Uptake of chylomicron remnants by the liver: further evidence for the modulating role of phospholipids

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Abstract Rat lymph chylomicrons were treated with rat heparin-releasable hepatic lipase (HL) or with bovine milk lipoprotein lipase (LPL). The ability of the resulting particles to be taken up by the liver in vivo was assessed following their infusion into the portal vein of partially hepatectomized animals. The following observations were made: *a*) the rate of phospholipid depletion, relative to the rate of triglyceride hydrolysis, induced by HL was two- to threefold higher than that observed for LPL; *b*) the depletion of at least 57% of phospholipids from the surface of HL-treated chylomicrons caused no major alterations in the apoprotein profile of the particles; *c*) for the same extent of triglyceride hydrolysis, HL-treated chylomicrons were taken up by liver at a rate significantly higher ($P < 0.005$) than LPL-treated particles; *d*) the liver uptake of HL-treated chylomicrons was competitively inhibited by endogenously generated chylomicron remnants, indicating that these two types of lipoproteins share the same process of recognition and uptake by liver cells. It is concluded that the in vivo changes in phospholipid content, or composition, on the surface of chylomicrons during their transformation into remnants, modulate the differentiation of these two particles by the hepatic remnant receptor.—**Borensztajn, J., G. S. Getz, and T. J. Kotlar.** Uptake of chylomicron remnants by the liver: further evidence for the modulating role of phospholipids. *J. Lipid Res.* 1988. **29:** 1087–1096.

Supplementary key words hepatic lipase • lipoprotein lipase • hepatic remnant receptor

Chylomicron remnants are lipoproteins formed in the vascular space of extrahepatic tissues as a result of the partial catabolism of chylomicrons by lipoprotein lipase (1). Once formed, remnants are rapidly removed from the circulation by the liver (2), a process of major importance in the regulation of plasma cholesterol levels. Remnants carry to the liver the plasma cholesterol of dietary origin that is absorbed associated with the chylomicrons (3). This cholesterol may suppress the expression of LDL receptors on liver and thus affect plasma LDL concentrations (3) and it may cause the suppression of hepatic cholesterol synthesis (4–7), thus reducing the amount of endogenous cholesterol that is secreted into the plasma.

The hepatic uptake of remnants occurs by endocytosis following their binding to receptors on the sinusoidal surface of liver cells (8, 9). Studies with the isolated perfused rat liver (10–12) and isolated liver membranes (11, 13, 14) have suggested that the hepatic remnant receptor recognizes the apoprotein E (apoE) normally present on the surface of the lipoprotein. Two types of apoE-binding receptors have been reported present on the liver cell membranes. One is the well-characterized LDL (B/E) receptor which also binds apoB (15). The other receptor appears to be specific for apoE alone (10, 12, 16, 17). Chylomicron remnants have been reported to bind with high affinity to the LDL (B/E) receptor of isolated hepatocytes (18) and isolated liver membranes (19, 20). However, studies in vivo and with the isolated perfused liver have shown that the hepatic uptake of chylomicron remnants is not mediated by this receptor. In man and experimental animals with absent, defective, or reduced numbers of LDL receptors, the capacity of the liver to take up chylomicron remnants is unchanged (8, 20–23), suggesting that a different receptor mediates this uptake, presumably the receptor that specifically binds apoE (24).

Chylomicrons, unlike their remnants, are not readily removed from the circulation by the liver, indicating that the specificity of the remnant receptor does not extend to the apoE on the surface of the remnant precursor (3, 25–27). It is only after chylomicrons are partially degraded that the apoE on the surface of the resulting remnant particle binds with high affinity to the receptor. This differentiation by the receptor between the apoE of chylomicrons and their remnants suggests that composition, and perhaps conformation, changes on the surface of chylomicrons during their transformation into remnants "expose" the apoprotein for

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recognition by the receptor. These surface changes involve the loss, by transfer to other lipoproteins, of phospholipids, as well as apoproteins A-I, A-IV and C (28, 29). Some phospholipids, in addition, are hydrolyzed by the phospholipase A_1 action of lipoprotein lipase.

The process whereby the liver remnant receptor differentiates between chylomicrons and their remnants has been the subject of considerable investigation (11, 12, 16, 17, 30, 31). Work from this laboratory showed that this distinction is not determined by the differences in apoprotein composition of the particles (32). We have proposed that it is the difference in the content and/or composition of the surface phospholipids of the particles that determines how they are discriminated by the liver (33, 34). This suggestion was based on the observations that chylomicrons exposed to phospholipase A_2, and which appear to retain their apoprotein components are, like chylomicron remnants, readily taken up by the liver in vivo or during perfusion of the isolated organ.

In the present investigation we used the more physiological heparin-releasable hepatic lipase, which like lipoprotein lipase has triglyceride hydrolase and phospholipase A_1 activities (35), to examine further the role of phospholipids in the hepatic differentiation of chylomicrons and their remnants. Chylomicrons were treated with either hepatic lipase or lipoprotein lipase and the capacity of the resulting particles to be taken up by the liver in vivo was assessed. We report the following observations. *a*) The rate of hydrolysis of chylomicron phospholipids by hepatic lipase, relative to the rate of hydrolysis of triglycerides, was significantly greater than that observed with lipoprotein lipase. *b*) Chylomicrons exposed to either enzyme were taken up by the liver. However, the magnitude of their uptake was not dependent on the degree of core triglyceride hydrolysis, nor did the uptake require a change in the apoprotein profile of the particles. *c*) The in vivo uptake of hepatic lipase-treated chylomicrons by the liver was competitively inhibited by chylomicron remnants. These results provide compelling evidence that changes in the phospholipid composition on the surface of the particles modulate the hepatic differentiation between chylomicrons and their remnants by the remnant receptor. The role of hepatic lipase in the generation and uptake of chylomicron remnants by the liver is discussed.

MATERIALS AND METHODS

Preparation of chylomicrons and chylomicron remnants

The thoracic ducts of male Sprague-Dawley rats (180–220 g) were cannulated and the animals were fed 3 ml of a mixture of corn oil (1 ml) and egg yolk (2 ml) by stomach intubation. Unless otherwise specified, 60 μCi of [1,2-^{3}H]cholesterol (sp act 50 Ci/mol; Amersham Corp.) and 30 μCi of [1-^{14}C]palmitic acid (sp act 58 Ci/mol; Amersham Corp.) were added to the mixture. The lymph was collected during the following 12 hr at room temperature in the absence of preservatives. Large ($S_f > 400$) and small ($S_f < 400$) chylomicrons were separated from the lymph as previously described (34). Remnants of large chylomicrons were prepared in vivo in functionally hepatectomized rats essentially as previously described (32) except that the hepatectomized rats were injected with 50 mg of chylomicron triglycerides (instead of 100 mg) and exsanguinated 20 min later.

Enzymes

To obtain hepatic lipase, male Sprague-Dawley rats (200–220 g) were anesthetized with sodium pentobarbital (45 mg/kg, injected intraperitoneally), and their livers were isolated and perfused through the portal vein with 0.15 M NaCl at 37°C for 2 min in order to remove blood from the organ. This was followed by a 1-min perfusion with the same solution containing 10 U of heparin/ml, at a flow rate of 14 ml/min. The effluent containing the heparin-releasable hepatic lipase was collected from the superior vena cava and used immediately. Bovine milk lipoprotein lipase, purified by affinity chromatography on heparin-Sepharose (36), was a generous gift from Dr. N. M. Reddy.

Lipolysis of chylomicrons

Small chylomicrons ($S_f < 400$) were treated with the lipolytic enzymes in assay systems containing the following. For hepatic lipase, 4.2 mg chylomicron triglycerides; 58 mg bovine serum albumin (Sigma); 0.15 ml 0.7 M Tris–HCl buffer, pH 8.6; 0.16 ml liver perfusate as the source of hepatic lipase; and 0.15 M NaCl to 1 ml. For milk lipoprotein lipase, 3 mg chylomicron triglyceride; 114 mg bovine serum albumin; 0.15 ml Tris–HCl buffer, pH 8.6; 0.15 ml barbital buffer (5 mM, pH 7.4) containing the purified enzyme; 1.6 U of heparin; 0.15 M NaCl to 1 ml. Control incubations contained all assay components except the lipolytic enzymes. The enzyme reaction was started by adding the enzyme to the reaction mixture and incubation was carried out at 37°C in a shaking water-bath. Preliminary experiments established that the assay conditions used were optimal for each enzyme and that the albumin concentrations did not limit the rate of lipolysis. At specified time intervals aliquots were removed and, where indicated, mixed with 10% (v/v) rat serum and immediately chromatographed on a gel filtration column (1.5 × 50 cm) of 2% agarose (Bio-Gel A-50 m, 50–100 mesh, Bio-Rad). The eluant was 0.15 M NaCl, and the pressure head was 60 cm. The chylomicrons eluted with the void volume.

Denaturation of chylomicrons

This was achieved by incubating 1 volume of small chylomicrons (15 mg triglycerides/ml) with 1 volume of 50%

glutaraldehyde for 2 hr at 37°C. The mixture was then extensively dialyzed for 18 hr against distilled water followed by dialysis against 0.15 M NaCl. The glutaraldehyde-treated chylomicrons did not coalesce or precipitate and remained in a homogeneous suspension. Although by these gross criteria these particles were indistinguishable from untreated chylomicrons, they were clearly altered as shown by their dramatically increased hepatic clearance (see Results).

Hepatic uptake of chylomicrons and remnants

Intact and partially hepatectomized rats (150–180 g) anesthetized with sodium pentobarbital were used. In the experiments using intact rats, the animals were injected through a tail vein with the labeled lipoproteins and, at specified times, their livers were perfused through the portal vein with 0.15 M NaCl for 1 min at room temperature to remove lipoproteins that might have been trapped in the sinusoidal spaces. The entire right lateral lobe of the liver was then taken for the determination of the radioactivity incorporated into the tissue. In preliminary experiments we determined that the radioactivity distribution was homogeneous throughout the liver.

Functional partial hepatectomy was achieved by ligating the blood vessels supplying the median and caudate liver lobes of ad libitum fed animals. Only the right lateral lobe, accounting for about 20% of the total liver weight, remained with its circulation intact. The uptake of small chylomicrons or remnants by the right lateral lobe was measured by infusing the labeled lipoproteins directly into the portal vein. To maximize mixing with the circulating blood, the lipoproteins were infused (0.5 ml in 30 seconds) against the blood flow, and at a point about 1 cm from the hilum. At the end of the infusion, carried out with the aid of a peristaltic pump (Gilson, Middleton, WI), 30 sec was allowed to elapse before the circulation was cut off and the lobe was perfused with 0.15 M NaCl for 30 sec through the portal vein in order to wash out entrapped particles. The whole right lateral lobe was then taken for the measurement of radioactivity incorporation. In certain experiments large chylomicrons were injected through the tail vein of partially hepatectomized rats 1 min after the operation, followed 8 min later by infusion of labeled lipoproteins into the portal vein, as described above.

Other procedures

Analytical electrophoresis of the apoproteins was carried out on SDS/10% (w/v) polyacrylamide gels (37). Densitometric scanning of the gels was done at 633 nm using a laser densitometer (LKB Ultrascan LX; Bromma, Sweden). Triglycerides were measured using a modification of the method of Bucolo and David (38) (Lancer Stat kit; Lancer, St. Louis). Cholesterol and phospholipid contents were measured according to the methods of Abell et al. (39) and Bartlett (40), respectively. The incorporation of radioactivity into the livers was determined as previously described (33). The significance of differences between means was calculated using Student's t test.

RESULTS

Hydrolysis of chylomicron triglycerides and phospholipids by hepatic lipase and lipoprotein lipase

Fig. 1 shows the results obtained when a single batch of small chylomicrons was treated with heparin-releasable rat hepatic lipase or purified bovine milk lipoprotein lipase. Both enzymes displayed triglyceride hydrolase and phospholipase activities. The rate of phospholipid depletion induced by the hepatic lipase, relative to the rate of triglyceride hydrolysis, was significantly higher than that observed with lipoprotein lipase. Similar findings were obtained when, instead of milk lipoprotein lipase, the comparison was carried out between the heparin-releasable hepatic lipase and heparin-releasable rat heart lipoprotein lipase (results not shown). The proportions of triglycerides and phospholipids hydrolyzed by the lipases varied with different batches of chylomicrons. However, the rate of phospholipid depletion, relative to the rate of triglyceride hydrolysis, was invariably significantly higher in the particles treated with hepatic lipase than with lipoprotein lipase. Following treatment of chylomicrons with either lipase, the particles remained in a homogeneous suspension and did not aggregate or coalesce; they retained their spherical shape, as determined by electron microscopy (not shown); their apoprotein composition remained essentially unaltered (see below); and their elution characteristics when applied to a 2% agarose column (see Methods section) were indistinguishable from untreated particles.

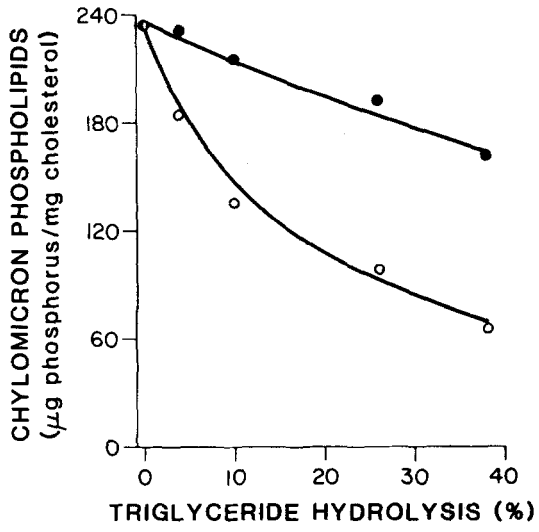

Fig. 1. Hydrolysis of chylomicron triglycerides and phospholipids by hepatic lipase (○) and lipoprotein lipase (●). Small chylomicrons from a single batch were treated with the lipases as described in Methods. Each point represents the average of two measurements.

Liver uptake of chylomicrons treated with hepatic lipase and lipoprotein lipase infused into the portal vein

Small chylomicrons doubly labeled with ^{14}C-labeled triglycerides and [^{3}H]cholesterol were treated with either hepatic lipase or lipoprotein lipase, infused into the portal vein, as described in Methods, and the uptake of the particles by the right lateral liver lobe was measured. The results (**Fig. 2**) show that chylomicrons treated with hepatic lipase were taken up by the liver significantly more efficiently than chylomicrons treated with lipoprotein lipase. For example, chylomicrons depleted of about 24% of their triglycerides by hepatic lipase were taken up fourfold more efficiently than control chylomicrons, whereas no significant difference in hepatic uptake was observed between control and lipoprotein lipase-treated chylomicrons depleted of about 26% of their triglycerides. A significant ($P < 0.05$), albeit small increase in uptake of lipoprotein lipase-treated chylomicrons was observed only when the particles were depleted of about 32% of their triglyceride core (Fig. 2). These findings indicate that the differences in liver uptake between chylomicrons treated with hepatic lipase and lipoprotein lipase cannot be ascribed to the extent of triglyceride depletion of the particles. Instead, the results in Fig. 2 indicate that this difference can be ascribed to the extent of phospholipid depletion (shown in parentheses) of the particles. A minimum phospholipid depletion of 25–30% was required before a significant increase in hepatic uptake of either lipase-treated chylomicrons could be demonstrated.

It is noteworthy that the ratio of [^{3}H]cholesterol/^{14}C-labeled fatty acid recovered in the livers was similar to that of the infused chylomicrons at all points measured, indicating that the lipoproteins were not degraded prior to their uptake, but were taken up as a unit. For example, the hepatic lipase-treated particles depleted of 24% of their triglycerides had a ^{3}H/^{14}C ratio of 3.0 ± 0.24. The ^{3}H/^{14}C ratio of counts recovered in the liver following infusion of the particles was 2.80 ± 0.24 (mean ± SD, n = 4).

Liver uptake of remnants and hepatic lipase-treated chylomicrons injected into the systemic circulation

The capacity of the liver to efficiently remove hepatic lipase-treated chylomicrons from the circulation could also be shown when these lipoproteins were injected into the systemic circulation of intact rats. The results in **Fig. 3** show that when hepatic lipase-treated chylomicrons (depleted of 54% of their surface phospholipids) were injected into a tail vein of rats, about 83% of the injected cholesterol label was recovered in the liver within 4 min (column A). Similar results were obtained when chylomicron remnants,

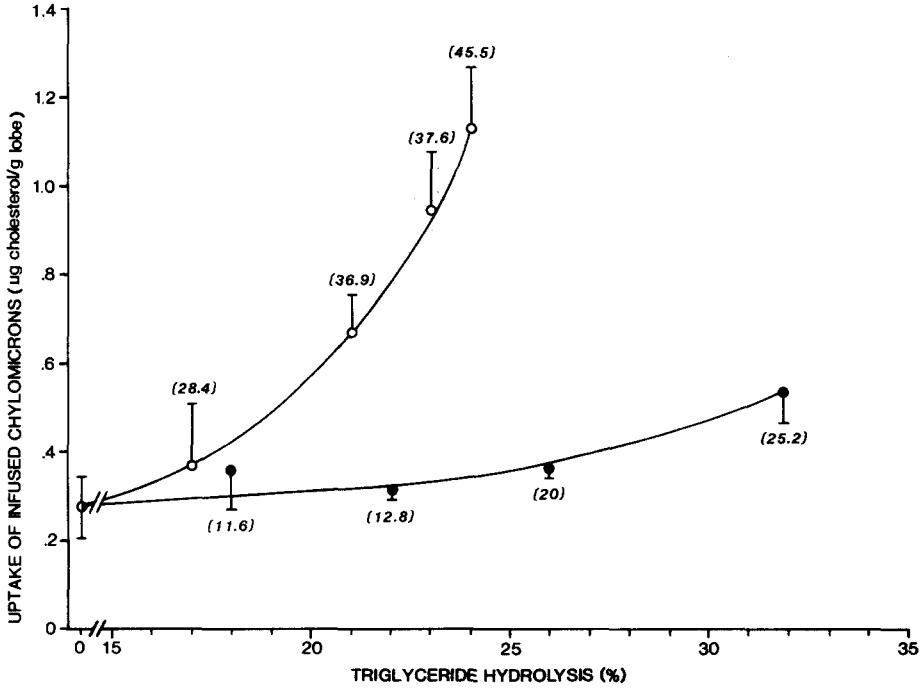

Fig. 2. Liver uptake of chylomicrons treated with hepatic lipase (○) or lipoprotein lipase (●). Control and lipase-treated chylomicrons doubly labeled with [^{3}H]cholesterol and ^{14}C-labeled fatty acids were infused (30 μg cholesterol) into the portal vein of partially hepatectomized rats, and the radioactivity incorporated into the intact right lateral lobe was measured as described in Methods. Each point is the result of infusions into four rats, and is expressed as the mean ± SD. The extent of the hydrolysis of chylomicron triglycerides and phospholipids (shown in parentheses) for each sample was determined in duplicate.

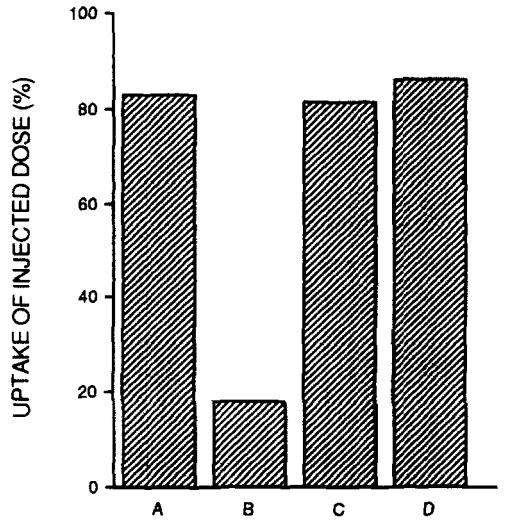

Fig. 3. Liver uptake of lipoproteins injected into the systemic circulation. (Doubly labeled ([^{3}H]cholesterol and ^{14}C-labeled fatty acid) hepatic lipase-treated chylomicrons (A), control chylomicrons (B), chylomicron remnants (C), and glutaraldehyde-treated chylomicrons (D) (in 0.7 ml of 0.15 M NaCl) were injected into a tail vein. Each dose contained 200 μg of cholesterol. Four minutes later, the ^{3}H-labeled cholesterol incorporated into the livers was measured as described in Methods. Each result represents the average of two livers.

prepared as described in the Methods section, were injected into the systemic circulation (column C). In contrast, when intact chylomicrons were injected into the rats, only 18% of the injected cholesterol label was recovered in the liver (column B). These observations alone are not sufficient to demonstrate that phospholipid-depleted chylomicrons are taken up by the same cell and mechanism as in vivo-generated remnants. Column D shows the equally rapid hepatic uptake (presumably by Kupffer cells) of chylomicrons denatured by incubation with glutaraldehyde. The extensive depletion of chylomicron surface phospholipids by hepatic lipase might have produced similarly "damaged" particles which could be taken up nonspecifically by the liver.

Inhibition of the liver uptake of hepatic lipase-treated chylomicrons by endogenously produced chylomicron remnants

It is well established that chylomicron remnants are removed from circulation mainly by hepatocytes through a receptor-mediated mechanism (see Introduction). To determine whether hepatic lipase-treated chylomicrons were taken up by the liver by a similar mechanism, or alternatively by a nonspecific process, we took advantage of the observation made in our laboratory (Borensztajn, J., and T. J. Kotlar, unpublished results) that, in partially hepatectomized rats injected intravenously with chylomicrons, all the remnants generated in the extrahepatic tissues are diverted to the hepatocytes of the remaining intact lobe. In these animals, injected with appropriate amounts of chylomicrons, the capacity of intact liver cells to take up remnants can become saturated.

Fig. 4 shows the results obtained when partially hepatectomized rats were injected intravenously with saline or intact chylomicrons, and 8 min later, when the endogenously generated remnants were being taken up by the liver cells at close to their maximum rate, doubly labeled hepatic lipase-treated chylomicrons, depleted of 45% of their phospholipids, were infused into the portal vein. At the dose infused, there was a 25% inhibition ($P < 0.005$) in uptake of the phospholipid-depleted chylomicrons by the intact right lateral lobe (column A). A similar inhibition ($P < 0.005$) was observed when, instead of phospholipid-depleted chylomicrons, isolated remnants were infused into the liver lobe (column B). These results indicate that the hepatic lipase-treated chylomicrons, like the infused remnants, were taken up by a common mechanism, and by the same cells, as the endogenous remnants. Had the hepatic lipase-treated chylomicrons been taken up by a non-specific mechanism, no inhibition of their uptake would have been observed. This conclusion is supported by the observation that when chylomicrons denatured by treatment with glutaraldehyde were infused into the intact liver lobe their uptake was not inhibited by the endogenously generated remnants (column C).

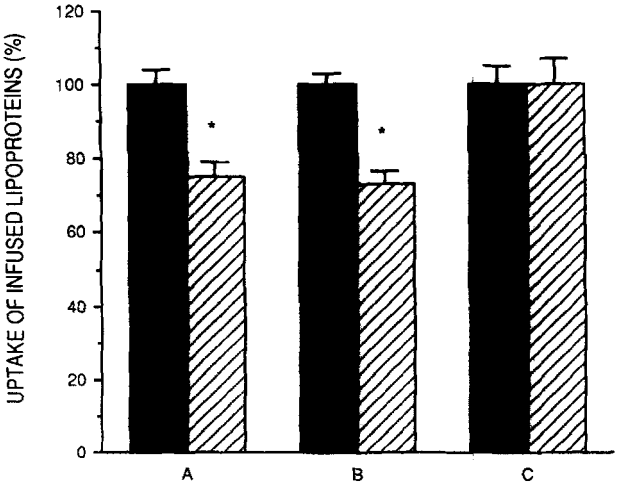

Fig. 4. Ability of chylomicron remnants, hepatic lipase-treated chylomicrons, and denatured chylomicrons to compete with endogenous remnants for uptake by the liver in vivo. Groups of five partially hepatectomized rats were injected through a tail vein with either saline (■) or unlabeled large chylomicrons (200 μg cholesterol) (▨). Eight minutes later, the animals were infused for 30 sec into the portal vein with [^{3}H]cholesterol-labeled hepatic lipase-treated chylomicrons (A), chylomicron remnants (B), and chylomicrons treated with glutaraldehyde (C), all at concentrations equivalent to 1 mg of intact chylomicron triglycerides. The 100% uptake values correspond to 92, 82, and 170 μg of intact chylomicron triglycerides taken up/g liver lobe, respectively. The radioactivity incorporated into the intact liver lobe was measured as described in Methods. The results are expressed as means ± SD. The * indicates a significant difference ($P < 0.005$) between saline- and chylomicron-injected groups.

Lipid and apoprotein composition of hepatic lipase-treated chylomicrons

The remnants used in this study had a phospholipid/cholesterol ratio of 0.09 compared to the corresponding ratio of 2.74 in the large chylomicrons from which the remnants were derived. This depletion of phospholipids was accompanied by marked changes in the apoprotein profile of the particles as previously reported (32). Other studies on the generation of chylomicron remnants in vivo have also shown that the depletion of phospholipids from the surface of the lipoproteins is accompanied by changes in their apoprotein composition (28, 29). We therefore examined whether the large depletion of phospholipids from the chylomicrons treated with hepatic lipase in vitro also resulted in changes in the apoprotein composition of the particles. **Table 1** shows the lipid ratios of control chylomicrons, and of chylomicrons depleted of 57% and 18% of their phospholipids by treatment with, respectively, hepatic lipase and lipoprotein lipase. In **Fig. 5** the apoprotein profiles of these particles are shown and in **Table 2** the ratios of apoproteins B/E and B/C obtained by scanning this gel. Because apoB is not removed from chylomicrons during lipolysis, it can be used as an internal standard to quantitate other apoproteins. It is apparent (Fig. 5 and Table 2) that the apoprotein profile of the phospholipid-depleted chylomicrons remained essentially unaltered, compared to control lipoproteins. It cannot be concluded from these observations, however, that, following infusion into the portal vein, the apoprotein profile of the hepatic lipase-treated chylomicrons (Fig. 3) remained unchanged. In this study chylomicrons were treated with hepatic lipase and lipoprotein lipase in an assay system devoid of plasma lipoproteins. The possibility could not be ruled out, therefore, that following their injection into the circulation the chylomicron apoprotein profiles would be altered by exchange with, or by transfer to, other plasma apolipoproteins. To examine this possibility we determined the apoprotein profiles of the control and phospholipid-depleted chylomicrons after incubation with rat serum. The results in Fig. 5 show that even in the presence of other plasma lipoproteins the apoprotein profile of the lipase-treated chylomicrons remained unchanged. The lipid ratios of the lipase-treated chylomicrons exposed to serum also remained unchanged (not shown). These results indicate that, following their injection into the circulation, the lipase-treated chylomicrons retained their lipid and apoprotein compositions, and that the rapid uptake of the phospholipid-depleted chylomicrons was not accompanied by changes in the apoprotein profile of the particles.

TABLE 1. Lipid weight ratios of chylomicrons treated with hepatic lipase and lipoprotein lipase

	Triglyceride/ Cholesterol	Phospholipid/ Cholesterol	Triglyceride/ Phospholipid
Control	29.6	5.85	5.06
Hepatic lipase-treated	21.9	2.50	8.76
Lipoprotein lipase-treated	21.9	4.80	4.56

Small chylomicrons from a single batch were treated with the lipases as described in Methods. Each value is the average of duplicate determinations.

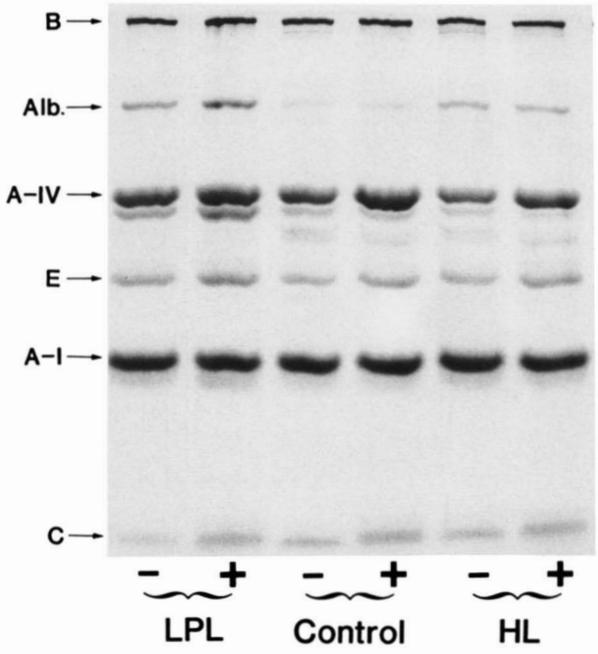

Fig. 5. SDS-polyacrylamide gel electrophoresis of apoproteins from chylomicrons treated with hepatic lipase or lipoprotein lipase. After the treatment of small chylomicrons with the lipases as described in Methods, rat serum was added to half of the incubation mixtures (10% (v/v)). The control and lipase-treated chylomicrons not exposed (−) or exposed (+) to serum were then separated by filtration chromatography as described in Methods. The protein from aliquots of particles containing 25 μg cholesterol was applied to each gel.

DISCUSSION

The search for the determinants of chylomicron remnant recognition by the liver has been pursued with the use of isolated liver membranes (19, 20), isolated hepatocytes (18) and the isolated perfused rat liver (11, 12, 16, 30, 31). Some of the results obtained using these isolated systems have not been consistent with observations made in vivo. For example, isolated liver membranes and isolated hepatocytes appear to bind chylomicron remnants with high affinity to the LDL (B/E) receptor, in contrast to the in vivo situation where the uptake of remnants is independent of this receptor (see Introduction). The isolated liver system perfused with plasma-free medium also has limitations for the investigation of remnant recognition, particularly when the role of

TABLE 2. Apoprotein ratios of chylomicrons treated with hepatic lipase or lipoprotein lipase

	Serum	ApoE/ApoB	ApoC/ApoB
Control	–	0.50	0.37
Control	+	0.50	0.37
Lipoprotein lipase	–	0.48	0.31
Lipoprotein lipase	+	0.55	0.31
Hepatic lipase	–	0.50	0.35
Hepatic lipase	+	0.52	0.34

The apoprotein ratios were determined by densitometric scanning of the gel shown in Fig. 5 as described in Methods.

apoproteins is being considered. Isolated livers continually secrete lipoproteins which, by exchange or transfer of their apoproteins, may alter the composition of the lipoproteins being perfused. Thus, the apoprotein composition of the lipoproteins that are taken up by the liver cells may be quite different from that of the particles present in the perfusate.

In the present investigation lipoproteins were infused directly into the portal vein of partially hepatectomized rats. This in vivo system offers several advantages for the study of the mechanism of hepatic recognition of chylomicron remnants: *a*) the remaining intact liver mass retains its anatomical and physiological integrity; *b*) the animal retains its capacity to generate remnants in the systemic vascular space; *c*) the remnants formed are diverted to, and can saturate the removal process of, the greatly reduced intact liver mass, thus allowing competition studies with exogenous lipoproteins; *d*) the infused lipoproteins can freely exchange their apoproteins and lipid components with the circulating plasma lipoproteins, and any resulting alteration in the composition of the particles can be established following their incubation with plasma in vitro.

The results obtained with this in vivo system are consistent with previous observations (33, 34) that indicated that phospholipids play a major role in modulating the process whereby the liver differentiates between chylomicrons and remnants. As a source of phospholipase activity we took advantage of the high phospholipase A_1 activity of heparin-releasable hepatic lipase (Fig. 1), in lieu of phospholipase A_2 used in previous studies, and confirmed that the removal of phospholipids from the surface of chylomicrons transforms these lipoproteins into remnant-like particles that are efficiently removed intact from the circulation by the liver. Because in the normal process of chylomicron degradation phospholipids are not preferentially removed from the surface, it was important to demonstrate that treatment with hepatic lipase did not denature or transform the lipoproteins into "foreign" particles that would be taken up by the Kupffer cells instead of the parenchymal cells. This was done by comparing the uptake of the hepatic lipase-treated particles with *a*) isolated chylomicron remnants and *b*) chylomicrons denatured by glutaraldehyde, all in the presence of endogenously generated chylomicron remnants. The uptake of the infused hepatic lipase-treated chylomicrons as well as that of isolated chylomicron remnants was inhibited by the endogeously generated chylomicron remnants, whereas, under the same experimental conditions, the uptake of the infused denatured chylomicrons was unaffected (Fig. 4). These results demonstrate that chylomicrons were not transformed into "foreign" particles by treatment with hepatic lipase, and that they were taken up by the same cells and by a similar mechanism as chylomicron remnants.

In the normal process of remnant formation in the vascular space, both apoproteins and phospholipids are lost from the chylomicron surface (28, 29). Our finding (Fig. 5 and Table 2) that the apoprotein composition is conserved when chylomicrons are depleted of at least 57% of their phospholipids by hepatic lipase is in agreement with the conclusion of previous reports that these two surface constituents can be released from lipoproteins independently (34, 41, 42). Furthermore, studies with VLDL exposed to membrane-bound lipoprotein lipase have indicated that the removal of surface apoproteins is related to the decrease in surface area induced by lipolysis of the triglyceride core (43). Presumably it is this reduction in surface area that is involved in the characteristic changes in apoprotein profile during the in vivo formation of remnants from chylomicrons. In the present study, a loss of 57% of surface phospholipids induced by hepatic lipase was accompanied by only about half as much loss of the triglyceride core. Assuming that the average particle had a core composed of 95% triglycerides, a shell thickness of 21.5 Å (44), and a diameter of 400 Å, it can be calculated that such a loss of triglyceride core would induce only a 16% reduction in surface area, which apparently was not sufficient to cause significant losses of apoproteins.

It has been claimed (11, 16, 17, 30, 31) that the differences between apoprotein compositions of chylomicrons and remnants (in particular the differences in their apoprotein C content) are responsible for their differential uptake by the liver. The results of this and a previous (32) investigation brings this suggestion into question. Hepatic lipase-treated chylomicrons, with apoprotein composition similar to that of control chylomicrons, were, as in vivo-generated remnants, efficiently taken up by the liver. Even after the lipase-treated chylomicrons were exposed to other plasma lipoproteins in vitro, their apoprotein composition remained unchanged (Fig. 5 and Table 2), indicating that no further changes occurred following their infusion into the portal circulation. These observations, together with the finding that chylomicron remnants compete with phospholipid-depleted chylomicrons for uptake by the liver (Fig. 4), do not support the thesis that the depletion of C apoproteins from chylomicrons during their transformation into remnants is responsible for facilitating their uptake by the liver. They do support, however, the thesis that the uptake of rem-

nants by the liver is facilitated by the depletion of their surface phospholipids. The mechanism whereby the progressive removal of phospholipids from the surface of chylomicrons in vivo or in vitro results in an increased affinity of the particle to the receptor is a matter for investigation. Apoprotein E is thought to be the ligand for the remnant receptor (24). It is conceivable that in the intact chylomicrons, with a full phospholipid complement, the receptor binding domain of apoprotein E is not exposed. As phospholipids are progressively removed, steric or conformational changes may occur (45) which allow access of the apoprotein to its receptor with increasing binding affinity.

In this study, hepatic lipase proved to be a useful tool to investigate the role that phospholipids play in the recognition of triglyceride-rich lipoproteins by the liver remnant receptor. However, the physiological function of hepatic lipase has not yet been conclusively established. Studies with rats and cynomolgus monkeys injected with hepatic lipase antisera (46, 47) reported that inhibition of the enzyme was accompanied by accumulation of triglyceride-rich lipoproteins in the circulation, suggesting a role for the triglyceride hydrolase activity of hepatic lipase in the catabolism of these lipoproteins. Berry et al. (48) reported that isolated VLDL perfused for long periods through an isolated liver preparation were catabolized by the hepatic lipase, and the resulting particles were taken up by the liver. In the presence of other plasma lipoproteins, however, the hepatic lipase action on the triglyceride-rich lipoproteins was inhibited. Thus, these authors concluded that, in vivo, hepatic lipase does not function in the catabolism of triglyceride-rich lipoproteins. Other studies in which rats were injected with antisera against hepatic lipase have suggested that the physiological function of this enzyme is associated with its phospholipase A_1 activity. These studies have suggested that hepatic lipase functions in the conversion of HDL_2 to HDL_3 (49, 50), and in the metabolism of chylomicron and HDL phosphatidylethanolamine (51). More recently, Daggy and Bensadoun (52) reported that, in rats fed corn oil, the in vivo inhibition of the heparin-releasable hepatic lipase by a monospecific antiserum resulted in the accumulation, in the plasma LDL fraction, of phospholipid-rich lipoproteins containing the lower molecular weight apoprotein B, which is mainly of intestinal origin (53). This lipoprotein fraction presumably corresponded to partially degraded chylomicrons enriched in phospholipids. On the basis of these findings, these authors suggested that hepatic lipase may function, through its phospholipase activity, in facilitating the removal of chylomicron remnants from the circulation. The results of the present investigation are consistent with this interpretation and suggest that the normal removal of chylomicrons from the circulation may involve the coordinated actions of lipoprotein lipase and hepatic lipase. In a first step, the surface to core ratio of chylomicrons would be progressively increased by the triglyceride hydrolase action of lipoprotein lipase in the extrahepatic vascular bed. In a second step, the resulting phospholipid-enriched lipoproteins, when circulated through the liver, would be acted upon by the phospholipase action of the hepatic lipase, thus exposing the receptor-binding domains of the apoprotein E on the particle. According to this model, hepatic lipase would only function to increase the rate at which chylomicrons are removed from circulation. Inasmuch as lipoprotein lipase alone can also cause the depletion of chylomicron phospholipids, albeit more slowly than hepatic lipase, inhibition or absence of the latter enzyme would delay, but not prevent, the removal of chylomicrons from circulation.

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