Protection From Obesity in Mice Lacking the VLDL Receptor

Jeltje R. Goudriaan, Paul J. Tacken, Vivian E.H. Dahlmans, Marion J.J. Gijbels, Ko Willems van Dijk, Louis M. Havekes, Miek C. Jong

Abstract—It has previously been reported that mice lacking the VLDL receptor (VLDLR−/−) exhibit normal plasma lipid levels and a modest decrease in adipose tissue mass. In the present study, the effect of VLDLR deficiency on profound weight gain was studied in mice. Obesity was induced either by feeding of a high-fat, high-calorie (HFC) diet or by crossbreeding mice onto the genetically obese ob/ob background. After 17 weeks of HFC feeding, VLDLR−/− mice remained lean, whereas their wild-type littermates (VLDLR+/+) became obese. Similarly, the weight gain of ob/ob mice was less profound in the absence of the VLDLR. Moreover, VLDLR deficiency led to increased plasma triglycerides after HFC feeding. The protection from obesity in VLDLR−/− mice involved decreased peripheral uptake of fatty acids, because VLDLR−/− mice exhibited a significant reduction in whole-body free fatty acid uptake, with no clear differences in food intake and fat absorption. These observations were supported by a strong decrease in average adipocyte size in VLDLR−/− mice of both obesity models, implying reduced adipocyte triglyceride storage in the absence of the VLDLR. These results suggest that the VLDLR plays a role in the delivery of VLDL-derived fatty acids into adipose tissue. (Arterioscler Thromb Vasc Biol. 2001;21:1488-1493.)

Key Words: VLDL receptor ■ obesity ■ fatty acid transport ■ triglyceride metabolism ■ transgenic mice

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 (1) 8 ligand-binding repeats instead of 7 and (2) its expression pattern among tissues. The VLDLR is highly expressed in 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

A role for the VLDLR in lipoprotein metabolism has been suggested by in vitro experiments showing that the VLDLR binds and internalizes particles that are rich in apolipoprotein (apo) E, such as VLDL, IDL, and chylomicrons.1,3 The binding of these lipoprotein particles to the VLDLR was stimulated by lipoprotein lipase (LPL)3,4 and inhibited by a 39-kDa protein named the receptor-associated protein (RAP).5 In addition to lipoproteins, the VLDLR has been shown to bind several other ligands, including urokinase complexed to its inhibitor, plasminogen activator inhibitor type 1,6 and thrombospordin-1.7

Based on its binding characteristics, endothelial localization,9 and tissue expression pattern, it is hypothesized that the VLDLR facilitates the binding of triglyceride (TG)-rich particles in the capillary bed and subsequent delivery of free fatty acids (FFAs) to tissues active in fatty acid metabolism.9,10 In line with this hypothesis, it was shown in mice that VLDLR mRNA levels are upregulated in heart and downregulated in adipose tissue after prolonged fasting.11 Reciprocally, VLDLR mRNA levels were downregulated in heart and upregulated in adipose tissue of LDLR-deficient mice fed an atherogenic diet.12

To directly investigate a role for the VLDLR in lipid metabolism, mice were generated lacking the VLDLR by gene targeting.13 VLDLR−/− mice exhibited no differences in plasma lipoproteins, and the sole abnormality detected was a modest decrease in body weight (15% to 20%).13 Recently, VLDLR−/− mice were crossbred with apoE receptor 2 (apoER2)−deficient mice.14 These double-knockout mice exhibited a striking disorganization of neurons in the cerebellum, indicating that the presence of the VLDLR in the brain, in combination with the apoER2, is required in a signaling pathway that regulates neuronal migration.14

Thus, in addition to its function in the brain, a clear role for the VLDLR in lipid metabolism remains to be established. Because it has been hypothesized that the VLDLR plays a role in the delivery of FFAs to peripheral tissues, we investigated whether the absence of the VLDLR, under conditions of severe dietary stress, might introduce a rate-limiting step in the delivery of FFAs to peripheral tissues. Therefore, VLDLR−/− mice and wild-type littermates (VLDLR+/+) were either fed a high fat, high-calorie (HFC) diet to enhance the intake of fatty acids or crossbred onto the genetically determined obese ob/ob background. We found that VLDLR deficiency led to less obesity and insulin resistance. The protection from obesity with the VLDLR
deficiency is most likely due to impaired uptake of fatty acids by adipose tissue, because whole-body FFA uptake was strongly decreased in VLDLR−/− mice, with no significant differences in food intake and fat absorption. These results suggest that the VLDLR plays a role in the delivery of VLDL-derived fatty acids into adipose tissue.

Methods

Animals

VLDLR−/− mice were obtained from Jackson Laboratories, Bar Harbor, Me. Nontransgenic littermates (VLDLR+/+) were used as controls. Heterozygous ob/o (Jackson Laboratories, Bar Harbor, Me) were crossbred with VLDLR+/− mice to obtain VLDLR−/− mice on a homozygous ob/ob background (VLDLR−/− ob/ob). ob/ob littermates (VLDLR+/+ ob/ob) were used as controls. All mice in the study were males on the C57BL/6J background and were housed individually under standard conditions with free access to water and food. Food was withdrawn at 9 AM, and the experiments were performed either at 1 PM (4-hour fasting) or at 9 AM the next day (24-hour fasting).

Diet and Dietary Treatment

After being weaned, the mice were fed a standard rat/mouse chow diet. After they reached 6 months of age, 10 VLDLR−/− and 12 VLDLR+/+ mice were given an HFC diet (46.2% of calories from fat) for a period of 17 weeks. The HFC diet contained 24% corn oil, 24% casein, 20% cerelose, 18% cornstarch, and 6% cellulose by weight (Hope Farms). Seven VLDLR−/− ob/ob and 8 VLDLR+/+ ob/ob mice were fed the standard rat/mouse chow diet (17.2% of calories from fat) and were followed up over time. Food intake was monitored individually.

Tissue and Biochemical Analyses

Histological Analysis of Adipose Tissue

After formalin fixation, adipose tissues were cut into sections (3 μm) and stained with hematoxylin-phloxine-saffron (see details online at http://atvb.ahajournals.org).

Plasma Lipid and Lipoprotein Analyses

For details, see Zambon et al15 and online at http://atvb.ahajournals.org.

Plasma Glucose and Insulin Assays

For details, see online at http://atvb.ahajournals.org.

Glucose Tolerance Test

Animals were fasted for 4 hours and injected intraperitoneally with glucose (2 g/kg). Blood samples were collected at the indicated time points (for details, see online at http://atvb.ahajournals.org).

Fat Absorption

Fat absorption was measured by comparing the amount and kind of fatty acids present in the HFC diet with those in feces. For details, see Lepage and Roy16 and online at http://atvb.ahajournals.org.

Hepatic VLDL Production

Mice were fasted for 24 hours and injected intravenously with Triton WR1339. For details, see Aalto-Setala et al17 and online at http://atvb.ahajournals.org.

[3H]FFA Turnover Experiments

VLDLR−/− and VLDLR+/+ mice were fasted for 24 hours and anesthetized. An indwelling catheter was then inserted in the right jugular vein, and 3H-labeled fatty acids were infused. Blood samples (100 μL) were collected from the tip of the tail at 20, 40, and 60 minutes after the start of infusion. For details, see Bonadonna et al,18 Shimabukuro et al,19 and online at http://atvb.ahajournals.org.

Statistical Analysis

The Mann-Whitney nonparametric test for 2 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 mean±SD.

Results

To investigate the effects of VLDLR deficiency under conditions of severe dietary stress, VLDLR−/− and VLDLR+/+ mice were fed an HFC diet for a period of 17 weeks. During this period, male VLDLR−/− mice gained little body weight with HFC feeding, whereas the body weight of male VLDLR+/+ mice continued to increase (Figures 1 and 2A). Similar findings were observed with females (results not shown). The difference in body weight between VLDLR−/− and VLDLR+/+ mice was reflected by a marked reduction in both subcutaneous (≈80%) and visceral (≈20%) adipose tissue.

Figure 1. Growth curves of VLDLR−/− and VLDLR+/+ mice on an HFC diet. At 6 months of age, mice were fed an HFC diet for a period of 17 weeks and were weighed weekly. Values represent the mean±SD of 10 VLDLR−/− and 12 VLDLR+/+ mice. *P<0.05 at all time points, indicating the difference between VLDLR−/− and VLDLR+/+ mice by nonparametric Mann-Whitney tests.
Comparison of Biochemical Parameters in VLDLR−/− and VLDLR+/+ Mice on an HFC Diet

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>VLDLR−/−</th>
<th>VLDLR+/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>9.8±1.7</td>
<td>6.6±3.7*</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
<td>4.6±2.5</td>
<td>1.8±0.5*</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>3.2±0.5</td>
<td>3.3±0.3</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.5±0.2</td>
<td>1.2±0.2*</td>
</tr>
<tr>
<td>FFA, mmol/L</td>
<td>0.8±0.1</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Food intake, g/24 h</td>
<td>3.3±0.9</td>
<td>2.7±0.9</td>
</tr>
<tr>
<td>Fat absorption, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16</td>
<td>98.7±0.7</td>
<td>99.2±0.3</td>
</tr>
<tr>
<td>C18:1</td>
<td>99.7±0.2</td>
<td>99.8±0.1</td>
</tr>
<tr>
<td>C18:2</td>
<td>99.8±0.2</td>
<td>99.9±0.1</td>
</tr>
<tr>
<td>Hepatic VLDL TG</td>
<td>116.8±24.1</td>
<td>129.5±20.3</td>
</tr>
<tr>
<td>production, μmol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG · h−1 · kg−1</td>
<td></td>
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</tr>
</tbody>
</table>

VLDLR−/− and VLDLR+/+ mice were fed an HFC diet for 17 weeks. Plasma glucose, insulin, cholesterol (TC), TG, and FFA levels were measured after 24 hours of fasting as described in Methods. Food intake was monitored individually and represents the average at 17 weeks of HFC feeding. Fat absorption was calculated as the amount of fatty acids (%) from the diet that did not appear in the feces (gas chromatographic analysis). Hepatic VLDL TG production was measured as described in Methods after 7 weeks of the HFC diet and 24 hours of fasting. Values represent the mean ± SD of 10 VLDLR−/− and 12 VLDLR+/+ mice (plasma parameters and food intake), 5 VLDLR−/− and 7 VLDLR+/+ mice (fat absorption), and 6 VLDLR−/− and 7 VLDLR+/+ mice (hepatic VLDL TG production).

*P<0.05 by nonparametric Mann-Whitney tests.

visceral (~70%) adipose tissue mass in VLDLR−/− mice (Figure 2B), indicating that VLDLR−/− mice are strongly protected from diet-induced obesity. Histological analysis of epididymal fat pads (Figure 2C) revealed that adipocytes from VLDLR−/− mice were significantly smaller than those from VLDLR+/+ mice (3679±453 vs 7889±572 μm2, P=0.009) under condition of HFC feeding. Similar differences were found for subcutaneous fat as well (not shown).

Diet-induced obesity is frequently associated with insulin resistance, so we next investigated whether depletion of the VLDLR would affect insulin sensitivity. Plasma glucose and insulin levels were significantly reduced in VLDLR−/− mice compared with those in VLDLR+/+ mice after 17 weeks of HFC diet feeding (the Table). These results indicated that VLDLR−/− mice remained insulin-sensitive, whereas VLDLR+/+ mice had indeed become insulin-resistant after HFC diet feeding. This result was sustained by an intraperitoneal glucose tolerance test (Figure 3A). VLDLR−/− mice on an HFC diet were much more efficient in their ability to clear an intraperitoneally injected bolus of glucose than were VLDLR+/+ mice, demonstrating that the absence of the VLDLR significantly hampered the development of insulin resistance on introducing diet-induced obesity.

We next wanted to investigate whether the protection from insulin resistance in VLDLR−/− mice was either directly due to the absence of the VLDLR or indirectly related to the less severe obesity in these mice. Therefore, we performed a glucose tolerance test in VLDLR−/− and VLDLR+/+ mice that had been maintained on a regular chow diet and that were exactly matched for body weight. Figure 3B shows that there were no obvious differences in glucose tolerance between VLDLR−/− and VLDLR+/+ mice when matched for body weight, indicating that the improved insulin sensitivity in VLDLR−/− mice was directly related to reduced obesity.

To investigate whether the protection from diet-induced obesity and insulin resistance in VLDLR deficiency was associated with changes in plasma lipid metabolism, several lipid parameters were measured in plasma after 17 weeks of HFC feeding. Plasma levels in VLDLR−/− mice were significantly higher than those in VLDLR+/+ mice (the Table). The elevated levels of plasma TGs in VLDLR−/− mice were mainly due to an accumulation of TGs in the VLDL-size fractions (results not shown). No significant differences were found in plasma FFA and cholesterol levels (the Table).

To investigate the mechanisms underlying the protection from HFC diet–induced obesity in VLDLR−/− mice, food intake, intestinal fat absorption, hepatic VLDL production, (the Table), and whole-body FFA uptake (Figure 4) were determined. Average food intake was similar in VLDLR−/− mice compared with VLDLR+/+ mice. VLDLR−/− mice showed almost complete absorption of the main fatty acids in the HFC diet, comparable to that in VLDLR+/+ mice. Also, no difference in hepatic VLDL TG production was found.
between VLDLR−/− and VLDLR+/+ mice. Turnover studies in VLDLR−/− and VLDLR+/+ mice with continuous infusion of [3H]FFA to estimate whole-body FFA uptake showed a significant reduction in this parameter and in peripheral FFA oxidation in VLDLR−/− mice, indicative of a decreased flux of fatty acids into peripheral tissues (Figure 4A). No significant differences in FFA whole-body storage were found between VLDLR−/− and VLDLR+/+ mice (Figure 4A). Furthermore, the appearance of [3H]FFAs in plasma TGs was significantly increased in VLDLR−/− and VLDLR+/+ mice, by nonparametric Mann-Whitney tests.

We wondered whether the protection from development of obesity in VLDLR−/− mice maintained on the HFC diet also held true for VLDLR−/− mice on a genetic ob/ob background. The growth curve and gross appearance (Figure 5) of these mice clearly showed that VLDLR−/− ob/ob mice gained less weight compared with VLDLR+/+ ob/ob mice, as reflected by a reduction in both subcutaneous (∼60%) and visceral (∼55%) adipose tissue mass in VLDLR−/− ob/ob mice. Histological analysis of epididymal fat pads revealed that similar to the situation with HFC feeding, adipocytes from VLDLR−/− ob/ob mice were significantly smaller than those from VLDLR+/+ ob/ob mice (5682±177 vs 9860±1348 μm², *P=0.014). Analysis of subcutaneous fat also showed a smaller average adipocyte area for VLDLR−/− ob/ob mice compared with VLDLR+/+ ob/ob mice (results not shown).

Discussion

Earlier studies have reported that VLDLR−/− mice at the age of 5 months have slightly lower body weights on both a chow and a high-sucrose diet due to a 50% decrease in adipose tissue mass. However, it still remained to be determined whether this was due to either early developmental differences or altered adipocyte FFA metabolism. In the present study, VLDLR−/− mice at the age of 6 months also displayed a slightly reduced body weight compared with VLDLR+/+ controls when fed a chow diet. Strikingly, on switching to the HFC diet containing predominantly linoleic acid (corn oil), VLDLR−/− mice failed to gain any further weight. Furthermore, the average adipocyte size of VLDLR−/− mice was significantly reduced compared with that of their wild-type littermates, implying a reduction in cellular TG storage. The reduction in weight gain, adipose tissue mass, and adipocyte size in the absence of the VLDLR also holds true for more severe forms of obesity, as in the genetically obese ob/ob model. Altogether, these data indicate that in the absence of the VLDLR, mice are protected from the development of obesity.

In addition to protection from diet-induced obesity, VLDLR−/− mice are protected from diet-induced insulin resistance. This feature was reflected by lower plasma glucose and insulin levels compared with those in VLDLR+/+ mice. Moreover, VLDLR−/− mice effectively cleared a glucose bolus that was administrated intraperitoneally, whereas VLDLR+/+ mice stayed hyperglycemic. Most likely, protection from insulin resistance in VLDLR−/− mice is secondary to protection from obesity, because no significant differences were found in the glucose tolerance tests between chow-fed, nonobese, wild-type mice and VLDLR−/− mice that were matched for body weight (Figure 3B). In contrast to VLDLR−/− mice on a wild-type background, no obvious improvements in insulin sensitivity were observed in VLDLR−/− mice on the ob/ob background (data not shown). This finding could be explained by the fact that VLDLR−/− ob/ob mice are still overtly obese compared with age-matched VLDLR+/+ wild-type mice (compare Figure 1, t=0 with Figure 5, t=25), although their body weight is significantly lower than that of VLDLR+/+ ob/ob mice (Figure 5).
There are at least 3 possible explanations for the protection from obesity in the absence of the VLDLR. First, there is an increase in energy expenditure: in ob/ob mice it has been shown that obesity results partly from decreased energy metabolism reflected by a strong reduction in core body temperature. Body temperature in VLDLR+/− mice was similar to that in VLDLR+/+ mice, both on the HFC diet as well as on the ob/ob background (37.0 ± 0.2°C vs 37.1 ± 0.2°C and 36.4 ± 0.6°C vs 35.9 ± 0.4°C, respectively), suggesting that the protection from obesity in the absence of the VLDLR is not likely to involve increased energy expenditure. However, body temperature does not represent a direct and accurate measure of energy expenditure, because body temperature can be maintained by adaptations in exercise. Hence, no definitive conclusion can be made on energy expenditure.

Second, protection from obesity in the absence of the VLDLR could also be due to fat malabsorption or reduced food intake (the Table). As shown in the Table, current results clearly exclude these possibilities. Because the VLDLR is predominantly expressed in tissues active in fatty acid metabolism, such as the heart, muscle, and adipose tissue, a third explanation for the protection from obesity in the absence of the VLDLR might be that the VLDLR plays a role in the delivery of fatty acids to peripheral tissues. To study the possibility of an altered flux of fatty acids into peripheral tissues with VLDLR deficiency, we performed FFA turnover experiments with a continuous infusion of [3H]palmitate. These experiments indeed revealed a significant reduction in whole-body FFA uptake in VLDLR+/− mice, which was reflected by a significant decrease in peripheral FFA oxidation (Figure 4A). Although this method does not allow exact identification of the affected peripheral tissues, these data strongly suggest that absence of the VLDLR indeed impairs fatty acid transport from the circulation into adipose tissue and as such, contributes to the protection from obesity.

On a chow diet, no differences in plasma lipids were found between VLDLR+/− and VLDLR+/+ mice. By stressing these mice with an HFC diet, a clear phenotype with respect to plasma lipids was detected. Although no significant differences in plasma FFA levels were found between VLDLR+/− and VLDLR+/+ mice after 17 weeks of HFC feeding (the Table), VLDLR-deficient mice showed a significant increase in plasma TG levels. A similar pronounced elevation in plasma TG levels was also observed in VLDLR+/− mice on a genetically obese ob/ob background (results not shown). Furthermore, recent studies have reported that on an LDLR-deficient background in combination with high-fat feeding or prolonged fasting, VLDLR deficiency results in increased plasma TG levels as well. Thus, the effect of the VLDLR on VLDL TG metabolism is only revealed under conditions of severe stress, ie, fasting, a high-fat diet, or an LDLR-deficient or ob/ob background.

The mechanisms underlying the development of hypertriglyceridemia in VLDLR+/− mice may involve either a decreased clearance of lipoprotein particles by the liver, an enhanced VLDL TG production, and/or a decreased VLDL TG lipolysis in peripheral tissues. Studies with adenoviral vectors conducted by Jong et al and Kobayashi et al have shown that ectopic expression of the VLDLR in mouse liver results in enhanced internalization of lipoproteins. Thus, when expressed in the liver, the VLDLR seems to act as a clearance receptor for whole lipoprotein particles, similar to the LDLR and LDLR-related protein (LRP). A decreased clearance of lipoprotein particles by the liver in VLDLR+/− mice is, however, unlikely, because the VLDLR is normally not expressed in the liver. An argument in favor of an enhanced VLDL TG production as a cause for increased plasma TG levels is that decreased peripheral uptake of FFAs due to VLDLR deficiency as presented in Figure 4 may lead to an increased flux of FFAs to the liver, which subsequently may result in an increased VLDL TG production. However, no differences in hepatic VLDL TG production were found between VLDLR+/− and VLDLR+/+ mice (the Table). Recent studies by Tacken et al in LDLR+/− mice also showed that VLDLR deficiency does not affect VLDL TG production. Thus, next to increased clearance by the liver, an increased VLDL TG production is also not likely to be the cause of the hypertriglyceridemia observed in VLDLR-deficient mice.

In coordination with LPL, the VLDLR is proposed to act as a docking protein for efficient VLDL TG lipolysis. LPL is the main enzyme involved in the hydrolysis of TGs and has been shown to bind to the VLDLR. Furthermore, LPL stimulates the binding of lipoprotein particles to the VLDLR through a bridging function between extracellular heparan sulfate proteoglycans and lipoprotein particles. This suggests that at its natural site of expression, the VLDLR facilitates hydrolysis of TGs instead of clearing entire lipoprotein particles and as such, affects plasma TG levels. In a previous study, we analyzed the VLDL composition in VLDLR+/− mice on an LDLR+/− background. In that study, it appeared that in VLDLR+/− mice, the VLDL particles were more enriched in TGs, also indicating that in the absence of the VLDLR, lipolysis of VLDL TGs is hampered. Thus, the increased amount of [3H]FFAs in VLDL TGs of VLDLR+/− mice (Figure 4B) was most likely due to impaired hydrolysis of VLDL TG rather than enhanced VLDL TG production or decreased particle clearance.

The hypothesis that an impaired VLDL TG lipolysis and a subsequent decrease in FFA generation and peripheral uptake may underlie protection from obesity in VDLR+/− mice is supported by data from Weinstock et al. In that study, it was shown that decreased FFA generation in ob/ob mice, due to a deficiency of LPL in adipose tissue, impaired the growth rate and adipose tissue accretion, very similar to observations in VLDLR+/− ob/ob mice. Furthermore, a reduced uptake in the periphery of albumin-bound FFAs in VLDLR+/− mice (as presented in Figure 4) suggests that the VLDLR is also involved in albumin-bound FFA uptake. We hypothesize therefore that next to the above-mentioned docking function, the VLDLR may also act as a gate keeper for cellular uptake of FFAs derived from VLDL TG hydrolysis as well as from albumin-bound plasma FFAs. The mechanism underlying the exact role of the VLDLR in peripheral FFA uptake is currently under investigation.

RAP is known to efficiently inhibit the binding of lipoproteins to the LRP. Overexpression of RAP in LDLR-deficient mice causes hypertriglyceridemia that is much more severe than that found in mice that are deficient in both the LDLR and LRP. These results suggest the existence of an additional RAP-sensitive pathway in TG removal, next to the LDLR and LRP. Because previous studies have shown that RAP also inhibits lipoprotein binding to the VLDLR in vitro, the VLDLR might...
represent this additional pathway. We have observed that apoC1 also strongly inhibits lipoprotein binding to the VLDLR.22 Similar to RAP overexpression in LDLR-deficient mice, overexpression of apoC1 in LDLR-deficient mice causes a more severe hypertriglyceridemia compared with that in LDLR/LRP double-deficient mice.29 These facts, together with the observation that overexpression of apoC1 is also associated with diminished adipose tissue mass,30 strongly sustain our conclusion that the VLDLR plays a role in VLDL TG metabolism and the development of obesity.

In summary, VLDLR deficiency protects mice from diet-induced as well as genetically determined obesity, possibly through interference with the entry of fatty acids into adipose tissue. As such, these data represent the first demonstration that the VLDLR participates in the regulation of adipose tissue formation and suggests that selective inhibition of the VLDLR may offer a therapeutic target for treatment of obesity.

Acknowledgments

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References

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**Materials and methods**

*Animals.* VLDLR -/- mice were obtained from the Jackson Laboratories. Non-transgenic littermates (VLDLR +/+) were used as controls. Heterozygous ob/- mice (Jackson Laboratories) were crossbred with VLDLR +/- mice to obtain VLDLR -/- mice on a homozygous ob/ob background (VLDLR -/- ob/ob). Ob/ob littermates (VLDLR +/- ob/ob) were used as controls. All mice in the study were males on the C57BL/6J background and were housed individually under standard conditions with free access to water and food. Food was withdrawn at 9:00 a.m. and the experiments were either performed at 1:00 p.m. (4 hours fasting) or at 9:00 a.m. the next day (24 hours fasting).

*Diet and dietary treatment.* After weaning, mice were fed a standard-rat-mouse chow diet. After 6 months of age, 10 VLDLR -/- and 12 VLDLR +/- mice were given a high-fat, high-caloric (HFC) diet (46.2% of the calories as fat) for a period of 17 weeks. The HFC diet contained 24% corn oil, 24% casein, 20% cerelose, 18% cornstarch and 6% cellulose, by weight (Hope Farms, Woerden, the Netherlands). 7 VLDLR -/- ob/ob and 8 VLDLR +/- ob/ob mice were fed standard-rat-mouse chow diet (17.2% of the calories as fat) and were followed in time. Food intake was monitored individually.
**Plasma lipid and lipoprotein analysis.** In all experiments, blood was collected by tail bleeding in chilled paraoxonized capillary tubes (15). These tubes were placed in ice and immediately centrifuged at 4 °C. Levels of total plasma cholesterol, plasma TG (without free glycerol) and free fatty acids (FFAs) were determined using enzymatically available kits #236691 (Boehringer Mannheim GmbH), #337-B (Sigma GPO-Trinder kit, St. Louis, MA, USA) and a Nefa-C kit (Wako Chemicals GmbH, Neuss, Germany), respectively.

**Plasma glucose and insulin assays.** Levels of plasma glucose were determined using the enzymatically available kit, #315-500 (Sigma). Plasma insulin levels were measured using a radioimmunoassay kit (Sensitive Rat Insulin Assay; Linco Research Inc., St. Charles, Missouri, USA).

**Glucose tolerance test.** Glucose tolerance tests were performed in 7 VLDLR -/- and 7 VLDLR +/- mice fed a HFC diet for a period of 17 weeks, in 5 VLDLR -/- and 5 VLDLR +/- mice on a chow diet and in 7 VLDLR -/- ob/ob and 7 VLDLR +/- ob/ob mice. Animals were fasted for 4 hours and a basal blood sample was collected from the tip of the tail (t = 0 minutes). Mice were subsequently injected i.p. with glucose (2 g/kg), and additional blood samples were collected at 15, 30, 60 and 120 min. All
drying and mechanically homogenizing, aliquots (15 mg) of HFC diet and feces were extracted (methanol-hexane 4:1 (v/v) with 0.01% butylated hydroxytoluene), hydrolyzed and methylated (16). Heptadecanoic acid (C17:0) was added to all samples as an internal standard before the extraction and methylation procedure. Fatty acid methyl esters were separated and quantified by gas liquid chromatography on a Varian gas chromatograph (model 3800) equipped with a CP-Sil88 column (50 m x 0.25 mm [inner diameter]) and a flame ionization detector. Dietary and fecal fatty acids were quantified by peak area comparison with the internal standard.

**Hepatic VLDL production.** After 7 weeks on a high fat diet, mice were fasted (24 hours) and injected intravenously with 500 mg Triton WR 1339 per kg body weight as a 15 % solution in 0.9% NaCl. Serum VLDL clearance is virtually completely inhibited under these circumstances (17). Blood samples were drawn 0, 30, 60, 90, 120 and 180 min after the Triton injection and serum TGs were determined and related to body mass of the mice. The production rate of hepatic TGs was calculated from the slope of the curve and expressed as mmol/h per kg body weight.

**3H-FFA turnover experiments.** To study the *in vivo* fatty acid turnover, VLDLR −/− and VLDLR +/+ mice after 4 weeks on HFC diet, were fasted (4 hours) and anesthetized.
of the mice. Blood samples (100 µl) were collected from the tip of the tail at 20, 40 and 60 minutes after the start of infusion. At 40 minutes steady state was reached. The whole body uptake of \(^3\)H-FFA in plasma was determined as previously described (18, 19). Briefly, basal plasma FFA concentrations were measured enzymatically as described above. The amount of plasma radioactivity in the FFA and TG fraction was determined after lipid extraction and separation from the other lipid components by thin layer chromatography (TLC) on silica gel plates (Merck) by using hexane/diethylether/acetic acid (83:16:1, vol/vol/vol) as resolving solution. \(^{14}\)C-trioleate and \(^{14}\)C-palmitic acid (Amersham) were used as internal standards. \(^3\)H-palmitate oxidation was assessed by measuring \(^3\)H-H\(_2\)O (19). This was done by subtracting the dpm in an aliquot (10 µl) of plasma, which had been evaporated to dryness and then resolved in H\(_2\)O, from the dpm of an unevaporated aliquot. The amount of storage (esterification) of FFA was calculated by subtracting the FFA oxidation from the FFA turnover (19).

**Statistical analysis.** The Mann-Whitney nonparametric test for 2 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.