LDL receptor deficiency unmasks altered VLDL triglyceride metabolism in VLDL receptor transgenic and knockout mice

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Abstract **The very low density lipoprotein receptor (VLDLR) has been proposed to play a role in the delivery of fatty acids to peripheral tissues. However, despite reduced adipose tissue mass in VLDLR-deficient (*VLDLR*$^{-/-}$) mice, this has been difficult to substantiate. In the present study, VLDLR-deficient and VLDLR-overexpressing (PVL) mice were cross-bred onto a low density lipoprotein receptor knockout (*LDLR*$^{-/-}$) background to study the VLDLR under conditions of relatively high serum VLDL and triglyceride levels. Absence of the VLDLR resulted in a significant increase in serum triglyceride levels (1.9-fold) when mice were fed a high fat diet. In contrast, overexpression of the VLDLR resulted in a significant decrease in serum triglyceride levels (2.0-fold) under similar conditions. When kept on a chow diet, a period of prolonged fasting revealed a significant increase in serum triglyceride levels in *VLDLR*$^{-/-}$; *LDLR*$^{-/-}$ mice (2.3-fold) as compared with *LDLR*$^{-/-}$ controls. This could not be attributed to altered apolipoprotein B and VLDL triglyceride production rates. Furthermore, no major differences in nascent VLDL triglyceride content were found between *VLDLR*$^{-/-}$; *LDLR*$^{-/-}$ mice and *LDLR*$^{-/-}$ controls. However, the triglyceride content of circulating VLDL of *VLDLR*$^{-/-}$; *LDLR*$^{-/-}$ mice (63%) was relatively high as compared with *LDLR*$^{-/-}$ controls (49%). These observations suggest that the VLDLR affects peripheral uptake of VLDL triglycerides. In conclusion, under conditions of LDLR deficiency in combination with high fat feeding or prolonged fasting, the effect of the VLDLR on VLDL triglyceride metabolism was revealed.**—Tacken, P. J., B. Teusink, M. C. Jong, D. Harats, L. M. Havekes, K. Willems van Dijk, and M. H. Hofker. **LDL receptor deficiency unmasks altered VLDL triglyceride metabolism in VLDL receptor transgenic and knockout mice.** ***J. Lipid Res.*** **2000.** 41: **2055–2062.**

Supplementary key words lipoprotein receptor • lipid metabolism • fatty acids

The very low density lipoprotein receptor (VLDLR) is a member of the low density lipoprotein receptor (LDLR) family (1). It has been described to bind apolipoprotein E (apoE)-rich VLDL (1, 2) and lipoprotein lipase (LPL) (3), suggesting a role for the VLDLR in lipid metabolism. However, other ligands such as receptor-associated protein (RAP) (4) and urokinase-type plasminogen activator-plasminogen activator inhibitor complexes (5) were also described to bind to the VLDLR. In addition, the VLDLR was found to play a role in transmission of extracellular signals to intracellular signaling processes, in a molecular pathway that regulates neuronal migration (6).

The VLDLR is expressed in blood vessels, where expression is located in endothelial and smooth muscle cells (7). Furthermore, expression levels are high in heart, skeletal muscle, and adipose tissue, but expression is absent from liver. Heart, skeletal muscle, and adipose tissue are active in fatty acid metabolism. Together with the observation that the VLDLR can bind apoE-rich VLDL, this has led to the hypothesis that the VLDLR is involved in delivery of fatty acids to extrahepatic organs. This hypothesis is supported by observations in mice showing upregulation of VLDLR mRNA levels in heart and downregulation in adipose tissue after prolonged periods of fasting (8). In addition, *LDLR*$^{-/-}$ mice fed an atherogenic diet show downregulation of VLDLR mRNA levels in heart and upregulation in adipose tissue (9). However, plasma cholesterol and triglyceride levels were not affected in *VLDLR*$^{-/-}$ mice. So

Abbreviations: apo, apolipoprotein; EDTA, ethylenediaminetetraacetic acid; FPLC, fast protein liquid chromatography; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LDLR, LDL receptor; LPL, lipoprotein lipase; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; RAP, receptor-associated protein; RT, reverse transcriptase; VLDL, very low density lipoprotein; VLDLR, VLDL receptor.

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far the only in vivo evidence implying a role for the VLDLR in lipid metabolism is the observation that *VLDLR*$^{-/-}$ mice show reduced adipose tissue mass (10).

In wild-type as well as in *VLDLR*$^{-/-}$ mice, high density lipoprotein (HDL) is the predominant lipoprotein in plasma, while plasma levels of VLDL are relatively low. Thus differences in VLDL metabolism between wild-type and *VLDLR*$^{-/-}$ mice are difficult to detect. We chose to study the VLDLR in the *LDLR*$^{-/-}$ mouse model, which displays relatively high serum VLDL and triglyceride levels (11). Both the *VLDLR*$^{-/-}$ mouse and a newly generated transgenic mouse model that expresses the human VLDLR in endothelial and smooth muscle cells (PVL mouse) were used. These mice were fed either a chow or a high fat diet, and serum lipid levels were studied both after 4 h and after overnight fasting.

LDLR deficiency in combination with a high fat diet or overnight fasting led to elevated serum triglyceride levels in the absence of the VLDLR. In contrast, overexpression of the VLDLR led to lowering of serum triglyceride levels. While there was no major difference in nascent VLDL triglyceride content between *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice and *LDLR*$^{-/-}$ controls, *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice showed a relatively high circulating triglyceride content. This suggests the VLDLR has an effect on the peripheral uptake of VLDL triglycerides.

EXPERIMENTAL PROCEDURES

Generation of transgenic mice

To express an additional VLDLR in the endothelium, an expression construct was generated using the human VLDLR cDNA in combination with the preproendothelin-1 promoter. This promoter was previously shown in vivo to express a luciferase reporter gene mainly in endothelial cells, and to a lesser extent in smooth muscle cells of the vessel wall of transgenic mice (12). The pHV58 plasmid, carrying the human VLDLR cDNA, was generously provided by L. Chan (13). The HL3 reporter plasmid, carrying the firefly luciferase gene under control of the 5.9-kb promoter region of the endothelin-1 gene, was described previously as p5.9mPPET-LUC (12). The HL3 reporter plasmid was digested with *Not*I to remove the luciferase reporter gene. The pHV58 plasmid was digested with *Not*I to isolate the human VLDLR cDNA, which was subsequently subcloned in the *Not*I-digested HL3 reporter plasmid, generating the PVL plasmid containing the human VLDLR cDNA under the control of 5.9-kb preproendothelin-1 promoter region (see **Fig. 1**).

The PVL plasmid DNA was digested with *Xho*I to obtain the insert, and subsequently was layered onto a continuous linear gradient of 10% to 40% sucrose in 1.0 M NaCl, 10.0 mM Tris-HCl (pH 8.0), 1 mM ethylenediaminetetraacetic acid (EDTA), and centrifuged at 35,000 rpm. Gradient fractions were collected to determine which fractions contained only the PVL insert, and these were pooled. Gradient medium was removed by five successive rinses with injection buffer [10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA] in a Centricon 100 microconcentrator (Amicon, Danvers, MA). The PVL insert was diluted in injection buffer to a concentration of 6 ng/ml. This mixture was injected into B:CBA zygotes to generate transgenic mice according to standard procedures (14).

To screen for transgenic mice, offspring were characterized by polymerase chain reaction (PCR) on tailtip DNA with forward primer U19 (CCTTTGAGGTCTAAACAAAT) and reverse primer L19 (TTTACAGATGGCCTATACAA), both localized in exon 19 of the human VLDLR gene. The size of the inserted construct was verified by digesting tailtip DNA with *Xho*I, followed by Southern blot analysis using an exon 6 to 14-spanning *Hin*cII restriction fragment of the pHV58 cDNA, pHV58.H, as a probe (Fig. 1B). Eight of 40 offspring that were characterized by PCR were shown to carry the transgene (data not shown). Six of the founder mice produced transgenic offspring. All experiments in the present article were performed with the mouse line that showed the highest human-versus-mouse VLDLR expression as was determined by semiquantitative reverse transcriptase (RT)-PCR (data not shown).

Semiquantitative analysis of human-versus-mouse VLDLR expression

To measure the level of transgene expression relative to endogenous mouse VLDLR expression, a semiquantitative RT-PCR

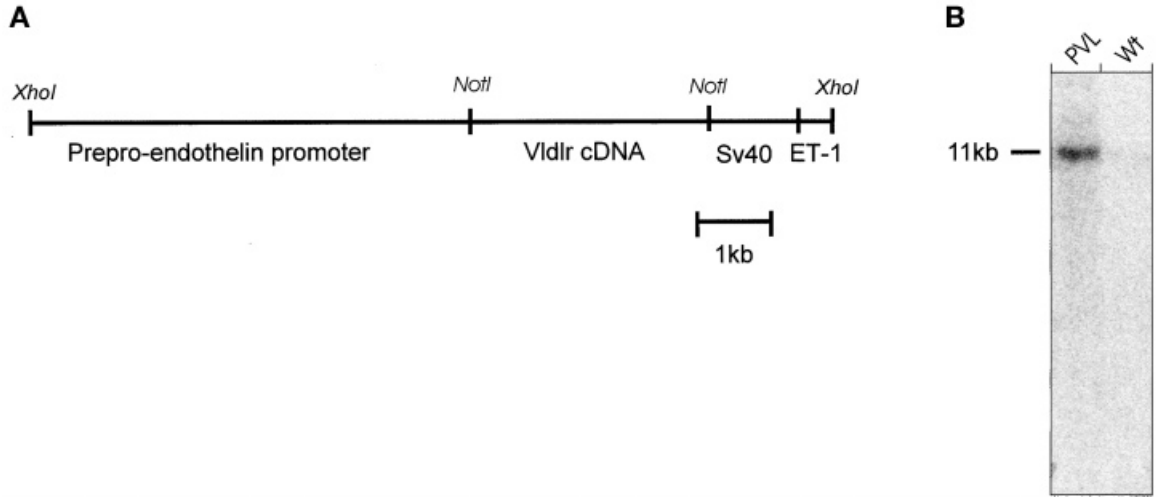

Fig. 1. Generation and Southern blot analysis of PVL transgenic mice. Schematic representation of the generation of the PVL construct (A). Genomic DNA of wild-type and PVL mice was digested with *Xho*I and subjected to electrophoresis. PVL construct was detected with the ^{32}P-labeled pHV58.H probe (B).

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method was developed. Reverse primer VR1 (TTCAAGTACA CAGGATTGTC) shows 100% sequence identity to the human (bp 2610 to 2629) and mouse (bp 2678 to 2697) VLDLR cDNA sequence. Forward primer MVR1 (ACAGTGAGACAAAAG ATATC, bp 2454 to 2473) is specific for mouse, and forward primer HVR5 (AACTCAACAGAAATTTCCAGC, bp 2406 to 2425) is specific for human VLDLR cDNA sequence. Both the MVR1 and HVR5 primers will compete for the VR1 primer in a PCR. MVR1 and VR1 amplify 244 bp of mouse VLDLR sequence, while HVR5 and VR1 amplify 224 bp of human VLDLR sequence. Calibration of the RT-PCR, using human and mouse VLDLR cDNA mixed in different relative amounts, proved that both amplifications were equally efficient (data not shown). Semiquantitative RT-PCR was performed on total RNA isolated from various mouse tissues for 34 cycles at 52°C. PCR products were separated by electrophoresis on an ethidium-stained 3% agarose gel (agarose MP; Boehringer Mannheim, Mannheim, Germany). The gel was photographed with an ImaGo™ (B&L Systems, Maarssen, The Netherlands) and the relative amount of human-versus-mouse product was calculated with ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Northern blot analysis of 10 μg of total heart RNA of PVL mice and wild-type littermates was performed, using pHV58.H as a probe. Membranes were reprobed with rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA (15).

RNA of mouse tissues was isolated with RNAzol™ (Biotecx Laboratories, Houston, TX), and RT-PCR was performed with the use of Superscript™ II RNase H reverse transcriptase (GIBCO-BRL, Gaithersburg, MD), both according to the manufacturer protocol.

Antibody

The rabbit polyclonal anti-human antibody, raised against amino acids 1–724 of the human VLDLR, was generously provided by H. Hobbs (University of Texas Southwestern Medical Center, Dallas, TX). The antibody has also been shown to recognize mouse VLDLR protein (16).

Western blot analysis

Membrane protein fractions of mouse heart were isolated. Therefore, hearts were frozen in liquid nitrogen, homogenized with a tight-fitting pestle in a solution of 0.25 M sucrose, 50 mM Tris (pH 7.4) and Complete™ EDTA-free MiniProtein inhibitor cocktail (Boehringer Mannheim). Subsequently the suspension was centrifuged at 600 *g*, followed by centrifugation of the supernatant at 10,000 *g*. Finally, the supernatant was centrifuged at 130,000 *g*. The resulting pellet containing the membrane proteins was resuspended in TBS (10 mM Tris, 150 mM NaCl, 2 mM $CaCl_2$, and 2 mM $MgCl_2$). Protein concentration was determined with the Pierce (Rockford, IL) bicinchoninic acid protein assay according to the manufacturer protocol. Eighty micrograms of protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 6% polyacridamide gel. Proteins were blotted onto Hybond™ ECL™ nitrocellulose membrane (Amersham Pharmacia Biotech, Little Chalfont, UK). The membrane was washed extensively in wash buffer, containing 0.1% (v/v) Tween 20 (Sigma, St. Louis, MO) in phosphate-buffered saline (PBS). Subsequently the membrane was blocked with blockbuffer, containing 0.1% (v/v) Tween-20 and 5% nonfat milk (w/w) in PBS, followed by incubation with rabbit polyclonal anti-human VLDLR antibody in blocking buffer. The membrane was then extensively washed in blocking buffer, followed by incubation with a peroxidized anti-rabbit IgG antibody (NIF824; Amersham Pharmacia Biotech). Finally the membrane was washed with wash buffer, followed by washing with PBS and ECL™ detection according to the manufacturer protocol (Amersham Pharmacia Biotech).

Mice

VLDLR$^{-/-}$ mice were obtained from Jackson Laboratories (Bar Harbor, ME) and were bred to *LDLR*$^{-/-}$ mice to produce *VLDLR*$^{+/-}$;*LDLR*$^{+/-}$ mice. *VLDLR*$^{+/-}$;*LDLR*$^{+/-}$ mice were subsequently bred to *LDLR*$^{-/-}$ mice to produce *VLDLR*$^{+/-}$;*LDLR*$^{-/-}$ mice. *VLDLR*$^{+/-}$;*LDLR*$^{-/-}$ mice were bred to produce *VLDLR*$^{-/-}$; *LDLR*$^{-/-}$ and *LDLR*$^{-/-}$ littermates. PVL mice were crossed back onto a C57BL6/J background, and generation N3 mice were used. PVL mice were bred to *LDLR*$^{-/-}$ mice to produce PVL; *LDLR*$^{+/-}$ mice. PVL;*LDLR*$^{+/-}$ mice were bred to *LDLR*$^{-/-}$ mice to generate PVL;*LDLR*$^{-/-}$ mice. PVL;*LDLR*$^{-/-}$ mice were bred to *LDLR*$^{-/-}$ mice to produce PVL;*LDLR*$^{-/-}$ and *LDLR*$^{-/-}$ littermates. For protein expression analysis, PVL mice were bred onto a *VLDLR*$^{-/-}$ background, using a strategy similar to that used to generate PVL;*LDLR*$^{-/-}$ mice.

All studies in this article were performed using 10- to 20-week-old females. Littermates were housed in groups. Mice were fed either a regular breeding chow diet or a high fat diet. The chow diet contained 6.2% fat (Hope Farms, Woerden, The Netherlands). The high fat diet contained 15% cacao butter, 0.25% cholesterol, 40.5% sucrose, 10% cornstarch, 1% corn oil, and 5.95% cellulose (w/w) (Hope Farms).

Serum cholesterol and triglyceride analysis

Mice were fasted for 4 h (9 AM to 1 PM) or overnight (5 PM to 9 AM), and blood was collected in Microvette CB 300 tubes (Sarstedt, Nuernbrecht, Germany), which were immediately placed on ice. Tubes were spun in an Eppendorf centrifuge at 4°C at 4,000 rpm. Serum cholesterol and triglyceride levels were determined with commercially available kits (kit 236,691 from Boehringer Mannheim and kit 337-B from Sigma, respectively).

Serum fast protein liquid chromatography analysis

For the analysis of the distribution of cholesterol and triglycerides over the different lipoprotein fractions pooled samples of mouse serum were analyzed on a Smart system (Pharmacia, Uppsala, Sweden) with a Superose 6 column as described previously (17).

VLDL production

The VLDL triglyceride production rate was determined essentially as described previously (18). Groups of six *VLDLR*$^{-/-}$; *LDLR*$^{-/-}$ mice and six *LDLR*$^{-/-}$ control mice fed a chow diet were fasted overnight. Tran ^{35}S-label™ (ICN Pharmaceuticals, Irvine, CA) was dissolved in PBS and 100 μCi was injected into the tail vein. After 30 min, Triton WR1339 (Sigma) was injected into the tail vein. At 0, 30, 60, and 90 min after Triton injection blood samples were collected in Microvette tubes (Sarstedt), and were placed on ice. Serum triglyceride levels were measured as described above, and a curve fit was performed using GraphPad Prism (GraphPad Software, San Diego, CA). VLDL was isolated by ultracentrifugation ($d < 1.006$ g/ml) from blood samples collected 90 min after Triton injection. Part of the VLDL was used to determine the apoB production rate. ApoB was specifically precipitated by isopropanol precipitation (19). The pellet was dissolved at 60°C in a 20% SDS solution, and radioactivity of the sample was determined.

VLDL lipid analysis

To determine the lipid composition of circulating and nascent VLDL, VLDL lipid content was measured both before and 90 min after Triton treatment. Analysis of mouse serum that was incubated with Triton showed that Triton treatment does not affect lipid composition of the different lipoprotein fractions (data not shown). Therefore, the lipid composition of nascent VLDL can be calculated from the difference in VLDL lipid con-

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tent before and after Triton treatment. VLDL total cholesterol content was determined as described above. VLDL triglyceride, free cholesterol, and phospholipid content were determined with enzymatic assay kits (701,904 and 310,328 from Boehringer Mannheim) and an analytical kit B (Wako Chemicals, Neuss, Germany), respectively. VLDL cholesteryl ester content was calculated by subtracting free cholesterol from total cholesterol content.

RESULTS

Characterization of VLDLR transgenic mice (PVL)

To overexpress the VLDLR in mouse endothelium, transgenic mice (PVL) were generated using the human VLDLR cDNA under the control of the preproendothelin-1 promoter. A semiquantitative RT-PCR was developed to determine the level of human VLDLR expression relative to endogenous mouse VLDLR expression in PVL mice. Semiquantitative RT-PCR performed on RNA isolated from PVL mouse tissues revealed that mouse and human VLDLR expression levels were in the same range in heart, aorta, and kidney. In skeletal muscle, human VLDLR expression levels were lower than endogenous mouse VLDLR expression levels, whereas the opposite was true for testis and liver (**Fig. 2A**). Northern blot analysis of PVL mouse heart RNA, using the pHV58.H probe, revealed expression of a 3.8-kb message (Fig. 2B). To assess the expression of the human VLDLR protein, heart membrane protein fractions were isolated from wild-type, PVL, *VLDLR*$^{-/-}$, and PVL;*VLDLR*$^{-/-}$ mice. Western blot analysis with a rabbit polyclonal anti-human VLDLR antibody revealed two bands in wild-type mice that were absent in the *VLDLR*$^{-/-}$ mouse. These two protein bands, which have been described previously (10), presumably represent the precursor and the mature form of the VLDLR. Expression of the human VLDLR protein was detected in PVL;*VLDLR*$^{-/-}$ mice, and revealed two protein bands of the same size as those of the endogenous VLDLR. The intensity of the bands detected in the PVL mice was stronger than in wild-type littermates (Fig. 2C), implying that total VLDLR expression is higher in PVL than in wild-type mice.

Serum lipid levels of *VLDLR*$^{-/-}$ and PVL mice on *LDLR*$^{-/-}$ background

When fed a standard chow diet, PVL (data not shown) and *VLDLR*$^{-/-}$ (10) mice show normal serum lipid levels. To study the VLDLR under conditions of relatively high serum VLDL and triglyceride levels, both PVL and *VLDLR*$^{-/-}$ mice were first crossed onto an *LDLR*$^{-/-}$ background. Subsequently, serum lipid levels were studied on chow and high fat diet, both after 4 h and after overnight fasting (**Table 1**). *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice showed a significant elevation of serum triglyceride levels (2.3-fold) on a chow diet after overnight fasting when compared with *LDLR*$^{-/-}$ controls. When fed a high fat diet for 6 weeks, *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice showed significantly elevated serum triglyceride levels both after 4 h (1.9-fold) and after overnight fasting (2.4-fold) when compared with controls. In contrast, after 4 h of fasting, PVL;*LDLR*$^{-/-}$ mice displayed a significant decrease in serum triglyceride levels

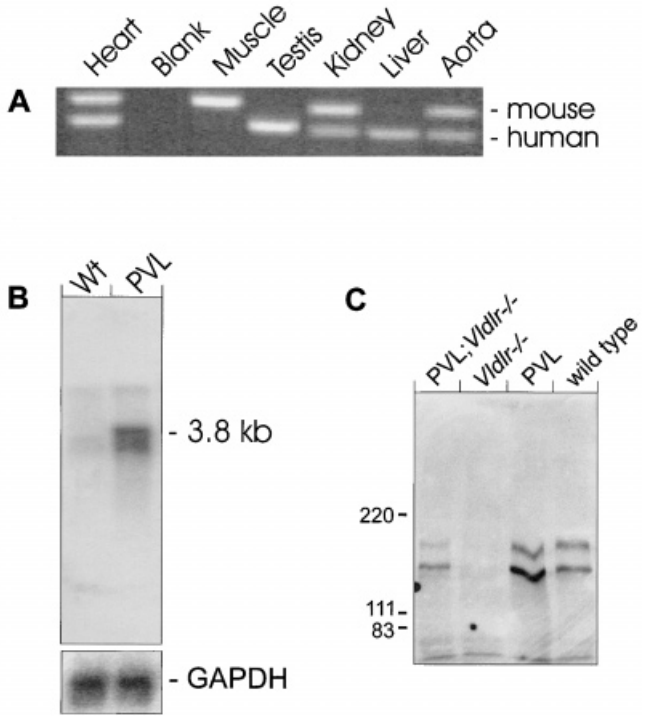

Fig. 2. Expression analysis of PVL transgenic mice. To determine human-versus-mouse VLDLR RNA expression levels, semiquantitative RT-PCR was performed on total RNA isolated from various PVL mouse tissues. PCR products were separated by electrophoresis on an ethidium-stained 3% agarose gel (A). Northern blot analysis of PVL and wild-type (Wt) RNA was performed with the ^{32}P-labeled pHV58.H probe. Membranes were reprobed with a ^{32}P-labeled GAPDH cDNA probe (B). Membrane protein fractions were isolated from PVL;*VLDLR*$^{-/-}$, *VLDLR*$^{-/-}$, PVL, and wild-type mouse heart. Eighty micrograms of protein was subjected to SDS-PAGE on a 6% polyacrylamidegel. VLDLR protein was detected with a rabbit polyclonal anti-human VLDLR antibody (C).

both on chow (1.2-fold) and on a high fat diet (2.0) when compared with *LDLR*$^{-/-}$ controls. No significant differences in serum triglyceride levels were found between PVL;*LDLR*$^{-/-}$ mice and *LDLR*$^{-/-}$ controls on a chow diet after overnight fasting. No significant differences in total serum cholesterol levels were found between *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice and *LDLR*$^{-/-}$ controls or between PVL;*LDLR*$^{-/-}$ and *LDLR*$^{-/-}$ controls under all circumstances studied.

Serum fast protein liquid chromatography (FPLC) analysis showed that the effect of the VLDLR on serum triglyceride levels was mainly due to an effect on VLDL triglycerides. While *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice show elevated VLDL triglyceride levels, PVL;*LDLR*$^{-/-}$ mice showed a lowering of VLDL triglyceride levels when compared with their controls. The extent of the effect of the VLDLR on VLDL triglycerides depended on the diet or the duration of the fasting period (**Fig. 3**).

VLDL production

To determine whether the relatively high serum triglyceride levels in *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice were due to enhanced VLDL production, apoB and triglyceride produc-

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TABLE 1. Serum cholesterol and triglyceride levels of *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$, PVL;*LDLR*$^{-/-}$, and *LDLR*$^{-/-}$ controls

Mouse Model	Diet	n	4-h Fast		Overnight Fast	
			Cholesterol	Triglycerides	Cholesterol	Triglycerides
			mM		*mM*	
VLDLR$^{-/-}$;*LDLR*$^{-/-}$	Chow	15	6.22 ± 1.78	0.73 ± 0.27	8.44 ± 1.32	4.18 ± 1.73[a]
LDLR$^{-/-}$[b]	Chow	19	5.25 ± 1.18	0.54 ± 0.22	7.88 ± 1.41	1.79 ± 0.63
VLDLR$^{-/-}$;LDLR$^{-/-}$	W	18	44.76 ± 14.04	5.47 ± 2.51[a]	42.76 ± 14.04	1.23 ± 0.16[a]
LDLR$^{-/-}$[b]	W	20	38.57 ± 14.79	2.91 ± 1.59	38.57 ± 14.79	0.52 ± 0.11
PVL;*LDLR*$^{-/-}$	Chow	16	5.33 ± 0.54	0.57 ± 0.15[a]	6.52 ± 0.89	1.53 ± 0.66
LDLR$^{-/-}$[b]	Chow	20	5.70 ± 1.09	0.71 ± 0.25	6.44 ± 0.69	1.72 ± 0.42
PVL;*LDLR*$^{-/-}$	W	9	47.16 ± 10.28	1.74 ± 0.55[a]	ND	ND
LDLR$^{-/-}$[b]	W	13	49.92 ± 10.07	3.47 ± 1.70	ND	ND

Serum cholesterol and triglyceride concentrations were measured in *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice and *LDLR*$^{-/-}$ littermates and in PVL;*LDLR*$^{-/-}$ mice and *LDLR*$^{-/-}$ littermates. All littermates were housed in groups. Lipid levels were measured in mice that were fasted for 4 h or were fasted overnight. Mice were kept on a chow diet or on a high fat diet (W). Values shown are means ± SD. Abbreviations: ND, not determined.

[a] Significantly different from *LDLR*$^{-/-}$littermates ($P < 0.05$), using the Student's *t*-test.

[b] Note that *LDLR*$^{-/-}$ control groups for PVL and *VLDLR*$^{-/-}$ mice differ in their genetic background.

tion rates were determined in *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ and *LDLR*$^{-/-}$ controls that were fed a chow diet and were fasted overnight. Mice were injected with Triton WR1339 to impair lipolysis and VLDL clearance, and the accumulation of triglycerides and the radiolabeled apoB was determined in the serum over a period of 90 min. No significant differences were found between *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice and *LDLR*$^{-/-}$ controls for both triglyceride (76.1 ± 9.4 and 62.4 ± 18.5 μmol/h per kg) and apoB (1.39 ± 0.34 and 1.00 ± 0.44 arbitrary units/h per kg) production

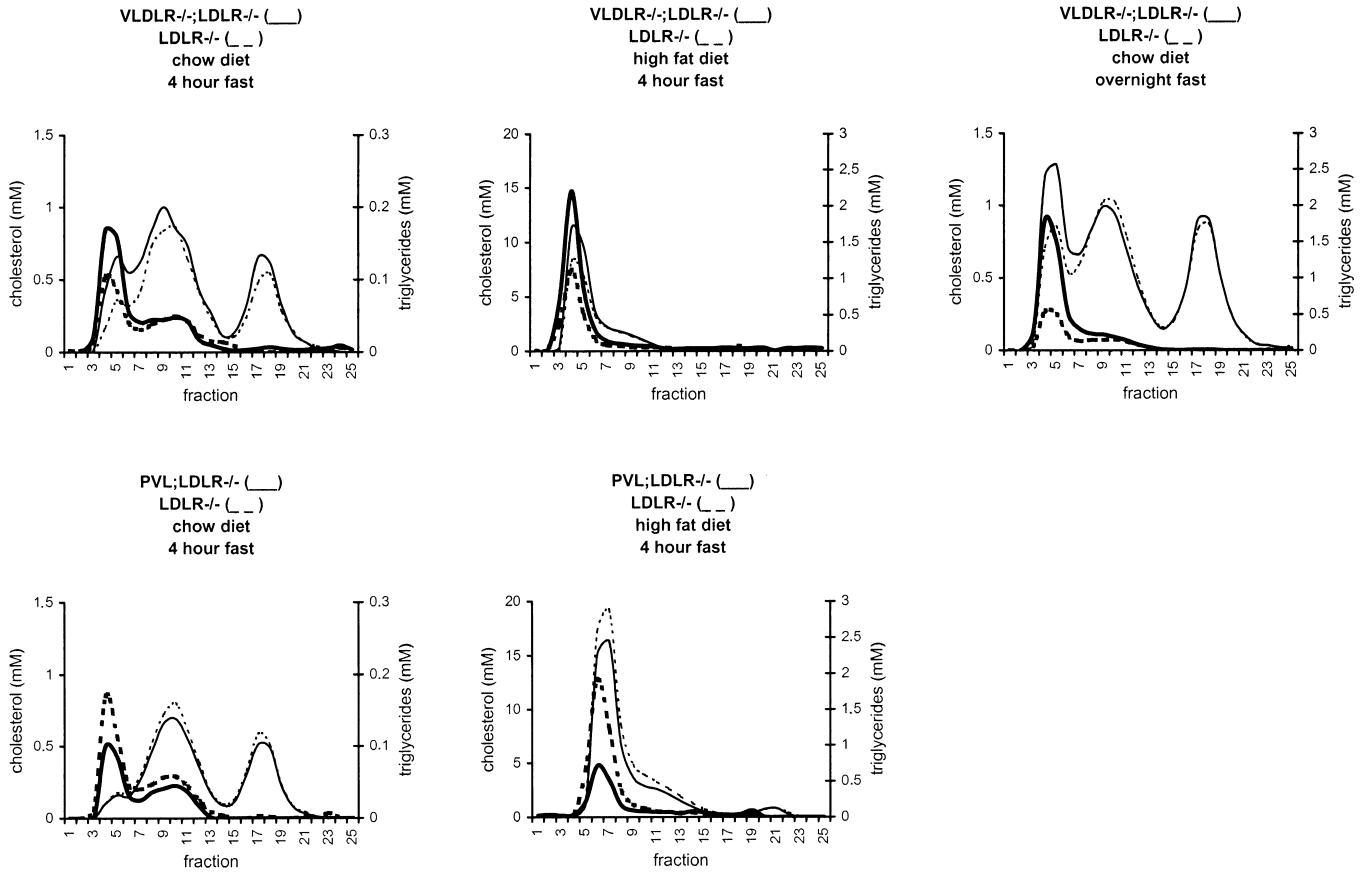

Fig. 3. Serum FPLC profiles of *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$, PVL;*LDLR*$^{-/-}$, and *LDLR*$^{-/-}$ controls. Mouse sera isolated from various mouse models under different dietary conditions were subjected to FPLC fractionation. Cholesterol levels (thin lines) and triglyceride levels (thick lines) of each fraction were determined as described in Materials and Methods. Pooled sera were analyzed from 15 *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ and 19 *LDLR*$^{-/-}$ littermates that were kept on a chow diet and were fasted for 4 h or overnight, from 18 *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ and 20 *LDLR*$^{-/-}$ littermates that were kept on a high fat diet and were fasted for 4 h, from 16 PVL;*LDLR*$^{-/-}$ and 20 *LDLR*$^{-/-}$ littermates that were kept on a chow diet and were fasted for 4 h, and from 9 PVL;*LDLR*$^{-/-}$ and 13 *LDLR*$^{-/-}$ mice that were kept on a high fat diet and were fasted for 4 h.

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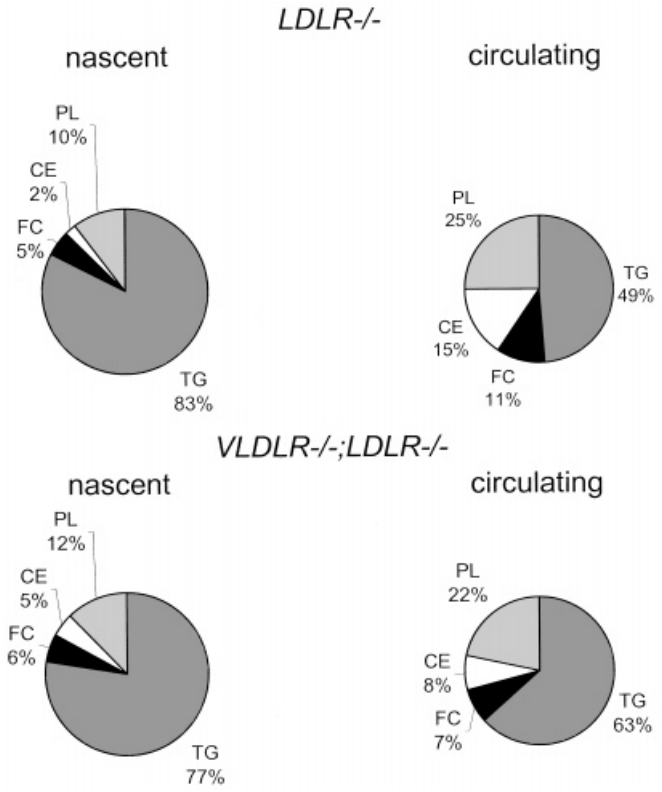

Fig. 4. VLDL lipid content of *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice and *LDLR*$^{-/-}$ controls. Serum was collected from the overnight-fasted mice in the VLDL production experiments (see Materials and Methods), both before and 90 min after Triton WR1339 injection. VLDL was isolated from pooled serum by ultracentrifugation ($d < 1.006$ g/ml). VLDL phospholipid (PL), cholesteryl ester (CE), free cholesterol (FC), and triglyceride (TG) content were determined as described in Materials and Methods. Composition of nascent VLDL was determined by subtracting the amount of VLDL lipids before Triton treatment from those 90 min after treatment. VLDL composition of nascent VLDL and circulating plasma VLDL was determined in *LDLR*$^{-/-}$ mice and *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice.

rates. Furthermore, nascent VLDL triglyceride content of *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice (77%) was comparable to that of *LDLR*$^{-}$/− controls (83%). However, circulating serum VLDL of *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice contained 63% triglycerides, compared with 49% in *LDLR*$^{-/-}$ controls (**Fig. 4**). This implies that the clearance of triglycerides from circulating VLDL is disturbed in *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice.

DISCUSSION

The purpose of this study was to gain insight into the role of the VLDL receptor in lipoprotein metabolism. The in vivo evidence that supports a role for the VLDLR in lipid metabolism is limited to the observation that *VLDLR*$^{-/-}$ mice show reduced adipose tissue mass (10). Thus far, *VLDLR*$^{-/-}$ mice were not reported to display altered serum lipid levels. However, mice have low serum VLDL and triglyceride levels. To study the VLDLR under conditions of relatively high serum VLDL and triglyceride levels, both VLDLR-deficient and VLDLR-overexpressing mice were cross-bred onto an *LDLR*$^{-/-}$ background. Absence of the VLDLR was associated with elevated serum triglyceride levels under conditions of dietary stress. In contrast, overexpression of the VLDLR under similar conditions was associated with lowering of serum triglyceride levels. Thus the variation in serum triglyceride levels is consistent with the variation in VLDLR expression in both mouse models.

In *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice, the increase in serum triglycerides was not accompanied by a significant increase in VLDL production rate when compared with *LDLR*$^{-/-}$ controls. In addition, no significant differences were found in serum cholesterol levels between *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice and *LDLR*$^{-/-}$ controls. Therefore it does not seem likely that the VLDLR influences serum triglyceride levels by affecting clearance of whole lipoprotein particles by the liver. Analysis of VLDL lipid composition showed that the triglyceride content of circulating VLDL in *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice is relatively high as compared with *LDLR*$^{-/-}$ controls. This implies that the clearance of triglycerides from circulating VLDL is disturbed in *VLDLR*$^{-/-}$;*LDLR*$^{-/-}$ mice.

The effect of the VLDLR on VLDL triglyceride metabolism could be explained by an effect on lipolysis. The principal enzyme responsible for the hydrolysis of triglycerides of circulating lipoproteins is lipoprotein lipase (LPL). It has been described to perform a bridging function between extracellular heparan sulfate proteoglycans and lipoprotein particles (20, 21). In addition, LPL has been reported to bind to receptors of the LDLR family, including the VLDLR, thereby enhancing their affinity for lipoproteins (3, 5). We hypothesize that the VLDLR and LPL together promote retention of circulating VLDL on the vessel wall. Subsequently, LPL could exert its lipolytic effect on triglycerides residing in the VLDL particle without affecting VLDL cholesterol. Thus, VLDLR overexpression would lead to an increase, while VLDLR deficiency would lead to a decrease in the rate of lipolysis of VLDL triglycerides.

In contrast to our present results, studies expressing the VLDLR ectopically in the liver of *LDLR*$^{-/-}$ mice fed a high cholesterol diet showed no effects on serum triglyceride levels, while serum cholesterol levels were reduced by 50%. Furthermore, VLDLR expression in liver affected intermediate density lipoprotein (IDL) and LDL instead of VLDL fractions, and was shown to facilitate the clearance of IDL particles (22). Apparently, when expressed in liver, the VLDLR can be engaged in uptake of IDL/LDL via lipoprotein receptor-mediated endocytosis. Thus VLDLR-mediated binding and processing of lipoproteins seems to depend on the site of VLDLR expression.

The *VLDLR*$^{-/-}$ mouse was previously reported to have normal serum lipid levels (10). In the present article we show that the VLDLR does affect serum triglyceride levels when specific conditions are applied. LDLR deficiency in combination with conditions of fasting, or feeding a high fat diet, revealed the effect of the VLDLR

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on VLDL triglyceride metabolism. Fasting and high fat feeding were previously reported to affect the regulation of VLDLR expression. VLDLR expression in mice was shown to increase in heart and decrease in adipose tissue under conditions of prolonged fasting (8). In contrast, feeding a high fat diet was reported to downregulate VLDLR mRNA expression in heart and upregulate expression in adipose tissue in *LDLR*$^{-/-}$ mice (9). These observations are in line with the hypothesis that the VLDLR is involved in the delivery of fatty acids to peripheral tissues. On fasting, fatty acid demand in tissues such as the heart will increase, while there will be less fatty acid storage in adipose tissue. On feeding a high fat diet, the reverse will be true. We hypothesize that in the present study the effect of the VLDLR on serum triglyceride levels was revealed by the effect of dietary status on fatty acid fluxes. Apparently, triglyceride metabolism is tightly regulated, and backup mechanisms can compensate for VLDLR deficiency. Only when specific dietary conditions are applied do these backup mechanisms fail to compensate for VLDLR deficiency or VLDLR overexpression.

Similar to the effect of the VLDLR on VLDL triglyceride content, the reduction in adipose tissue mass observed in *VLDLR*$^{-/-}$ mice (10) could be explained by impaired hydrolysis of VLDL triglycerides. A second mouse model that exhibits reduced adipose tissue mass is the apolipoprotein C-I (apoC-I) transgenic mouse (23). Interestingly, apoC-I was reported to strongly inhibit lipoprotein binding to the VLDLR (24), although it does not impair LPL lipolysis activity in vitro (25). RAP was also reported to inhibit lipoprotein binding by the VLDLR. Intriguingly, adenovirus-mediated overexpression of RAP in the circulation of mice that lacked both the LDLR and the LRP in their livers showed an increase in serum triglycerides. Adenovirus-mediated RAP expression led to high serum levels of RAP. The effect of RAP on the metabolism of triglyceride-rich lipoproteins could not be attributed to RAP-binding proteins in the liver (26). Considering the results of the present study, it could be hypothesized that the extrahepatic effect of RAP is due to blocking of lipoprotein binding to receptors such as the VLDLR in peripheral tissues.

In summary, our results suggest a role for the VLDLR in peripheral uptake of VLDL triglycerides. Apparently, backup mechanisms can compensate for VLDLR deficiency or overexpression. Only under stressed conditions of LDLR deficiency in combination with high fat feeding or prolonged fasting periods is the effect of the VLDLR on VLDL triglyceride metabolism revealed.

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