An Extrahepatic Receptor-associated Protein-sensitive Mechanism Is Involved in the Metabolism of Triglyceride-rich Lipoproteins*

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We have used adenovirus-mediated gene transfer in mice to investigate low density lipoprotein receptor (LDLR) and LDLR-related protein (LRP)-independent mechanisms that control the metabolism of chylomicron and very low density lipoprotein (VLDL) remnants in vivo. Overexpression of receptor-associated protein (RAP) in mice that lack both LRP and LDLR (MX1cre+LRP/–/–LDLR/–/–) in their livers elicited a marked hypertriglyceridemia in addition to the pre-existing hypercholesterolemia in these animals, resulting in a shift in the distribution of plasma lipids from LDL-sized lipoproteins to large VLDL-sized particles. This dramatic increase in plasma lipids was not due to a RAP-mediated inhibition of a unknown hepatic high affinity binding site involved in lipoprotein metabolism, because no RAP binding could be detected in livers of MX1cre+LRP/–/–LDLR/–/– mice using both membrane binding studies and ligand blotting experiments. Remarkably, RAP overexpression also resulted in a 7-fold increase (from 13.6 to 95.6 ng/ml) of circulating, but largely inactive, lipoprotein lipase (LPL). In contrast, plasma hepatic lipase levels and activity were unaffected. In vitro studies showed that RAP binds to LPL with high affinity (Kd = 5 nM) but does not affect its catalytic activity, in vitro or in vivo. Our findings suggest that an extrahepatic RAP-sensitive process that is independent of the LDLR or LRP is involved in metabolism of triglyceride-rich lipoproteins. Thus, RAP may affect the functional maturation of LPL, thus causing the accumulation of triglyceride-rich lipoproteins in the circulation.

Hypertriglyceridemia, combined with the accumulation of remnant lipoproteins in the circulation, is a major risk factor for atherosclerosis and coronary artery disease. The genetic bases of this clinically important syndrome are complex and incompletely understood. Two endocytic receptor systems are known to remove the lipolyzed remnants of chylomicrons and very low density lipoproteins (VLDL) from the circulation. They are the low density lipoprotein (LDL) receptor and the LDL receptor-related protein (LRP) (1, 2). Following lipolysis in the peripheral capillaries of muscle, heart, and adipose tissue, where chylomicrons deliver most of the triglyceride load they carry, the remnants have shrunk to a size at which they can permeate the fenestrated endothelium separating the hepatocyte surface and the space of Disse from the circulation (for review see Ref. 3). LRP and LDL receptors at the surface of hepatocytes bind and clear remnant lipoproteins from an intermediate binding site. This intermediate compartment is created by interactions of heparan sulfate proteoglycans (4), hepatic lipase (5, 6), lipoprotein lipase (7), and apoE (8) with the remnants.

Gene knockout and gene transfer experiments in mice have defined the roles of the receptors, apoproteins, and lipases in the remnant clearance process. Although the LDL receptor efficiently removes apoB100-containing LDL, as well as apoB48-containing remnants through interaction with apoE (9, 10), from the bloodstream, LRP binds B48-containing remnants exclusively through apoE (11–13).

The LRP receptor-associated protein (RAP), a specialized chaperone that is required for biosynthesis of LRP, blocks the binding function of this receptor in vivo and has been successfully used to transiently inactivate LRP in adult mice (14). These experiments have revealed a physiological role of the LDL receptor and LRP in remnant removal. In the absence of functional LDL receptor in knockout mice, inhibition of LRP by adenovirus-mediated gene transfer and overexpression of RAP resulted in the accumulation of large, triglyceride- and cholesterol-rich apoB48-containing remnants.

In another gene knockout model generated in mice, LRP has recently been inactivated by inducible tissue-specific techniques using the Cre-lox recombination system (2). By this approach, it was possible to circumvent the early embryonic lethal phenotype caused by conventional gene disruption of LRP (15, 16). LRP inactivation was initiated in adult mice following interferon induction, which in turn led to expression of the cre recombinase from the interferon inducible MX1 promoter (17). Recombination of the loxP flanked (floxed) LRP gene was essentially complete in hepatocytes and other cell types exposed to the circulating interferons.

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As in the RAP overexpression experiments (14), LRP gene disruption in LDL receptor-deficient mice did cause the accumulation of cholesterol-rich, apoB48-containing remnants. However, these remnants were smaller and contained significantly less triglyceride than those that accumulated in the RAP overexpressing animals. These observations suggest that a novel and hitherto unsuspected RAP-sensitive process is involved in the metabolism of triglyceride-rich lipoproteins. This could involve other RAP-sensitive receptors, such as the hypothetical lipolysis stimulated receptor (18), or a direct or indirect effect of RAP on lipase-mediated conversion of chylomicrons to the smaller remnants.

In this study we have addressed this question by measuring the effect of RAP overexpression on remnant removal in animals in which the LDL receptor, LRP, or both proteins had been inactivated. We have also investigated the presence of other potential RAP-binding sites in LRP and LDL receptor-deficient mouse liver membranes. Furthermore, we have determined the protein mass and catalytic activity of hepatic lipase and lipoprotein lipase in RAP overexpressing mice. Our results suggest that RAP affects the conversion of large triglyceride-rich chylomicrons to smaller remnants by interfering with LPL activation in the periphery and rule out the contribution of other major RAP-binding proteins in the liver. The biochemical basis underlying this process may play a role in some of the complex genetic traits that cause hypertriglyceridemia in man.

**Experimental Procedures**

**Transgenic Animals—**Mice in which the LRP alleles have been altered by introduction of loxP sites (LRPlox/lox) were generated by homologous recombination of the LRP allele in embryonic stem cells and have been described previously (19). Mice transgenic for the MX1cre expression construct were generated by pronuclear injection of hybrid (SJLxC57BL/6J) mice (2). LDL receptor-deficient (LDLR−/−) mice were generated by homologous recombination of the LDLR allele in embryonic stem cells and have been described previously (10). Six genetically distinct strains of animals were used: mice that were wild type at both LRP loci, deficient for the LDL receptor (LDLR−/−), homozygous for the floxed LRP allele (LRPfl/−/−), homozygous for the floxed LDLR allele (LDLRfl/−/−), and homozygous for the floxed LRP allele (LRPfl/−/−), deficient for the LDL receptor, and transgenic for the MX1cre transgene (MX1cre-1 LRPfl/−/−). After a 5 h fasting period, mice were anesthetized by intraperitoneal injection of Nembutil (80 μg/g body weight). Mice were injected intravenously with Triton WR1339 (500 mg/kg body weight) using 15% (v/v) Triton solution in 0.9% NaCl (23). At 1, 15, and 30 min after injection, blood samples were drawn from the tail vein and analyzed for triglycerides as described above.

**Assay of Lipoprotein Lipase and Hepatic Lipase Mass in Mouse Liver Membranes—**Lipozyme mass was measured by ELISA developed for rat HL (24). Mouse LPL was also measured by a sandwich ELISA. A full-length mouse LPL cDNA kindly provided by Michael Schotz (25) was subcloned into pQE32 vector for expression in bacteria. The His6-tagged lipase was used to generate antibodies in a goat and to construct a column of mouse LPL Affi-Prep 10 for affinity purification of the antibodies. The conditions for the assay were the same as described for chicken LPL ELISA (26) with the following exceptions. The initial incubation of samples with the capture antibodies coated on microtiter plates was conducted at 4°C in 0.8M NaCl, 1% bovine serum albumin, 0.05% Tween-20, 10 mM sodium phosphate, pH 7.4. The standard curves ranged from 0.05 to 1.8 ng/well. At 1 ng/well the reading was 0.230 optical density units, and the correlation coefficient was larger than 0.99. Catalytic activity of LPL and HL in post-heparin plasma were determined as described below on 20 μl of plasma.

**Solid Phase Assay of Interaction of RAP and LPL or HL—**To preserve the integrity of the lipoproteins, all steps were conducted at 4°C. Microtiter plates (24-well) were coated with highly purified lipases (27, 28) or native HL in 0.1 M of Tris-buffered saline, containing 2 mg/ml albumin and protease inhibitor mixture. Immediately prior to use, this fraction was sonicated (Biblok Scientific Vibracell, 30 s, power 2.5, 25% pulse). 100 μg of membranes was incubated overnight at 4°C with increasing amounts of 125I-RAP either in the absence or in the presence of a 100 μg/ml excess of unlabeled RAP (final incubation volume, 250 μl; n = 4). Membrane bound 125I-RAP was separated from unbound RAP by layering a 200 μl solution of 5% (w/v) BSA and centrifuging at 13,200 g for 5 min at 4°C. The supernatant was carefully removed, and the bottoms of the vials were cut and counted to measure the amount of membrane bound 125I-RAP.

**Ligand Blotting—**Membranes were prepared from mouse livers as described above, and proteins separated by nondenaturing, nonreducing SDS gel electrophoresis on 4–15% polyacrylamide gels (50 μg protein/lane). After separation, the proteins were transferred to nitrocellulose. The nitrocellulose membranes were blocked for 30 min at room temperature in PBS containing, 0.5% Tween, 2% BSA, and 5% powdered milk, pH 7.4, followed by incubation for 60 min at room temperature with 10 μg/ml peroxidase-conjugated RAP in PBS in blocking buffer either in absence or presence of an excess of nonconjugated RAP (100 μg/ml). The nitrocellulose membranes were washed three times with PBS containing 0.5% Tween and 2% deoxycholic acid with buffer changes each 5 min. Bound peroxidase-labeled RAP was detected using the ECL system.

As a result of RAP overexpression in the RAP overexpressing experiments (14), LRP gene disruption in LDL receptor-deficient mice did cause the accumulation of cholesterol-rich, apoB48-containing remnants. However, these remnants were smaller and contained significantly less triglyceride than those that accumulated in the RAP overexpressing animals. These observations suggest that a novel and hitherto unsuspected RAP-sensitive process is involved in the metabolism of triglyceride-rich lipoproteins. This could involve other RAP-sensitive receptors, such as the hypothetical lipolysis stimulated receptor (18), or a direct or indirect effect of RAP on lipase-mediated conversion of chylomicrons to the smaller remnants.
Adult LRPfloxFlox and LRPfloxFloxLDLR−/− mice either transgenic or nontransgenic for the MX1cre transgene were injected (three times, intraperitoneally) with 250 μg of pcPc at 2-day intervals. Similarly, regular wild type and LDLR−/− mice were treated and included as extra controls. Four weeks after the last pcPc injection, the mice were injected intravenously with 2 × 10⁶ PFU of Ad-RAP. As a control similar mice were injected with 2 × 10⁶ PFU of Ad-Gal. Before and 5 days after adenovirus injection plasma was obtained and analyzed for cholesterol and triglycerides. Values are represented as the means ± S.D. (±, range, n = 2).

<table>
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<tr>
<th>Genotype</th>
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<th>n</th>
<th>Immediately after adenovirus injection</th>
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<td>Cholesterol (mg/dl)</td>
<td>Triglycerides (mg/dl)</td>
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<td>90 ± 6</td>
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<tr>
<td>Wild type</td>
<td>Ad-RAP</td>
<td>3</td>
<td>155 ± 20</td>
<td>107 ± 6</td>
</tr>
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<td>516 ± 202</td>
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<tr>
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<td>242 ± 44</td>
<td>109 ± 25</td>
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* p < 0.05, significantly different from Ad-β-Gal injected mice, using nonparametric Mann-Whitney tests.
levels of approximately 30%, and total plasma triglyceride levels increased approximately 2-fold as compared with Ad-β-Gal-injected mice of the same genotype (Table I). Although cholesterol levels were only slightly elevated, Ad-RAP-injected MX1cre<sup>−/−</sup>LRP<sup>125I</sup>LDLR<sup>−/−</sup> mice showed a dramatic shift in cholesterol distribution from LDL-sized lipoproteins to large VLDL-sized lipoproteins, with a concomitant reduction in high density lipoprotein cholesterol (Fig. 1B, panel f). Total plasma lipid levels and the lipoprotein profile of Ad-RAP-injected MX1cre<sup>−/−</sup>LRP<sup>125I</sup>LDLR<sup>−/−</sup> mice closely resembled that of Ad-RAP-injected LRP<sup>125I</sup>LDLR<sup>−/−</sup> or LDLR<sup>−/−</sup> mice (Fig. 1B, panels d and e).

Total plasma cholesterol and triglyceride concentrations were only slightly elevated in Ad-RAP-injected MX1cre<sup>−/−</sup>LRP<sup>125I</sup> mice compared with Ad-β-Gal-injected mice of the same genotype (Table I). However, plasma lipid levels in Ad-RAP-injected MX1cre<sup>−/−</sup>LRP<sup>125I</sup> mice were not different from Ad-RAP-injected LRP<sup>125I</sup> or wild type mice. In these mice, the slight elevation in plasma cholesterol was caused mainly by the accumulation of large, remnant-sized lipoproteins (Fig. 1B, panels a–c).

**Immunoblot Analysis of Plasma Apoproteins after Adenovirus-mediated RAP Gene Transfer**—The effect of RAP overexpression on plasma concentrations of apolipoproteins B100, B48, E, and AI in the pI:pC-induced wild type, LRP<sup>125I</sup>LDLR<sup>−/−</sup>, LRP<sup>125I</sup>LDLR<sup>−/−</sup> mice, and MX1cre<sup>−/−</sup>LRP<sup>125I</sup>LDLR<sup>−/−</sup> mice is shown in Fig. 2. Before adenovirus injections, MX1cre<sup>−/−</sup>LRP<sup>125I</sup>LDLR<sup>−/−</sup> mice had elevated levels of apoB (100+48) and apoE as compared with nontransgenic controls (LRP<sup>125I</sup>LDLR<sup>−/−</sup> and LDLR<sup>−/−</sup> mice (Fig. 2A, lanes 4–6). Plasma apolipoprotein levels in MX1cre<sup>−/−</sup>LRP<sup>125I</sup> mice were comparable with those of LRP<sup>125I</sup> and wild type controls (Fig. 2A, lanes 1–3).

Upon Ad-RAP injection, plasma apoB48 and apoE levels were elevated in MX1cre<sup>−/−</sup>LRP<sup>125I</sup>LDLR<sup>−/−</sup> mice (Fig. 2B, right panel, lane 6) but were not different from apolipoprotein levels of Ad-β-Gal-injected mice of the same genotype (Fig. 2B, left panel, lane 6). In addition, plasma apolipoprotein levels in Ad-RAP-injected MX1cre<sup>−/−</sup>LRP<sup>125I</sup>LDLR<sup>−/−</sup> mice were almost identical to those of Ad-RAP-injected LRP<sup>125I</sup>LDLR<sup>−/−</sup> and LDLR<sup>−/−</sup> mice (Fig. 2B, right panel, lanes 4–6). Consistent with the increase in high density lipoprotein cholesterol (Fig. 1B, panels D–F), plasma apoA-I levels were also decreased in LDLR-deficient mice injected with Ad-RAP (Fig. 2B, right panel, lanes 4–6).

Ad-RAP-injected MX1cre<sup>−/−</sup>LRP<sup>125I</sup> showed only a slight elevation in plasma apoB48 and apoE levels as compared with Ad-β-Gal-injected mice of the same genotype (Fig. 2B, lanes 3, right and left panels, respectively). Plasma apolipoprotein levels were also not different from Ad-RAP-injected LRP<sup>125I</sup> or wild type mice (Fig. 2B, right panel, lanes 1 and 2).

**Binding of 125I-Labeled RAP and Peroxidase-labeled RAP to Liver Membranes**—The striking increase in plasma lipids and shift in lipoprotein profile in Ad-RAP-injected MX1cre<sup>−/−</sup>LRP<sup>125I</sup>LDLR<sup>−/−</sup> mice indicates that RAP acts on another process besides the LDL receptor and RAP that is also involved in the metabolism of triglyceride-rich lipoproteins. To investigate whether another RAP-binding protein might exist on liver membranes, we determined the binding of 125I-labeled RAP to liver membranes from pI:pC-induced adult LRP<sup>125I</sup>, MX1cre<sup>−/−</sup>LRP<sup>125I</sup>, LRP<sup>125I</sup>LDLR<sup>−/−</sup>, and MX1cre<sup>−/−</sup>LRP<sup>125I</sup>LDLR<sup>−/−</sup> mice. As shown in Fig. 3, liver membranes from mice expressing LRP (i.e. LRP<sup>125I</sup> and LDLR<sup>−/−</sup>LRP<sup>125I</sup>, Fig. 3, A and C) bound RAP with high affinity. In contrast, membranes from

![Image](http://www.jbc.org/ccc/2017/05/01/fig1.png)
mice lacking LRP (i.e. MX1cre<sup>−/−</sup>LRP<sup>flk</sup>/<sup>flk</sup> and MX1cre<sup>−/−</sup>LRP<sup>flk</sup>/<sup>flk</sup>LDLR<sup>−/−</sup>; Fig. 3, B and D) did not bind RAP specifically. These results show that LRP is the only liver membrane protein that binds RAP with high affinity.

A similar result was obtained when we determined the ability of RAP to bind to liver membrane proteins by ligand blotting using peroxidase-labeled RAP (Fig. 4, lower panel). The presence of LRP was detected by immuno blotting with antibodies directed against the 85-kDa subunit of LRP (Fig. 4, upper panel). A prominent band of approximately 515 kDa that bound RAP was present in the livers of mice expressing LRP and absent from livers lacking this receptor. No other RAP-binding protein was detected in these ligand blotting experiments. These findings further show that LRP is the only high affinity RAP-binding protein in liver membranes and suggest that the hyperlipidemia caused by overexpression of RAP in MX1cre<sup>−/−</sup>LRP<sup>flk</sup>/<sup>flk</sup>LDLR<sup>−/−</sup> is not due to inhibition of an LRP-independent RAP-binding protein.

Effect of Adenovirus-mediated RAP Gene Transfer on Triglyceride Metabolism—The predominant increase in plasma triglyceride rather than cholesterol levels in MX1cre<sup>−/−</sup>LRP<sup>flk</sup>/<sup>flk</sup>LDLR<sup>−/−</sup> following Ad-RAP-mediated gene transfer suggests that RAP may directly interfere with triglyceride metabolism. This effect of RAP overexpression on triglyceride levels may take place at the level of VLDL-triglyceride production or result from direct inhibition of triglyceride lipolysis by LPL and/or HL.

Production of VLDL-triglycerides was measured by determining the rate of triglyceride secretion in pI:pC-induced MX1cre<sup>−/−</sup>LRP<sup>flk</sup>/<sup>flk</sup>LDLR<sup>−/−</sup> and wild type control mice 5 days after injection of 2×10<sup>9</sup> PFU of Ad-RAP or Ad-β-Gal (Fig. 5). VLDL-triglyceride production rate was similar in all groups of mice, indicating that RAP overexpression did not affect hepatic VLDL-triglyceride production.

To determine whether RAP interferes with triglyceride metabolism through a direct effect on LPL and/or HL-mediated triglyceride hydrolysis, wild type mice were injected with Ad-
RAP or Ad-β-Gal. Pre- and post-heparin plasma LPL and HL levels and activities were determined 5 days after adenovirus injection. As shown in Table II, plasma HL levels and activity in pre- and post-heparin plasma of Ad-RAP-injected mice were similar to those of Ad-β-Gal-injected mice. In contrast, LPL concentrations (protein mass) were increased approximately 7-fold in pre-heparin plasma of Ad-RAP-injected mice as compared with Ad-β-Gal-injected animals. Surprisingly, the accumulating LPL was almost completely enzymatically inactive. Upon heparin injection, plasma LPL levels increased by the same amount in animals injected with either virus.

We next determined, in vitro, whether the effect of RAP on plasma LPL, but not HL, levels and activity may result from a direct effect of RAP on the activity of these lipases. Although RAP bound with high affinity to both HL and LPL (Kd ≈ 8 and 5 nM, respectively; data not shown), it did not affect lipolytic activity when both were assayed in solution using Triton X-100 stabilized triolein emulsions (Table III). There was also no effect of RAP on lipolysis when bovine milk LPL was bound to heparan sulfate proteoglycans, and the substrate employed was human d1-1.006 lipoproteins (Table III). Thus, RAP overexpression apparently affects the biological activity of LPL in vivo, although probably not by direct inhibition of enzyme activity.

To study whether RAP can acutely affect triglyceride hydrolysis by LPL in vivo, we determined plasma triglyceride decay in functionally hepatectomized mice. As shown in Fig. 6 the plasma decay of [14C]-triolaete labeled neohylocromics was not different for hepatectomized mice that were co-injected with a high dose of RAP (1 mg/mouse) and control-injected mice. This indicates that the effect of hepatic RAP overexpression on LPL specific activity in the circulation could not be mimicked in vivo by intravenous injection of a bolus of RAP and that the effect of RAP on LPL activity requires a prolonged overexpression of this protein.

**DISCUSSION**

In the present study, we demonstrated that a RAP-sensitive process, independent of the LDL receptor and the LRP, is involved in the metabolism of triglyceride-rich lipoproteins. This is illustrated by the fact that adenovirus-mediated overexpression of RAP increased plasma lipid and lipoprotein levels in MX1cre-LRPlox/loxLDLR−/− mice. The presence of this RAP-sensitive site explains the difference in lipid levels and lipoprotein profile of LDL receptor-deficient mice in which LRP was inactivated transiently by RAP overexpression (14) and animals in which the LRP gene was disrupted by inducible Cre/loxP-mediated recombination (2).

Our binding and ligand blotting studies with LRP- and LDL-receptor-deficient mouse liver membranes have shown that the RAP-mediated effect on the metabolism of triglyceride-rich lipoproteins was not due to inhibition of an unknown RAP-sensitive hepatic lipoprotein receptor. Others have postulated that the uptake of chylomicrons and/or VLDL may also involve hepatic lipoprotein receptors other than the LDL receptor and the LRP, for instance a hypothetical lipolysis-stimulated receptor (34) and remnant receptor (35). However, our findings rule out the possibility of other major RAP-binding proteins in the liver that may participate in this process.

RAP overexpression strongly affected triglyceride metabolism. This was not due to a RAP-mediated stimulation of hepatic VLDL-triglyceride production. RAP overexpression resulted in an almost complete inactivation of (postheparin) plasma LPL. Thus, RAP has a direct or indirect effect on lipase-mediated conversion of chylomicrons to the smaller remnants, leading to the observed accumulation of large triglyceride-rich particles. This also explains the lack of an effect of RAP on apolipoprotein levels (Fig. 2B).

RAP binds with high affinity to LPL. Because RAP does not affect LPL activity in vitro (Table III), we can conclude that RAP does not bind to the domains essential for the catalytic activity of the enzyme. Because a high dose of intravenous RAP protein had no effect on liver-independent triglyceride removal (Fig. 6), we can also conclude that RAP had no direct effect on LPL activity within the vascular bed. The heparin-releasable LPL mass, that is the increment above the pre-heparin level, was not significantly different in Ad-RAP- or Ad-β-Gal-injected mice, suggesting that RAP overexpression did not affect the amount of LPL bound to the endothelium. In addition, RAP does not compete for binding of apolipoprotein CII with VLDL or LDL, because no such effect was detectable, in vitro, even at concentrations of 200–500 μg RAP/ml.

It has been suggested that the VLDL receptor may play a role in peripheral triglyceride metabolism. The VLDL receptor and LPL are expressed and localized in peripheral tissues involved in triglyceride metabolism. Furthermore, the VLDL receptor binds RAP and LPL with high affinity (36, 37). RAP may affect the role of the VLDL receptor in VLDL-mediated lipolysis. However, VLDL receptor-deficient mice have a normal lipoprotein profile (38) and display normal plasma triglyceride removal rate and normal lipoprotein uptake by peripheral tissues (data not shown), suggesting that the RAP-mediated effect on LPL activity is not related to the VLDL receptor activity.

RAP gene transfer resulted in greatly elevated levels of inactive LPL in pre- and post-heparin plasma. The high concentration of inactive LPL in plasma may result from an overproduction of LPL or a defect in its removal. LPL has been shown to bind LR both by solid phase assays with purified LRP (39) and by Western blotting of liver membranes extracts (40). In addition, in cell culture systems, LRP antibodies have been shown to inhibit LPL degradation (39). Thus, inactivation of LRP by RAP is a likely cause for the accumulation of LPL in the plasma, even in the absence of marked hypertriglyceridemia in the wild type mice that received Ad-RAP intravenously.

The effect of hepatic RAP overexpression on LPL specific activity in the circulation could not be mimicked in vivo by adding RAP to a VLDL lipolysis assay (Table III) or in vivo by intravenous injection of a bolus of RAP (Fig. 6). This suggests that RAP may have a function in LPL processing in the capillary bed, possibly by associating with Sortilin (41).
Adult wild type mice (n = 5–6 per group) were injected with 2 × 10² PFU of Ad-RAP or Ad-β-Gal. At 5 days after adenovirus injection, mice were injected intravenously with heparin (100 units/kg body weight). Immediately before and 15 min after heparin blood samples were drawn, and plasma was analyzed for hepatic lipase and lipoprotein lipase levels and activities (see methods). Values are represented as the means ± S.D.

**Table II**

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<th>Genotype</th>
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<th>Lipoprotein lipase</th>
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<td>Pre-heparin levels</td>
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<td>Ad-RAP</td>
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*p < 0.05, significantly different from Ad-β-Gal injected mice, using nonparametric Mann-Whitney tests.

**Figure 6.** The effect of intravenous RAP on plasma decay of [³H]trioleate-labeled neo-chylomicrons in hepatectomized mice. Functionally hepatectomized wild type mice were injected with 500,000 dpm of [³H]trioleate neo-chylomicrons either without (open circles) or with an excess of RAP (1 mg RAP/mouse; black circles). Blood was drawn at 1, 3, 5, 8, 11, and 15 min after injections and the radioactivity was determined. Values are the means (+ S.D.) of five animals/group and are expressed as a percentage of the radioactivity present in 1 min serum sample.

The RAP-mediated inhibition of LPL activity resulted in massive hypertriglyceridemia in mice that lack the LDL receptor or both LDL receptor and LRP. However, wild type mice and LRP-deficient mice, both having normal LDL receptor expression, did not display hypertriglyceridemia upon inactivation of LPL. This is consistent with the observation that mice lacking both the apoE and LDL receptor genes and not mice lacking only the apoE gene display massive hypertriglyceridemia upon apoE-induced inhibition of lipolysis (42). These data demonstrate that in contrast to LDL receptor-independent pathways, the LDL receptor is capable of removing triglyceride-rich lipoproteins from the circulation, even when the lipoproteins are poorly lipolyzed.

In summary, our results suggest that RAP affects the con-
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