apoE isoform-specific effects that are mediated by HSPG-LRP.

We have shown that apoE3 plus a source of lipid stimulates, whereas apoE4 plus a source of lipid inhibits, neurite extension in rabbit dorsal root ganglion neurons (119) and Neuro-2a cells (120). Holtzman and associates (121) and Fagan et al. (122) also reported that apoE3 stimulates neurite outgrowth. Apolipoprotein E alone had no effect, and lipid alone caused branching but little extension of neurites. These isoform-specific effects were abolished by heparinase treatment (120). One difference between apoE3 and apoE4 involves microtubule stability, apoE3 supports microtubule stability and apoE4 does not (123). In addition, we have shown a difference in the accumulation of apoE3 versus apoE4 in Neuro-2a cells (123). When added with a source of lipid, apoE3 accumulated throughout the cell bodies and neurites, whereas apoE4 displayed little accumulation in the cell bodies (**Fig. 9**). Similar results were obtained with skin fibroblasts and hepatocytes, where apoE3 accumulated at levels 2- to 4-fold greater than apoE4 (124).

To determine the relative importance of the different uptake mechanisms, the LDL receptor, the LRP, and HSPG, responsible for the differential intracellular accumulation of apoE, we compared the accumulation of iodinated apoE3 and apoE4 in human FH fibroblasts lacking the LDL receptor and in mouse fibroblasts lacking the LRP (**Fig. 10**) (124). In fibroblasts lacking LDL receptors, the accumulation of apoE3 was 3-fold greater than that of

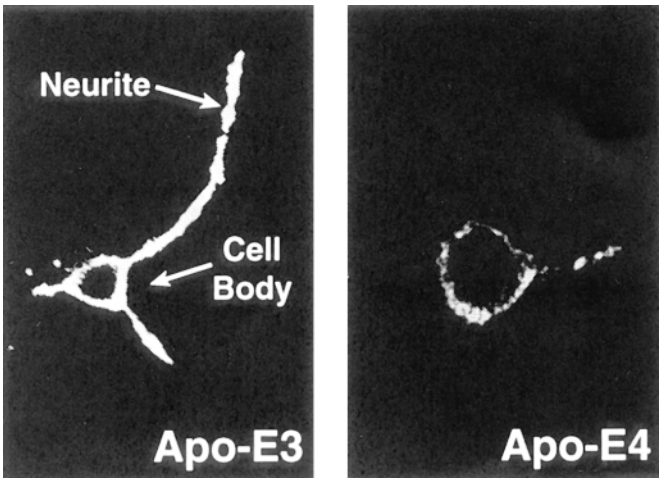

Fig. 9. Immunocytochemical localization of human apoE in Neuro-2a cells. Apolipoprotein E3 accumulated to a much greater extent than apoE4 in the cell body and neurites.

apoE4. Identical results were obtained in normal fibroblasts. Thus, the differential accumulation is unrelated to LDL receptor activity. In fibroblasts lacking the LRP (kindly provided by Dr. Joachim Herz, University of Texas Southwestern Medical School, Dallas, TX), apoE3 and apoE4 also accumulated differentially. Therefore, the LRP is not primarily involved, although it may have a limited effect. However, heparinase treatment of fibroblasts lacking LDL receptors abolished the differential uptake, resulting in very low uptake of both isoforms. Almost all of the cellular accumulation of apoE3 and apoE4 was abolished in the fibroblasts lacking the LDL receptor and the LRP plus heparinase treatment (124). Thus, the differential accumulation is related primarily to a difference in how apoE3 and apoE4 are handled by cell-surface HSPG (124). Although these data do not reveal the exact mecha-

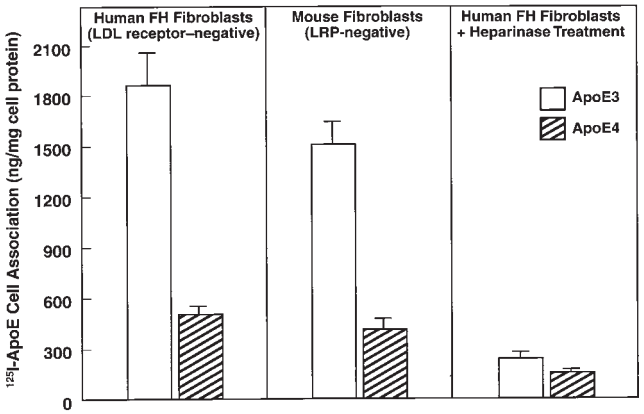

Fig. 10. The binding and internalization of β-VLDL enriched with ^{125}I-labeled apoE3 or apoE4 to human fibroblasts lacking the LDL receptor (FH fibroblasts), mouse fibroblasts lacking the LRP (LRP-negative), and human FH fibroblasts after heparinase treatment. The absence of the LDL receptor or the LRP did not alter the differential apoE3/apoE4 ratio, but treatment of FH cells with heparinase decreased the uptake of both apoE3 and apoE4 and abolished the isoform-specific differential intracellular accumulation.

nism of the differential accumulation of apoE3 and apoE4, they do establish the importance and uniqueness of the HSPG pathway in lipoprotein metabolism and shed light on this alternate pathway involved in the binding and uptake of apoE-containing lipoproteins.

Other lipoprotein studies have also established that HSPG can function as a receptor. Fuki et al. (88) have shown that LPL associated with lipoproteins can bind and be taken up via HSPG. They demonstrated that the syndecan family of HSPG mediates the uptake of LPL·LDL and that the HSPG clearance mechanism is a "slow" uptake pathway, whereas lipoprotein receptors, such as the LDL receptor mechanism, are a "fast" pathway. Thus, evidence supporting a unique role for HSPG is accumulating.

ApoE and HL: similarities in remnant metabolism

To elucidate the role of HL in mediating the binding and uptake of remnant lipoproteins, we stably transfected McA-RH7777 rat hepatoma cells with a human HL cDNA construct that generates liver-specific expression (29). These cells secreted about 50–80 ng of enzymatically active human HL per milligram of cell protein per 4 h. The secreted HL increased the binding and uptake of radiolabeled canine chylomicrons, chylomicron remnants, and β-VLDL by transfected cells compared with nontransfected cells (**Fig. 11**). Treatment with heparinase decreased the enhanced binding mediated by HL, reducing it nearly to the basal level of binding seen in the nontransfected cells (**Fig. 12**). We have now established that most of the enhanced binding of remnants by HL-transfected cells is mediated by HSPG. Direct visualization of the uptake of DiI-labeled β-VLDL by nontransfected and HL-transfected cells clearly showed that HL facilitates remnant uptake (29).

These studies demonstrate the involvement of HL, possibly by facilitating the binding of remnants to HSPG. But does HL serve primarily as a ligand or does it process the

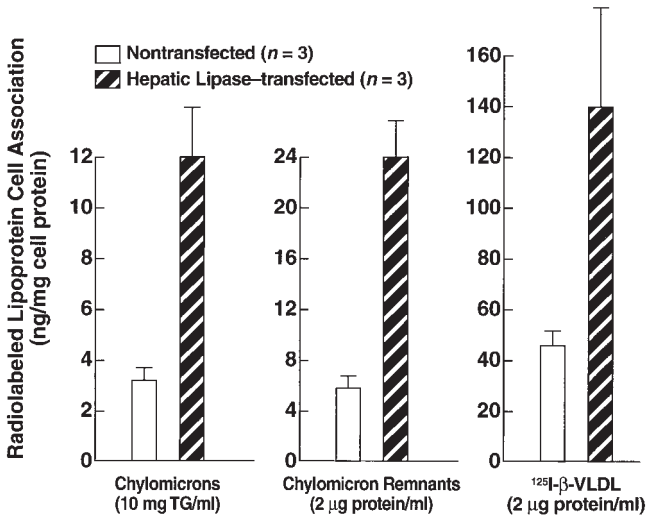

Fig. 11. Binding and uptake of chylomicrons, chylomicron remnants, and β-VLDL by nontransfected or human HL–transfected McA-RH7777 hepatocytes. Synthesis and secretion of HL by these cells stimulated the cell association of the lipoproteins. TG, triglyceride.

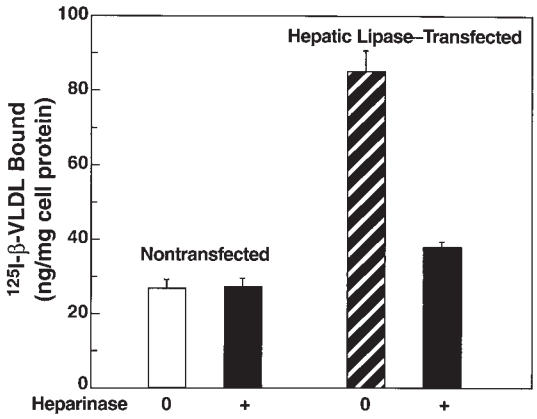

Fig. 12. Enhanced ^{125}I-labeled β-VLDL binding to HL-transfected McA-RH7777 cells was abolished by heparinase treatment of the cells.

remnants and facilitate their uptake? To answer this question, we expressed wild-type HL, catalytically inactive HL (HL-CAT$^-$), and LRP binding-defective HL (HL-LRP$^-$) in McA-RH7777 cells (108). Characterization of the transfected cells showed that the wild-type HL was catalytically active and bound normally to heparin (HSPG) and the LRP. The HL-CAT$^-$(Ser$_{145}$→Gly) lacked catalytic activity but retained heparin- and LRP-binding activities, and the HL-LRP$^-$(Lys$_{433}$→Ala) showed only 60% of LRP-binding activity compared with wild-type HL. The binding and uptake of rabbit ^{125}I-labeled β-VLDL by cells transfected with wild-type HL were enhanced about 3-fold compared with nontransfected controls. This enhancement was significantly reduced by 20–35% in cells transfected with HL-LRP$^-$ and HL-CAT$^-$. Furthermore, most of the enhanced binding and uptake was abolished after treatment with heparinase. Therefore, HL can enhance the uptake of remnants. Some of this enhanced uptake is mediated by the LRP because HL can bind directly to the LRP and initiate internalization (64, 66); another component of the enhanced uptake may be facilitated by lipolytic processing. Thus, almost all of the uptake is linked directly or indirectly with HSPG.

Transgenic mice expressing wild-type human HL show a marked reduction in apoB-containing remnant lipoproteins when fed a high-fat, high-cholesterol diet, when they overexpress human apoB, or when they are bred onto the apoE-null background (125). Furthermore, the overexpression of HL-CAT$^-$ in the apoE-null mice also decreases remnant lipoproteins by ~60% (125). Therefore, HL appears to contribute to remnant metabolism by catalytic processing of the lipoproteins. However, HL can also serve as a ligand facilitating remnant lipoprotein removal from the plasma independently of its catalytic activity. This has been demonstrated in both in vivo and in vitro studies (68, 108, 114, 125, 126).

Summary

In summary, the model of chylomicron remnant clearance via the LDL receptors, the HSPG-LRP pathway, and

HSPG alone appears to be valid (Fig. 1). Apolipoprotein E serves as the major ligand facilitating remnant lipoprotein catabolism. The HSPG in the space of Disse may well serve as a reservoir to allow apoE to accumulate and thereby participate in the sequestration and capture of the lipoproteins. In addition, the HSPG may serve as a receptor, either alone or as part of the HSPG-LRP complex, and may be responsible for endocytosis by hepatocytes. Along with the LDL receptor and the HSPG-LRP pathway, HSPG function as an alternate pathway. Hepatic lipase appears to function as a ligand, facilitating sequestration and uptake; it also appears to facilitate uptake by further processing of the remnants in the space of Disse. LPL may serve a similar role. However, HL or LPL at normal physiological concentrations cannot completely substitute for apoE. In apoE-null mice, remnants accumulate in the plasma, and in patients with type III hyperlipoproteinemia the critical abnormality is the presence of a mutant form of apoE defective in mediating plasma clearance of remnants, and the hyperlipidemia occurs despite normal HL and LPL activities. Thus, apoE is the essential ligand controlling remnant catabolism by mediating hepatocyte uptake by the three major pathways described.

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