Initial hepatic removal of chylomicron remnants is unaffected but endocytosis is delayed in mice lacking the low density lipoprotein receptor

(low density lipoprotein-related protein/receptor-associated protein/cholesteryl esters)

JOACHIM HERZ*, SHI-QIANG QIU†, ADAIR OESTERLE†, HARSHINI V. DESILVA†, SHAHIDA SHAFI†,
AND RICHARD J. HAVEL†‡§

*Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX 75235; and †Cardiovascular Research Institute and
‡Department of Medicine, University of California, San Francisco, CA 94143

Contributed by Richard J. Havel, February 2, 1995

ABSTRACT   Two endocytic receptors, the low density lipoprotein (LDL) receptor (LDLR) and the LDLR-related protein (LRP), are thought to act in concert in the hepatic uptake of partially metabolized dietary lipoproteins, the chylomicron remnants. We have evaluated the role of these two receptors in the hepatic metabolism of chylomicron remnants in normal mice and in LDLR-deficient [LDLR (−/−)] mice. The rate of chylomicron remnant removal by the liver was normal up to 30 min after intravenous injection of chylomicrons into LDLR (−/−) mice and was unaffected by receptor-associated protein (RAP), a potent inhibitor of ligand binding to LRP. In contrast, endocytosis of the remnants by the hepatocytes, measured by their accumulation in the endosomal fraction and by the rate of hydrolysis of component cholesteryl esters, was dramatically reduced in the absence of the LDLR. Coadministration of RAP prevented the continuing hepatic removal of chylomicron remnants in LDL (−/−) mice after 30 min, consistent with blockade of the slow endocytosis by a RAP-sensitive process. Taken together with previous studies, our results are consistent with a model in which the initial hepatic removal of chylomicron remnants is primarily mediated by mechanisms that do not include LDLR or LRP, possibly involving glycosaminoglycan-bound hepatic lipase and apolipoprotein E. After the remnants bind to these alternative sites on the hepatocyte surface, endocytosis is predominantly mediated by the LDLR and also by a slower and less efficient backup process that is RAP sensitive and therefore most likely involves LRP.

Chylomicron remnants are derived from lymph chylomicrons that carry dietary fat and cholesterol into the blood. Chylomicron remnants are produced in extrahepatic tissues by hydrolysis of component triglycerides and some phospholipids by lipoprotein lipase, and after entering the liver are taken up into parenchymal cells by receptor-mediated endocytosis (1). Previous observations and experiments have strongly suggested that this process involves at least two receptors on the surface of hepatocytes (1, 2). Binding of the remnants to these receptors is mediated by apolipoprotein E (apoE) (1, 3). One of the receptors thought to be involved in remnant uptake is the low density lipoprotein (LDL) receptor (LDLR), which strongly binds apoE. Considerable indirect evidence suggests that another is the LDLR-related protein (LRP), which mediates the cellular uptake of apoE-enriched remnant lipoproteins (4).

LRP is a multifunctional protein that also binds α₂-macroglobulin and a variety of other macromolecules (5). Its role in cellular lipoprotein metabolism has been investigated in great detail, but the physiological importance of this function

has been difficult to evaluate in vivo because mouse embryos lacking LRP die in utero (6). LDLR-deficient [LDLR (−/−)] mice, in contrast, develop normally and do not accumulate large amounts of chylomicron remnants (7), consistent with observations in LDLR-deficient rabbits (8) and humans (9).

To dissect the respective contributions of LDLR and LRP to the removal of chylomicron remnants by the liver, we have in previous studies (10, 11) used recombinantly produced 39-kDa receptor-associated protein (RAP). RAP copurifies with LRP (12) and blocks the interaction of all known ligands with LRP by binding to multiple sites on the receptor (5). RAP effectively blocks the endocytosis of apoE-enriched chylomicron particles by the isolated perfused rat liver over a period of 15 min. To obtain this blockade, it was necessary to add a large excess of RAP to the liver perfusate, because RAP itself was rapidly endocytosed via binding to LRP. This amount of RAP was also found to block uptake of LDL via the LDLR (10), which binds RAP with lower affinity than LRP (10). Therefore it was impossible to determine whether the blockade of endocytosis of the chylomicron particles by RAP was a result of binding to the LDLR, LRP, or both. In these rat experiments, RAP caused only partial inhibition of the clearance of the chylomicron particles by the liver, consistent with other data that the initial removal of chylomicron remnants by the liver is mediated by interaction with other macromolecules, such as hepatic lipase (1, 13), or by interaction with apoE bound to extracellular proteoglycans (13–15).

In another study Willnow et al. (11) used adenovirus-mediated gene transfer to overexpress RAP in the livers of normal and LDLR (−/−) mice. RAP overexpression completely blocked LRP function and resulted in the massive accumulation of chylomicron remnants in LDLR (−/−) animals, whereas these lipoproteins were only modestly increased in wild-type mice. Taken together with the effects of RAP on hepatic endocytosis of apoE-enriched chylomicrons (10), these results conclusively demonstrate that the LDLR and another RAP-sensitive pathway, most likely involving LRP, are responsible for the hepatic endocytosis of chylomicron remnants.

In the current experiments, we have investigated the kinetics of the initial removal of chylomicron remnants by the livers of normal and LDLR (−/−) mice. We have further evaluated the respective quantitative contributions of LDLR and LRP to the subsequent endocytosis of the remnant particles. Our results show that the rate of endocytosis of chylomicron remnants is

much slower in LDLR $(-/-)$ mice, indicating that the LDLR is mainly responsible for the prompt endocytosis of chylomicron remnants in normal mice.

EXPERIMENTAL PROCEDURES

Materials. Intestinal lymph was collected from male Sprague–Dawley rats during intraduodenal infusion of 10% glucose in 0.15 M NaCl containing [³H]cholesterol suspended in bovine serum albumin (16). Chylomicrons were separated from lymph serum by ultracentrifugation and used within 48 h. Human α_2-macroglobulin was prepared and activated with 200 mM methylamine, and then radioiodinated as described (10). Bacterial glutathione S-transferase (GST) and fusion protein of GST with rat 39-kDa LRP-associated protein (GST-RAP) were prepared as described (17).

Mice and Experimental Protocols. LDLR-"knockout" mice [LDLR $(-/-)$] were created from hybrids of C57BL/6J and 129SvJ strains as described (7). Control mice were normal littermates or C57BL/6J mice of the same sex and similar age. All mice were raised on normal chow diet and fed the same chow for at least 1 week before study. Unless otherwise indicated, all experiments were performed upon male animals weighing 20–30 g, anesthetized with sodium pentobarbital (0.09 mg/g of body weight) administered intraperitoneally. Additional anesthetic was injected as needed to maintain light surgical anesthesia. Test materials were injected into an exposed jugular vein in a total volume of <250 μl. Blood samples of ≈ 50 μl were obtained from the orbital plexus, anticoagulated with EDTA as described (7), and placed on ice. At the end of experiments, the abdominal cavity was exposed via a midline incision and a blood sample was drawn from the inferior vena cava. The portal vein was then cannulated with a 21-gauge needle, which was tied in place. The vena cava above the liver was then incised and the liver was flushed with 3 ml of cold 0.15 M NaCl under a pressure of 50 cm of water. The liver was then removed and weighed. A mixed endosome fraction was prepared by minor modifications of methods developed for rat liver (18). Two livers were pooled and homogenized in 0.5 M sucrose, and an endosome-enriched subcellular fraction was obtained after several steps by centrifugation in a Percoll gradient. This material was diluted with 2 vol of 0.15 M NaCl, and the endosomes were obtained by centrifugation onto a sucrose cushion. The fluffy white material just above the sucrose cushion was harvested with a Pasteur pipette, resuspended in 0.15 M NaCl, and recentrifuged as above. The purified endosomes were again harvested from just above the sucrose cushion for analysis. Their appearance by electron microscopy closely resembled that shown previously for hepatocytic endosomes from rats (18). At each step of the fractionation procedure, samples were taken for analysis of ³H.

Analysis. Lipids were extracted from samples of blood plasma, liver, and subcellular fractions into chloroform/methanol (19) and ³H was estimated by liquid scintillation spectrometry in the extracts and in cholesterol and cholesteryl esters (CE) separated by thin-layer chromatography (20). ¹²⁵I was estimated in plasma and tissue samples by scintillation

spectrometry. Content of ³H was calculated for total plasma, estimated as 4.5% of body weight, and for total liver, with appropriate corrections for samples removed during subcellular fractionation. For analysis of concentration of plasma lipoprotein components, blood plasma pooled from samples obtained by exsanguination of 8–10 anesthetized mice from the inferior vena cava was subjected to sequential ultracentrifugation at densities of 1.006, 1.019, 1.055, 1.085, and 1.21 g/ml to obtain very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), LDL, and light and heavy high density lipoproteins (HDL_2 and HDL_3), respectively (21). Total cholesterol (22) and triglycerides (23) were estimated in plasma and lipoprotein fractions and their concentrations in original plasma were calculated as mg/dl. The concentrations of apolipoproteins B-100, B-48, and E were estimated by a quantitative SDS gel electrophoretic method developed for human apolipoproteins (24). Chromogenicity factors for human apoB-100 and apoB-48 (but not apoE) have been shown to apply to the rat proteins (24). These chromogenicity factors were used here.

Calculations. Differences between experimental groups were evaluated by one-way ANOVA. Results are given as mean of n experiments $\pm$ SD.

RESULTS

LDLR $(-/-)$ mice had increased levels of cholesterol in LDL, as reported previously (7), and also in IDL, but only a slight increase in VLDL-cholesterol (Table 1). The concentrations of apoB-100 and apoB-48 in VLDL of normal and LDLR $(-/-)$ mice were similar. The total concentration of apoB-100 in plasma, however, was increased almost 4-fold, mainly in LDL, and that of apoB-48 was increased only about 1.4-fold, also mainly in LDL, with a lesser increase in IDL. The concentration of apoE was increased 2.5-fold, predominantly in the LDL and IDL fractions, with a minor increase in VLDL.

To determine the effectiveness of GST-RAP in blocking removal of activated α_2-macroglobulin by the liver, the hepatic content of ¹²⁵I was determined at intervals after intravenous injection of ¹²⁵I-labeled α_2-macroglobulin together with GST-RAP (1.5 mg) or an equimolar amount of GST (0.9 mg) into normal and LDLR $(-/-)$ mice. Blockade by GST-RAP was $>97\%$ 20 min after injection (Table 2). Hepatic uptake began after about 40 min and was extensive by 60 min (data not shown). In two experiments, mean recovery of ¹²⁵I in hepatic endosomes 20 min after injection was 4.24% and 6.07% of that injected in normal and LDLR $(-/-)$ mice given GST and 0.07% and 0.02%, respectively, in mice given GST-RAP.

In normal and LDLR $(-/-)$ mice given GST together with injection of cholesterol-labeled chylomicrons, [³H]CE were cleared from blood plasma at comparable rates and more than 50% was recovered in the liver 20 min after intravenous injection (Table 2). In normal mice, injection of GST-RAP inhibited hepatic recovery of [³H]CE by 34% ($P < 0.01$), whereas in LDLR $(-/-)$ mice recovery of [³H]CE was reduced by only 17% (not significant). At this time, recovery of [³H]CE in hepatic endosomes of LDLR $(-/-)$ mice injected with GST was only about 1/10 that found in normal

Table 1. Concentrations (mg/dl) of plasma lipoprotein components in normal and LDLR $(-/-)$ male mice

Lipoprotein	Normal mice					LDLR $(-/-)$ mice				
	TC	TG	ApoB-100	ApoB-48	ApoE	TC	TG	ApoB-100	ApoB-48	ApoE
VLDL	4.7	54.1	0.9	1.2	1.1	8.2	46.7	0.8	0.6	1.4
IDL	2.1	6.1	0.8	0.2	0.2	17.2	8.8	2.4	0.6	1.5
LDL	19.4	14.7	6.0	0.8	1.5	134.6	14.3	25.4	3.2	8.7
HDL₂	9.6	2.0	0.3	0.9	1.5	15.5	1.8	0.8	0.7	1.2
HDL₃	48.5	1.4	—	0.5	1.8	87.2	1.5	—	—	2.4

TC, total cholesterol; TG, triglycerides.

Medical Sciences: Herz *et al.*

Proc. Natl. Acad. Sci. USA 92 (1995)   4613

Table 2. Hepatic recovery of [^{3}H]CE and ^{125}I 20 min after intravenous injection of [^{3}H]cholesterol-labeled chylomicrons and ^{125}I-labeled α_2-macroglobulin into normal and LDLR ($-/-$) male mice: Effect of GST and GST-RAP

| Injected material | Hepatic recovery, % of injected [^{3}H]CE or ^{125}I | | | |
| | Normal mice ($n = 8$ or 4) | | LDLR ($-/-$) mice ($n = 6$ or 4) | |
	GST	GST-RAP	GST	GST-RAP
Chylomicrons	57.82 ± 5.98	38.07 ± 13.73*	54.58 ± 5.24	45.07 ± 13.18
α_2-Macroglobulin	66.12 ± 7.21	1.24 ± 0.11*	56.96 ± 1.81	1.56 ± 0.50*

Mice received chylomicrons ($\approx$300 μg of triglycerides) and activated α_2-macroglobulin (3 μg) intravenously together with 0.9 mg of GST or 1.5 mg of GST-RAP. Livers were perfused and homogenized as described in the text. Values are means ± SD. Chylomicrons were analyzed in 8 normal mice and 6 LDLR ($-/-$) mice; α_2-macroglobulin was analyzed in 4 mice in all cases. *, $P < 0.01$ (vs. GST).

mice (Table 3). In normal mice GST-RAP reduced endosomal recovery of [^{3}H]CE by 78%, expressed as percent of [^{3}H]CE in liver and by 85%, expressed as percent of injected [^{3}H]CE ($P < 0.05$ in both cases). In LDLR ($-/-$) mice, GST-RAP had no effect upon endosomal recovery of [^{3}H]CE. These findings suggested that initial removal of chylomicron remnants by the liver was unimpaired in mice lacking the LDLR, but subsequent endocytosis was markedly impeded. Recovery of [^{3}H]CE in hepatic endosomes of LDLR ($-/-$) mice remained low at later intervals up to 1 h after injection of labeled chylomicrons (data not shown). In two experiments, endosomal uptake 35 min after injection (0.85% and 1.02% of hepatic [^{3}H]CE) was inhibited by 72% and 82% by injection of GST-RAP.

To gain additional information about endocytosis of chylomicron remnants in LDLR ($-/-$) mice, the rate of accumulation of ^{3}H in unesterified cholesterol (UC) in the liver was evaluated as a measure of lysosomal hydrolysis of the [^{3}H]CE by acid lipase (25). In the injected chylomicrons 13–18% of the [^{3}H]cholesterol was unesterified. Therefore, the increase in the percentage of ^{3}H in UC in liver above that found in the injected chylomicrons was taken as a measure of hydrolysis of [^{3}H]CE removed by the liver. As shown in Table 4, a progressive increase in the percentage of ^{3}H in UC was observed in livers of normal mice after injection of labeled chylomicrons, to a level of 45% above that in the injected chylomicrons after 60 min. Little increase was evident in LDLR ($-/-$) mice at 20 and 40 min after injection, but a value of 17% above that in the injected chylomicrons was observed after 60 min, significantly less than the increase found in normal mice ($P < 0.01$). This estimate of the rate of hydrolysis could be imprecise for two reasons: first, [^{3}H]UC was removed from blood plasma at a somewhat greater rate than that of [^{3}H]CE, reflecting exchange with cell membranes of liver and erythrocytes (26); second, some of the UC released by hydrolysis of CE in liver could reenter the blood by exchange with lipoprotein UC or be excreted into the bile.

The slower rate of hydrolysis of hepatic [^{3}H]CE in LDLR ($-/-$) mice was reflected in persistent accumulation of [^{3}H]CE in liver, up to 43% of that injected after 60 min (Table

5). By contrast, the more rapid hydrolysis of chylomicron CE in livers of normal mice was reflected in lower rates of accumulation in liver, with maximal recovery of 32% of that injected after 40 min and only 19% after 60 min.

Injection of GST-RAP together with and 30 min after injection of labeled chylomicrons into female LDLR ($-/-$) mice reduced recovery of injected [^{3}H]CE in the livers 60 min after injection to 29.1 ± 11.5%, whereas recovery was 44.3% ± 8.1% after injection of GST ($P < 0.01$). Although clearance of [^{3}H]cholesterol from the blood was similar in animals given GST or GST-RAP 30 min after injection (Fig. 1), no further clearance was observed after injection of GST-RAP during the next 30 min (in some animals, recovery of [^{3}H]cholesterol in the blood plasma increased between 30 and 60 min). In animals given GST, clearance of [^{3}H]cholesterol invariably continued during this interval.

DISCUSSION

The current studies with LDLR-deficient mice indicate that the initial removal of chylomicron remnants from the circulation by mouse liver is largely independent of the LDLR and of the LRP, which can also internalize chylomicron remnants in an apoE-dependent manner. Rather, remnants appear to bind predominantly to other distinct sites on the hepatocyte surface. Endocytosis of these surface-bound remnants is, however, mediated primarily by the LDLR, as the rate of chylomicron remnant accumulation in endosomes was substantially reduced in LDLR ($-/-$) mice. In the absence of LDLR, endocytosis proceeds by means of a RAP-sensitive process involving LRP, albeit at a much slower rate.

These results and the proposed mechanism of delayed hepatic remnant endocytosis in the absence of functional LDLRs are consistent with previous observations in LDLR-deficient rabbits (8) and humans (9). As expected, LDLR ($-/-$) mice had grossly elevated concentrations of LDL and IDL. There was no appreciable increase in particle number in the VLDL fraction in these mice, as assessed from the concentration of apoB-100 and apoB-48. The VLDL were, however, enriched with cholesterol and apoE, suggesting a limited

Table 3. Recovery of chylomicron CE in hepatic endosomes 20 min after intravenous injection of [^{3}H]cholesterol-labeled chylomicrons into normal and LDLR ($-/-$) male mice

| Mice | % of [^{3}H]CE | | | |
| | In liver ($n = 4$) | | Injected ($n = 3$) | |
	GST	GST-RAP	GST	GST-RAP
Normal	4.90 ± 3.06	1.10 ± 0.42*	2.85 ± 1.94	0.43 ± 0.19*
LDLR ($-/-$)	0.52 ± 0.091†	0.56 ± 0.46	0.29 ± 0.032†	0.24 ± 0.20

Data are from the same experiments as shown in Table 2. Endosomes were isolated from homogenates of livers from pairs of mice. Values are means ± SD. *, $P < 0.05$ (vs. GST); †, $P < 0.01$ (vs. normal mice).

Table 4. Hydrolysis of CE of chylomicrons taken up by liver of normal and LDLR ($-/-$) male mice

| Time after injection, min | % increase in [^{3}H]UC above that in injected chylomicrons | |
	Normal mice ($n = 6$)	LDLR ($-/-$) mice ($n = 6$)
20	9.2 ± 9.7	2.0 ± 2.2
40	17.3 ± 14.3	4.8 ± 3.8
60	44.5 ± 15.9*	16.7 ± 6.7*†

Mice were injected with chylomicrons (300 μg of triglycerides) intravenously. At indicated times, livers were perfused and homogenized. Values are means ± SD. *, $P < 0.01$ (vs. 40 and 20 min); †, $P < 0.01$ (vs. normal mice).

Table 5. CE in liver after injection of chylomicrons into normal and LDLR $(-/-)$ male mice

Time after injection, min	[³H]CE in liver, % of injected [³H]CE	
	Normal mice ($n = 6$)	LDLR $(-/-)$ mice ($n = 6$)
20	25.7 ± 7.1	36.6 ± 15.6
40	31.6 ± 11.4	41.6 ± 12.3
60	18.9 ± 6.1*	42.9 ± 6.6†

Data are from the same experiments as shown in Table 4. Values are means ± SD. *, $P < 0.01$ (vs. 40 min); †, $P < 0.01$ (vs. normal mice).

accumulation of remnants of hepatogenous VLDL and chylomicrons.

Endocytosis of remnant particles by hepatocytes was readily detectable in normal mice expressing functional LDLRs, but it was strikingly impeded in LDLR $(-/-)$ mice. Failure of [³H]CE in the injected bolus of chylomicrons to accumulate in endosomes of LDLR $(-/-)$ mice could reflect a slow rate of endocytosis such that transit through endosomal compartments to lysosomes occurred as rapidly as entry into these compartments. This interpretation is consistent with the much slower rate of hydrolysis of the [³H]CE contained in the chylomicrons after initial removal of the remnants by the liver of LDLR $(-/-)$ mice. Such a slow rate of endocytosis is, however, evidently sufficient to prevent gross accumulation in plasma of remnant particles containing apoB-48, in contrast with particles derived from the liver that contain apoB-100.

Our results do not speak to the effect of deficiency of the LDLR on the hepatic removal and endocytosis of remnants derived from hepatogenous VLDL containing apoB-48, but it may be speculated that the endocytosis of these particles may be a function of their size: large particles may be susceptible to endocytosis via LRP as well as the LDLR (1). The injected chylomicrons in the current study mainly had diameters in the range of 400–800 Å (16). In LDLR-deficient rabbits, hepatogenous VLDL particles (greater than 500 Å in diameter) containing apoB-100 are removed from the blood at a normal rate, whereas clearance of smaller VLDL is substantially impeded (27). Smaller VLDL containing apoB-48, like those containing apoB-100, may be converted to LDL in normal mice, and this conversion is likely to be increased in LDLR
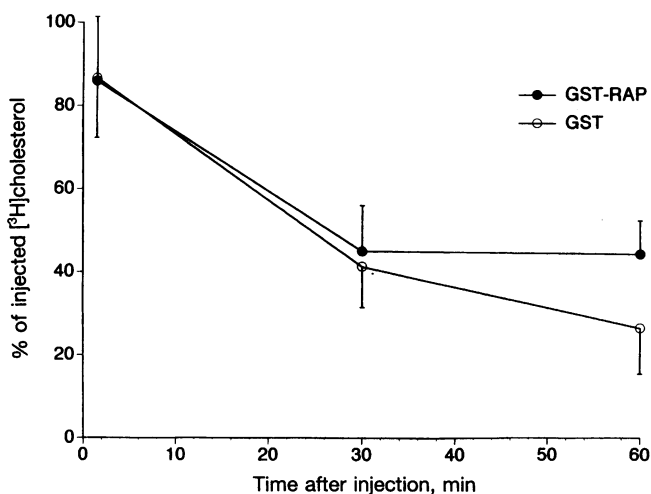

FIG. 1. Effect of administration of GST and GST-RAP upon the clearance of [³H]cholesterol in chylomicrons from blood plasma of female LDLR $(-/-)$ mice. GST (0.9 mg) and GST-RAP (1.5 mg) were injected together with the chylomicrons and again 30 min later. Values are means for 11 mice given GST and 10 mice given GST-RAP. Bars indicate 1 SD. The values for the two groups, calculated for a plasma volume equal to 4.5% of body weight, differ at 60 min after injection ($P < 0.001$).

$(-/-)$ mice, given the increased concentration of apoB-48 in their LDL.

As in normal rats (10), recovery of [³H]CE in the liver 20 min after injection of labeled chylomicrons into normal mice was reduced about 30% by injection of a saturating amount of GST-RAP, and accumulation of the [³H]CE in endosomes was largely prevented. By contrast, GST-RAP had little effect on hepatic removal of [³H]CE in LDLR $(-/-)$ mice. This may reflect the normal contribution of the LDLR to the initial binding of chylomicron remnants at the cell surface as well as its primary role in their endocytosis.

Most of the initial removal of [³H]CE by the liver of normal as well as LDLR $(-/-)$ mice appears to be mediated by binding of the remnants to sites other than LDLR. This binding could involve hepatic lipase, as demonstrated in normal rats (13). It must be recalled, however, that only a fraction of hepatic lipase in mice is bound to liver cell surfaces, while the remainder is present in blood plasma (28). The initial removal does not involve binding to LRP in any significant amount, however, because it was largely unaffected by GST-RAP in the animals lacking the LDLR. Hepatic lipase and other macromolecules that could bind chylomicron remnants and thus mediate the initial removal, such as glycosaminoglycans (29), were evidently affected little by the large amount of RAP to which the livers of the LDLR $(-/-)$ mice were exposed.

Given the slow rate of endocytosis of chylomicron remnants in the LDLR $(-/-)$ mice, it was difficult to demonstrate an effect of GST-RAP upon this process. Maintenance of saturating amounts of GST-RAP in the circulation for 1 h did, however, prevent the continuing removal of the injected [³H]CE from the blood after 30 min. On average, clearance between 30 and 60 min was abolished, and in some animals, [³H]CE evidently reentered the plasma compartment during this time. This phenomenon could reflect blockade of the slow hepatic endocytosis by GST-RAP, leading to saturation of remnant particles at the sites of initial binding to liver cells and subsequent displacement by other particles entering the blood. These observations suggest that removal of chylomicron remnants from the blood in the steady state could be more easily saturated in the absence of the LDLR, resulting in their delayed postprandial removal from plasma. In our experiments, we injected only 300 μg of chylomicron triglycerides, which yielded an initial concentration in plasma of ≈30 mg/dl.

It is of interest to relate our results to those obtained by overexpressing RAP in normal and LDLR $(-/-)$ mice (11). In both situations, RAP was secreted into the blood to give steady-state concentrations of up to 0.3 mg/ml of blood plasma (considerably less than the level of about ≈1.5 mg/ml initially achieved in the current studies). In normal mice, this led to a modest accumulation of particles the size of chylomicron remnants which were enriched in apoB-48. These particles could represent either chylomicron remnants or remnants derived from hepatogenous VLDL, because the mouse liver secretes apoB-48 as well as apoB-100. In LDLR $(-/-)$ mice, however, this level of circulating RAP was associated with a gross accumulation of particles resembling chylomicron/VLDL remnants containing apoB-48. Given the lower affinity of RAP for the LDLR than for LRP, it appears that a partially functioning LDLR can prevent gross accumulation of such particles when LRP's function is essentially completely blocked. This result is consistent with our conclusion that the LDLR normally mediates the prompt endocytosis of chylomicron remnants into mouse hepatocytes. LRP (or conceivably another receptor whose function is blocked by RAP) thus emerges as a rather inefficient substitute for endocytosis via the LDLR, but one which can prevent gross accumulation of chylomicron remnants.

The delay in the hepatic endocytosis of chylomicron remnants when the LDLR is absent may be explained by the

requirement for enrichment of remnant lipoproteins with additional apoE before they become a substrate for binding to LRP (4, 30). Only after addition of exogenous apoE are β-VLDL, a remnant lipoprotein from cholesterol-fed rabbits, taken up by endocytosis via LRP and delivered to lysosomes (4). ApoE is known to be concentrated on the sinusoidal surface of rat hepatocytes (14). This raises the possibility that the additional apoE required for binding to LRP is acquired by chylomicron remnants after the particles bind to other sites at or near the cell surface (1, 4, 14). Inhibition of cell surface hepatic lipase by specific antibody substantially reduces the initial removal of chylomicron remnants by rat liver but not the endocytosis of those remnants taken up (13), consistent with an important role for hepatic lipase in the initial binding as well as the role demonstrated here for the LDLR in the endocytosis of chylomicron remnants. This model is consistent with *in vitro* findings of enhanced binding of β-VLDL by cultured cells that had been transfected with apoE (15) or with hepatic lipase (31). In both cases, treatment of the cells with heparinase abrogated the effect, suggesting that glycosaminoglycans are also involved. Acquisition of additional apoE by chylomicron remnants or altered exposure of apoE on the particle (32) may be promoted by hydrolysis of component lipids by hepatic lipase; hence it would be of interest to evaluate the effect of inhibiting this enzyme in LDLR $(-/-)$ mice.

1. Havel, R. J. (1992) *Semin. Liver Dis.* **12**, 356–363.
2. Ishibashi, S., Herz, J., Maeda, N., Goldstein, J. L. & Brown, M. S. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 4431–4435.
3. Mahley, R. W. (1988) *Science* **240**, 622–630.
4. Kowal, R. C., Herz, J., Goldstein, J. L., Esser, V. & Brown, M. S. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5810–5814.
5. Krieger, M. & Herz, J. (1994) *Annu. Rev. Biochem.* **63**, 601–637.
6. Herz, J. (1993) *Curr. Opin. Lipidol.* **4**, 107–113.
7. Ishibashi, S., Brown, M. S., Goldstein, J. L., Gerard, R. D., Hammer, R. E. & Herz, J. (1993) *J. Clin. Invest.* **92**, 883–893.
8. Kita, T., Goldstein, J. L., Brown, M. S., Watanabe, Y., Hornick, C. A. & Havel, R. J. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3623–3627.
9. Rubinsztein, D. C., Cohen, J. C., Berger, G. M., van der Westhuyzen, D. R., Coetzee, G. A. & Gevers, W. (1990) *J. Clin. Invest.* **86**, 1306–1312.
10. Mokuno, H., Brady, S., Kotite, L., Herz, J. & Havel, R. J. (1994) *J. Biol. Chem.* **269**, 13238–13243.
11. Willnow, T. E., Sheng, Z., Ishibashi, S. & Herz, J. (1994) *Science* **264**, 1471–1474.
12. Strickland, D. K., Ashcom, J. D., Williams, S., Burgess, W. H., Migliorini, M. & Argraves, W. S. (1990) *J. Biol. Chem.* **265**, 17401–17404.
13. Shafi, S., Brady, S. E., Bensadoun, A. & Havel, R. J. (1994) *J. Lipid Res.* **35**, 709–720.
14. Hamilton, R. L., Moorehouse, A. & Havel, R. J. (1991) *J. Lipid Res.* **32**, 529–543.
15. Ji, Z.-S., Brecht, W. J., Miranda, R. D., Hussain, M. M., Innerarity, R. L. & Mahley, R. W. (1993) *J. Biol. Chem.* **268**, 10160–10167.
16. Windler, E., Chao, Y.-S. & Havel, R. J. (1980) *J. Biol. Chem.* **255**, 5475–5480.
17. Herz, J., Goldstein, J. L., Strickland, D. K., Ho, Y. K. & Brown, M. S. (1991) *J. Biol. Chem.* **266**, 21232–21238.
18. Belcher, J. D., Hamilton, R. L., Brady, S. E., Hornick, C. A., Jaeckle, S., Schneider, W. J. & Havel, R. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6785–6789.
19. Bligh, E. G. & Dyer, W. J. (1959) *J. Biochem. Physiol.* **37**, 911–917.
20. Fielding, C. J. (1979) *Biochim. Biophys. Acta* **573**, 255–265.
21. Havel, R. J., Eder, H. & Bragdon, J. (1955) *J. Clin. Invest.* **34**, 1345–1353.
22. Allain, C. C., Poon, L. C., Chan, C. S. G., Richmond, W. & Fu, P. C. (1974) *Clin. Chem.* **20**, 470–475.
23. Buccolo, G. & David, H. (1973) *Clin. Chem.* **19**, 476–482.
24. Kotite, L., Bergeron, N. & Havel, R. J. (1995) *J. Lipid Res.* **36**, 890–900.
25. Runquist, E. A. & Havel, R. J. (1991) *J. Biol. Chem.* **266**, 22557–22563.
26. Goodman, D. S. (1962) *J. Clin. Invest.* **41**, 1886–1896.
27. Yamada, N., Shames, D. M., Takahashi, K. & Havel, R. J. (1988) *J. Clin. Invest.* **82**, 2106–2113.
28. Peterson, J., Bengtsson-Olivecrona, G. & Olivecrona, T. (1986) *Biochim. Biophys. Acta* **878**, 65–70.
29. Jackson, R. L., Busch, S. J. & Cardin, A. D. (1991) *Physiol. Rev.* **71**, 481–539.
30. Kowal, R. C., Herz, J., Weisgraber, K. H., Mahley, R. W., Brown, M. S. & Goldstein, J. L. (1990) *J. Biol. Chem.* **265**, 10771–10779.
31. Ji, Z.-S., Lauer, S. J., Fazio, S., Bensadoun, A., Taylor, J. M. & Mahley, R. W. (1990) *J. Biol. Chem.* **269**, 13429–13436.
32. Brasaemle, D. L., Cornely-Moss, K. & Bensadoun, A. (1993) *J. Lipid Res.* **34**, 455–465.