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Triglyceride-rich lipoprotein metabolism in unique VLDL receptor, LDL receptor, and LRP triple-deficient mice

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Abstract The very low density lipoprotein receptor (VLDLR), low density lipoprotein receptor (LDLR), and low density lipoprotein receptor-related protein (LRP) are the three main apolipoprotein E-recognizing endocytic receptors involved in the clearance of triglyceride (TG)-rich lipoproteins from plasma. Whereas LDLR deficiency in mice results in the accumulation of plasma LDL-sized lipoproteins, VLDLR or LRP deficiency alone only minimally affects plasma lipoproteins. To investigate the combined effect of the absence of these receptors on TG-rich lipoprotein levels, we have generated unique VLDLR, LDLR, and LRP triple-deficient mice. Compared with wild-type mice, these mice markedly accumulated plasma lipids and lipases. These mice did not show aggravated hyperlipidemia compared with LDLR and LRP double-deficient mice, but plasma TG was increased after high-fat diet feeding. In addition, these mice showed a severely decreased postprandial TG clearance typical of VLDLR-deficient (VLDLR$^{-/-}$) mice. Collectively, although VLDLR deficiency in LRP^{-} and LDLR$^{-/-}$ mice does not aggravate hyperlipidemia, these triple-deficient mice represent a unique model of markedly delayed TG clearance on a hyperlipidemic background.—Espirito Santo, S. M. S., P. C. N. Rensen, J. R. Goudriaan, A. Bensadoun, N. Bovenschen, P. J. Voshol, L. M. Havekes, and B. J. M. van Vlijmen. **Triglyceride-rich lipoprotein metabolism in unique VLDL receptor, LDL receptor, and LRP triple-deficient mice. *J. Lipid Res.* 2005.** 46: **1097–1102.**

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The very low density lipoprotein receptor (VLDLR) is expressed in tissues active in fatty acid metabolism (i.e., heart, muscle, adipose) and macrophages (1, 2). In vitro studies show that the VLDLR binds apolipoprotein E (apoE) but not apoB-100 and that LPL modulates the binding of triglyceride (TG)-rich lipoprotein particles to the VLDLR and vice versa (3, 4). Frykman et al. (5) generated VLDLR-deficient (VLDLR$^{-/-}$) mice and showed that these mice present normal plasma lipoprotein levels when fed a chow diet. Interestingly, only when the TG metabolism was stressed either by a high-fat diet (HFD) or by cross-breeding on a background of obesity (*ob/ob*) or low density lipoprotein receptor (LDLR) deficiency (6, 7) did VLDLR deficiency result in moderate accumulation of plasma TG-rich lipoproteins. In agreement with these observations, we recently demonstrated that the postprandial TG response after an oral fat load is strongly increased in VLDLR$^{-/-}$ mice (8). Hence, the VLDLR plays a role in TG-rich lipoprotein metabolism that becomes apparent only after severely stressing TG metabolism.

The LDLR and the low density lipoprotein receptor-related protein (LRP) are two other major members of the LDLR family that act in concert in the hepatic clearance of plasma lipoproteins (9). Absence of the LDLR in mice results in the accumulation of LDL-sized lipoproteins in plasma, whereas LRP deficiency does not affect plasma lipoprotein levels (10, 11). Strikingly, LRP deficiency on an LDLR$^{-/-}$ background in mice results in aggravated hyperlipidemia attributable to the accumulation of TG-rich lipoprotein remnants (9). This indicates that the role of

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Abbreviations: apoE, apolipoprotein E; AUC, area under the curve; HFD, high-fat diet; LDLR, low density lipoprotein receptor; LRP, low density lipoprotein receptor-related protein; RAP, receptor-associated protein; TG, triglyceride; VLDLR, very low density lipoprotein receptor.

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LRP in lipoprotein metabolism in vivo is masked by the LDLR, the presence of which can apparently fully compensate for the absence of the LRP (9). Whether a similar interaction exists between the VLDLR and LDLR and/or LRP is not yet known.

Because we hypothesized that the addition of the absence of the VLDLR to the absence of the LDLR and hepatic LRP would severely aggravate the hyperlipidemic phenotype of LDLR$^{-/-}$/LRP^{-} mice, we generated unique VLDLR, LDLR, and conditional LRP triple-deficient mice. It appeared that VLDLR deficiency in LDLR$^{-/-}$ and LRP^{-} mice does not aggravate hyperlipidemia on a chow diet. However, we do show that the additional absence of the VLDLR does lead to an aggravated phenotype on stressing TG metabolism, either by high-fat feeding or by giving an intragastric olive oil bolus, although these results may be attributed to the cumulative effects of the phenotypes of the individual mice (i.e., LRP^{-}, LDLR$^{-/-}$, and VLDLR$^{-/-}$). In summary, LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$ triple-deficient mice represent a unique model of markedly delayed TG clearance on a hyperlipidemic background.

MATERIALS AND METHODS

Transgenic animals and diet

We cross-bred VLDLR$^{-/-}$ mice (6) with mice conditionally lacking the LRP on an LDLR$^{-/-}$ background (MX1Cre:LRP$^{lox/lox}$ LDLR$^{-/-}$; referred to hereafter as LRP^{-}LDLR$^{-/-}$) (10), yielding a unique triple-knockout mouse model that lacks (conditionally) LRP, LDLR, and VLDLR (MX1Cre:LRP$^{lox/lox}$LDLR$^{-/-}$ VLDLR$^{-/-}$; referred to hereafter as LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$). Mice were genotyped by PCR analysis on tail tip DNA for the presence of the "floxed" LRP allele, the MX1Cre transgene, and the disrupted LDLR and VLDLR allele as described previously (7, 10). For experiments, 8–10 week old male VLDLR$^{-/-}$ (n = 8), VLDLR$^{+/+}$ (n = 8), LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$ (n = 10), and LRP^{-}LDLR$^{-/-}$VLDLR$^{+/+}$ (n = 10) mice were used. To induce LRP deficiency, all mice (including VLDLR$^{-/-}$ and VLDLR$^{+/+}$) received three intraperitoneal injections of 250 μl of a 1 mg/ml solution of polyinosinic:polycytidylic ribonucleic acid (pI:pC; Sigma, St. Louis, MO) at 2 day intervals as described previously (10). PCR analysis for the presence of the disrupted LRP allele and immunoblot analysis with antibodies directed against the 85 kDa subunit of LRP from liver tissue were performed as described previously (9, 12). Mice were kept for 12 weeks on a standard rat/mouse chow diet (SRM-A; Hope Farms, Woerden, The Netherlands), followed by 10 weeks of HFD containing 24% corn oil, 24% casein, 18% corn starch, and 6% cellulose (Hope Farms). Diet and water were given ad libitum to the animals. The institutional committee on animal welfare of Netherlands Organization for Applied Scientific Research-Quality of Life approved all animal experiments.

Plasma analysis

Blood was collected by tail bleeding into chilled paraoxon-coated capillaries (13) 4 weeks after induction of LRP deficiency either after 4 h of fasting from 7:00 to 11:00 AM or after overnight fasting from 5:00 PM to 7:00 AM. Plasma was isolated and assayed for total cholesterol, TG, and glucose levels using enzymatic kits C0534, 337-B, and 315-500 (Sigma Diagnostics, Deisenhofen, Germany) and for FFA using the enzymatic kit Nefa-C (Wako Chemicals GmbH, Neuss, Germany). Plasma lipid distribution over lipoproteins was determined by size fractionation using fast-performance liquid chromatography (12). Plasma concentrations of mouse apoB-48 and apoB-100, apoE, and apoA-I were determined by immunoblotting using mouse apolipoprotein-specific polyclonal rabbit antiserum as described previously (12). Plasma levels of HL activity, LPL activity, and LPL mass were determined after 4 h of fasting as described previously (9, 14).

Postprandial TG response

Mice were fasted for 4 h. After a basal blood sample was taken by tail bleeding, the animals received an intragastric load of 200 μl of olive oil. Subsequently, blood was drawn at the indicated times after olive oil administration. Plasma was isolated and TG levels were determined as described above and are presented as relative increases from time 0.

Statistical analysis

All data are presented as means ± SD. Data were analyzed using the Mann-Whitney *U*-test. $P < 0.05$ was regarded as statistically significant.

RESULTS

General parameters

Treatment of LDLR$^{-/-}$VLDLR$^{+/+}$ and LDLR$^{-/-}$ VLDLR$^{-/-}$ mice with pI:pC resulted in the presence of the disrupted LRP allele in tail tip DNA and in the complete absence of LRP protein in liver membrane extracts (**Fig. 1**). Upon LRP inactivation, the LRP^{-}LDLR$^{-/-}$ VLDLR$^{-/-}$ mice appeared healthy and displayed no signs of abnormalities, but throughout their life span they had a slightly lower body weight compared with LRP^{-}LDLR$^{-/-}$VLDLR$^{+/+}$ mice (19.6 ± 1.8 g vs. 22.4 ± 0.8 g; $P = 0.02$). However, reduced body weight was also observed in VLDLR deficiency only compared with wild-type controls (18.7 ± 0.9 g vs. 23.8 ± 2.7 g; $P = 0.001$), which is in agreement with previous reports (5, 6).

Plasma lipids on a chow diet

On a regular chow diet, single VLDLR deficiency resulted in increased plasma TG levels after an overnight

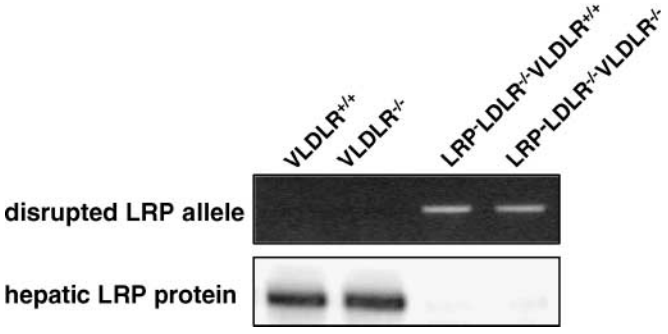

Fig. 1. Low density lipoprotein receptor-related protein (LRP) deficiency. Mice were treated with polyinosinic:polycytidylic ribonucleic acid, and LRP deficiency was assessed by the presence of the disrupted LRP allele by PCR analysis of tail tip DNA (upper panel) and by the absence of the 85 kDa subunit of the LRP protein in the liver (lower panel). LDLR, low density lipoprotein receptor; VLDLR, very low density lipoprotein receptor.

TABLE 1. Plasma lipid and glucose levels on a chow diet after overnight fasting

Animals	Chow Diet				High-Fat Diet			
	Cholesterol	TGs	FFA	Glucose	Cholesterol	TGs	FFA	Glucose
	mM							
Wild-type background								
VLDLR$^{+/+}$	1.8 ± 0.2	0.3 ± 0.1	n.d.	n.d.	2.9 ± 0.4	0.4 ± 0.3	n.d.	n.d.
VLDLR$^{-/-}$	1.9 ± 0.3	1.2 ± 0.3[a]	n.d.	n.d.	3.2 ± 0.3	1.1 ± 0.2[a]	n.d.	n.d.
LRP^{-}LDLR$^{-/-}$ background								
VLDLR$^{+/+}$	32.4 ± 6.5	8.6 ± 1.9	1.3 ± 0.2	6.0 ± 1.4	32.4 ± 6.1	6.3 ± 2.5	1.1 ± 0.2	5.8 ± 0.9
VLDLR$^{-/-}$	34.1 ± 3.6	8.9 ± 2.3	1.5 ± 0.5	5.6 ± 1.6	39.4 ± 7.4	12.5 ± 5.0[a]	1.5 ± 0.3	6.5 ± 0.9

LDLR, low density lipoprotein receptor; LRP, low density lipoprotein receptor-related protein; n.d., not determined; TG, triglyceride; VLDLR, very low density lipoprotein receptor. Values represent means ± SD of 8–10 mice per group.

[a] $P < 0.05$.

fast (**Table 1**). Under these conditions, VLDLR deficiency did not affect plasma cholesterol, FFA, and glucose levels or plasma lipoprotein distribution (**Fig. 2**). The deletion of both LRP and LDLR (LRP^{-}LDLR$^{-/-}$) elicited severe hyperlipidemia as a result of the accumulation of VLDL/LDL-sized lipoproteins. Interestingly, VLDLR deficiency on this LRP^{-}LDLR$^{-/-}$ background did not influence plasma lipids, glucose (Table 1), and lipoprotein profiles (Fig. 2). In addition, levels of plasma apoB-100 (90 ± 43% vs. 100 ± 23%; $P = 0.818$), apoB-48 (95 ± 20% vs. 100 ± 15%; $P = 0.394$), apoE (125 ± 12% vs. 100 ± 13%; $P = 0.100$), and apoA-I (102 ± 46% vs. 100 ± 29%; $P = 0.589$) were not altered in LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$ vs. LRP^{-}LDLR$^{-/-}$VLDLR$^{+/+}$ mice, respectively.

We next investigated the effect of VLDLR status in the presence or absence of the LDLR and LRP on plasma lipids after 4 h of fasting. As shown in **Table 2**, for both the wild-type and LRP^{-}LDLR$^{-/-}$ backgrounds, the effects of VLDLR deficiency on plasma lipid levels were comparable to those observed for the overnight fasting state. Single VLDLR$^{-/-}$ mice showed only modestly increased plasma TG levels and no effects on plasma cholesterol, FFA, and glucose levels (Table 2). Again, VLDLR deficiency on an LRP^{-}LDLR$^{-/-}$ background did not affect plasma lipids and glucose levels.

Plasma HL, LPL activity, and LPL mass

Yagyu et al. (15) reported that the increase in plasma TG levels in VLDLR$^{-/-}$ mice is associated with reduced LPL activity in these mice. As shown in Table 2, single VLDLR$^{-/-}$ mice indeed showed a significant 19% decrease in LPL activity ($P = 0.02$) and a 23% decrease in LPL mass ($P = 0.03$) compared with control VLDLR$^{+/+}$ mice. Interestingly, LPL activity and mass levels were ~1.8-fold and 2.4-fold higher for mice on an LRP^{-}LDLR$^{-/-}$ background compared with mice on a wild-type background, respectively. The LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$ mice also had a significant 30% lower plasma LPL activity ($P =$

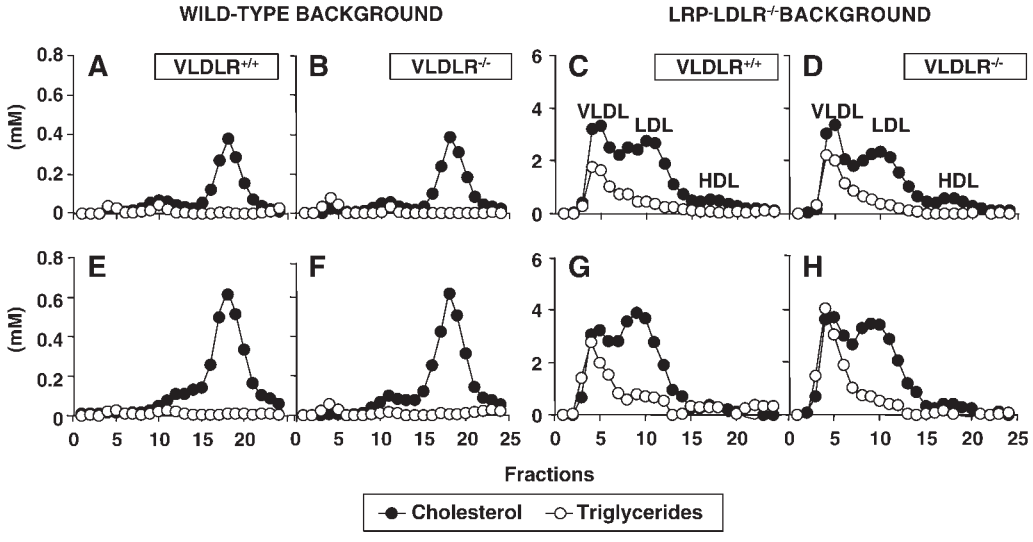

Fig. 2. Lipoprotein distribution. Plasma was obtained from mice on a wild-type background, VLDLR$^{+/+}$ (A, E) and VLDLR$^{-/-}$ (B, F), and on a LRP^{-}LDLR$^{-/-}$ background, LRP^{-}LDLR$^{-/-}$VLDLR$^{+/+}$ (C, G) and LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$ (D, H), after 4 weeks of inducing LRP deficiency on a chow diet (upper panels) and after 10 weeks on a high-fat diet (lower panels) with overnight fasting. Lipoproteins in pooled plasma were size-fractionated by fast-performance liquid chromatography, and the plasma cholesterol (closed circles) and triglyceride (TG; open circles) contents of the individual fractions were determined.

TABLE 2. Plasma lipids, glucose, and lipolytic enzymes on a chow diet after 4 h of fasting

Animals	Cholesterol	TGs	FFA	Glucose	HL Activity	LPL Activity	LPL Mass
	mM				*μmol FFA/h/ml*		*ng/ml*
Wild-type background							
VLDLR$^{+/+}$	2.2 ± 0.3	0.6 ± 0.2	n.d.	n.d.	8.2 ± 1.3	21.0 ± 3.5	76.6 ± 3.1
VLDLR$^{-/-}$	2.0 ± 0.3	1.3 ± 0.6[a]	n.d.	n.d.	7.0 ± 0.8	17.1 ± 2.1[a]	59.3 ± 7.9[a]
LRP^{-}LDLR$^{-/-}$ background							
VLDLR$^{+/+}$	24.9 ± 5.6	6.4 ± 2.4	0.8 ± 0.2	10.5 ± 1.3	14.2 ± 2.2	39.5 ± 3.6	180.6 ± 2.8
VLDLR$^{-/-}$	19.8 ± 4.3	4.6 ± 1.7	0.7 ± 0.1	11.1 ± 1.4	14.6 ± 1.2	27.7 ± 4.1[a]	151.6 ± 10.5[a]

Values represent means ± SD of 8–10 mice per group.
[a] $P < 0.05$.

0.001) and a 16% decrease in plasma LPL mass ($P = 0.04$) compared with control LRP^{-}LDLR$^{-/-}$VLDLR$^{+/+}$ mice. HL activity was not affected upon deletion of the VLDLR on both wild-type and LRP^{-}LDLR$^{-/-}$ backgrounds. However, as for LPL activity, HL activity levels were increased ~2-fold in mice on an LRP^{-}LDLR$^{-/-}$ background compared with mice on a wild-type background (Table 2).

Postprandial TG response

Stressing TG metabolism by forced feeding through an intragastric load of olive oil proved to be very effective at evoking a clear effect of VLDLR deficiency on plasma TG. Single VLDLR$^{-/-}$ mice had a strong increase in postprandial TG response compared with controls (**Fig. 3A**), as indicated by a 12-fold increased area under the curve (AUC) [218 ± 170 mM TG/h vs. 12 ± 5 mM TG/h ($P = 0.01$) for VLDLR$^{-/-}$ and VLDLR$^{+/+}$, respectively], which is in agreement with our recent report (8). Likewise, LRP^{-}LDLR$^{-/-}$ VLDLR$^{-/-}$ mice had a strong increase in postprandial TG response compared with control LRP^{-}LDLR$^{-/-}$VLDLR$^{+/+}$ mice (Fig. 3B) [AUC = 411 ± 107 mM TG/h vs. 163 ± 85 mM TG/h ($P = 0.002$) for LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$ and LRP^{-}LDLR$^{-/-}$VLDLR$^{+/+}$, respectively]. The total TG response in VLDLR$^{-/-}$ and LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$ mice was similar, as indicated by a similar mean increase in AUC compared with their respective controls (206 and 248 mM TG/h, respectively). However, the TG response in the LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$ mice was remarkably prolonged. Whereas TG levels in VLDLR$^{-/-}$ mice reached baseline TG levels within 24 h after gavage, TG levels were still significantly increased at 48 h after gavage in LRP^{-}LDLR$^{-/-}$ VLDLR$^{-/-}$ mice, which is probably related to the high remnant levels in plasma that compete for the binding of the nascent chylomicrons to LPL.

Plasma lipids under HFD

Finally, TG metabolism was assessed by challenge of mice with HFD (Table 1). As observed on a chow diet, VLDLR$^{-/-}$ mice had ~3-fold higher plasma TG levels ($P = 0.02$) compared with wild-type mice, whereas plasma cholesterol was not different. Remarkably, on this diet, LRP^{-}LDLR$^{-/-}$ VLDLR$^{-/-}$ mice also displayed 2-fold higher plasma TG levels ($P = 0.032$) but no change in plasma cholesterol, FFA, and glucose compared with the control LRP^{-}LDLR$^{-/-}$ VLDLR$^{+/+}$ mice. Thus, under high-fat feeding conditions, the role of the VLDLR in TG-rich lipoprotein metabolism becomes evident in the absence of the LRP and LDLR.

DISCUSSION

Studies in VLDLR$^{-/-}$ mice only revealed a role of this receptor specifically in postprandial TG-rich lipoprotein metabolism after severely stressing TG metabolism (5–8). Interestingly, the role of the LRP in TG-rich lipoprotein metabolism is fully compensated for by the presence of the LDLR (9). We wondered whether a prominent role of the VLDLR would be similarly overtaken by the LDLR or

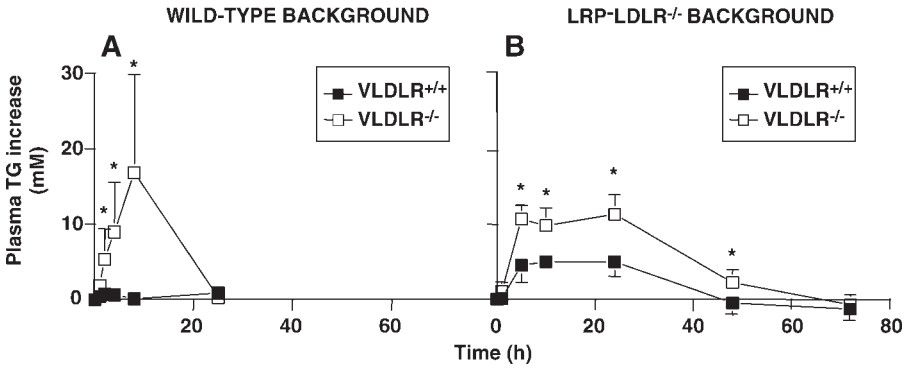

Fig. 3. Postprandial TG response. Overnight fasted VLDLR$^{+/+}$ (closed squares) and VLDLR$^{-/-}$ (open squares) mice on a wild-type (A) or LRP^{-}LDLR$^{-/-}$ (B) background were given an intragastric bolus of 200 μl of olive oil. Blood samples were drawn at the indicated times after gavage. Plasma TG concentrations were determined and corrected for time 0 values. Values represent means ± SD of eight mice per group. * $P < 0.05$.

LRP. Here, we show that the absence of a phenotype for VLDLR$^{-/-}$ mice with respect to TG-rich lipoprotein remnant levels is not attributable to backup activity of the LRP/LDLR pathway. Therefore, it seems reasonable to conclude that the contribution of VLDLR to the clearance of TG-rich lipoproteins is not rate-limiting under physiological circumstances but becomes apparent after stressing TG metabolism by high-fat feeding or giving a large TG bolus.

Previously, we demonstrated that adenovirus-mediated overexpression of the LDLR family antagonist receptor-associated protein (RAP) in LRP/LDLR double-deficient mice elicits marked hyperlipidemia in addition to the pre-existing hypercholesterolemia in these animals and decreases LPL activity (9). Because RAP binds to the VLDLR with high affinity (16), we speculated that it was possible that a RAP-mediated inhibition of the VLDLR underlies the observed impaired LPL-mediated lipolysis and subsequent hypertriglyceridemia. However, because LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$ and LRP^{-}LDLR$^{-/-}$VLDLR$^{+/+}$ mice have comparable hyperlipidemia, we can now conclude that RAP-induced hypertriglyceridemia does not directly involve the VLDLR. This is further supported by our observation that adenovirus-mediated overexpression of RAP still elicits marked hypertriglyceridemia and decreases LPL activity in LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$ mice (data not shown).

LRP^{-}LDLR$^{-/-}$VLDLR$^{+/+}$ and LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$ mice present higher plasma lipid levels after overnight fasting compared with 4 h of fasting, as observed for LDLR$^{-/-}$VLDLR$^{-/-}$ mice (7). This effect is probably caused by an increased hepatic production of VLDL-TG, which is the primary source of FFA for peripheral tissues in the absence of chylomicrons in the fasted state.

Yagyu et al. (15) and Goudriaan et al. (8) showed that impaired TG-rich lipoprotein catabolism in VLDLR$^{-/-}$ mice is associated with reduced LPL activity. This has been explained by reduced translocation of LPL over endothelial cells as related to the chaperone function of the VLDLR (17). Likewise, we now show that VLDLR deficiency also reduces LPL protein and activity on an LRP^{-}LDLR$^{-/-}$ background. In addition, the LRP^{-}LDLR$^{-/-}$ background resulted in increased LPL and HL activities in postheparin plasma, irrespective of VLDLR status. LPL and HL are both well-established ligands for LRP (18), and plasma LPL mass is increased in LRP^{-} mice (12). However, as LPL activity levels are not affected in LRP^{-} mice (12), it is uncertain whether LRP deficiency contributes to increased LPL protein and activity levels in LRP^{-}LDLR$^{-/-}$ mice. Most likely, the increase in LPL is a direct consequence of the severe hyperlipidemia in LRP^{-}LDLR$^{-/-}$ mice, because both total HDL and LPL are also increased in genetically unrelated severely hypertriglyceridemic mice as a result of apoC-I expression (19).

The unique triple-deficient mouse model (LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$) enabled us to conclude that the LRP/LDLR pathway does not mask a prominent role for the VLDLR in TG-rich lipoprotein metabolism. Apart from the LDLR family members, other mechanisms also have been identified that contribute to TG-rich lipoprotein uptake and degradation, such as LRP5 (20), apoB-48 receptor (21), LR11 (22), heparan sulfate proteoglycans (23), and scavenger receptor class B type I (24). Our LRP^{-}LDLR$^{-/-}$VLDLR$^{-/-}$ mouse model serves as a unique tool to elucidate the contributions of these pathways in TG-rich lipoprotein clearance in the absence of the three quantitatively important main apoE-recognizing receptors. This will further advance our understanding of the mechanisms by which plasma levels of TG-rich lipoproteins are regulated in vivo.

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