Intracellular Localization and Metabolism of Chylomicron Remnants in the Livers of Low Density Lipoprotein Receptor-deficient Mice and ApoE-deficient Mice

EVIDENCE FOR SLOW METABOLISM VIA AN ALTERNATIVE apoE-DEPENDENT PATHWAY*

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The metabolism of chylomicron remnants in mice deficient in low density lipoprotein receptor (LDLr) or apolipoprotein E (apoE) was compared with that of control C57BL/6J mice. Mice were injected intravenously with chylomicron-like emulsions labeled with radioactive lipids. Blood samples were taken at fixed time intervals from the retro-orbital sinus, and clearance rates of the lipoproteins were assessed from the decline in plasma radioactivities. To follow the intracellular pathway of remnants in the liver, emulsions labeled with a fluorescent cholesteryl ester (BODIPY) were injected, and liver sections were processed and assayed by laser confocal microscopy. Catabolism of remnant cholesteryl esters was assessed by injecting emulsions labeled with cholesteryl[L-14C]oleate and measuring the expired CO2 from each animal.

In apoE-deficient mice, remnant removal from plasma was totally impeded, while the clearance of remnants in LDLr-deficient mice was similar to that in C57BL/6J control mice. The confocal micrographs of livers 20 min after injection of fluorescent chylomicron-like emulsions showed evenly distributed fluorescent particles in the hepatocytes from control mice. In contrast, the fluorescent particles were mainly located in sinusoidal spaces in LDLr-deficient mice. Three hours after injection the liver sections from control mice showed few fluorescent particles, indicating that remnants have been catabolized, while the sections from LDLr-deficient mice were still highly fluorescent. Micrographs from apoE-deficient mice showed no fluorescent particles in the liver at any time after injection. Measurement of expired radioactive CO2 after injection of emulsions labeled in the fatty acid moiety of cholesteryl oleate indicated that remnant metabolism was slower in the LDLr-deficient mice and essentially nil in the apoE-deficient mice. Control mice had expired 50% of the injected label by 3 h after injection.

We conclude that under normal circumstances, chylomicron remnants are rapidly internalized by LDLr and catabolized in hepatocytes, with a critical requirement for apoE. When LDLr is absent, remnants are taken up by a second apoE-dependent pathway, first to the sinusoidal space of the liver, with subsequent slow endocytosis and slow catabolism. Hepatic clearance via this second pathway is increased by heparin, inhibited by lactoferrin, heparinase, and suramin, and down-regulated by feeding a high fat diet.

Chylomicrons are responsible for transporting most dietary lipids from the intestinal tract into the bloodstream. Along with the bulk of lipids in the form of triacylglycerols (triglycerides) and phospholipids, a small proportion of the total mass of chylomicron is made up of cholesterol and cholesteryl esters. In the circulation, chylomicrons are metabolized by a two-stage process. Initially, the majority of the triglyceride is hydrolyzed by the action of lipoprotein lipase and taken up by extrahepatic tissues. The resulting smaller particles, known as chylomicron remnants, contain the residual triglyceride and all of the cholesterol and cholesteryl ester. The remnants have acquired apolipoprotein E (apoE) and are removed rapidly from the plasma by the liver into hepatocytes and delivered to lysosomes (1). After hydrolysis of the cholesteryl esters by cholesterol ester hydrolase, the liberated fatty acids enter the pathways of oxidative metabolism and carbon dioxide is formed. The exhaled CO2 can be quantified (2) to provide a measure for the internalization and catabolism of remnants.

The central role played by apoE in the metabolism of remnant lipoproteins has been well established by several lines of evidence. In type III hyperlipidemia, the presence of defective apoE, which does not bind to the liver receptors, leads to an accumulation of β-very low density lipoproteins (β-VLDL) (3). For mice in which the apoE gene was targeted and nullified by homologous recombination (apoE knock-out), remnant lipoproteins accumulated in the plasma, and the mice developed hypercholesterolemia and premature atherosclerosis even on a chow (low fat) diet (4). In contrast, transgenic mice overexpressing rat apoE (5) or human apoE-3 (6) showed enhanced remnant clearance and were protected from diet-induced hypercholesterolemia. Transgenic mice overexpressing human apoE-4 were less protected against hypercholesterolemia, and chylomicron clearance was enhanced when mice were fed a low fat diet but not when fed a high fat diet (7). These findings have confirmed the critical role of apoE in directing the clearance of remnants from plasma and show that remnant clearance is modulated by the isoform of apoE as well as by the amount of apoE available.

Although the necessity of apoE for hepatic uptake of chyl-

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1 The abbreviations used are: apoE, apolipoprotein E; LDLr, low density lipoprotein receptor; VLDL, very low density lipoprotein; LRP, low density lipoprotein receptor related protein; GST, glutathione S-transferase; RAP, receptor-associated protein; HSPG(s), heparan sulfate proteoglycans.
micron remnants is well established, the putative hepatic receptor that mediates this uptake remains controversial. The frequently cited remnant (apoE) receptor (8) has not been identified. However, it is now well accepted that under normal circumstances the bulk of chylomicron remnants are cleared by the apoE/E receptor, also known as the low density lipoprotein receptor (LDLR) (9, 10). Apart from LDLr, both the low density lipoprotein receptor-related protein (LRP) (11) and a newly defined lipolysis-stimulated receptor (12) have been proposed to serve as receptors mediating the removal of chylomicron remnants from the circulation. Recently, an important contributory role for heparan sulfate proteoglycans (HSPG) in remnant uptake has been clarified. The LDLr knockout mice provide a unique model for testing the contributions by receptors such as LRP or lipolysis-stimulated receptor and HSPG for remnant clearance in vivo.

Using the $^3$H-retinol-fat tolerance test in apoE-deficient mice, Chang et al. (13) recently described an apoE-independent pathway for the hepatic clearance of chylomicron remnants. By 4–6 h after a fat meal containing $^3$H-retinol, only 17–20% of the label remained in plasma, and up to 53% of the radioactivity was recovered in the liver. These findings are inconsistent with previous reports of hypercholesterolemia caused by the accumulation of triglyceride-rich lipoproteins (d < 1.006 g/ml) and the high incidence of atherosclerosis found in apoE-deficient mice (4, 14) and also fail to explain the high concentration of apoB-48 in apoE deficiency shown by Ishibashi et al. (9).

In the present investigation, we measured plasma clearance and liver uptake of injected chylomicron-like emulsions in apoE-deficient mice and LDLr-deficient mice. Findings were compared with control C57BL/6J mice. Chylomicron-like emulsions mimic the metabolism of lymph chylomicrons (7, 15) and were preferred for this study to avoid injecting exogenous apolipoproteins that would complicate interpretation of results. To establish the intracellular pathway of the remnants, emulsions were labeled with a fluorescent cholesteryl ester (BODIPY). The emulsions were injected into the knockout and control mice, and the liver sections were processed for confocal microscopic images. We also traced the catabolism of the remnants by quantifying the exhaled CO$_2$ from both LDLr knockout and apoE knockout mice for comparison with controls, following injection of emulsions labeled with $^{14}$C on the fatty acid moiety of cholesteryl oleate.

**EXPERIMENTAL PROCEDURES**

Materials—Egg yolk phosphatidylcholine was purchased from Lipid Products (Surrey, UK). Cholesterol, cholesteryl oleate, and triglyceride were from Nu-Chek-Prep. Radiochemicals, including $[7-^3$H]cholesterol, glycerol tri-[$^{14}$C]oleate, and cholesteryl [$^{14}$C]oleate, were purchased from Amersham Corp. As described earlier, $^{3}$H-cholesteryl oleate was synthesized from [9,10-$^{3}$H]oleic acid (Amersham) and cholesterol (15). The fluorescent probes, BODIPY and Texas Red dextran (lysine-fixable, with M, 70,000) were purchased from Molecular Probes Inc. Bovine lactoferrin and heparin were purchased from Sigma, and suramin was obtained from Bayer. Heparinase (EC 4.2.2.7, Sigma, Heparinase I, H 2510) was solubilized in 0.15M NaCl. As described earlier, GST-RAP was purified from human plasma by zinc chelate chromatography according to the method of Porath et al. (16) and activated according to Vassiliou and Stanley (17). The glutathione S-transferase and rat 39-kDa receptor-associated fusion protein (GST-RAP) was purified from a pGEX-GST-RAP construct generously supplied by Dr. K. Stanley and originally from Dr. J. Herz.

Animals—Colonies of apoE knockout mice and LDLr receptor knockout mice were established from progenitor stocks obtained from the Jackson Laboratories (Bar Harbor, ME). The mice had been bred by sibling matings to obtain animals homozygous for the null mutation. C57BL/6J mice and Wistar male rats were obtained from the Animal Resources Centre (Murdoch, Australia). Male mice ranging in age from 9 to 12 weeks were used for this study. Animals were fed a pelleted diet containing approximately 5% fat unless otherwise specified.

Preparation of Chylomicrons—Male Wistar rats weighing approxi-
Preparation of Chylomicron-like Lipid Emulsions—Chylomicron-like lipid emulsions labeled with cholesteryl [9,10-3H]oleate and glycerol tri-[1-14C] oleate were prepared by sonicating a mixture of pure triolein, cholesteryl oleate, egg lecithin, and free cholesterol in 0.154 M NaCl, 10 mM HEPES (pH 7.4). Chylomicron-size particles of diameter 135–150 nm were purified from the sonicated mixture by serial ultracentrifugation in a density gradient. Details of the procedures and characterization of the emulsion particles have been given previously (15).

Clearance—Anesthesia was induced in the mice by intraperitoneal injection of avertin (tribromoethanol). Exactly 50 μl of emulsion or lymph chylomicrons, containing approximately 250–300 μg of total lipid, was injected via a 30-gauge needle into a tail vein. The effects of other ligands on remnant clearance were tested by injecting a solution containing the effectors (lactoferrin, heparin, heparinase, RAP, α2-macroglobulin, or suramin) 1–5 min prior to emulsion injection. Three blood
samples of approximately 75 μl were taken from a retro-orbital venous sinus of each animal at fixed time intervals following emulsion injection. Radioactivity in plasma was measured by liquid scintillation spectrometry. After collection of the final blood sample, the animal was exsanguinated, and the liver was excised for extraction of lipids and measurement of radioactivity.

Measurement of Remnant Metabolism from Carbon Dioxide—Fig. 1A compares the plasma clearance of a chylomicron-like emulsion in the control, apoE-deficient, and LDLr-deficient mice. At 30 min after the injection, more than 85% of the injected emulsion was cleared from the plasma of the control and LDLr-deficient mice. In contrast, over 60% of the injected emulsion remained in the plasma of apoE-deficient mice. The differences in clearance were statistically significant (p < 0.05 at 10 min and p < 0.0001 at 20 and 30 min). The hepatic uptake of the emulsion is shown in Fig. 1B. Approximately 60% of the injected radioactivity was recovered in the liver of the control and LDLr-deficient mice, but only about 5% was recovered in the liver of the apoE-deficient mice. The removal of emulsion triglyceride in apoE-deficient mice was normal and comparable with triglyceride clearance in normal mice (results not shown). Triglyceride removal in LDLr-deficient mice was also not significantly different from that in control mice.

To confirm the results obtained with chylomicron-like emulsions, clearances of lymph chylomicrons were also measured. Plasma clearances of endogenously labeled rat lymph chylomicrons in control and LDLr-deficient mice were similar to those of chylomicron-like emulsions. Clearance of lymph chylomicrons in apoE-deficient mice was not measured, as the chylomicrons contained endogenous apoE from the donor rats.

Fluorescent Localization of Remnants in Liver Cells—Fig. 2A shows a series of confocal images of liver sections. As shown in the figures, 5 min after injection of the emulsion, fluorescent labeled remnants were associated with the liver cells in both the control and the LDLr-deficient mice but not with apoE-deficient mice. However, in the LDLr-deficient mice, remnants accumulated at the boundary of the sinusoidal spaces. By 20 min after injection, the fluorescent label remained evenly dis-
tributed in the hepatocytes of control mice, whereas in the LDLr-deficient mice, streaks of fluorescent remnants again accumulated in the sinusoidal spaces. Using Texas Red associated with dextran (Mr 70,000) (a second fluorescent probe, which remained confined to the sinusoidal space), we confirmed that the accumulated remnant particles in the LDLr-deficient mice were localized to the sinusoidal spaces (Fig. 2B). At 3 h after the injection, little fluorescence was detected in the liver sections of the control mice, indicating that by this time remnants had been catabolized completely in the hepatocytes of control mice. In contrast, in LDLr-deficient mice, 3 h after injection of emulsion, fluorescence was now evenly distributed in the hepatocytes.

It was of interest to compare the hepatic uptake of the fluorescent label with the hepatic uptake of radioactivity after injection of the emulsion labeled with 3H-cholesteryl oleate (CO) and 14C-triolein (TO) to trace the remnant clearance and triglyceride removal of the injected particles. An aliquot of heparin (5 units/mouse) was injected 1 min before the injection of emulsions. Closed circle, control mice; open square, LDLr-deficient mice; diamond, control with heparin; cross, LDLr-deficient mice with heparin. A, plasma clearance of emulsion 3H-cholesteryl oleate; B, plasma clearance of 14C-triolein; C, 3H-cholesteryl oleate recovered in the livers; D, 14C-triolein recovered in the liver of control C57BL/6 and LDLr-deficient mice.

The catabolism of remnants by the liver was quantified by measuring the output of 14CO2 in the expired breath of the control, LDLr-deficient and apoE-deficient mice (19). The rate of expiration of 14CO2 was much slower in the apoE-deficient mice than in the controls (p < 0.0001). The radioactivity in expired breath was also significantly less in the LDLr-deficient mice compared with controls (p < 0.0001), indicating a defect or delay in the catabolism of remnants removed from the plasma.

The effect of lactoferrin, heparinase, heparin, RAP, suramin and a2-macroglobulin on remnant clearance in control and LDLr-deficient mice—To elucidate the nature of the hepatic remnant uptake in the absence of LDLr, we compared the effects of lactoferrin (2 mg/mouse), heparin (5 units/mouse), heparinase (30 units/mouse), RAP-GST (1 mg/mouse), suramin (0.2 mg/mouse and 1 mg/mouse) and a2-macroglobulin (1 mg/mouse) on remnant clearance in control and LDLr-deficient mice.

In previous studies lactoferrin has been shown to delay clearance (21) and to inhibit the endocytosis of chylomicron remnants (22) in the rat. Fig. 3A shows that injection of both lactoferrin and heparinase prior to emulsion injection inhibited the clearance of chylomicron remnants similarly in control and LDLr-deficient mice. Heparinase appeared to be a more effective inhibitor of remnant removal than lactoferrin, with about 80% of the injected radioactivity remaining in plasma at 30 min (Fig. 3A). The removal of emulsion triglyceride closely mirrored that of cholesteryl oleate. Triglyceride clearance was fast in both control and LDLr-deficient mice but severely retarded in the presence of lactoferrin or heparinase. Heparinase also appeared to be more potent than lactoferrin in inhibiting triglyceride clearance (Fig. 3B). Consistent with the clearance data, the hepatic recovery of labeled emulsion cholesteryl oleate was reduced from about 60 to 12% (p < 0.0005) in both control and LDLr-deficient mice, in the presence of lactoferrin or heparinase (Fig. 3C). The hepatic recovery of labeled triglyceride was
similar with and without the inhibitors.

In contrast to lactoferrin and heparinase, heparin increased the rates of lipolysis and remnant clearance of the injected emulsion in both control and LDLr-deficient mice (Fig. 4A). Heparin is known to release lipoprotein lipase from the vascular endothelial surface, and hence an increase in lipolysis is expected. As shown in Fig. 4A, 10 min after emulsion injection, the amount of radioactive cholesteryl oleate remaining in the plasma was about 10% in the presence of heparin, compared with 40% without heparin (p < 0.0005). Similarly, the amount of 14C-labeled triolein remaining in plasma decreased from about 25 to about 5% accompanied by a significant (p < 0.0005) increase in the liver uptake of 14C-label (35% with heparin compared with 25% without) at 30 min after emulsion injection (Fig. 4D). Heparin also increased the uptake of cholesteryl oleate (Fig. 4C) in the liver of control and LDLr mice.

The clearance of chylomicron remnants in the presence of activated α2-macroglobulin was also compared in control and LDLr-deficient mice. Consistent with Choi and Cooper (23), in control mice unlabeled activated α2-macroglobulin slightly inhibited (difference not significant) the plasma clearance of chylomicron remnants (results not shown). However, the slight inhibition was not observed in LDLr-deficient mice. RAP, the 39-kDa protein that co-purifies with α2-macroglobulin, was injected as a fusion protein conjugated to GST. Because of the fast metabolism of RAP, the clearance of emulsion was measured at 5, 10, and 20 min. 1 mg/mouse of GST-RAP injected prior to emulsion injection did not affect the clearance of chylomicrons in control mice but slightly delayed the remnant clearance in LDLr-deficient mice at 10 min (p < 0.05).
The liver uptake of emulsion cholesteryl oleate in the high trol and LDLr-deficient mice are summarized in Table I. With a high fat diet for 3 weeks, the plasma clearance of the glyceride was accelerated (7). In LDLr-deficient mice consuming a high fat, high cholesterol, atherogenic diet in control mice. When the suramin dose was 1 mg/mouse did not affect remnant clearance in the control mice. The inhibitory effect on emulsion clearance was more profound in the earlier time points, particularly at 5 and 10 min after the injection of emulsion, possibly due to the fast metabolism of suramin in vivo. The effects on emulsion remnant clearance of these competitors and inhibitors in the control and LDLr-deficient mice are summarized in Table I.

| Effectors | Control remnant clearance | LDLr-deficient remnant clearance | Radioactivity in liver |
|-----------|---------------------------|--------------------------------|------------------------|
| No competitor | Decreased | Decreased | 62 ± 2.3 (24) |
| Lactoferrin | Decreased | Decreased | 13 ± 1.5 (8) |
| Heparinase | Decreased | Decreased | 14 ± 4.1 (6) |
| Heparin | Increased | Increased | 78 ± 2.6 (4) |
| RAP | No change | Slight decreased | 58 ± 3.4 (4) |
| α2-Macroglobulin | No change | No change | 64 ± 3.7 (3) |
| Suramin (low dose) | No change | No change | 59 ± 3.0 (6) |
| Suramin (high dose) | Decreased | Decreased | 26 ± 2.1 (5) |
| High fat diet | Decreased | Decreased | ND |

* Numbers in parentheses represent the number of animals.

Our results confirm the critical requirement for apoE in the hepatic clearance of chylomicron remnants. In mice deficient in apoE, more than 60% of the injected 3H-cholesteryl oleate, which traces emulsion remnants, remained in plasma 30 min after injection, compared with 15% in the control or LDLr-deficient mice (Fig. 1A). Moreover, apoE-deficient mice had negligible hepatic recovery of the labeled cholesteryl from the clearance study (Fig. 1B) and expired little radioactive carbon dioxide following injection of 14C-cholesteryl ester-labeled emulsion. The confocal images of liver sections following injection of fluorescent emulsions confirmed the delayed internalization of remnants into the hepatocytes. Our findings are contradictory to Chang et al. who recently reported the fast hepatic clearance of chylomicron remnants in apoE-deficient mice 4–6 h after a vitamin fat load labeled with 3H-retinol (13). In our experiments, chylomicron remnants were not cleared or metabolized in these mice for up to 3 h. The discrepancy is probably due to different methods used in the studies. Retinyl palmitate had been shown to clear differently from triglyceride lipoproteins (25). Alternatively, the longer period of experimental time may have resulted in the lipoprotein particles being depleted of all core triglycerides and entering the liver via a totally different pathway independent of apoE, since we know that lipolysis is fast, and lipase activity is intact in these mice. Moreover, the efficient remnant clearance from plasma in the apoE-deficient mice shown by Chang et al. is inconsistent with earlier reports of hypercholesterolemia, atherogenesis (4, 14), and increased plasma concentration of apoB-48 (9).

The rates of chylomicron remnant clearance from plasma were similar in the control and LDLr-deficient mice, with sim-
accumulated in sinusoidal spaces of the liver prior to internalization into the hepatocytes; these remnants also remained within the hepatocytes for a longer period (up to 3 or 4 h) in the LDLr-deficient mice. These findings are consistent with Herz et al. (26) who compared the endosomal uptake of radioactive labels to show a delay in the endocytosis of chylomicron remnants in LDLr-deficient mice.

The finding that lactoferrin inhibits remnant clearance in LDLr-deficient mice (Fig. 3A) is consistent with the inhibition of apoE-enriched β-VLDL uptake by lactoferrin in human fibroblasts lacking LDLr (27). Ji and Mahley (28) showed that lactoferrin bound to HSPG and to LRP in Chinese hamster ovary cells. Recently, Mellinger et al. found a stable lactoferrin-LRP complex in isolated endosome fractions (29). In the LDLr-deficient mice, it is not known if the inhibition of remnant clearance is due to the binding of lactoferrin to cell surface HSPG or to LRP.

Similarly, injection of heparinase blocked hepatic clearance of remnants from plasma in both the control and the LDLr-deficient mice (Fig. 3A). Ji et al. recently showed that heparinase decreased the amount of liver HSPG and inhibited the hepatic clearance of chylomicrons, apoE-enriched chylomicron remnants, and apoE-enriched β-VLDL (30) but not the clearance of LDL and α2-macroglobulin. This reinforces the importance of HSPG and apoE in hepatic chylomicron remnant clearance via the receptor pathway. Thus, results from the lactoferrin and heparinase studies suggest the involvement of HSPG, apoE, and receptors in the hepatic clearance of chylomicron remnants in both control and LDLr-deficient mice.

TABLE II
Plasma lipids in LDLr-deficient mice after consuming a high fat diet for 3 weeks

| Diet            | Total triglyceride* | Total cholesterol | HDL-cholesterol |
|-----------------|---------------------|-------------------|-----------------|
| Chow            | 94 ± 14 (12)        | 297 ± 9.2 (6)     | 113 ± 9.9 (4)   |
| High-fat        | 238 ± 47 (4)        | 1550 ± 16.6 (4)   | 38 ± 2.2 (4)    |

* Numbers in parentheses represent the number of animals.

The mechanism for the slower removal of emulsion triglyceride in the presence of lactoferrin and heparinase is unclear (Fig. 3B). The finding that lactoferrin inhibits remnant clearance in LDLr-deficient mice (Fig. 3A) is consistent with the inhibition of apoE-enriched β-VLDL uptake by lactoferrin in human fibroblasts lacking LDLr (27). Ji and Mahley (28) showed that lactoferrin bound to HSPG and to LRP in Chinese hamster ovary cells. Recently, Mellinger et al. found a stable lactoferrin-LRP complex in isolated endosome fractions (29). In the LDLr-deficient mice, it is not known if the inhibition of remnant clearance is due to the binding of lactoferrin to cell surface HSPG or to LRP.

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In contrast, injection of heparin increased the removal of both emulsion triglyceride and remnants (Fig. 4, A and B). The increase in remnant clearance in the presence of heparin is probably due to the faster remnant production; however, the increase in the rate of lipolysis is not enough to account for the increased remnant removal. Heparin has been known to increase the uptake of chylomicron remnants by isolated rat hepatocytes (31) and to increase remnant clearance in streptozotocin diabetic rats (32) and also in rats consuming a high cholesterol diet (21), possibly by stabilizing hepatic lipase and increasing its secretion. Hepatic lipase has been reported to be involved in the binding of chylomicron remnants to receptors and the endocytosis of the remnants (33, 34). The alternative explanation is that in the presence of heparin, binding sites in HSPG molecules originally occupied by the lipases are now available for the binding of remnants. The availability of HSPG has been reported to be directly related to the clearance of lipoprotein remnants. For example, in diabetes mellitus it has been reported that a decrease in concentration of HSPG led to poor chylomicron clearance and hypercholesterolemia (35).

Consistent with the studies of Mokuno et al. (36) in the rat, we did not find any significant effect of α2-macroglobulin in plasma clearance or the hepatic uptake of chylomicron remnants. In contrast, while RAP did not affect the remnant clearance from plasma in control mice, it delayed (p < 0.05) the
remnant and triglyceride clearance in LDLr-deficient mice, suggesting the involvement of LRP. RAP has been known to block the binding of ligands to LRP but only interacts weakly with LDLr (36). In the presence of LDLr, RAP did not inhibit remnant uptake, as shown by Willnow et al. (37). Herz et al. (38) reported that in FH fibroblasts lacking LDLr, RAP inhibited up to 80% of cholesterol esterification stimulated by the apoe-enriched β-VLDL, but RAP had no effect on cholesteryl ester formation in normal fibroblasts. The effect of RAP on remnant clearance is relatively less marked in vivo, probably because of its short half-life when compared with remnants. When the concentration of RAP was sustained by gene transfer using an adenoviral vector to the liver of LDLr-deficient mice, hepatic remnant uptake was effectively inhibited (39). Mokuno et al. (36) also demonstrated that GST-RAP produced a weak inhibitory effect on the hepatic uptake of apoE-enriched chylomicrons.

Suramin is a highly sulfated compound, structurally unrelated to heparin or dextran sulfate. The inhibition of lipoprotein uptake has been ascribed to its polyanionic nature. Due to its toxicity, suramin was initially tested at a low dose (0.2 mg in each animal) without producing effects on clearance or hepatic uptake. When the dose was increased to 1 mg/ml, suramin effectively inhibited the clearance of both triglyceride and remnants (Fig. 6, A and B) in LDLr mice and, to a lesser extent, in control mice. The mechanism by which suramin inhibits remnant clearance is still unclear but could probably be attributable to its binding to the lipases, the ligands, or the receptors, although the more profound inhibitory effect produced in LDLr-deficient mice cannot be determined without further studies.

A high fat, high cholesterol, atherogenic diet is known to retard chylomicron remnant clearance in normal and apoE−4 transgenic mice, while the removal of emulsion triglyceride was increased due to an increase in lipase concentration (7). In LDLr-deficient mice consuming a high fat diet, plasma clearance and liver uptake of remnants are greatly retarded (Fig. 7). Lipolysis of emulsion triglyceride increased, but not to the extent observed for the normal and transgenic mice expressing apoE−4 (7). The high fat diet elevated the plasma levels of apoB-48, apoB-100, and apoE, consistent with Ishibashi et al. (9). In addition, a lower HDL-cholesterol and apoA-I concentration was also observed in the LDLr mice after high fat feeding.

In conclusion, LDLr and apoE are both required for the normal fast internalization and metabolism of chylomicron remnants by the liver. An alternative apoE-dependent pathway operates to internalize chylomicron remnants in the absence of LDLr. The catabolism of remnants via this pathway is significantly slower when compared with the LDLr endocytotic pathway, probably due to the slow internalization of remnants. From the competition studies with lactoferrin, heparinase, heparin, and RAP, it appears that both HSPG and LRP are required for remnant clearance. Although the more profound inhibitory effect produced in LDLr-deficient mice cannot be determined without further studies, in conclusion, LDLr and apoE are both required for the normal fast internalization and metabolism of chylomicron remnants by the liver. An alternative apoE-dependent pathway operates to internalize chylomicron remnants in the absence of LDLr. The catabolism of remnants via this pathway is significantly slower when compared with the LDLr endocytotic pathway, probably due to the slow internalization of remnants. From the competition studies with lactoferrin, heparinase, heparin, and RAP, it appears that both HSPG and LRP are required in this pathway. Whether or not LRP is involved synergistically or by independent to the HSPG needs further investigation.

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