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The role of lipoprotein lipase and apoprotein E in the recognition of chylomicrons and chylomicron remnants by cultured isolated mouse hepatocytes

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Lipoprotein lipase (LPL) has been proposed to play a role in the uptake of chylomicron remnants by hepatocytes by mediating the binding of these lipoproteins to cell-surface glycosaminoglycans and to the low-density-lipoprotein receptor-related protein (LRP). This proposal is based on studies that examined the binding of chylomicrons to HepG2 cells, fibroblasts and Chinese hamster ovary cells in culture, in the presence of large amounts of LPL [Beisiegel (1995) Curr. Opin. Lipidol. **6**, 117–122]. We have investigated whether LPL attached to the surface of chylomicrons enhances the binding and uptake of these lipoproteins to isolated hepatocytes maintained in culture. Bovine milk LPL was bound to mouse chylomicrons, double-labelled *in vivo* with [^{3}H]retinol (in retinyl esters) and with [^{14}C]palmitic acid (in triacylglycerols), collected from the mesenteric lymph of normal mice and from mice lacking the apoprotein E (apo E) gene. Normal chylomicrons (containing apo E) and apo E-free chylomicrons, with or without bound LPL, were incubated with cultured hepatocytes isolated from mice lacking the apo E gene. At 0 °C LPL did not enhance the binding of the normal or apo E-free chylomicrons by the hepatocytes. When incubations were performed at 37 °C the triacylglycerols of normal and apo E-free chylomicrons were hydrolysed by LPL and there was a significant uptake of [^{14}C]fatty acids and [^{3}H]retinol by the hepatocytes. The addition of heparin or lactoferrin, a known inhibitor of hepatic uptake of chylomicron remnants, to the incubation medium inhibited the uptake of [^{3}H]retinol, present in the lipoprotein core, but not the uptake of the [^{14}C]fatty acids. We conclude that: (1) LPL attached to chylomicrons in amounts sufficient to effectively hydrolyse their core triacylglycerols does not enhance the binding of these lipoproteins to the surface of isolated hepatocytes; (2) the recognition and uptake of chylomicrons by hepatocytes requires that these lipoproteins be first hydrolysed by LPL; and (3) the uptake of lipolysed chylomicrons (remnants) by hepatocytes does not require the mediation of apo E.

INTRODUCTION

Chylomicrons are triacylglycerol-rich lipoproteins responsible for the transport, in the blood plasma, of cholesterol and vitamin A absorbed in the intestine to the liver. The chylomicrons themselves are not taken up by the liver cells. To deliver their cholesterol and vitamin A to the liver the chylomicrons must first be transformed into lipoproteins that can be recognized and endocytosed by hepatocytes: the chylomicron remnants [1,1a].

In the process of being transformed into remnants, chylomicrons undergo changes in the composition of their surface apoproteins and phospholipids, and they may acquire new surface components. Because only remnants, but not intact chylomicrons, are rapidly removed from circulation by the liver it is clear that one or more of the alterations in apoprotein and/or phospholipid composition, or one or more of the newly acquired surface components, must be instrumental in modulating their recognition by liver cells. In 1975 Felts et al. [2] hypothesized that lipoprotein lipase (LPL) acquired by chylomicrons during their transformation into remnants might be the signal that allows the liver to recognize the latter particles specifically. Arguments supporting this hypothesis have been made on the basis of results of studies that have examined the binding of chylomicrons to HepG2 cells, fibroblasts and Chinese hamster ovary cells in culture, in the presence of large amounts of LPL [3–5]. No evidence is currently available, however, on the effects of LPL on the recognition of remnants by hepatocytes. In a recent study with an isolated rat liver preparation perfused with [^{3}H]retinol-labelled chylomicrons, Skottova et al. [6] reported that these lipoproteins were cleared from the perfusion medium on the addition of an excess of catalytically inactive LPL. Skottova et al. were unable to demonstrate conclusively, however, that the clearance of hydrolysed chylomicrons (remnants) by the perfused liver involved the direct mediation of LPL.

Apoprotein E (apo E) is also believed to play an essential role in the hepatic recognition and uptake of chylomicron remnants [7]. However, its role in the postulated LPL-mediated cellular recognition of chylomicron remnants has not been defined. It has been speculated that the LPL-mediated attachment of lipoproteins to the cell surface facilitates the interaction of apo E, on the lipoprotein surface, with specific cellular receptors, leading to endocytosis of the lipoprotein–receptor complex [3–5].

In the present study we used isolated mouse hepatocytes to examine the role of LPL in mediating the cellular binding of chylomicrons. To examine also the role of apo E in the uptake of these lipoproteins, the hepatocytes in addition to the chylomicrons were obtained from apo E-gene 'knock-out' mice.

Abbreviations used: apo E, apoprotein E; LPL, lipoprotein lipase; LRP, low-density-lipoprotein receptor-related protein.
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MATERIALS AND METHODS

Animals

Mice homozygous for the inactivated apo E gene used in this study were progeny of those initially generated by Piedrahita et al. [8]. They ranged in age from 15 to 50 weeks. Normal mice (C57 BL/6J) were used as controls.

Chylomicron collection

The main mesenteric duct of normal and apo E-deficient mice was cannulated, and the animals were then fed by stomach tube with a bolus of 0.1 ml of corn oil containing 50 μCi of [11,12(n)-^{3}H]retinol (New England Nuclear) and 20 μCi of [1-^{14}C]palmitic acid (Amersham). The animals were allowed free access to food and water. Lymph was collected overnight in the presence of penicillin and streptomycin. Chylomicrons were isolated by ultracentrifugation of lymph through 0.15 M NaCl at 15 °C in a SW 55 Ti rotor (Beckman Instruments) at 80000 ***g*** for 1 h. Except for the presence of apo E, the chylomicrons collected from the the apo E-deficient mice had lipid and apoprotein compositions similar to those of chylomicrons collected from normal mice. The $^{14}C/^3H$ ratio of the chylomicrons collected from normal and apo E-deficient mice ranged from 0.7 to 1.2.

Liver cell isolation

Hepatocytes were isolated from mice deficient in apo E, essentially as described by Seglen [9] except that the perfusion with the calcium-free buffer was performed without recirculation for 2 min, followed by perfusion with buffer containing calcium, collagenase (84 units/ml) and soybean trypsin inhibitor (13 mg/ml) for 4 min. The viability of the cells was greater than 75 %.

Liver cell culture

The isolated hepatocytes were suspended in Dulbecco's modified Eagle's medium/Ham's F-12 (Gibco) supplemented with a mixture of insulin, transferrin, selenium (ITS; Collaborative Research), 50 μg/ml gentamycin (Sigma) and 10 % (v/v) fetal bovine serum, and seeded in six-well plates at a density of 8×10^5 viable cells per well. After incubation for 2 h at 37 °C in a humidified incubator (air/CO_2, 19:1), the medium was replaced with fresh medium and cells incubated for 22 h before being used for the experiments.

Binding and uptake assays

For the binding and uptake experiments, the cells were washed twice with 2 ml of serum-free medium, containing 0.2 % BSA. The cells were then incubated with 1 ml of the same medium, containing double-labelled chylomicrons (0.5 mg of triacylglycerol) with or without bound LPL and, where indicated, with 5 mg of heparin (130 units/mg) or lactoferrin (5 mg). In all experiments incubations were for 4 h. For experiments performed at 0 °C the cells were prechilled on ice for 30 min before washing and incubation with chylomicron-containing medium on ice. In some experiments, cells were incubated with 1 ml of PBS containing 5 mg of heparin for 20 min at 37 °C and washed as described above, before the addition of the chylomicron-containing medium. After incubation with chylomicrons, the medium was removed and the cells were washed twice with 2 ml of ice-cold PBS containing 0.2 % BSA followed by two more washes with 2 ml of ice-cold PBS. For the determination of surface-bound radioactivity, 0.5 ml of 0.05 % trypsin/0.02 % EDTA was added to the cells and incubated at 37 °C for 5–10 min. Trypsin inhibitor was then added to stop the proteolytic reaction, and the medium removed for radioactivity measurements. The cells were lysed by the addition of 1 M NaOH and the lysate was collected for the measurement of protein and radioactivity taken up by the cells. The amount of protein per well [2.14 ± 0.22 mg (S.D.)] was comparable in all experiments.

LPL

LPL was purified from bovine milk by using heparin–Sepharose affinity chromatography [10]. The purity of the enzyme was established by SDS/PAGE. In all experiments the LPL added to the hepatocyte incubation medium was bound to chylomicrons. This was done by incubating LPL with normal or apo E-deficient chylomicrons (4.8 μg of protein per mg of chylomicron triacylglycerol) at 4 °C for 20 min. The LPL/chylomicron mixture was then applied to a column (50 cm × 1.4 cm) of 2 % (w/v) agarose (BioGel A-50m, 50–100 mesh; Bio-Rad) and eluted with 0.15 M NaCl. The chylomicrons that were eluted with the void volume were then concentrated by ultracentrifugation at 4 °C as described above, redispersed and added to the cultured hepatocytes. In preliminary experiments the LPL activity that became bound to the chylomicrons was measured as previously described [11] and found to be 4–6 units/mg of triacylglycerol, 1 unit being defined as 1 μmol of unesterified fatty acid released per h.

Other methods

Chylomicron triacylglycerol was measured with the Synchron CX-7 Analyser (Beckman). Protein was measured by the method of Lowry et al. [12].

RESULTS

Effect of LPL on the recognition of normal chylomicrons by hepatocytes incubated at 0 °C

The results in Table 1 show the hepatocyte-associated radioactivity after incubation at 0 °C with chylomicrons obtained from normal mice, double-labelled with [^{3}H]retinol and [^{14}C]palmitic acid, in the presence or absence of LPL. After 4 h of incubation, the ^{3}H radioactivity released by trypsin from the cells incubated in the presence of LPL was similar to that of cells incubated in the absence of the enzyme. These results indicate that LPL did not enhance the binding of the chylomicrons to the liver cell surface. In parallel experiments the ^{3}H radioactivity released by heparin, instead of trypsin, yielded similar results

Table 1 Association of chylomicron [^{14}H]retinol and [^{14}C]fatty acids with hepatocytes incubated at 0 °C

Hepatocytes isolated from mice lacking the apo E gene were incubated in serum-free medium with double-labelled normal chylomicrons (containing apo E) (0.5 mg/ml triacylglycerol), with and without bound bovine milk LPL. After 4 h of incubation, cell-surface binding and uptake of chylomicrons were determined as described in the Materials and methods section. The results are expressed as a percentage of the radioactivity added to the incubation medium. They are the means ± S.D. for the combined results of two separate experiments, each performed in triplicate. * $P < 0.005$.

	[^{3}H]Retinol		[^{14}C]Fatty acids	
	Surface	Uptake	Surface	Uptake
−LPL	2.43 ± 0.5	1.55 ± 0.15	2.08 ± 0.52	1.05 ± 0.1
+LPL	1.42 ± 0.22	2.11 ± 0.8	0.95 ± 0.29	3.95 ± 0.6*

Table 2 Association of chylomicron [^{14}H]retinol and [^{14}C]fatty acids to hepatocytes incubated at 37 °C

Conditions were as in Table 1, except that the incubations were performed at 37 °C. * $P <$ 0.005.

	[^{3}H]Retinol		[^{14}C]Fatty acids	
	Surface	Uptake	Surface	Uptake
−LPL	0.65 ± 0.19	3.51 ± 0.18	0.88 ± 0.5	7.1 ± 0.9
+LPL	1.55 ± 0.18	11.7 ± 1.8*	1.71 ± 0.37	26.3 ± 1.6*

Table 3 Effect of heparin and lactoferrin on the association of chylomicron [^{14}H]retinol and [^{14}C]fatty acids to hepatocytes incubated at 37 °C

Conditions were as in Table 2, except that heparin (5 mg/ml) or lactoferrin (5 mg/ml) was added to the serum-free incubation medium. *$P < 0.005$

	[^{3}H]Retinol		[^{14}C]Fatty acids	
	Surface	Uptake	Surface	Uptake
−LPL	0.47 ± 0.02	3.63 ± 0.03	0.44 ± 0.02	6.41 ± 0.39
+LPL	1.62 ± 0.26	10.2 ± 0.4*	1.47 ± 0.18	25.5 ± 1.34*
+LPL + heparin	1.11 ± 0.08	5.53 ± 0.05	1.54 ± 0.18	23.7 ± 0.2*
+LPL + lactoferrin	0.42 ± 0.09	4.7 ± 0.09	1.51 ± 0.22	29.0 ± 1.65*

(results not shown). A small non-significant increase in the cell-associated radioactivity (not releasable by trypsin) was observed in the hepatocytes that had been incubated with chylomicrons with attached LPL.

Table 1 also shows the ^{14}C radioactivity that became bound to and taken up by the hepatocytes. As with the [^{3}H]retinol label, the presence of LPL in the incubation medium did not result in an increase in the amounts of ^{14}C radioactivity releasable by trypsin from the cells, consistent with the conclusion that under the conditions used this enzyme did not enhance the binding of chylomicrons to the cell surface. There was, however, a significant increase ($P < 0.005$) in cell-associated ^{14}C radioactivity that was not released by trypsin, suggesting that even at 0 °C some lipolytic activity had occurred and that ^{14}C-labelled unesterified fatty acids generated had been taken up by the cells.

Effect of LPL on the recognition of normal chylomicrons by hepatocytes incubated at 37 °C

In Table 2 are shown the results obtained when LPL bound to chylomicrons was added to the culture medium of hepatocytes incubated at 37 °C. The presence of LPL in the culture medium did not result in an increase in either ^{3}H or ^{14}C radioactivity bound to the cell surface. The amounts of surface-bound ^{3}H and ^{14}C radioactivities were similar to those observed in cells incubated at 0 °C (see Table 1). In contrast, the presence of LPL in the incubation medium resulted in a greater than 3-fold increase in the amounts of hepatocyte-associated ^{3}H radioactivity and ^{14}C radioactivity (Table 2) that were not releasable by trypsin. These latter findings suggest that at 37 °C the labelled chylomicrons' triacylglycerols were hydrolysed by LPL more rapidly than at 0 °C, and that the unesterified ^{14}C-labelled fatty acids, as well as the resulting [^{3}H]retinyl ester-labelled lipolysed particles (remnants), were taken up by the cells.

Table 4 Association of apo E-free chylomicron [^{14}H]retinol and [^{14}C]fatty acids with hepatocytes incubated at 0 °C

Hepatocytes isolated from mice lacking the apo E gene were incubated in serum-free medium with double-labelled chylomicrons, collected from mice lacking the apo E gene (0.5 mg/ml triacylglycerol), with and without bound bovine milk LPL. After 4 h of incubation, cell-surface binding and uptake of chylomicrons were determined as described in the Materials and methods section. The results are expressed as a percentage of the radioactivity added to the incubation medium. They are the means ± S.D. for the combined results of two separate experiments, each performed in triplicate.

	[^{3}H]Retinol		[^{14}C]Fatty acids	
	Surface	Uptake	Surface	Uptake
−LPL	2.1 ± 0.64	1.54 ± 0.17	1.82 ± 0.62	1.09 ± 0.2
+LPL	1.32 ± 0.17	2.04 ± 0.61	0.84 ± 0.25	3.74 ± 0.5

Effect of lactoferrin and heparin on the LPL-enhanced recognition of normal chylomicrons by hepatocytes

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 [13–16]. Table 3 shows the results obtained when hepatocytes were incubated at 37 °C with normal chylomicrons and LPL, in the presence or absence of lactoferrin in the medium. At the concentration used, lactoferrin caused a 50 % inhibition in ^{3}H radioactivity taken up by the cells, but had no effect on the uptake of ^{14}C radioactivity. Similar results were observed when heparin, instead of lactoferrin, was present in the incubation medium (Table 3). It may be concluded from these findings that the [^{14}C]fatty acids and [^{3}H]retinol were taken up by the hepatocytes independently of each other. Lactoferrin and heparin did not affect the cellular uptake of the ^{14}C-labelled unesterified fatty acids generated by the hydrolysis of chylomicron triacylglycerols, but did inhibit the uptake of [^{3}H]retinol, presumably by interfering with the cellular recognition of the chylomicron remnants generated in the incubation medium.

Effect of LPL on the recognition of apo E-free chylomicrons by hepatocytes incubated at 0 °C

The results of the experiments performed at 0 °C (Table 1) and 37 °C (Tables 2 and 3) clearly demonstrate that LPL did not mediate the binding of normal chylomicrons to the hepatocyte cell surface. However, LPL was essential, through its lipolytic activity, for the uptake of [^{3}H]retinol by the hepatocytes, presumably as a result of the cellular uptake of the lipolysed chylomicrons (remnants). This uptake might have been mediated by the apo E present on the surface of the lipoproteins. Previous work from this laboratory suggested, however, that apo E might not be necessary for the uptake of chylomicron remnants by hepatocytes [17,18]. To investigate this possibility further, hepatocytes isolated from mice lacking apo E were incubated with labelled apo E-free chylomicrons, also obtained from mice lacking apo E, in the presence and absence of LPL. The results obtained when the cells were incubated at 0 °C (Table 4) are similar to those observed in experiments with normal chylomicrons (Table 1). There was no significant increase in the amounts of ^{3}H and ^{14}C radioactivity releasable by trypsin from cells incubated in the presence of LPL, indicating that this

Table 5 Association of apo E-free chylomicron [^{14}H]retinol and [^{14}C]fatty acids with hepatocytes incubated at 37 °C

Conditions were as in Table 4, except that incubations were performed at 37 °C. * $P < 0.005$.

	[^{3}H]Retinol		[^{14}C]Fatty acids	
	Surface	Uptake	Surface	Uptake
−LPL	2.26 ± 0.04	1.06 ± 0.33	0.43 ± 0.16	5.7 ± 0.5
+LPL	2.15 ± 0.32	10.0 ± 1.23*	0.94 ± 0.35	23.4 ± 1.5*

Table 6 Effect of heparin and lactoferrin on the association of apo E-free chylomicron [^{14}H]retinol and [^{14}C]fatty acids with hepatocytes incubated at 37 °C

Conditions were as in Table 4, except that heparin (5 mg/ml) or lactoferrin (5 mg/ml) was added to the serum-free medium. * $P < 0.005$.

	[^{3}H]Retinol		[^{14}C]Fatty acids	
	Surface	Uptake	Surface	Uptake
−LPL	0.29 ± 0.02	1.87 ± 0.15	0.34 ± 0.02	4.48 ± 0.32
+LPL	1.35 ± 0.06	8.88 ± 0.50*	1.26 ± 0.12	22.6 ± 1.7*
+LPL + heparin	0.35 ± 0.03	5.0 ± 0.21	1.41 ± 0.25	22.0 ± 0.85*
+LPL + lactoferrin	0.35 ± 0.01	3.83 ± 0.82	1.57 ± 0.24	26.9 ± 5.3*

enzyme did not enhance the binding of chylomicrons to the surface of the hepatocytes. There was, however, a small, but significant increase ($P < 0.005$) in cell-associated ^{14}C radioactivity that was not released by trypsin, suggesting that some lipolytic activity had occurred and that ^{14}C-labelled fatty acids generated had been taken up by the hepatocytes.

Effect of LPL on the recognition of apo E-free chylomicrons by hepatocytes incubated at 37 °C

As shown in Table 5, the results obtained with apo E-free chylomicrons after incubation at 37 °C were similar to those observed when hepatocytes were incubated with normal chylomicrons (Table 2). Chylomicron binding to the cell surface (trypsin-releasable ^{3}H and ^{14}C radioactivity) was not enhanced by the presence of LPL. There was, however, a significantly higher incorporation of both [^{3}H]retinol and [^{14}C]fatty acids into the cells incubated in the presence of LPL, indicating that the chylomicrons' triacylglycerols had been hydrolysed and that the ^{14}C-labelled unesterified fatty acids generated, as well as the [^{3}H]retinol of the lipolysed particles, were taken up by the cells.

Effect of lactoferrin and heparin on the LPL-enhanced recognition of apo E-free chylomicrons by hepatocytes

The results in Table 6 show that, as in the experiments with normal chylomicrons (Table 3), the addition of lactoferrin or heparin to the incubation medium resulted in a significant inhibition in the cellular uptake of [^{3}H]retinol from the apo E-free chylomicrons, but not in the cellular uptake of [^{14}C]fatty acids generated by the lipolysis of the apo E-free chylomicron triacylglycerol.

Table 7 Effect of preincubation of hepatocytes with heparin on the binding and uptake of apo E-free chylomicrons double-labelled with [^{14}H]retinol and [^{14}C]fatty acids

Hepatocytes isolated from mice lacking the apo E gene were incubated in PBS containing heparin (5 mg/ml) for 20 min at 37 °C. At the end of the incubation the heparin-containing medium was removed and the cells were washed twice with 2 ml of serum-free medium without heparin. Fresh heparin-free medium containing double-labelled apo E-free chylomicrons (0.5 mg/ml triacylglycerol) with bound bovine milk LPL was then added to the cells and incubated for 4 h at 37 °C. The results are expressed as a percentage of the radioactivity added to the incubation medium. They are the means ± S.D. for the combined results of two separate experiments, each performed in triplicate.

	[^{3}H]Retinol		[^{14}C]Fatty acids	
	Surface	Uptake	Surface	Uptake
−Heparin	1.48 ± 0.02	13.2 ± 1.0	1.96 ± 0.37	27.1 ± 1.61
+Heparin	1.32 ± 0.12	11.1 ± 1.0	1.43 ± 0.15	23.7 ± 1.2

Effect of preincubation with heparin on the recognition of apo E-free chylomicrons by hepatocytes incubated at 37 °C

All experiments described above were performed with apo E-free chylomicrons and hepatocytes, incubated in serum-free medium. Thus the results presented in Table 5 and 6 are consistent with the notion that the uptake of lipolysed chylomicrons by hepatocytes does not require the mediation of apo E. However, after their isolation, the hepatocytes were maintained in culture medium containing 10 % (v/v) fetal calf serum (see the Materials and methods section). The possibility therefore had to be considered that apo E present in the fetal calf serum might have become bound to the surface of the cells and thus participate in the uptake of the chylomicron remnants. Because apo E can be released from the surface of cells by heparin [19,20], we performed an experiment in which the hepatocytes were exposed to heparin before incubation with the labelled chylomicrons. The results in Table 7 show that the incorporation of [^{3}H]retinol by the hepatocytes preincubated with heparin was similar to that of the control hepatocytes.

DISCUSSION

According to the hypothesis first proposed by Felts et al. [2], LPL attached to remnants is the signal that allows the liver to recognize remnants from chylomicrons. The corollary of this hypothesis is that in the absence of LPL the ability of remnants to be recognized by the liver should be impaired. Previous work from this laboratory has, however, provided evidence that the absence of LPL from the surface of remnants does not affect their ability to be recognized by the liver. When chylomicron remnants generated *in vivo* were treated with proteases to digest all surface proteins including, presumably, acquired LPL, they retained unaltered their ability to be taken up by isolated perfused rat livers [17,21], and to bind to isolated rat liver membranes [17]. These observations clearly indicate that LPL does not play a role in the hepatic recognition of remnants. The results of the present investigation, with a different experimental approach, are consistent with this conclusion. The addition of LPL to chylomicrons did not enhance their binding to hepatocytes (Tables 1 and 2).

Unlike the results of the present investigation, other studies reported that the addition of LPL to chylomicrons enhanced their binding to cultured cells [3–5]. However, the experimental conditions used in those studies differed in two important aspects

from those described in this paper. First, we used isolated hepatocytes instead of HepG2 cells, fibroblasts or Chinese hamster ovary cells [3–5]. The isolated hepatocytes were obtained from apo E-deficient mice, but similar results were obtained when we used hepatocytes isolated from livers of normal mice, or rats (S. Chang, N. Maeda and J. Borensztajn, unpublished work). Secondly, in the studies on the binding of human chylomicrons to HepG2 cells, fibroblasts and Chinese hamster ovary cells, large amounts of LPL were added to the culture medium or to the cells. LPL is known to bind to lipoprotein lipids as well as to proteoglycans and low-density-lipoprotein receptor-related protein (LRP) [3,5,22–24]. It is therefore not unexpected that when present in large quantities LPL can mediate the cellular binding of chylomicrons by serving as a bridge between these lipoproteins and the proteoglycans, as well as LRP, present on the cell surface. In the present study, to test the hypothesis of Felts et al. [2] more directly, the LPL added to the culture medium was prebound to chylomicrons. In the post-prandial state, only a small fraction of the LPL mass present in the plasma is associated with triglyceride-rich particles [25]. The amount of LPL that can become attached to chylomicrons is not known. Previous work that examined the protein composition of chylomicron remnants generated *in vivo* by electrophoresis did not detect the presence of a protein band corresponding to LPL [21,26]. It is therefore very likely that LPL *in vivo* becomes attached to chylomicron remnants in even smaller quantities than those used in the present study. Such quantities, as indicated by the present results, would be insufficient to mediate the binding of remnants to hepatocytes.

When incubations were performed at 37 °C, more than 20% of the normal chylomicron [^{14}C]fatty acids became associated with the cells (non-releasable by trypsin), indicating that the LPL bound to the chylomicrons was capable of sustaining lipolysis. The amounts of cell-associated ^{14}C radioactivity probably underestimate the extent of lipolysis because some of the unesterified [^{14}C]fatty acids released might have remained associated with the albumin present in the incubation medium. As a result of the LPL-mediated lipolysis, there was a significant uptake by the hepatocytes of the [^{3}H]retinol present in the lipoprotein core. This finding suggests that, as a result of LPL action, remnant particles were formed in the incubation medium and taken up by the hepatocytes. The finding that lactoferrin also inhibited the cellular incorporation of [^{3}H]retinol, without inhibiting the hydrolysis of the ^{14}C-labelled triacylglycerols, further indicates that remnant particles were generated in the incubation medium and subsequently taken up by the hepatocytes. In a recent study, Skottova et al. [6] used the isolated rat liver preparation to examine the effect of LPL in the clearance of chylomicrons, double-labelled with [^{14}C]fatty acids and [^{3}H]retinol, from circulation. In agreement with the results reported here, they observed that LPL caused lipolysis of the chylomicron triacylglycerols, as evidenced by increased levels of ^{14}C-labelled fatty acids in the perfusate. They further observed an increased incorporation of [^{3}H]retinol by the livers, which they also interpreted as indicating uptake of remnants generated in the perfusate. Together, the present results obtained with isolated hepatocytes and those obtained with the isolated perfused rat liver [6] indicate that the role of LPL in the clearance of chylomicrons is in catalysing the lipolysis of the particle. Skottova et al. [6] suggested that in addition to its catalytic action LPL might also mediate the uptake of chylomicrons by acting as a ligand to LRP. However, as with the studies with cultured cells [3–5], this ligand effect might have resulted from the large quantity of LPL added to the perfusion medium.

Because, as demonstrated in this study, LPL did not play a role in the binding of normal chylomicrons by the hepatocytes, the recognition and endocytosis of the lipolysed particles might have been mediated by the apo E present on the lipoprotein surface. Previous work from this laboratory had suggested, however, that the hepatic uptake of chylomicron remnants can occur by an apo E-independent mechanism [17,18]. The availability of chylomicrons isolated from apo E-deficient mice allowed us to investigate this possibility further. The results obtained when the apo E-free chylomicrons were incubated with apo E-free hepatocytes, in the presence or absence of LPL (Tables 4 and 5), were similar to those obtained when normal (apo E-containing) chylomicrons were used (Tables 1 and 2). These findings are consistent with the notion that chylomicron remnants formed in the incubation medium were subsequently taken up by hepatocytes through an apo E-independent mechanism. The nature of such a mechanism remains to be determined. It has been suggested that hepatic lipase may function in the uptake of chylomicron remnants by the liver by serving as a glycosaminoglycan-anchored ligand for remnant binding to the hepatocyte plasma membrane [27,28]. Hepatic lipase was presumably present on the surface of the isolated hepatocytes used in the present study. However, it is unlikely that it played a role in the uptake of the chylomicron remnants. Incubation of the cells with heparin, which releases hepatic lipase from the hepatocyte surface [20,27], did not affect the uptake of [^{3}H]retinol (Table 7).

Retinyl esters absorbed in the intestine are transported to the liver in association with chylomicron remnants [1], allowing them to be used as markers in studies on the uptake of chylomicron remnants by cells in culture [29] and on the clearance of chylomicrons from the circulation by the liver [30,31]. In the present study we also assumed that the ^{3}H radioactivity incorporated into the cells represented the uptake of chylomicron remnants containing ^{3}H-labelled retinyl esters in its core. In a recent study, however, Blaner et al. [32] reported that bovine milk LPL is able to catalyse the hydrolysis of retinyl ester in an artificial lipid emulsion and that the resulting unesterified retinol could be taken up by an adipocyte cell line in culture. It is conceivable that in the present study the [^{3}H]retinyl esters were hydrolysed by the chylomicron-bound milk LPL and that the cell-associated ^{3}H radioactivity represents unesterified [^{3}H]retinol taken up by the hepatocytes. However, the present results differ from those reported by Blaner et al. [32]. Whereas those authors observed that the incorporation of [^{3}H]retinol by the adipocytes was greatly stimulated by the presence of heparin in the incubation medium, in the present study heparin inhibited the uptake of [^{3}H]retinol by the hepatocytes (Figures 3 and 6). Further, the observation in the present study that the uptake of [^{3}H]retinol by the cells was also inhibited by lactoferrin is a further indication that the ^{3}H radioactivity present in the hepatocytes was derived from the uptake of [^{3}H]retinyl esters in the core of the chylomicron remnants.

In summary, we have demonstrated that LPL, attached to chylomicrons at a concentration that was sufficient to effectively hydrolyse their core triacylglycerols, did not enhance the binding of these lipoproteins to the surface of isolated hepatocytes. This observation is consistent with those of previous studies that demonstrated that chylomicron remnants can bind to liver membranes, and be taken up by the isolated perfused liver, in the absence of LPL [17,21]. We have also provided evidence that as a result of LPL-mediated lipolysis of chylomicrons lacking apo E, the [^{3}H]retinol present in the core of the lipoproteins was taken up by hepatocytes that also lacked apo E. These findings suggest that chylomicron remnants formed in the incubation medium were taken up by the hepatocytes by a process that does not involve medation by apo E. This conclusion is consistent

with that of a previous study that showed that in mice lacking apo E chylomicrons can be cleared from the circulation by the liver [18].

While the present study was being prepared for publication, our conclusion [18] that chylomicron remnants can be taken up by hepatocytes through an apo E-independent mechanism was questioned in a study by Mortimer et al. [33]. These authors injected apo E-deficient mice intravenously with a bolus of [^{3}H]cholesteryl ester-labelled chylomicron-like lipid emulsions, and measured the label incorporated into the livers 3 h later. They reported that less than 10 % of the injected dose was taken up by the livers. In our investigation [18] apo E-deficient mice were fed with [^{3}H]retinol, which is esterified in the intestine and absorbed in association with chylomicrons, and the radioactivity incorporated into the liver was measured 6 h later. We found that more than half of the absorbed [^{3}H]retinyl ester had been taken up by the livers. It is likely that the discrepancy between our observations *in vivo* and those of Mortimer et al. [33] are due to the differences in methods used in the studies. It is of interest that in the study by Mortimer et al. [33] the injected chylomicron-like particles were hydrolysed by LPL. The fact that the resulting remnants were not found associated with the liver indicates that, under the experimental conditions used, LPL was not involved in the recognition of remnants by the liver.

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