Hepatic lipase function and the accumulation of β -very-low-density lipoproteins in the plasma of cholesterol-fed rabbits

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The accumulation of cholesterol-rich β -very-low-density lipoproteins (β -VLDL) in the plasma of rabbits fed on a high-fat high-cholesterol diet is due to a defect in the clearance of these lipoprotein remnants from circulation by the liver. In view of the evidence that hepatic lipase participates in the process of rapid removal of remnants from circulation, and considering that rabbits are naturally deficient in hepatic lipase, we examined whether this defect in the clearance of β -VLDL could be reversed by exogenous hepatic lipase. We report that treatment *in vitro* of [3 H]cholesterol-labelled β -VLDL, or rat chylomicrons, with hepatic lipase resulted in the formation of particles that were rapidly cleared from circulation by the liver when injected intravenously into hypercholesterolaemic rabbits. These results are consistent with the notion that, in addition to the well-

established requirement for lipoprotein lipase activity, the generation of remnants capable of being efficiently taken up by the liver also requires the action of hepatic lipase. Lipoprotein lipase acts on triacylglycerol-rich lipoproteins to transform them into particles (remnants) which bind to the surface of liver cells, where they become accessible to hepatic lipase. Hepatocyte endocytosis of these remnants occurs only after further modification by hepatic lipase. According to this scheme, the results presented suggest that the accumulation of β -VLDL in the circulation of rabbits fed on a high-fat high-cholesterol diet is the result of the saturation of the available hepatic lipase by abnormally high levels of lipoprotein-lipase-generated chylomicron remnants.

INTRODUCTION

The clearance of chylomicrons from circulation is a multi-step process that begins with their partial degradation by lipoprotein lipase, and ends with the endocytosis of chylomicron remnants by hepatocytes. The initial step occurs primarily in the vascular space of skeletal muscle and adipose tissue, where the composition of chylomicrons is modified by lipoprotein lipase. These modifications convert chylomicrons into particles (remnants) that can readily bind to the surface of hepatocytes. This has been demonstrated by numerous studies which have shown that lipoprotein-lipase-generated remnants, but not intact chylomicrons, bind with high affinity to the surface of hepatocytes (Floren and Nilsson, 1978; van Dijk et al., 1992) and to isolated liver membranes (Carella and Cooper, 1979; Cooper et al., 1982; Kita et al., 1982; Hui et al., 1984; Windler et al., 1988; Nagata et al., 1988; Borensztajn et al., 1991; Sultan et al., 1991). Although these lipoprotein-lipase-generated remnants are capable of binding to the liver cell surface, several studies have indicated that endocytosis of these particles by hepatocytes appears to require further action by hepatic lipase. In studies with isolated hepatocytes, Sultan et al. (1989) reported that the uptake of chylomicrons that had been partially degraded by lipoprotein lipase *in vivo* was significantly increased when these particles were also exposed to hepatic lipase. Work from our laboratory has shown that treatment of chylomicrons with hepatic lipase alone generates particles that are rapidly removed from circulation by the liver and can compete with remnants generated *in vivo* for uptake by hepatocytes (Borensztajn et al., 1988). Other evidence has shown that, in the absence of hepatic lipase activity, the uptake of remnants by the liver is greatly inhibited. Sultan et al. (1990) showed that inhibition of hepatic

lipase in rats by specific antibodies inhibited the plasma clearance and hepatic uptake of chylomicrons that had been treated with lipoprotein lipase. Furthermore, Daggy and Bensadoun (1986) demonstrated that inhibition of hepatic lipase *in vivo* by specific antibodies in rats fed on corn oil resulted in the plasma accumulation of phospholipid-rich lipoproteins consisting of partially degraded lipoproteins of intestinal origin. Similarly, in rats (Murase and Itakura, 1981) and in cynomolgus monkeys (Goldberg et al., 1982), the inhibition of hepatic lipase has also been shown to result in the plasma accumulation of partially degraded triacylglycerol-rich lipoproteins. Comparable observations have been reported in human subjects with familial hepatic lipase deficiency (Breckenridge et al., 1982; Connely et al., 1990).

Recent work has demonstrated that the rabbit is naturally deficient in hepatic lipase (Clay et al., 1989; Warren et al., 1991). In view of the above evidence indicating the important role of hepatic lipase in the clearance of chylomicron remnants, it might be expected that, under certain conditions, the rabbit would demonstrate a defect in remnant clearance. Indeed, when rabbits are fed on a high-fat high-cholesterol diet, they develop, within a few days, a severe hypercholesterolaemia which is due primarily to the accumulation of cholesterol-rich β -very-low-density lipoproteins (β -VLDL). Ross and Zilversmit (1977) showed that in the hypercholesterolaemic rabbit the β -VLDL consist predominantly of chylomicron remnants, which accumulate due to a defect in their clearance from circulation. This accumulation of remnants in an experimental animal known to be deficient in hepatic lipase suggests that the hypercholesterolaemic rabbit would be a good model for further investigating the contribution of this enzyme to remnant clearance. We report here the results of experiments investigating the ability of hepatic lipase to enhance the hepatic clearance of β -VLDL and chylomicrons in the hypercholesterolaemic rabbit.

Abbreviation used: VLDL, very-low-density lipoproteins.

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MATERIALS AND METHODS

Animals

Male New Zealand White rabbits (1.5–2 kg) were maintained on standard chow or on chow supplemented with 2% cholesterol and 10% corn oil for 14 days. They were fasted overnight before their use as a source of post-heparin plasma, or in metabolic studies. The plasma cholesterol concentration of fasted normal rabbits was 60.5 ± 5.4 mg/dl (mean $\pm$ S.D., $n = 4$), whereas that of fasted rabbits on the high-fat high-cholesterol diet was 1117 ± 264 mg/dl (mean $\pm$ S.D., $n = 4$). Male Sprague–Dawley rats (180–200 g) were maintained on standard rat chow.

Preparation of lipoproteins

β -VLDL labelled *in vivo* was prepared by feeding rabbits maintained on the high-fat high-cholesterol diet with 1 mCi of [1,2- 3 H]cholesterol (sp. radioactivity 50 Ci/mol; Amersham Corp.) in 2 ml of corn oil, by stomach tube. The rabbits were then allowed to eat freely for 24 h before being bled. The blood, after clotting, was centrifuged at 2000 g for 20 min at 15 °C, and the serum was collected and centrifuged at 80000 g for 1 h at 15 °C in an SW 40 Ti rotor in a Beckman model L5-75 ultracentrifuge. The floating layer of chylomicrons was discarded, and the infranatant was spun for 18 h at 170000 g in an SW 40 Ti rotor. The floating [3 H]cholesterol-labelled lipoproteins were then collected and purified by gel-permeation chromatography on a 2% agarose column (Bio-Gel A-50m, 50–100 mesh), as previously described (Borensztajn et al., 1988). On agarose-gel electrophoresis more than 90% of the lipid in this lipoprotein fraction was contained in β -VLDL.

Rat lymph chylomicrons were obtained by cannulation of the thoracic lymph duct of male Sprague–Dawley rats (180–220 g). The animals were then fed, by stomach intubation, with a mixture of 1 ml of corn oil containing 100 μ Ci of [1,2- 3 H]cholesterol, and 3 ml of egg yolk. The lymph was collected during the following 16 h at room temperature in the absence of preservatives. The lymph was de-clotted, layered under normal saline and centrifuged in an SW-27 rotor at 80000 g for 60 min at 15 °C, after which the floating chylomicrons were harvested. In order to minimize any species differences in chyle apoproteins that might affect the results of the experiments, rat chylomicrons were treated with trypsin to remove rat apoproteins and further purified by gel-permeation chromatography, as previously described (Borensztajn et al., 1988).

Chylomicron remnants were prepared *in vitro* by using post-heparin plasma obtained from rabbits maintained on a normal chow diet. They were injected with 75 i.u. of heparin/kg and, after 10 min, the rabbits were exsanguinated via cardiac puncture, and the post-heparin plasma was separated by centrifugation of the blood at 2000 g for 20 min at 4 °C. The plasma was then centrifuged at 80000 g for 18 h at 4 °C and the floating lipoprotein was discarded. Trypsin-treated rat chylomicrons were incubated with rabbit post-heparin plasma (3 mg of triacylglycerol/ml of plasma) for 5 h at 37 °C in a shaking water bath. The incubation mixture was then adjusted to a density of 1.019 g/ml with NaCl, layered under an equal volume of NaCl ($d = 1.019$), and centrifuged at 80000 g for 18 h at 15 °C. The floating layer of remnants was collected and purified by gel-permeation chromatography. With this procedure, 60–70% of the triacylglycerols were hydrolysed.

Treatment of rat chylomicrons and rabbit β -VLDL with rat hepatic lipase was carried out as previously described (Borensztajn et al., 1988). Briefly, hepatic lipase was obtained by perfusing rat livers for 30 s with saline containing 10 units of

heparin/ml. The incubation mixture contained the following components (per ml): rat trypsin-treated chylomicrons (42 μ g of cholesterol); 0.7 M Tris/HCl buffer, pH 8.6 (146 μ l); 20% (w/v) BSA (290 μ l), pH 8.1; hepatic lipase (170 μ l); and 0.15 M NaCl to 1 ml. For hepatic-lipase-treated β -VLDL, the incubation mixture components were the same, except that the final concentration of β -VLDL in the incubation mixture was 3.5 mg of cholesterol/ml. The incubation was carried out at 37 °C for 90 min in a shaking water bath. The lipoproteins were then layered under saline and isolated by ultracentrifugation for 16 h at 15 °C, at 170000 g for the β -VLDL and 80000 g for the chylomicron remnants, and then purified by gel-permeation chromatography.

Metabolic studies

Normal and cholesterol-fed rabbits were fasted overnight before use for studies *in vivo*. The animals were anaesthetized with an intramuscular injection of Ketamine and Xylazine (47 and 7 mg/kg body wt. respectively). Lipoproteins were injected via the marginal ear veins, and blood samples (1–2 ml) were collected at intervals from alternate ears into EDTA-containing tubes. The blood samples were centrifuged at 1000 g for 10 min to separate the plasma, and samples were taken for radioactivity measurements.

After collection of the last blood sample, the rabbits were killed by an intravenous injection of sodium pentobarbital and the livers were perfused *in situ*, with saline through the portal vein, to remove the residual blood. The whole organ was then weighed, and four tissue samples (2 g each) were taken at random from all lobes and processed for measurement of radioactivity, as previously described (Borensztajn and Kotlar, 1984). Bone marrow was obtained by cutting the ends of the femur and extruding the marrow with a wooden applicator. The marrow was weighed and processed as for the liver. In specified experiments, the radioactivity incorporated into the spleen was also determined. For the calculation of radioactivity remaining in the plasma, the total plasma volume was estimated to be 3.5% of body weight (Hussain et al., 1989). For the calculation of percentage radioactivity recovered in the bone marrow, the total bone marrow weight was assumed to be 2.2% of body weight (Hussain et al., 1989).

Other procedures

Separation of β -VLDL phospholipids was carried out by t.l.c. as previously described (Borensztajn et al., 1980). The spots were revealed by iodine vapour, and scraped off the plate for phosphorus determination. The recovery of the separated phospholipids was greater than 96%. Lipoprotein cholesterol was measured by the method of Abell et al. (1952), triacylglycerols were measured with a Synchron CX Analyzer (Beckman Instruments), and phospholipid phosphorus was measured by the method of Bartlett (1959). Lipoprotein agarose electrophoresis was done with the Ciba–Corning electrophoretic kit.

RESULTS

Figure 1 shows the results of an experiment in which [3 H]cholesterol-labelled β -VLDL were injected into normal and hypercholesterolaemic rabbits, and the radioactivity cleared from circulation was measured. In agreement with previous observations (Ross and Zilversmit, 1977; Kovanen et al., 1981), the clearance of β -VLDL from the circulation of hypercholesterolaemic rabbits was much delayed compared with

Figure 1 Plasma clearance and tissue uptake of β -VLDL by control and hypercholesterolaemic rabbits

Rabbit β -VLDL labelled *in vivo* with [3 H]cholesterol were injected into two normal rabbits (7.0 mg of cholesterol/kg body wt.) and two cholesterol-fed rabbits (3.0 mg of cholesterol/kg body wt.). Blood samples (1–2 ml) were taken from alternate ear veins at the indicated times, and the radioactivity remaining in the plasma was measured (a). Values from both animals are shown, and the curve is drawn through the mean value at each time point. At 60 min after injection the rabbits were killed, and four tissue samples were taken from each of the two rabbits for radioactivity determination (b). The liver-uptake results are expressed as means $\pm$ S.D. ($n = 8$), and the bone-marrow-uptake results as the average of measurements from two rabbits.

the control animals. Figure 1(a) shows that at 60 min after injection 60% of the injected dose remained in the circulation of the hypercholesterolaemic rabbits, compared with only 10% in the control animals. This difference is particularly striking, since the control rabbits were injected with more than twice the amount of β -VLDL (7.0 mg of cholesterol/kg body wt.) as the hypercholesterolaemic rabbits (3.0 mg of cholesterol/kg body wt.). The higher dose was injected into the control rabbits in

order to emphasize that the delay in β -VLDL clearance in cholesterol-fed rabbits was not due to the quantity injected, but rather to pre-existing hypercholesterolaemia. A major difference was also observed in the amount of radioactivity cleared from circulation by the livers. Figure 1(b) shows that, 60 min after injection of the [3 H]labelled β -VLDL, 17% of the radioactivity was recovered in the livers of the hypercholesterolaemic rabbits and 66.2% in that of the control animals. Because in the rabbit the bone marrow has been reported to participate in the uptake of remnants (Hussain et al., 1989), we also measured the radioactivity present in this tissue. The results in Figure 1(b) show that 14% and 2% of the injected dose was recovered in the bone marrow of the cholesterol-fed and control rabbits respectively. This difference in bone-marrow uptake between control and cholesterol-fed rabbits was not consistently observed when other types of remnant preparations were injected (see below).

To investigate the hypothesis that the reported rabbit deficiency in hepatic lipase activity could account for the defect in β -VLDL clearance from circulation by the liver, [3 H]cholesterol-labelled β -VLDL were treated with rat hepatic lipase, as described in the Materials and methods section, and re-injected with hypercholesterolaemic rabbits. Table 1 shows the lipid composition of the particles before and after exposure to hepatic lipase. In agreement with previous observations with rat chylomicrons (Borensztajn et al., 1988), hepatic lipase hydrolysed a large proportion of β -VLDL phospholipids (39%), whereas only 7.4% of the triacylglycerols were hydrolysed. Of particular interest was the finding that, whereas phosphatidylcholine and phosphatidylserine were markedly decreased, the lysophosphatidylcholine content of the particles increased 6-fold. Figure 2(a) shows that the hepatic-lipase-treated β -VLDL were rapidly cleared from circulation. At 60 min, only 19% of the injected dose remained in circulation, whereas 67.2 $\pm$ 17.7% of the dose was recovered in the liver and 8.4% in the bone marrow (Figure 2b).

Although β -VLDL consist predominantly of chylomicron remnants, we considered the possibility that the results obtained with these lipoproteins (Figure 1) might not necessarily be reproduced using other remnant preparations. Most studies aimed at elucidating the mechanism of chylomicron-remnant clearance from circulation by the liver have employed remnants prepared either by injecting chylomicrons into hepatectomized animals or by exposing chylomicrons to lipoprotein lipase *in vitro* (Guldur and Mayes, 1991). To determine whether the results obtained with β -VLDL could be reproduced by using chylomicron remnants generated *in vitro*, [3 H]cholesterol-labelled

Table 1 Composition of control and hepatic-lipase-treated β -VLDL

The ratio values and the phospholipid composition, expressed as % of the total phospholipids, are from measurements carried out in duplicate. Individual values as well as the means are shown. Abbreviations: PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; Sph, sphingomyelin; LPC, lysophosphatidylcholine.

β -VLDL	Phospholipid/cholesterol	Triacylglycerol/cholesterol	Protein/cholesterol	Phospholipid composition (%)				
				PC	PS	PE	Sph	LPC
Control	0.169	0.135	0.057	72.2	4.2	2.5	19.4	1.8
	0.161	0.129	0.055	72.0	4.0	2.7	19.7	1.6
	Mean	0.165	0.132	72.1	4.1	2.6	19.6	1.7
Hepatic lipase-treated	0.103	0.125	0.062	54.6	4.8	3.7	26.2	10.6
	0.099	0.120	0.060	48.4	4.9	3.9	31.2	11.9
	Mean	0.101	0.122	51.5	4.8	3.8	28.7	11.2

Figure 2 Plasma clearance and tissue uptake of hepatic-lipase-treated β -VLDL by hypercholesterolaemic rabbits

Rabbit β -VLDL labelled *in vivo* with [3 H]cholesterol were treated with rat hepatic lipase and injected into two cholesterol-fed rabbits (3.0 mg of cholesterol/kg body wt.). At various times after injection, blood samples were taken for determination of radioactivity in the plasma (a). Each point is the average of measurements carried out in two rabbits. At 60 min after injection the rabbits were killed and four tissue samples were taken from each of the two rabbits for radioactivity determination (b). The liver-uptake results are expressed as means $\pm$ S.D. ($n = 8$), and the bone-marrow-uptake results as the average of measurements from two rabbits.

Figure 3 Plasma clearance and tissue uptake of post-heparin-plasma-generated chylomicron remnants by control and hypercholesterolaemic rabbits

[3 H]Cholesterol-labelled rat chylomicrons were treated with rabbit post-heparin plasma and injected into two control and two cholesterol-fed rabbits (250 μ g of cholesterol/kg body wt.). Blood samples were taken at the indicated times, and plasma samples were counted for radioactivity (a). Values from both animals are shown, and the curve is drawn through the mean value at each time point. At 60 min after injection the rabbits were killed, and four tissue samples were taken from each of the two rabbits for radioactivity determination (b). The liver-uptake results are expressed as means $\pm$ S.D. ($n = 8$), and the bone-marrow-uptake results as the average of measurements from two rabbits.

Figure 4 Plasma clearance and tissue uptake of hepatic-lipase-treated chylomicrons by control and hypercholesterolaemic rabbits

[3 H]Cholesterol-labelled rat chylomicrons were treated with rat hepatic lipase and injected into two control and two hypercholesterolaemic rabbits (250 μ g of cholesterol/kg body wt.). The experimental conditions and the expression of results are as described in the legend of Figure 2.

rat lymph chylomicrons were treated with rabbit post-heparin plasma and the clearance of the resulting remnants from the circulation of control and hypercholesterolaemic rabbits examined. The results in Figure 3 demonstrate that, as was the case with β -VLDL (Figure 1), the clearance from the plasma, and hepatic uptake of the post-heparin plasma-generated chylomicron remnants, were impaired in the hypercholesterolaemic rabbits. Figure 3(a) shows that, after 60 min, only 21.5% of the injected radioactivity remained in circulation of the control animals, compared with 78% in the circulation of the hypercholesterolaemic rabbits. Figure 3(b) shows that the radioactivity recovered in the livers of the control and hypercholesterolaemic rabbits was respectively 62% and 12% of the injected dose. Less than 1% of the injected radioactivity was recovered in the spleens of both groups of animals, indicating that the lipoproteins were not removed from circulation by a non-specific mechanism. It is noteworthy that, whereas both lipoprotein lipase and hepatic lipase activities are normally present in post-heparin plasma, the activity of the hepatic lipase towards chylomicrons is inhibited by other plasma lipoproteins, especially high-density lipoproteins (Kubo et al., 1980). Thus remnants prepared with post-heparin plasma are essentially lipoprotein-lipase-generated remnants and, in light of the results with β -VLDL (Figures 1 and 2), their delayed clearance in the cholesterol-fed animals may also be ascribed to the rabbit's deficiency in hepatic lipase activity.

Previous work from this laboratory has shown that treatment of chylomicrons with hepatic lipase alone is capable of generating remnant-like particles that are readily removed from circulation by the liver and which can compete with remnants generated *in vivo* for uptake by hepatocytes (Borensztajn et al., 1988). In Figure 4 are shown the results of an experiment in which [3 H]cholesterol-labelled rat chylomicrons were treated with hepatic lipase, as described in the Materials and methods section, and then injected into normal and hypercholesterolaemic rabbits. Under the conditions used, approx. 40% of the chylomicron phospholipids were hydrolysed, as previously reported (Borensztajn et al., 1988). The clearance of the particles from circulation is shown in Figure 4(a). Like the hepatic-lipase-treated β -VLDL (Figure 2), the hepatic-lipase-treated chylomicrons were rapidly removed from the circulation of the

hypercholesterolaemic animals. After 60 min, $85 \pm 3.2\%$ of the injected dose was recovered in the livers and 13% in the bone marrow. Similar results were obtained when chylomicrons were treated with purified rat hepatic lipase, instead of heparin-releasable hepatic lipase (results not shown).

DISCUSSION

The data presented here show that treatment of β -VLDL *in vitro* with hepatic lipase reverses the clearance defect responsible for the accumulation of these remnants in hypercholesterolaemic rabbits. The results are in agreement with, and extend, the observations by Sultan et al. (1989, 1990), who reported that when lipoprotein-lipase-generated rat chylomicron remnants are treated with hepatic lipase the rate of their uptake by isolated rat hepatocytes, and by the liver *in vivo*, is greatly increased. Although Kovanen et al. (1981) suggested that the defective clearance of β -VLDL from circulation of hypercholesterolaemic rabbits could be explained by the saturation of down-regulated hepatic lipoprotein receptors, an alternative hypothesis is suggested by our results. As it has been established that the rabbit is deficient in hepatic lipase activity (Clay et al., 1989; Warren et al., 1991), it is conceivable that it is not the lipoprotein receptors, but the hepatic lipase, that is saturated by the high circulating lipoprotein levels in the cholesterol-fed rabbit.

Although the data presented here demonstrate that hepatic lipase *in vitro* is capable of converting β -VLDL (Figure 2) and chylomicrons (Figure 4) into particles that are efficiently taken up even by the liver of hypercholesterolaemic rabbits, they do not in themselves demonstrate a role for this enzyme in the clearance of remnants under physiological conditions *in vivo*. There is, however, ample evidence supporting such a role (see the introduction). Specifically, studies in which hepatic lipase activity is inhibited *in vivo* by specific antibodies show an inhibition of remnant clearance (Goldberg et al., 1982; Daggy and Bensadoun, 1986; Sultan et al., 1990). Our results presented here and elsewhere (Borensztajn et al., 1988) show that *in vitro* hepatic lipase alone is able to generate chylomicron remnants which are rapidly taken up by the liver. This does not appear to be the case *in vivo*. Studies with isolated rat livers perfused with intact chylomicrons (Gardner and Mayes, 1978; Borensztajn and Kotlar, 1984), as well as observations of individuals with hyperchylomicronaemia due to lipoprotein lipase or apolipoprotein CII deficiency (Taskinen, 1987), have clearly established that, *in vivo*, chylomicrons cannot be efficiently cleared from the circulation by the liver unless they are first acted on by lipoprotein lipase. Thus, both lipoprotein lipase and hepatic lipase would seem necessary for the efficient generation and hepatic clearance of chylomicron remnants *in vivo*. A reasonable hypothesis to explain the joint roles of lipoprotein lipase and hepatic lipase includes the sequential action of these enzymes in the generation of remnants before their endocytosis by hepatocytes. First, the action of lipoprotein lipase on chylomicrons on the surface of the capillary endothelium transforms the lipoproteins into particles that can bind with high affinity to the hepatocyte membrane within the space of Disse. Only when these particles become immobilized on the membrane surface, in proximity to hepatic lipase, is this enzyme capable of modifying them further to generate remnants which can undergo endocytosis by the hepatocyte. According to this scheme, in the hypercholesterolaemic rabbit the lipoprotein-lipase-modified chylomicrons are capable of binding to the hepatocyte surface, but, because of the deficiency in hepatic lipase activity, they are not further modified at a rate sufficient to enable them to be rapidly

internalized by the hepatocytes. Consequently they remain in circulation, where they are continually exposed to lipoprotein lipase, resulting in the formation of triacylglycerol-poor cholesterol-rich β -VLDL. Treating β -VLDL or chylomicrons with hepatic lipase *in vitro* allows these lipoproteins to by-pass the initial binding step and to be rapidly internalized by the liver cells (Figures 2 and 4).

The hepatic-lipase-mediated changes of remnants bound to the hepatocyte membrane leading to their endocytosis are not known. The results in Table 1 show that treatment of β -VLDL with hepatic lipase resulted in a significant depletion of the phospholipid content of the particles. Similar phospholipid depletion is also observed when rat chylomicrons are treated with hepatic lipase (Borensztajn et al., 1988). We have previously proposed (Borensztajn et al., 1988) that, after treatment of chylomicrons with hepatic lipase, receptor-binding domains of the apoprotein E on the surface of the chylomicrons become exposed. As a result these particles would more readily interact with specific receptors on the surface of the hepatocytes, leading to their endocytosis. The validity of this hypothesis, as well as the identity of the putative receptor(s), remains to be determined. The possibility that hepatic lipase bound to the surface of lipoproteins (e.g. after treatment of chylomicrons or β -VLDL with hepatic lipase or post-heparin plasma) may serve as the ligand to binding sites on the surface of hepatocytes is unlikely. Work from our laboratory has shown that emulsions of lipids extracted from hepatic-lipase-treated chylomicrons, which are free of hepatic lipase, as well as other surface proteins, are readily cleared from circulation by the liver (Borensztajn and Kotlar, 1990). Further, hepatic-lipase-treated chylomicrons that are subsequently exposed to Pronase to remove all their surface proteins retain unchanged their capacity to be rapidly taken up by the liver (S. Chang and J. Borensztajn, unpublished work).

In conclusion, although it is well established that lipoprotein lipase activity *in vivo* is necessary for the generation of chylomicron remnants, the results of the present investigation support the notion that their efficient clearance from circulation by the liver requires, in addition, the action of hepatic lipase. Recently mice unable to express hepatic lipase, as a result of the inactivation of their hepatic lipase gene, have been generated (N. Maeda, personal communication). These experimental animals should be of great value in helping to elucidate the role of this enzyme in chylomicron-remnant uptake by the liver.

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