

Plasma clearance and liver uptake of chylomicron remnants generated by hepatic lipase lipolysis: evidence for a lactoferrin-sensitive and apolipoprotein E-independent pathway

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Abstract Chylomicrons labeled with [³H]cholesterol and [¹⁴C]triglyceride fatty acids were lipolyzed by hepatic lipase (HL) *in vitro* and then injected intravenously into normal mice fed low- or high-fat diets, and into apolipoprotein (apo) E-deficient mice. In normal mice fed the high-fat diet and injected with non-lipolyzed chylomicrons, the plasma clearance and hepatic uptake of the resulting [³H]cholesterol-labeled remnants was markedly inhibited. In contrast, chylomicrons lipolyzed by HL were taken up equally rapidly by the livers of mice fed the low- and high-fat diets. The removal of non-lipolyzed chylomicrons lacking apoE from the plasma of apoE-deficient mice was inhibited, but not the removal of chylomicrons lipolyzed by HL. Pre-injection of lactoferrin into normal mice inhibited the plasma clearance of both non-lipolyzed chylomicrons and chylomicrons lipolyzed by HL. The removal of HL from the surface of the lipolyzed particles by proteolytic digestion did not affect their rapid uptake, indicating that the hepatic recognition of the lipoproteins was not mediated by HL. These observations support previous findings that phospholipolysis of chylomicrons by hepatic lipase generates remnant particles that are rapidly cleared from circulation by the liver. They also support the concept that chylomicron remnants can be taken up by the liver by an apolipoprotein E-independent mechanism. We hypothesize that this mechanism is modulated by the remnant phospholipids and that it may involve their interaction with a phospholipid-binding receptor on the surface of hepatocytes such as the class B scavenger receptor B1.—Crawford, S. E., and J. Borensztajn. Plasma clearance and liver uptake of chylomicron remnants generated by hepatic lipase lipolysis: evidence for a lactoferrin-sensitive and apolipoprotein E-independent pathway. *J. Lipid Res.* 1999. 40: 797–805.

Supplementary key words chylomicrons • remnants • hepatic lipase • lactoferrin • scavenger receptor • liver • lipoprotein lipase • phospholipids

Chylomicrons are triglyceride-rich plasma lipoproteins responsible for the transport through the blood plasma of most of the cholesterol absorbed in the intestine to hepa-

toocytes. The chylomicrons themselves do not deliver their cholesterol load to the liver cells. In order to do so, they must first undergo changes in composition transforming them into remnant particles capable of being recognized and endocytosed by hepatocytes (1).

The transformation of chylomicrons into remnants is a complex process that begins in the vascular bed of extrahepatic tissues through the action of lipoprotein lipase (LPL). This enzyme hydrolyzes triglycerides in the core of the chylomicrons and, through its phospholipase A₁ activity, surface phospholipids. As a result of LPL action the chylomicrons become smaller and the composition of their surface phospholipids, as well as their surface apoproteins, may become significantly altered. The lipolyzed (remnant) particles are then carried through the blood stream to the liver where they penetrate the fenestrated endothelium and bind to the surface of hepatocytes. Available evidence indicates that while bound to the surface of the hepatocytes the remnants undergo further remodeling of their surface, a process that facilitates their subsequent endocytosis. This remodeling has been postulated to involve: *a*) the acquisition of apoE, allowing the remnants to be recognized by one or more apoE-binding receptors on the surface of the hepatocytes, and *b*) lipolysis by hepatic lipase (HL), a 66-kDa lipolytic enzyme that preferentially hydrolyzes lipoprotein phospholipids (1).

The involvement of HL in facilitating the liver uptake of remnants is well documented. Sultan et al. (2) reported that inhibition of HL by specific antibodies inhibited the plasma clearance and hepatic uptake of chylomicron remnants in the rat. Daggy and Bensadoun (3) found that in-

Abbreviations: HL, hepatic lipase; LPL, lipoprotein lipase; apo E, apolipoprotein E; β -VLDL, β -very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LRP, low density lipoprotein receptor-related protein; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; HSPG, heparan sulfate proteoglycans; SR-B1, scavenger receptor B1.

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inhibition of HL *in vivo* by specific antibodies in rats fed corn oil resulted in the plasma accumulation of partially degraded phospholipid-rich lipoproteins, presumably of intestinal origin. Shafi et al. (4) showed that antibody inhibition of HL reduced the uptake of chylomicron remnants by the isolated perfused rat liver. In cynomolgous monkeys, antibody-mediated inhibition of HL also resulted in the plasma accumulation of remnant particles (5). Accumulation of lipoprotein remnants also occurs in some human subjects with HL deficiency (6). In mice, HL deficiency does not result in an apparent defect in chylomicron remnant clearance from the plasma (7). The defect becomes apparent, however, when the mice are fed a very high fat load (7). The involvement of HL in remnant metabolism was also demonstrated in studies with homozygous apoE-deficient mice. The characteristic hypercholesterolemia that results from the large accumulation of remnants in the plasma of these animals is almost doubled if they have a combined HL deficiency (8). In aggregate, these studies suggest that catalytically active HL has an important function in the transformation of LPL-generated remnants into particles that can be recognized by hepatocytes. Other studies have suggested, however, that HL need not be catalytically active in order to affect remnant uptake by the liver. In isolated cultured cells (9–11) and transgenic mice overexpressing HL (12) this enzyme has been shown to act as a ligand that mediates the cellular binding and uptake of remnants. This ligand function is apparent, however, only when HL is attached in excessive amounts either to the remnant particle (9, 11) or to the surface of hepatocytes (12).

Work from this laboratory has provided evidence that is consistent with a role of catalytically active HL in the generation of chylomicron remnants. We have shown that lipolysis of chylomicrons by HL *in vitro* transforms these lipoproteins into remnant-like particles that, when injected intravenously into rats (13, 14) or rabbits (15), are rapidly cleared from circulation by the liver. The preferential uptake of chylomicrons lipolyzed by HL was also demonstrated in murine hepatocytes in culture (16). In addition, we demonstrated that chylomicron-like emulsions prepared with phospholipids obtained from chylomicrons lipolyzed by HL were taken up by the rat liver in preference to emulsions prepared with phospholipids obtained from intact chylomicrons (14). Based on these and other observations (17) we postulated that phospholipolysis, catalyzed first by LPL and then by HL, is a necessary requirement for the generation of remnant particles that can be recognized and taken up by the liver. To explain how phospholipolysis transforms chylomicrons into remnant-like particles we also postulated that, unlike the chylomicron phospholipids, the remnant phospholipids could act as determinants of hepatocyte remnant recognition and that this process could occur independently of apoE (14, 18).

In this study we used mice to investigate the mechanism by which chylomicrons lipolyzed by HL are recognized and taken up by the liver. Specifically we examined whether chylomicrons lipolyzed by HL are taken up by the livers by

a mechanism similar to that of endogenously generated remnants. For this purpose we used normal mice maintained either on a normal diet or a high-fat/high-cholesterol atherogenic diet known to cause a major defect in the plasma clearance and hepatic uptake of remnants (19, 20). In addition, we used mice and chylomicrons deficient in apoE to examine the role played by this apoprotein in mediating the plasma clearance and liver uptake of HL-treated chylomicrons.

MATERIALS AND METHODS

Animals

Mice (5–6 months old) homozygous for the inactivated apoE gene were progeny of those initially generated by Piedrahita et al. (21). Normal male C57BL/6J mice (5–6 months old) were purchased from Harlan Sprague-Dawley Inc. (Indianapolis, IN). The animals were fed a normal rodent chow diet that contained 4% (w:w) fat and 0.4% (w:w) cholesterol, purchased from Harlan Teklad, Madison, WI. Some of the normal mice were also fed, for 10 days, an atherogenic diet that contained 1.25% (w:w) cholesterol, 7.5% cocoa butter, and 0.5% sodium cholate (Teklad diet TD 78399).

Collection of small chylomicrons

The main mesenteric duct of anesthetized normal and homozygous apoE-deficient mice was cannulated and the animals were then fed by stomach tube with a bolus of 0.1 ml corn oil containing 100 μ Ci [1,2-³H]cholesterol alone or with added 30 μ Ci [1-¹⁴C]palmitic acid, purchased from Amersham. Lymph was collected overnight, at room temperature, in tubes containing penicillin and streptomycin. During lymph collection the animals had free access to regular chow and water. The collected lymph was centrifuged at 80,000 *g* for 30 min at 15°C. The floating layer of large chylomicrons was removed and the infranatant was centrifuged further at 80,000 *g* for 16 h at 15°C. The floating pellet, containing small chylomicrons, was dispersed in saline, stored at 4°C, and used within 24 h of collection.

Hepatic lipase

Normal mice were anesthetized with Metofane and their livers were isolated and perfused through the portal vein with 0.15 M NaCl at 37°C for 1 min in order to remove blood from the organ. This was followed by a 0.5-min perfusion with the same solution containing 10 U heparin/ml, at a flow rate of 4 ml/min. The effluent containing heparin-releasable HL was collected from the superior vena cava and used within 30 min of collection. Because heparin may also release apoE from its binding sites in the liver, experiments requiring the complete absence of this apoprotein were carried out using HL obtained from livers of apoE-deficient mice, as described above.

Lipolysis of chylomicrons

The conditions used for the lipolysis of the small chylomicrons by HL were essentially as previously described (13), except that Tris buffer was omitted. Briefly, chylomicrons (5 mg triglyceride) were mixed with an albumin solution (60 mg, pH 8.5), 0.15 ml of liver perfusate obtained as described above, and 0.15 M NaCl to 1 ml. Control (non-lipolyzed) chylomicrons were incubated under the same conditions, except that albumin was omitted from the assay medium. Lipolysis was carried out at 37°C in a shaking water bath for 90 min. To determine the extent of lipolysis, chylomicrons were separated from the other assay constituents by applying aliquots of the assay medium to columns (1.5 \times 50 cm) of 2%

agarose (Bio-Gel A-50m, 50–100 mesh, Bio-Rad). The eluant was 0.15 M NaCl and the chylomicrons eluted with the void volume.

Chylomicron clearance

All experiments were carried out between 7 am and 11 am. Mice fed ad lib were anesthetized with Nembutal intraperitoneally (0.65 mg/10 g body weight) and injected intravenously through a tail vein, with 0.2 ml of non-lipolyzed or lipolyzed chylomicrons (25 µg cholesterol) labeled with [1,2-³H]cholesterol only, or double labeled with [1,2-³H]cholesterol and [1-¹⁴C] palmitic acid. When indicated, the mice were first injected intravenously with bovine lactoferrin (Sigma) (2 mg/mouse, dissolved in 0.1 ml of saline) and, after 1 min, with the labeled chylomicrons as described above. At 3, 10, 15, and 20 min after injection, 50-µl blood samples were collected from the tail into heparinized capillary tubes. The tubes were centrifuged and known volumes of plasma were taken for radioactivity measurement. The radioactivity in the whole animal plasma compartment was calculated by assuming a blood volume of 5.5% body weight (22), and using the hematocrit value for each blood sample. At the end of the experiment, after the last sample of blood had been collected, the livers were perfused for 1 min with 0.15 M NaCl through the inferior vena cava, in order to remove residual blood from the organ. The livers were then homogenized in 30 ml chloroform-methanol 2:1 (v:v) for the determination of tissue radioactivity, as previously described (13).

Other procedures

Chylomicron triglycerides were measured with the Synchron CX-7 Analyzer (Beckman). Cholesterol was measured using assay kits from Sigma. Thin-layer chromatography (Silica gel G) with chloroform-methanol 97:3 (v:v) was used to separate chylomicron phospholipids from the other lipids, followed by the separation of the individual phospholipids with chloroform-methanol-acetic acid-water 25:15:4:2 (by volume). Phospholipids were measured using the method of Bartlett (23). Proteolytic digestion of HL, as well as other proteins, on the surface of the chylomicrons was carried out with trypsin (Sigma), as previously described (18).

RESULTS

In vitro, solubilized HL readily binds to the surface of chylomicrons but does not hydrolyze the lipoprotein surface phospholipids and core triglycerides unless fatty acid acceptors (e.g., albumin) are also present in the medium (24). In this study mouse lymph chylomicrons were incubated with mouse HL in the presence or in the absence of albumin in the assay medium. In the presence of albumin, under the conditions used, 52 ± 6% of the chylomicron phospholipids and 18 ± 3% of the triglycerides (mean ± SD, n = 6) were hydrolyzed and removed from the lipoproteins. The chylomicrons lipolyzed by HL under the conditions here used remained in a visually homogeneous suspension, did not aggregate or coalesce, and their elution characteristics when applied to a 2% agarose column were indistinguishable from the control particles. Previous work has shown (13) that chylomicrons lipolyzed by HL retained their spherical shape, as determined by electron microscopy, and their apoprotein composition remained essentially unchanged. The phospholipid distribution in the non-lipolyzed and lipolyzed chylomicrons is

TABLE 1. Phospholipid composition of non-lipolyzed and lipolyzed chylomicrons

	PC	Lyso-PC	PE	PS/PI	Sph.
Non-lipolyzed	78	1	8	11	2
Lipolyzed	60	6	10	20	4

Results are expressed as % of the total phospholipids, and are the average of two separate determinations. Abbreviations: PC, phosphatidylcholine; lyso-PC; lyso-phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; Sph., sphingomyelin.

shown in Table 1. The relative proportion of all phospholipids in the lipolyzed chylomicrons was increased at the expense of phosphatidylcholine (PC). While apoE has been reported to enhance the HL hydrolysis of lipid monolayers (25), the lipolysis of chylomicrons obtained from normal mice was similar to that of chylomicrons lacking apoE (results not shown), indicating that this apoprotein, when present on a physiological substrate, does not modulate the HL catalytic activity. In the absence of albumin in the incubation medium, the HL became attached to the chylomicrons but no lipolysis occurred, and the phospholipid and triglycerides contents of the lipoproteins were not significantly different from that of chylomicrons that had not been exposed to the enzyme. Attachment of HL to the chylomicrons was confirmed by isolating the chylomicrons from the albumin-free incubation media by ultracentrifugation followed by their reincubation in an albumin-containing medium. Under those conditions, hydrolysis of the chylomicron phospholipids and triglycerides was readily detected, indicating that HL was bound to the surface of the chylomicrons.

Figure 1 compares the plasma clearance of [³H]cholesterol-labeled chylomicrons injected into mice fed either a normal or a high-fat diet. The injected chylomicrons were: a) untreated (control); b) exposed to HL in the absence of albumin (non-lipolyzed chylomicrons); and c) exposed to HL in the presence of albumin (lipolyzed chylomicrons). In the mice fed a normal diet, the plasma clearance of the control and the non-lipolyzed chylomicrons was very similar at all time points examined. At 3 min after the injection, 60 ± 5.3 and 58 ± 5%, respectively, of the injected [³H]cholesterol associated with the control chylomicrons and the non-lipolyzed chylomicrons remained in the plasma, and by 20 min, only about 6% of the radioactivity of both types of chylomicrons was detected in the plasma (panels a and b). In contrast to these findings, the [³H]cholesterol-labeled chylomicrons that had been lipolyzed by HL in vitro were cleared from circulation much more rapidly and only about 25 ± 2% of the radioactivity remained in the plasma 3 min after the injection (panel c). The differences in clearance at 3 min between the lipolyzed chylomicrons and the other two types of chylomicrons were statistically significant (*P* < 0.01). In the mice fed the high-fat diet, the plasma clearance of the control and non-lipolyzed [³H]cholesterol-labeled chylomicrons was greatly inhibited (panels a and b). By 20 min after injection, about 57% of the injected dose was still present in the circulation. In

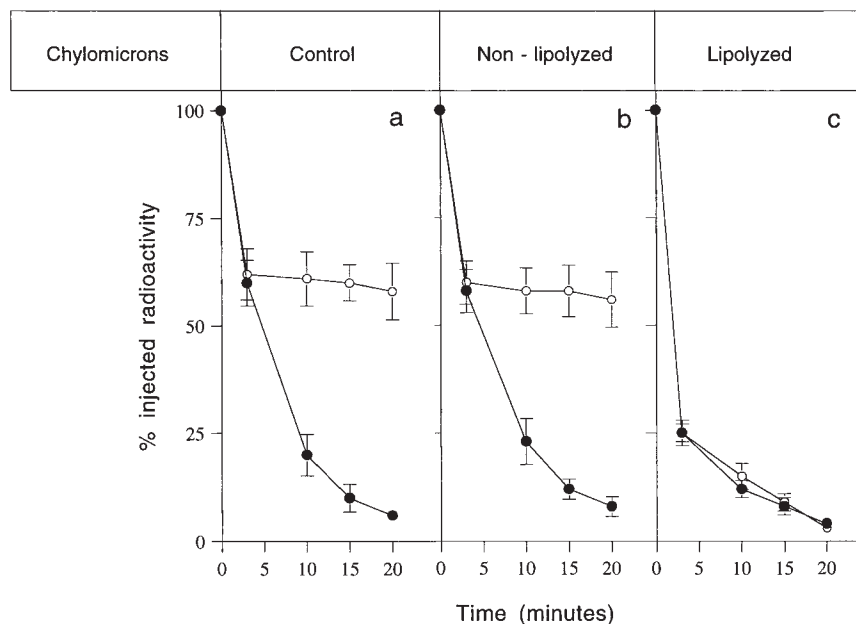


Fig. 1. Plasma clearance of HL-lipolyzed chylomicrons in normal mice. Mice fed a low-fat diet or a high-fat/high-cholesterol diet for 10 days were injected intravenously with 0.2 ml of [^3H]cholesterol-labeled chylomicrons (25 μg cholesterol) that were untreated, incubated with HL but not lipolyzed, or lipolyzed by HL as described in Materials and Methods. The results at each point are means \pm SD for 4 animals/group; \bullet , normal diet; \circ , high-fat diet.

marked contrast to this clearance inhibition, the plasma clearance of the lipolyzed chylomicrons was very rapid and similar to that observed in the animals on the chow diet (panel c). By 3 min after injection, only $25 \pm 3\%$ of the radioactivity was recovered from the circulation. The injected radioactivity taken up by the livers is shown in **Fig. 2**. In the

mice fed a normal diet, between 70 and 80% of the injected radioactivity was recovered in the livers of all three groups, indicating that the HL-treated chylomicrons, like the control and non-lipolyzed chylomicrons, were cleared from the plasma by the liver. In the mice fed a high-fat diet, consis-

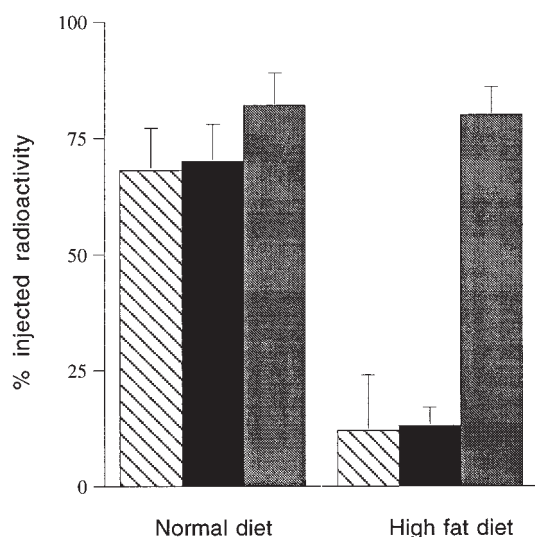


Fig. 2. Liver uptake of HL-lipolyzed chylomicrons in normal mice. Twenty minutes after the intravenous injection of the [^3H]cholesterol-labeled chylomicrons (see legend for Fig. 1), the radioactivity incorporated into the livers of the injected mice was measured as described in Materials and Methods. The results are the means \pm SD for 4 livers/group; ▨ , control; \blacksquare , non-lipolyzed; ▩ , lipolyzed.

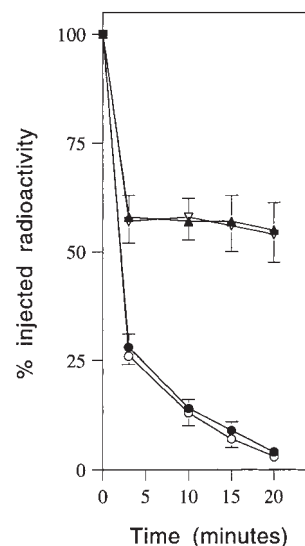


Fig. 3. Plasma clearance of chylomicrons devoid of HL by treatment with trypsin. Non-lipolyzed or HL-lipolyzed chylomicrons labeled with [^3H]cholesterol were treated with trypsin, as described in Materials and Methods, in order to remove all their surface proteins, including HL. They were then injected intravenously into mice fed a high-fat/high-cholesterol diet for 10 days. The results at each point are means \pm SD for 4 animals/group; \bullet , lipolyzed; \circ , lipolyzed and trypsin; \blacktriangle , non-lipolyzed and trypsin.

tent with the clearance results, the hepatic recovery of the radioactivity associated with the control and non-lipolyzed chylomicrons was only about 13% of the injected dose. In the animals injected with lipolyzed chylomicrons, 80% of the radioactivity was recovered in the liver.

Studies with cultured cells (9, 11) and with mice overexpressing HL (12) have documented that this enzyme can serve as a ligand that mediates the cellular binding and uptake of triglyceride-rich lipoproteins. In this study, non-lipolyzed chylomicrons with bound HL were not removed from circulation as rapidly as the lipolyzed chylomicrons (Fig. 1), suggesting that the enzyme attached to the surface of the lipoproteins was not involved in their removal from the plasma. To confirm that under the conditions used in this study, the HL bound to the chylomicrons had no role in the removal of chylomicrons from circulation, chylomicrons that had been lipolyzed by HL were stripped of their surface proteins by trypsin treatment, as described in Methods and then injected into the circulation of mice fed the high-fat diet. **Figure 3** shows that the plasma clearance of the trypsin-treated lipolyzed chylomicrons, which had no bound HL, was similar to that of HL-lipolyzed chylomicrons that were not treated with trypsin. As anticipated, trypsin treatment of control chylomicrons did not affect their clearance from circulation.

Studies with rats (26, 27) and mice (20) have demonstrated that when these animals are pre-injected with lactoferrin and then injected with chylomicrons or chylomicron-like lipid emulsions, the clearance of the resulting remnants generated in the vascular space is greatly inhibited.

To examine the effect of lactoferrin on the plasma clearance of HL-lipolyzed chylomicrons, mice maintained on the normal diet were injected intravenously with either saline or lactoferrin and 1 min later with non-lipolyzed or HL-lipolyzed chylomicrons double-labeled with ^{14}C -labeled fatty acid and ^3H cholesterol. In agreement with other reports (20), **Fig. 4** shows that the pre-injection of lactoferrin caused a marked inhibition in the clearance of ^3H cholesterol of non-lipolyzed chylomicrons (panel b), compared to animals pre-injected with saline (panel a). Twenty minutes after administration of the chylomicrons, about 70% of the injected ^3H cholesterol label was present in the plasma of the animals pre-injected with lactoferrin, compared to less than 8% of the ^3H cholesterol label injected into the animals pre-injected with saline. The clearance of the ^{14}C -radioactivity from the plasma of the mice pre-injected with lactoferrin (panel a) was not different from that of animals pre-injected with saline (panel b), indicating that lactoferrin did not interfere with the LPL-mediated hydrolysis of the TG core of the particles. In the animals pre-injected with saline and then injected with lipolyzed chylomicrons (panel c), both the ^3H cholesterol and ^{14}C -labeled fatty acids were rapidly cleared from circulation, indicating that the injected particles were removed as a whole by the liver. In mice pre-injected with lactoferrin (panel d), the clearance of ^3H cholesterol and ^{14}C -labeled fatty acids was similar to that observed after injection of non-lipolyzed chylomicrons (panel b). Twenty minutes after injection of the HL-lipolyzed chylomicrons, about 70% of the ^3H cholesterol was re-

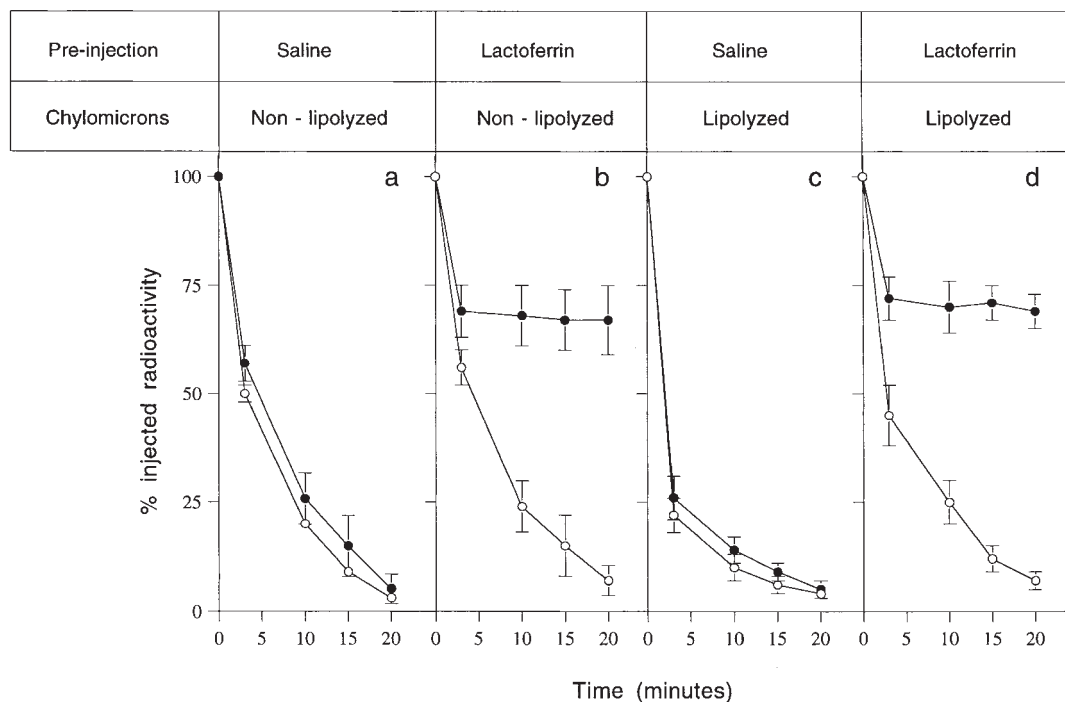


Fig. 4. Effect of lactoferrin on the plasma clearance of chylomicrons in normal mice. Mice maintained on a normal low-fat diet were injected intravenously with 0.1 ml of saline or lactoferrin (2 mg/mouse) and 1 min later with non-lipolyzed and HL-lipolyzed chylomicrons double-labeled with ^3H cholesterol and ^{14}C fatty acids. The results at each point are means \pm SD for 5 animals/group; \circ , ^{14}C fatty acids; \bullet , ^3H cholesterol.

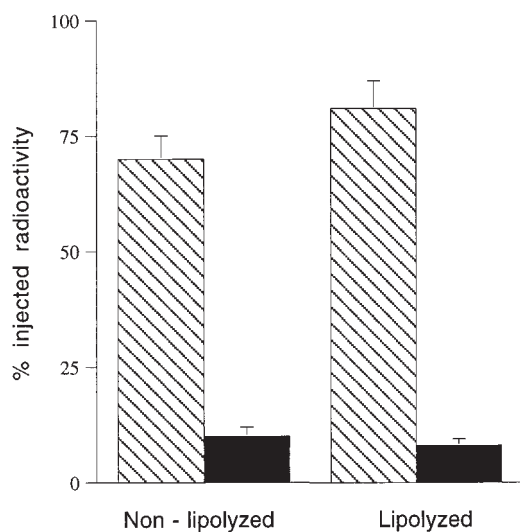


Fig. 5. Effect of lactoferrin on the liver uptake of chylomicrons in normal mice. Twenty minutes after the intravenous injection of the chylomicrons double-labeled with [^3H]cholesterol and [^{14}C]fatty acids (see legend for Fig. 4), the radioactivity incorporated into the livers of the injected mice was measured as described in Materials and Methods. The results are the means \pm SD for 5 livers/group; \square , saline; \blacksquare , lactoferrin.

tained in the plasma whereas the ^{14}C -labeled fatty acids were readily cleared. These results demonstrate that in the animals injected with lactoferrin, the HL-treated chylomicrons remained in the plasma and were subjected to LPL-mediated lipolysis with the resulting [^3H]cholesterol remnants remaining in circulation whereas the ^{14}C -labeled fatty acids released were then cleared from the plasma. Consistent with the clearance data, the hepatic recovery of the [^3H]cholesterol label was reduced from about 75% in the animals injected with saline, to about 10% in the animals injected with lactoferrin (Fig. 5).

To investigate how the lack of apoE might affect the plasma clearance and hepatic uptake of chylomicrons lipolyzed by HL, apoE-deficient chylomicrons double-labeled with [^3H]cholesterol and ^{14}C -labeled fatty acids were incubated with HL in the presence or the absence of albumin and then injected into mice that also lacked apoE. Figure 6A shows that in the animals injected with non-lipolyzed chylomicrons the ^{14}C label was readily removed from the plasma indicating that the ^{14}C -labeled triglycerides had been hydrolyzed by LPL *in vivo* and the resultant unesterified ^{14}C -labeled fatty acids had been removed from circulation. In contrast, the plasma clearance of the ^3H -labeled cholesterol-containing remnants was markedly inhibited, in agreement with the results of previous studies (20). In the animals injected with chylomicrons that had been lipolyzed by HL, [^3H]cholesterol did not accumulate in the plasma. Twenty minutes after injection, only about 8% of the injected ^3H label and the ^{14}C label remained in the plasma, indicating that the particles were removed as a whole from circulation. Consistent with the clearance data, in the animals injected with the non-lipolyzed chylomicrons, the hepatic recovery of the [^3H]cholesterol label

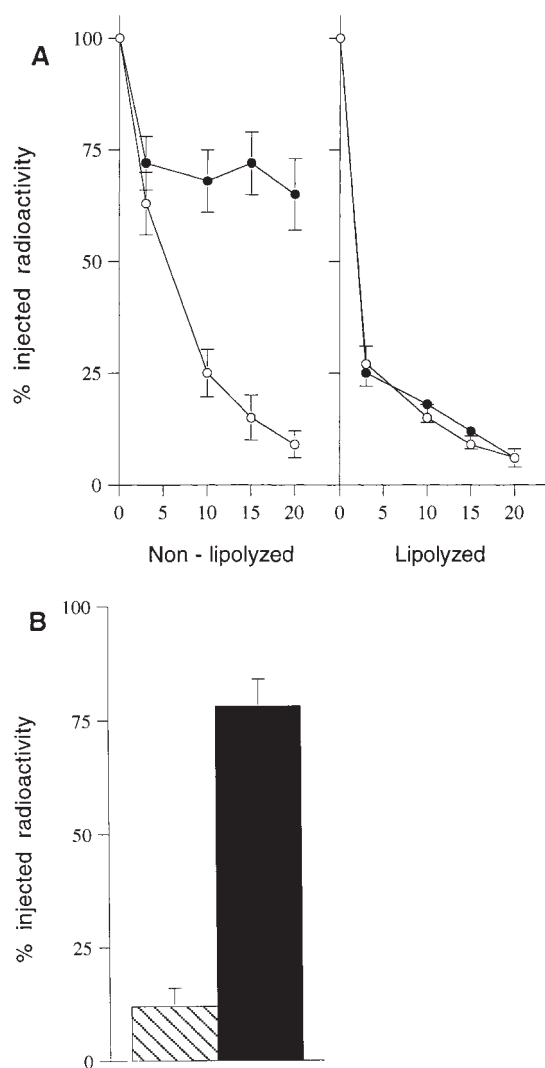


Fig. 6. Plasma clearance and hepatic uptake of apoE-free chylomicrons in mice lacking apoE. Mice lacking apoE fed on a normal low-fat diet were injected intravenously with non-lipolyzed or HL-lipolyzed chylomicrons double-labeled with [^3H]cholesterol and [^{14}C]fatty acids. The radioactivity in the plasma, and in the livers 20 min after the injections was determined as described in Materials and Methods. The results are means \pm SD for 4 mice/group; A: \circ , [^{14}C]fatty acids; \bullet , [^3H]cholesterol. B: \square , non-lipolyzed; \blacksquare , lipolyzed.

was only $12 \pm 4\%$ of the injected dose whereas in the mice injected with the lipolyzed chylomicrons the radioactivity recovered from the livers was $78 \pm 6\%$ of the injected dose (Fig. 6B).

DISCUSSION

The results of the present investigation carried out using mice confirm previous observations in the rat (13, 14) and rabbit (15) that the *in vitro* lipolysis of chylomicrons by HL generates lipoprotein remnants that, when injected intravenously, are rapidly cleared from circulation by the liver. *In vivo*, under normal conditions, chylomicrons are not lipolyzed by HL, presumably because these lipopro-

teins do not gain access to the enzyme on the surface of hepatocytes. In order to come in contact with HL, chylomicrons must first be acted upon by LPL in the extrahepatic tissues and be transformed into remnant particles that can penetrate the space of Disse. Although HL has been reported to be present in the plasma of mice (28), it is unlikely that it functions in the lipolysis of chylomicrons in the circulation. Thus HDL, the main lipoprotein in the mouse plasma, is the preferred substrate for HL and it readily inhibits HL action on chylomicron triglycerides (24). Although in this study we used only intact chylomicrons, other studies have examined the effects of HL on remnants (β -VLDL) (15) and chylomicron remnant-like emulsions (9). The results obtained showed that *in vitro* lipolysis by HL also accelerated the uptake of these particles by, respectively, the liver *in vivo* and by isolated hepatocytes in culture. Thus, chylomicrons lipolyzed by HL *in vitro* can be advantageously used as a model to investigate the function of this enzyme in modulating the plasma clearance of remnants by the liver *in vivo*.

Studies with cultured cells (9, 11) and with transgenic mice overexpressing human HL (12) have provided compelling evidence that HL, independently of its catalytic activity, can function as a ligand that mediates the cellular binding and uptake of lipoproteins. It is not known, however, whether HL, when expressed in physiological amounts, functions as a lipoprotein ligand. In the present study we demonstrated that HL did not act as a ligand. First, although both lipolyzed and non-lipolyzed chylomicrons contained HL, only the former lipoproteins were rapidly cleared from circulation by the liver (Figs. 1 and 2). Second, the removal of HL from the surface of the lipolyzed chylomicrons by proteolytic digestion did not inhibit their clearance from the plasma by the liver (Fig. 3). These results are consistent with those of a previous study with rats in which we compared the hepatic uptake of chylomicrons lipolyzed by HL and emulsions prepared with lipids extracted from these lipolyzed lipoproteins (14). Although the protein-free emulsions contained no HL, they were taken up by the liver as efficiently as the lipolyzed chylomicrons. Together, these observations demonstrate that, under the experimental conditions used, the catalytic activity of HL was essential for modulating the rapid uptake of chylomicrons by the liver. HL has acyl glycerol hydrolase activity, responsible for the hydrolysis of chylomicron triglycerides, as well as phospholipase A_1 activity responsible for remodeling the phospholipids on the surface of the chylomicrons. In previous studies we provided substantial evidence demonstrating that it is the phospholipolysis action of HL that is responsible for transforming the chylomicrons into lipoproteins that are readily cleared from the plasma by the liver (13–16, 17).

The results obtained in this investigation, using normal mice fed high-fat/high-cholesterol diets and mice lacking apoE, support the concept that two different mechanisms participate in the removal of plasma chylomicrons by the liver: one that is dependent on apoE, and a second that is apoE-independent and requires the mediation of catalytically active HL. In both animal models used in this study,

the removal of LPL-generated chylomicron remnants by the liver was severely impaired. In the mice consuming the high-fat/high-cholesterol diet, plasma apoE levels are similar to, or higher than in mice fed a regular diet (29, 30). Therefore, their defect in removing remnants from the plasma must be at the level of apoE recognition by binding sites in the liver. Current models for the hepatic uptake of remnants suggest that these lipoproteins, upon their entry into the space of Disse, first bind to heparan sulfate proteoglycans (HSPG) and then to the LDL receptor, the LDL receptor-related protein (LRP), and/or the lipolysis-stimulated receptor (LSR). The role which each of these apoE-binding receptors plays in the uptake of remnants is still a matter of controversy (1). In animals fed a high-fat/high-cholesterol diet, the hepatic LDL receptor is down-regulated, which might explain, at least in part, the impairment of remnant removal by the liver (31, 32). Under the same dietary conditions, the LRP is not down-regulated but is, apparently, unable to cope with the increased load of remnants (33). Regardless of the role that these receptors play in remnant removal, the present results demonstrating the rapid uptake of chylomicrons lipolyzed by HL by the livers of mice fed the high-fat/high-cholesterol diet, indicate that these particles do not depend on remnant apoE receptors for their removal. This conclusion is clearly substantiated by the demonstration that chylomicrons lipolyzed by HL are rapidly removed from the plasma by the liver even in the complete absence of apoE (Fig. 6).

Although the LPL-generated chylomicron remnants and HL-lipolyzed chylomicrons are clearly removed from the plasma by the liver through different mechanisms, the observation that lactoferrin inhibits the hepatic uptake of both types of particles (Figs. 4 and 5) indicates that, at some stage of the removal process, they must also share a common pathway. Lactoferrin is a 70 kDa glycoprotein that has been shown to inhibit the recognition and uptake of chylomicron remnants by liver cells *in vivo*, by freshly isolated liver cells, and by hepatocytes maintained in culture (16, 26, 27, 34). The mechanism whereby lactoferrin exerts its inhibitory effect is not known. Using HepG2 hepatocytes and Chinese hamster ovary cells in culture, Ji and Mahley (35) reported that lactoferrin interacts with HSPG and with the LRP. They suggested that lactoferrin may inhibit remnant uptake by the liver *in vivo* by preventing their apoE-mediated binding to HSPG and their subsequent transfer to the LRP. Ziern et al. (36) have argued that the LRP is not involved in remnant recognition and have suggested that lactoferrin competes with remnants for binding to a still unidentified recognition site. In the present study chylomicrons lipolyzed by HL were taken up by the livers in the complete absence of apoE (Fig. 6) indicating that it is unlikely that the lactoferrin inhibitory effect resulted from its binding to HSPG or the LRP.

What mechanism is responsible for the rapid plasma removal and liver uptake of chylomicrons lipolyzed by HL? One possibility is that, as a result of their lipolysis by HL, the chylomicrons became “damaged” (e.g., formed micro aggregates) and were removed from circulation by the

liver Kupffer cells. This possibility is unlikely, however, in light of the results obtained with the animals injected with lactoferrin (Fig. 5). van Dijk, Ziere, and van Berkel (27) showed that the rapid uptake of "damaged" remnants by rat Kupffer cells in vivo could not be inhibited by pre-treating the animals with lactoferrin. The present results, in contrast, showed that this compound caused a marked inhibition in the uptake of the particles. Further, had the chylomicrons been "damaged" by HL-mediated lipolysis, it is very likely that they would have also been removed from circulation by macrophages in other organs, e.g., spleen. Such non-specific uptake would not be prevented by lactoferrin, as demonstrated by Huettinger et al. (26). However, in the present study, in the animals injected with lactoferrin the HL-lipolyzed particles remained in the circulation and served as substrate for the endothelium-bound LPL (Fig. 4). Thus, if the lipolyzed chylomicrons were taken up by liver cells, it is very likely that hepatocytes were responsible for this process. However, the present results measuring radioactivity incorporated into the livers do not allow us to determine whether the lipoproteins were, in fact, taken up by the cells or simply sequestered in the extracellular space. Still another possibility to explain our findings is that, as a result of HL action, the radioactive cholesterol was selectively transferred from the lipolyzed chylomicrons to the liver. Such a mechanism is known to occur with HDL, when this lipoprotein binds to its receptor, the scavenger receptor BI (SR-BI) (37).

In previous work we reported that the binding of HL-treated chylomicrons by a murine hepatocyte cell line in culture occurred in a specific and saturable manner, consistent with the existence of a receptor-mediated mechanism (16). We have also suggested that phospholipids on the surface of the chylomicrons lipolyzed by HL may mediate the recognition of the resulting particles by the liver (14). It is noteworthy that cell surface receptors capable of binding phospholipids have been reported by several investigators (38, 39). Of particular significance to the present investigation, Rigotti, Acton, and Krieger (40) have demonstrated that the scavenger receptor BI (SR-BI), expressed in the liver, and identified as the HDL receptor, binds anionic phospholipids with great affinity. Further, Fluiter and van Berkel (41) reported that liposomes of anionic phospholipids can inhibit the SR-BI-mediated binding of HDL to isolated rat hepatocytes. Although SR-BI has been proposed to function mainly as an HDL receptor, it can also bind LDL as well as other modified lipoproteins (37). It is possible, therefore, that anionic phospholipids on the surface of HL-lipolyzed lipoproteins may also mediate their binding to this receptor. HL-mediated lipolysis of chylomicrons involves mainly the hydrolysis of phosphatidylcholine (PC), the most abundant phospholipid on the surface of chylomicrons, generating lysophosphatidylcholine (LPC), most of which is removed from the surface of the particle by transfer to albumin (13). As a result of the PC hydrolysis, the remnants become relatively enriched in the other phospholipids that remain on the particle, including anionic phospholipids (e.g., phos-

phatidylserine) (Table 1). Whether SR-BI can also function as a remnant receptor by binding anionic phospholipids on the surface of the particles remains to be determined. At the present time, available evidence indicates that SR-BI facilitates the selective transfer of cholesteryl ester from lipoproteins to cells, without mediating the uptake of the particles (37). Such a role for SR-BI in remnant metabolism would be consistent with observations in mice deficient in HL and apoE (8). In these animals the remnants that accumulate in the circulation appear to be enriched in cholesterol, an observation that can be interpreted to indicate that in the absence of HL-mediated remodeling of phospholipids the remnants cannot interact with SR-BI for transfer of their cholesterol moiety to the cells.

In conclusion, the present findings are consistent with those of other studies suggesting a role for catalytically active HL in chylomicron metabolism, and with the concept that phospholipids on the surface of chylomicron remnants may be determinants of their recognition by the liver. In mice, the experimental animals used in this study, liver HL activity is relatively low and the extent of its involvement in chylomicron metabolism may depend on the metabolic status of the animal. Under normal conditions, the HL-mediated mechanism of remnant clearance from circulation might only complement the normally efficient apoE-dependent mechanism of remnant uptake by the liver. However, under conditions in which apoE is absent or cannot bind to its receptors, catalytically active HL may play a more prominent role. ■

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REFERENCES

1. Cooper, A. D. 1997. Hepatic uptake of chylomicron remnants. *J. Lipid Res.* **38**: 2173-2192.
2. Sultan, F., D. Lagrange, H. Jansen, and S. Griglio. 1990. Inhibition of hepatic lipase activity impairs chylomicron remnant-removal in rats. *Biochim. Biophys. Acta.* **1042**: 150-152.
3. Daggy, B. P., and A. Bensadoun. 1986. Enrichment of apolipoprotein B-48 in the LDL density class following in vivo inhibition of hepatic lipase. *Biochim. Biophys. Acta.* **877**: 252-261.
4. Shafi, S., S. E. Brady, A. Bensadoun, and R. J. Havel. 1994. Role of hepatic lipase in the uptake and processing of chylomicron remnants in rat liver. *J. Lipid Res.* **35**: 709-720.
5. Goldberg, I. J., N-A. Le, J. R. Paterniti, Jr., and H. N. Ginsberg. 1982. Lipoprotein metabolism during acute inhibition of hepatic triglyceride hydrolase in the cynomolgus monkey. *J. Clin. Invest.* **70**: 1184-1192.
6. Hegele, R. A., J. A. Little, C. Vezina, G. F. Maguire, L. Tu, T. S. Wolever, D. J. A. Jenkins, and P. W. Connelly. 1993. Hepatic lipase deficiency. Clinical, biochemical, and molecular genetic characteristics. *Arterioscler. Thromb.* **13**: 720-728.
7. Homanics, G. E., H. V. de Silva, J. Osada, S. H. Zhang, H. Wong, J. Borensztajn, and N. Maeda. 1995. Mild dyslipidemia in mice following targeted inactivation of the hepatic lipase gene. *J. Biol. Chem.* **270**: 2974-2980.
8. Mezdoor, H., R. Jones, C. Dengremont, G. Castro, and N. Maeda. 1997. Hepatic lipase deficiency increases plasma cholesterol but reduces susceptibility to atherosclerosis in apolipoprotein E-deficient mice. *J. Biol. Chem.* **272**: 13570-13575.

9. Diard, P., M. I. Malewiak, D. Lagrange, and S. Griglio. 1994. Hepatic lipase may act as a ligand in the uptake of artificial chylomicron remnant-like particles by isolated rat hepatocytes. *Biochem. J.* **299**: 889–894.
10. Ji, Z. S., S. J. Lauer, S. Fazio, A. Bensadoun, J. M. Taylor, and R. W. Mahley. 1994. Enhanced binding and uptake of remnant lipoproteins by hepatic lipase-secreting hepatoma cells in culture. *J. Biol. Chem.* **269**: 13429–13436.
11. Ji, Z. S., H. L. Dichek, R. D. Miranda, and R. W. Mahley. 1997. Heparan sulfate proteoglycans participate in hepatic lipase and apolipoprotein E-mediated binding and uptake of plasma lipoproteins, including high density lipoproteins. *J. Biol. Chem.* **272**: 31285–31292.
12. Dichek, H. L., W. Brech, J. Fan, Z-S. Ji, S. P. A. McCormick, S. G. Young, J. M. Taylor, and R. W. Mahley. 1998. Overexpression of hepatic lipase in transgenic mice decreases apolipoprotein B-containing and high density lipoproteins. *J. Biol. Chem.* **273**: 1896–1903.
13. Borensztajn, J., G. S. Getz, and T. J. Kotlar. 1988. Uptake of chylomicron remnants by the liver: further evidence of the modulating role of phospholipids. *J. Lipid Res.* **29**: 1087–1096.
14. Borensztajn, J., and T. J. Kotlar. 1990. Phospholipids as modulators of hepatic recognition of chylomicron remnants. Observations with emulsified lipoprotein lipids. *Biochem. J.* **269**: 539–542.
15. Chang, S., and J. Borensztajn. 1993. Hepatic lipase function and the accumulation of β -very low density lipoproteins in the plasma of cholesterol-fed rabbits. *Biochem. J.* **293**: 745–750.
16. Chang, S., and J. Borensztajn. 1995. Uptake of chylomicron remnants and hepatic lipase-treated chylomicrons by a non-transformed murine hepatocyte cell line in culture. *Biochim. Biophys. Acta.* **1256**: 81–87.
17. Borensztajn, J., and T. J. Kotlar. 1984. Liver uptake of chylomicron remnants with high and low apoprotein E:C ratio. *Proc. Natl. Acad. Sci. USA.* **81**: 5863–5866.
18. Borensztajn, J., T. J. Kotlar, and S. Chang. 1991. Apoprotein-independent binding of chylomicron remnants to rat liver membranes. *Biochem. J.* **269**: 539–542.
19. Mortimer, B-C., T. G. Redgrave, E. A. Spangler, J. G. Verstuyft, and E. M. Rubin. 1994. Effect of human apoE4 on the clearance of chylomicron-like lipid emulsions and atherogenesis in transgenic mice. *Arterioscler. Thromb.* **14**: 1542–1552.
20. Mortimer, B-C., D. J. Beveridge, I. J. Martins, and T. G. Redgrave. 1995. Intracellular localization and metabolism of chylomicron remnants in the livers of low density lipoprotein receptor-deficient mice and apoE-deficient mice. *J. Biol. Chem.* **270**: 28767–28776.
21. Piedrahita, J. A., S. H. Zhang, J. R. Hagaman, P. M. Oliver, and N. Maeda. 1992. Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. *Proc. Natl. Acad. Sci. USA.* **89**: 4471–4475.
22. Green, E. L. 1968. *Biology of the Laboratory Mouse*. Dover Publications, New York.
23. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**: 466–468.
24. Ehnholm, C., and T. Kuusi. 1986. Preparation, characterization, and measurement of hepatic lipase. *Methods Enzymol.* **129**: 716–737.
25. Thuren, T., R. W. Wilcox, P. Sisson, and M. Waite. 1991. Hepatic lipase hydrolysis of lipid monolayers—regulation by apolipoproteins. *J. Biol. Chem.* **266**: 4853–4861.
26. Huettinger, M., H. Retzek, M. Eder, and H. Goldenberg. 1988. Characteristics of chylomicron remnant uptake into rat liver. *Clin. Biochem.* **21**: 87–92.
27. van Dijk, M. C., G. J. Ziere, and T. J. van Berkel. 1992. Characterization of the chylomicron-remnant-recognition sites on parenchymal and Kupffer cells of rat liver. Selective inhibition of parenchymal cell recognition by lactoferrin. *Eur. J. Biochem.* **205**: 775–784.
28. Peterson, J., G. Bengtsson-Olivecrona, and T. Olivecrona. 1986. Mouse preheparin plasma contains high levels of hepatic lipase with low affinity for heparin. *Biochim. Biophys. Acta.* **878**: 65–70.
29. Reue, K. L., D. H. Quon, K. A. O'Donnell, G. L. Dizikes, G. C. Fareed, and A. J. Lusis. 1984. Cloning and regulation of mouse apolipoprotein E. *J. Biol. Chem.* **259**: 2101–2107.
30. Ishida, B. Y., P. J. Blanche, A. V. Nichols, M. Yashar, and B. Paigen. 1991. Effects of atherogenic diet consumption on lipoproteins in mouse strains C57BL/6 and C3H. *J. Lipid Res.* **32**: 559–568.
31. Grundy, S. M., and M. A. Denke. 1990. Dietary influences on serum lipids and lipoproteins. *J. Lipid Res.* **31**: 1149–1172.
32. Srivastava, R., S. Jiao, J. J. Tang, B. A. Pflieger, R. T. Kitchens, and G. Schonfeld. 1991. In vivo regulation of low density lipoprotein receptor and apolipoprotein-B gene expressions by dietary fat and cholesterol in inbred strains of mice. *Biochim. Biophys. Acta.* **1086**: 29–43.
33. Ishibashi, S., J. Herz, N. Maeda, J. L. Goldstein, and M. S. Brown. 1994. The two-receptor model of lipoprotein clearance: tests of the hypothesis in “knockout” mice lacking the low density lipoprotein receptor, apolipoprotein E, or both proteins. *Proc. Natl. Acad. Sci. USA.* **91**: 4431–4435.
34. Huettinger, M., H. Retzek, M. Hermann, and H. Goldenberg. 1992. Lactoferrin specifically inhibits endocytosis of chylomicron remnants but not α -macroglobulin. *J. Biol. Chem.* **267**: 18551–18557.
35. Ji, Z-S., and R. W. Mahley. 1994. Lactoferrin binding to heparan sulfate proteoglycans and the LDL receptor-related protein. *Arterioscler. Thromb.* **14**: 2025–2032.
36. Ziere, G. J., J. K. Kruijt, M. K. Bijsterbosch, and T. J. C. van Berkel. 1996. Recognition of lactoferrin and aminopeptidase M-modified lactoferrin by the liver: involvement of proteoglycans and the remnant receptor. *Biochem. J.* **313**: 289–295.
37. Rigotti, A., B. Trigatti, J. Babbitt, M. Penman, S. Xu, and M. Krieger. 1997. Scavenger receptor BI—a cell surface receptor for high density lipoprotein. *Curr. Opin. Lipidol.* **8**: 181–188.
38. Nishikawa, K., H. Arai, and K. Inoue. 1990. Scavenger receptor-mediated uptake and metabolism of lipid vesicles containing acidic phospholipids by mouse peritoneal macrophages. *J. Biol. Chem.* **265**: 5226–5231.
39. Fukasawa, M., K. Hirota, H. Adachi, K. Mimura, K. Murakami-Murofushi, M. Tsujimoto, H. Arai, and K. Inoue. 1995. Chinese hamster ovary cells expressing a novel type of acetylated low density lipoprotein receptor. Isolation and characterization. *J. Biol. Chem.* **270**: 1921–1927.
40. Rigotti, A., S. L. Acton, and M. Krieger. 1995. The class B scavenger receptors SR-BI and CD36 are receptors for anionic phospholipids. *J. Biol. Chem.* **270**: 16221–16224.
41. Fluiter, K., and T. J. C. van Berkel. 1997. Scavenger receptor BI (SR-BI) substrates inhibit the selective uptake of high-density-lipoprotein cholesteryl esters by rat parenchymal liver cells. *Biochem. J.* **326**: 515–519.