The VLDL receptor plays a major role in chylomicron metabolism by enhancing LPL-mediated triglyceride hydrolysis

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Abstract **The VLDL receptor (VLDLr) is involved in tissue delivery of VLDL-triglyceride (TG)-derived FFA by facilitating the expression of lipoprotein lipase (LPL). However, *vldlr*$^{-/-}$ mice do not show altered plasma lipoprotein levels, despite reduced LPL expression. Because LPL activity is crucial in postprandial lipid metabolism, we investigated whether the VLDLr plays a role in chylomicron clearance. Fed plasma TG levels of *vldlr*$^{-/-}$ mice were 2.5-fold increased compared with those of *vldlr*$^{+/+}$ littermates (1.20 ± 0.37 mM vs. 0.47 ± 0.18 mM; $P < 0.001$). Strikingly, an intragastric fat load led to a 9-fold increased postprandial TG response in *vldlr*$^{-/-}$ compared with *vldlr*$^{+/+}$ mice (226 ± 188 mM/h vs. 25 ± 11 mM/h; $P < 0.05$). Accordingly, the plasma clearance of [^{3}H]TG-labeled protein-free chylomicron-mimicking emulsion particles was delayed in *vldlr*$^{-/-}$ compared with *vldlr*$^{+/+}$ mice (half-life of 12.0 ± 2.6 min vs. 5.5 ± 0.9 min; $P < 0.05$), with a 60% decreased uptake of label into adipose tissue ($P < 0.05$). VLDLr deficiency did not affect the plasma half-life and adipose tissue uptake of albumin-complexed [^{14}C]FFA, indicating that the VLDLr facilitates postprandial LPL-mediated TG hydrolysis rather than mediating FFA uptake. We conclude that the VLDLr plays a major role in the metabolism of postprandial lipoproteins by enhancing LPL-mediated TG hydrolysis.—** Goudriaan, J. R., S. M. S. Espirito Santo, P. J. Voshol, B. Teusink, K. W. van Dijk, B. J. M. van Vlijmen, J. A. Romijn, L. M. Havekes, and P. C. N. Rensen. **The VLDL receptor plays a major role in chylomicron metabolism by enhancing LPL-mediated triglyceride hydrolysis. *J. Lipid Res.* 2004.** 45: **1475–1481.**

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The VLDL receptor (VLDLr) is a member of the LDL receptor (LDLr) family (1). The most striking features that distinguish the VLDLr from the LDLr are its ligand-binding properties and its tissue distribution (1, 2). The VLDLr is, like lipoprotein lipase (LPL), uniquely expressed at sites that are involved in the peripheral metabolism of triglyceride (TG)-rich lipoproteins. The VLDLr is highly expressed on the capillary endothelium (3) of skeletal muscle, heart, and adipose tissue and only in trace amounts in the liver, whereas the LDLr is abundantly expressed in the liver (1, 2). Initial in vitro studies have demonstrated that the VLDLr is a receptor for several apolipoprotein E (apoE)-containing lipoprotein ligands, such as VLDL, intermediate density lipoproteins, and chylomicrons (2, 4). The binding of these lipoprotein particles to the VLDLr is stimulated by apoE and LPL (4, 5) and inhibited by the 39 kDa receptor-associated protein (6). Based on these studies, it has initially been suggested that the VLDLr could play a role in the peripheral binding and subsequent internalization of TG-rich lipoproteins (4, 5). More recently, Obunike et al. (7) showed in vitro that the VLDLr is involved in the transcytosis of active LPL across endothelial cells. Therefore, it is now believed that the VLDLr may possibly function mainly by facilitating the binding of TG-rich lipoproteins in the capillary bed in concert with LPL, leading to the subsequent delivery of TG-derived FFAs to the underlying tissues active in FFA metabolism (5, 8).

To establish whether the VLDLr plays a role in lipid metabolism in vivo, Frykman et al. (9) generated mice lack-

Abbreviations: apoE, apolipoprotein E; HL, hepatic lipase; LDLr, LDL receptor; LPL, lipoprotein lipase; TG, triglyceride; VLDLr, VLDL receptor.

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ing the VLDLr by gene targeting. Although the initial in vitro data suggested that the VLDLr may play a prominent role in TG metabolism (4, 5), these *vldlr*$^{-/-}$ mice exhibited no differences in plasma lipoproteins on a normal chow diet (9). However, stressing the FA metabolism by prolonged fasting (10), a high-fat diet (10), or crossbreeding on a hyperlipidemic *ob/ob* (10) or *ldlr*$^{-/-}$ (11) background, VLDLr deficiency led to a hypertriglyceridemic phenotype in mice. In addition, under conditions of dietary stress, we showed that despite the development of hypertriglyceridemia, *vldlr*$^{-/-}$ mice were protected from diet-induced obesity (10). Recently, Yagyu et al. (12) showed that the VLDLr can play a role in the delivery of VLDL-TG-derived FFAs to adipose tissue, because VLDLr deficiency resulted in defective VLDL catabolism in vivo associated with reduced activity of LPL, albeit under prolonged fasting conditions. Compared with the fasting state, LPL activity in adipose tissue is increased postprandially (13), and the flux of TG-derived FFA to adipose tissue is greatly enhanced during the postprandial state (14). Therefore, we hypothesize that the VLDLr plays a crucial role in postprandial lipoprotein metabolism.

Thus, the aim of the present study was to evaluate the role of the VLDLr in chylomicron metabolism. We show that VLDLr deficiency in mice severely impairs chylomicron catabolism. Furthermore, postprandial TG-derived FFA uptake in adipose tissue is severely impaired, indicating that the VLDLr is required for optimal LPL-mediated chylomicron processing in vivo. These findings can fully explain our earlier observation that *vldlr*$^{-/-}$ mice are protected against diet-induced and genetically induced obesity (10). From the present study, we conclude that the VLDLr plays a major role in postprandial lipoprotein metabolism by facilitating LPL-mediated TG hydrolysis.

MATERIALS AND METHODS

Animals

Vldlr$^{-/-}$ mice originated from the Jackson Laboratories and were bred in our animal facility in Leiden. Nontransgenic littermates (*vldlr*$^{+/+}$) were used as controls. All mice used in this study were housed under standard conditions with free access to water and food and a normal 12 h light/dark cycle (lights on at 7:00 AM and off at 7:00 PM). Principles of laboratory animal care were followed, and the animal ethics committee of our institute approved all animal experiments.

Plasma lipid analysis

To determine postprandial and fasting lipid levels, tail vein blood was collected from male *vldlr*$^{-/-}$ and *vldlr*$^{+/+}$ mice at 7:00 AM just after the end of the dark cycle (fed state) and at 11:00 AM (after a 4 h fasting period) into chilled paraoxon-coated capillary tubes to prevent ongoing lipolysis (15). These tubes were placed on ice and immediately centrifuged at 4°C. Plasma levels of total cholesterol, TG (corrected for free glycerol), and FFA were determined enzymatically using commercially available kits and standards (236691, Boehringer, Mannheim, Germany; 337-B GPO-Trinder kit, Sigma, St. Louis, MO; Nefa-C kit, Wako Chemicals GmbH, Neuss, Germany).

LPL and hepatic lipase enzyme activity

Female *vldlr*$^{-/-}$ and *vldlr*$^{+/+}$ mice were fasted for 4 h, and postheparin plasma was obtained 10 min after a tail vein injection of heparin (0.1 U/g body weight) (Leo Pharma BV, Weesp, The Netherlands). The LPL activity was determined in postheparin plasma as described (16).

Intragastric fat load

To investigate the handling of postprandial TG, *vldlr*$^{-/-}$ and *vldlr*$^{+/+}$ mice were given an intragastric 200 μl olive oil bolus after an overnight fast. Blood samples were drawn at the indicated time points after administration. TG concentrations were determined in plasma as described above and are presented as relative increase from time 0.

Preparation of emulsion particles

Protein-free chylomicron-mimicking TG-rich emulsion particles were prepared essentially as described by Rensen et al. (17) from 100 mg of total lipid at a weight ratio of triolein (Sigma)-egg yolk phosphatidylcholine (Lipoid, Ludwigshafen, Germany)-lysophosphatidylcholine (Sigma)-cholesteryl oleate (Sigma)-cholesterol (Sigma) of 70:22.7:2.3:3.0:2.0 in the presence of 200 μCi of glycerol tri[9,10(n)-^{3}H]oleate ([^{3}H]TG) (Amersham, Little Chalfont, UK). Lipids were hydrated in 10 ml of 2.4 M NaCl, 10 mM HEPES, and 1 mM EDTA, pH 7.4, and sonicated for 30 min at 10 μm output using a Soniprep 150 (MSE Scientific Instruments) equipped with a water bath for temperature (54°C) maintenance. Subsequently, the emulsion was separated into fractions with different average sizes by density gradient ultracentrifugation (17).

In vivo lipolysis: bolus experiment

Fed female *vldlr* $^{-/-}$ and *vldlr* $^{+/+}$ mice were anesthetized (0.5 mg/kg Domitor, Pfizer Animal Health, Capelle a/d IJssel, The Netherlands; 0.05 mg/kg Fentanyl Bipharma, Pharma Hameln, Hameln, Germany; and 5 mg/kg midazolam, Roche, Mijdrecht, The Netherlands), and the abdomens were opened. [^{3}H]TG-labeled emulsion particles were injected via the vena cava inferior at t = 0 as a large bolus (1.0 mg of TG), resulting in plasma TG levels well above the endogenous TG concentration. At the time points indicated after injection, small blood samples (30 μl) were taken from the vena cava inferior and serum ^{3}H-radioactivity was measured. At 15 min, the mice were sacrificed, and their organs excised and weighed. Radioactivity was counted after the tissues were dissolved in Solvable (Packard Bioscience, Groningen, The Netherlands), and corrections were made for the serum radioactivity in the tissues as described by Rensen et al. (17).

In vivo lipolysis: infusion experiment

After 2 weeks on a high-fat diet (46.2% of the calories as fat; Hope Farms, Woerden, The Netherlands), fed male *vldlr*$^{-/-}$ and *vldlr*$^{+/+}$ mice were anesthetized and an infusion needle, connected to a Harvard microdialysis low-flow 11 minipump (Holliston, MA), was inserted into the tail vein. [^{3}H]TG-labeled emulsion particles (1.0 mg TG/ml) and a trace amount of [^{14}C]palmitic acid (Amersham) complexed to BSA (2%) in the presence of citrate (3 μg/ml) were infused at a rate of 0.2 ml/h for 2 h to achieve steady-state TG levels. After 1.5 and 2 h, a 150 μl blood sample was taken by tail bleeding. Subsequently, the mice were killed and their organs quickly removed and frozen in liquid nitrogen. Plasma levels of TG and FFA were determined as described above. Lipids were extracted from plasma according to Bligh and Dyer (18). The lipid fraction was dried under nitrogen, dissolved into chloroform-methanol (5:1, v/v), and subjected to TLC (LK5D gel 150; Whatman) using hexane-diethylether-acetic acid (83:16:1, v/v/v) as mobile phase. Lipid spots

TABLE 1. Effect of VLDLr deficiency on fed and 4 h fasting plasma lipid levels

	TGs		Free Fatty Acids		Total Cholesterol	
Mouse	Fed	Fasted	Fed	Fasted	Fed	Fasted
			mM			
$vldlr^{+/+}$	0.47 ± 0.18[a]	0.27 ± 0.06	0.64 ± 0.25[b]	0.64 ± 0.15	2.29 ± 0.35	2.37 ± 0.37
$vldlr^{-/-}$	1.20 ± 0.37[a]	0.44 ± 0.20	1.02 ± 0.15[b]	0.83 ± 0.29	2.08 ± 0.24	2.10 ± 0.23

TG, triglyceride; VLDLr, VLDL receptor. $Vldlr^{+/+}$ and $vldlr^{-/-}$ mice were fed a normal chow diet. Plasma levels of TGs, free fatty acids, and total cholesterol were measured in the fed state and after 4 h of fasting as described. Values represent means ± SD of eight $vldlr^{+/+}$ and eight $vldlr^{-/-}$ mice.

[a] $P < 0.001$.

[b] $P < 0.05$.

were visualized by I_2 vapor and scraped off, lipids were dissolved in hexane, and radioactivity was measured. Tissues were dissolved in 3 M KOH in 50% (v/v) ethanol at 70°C for 1 h. Retention of radioactivity in the tissues was measured per milligram of protein and corrected for the corresponding plasma-specific activities of [^{3}H]TG and [^{14}C]FFA.

Statistical analysis

The Mann-Whitney nonparametric test for two independent samples was used to define differences between $vldlr^{-/-}$ and $vldlr^{+/+}$ mice. The criterion for significance was set at $P < 0.05$. All data are presented as means ± SD.

RESULTS

VLDLr deficiency increases fed TG levels

To investigate the effect of VLDLr deficiency on postprandial lipid metabolism, plasma lipid levels were measured in fed and 4 h fasting male $vldlr^{-/-}$ and $vldlr^{+/+}$ mice (**Table 1**). Whereas no significant differences were found between these mice with respect to TG, FFA, and cholesterol levels after a 4 h fast, fed $vldlr^{-/-}$ mice did show significantly increased levels of TG (2.5-fold) and FFA (1.6-fold), but not cholesterol, compared with $vldlr^{+/+}$ mice. In fact, postprandial chylomicron-TG levels (i.e., the difference between fed and fasting TG levels) were even 4-fold increased upon VLDLr deficiency. The plasma lipid levels in female mice did not differ significantly from those in male mice, and fed TG levels were also consistently higher in female $vldlr^{-/-}$ mice (data not shown). These data suggest that both male and female $vldlr^{-/-}$ mice have reduced postprandial TG clearance under normal feeding conditions.

VLDLr deficiency decreases LPL activity

Because $vldlr^{-/-}$ mice showed no differences compared with $vldlr^{+/+}$ mice with respect to intestinal lipid absorption and hepatic VLDL production (10), the postprandial hypertriglyceridemia can be explained by the impaired lipolytic processing of chylomicrons. To confirm a decreased plasma lipolytic activity in $vldlr^{-/-}$ mice as shown by Yagyu et al. (12), hepatic lipase (HL) and LPL activities were measured in postheparin plasma. As shown in **Fig. 1**, the activity of LPL was significantly decreased (20%; $P < 0.05$) in $vldlr^{-/-}$ compared with wild-type mice, whereas no differences were observed with respect to HL activity.

VLDLr deficiency increases postprandial TG response

To investigate further the role of the VLDLr in postprandial lipoprotein metabolism, we administered an intragastric olive oil load by gavage to male $vldlr^{-/-}$ and $vldlr^{+/+}$ mice (**Fig. 2**). Strikingly, whereas wild-type mice showed only a mild increase in TG levels at 2 h after administration (from 0.3 ± 0.1 to 1.0 ± 0.4 mM), which reached baseline levels again by 8 h, $vldlr^{-/-}$ mice showed a severely increased and prolonged hypertriglyceridemia. The increase in TG was already 7-fold compared with the level in wild-type mice at 2 h and reached a maximum only at 8 h after the fat load. Therefore, the postprandial TG response in $vldlr^{-/-}$ compared with $vldlr^{+/+}$ mice was 9-fold increased (AUC: 226 ± 188 mM/h vs. 25 ± 11 mM/h), demonstrating that VLDLr deficiency leads to severe impairment of postprandial TG clearance. Again, similar findings were observed for female mice.

VLDLr deficiency decreases uptake of emulsion TG-derived FFA by adipose tissue

Subsequently, we investigated whether VLDLr deficiency affects the delivery of postprandial lipoprotein-derived FFA to tissues with the highest LPL expression in the fed state [i.e., adipose tissue and heart (19)]. To exclude interindividual variations with respect to intestinal lipid absorption, [^{3}H]TG-labeled emulsion particles that closely mimic chylomicrons were injected intravenously as a bo-

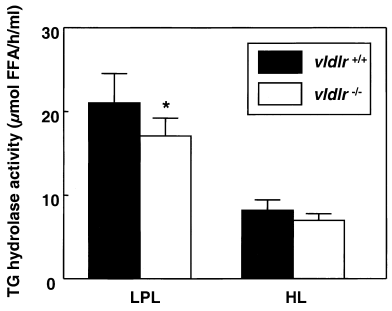

Fig. 1. Effect of VLDL receptor (VLDLr) deficiency on plasma lipoprotein lipase (LPL) and hepatic lipase (HL) activity. Postheparin plasma was obtained from $vldlr^{+/+}$ (closed bars) and $vldlr^{-/-}$ (open bars) mice, and triglyceride (TG) hydrolase activity was measured in the absence (i.e., LPL and HL) or presence (i.e., HL) of 1 M NaCl. Values represent means ± SD of 10 $vldlr^{+/+}$ and 8 $vldlr^{-/-}$ mice. * $P < 0.05$.

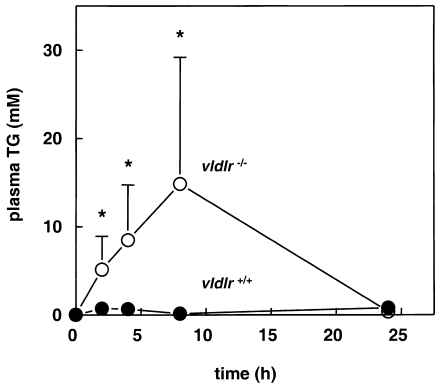

Fig. 2. Effect of VLDLr deficiency on postprandial TG response. Overnight-fasted male *vldlr*$^{+/+}$ (closed circles) and *vldlr*$^{-/-}$ (open circles) mice were given an intragastric 200 μl olive oil bolus. Blood samples were drawn at 0, 2, 4, 8, and 24 h after the bolus. TG concentrations were determined in plasma and corrected for TG values at time 0. Values represent means ± SD of seven *vldlr*$^{+/+}$ and eight *vldlr*$^{-/-}$ mice. * $P < 0.05$.

lus into mice of both genotypes (**Fig. 3**). Whereas an efficient monoexponential TG clearance was observed in wild-type mice with a serum half-life of 5.5 ± 0.9 min, *vldlr*$^{-/-}$ mice showed a delayed plasma TG clearance, as indicated by the 2.2-fold increased serum half-life (Fig. 3A, **Table 2**), confirming a disturbance in postprandial

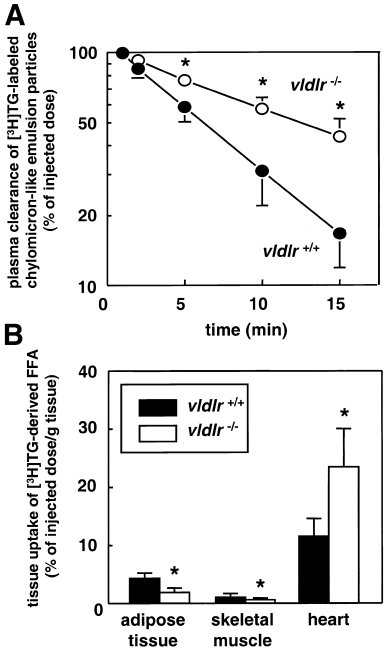

Fig. 3. Effect of VLDLr deficiency on the serum decay and tissue distribution of [^{3}H]TG-labeled emulsion particles. [^{3}H]TG-labeled chylomicron-like emulsion particles were injected intravenously into fed anesthetized *vldlr*$^{+/+}$ (closed symbols) and *vldlr*$^{-/-}$ (open symbols) mice. At the time points indicated, the serum decay was determined. A: The serum values obtained at 1 min after infection were set at 100%. B: At 15 min after injection, the uptake of [^{3}H]TG-derived radioactivity by adipose tissue, skeletal muscle, and heart was determined. Values represent means ± SD of four mice per group. * $P < 0.05$.

TABLE 2. Effect of VLDLr deficiency on the plasma half-life of [^{3}H]TG and [^{14}C]FFA after bolus injection and continuous infusion

	Bolus Injection	Steady-State Infusion	
Mouse	$t_{1/2}$ [^{3}H]TG	$t_{1/2}$ [^{3}H]TG	$t_{1/2}$ [^{14}C]FFA
		min	
vldlr$^{+/+}$	5.5 ± 0.9[a]	6.6 ± 1.2[a]	2.0 ± 0.4
vldlr$^{-/-}$	12.0 ± 2.6[a]	9.2 ± 2.3[a]	2.5 ± 0.2

After bolus injection of [^{3}H]TG-labeled chylomicron-like emulsion particles (described in the legend to Fig. 3) or during continuous infusion of these particles in the presence of [^{14}C]FFA (described in the legend to Fig. 4), blood samples were taken and plasma half-lives ($t_{1/2}$) were calculated from monoexponential decay curves (bolus) or from steady-state kinetics (infusion) according to Teusink et al. (14). Values represent means ± SD of four *vldlr*$^{-/-}$ and four *vldlr*$^{+/+}$ mice (bolus) or four *vldlr*$^{-/-}$ and six *vldlr*$^{+/+}$ mice (infusion).

[a] $P < 0.05$.

particle clearance. The delayed TG clearance in *vldlr*$^{-/-}$ mice was accompanied by a 60% decreased uptake of ^{3}H activity by adipose tissue and a 55% decreased uptake by skeletal muscle per mass unit (Fig. 3B). As depicted in Fig. 3B, the uptake of [^{3}H]TG-derived radiolabel by the heart per mass unit was higher in *vldlr*$^{-/-}$ mice. However, it was noted that the total heart weight of *vldlr*$^{-/-}$ mice was 25% reduced compared with that of wild-type mice (0.74 ± 0.06 g vs. 0.98 ± 0.09 g; $P < 0.05$). Therefore, the total uptake of ^{3}H radioactivity by the heart was not different ($P = 0.149$) when corrected for total heart weight.

VLDLr deficiency does not affect the uptake of albumin-bound FFAs by adipose tissue

To evaluate whether the VLDLr may function by transporting FFAs across the endothelial layer per se rather than by facilitating LPL-mediated lipolysis, [^{3}H]TG-labeled chylomicron-like emulsion particles were continuously infused together with albumin-bound [^{14}C]palmitate on a high-fat diet. On this diet, similar increasing effects of VLDLr deficiency on fed plasma TG levels were observed compared with the chow diet (1.10 ± 0.27 mM vs. 0.68 ± 0.15 mM for *vldlr*$^{-/-}$ mice and wild-type littermates, respectively; $P < 0.05$). Plasma half-lives of both [^{3}H]TG and [^{14}C]FFA were calculated from steady-state kinetics (14). Whereas the plasma clearance of [^{3}H]TG under these conditions was again delayed in *vldlr*$^{-/-}$ compared with *vldlr*$^{+/+}$ mice, the plasma half-life of [^{14}C]FFA was not affected significantly (Table 2). Accordingly, the uptake of [^{3}H]TG-derived activity by adipose tissue per mass unit was 60% decreased in *vldlr*$^{-/-}$ mice (**Fig. 4A**). The uptake of [^{3}H]TG-derived activity by the heart per mass unit was increased in *vldlr*$^{-/-}$ mice (Fig. 4B), but total uptake by the heart was unaffected. The uptake by skeletal tissue was also not affected (data not shown). No differences were found with respect to [^{14}C]FFA uptake by adipose tissue, skeletal muscle, or heart (Fig. 4B). These data demonstrate that the VLDLr does not seem to be involved in the transport and storage of albumin-bound FFAs to the underlying adipose tissue per se but rather enhances LPL-mediated TG hydrolysis.

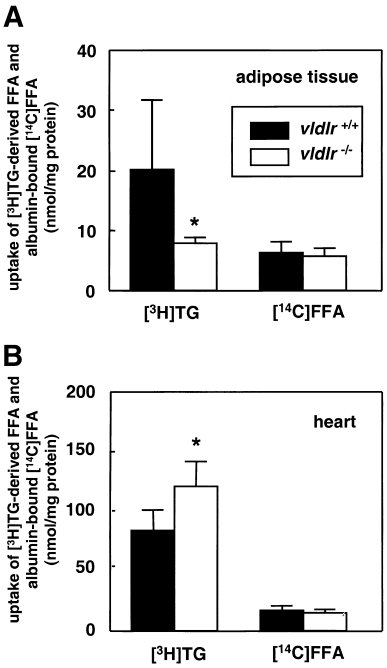

Fig. 4. Effect of VLDLr deficiency on the uptake of TG-derived and albumin-derived FFA by adipose tissue and heart. Fed *vldlr*$^{+/+}$ (closed bars) and *vldlr*$^{-/-}$ (open bars) mice were infused for 2 h with [^{3}H]TG-labeled chylomicron-like emulsion particles and albumin-bound [^{14}C]FFA. After 2 h, the mice were killed and the retention of [^{3}H]TG and [^{14}C]FFA was determined in adipose tissue (A) and heart (B) and corrected for the specific activities of both radiolabels in plasma. Values represent means ± SD of seven *vldlr*$^{+/+}$ and four *vldlr*$^{-/-}$ mice. * $P < 0.05$.

DISCUSSION

Although earlier studies have reported that the VLDLr is able to bind and internalize TG-rich lipoproteins as mediated via apoE and/or LPL in vitro (4, 5), *vldlr*$^{-/-}$ mice were initially reported to have no phenotype with respect to lipid and/or lipoprotein levels (9). Placing FFA metabolism under stress by feeding *vldlr*$^{-/-}$ mice a high-fat diet or cross-breeding these mice onto a hyperlipidemic background (10, 11) or by prolonged fasting (10, 12) did lead to a hypertriglyceridemic phenotype, which was not caused by increased hepatic VLDL production (10). A recent study has shown that a decreased VLDL catabolism in *vldlr*$^{-/-}$ compared with *vldlr*$^{+/+}$ mice was related to decreased LPL activity (12). These data were derived from mice that underwent a prolonged 16 h fasting period (12). However, because LPL is more crucial in postprandial lipoprotein metabolism, we hypothesized that the role of the VLDLr may be most prominent under postprandial conditions, during which the flux of TG-derived FFAs to adipose tissue is greatly enhanced (14). Therefore, we performed as yet unaddressed studies to evaluate a function of the VLDLr in chylomicron catabolism using *vldlr*$^{-/-}$ mice and wild-type littermates. Indeed, we found that the VLDLr has a prominent physiological function in the lipolysis of postprandial lipoproteins, with a subsequent decreased delivery of particle TG-derived FAs to adipose tissue, and not in the uptake of FAs per se.

After a 4 h fast, which allows the complete clearance of plasma chylomicrons, plasma lipid levels in *vldlr*$^{-/-}$ and *vldlr*$^{+/+}$ mice were not different, which is in agreement with reports by others (9). However, analysis of the plasma of mice just after the end of the dark cycle, which most closely resembles a normal fed condition, showed a 2.5-fold increase in TG levels in *vldlr*$^{-/-}$ mice along with a 1.6-fold increase in FFA levels. Taking account of the fact that postprandial TG levels include both VLDL-TG and chylomicron-TG, it could be calculated that postprandial chylomicron levels differed to an even larger extent (i.e., 4-fold). By forcing rapid and extensive intestinal chylomicron production by the intragastric administration of a large olive oil load, *vldlr*$^{-/-}$ mice showed a 9-fold increased TG response. Because we had previously excluded the possibility of decreased intestinal lipid absorption and hepatic VLDL production in *vldlr*$^{-/-}$ mice (10), these data confirm the hypothesis that VLDLr-deficient mice are unable to efficiently clear postprandial TG from the plasma. LPL is a major player in peripheral TG hydrolysis, and the LPL activity appeared to be 20% decreased ($P < 0.05$) in female *vldlr*$^{-/-}$ mice, which is in agreement with recent observations in male *vldlr*$^{-/-}$ mice by others (12). The decreased postheparin LPL activity is not solely attributable to decreased adipose tissue stores, because Yagyu et al. (12) showed that VLDLr deficiency also reduces the specific LPL activity in adipose tissue per mass unit.

The involvement of a reduced peripheral lipolysis of postprandial lipoproteins as part of the mechanism underlying the disturbed postprandial TG clearance was established by performing kinetic studies with [^{3}H]TG-labeled chylomicron-mimicking emulsion particles. These emulsions have previously been shown to be true chylomicron mimics with respect to rapid apolipoprotein acquisition from plasma, LPL-mediated remnant formation, and efficient apoE-mediated hepatic uptake of core remnants by the LDLr and LDLr-related protein (17). Both upon intravenous bolus injection and continuous infusion, the plasma half-life of TG was prolonged in *vldlr*$^{-/-}$ compared with *vldlr*$^{+/+}$ mice in the fed state. Accordingly, the distribution of [^{3}H]TG-derived FFAs into adipose tissue was 60% decreased, irrespective of the mode of administration, bolus or continuous infusion. Taking into account that the total fat pad mass is reduced in *vldlr*$^{-/-}$ mice compared with wild-type littermates (9, 10), the difference in total uptake of radiolabel by adipose tissue is even more pronounced. Our findings complement the data of Yagyu et al. (12), who showed decreased VLDL-TG catabolism in *vldlr*$^{-/-}$ mice that had fasted for 16 h. However, they observed no change in the uptake of VLDL-TG by adipose tissue and a reduced uptake by heart and muscle in VLDLr deficiency, whereas we find a significantly decreased uptake of chylomicron-like emulsion TG by adipose tissue with no consistent reduction in uptake of TG by heart or muscle. Although substrate variation (i.e., emulsion particles vs. native VLDL) may have contributed to the observed discrepancy, the differences between the two studies can be fully explained by the natural regulation of tissue LPL levels by the feeding state, as prolonged

fasting induces a gradually decreased expression of LPL by adipose tissue whereas that of heart and muscle is gradually increased (13, 19–23). Furthermore, under fed conditions, the flux of TG-derived FFAs to adipose tissue is greatly enhanced compared with the fasting state (14). The finding that the VLDLr is crucially involved in the uptake of chylomicron-TG-derived FFA by adipose tissue appears to fully explain the prevention of high-fat-diet-induced obesity in *vldlr*$^{-/-}$ mice as we described previously (10). Clearance of albumin-bound FFAs from the circulation did not seem to be affected by VLDLr deficiency, because the plasma half-life of albumin-bound FFAs was not significantly different between fed *vldlr*$^{-/-}$ mice and their littermate controls. However, a tendency toward a 25% increased half-life of FFAs in fed *vldlr*$^{-/-}$ mice was observed (Table 2), which is in agreement with the reduced FFA turnover we previously reported for fasted *vldlr*$^{-/-}$ mice (10). Taken together with the observation that plasma FFA levels were also significantly increased in fed mice (Table 1), we cannot exclude that the VLDLr may have an additional function in FFA turnover per se.

The effect of reduced peripheral lipolysis in VLDLr deficiency is thus likely to be one of the reasons underlying the postprandial hypertriglyceridemia, confirming previous reports on the crucial involvement of LPL in TG metabolism. First, LPL knockout mice do not survive, most probably because of extreme hypertriglyceridemia (24). Conversely, mice that overexpress LPL show a decreased postprandial TG response (25). Similarly, transgenic mice overexpressing the main endogenous LPL inhibitor apoC-III develop hypertriglyceridemia (26), whereas *apoc3*$^{-/-}$ mice exhibit reduced plasma TG levels and are protected from postprandial hypertriglyceridemia (26, 27).

Mice heterozygous for a mutation in the LPL gene (*lpl*$^{+/-}$) were shown to have a 43% decreased postheparin LPL activity (28). In these mice, plasma TG levels were increased during ad libitum feeding and after fasting for 12 h (28). In addition, the response to orally administered vitamin A-containing corn oil was impaired in *lpl*$^{+/-}$ mice compared with wild-type littermates, as shown by a 3-fold increased vitamin A response (24). Although comparison of *vldlr*$^{-/-}$ mice with *lpl*$^{+/-}$ mice reveals similarities with respect to its impeding effects on TG metabolism, the effects of VLDLr deficiency on chylomicron catabolism are much more severe and cannot be explained solely by a modest 20% reduced LPL expression. Despite less affected LPL levels, the increasing effects of VLDLr deficiency on the postprandial TG response is much more dramatic (i.e., 9-fold) than the effects of LPL heterozygosity (i.e., 5-fold) (our unpublished observations). These data indicate that besides affecting LPL expression by its chaperone function (7) or LPL binding properties (4), the VLDLr is likely to participate in chylomicron catabolism by bridging of particles to the endothelial surface ("docking function"), thereby facilitating the LPL-particle interaction. In addition, the involvement of the VLDLr in lipoprotein internalization, as has been shown by VLDLr-expressing cells in vitro (4, 5) and by hepatocytes after recombinant adenovirus-mediated transfer of the VLDLr in vivo (29), cannot be conclusively eliminated as an additional factor contributing to TG clearance.

To summarize, we have shown that VLDLr deficiency leads to a disturbed clearance of postprandial lipoproteins into peripheral tissues such as adipose tissue, which can explain our previous findings that *vldlr*$^{-/-}$ mice are protected from diet-induced obesity. We conclude that the VLDLr plays a major physiological role in postprandial lipoprotein metabolism by enhancing LPL-mediated TG hydrolysis, with the subsequent delivery of FFAs to adipose tissue.

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