VLDL receptor deficient mice have reduced lipoprotein lipase activity:
Possible causes of hypertriglyceridemia and reduced body mass with VLDL receptor deficiency

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Running title: VLDL receptor deficiency and reduced lipoprotein lipase
Abstract

Although very low density lipoprotein receptor (VLDLr) knockout mice have been reported to have no lipoprotein abnormalities, they develop less adipose tissue than control mice when fed a high calorie diet. Mice that are deficient in adipose-tissue expression of lipoprotein lipase (LpL) also have less fat, but only when crossed with ob/ob mice. We hypothesized that the VLDLr, a protein that will bind and transport LpL, is required for optimal LpL actions *in vivo* and that hypertriglyceridemia due to VLDLr deficiency is exacerbated by either LpL deficiency or VLDL overproduction. Fasted VLDLr knockout (VLDLr0) mice were more hypertriglyceridemic than controls, 2 fold greater triglyceride (TG) levels. The hypertriglyceridemia due to VLDLr0 was even more evident when VLDLr0 mice were crossed with heterozygous LpL deficient (LpL1) and human apolipoproteinB (apoB) transgenic mice. This was due to an increase in apoB48-containing VLDL. [3H]VLDL turnover studies showed that VLDL-TG clearance in VLDLr0/LpL1 mice was impaired by 50% compared to LpL1 mice. VLDLr0/LpL1 mice had less LpL activity in postheparin plasma, heart and skeletal muscle. Infection of mice with an adenovirus-expressing RAP, an inhibitor of the VLDLr, reduced LpL activity in wild type, but not VLDLr0 mice. Therefore, the VLDLr is required for normal LpL regulation *in vivo* and the disruption of VLDLr results in hypertriglyceridemia associated with decreased LpL activity.
Introduction

The very low density lipoprotein receptor (VLDLr) is one of a number of receptors in the low density lipoprotein (LDL) receptor superfamily. These receptors mediate the internalization and degradation of lipoproteins and a variety of other molecules (1,2). Recent data have also suggested that these receptors function as signaling (3) and transcytotic molecules (4). Despite the similarity of the VLDLr to other molecules that regulate circulating plasma lipoproteins, the role of this receptor in triglyceride (TG) and/or cholesterol metabolism in vivo is still unclear.

In an effort to understand the physiologic function of the VLDLr, a number of genetically modulated mice have been produced (5,6). When the VLDLr was knocked out, the resulting mice were fertile and had no obvious lipoprotein metabolic abnormalities (5). They did, however, appear to gain weight more slowly postnatally, a period in which pups ingest only milk and, therefore, have fat as their primary source of calories. In addition, VLDLr knockout (VLDLr0) mice were resistant to the development of obesity both when fed a high calorie diet (5) and when crossed with ob/ob mice (7). Furthermore, LDL receptor knockout mice that also had a knockout of the VLDLr developed increased plasma triglyceride (TG) levels when fed a high fat diet (6). Prolonged fasting in chow fed mice also led to hypertriglyceridemia (7). The mechanism responsible for the changes in obesity and the hypertriglyceridemia is unknown. In many respects, these phenotypes resemble those of mice with defects in LpL production. Mice that only produce LpL in the liver have neonatal growth retardation (8). Those expressing LpL in muscle, but not adipose tissue, are resistant to obesity (9).
Because VLDLr and LpL interact, we postulated that VLDLr deficiency leads to abnormal LpL regulation. Further support for this hypothesis come from studies of overexpression of receptor associated protein (RAP) in mice. RAP is a 39kDa protein that inhibits the LDL receptor related protein (LRP) and the VLDLr (10,11). Overexpression of RAP via adenoviral infection led to hypertriglyceridemia in a number of mouse models (12,13) including animals that had a knockout of LRP in the liver (14). This hypertriglyceridemia was associated with a decrease in postheparin plasma LpL activity. Thus, inhibition of the VLDLr or some other action of RAP was responsible for changes in LpL activity. RAP does not directly inhibit LpL activity (4,14). It does, however, block the removal of LpL protein from the bloodstream leading to high plasma levels of inactive LpL. The VLDLr is uniquely expressed in sites that are involved in regulation of LpL and, consequently, the peripheral metabolism of TG-rich lipoproteins. In most endothelial cells, the VLDLr is the most highly expressed of the LDL receptor family members (15). VLDLr is increased in hearts during fasting (16), a time when LpL activity increases and allows more delivery of fatty acids.

To test the hypothesis that the VLDLr regulates catabolism of TG-rich lipoproteins, we studied lipoproteins in VLDLr0 mice and then crossed these animals with mice that had a heterozygous deletion of LpL (denoted LpL1) and mice that overexpress human apoB (HuB). Previous studies showed that the effects of transgenic LpL expression on plasma lipoproteins were most evident when placed on the LpL1 background (17). This was presumably because normal mice produce so much LpL that it is not rate-limiting in most circumstances. We found that VLDLr0 leads to defective VLDL catabolism associated with reduced activity of LpL.
Methods

Mice and Diets: VLDLr0 (5), LpL knockout (18) and HuB transgenic mice (19) have been described. To obtain VLDLr0/LpL1 mice, VLDLr0 mice were crossbred with LpL1 mice to produce mice that were heterozygous for the disrupted alleles of both VLDLr (VLDLr1) and LpL (LpL1). VLDLr1/LpL1 mice were further bred to VLDLr1 littermates to produce a set of wild type, VLDLr0, LpL1 and VLDLr0/LpL1 mice.

To produce VLDLr0 mice overexpressing HuB (VLDLr0/HuB), VLDLr0 mice were bred with HuB transgenic mice. The resultant pups, which overexpressed the HuB transgene and were heterozygous for the mutant VLDLr locus, were further bred to VLDLr1 littermates to obtain VLDLr0 mice with the HuB transgene.

3-month old male mice fasted for 16 h were used for experiments and littermates were used as controls. The parent VLDLr0 mice were F2 hybrid between 129/Sv and C57BL/6; LpL1 and HuB mice were backcrossed to C57BL/6 seven times. Therefore, 75% of the genetic background of the mice was derived from C57BL/6 and 25% from 129/Sv strains. All mice were housed in a temperature-controlled (25°C) facility with 12 h light/dark cycle and given free access to food.

Two diets were used: i) normal chow diet that contained 4.5% (w/w) fat with 0.02% (w/w) cholesterol; ii) high fat/cholic acid diet containing 1.25% (w/w) cholesterol, 15% (w/w) fat and 0.5% (w/w) cholic acid (20).

Genotyping of induced mutant mice: The knockout LpL allele and HuB transgene were assessed using tail tip DNA by previously described PCR methods (8,21). Mutant and normal VLDLr alleles were detected by PCR using following primers: sense primer A, 5’-CCC TGG AGA AAA TCT GCG GGT TAA ATA-3’ located in intron 4 in the
VLDLr gene (5) and two antisense primers. Primer B, 5’-GAT TGG GAA GAC AAT AGC AGG CAT GC-3’ located in the neo cassette (22), and Primer C, 5’-CAC ACT GCT CAA GAG ACT CGT CTG AT-3’ located in the exon 5 in the VLDLr gene (23).

**Plasma lipid, lipoprotein and glucose analyses:** Blood was collected from the retroorbital plexus into tubes containing EDTA. Lipoproteins, VLDL (d < 1.006 g/ml), intermediate/low density lipoproteins (IDL/LDL) (d = 1.006-1.063 g/ml), and high density lipoproteins (HDL) (d = 1.063-1.21 g/ml), were isolated by sequential ultracentrifugation using a TLA 100 rotor (Beckman Instruments, Palo Alto, CA). Cholesterol and TG concentrations in plasma or lipoprotein fractions were determined enzymatically by kits (Sigma, St. Louis, MO) in duplicate. Fast performance liquid chromatography (FPLC) analyses of plasma lipoproteins were performed as described (21). Plasma free fatty acids (FFA) and glucose were measured as described (21, 24).

**Immunoblots:** ApoB in VLDL was detected by immunoblot analysis. VLDL equivalent to that in 0.75 µl of plasma were subjected to 5% SDS/PAGE under reducing conditions. After transfer to a nitrocellulose membrane (Hybond ECL™, Amersham), immunoblot analysis to detect mouse apoB was performed as described (25). LDL receptor protein in livers from fasted mice was determined as reported (26).

**VLDL turnover studies:** Sixteen LpL1 mice were injected intravenously with 200 µCi of [9, 10(n)-³H] palmitic acid (NEN, specific activity 36.3 Ci/mmol) complexed to fatty acid-free BSA (27). Blood was collected 45 min after the injection. [³H]VLDL was isolated from pooled plasma by ultracentrifugation at density of 1.006 g/ml. The lipoprotein was re-floated at the same density. TLC was performed on silica gel plates...
using a solvent system composed of hexane-diethylether-acetic acid solution (70:30:1) and 95% of total radioactive counts in the VLDL were in the TG fraction.

Fasted wild type, VLDLr0, LpL1 and VLDLr0/LpL1 mice were injected intravenously with 300,000 cpm of $[^3]$H]VLDL. Eighty $\mu$l of blood was obtained 0.5, 2 and 5 min after injection. Fifteen min after injection, mice were bled by cardiac puncture and then perfused with 10 ml PBS. VLDL fraction catabolic rate (FCR) and tissue uptakes were determined as described previously (17).

**Tissue and plasma LpL activity and mass:** Postheparin plasma was obtained from mice 5 min after a tail vein injection of heparin (300 units/kg) and stored and analyzed for LpL activity as described (24). LpL activity in homogenized tissues was measured as described by Hocquette *et al.* (28). Murine LpL protein was measured by ELISA (14).

**Northern blot analysis:** Total RNA was isolated from hearts of LpL1 and VLDLr0/LpL1 mice using TRIZOL reagent (GIBCO/BRL). Ten $\mu$g of total RNA was subjected to electrophoresis in 1% agarose gel containing formamide and transfer to a nylon filter (Hybond N, Amersham Pharmacia). A murine LpL cDNA probe was kindly provided by C. F. Semenkovich (Washington U., St Louis, MO) and radiolabeled with $[^\alpha-32]$P deoxy-CTP. After prehybridization for 2 h, blots were hybridized in a Rapidhyb$^R$ Buffer (Amersham Pharmacia) for 1 h at 65 °C with the probe (22). The same membrane was re-hybridized with a control, glyceraldehydes-3-phosphate dehydrogenase (GAPDH).

**Effect of RAP on LpL:** Recombinant adenovirus containing rat RAP cDNA (Ad-RAP) and antibody against RAP (Rb80) were generously provided by J. Herz (14) and D. Strickland (4), respectively. For Ad-RAP infection, $2.0 \times 10^9$ plaque-forming units in a
total volume of 100 µl (diluted with PBS) were injected into tail veins of wild type and VLDLr0 mice. Eighty µl of pre- and post-heparin blood was obtained 5 days before and after virus injection. Plasma lipids and postheparin plasma LpL activity were measured as described above. Western blot was performed to verify that each of these mice had a high level of RAP in the circulation.

Atherosclerosis analyses: At the age of 3 months, HuB and VLDLr0/HuB male mice were fed a high fat/cholic acid diet. Blood was taken after 16 weeks and lipoprotein profiles were determined. Mice were sacrificed at 16 weeks and atherosclerosis was determined as described previously (29).

Statistics: Data are represented as means ± SD. Student’s t-test and ANOVA were used to compare the mean values between two and four groups respectively.
Results

Lipid levels in VLDLr0/LpL1 and VLDLr0/HuB mice: Plasma lipids and lipoprotein levels were compared between wild type, VLDLr0, LpL1 and VLDLr0/LpL1 mice. VLDLr0 mice had 2.2 fold greater levels of plasma TG than wild type mice and VLDLr0/LpL1 mice had 1.8 fold higher TG levels than LpL1 mice (Table 1). Cholesterol levels were significantly higher in VLDLr0 than wild type mice, but not VLDLr0/LpL1 compared with LpL1 mice. FFA and glucose levels did not differ between the four genotypes (Table 2). VLDLr0/HuB mice had >2 fold greater TG levels than HuB mice, 340 versus 162 mg/dl (Table 1). Thus, VLDLr0 led to more TG when bred onto LpL1, a model of defective lipoprotein metabolism, and HuB, a model of lipoprotein overproduction.

We also assessed lipids in HuB and VLDLr0/HuB mice fed a high fat/cholic acid containing diet. Total plasma lipids in the two genotypes were not significantly different (Table 1).

Lipoprotein profiles in VLDLr0 mice: FPLC analyses on the pooled plasma collected from 3-5 mice are shown in Figure 1. Cholesterol and TG levels in the VLDL fraction were increased in VLDLr0 compared to wild type mice (Figure 1A and B). A similar effect of VLDLr deficiency was noted when VLDLr0/LpL1 mice were compared to LpL1 mice (Figure 1C and D). There were no changes in IDL/LDL and HDL fractions between the two types of mice. VLDLr0/HuB mice also had much greater cholesterol and TG levels in the VLDL fraction than HuB mice (Figure 1E and 1F). There were no changes in HDL. Despite the lack of changes in total plasma lipids with high fat feeding
(Table 1), lipoprotein distribution was altered in VLDLr0/HuB mice. They had less IDL/LDL cholesterol and more VLDL-TG than HuB mice (Figure 1G and H).

Lipoprotein analysis by ultracentrifugation was also performed as a second method of assessing the distribution of lipoproteins in plasma. TG levels in isolated VLDL were increased by 2.5 fold in VLDLr0/LpL1 mice compared to those in LpL1 mice (382 versus 152 mg/dl, \( P < 0.01 \)) (Table 3). VLDL-cholesterol levels were also significantly higher in VLDLr0/LpL1 mice (39 versus 22 mg/dl, \( P < 0.01 \)). Therefore the ratio of TG/cholesterol in VLDL increased from 6.9 to 9.8. There were no significant differences in HDL or IDL/LDL. Similarly, VLDLr0/HuB mice had >3 fold more VLDL-TG than HuB mice (258 versus 84 mg/dl, \( P < 0.01 \)), the ratio of VLDL-TG/cholesterol increased from 7 to 9.6 and there was no change in IDL/LDL or HDL (Table 3). With high fat feeding, the increase in VLDL and reduction of IDL/LDL noted by FPLC was confirmed (Table 3). Thus, VLDLr0 mice had a specific increase in VLDL that must have resulted from either increased VLDL production or decreased catabolism.

**Plasma apoB48/apoB100 levels:** The hypertriglyceridemia found in LpL1 and VLDLr0/LpL1 mice was associated with an increase in apoB48-containing VLDL (Figure 2). VLDLr0/LpL1 mice had more apoB48-VLDL than either LpL1 or wild type mice. This increase in apoB48 was not found in IDL/LDL and was not associated with a change in LDL receptor expression levels between LpL1 and VLDLr0/LpL1 mice (data not shown).

**VLDL turnover studies:** VLDLr deficiency led to slower clearance of radiolabeled VLDL. The most rapid turnover occurred in wild type mice (FCR 11.2±2.3 pools/h). VLDLr0 and LpL1 mice cleared the tracer at almost identical rates, 6.2±2.1 and 7.2±2.2
pools/h, respectively ($P = 0.4$). VLDL removal was significantly slower in VLDLr0/LpL1 mice than that in either VLDLr0 or LpL1 mice (Figure 3A) (FCR LpL1/VLDLr0 = 3.6±1.5 pools/h, $P < 0.03$ compared to VLDLr0 and $P < 0.01$ compared to LpL1). This corresponded to a significant reduction in tissue uptake of labeled VLDL by heart and skeletal muscle (Figure 3B); differences in tracer uptake into adipose and liver were not significant. It should be noted (see Figure 2) that most of the VLDL in this preparation contains apoB48. Thus, these changes were consistent with a defect in VLDL lipolysis leading to reduced muscle TG uptake.

**Plasma LpL activity and mass:** VLDLr0 mice had significantly less postheparin plasma LpL activity. VLDLr0 mice had 37% less activity than wild type, and VLDLr0/LpL1 mice had 26% less activity than LpL1 mice (Figure 4A). Postheparin plasma LpL mass paralleled these changes in activity (Figure 4B). VLDLr0/HuB mice also had lower LpL activity and mass than HuB mice (Figure 4C and D). Therefore, the VLDL increase with loss of the VLDLr corresponded to changes in LpL.

**Muscle and adipose LpL:** With fasting, muscle becomes relatively more important than adipose as a source of LpL activity (30). To determine if the reduced LpL was associated with changes in only one tissue, e.g. muscle, LpL activity was measured in tissues obtained from LpL1 and VLDLr0/LpL1 mice. Both heart and skeletal muscle LpL activities were significantly lower in VLDLr0/LpL1 than LpL1 mice; adipose LpL was also reduced by 26% but this difference did not reach statistical significance (Figure 5A). Although LpL protein in skeletal muscle and adipose could not be assessed due to its lower protein levels in both groups of mice, VLDLr0/LpL1 mice had significantly less LpL protein in heart than LpL1 mice (Figure 5B). Northern blot analysis showed no
difference in LpL mRNA in the heart (Figure 5C). Thus, reduced LpL appeared to be due to a posttranscriptional process.

**Effect of RAP on plasma TG and LpL:** Previous studies have shown that RAP leads to reduced LpL activity compared to control virus (14). To test whether this was due to inhibition of the VLDLr, changes in LpL with RAP were assessed in mice deficient in VLDLr. Infection with Ad-RAP led to a 31% decrease in LpL activity in wild type mice (Figure 6A), but no change in LpL activity in VLDLr0 mice.

The effects of RAP on plasma TG levels reflected, in part, the changes in LpL activity (Figure 6B). A greater increase in TG occurred in wild type mice that also had greater reduction of LpL. However, VLDLr0 mice also had an increase in plasma TG. Thus, RAP probably increases plasma lipids due both to changes in LpL and to effects on lipoprotein receptors.

**Atherosclerosis in VLDLr0/HuB mice:** It has been postulated that VLDLr expression in macrophages allows for foam cell development in atherosclerotic lesions (31). Atherosclerosis was, however, not different between HuB and VLDLr0/HuB mice (37,000 versus 27,000 µm², \( P = 0.5, \) figure 7) after 4 months of an atherogenic diet.
Discussion

In the present study, we provide a mechanism for the abnormality in lipoprotein metabolism due to a deficiency of the VLDLr. We investigated VLDLr deficiency in two genetically engineered mouse models: LpL1 mice, an animal with decreased lipoprotein hydrolysis, and HuB mice, a mouse with hypercholesterolemia due to increased lipoprotein production. In contrast to the initial report (5), we show that VLDLr deficiency alters lipoprotein profiles. The different results may result from the fasting protocol used in our experiments; we did not find differences in plasma lipids in non-fasting mice. Our data complement those of Tacken et al. (6) and Goudriaan et al. (7), and provide mechanistic information for their results. We show the following: 1) VLDLr deficiency increases plasma and VLDL TG. 2) This is associated with reduced LpL activity. 3) RAP inhibits LpL activity in mice that also express the VLDL receptor, but RAP is less effective in VLDLr0 animals. 4) Reduced LpL activity leads to an increase in mainly apoB48-VLDL. 5) VLDLr deficiency does not alter atherosclerosis in the HuB transgenic model.

VLDLr deficiency led to hypertriglyceridemia, however TG levels were highest in two different models in which lipoprotein metabolic pathways were stressed, LpL deficiency and overproduction of apoB-lipoproteins. Thus, the effects of these additional genetic mutations were additive to VLDLr deficiency. It should be noted that mice have a very active lipolytic system and mouse postheparin plasma contains 5-10 fold more LpL activity than human postheparin plasma. Moreover, plasma apoB levels in wild type mice are nearly an order of magnitude lower that those in humans. Thus, the role of the VLDLr
in modulation of plasma lipoproteins may be even more important in humans whose lipolytic system is more limited.

The development of hypertriglyceridemia with VLDLr deficiency was associated with reduced catabolism of VLDL. Kinetic studies in these mice showed that elevated VLDL was associated with reduced plasma catabolism and decreased peripheral uptake of the lipoprotein TG. Others have reported that VLDLr deficiency did not cause triglyceride overproduction in LDL receptor knockout mice (6), but did not provide a reason for the hypertriglyceridemia that occurred. As opposed to a defect merely in remnant uptake that primarily should affect liver, our metabolic study was most consistent with defective TG lipolysis leading to a reduction of heart and skeletal muscle VLDL uptake.

VLDLr0 led to less postheparin plasma LpL activity in all three types of mice studied: wild type, LpL1, and HuB. Both LpL activity and LpL mass were reduced. Moreover, we also documented a reduction of LpL activity in heart and skeletal muscle, and a reduction of LpL mass in the heart without an alteration of mRNA expression level. Therefore, the decrease of VLDL catabolism likely results from the decrease of LpL activity in tissues. Furthermore, it is reasonable to speculate that hypertriglyceridemia in VLDLr0 mice in the current study and other reports (6,7) is due to a reduction of LpL activity attributable to the disruption of VLDLr.

Changes in LpL are thought to be a major modulator of tissue lipid metabolism during feeding/fasting. In fasted animals, adipose LpL is reduced and plasma LpL mainly comes from skeletal muscle and heart (30). VLDLr expression is increased in mouse hearts with fasting (16), and this may be necessary to allow optimal lipolysis. For this
reason, our studies were performed in fasted animals where the actions of the VLDLr were likely to be most evident.

After Ad-RAP infection, postheparin plasma LpL activity was significantly decreased in wild type mice, whereas it was not significantly changed in VLDLr0 mice. The increase of plasma TG with RAP was more pronounced in wild type mice. These results are consistent with and explain, in part, observations reported in the literature. Venient et al. (12) showed that RAP increased the size and TG content of VLDL lipoproteins; a similar effect occurs when LpL-mediated lipolysis is inhibited (32). Van Vlijmen et al. (14) analyzed the effect of RAP overexpression in animals lacking the LDL receptor and liver-LRP. Even in the absence of these two receptors RAP markedly elevated plasma TG. In wild type, but not in these genetically altered mice, LpL activity and mass were measured. Not unexpectedly, LpL mass markedly increased because RAP blocks liver catabolism of LpL. For this reason, we did not do LpL mass measurements in RAP-overexpressing mice. LpL activity decreased with RAP and our studies were designed to assess whether this decrease was due to an effect of RAP on the VLDLr. From our data, we postulate that the extrahepatic process that is defective in the catabolism of TG-rich lipoproteins in RAP-overexpressing mice is reduced LpL activity due to blockade of the VLDLr. RAP inhibition of VLDLr and VLDLr deficiency both compromise LpL activity.

Why did VLDLr0 mice have less LpL activity? Because LpL mRNA levels were not changed, the differences were due to a posttranscriptional process. There are several possible abnormal processes: 1) VLDLr0 could have altered the movement of LpL from myocytes and adipocytes to the endothelial surface and led to increased cellular LpL
degradation. We had observed in vitro (4) that inhibition of the VLDLr reduced LpL transport across endothelial cells. Some LpL also crosses the endothelial cells via a “default” non-receptor mediated pathway and LpL on sites other than the endothelial surface may perform lipolysis and be released with heparin. Therefore, loss of the VLDLr compromised, but did not eliminate, active LpL. 2) A decrease in endothelial cell binding of LpL could have occurred. In addition to heparan sulfate proteoglycans, LpL also associates with plasma and presumably endothelial cell bound lipoproteins (33). Therefore, in VLDLr0 mice more endothelial LpL could have exchanged onto circulating TG-rich lipoproteins and been rapidly cleared from the bloodstream. Alternatively, less LpL may have associated with VLDLr-associated lipoproteins attached to the endothelial surface.

Remarkably the models of reduced LpL activity, LpL1 and VLDLr0/LpL1, led to an increase in apoB48-VLDL but not apoB100-VLDL. Various kinetic studies have suggested that the metabolism of apoB48-lipoproteins is more rapid than liver derived apoB100-containing VLDL (34). This has led to the hypothesis that chylomicrons are a preferred substrate for LpL (35). While such a conclusion may be warranted in most circumstances, the reason for this may be that average chylomicrons have a larger volume and more easily come into contact with LpL associated with the vascular wall (32). Unlike the situation in humans, fasted mice continue to produce apoB48-lipoproteins since apoB is edited in the rodent liver. We postulate that mouse hepatic apoB48-VLDL are a worse LpL substrate than large human intestinally derived apoB48-lipoproteins.

In our final experimental model, high fat/cholic acid diet fed HuB mice, the effects of VLDLr deficiency on lipoproteins and atherosclerosis was studied. Consistent
with mice fed a normal chow diet, VLDLr0/HuB mice had elevated VLDL-TG after fasting compared to HuB mice. Cholic acid decreases TG production (36), thus the expected TG elevations were not found. Interestingly, LDL particles were markedly reduced in VLDLr0/HuB mice. This phenotype likely reflects a defect in lipolysis. Although it has reported that VLDLr in macrophages is upregulated during monocyte-macrophage differentiation and that this receptor can induce macrophage foam cell formation (31), atherosclerosis did not differ between mice with and without the VLDLr in the current study. Although this suggests that independent vascular effects of this receptor play a minor role in lesion development, multiple animal models are needed to confirm this. Even then, it may be hazardous to extrapolate this conclusion to human disease.

In conclusion, the current study provides evidence of an important role of the VLDLr in catabolism of TG-rich lipoproteins via regulation of LpL. VLDLr modulates lipolysis and for this reason, its effects on neonatal growth and adipose tissue accumulation are similar to those of LpL deficiency. The mechanism for this effect may be similar to that found in previously published in vitro studies that implicated the VLDLr as a transport protein for LpL (4). Whether this or other mechanisms are involved, our data and that of others (6), demonstrate that the VLDLr, true to its name, modulates circulating levels of TG and VLDL.
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Footnotes

1Abbreviations used are: VLDLr, very low density lipoprotein receptor; VLDLr0, VLDLr homozygous knockout; LpL, lipoprotein lipase; LpL1, LpL heterozygous knockout; apoB, apolipoprotein B; HuB, human apoB; TG, triglyceride; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; RAP, receptor associated protein; LRP, LDL receptor related protein; FPLC, fast performance liquid chromatography; FFA, free fatty acids; FCR, fraction catabolic rate; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; Ad-RAP, recombinant adenovirus containing RAP cDNA; HF, high fat/cholic acid diet
References


Figure legends

Figure 1. Lipoprotein profiles of VLDLr deficient mice. Distribution of cholesterol (A, C, E, G) and triglyceride (B, D, F, H) in the plasma lipoproteins. (A, B) Wild type (open circles) and VLDLr0 mice (closed circles) fed a chow diet. (C, D) LpL1 (open circles) and VLDLr0/LpL1 mice (closed circles) fed a chow diet. (E, F) HuB (open circles) and VLDLr0/HuB mice (closed circles) fed a chow diet. (G, H) HuB (open circles) and VLDLr0/HuB mice (closed circles) fed a high fat/cholic acid diet. Two hundred µl of pooled plasma from 3-5 fasted mice was subjected to FPLC lipoprotein analyses, and cholesterol and triglyceride contents in effluents were measured enzymatically.

Figure 2. Immunoblot analysis of apoB. VLDL were isolated by ultracentrifugation and equivalent amounts to that in 0.75 µl of plasma were subjected to 5% SDS/PAGE electrophoresis. ApoB immunoblot analysis was performed using anti-human apoB antibody.

Figure 3. VLDL turnover studies in VLDLr deficiency mice. [3H]VLDL obtained from LpL1 mice was used in VLDL turnover studies. (A) Plasma clearance of [3H]VLDL. [3H]VLDL was injected into wild type (open circles and solid line, n = 6), VLDLr0 (open circles and dotted line, n = 7), LpL1 (closed circles and solid line, n = 8) and VLDLr0/LpL1 mice (closed circles and dotted line, n = 6). At the indicated times, blood was collected. The plasma count at 30 sec after injection was considered as injected dose (ID). (B) Fifteen minutes after injection, the mice were perfused with PBS and the indicated tissues were taken out and homogenized. Lipids were extracted from homogenates and counted. The data are shown as percentage of wild type mice (cpm/g tissue/ID). Wild type mice are shown with open bars, VLDLr0 with solid bars, LpL1 with
striped bars, and VLDLr0/LpL1 with dotted bars. Values are expressed as means ± SD. 

\[ A^P < 0.01 \text{ versus wild type}, \quad B^P < 0.01 \text{ versus VLDLr0}, \quad C^P < 0.01 \text{ versus LpL1}, \quad D^P < 0.01 \text{ versus VLDLr0/LpL1}, \quad E^P < 0.03 \text{ versus VLDLr0}, \quad F^P < 0.03 \text{ versus LpL1 and } \quad G^P < 0.05 \text{ versus VLDLr0/LpL1 mice.} \]

**Figure 4. Postheparin plasma LpL activity and mass.** (A) Postheparin plasma was collected and LpL activity was measured. VLDLr0 and VLDLr0/LpL1 mice had reduced LpL activity compared to wild type and LpL1 mice, respectively. Wild type \( n = 13; \) VLDLr0, LpL1 and VLDLr0/LpL1 \( n = 10 \) each group. (B) Postheparin plasma LpL mass was measured by ELISA. The data are shown as a percentage of wild type mice (100% = 246 ng/ml). LpL mass was reduced to 70, 73 and 53 % in VLDLr0 \( (n = 8), \) LpL1 \( (n = 8) \) and VLDLr0/LpL1 mice \( (n = 12) \) respectively compared to wild type mice \( (n = 6). \) (C, D) Postheparin plasma LpL activity and mass in HuB \( (n = 9) \) and VLDLr0/HuB mice \( (n = 8) \) are shown. Values are expressed as means ± SD. \( A^P < 0.05, \quad B^P < 0.02 \text{ and } \quad C^P < 0.01 \) for relationships described by brackets.

**Figure 5. Tissue LpL.** (A) Heart, quadriceps muscle and adipose were collected from fasted LpL1 \( (n = 7) \) and VLDLr0/LpL1 mice \( (n = 6). \) LpL activity in homogenates of heart and skeletal muscle was significantly reduced in VLDLr0/LpL1 mice (closed bars) compared to LpL1 mice (open bars). (B) The samples in A were used for measurements of heart LpL mass by ELISA. LpL1 mice had significantly more LpL than mice that were also deficient in VLDLr. Values are expressed as means ± SD. \( A^P \leq 0.01 \text{ versus LpL1 mice.} \) (C) Northern blot analysis. Ten \( \mu \text{g} \) of total RNA was isolated from heart, and subjected Northern blot analysis. Two probes were used: cDNA fragment containing the entire coding region of mouse LpL and GAPDH as a reference.
Figure 6. The effect of RAP expression on plasma lipids and postheparin plasma LpL activity. Pre- and post-heparin plasma was collected from fasted mice (n = 5, both groups) 5 days before and after infection with Ad-RAP. (A) Postheparin plasma LpL activity. Open and closed bars represent the LpL activity before and after Ad-RAP infection, respectively. Ad-RAP infection led to a significant reduction of LpL activity only in wild type mice. (B) Percent increase of plasma triglyceride after Ad-RAP infection. Plasma triglyceride levels were measured 5 days before and after Ad-RAP infection. Values are expressed as means ± SD. ^P < 0.05.

Figure 7. Effect of high fat/cholic acid diet on atherosclerosis in VLDLr0/HuB mice. Cross-sectional lesion area in the aortic sinus from HuB (open circles, n = 9) and VLDLr0/HuB mice (closed circles, n = 8). Fatty streak lesion areas were determined as described in Methods. Mean values are indicated by horizontal lines.
Table 1. Plasma lipids in VLDLr0, VLDLr0/LpL1 and VLDLr0/HuB mice

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Cholesterol mg/dl</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>chow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>8</td>
<td>104 ± 16</td>
<td>141 ± 48</td>
</tr>
<tr>
<td>VLDLr0</td>
<td>8</td>
<td>141 ± 34&lt;sup&gt;A&lt;/sup&gt;</td>
<td>316 ± 71&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>LpL1</td>
<td>9</td>
<td>146 ± 34&lt;sup&gt;A&lt;/sup&gt;</td>
<td>296 ± 117&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>VLDLr0/LpL1</td>
<td>13</td>
<td>151 ± 24&lt;sup&gt;B&lt;/sup&gt;</td>
<td>544 ± 125&lt;sup&gt;BCD&lt;/sup&gt;</td>
</tr>
<tr>
<td>chow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HuB</td>
<td>5</td>
<td>141 ± 22</td>
<td>162 ± 21</td>
</tr>
<tr>
<td>VLDLr0/HuB</td>
<td>9</td>
<td>135 ± 24</td>
<td>340 ± 125&lt;sup&gt;E&lt;/sup&gt;</td>
</tr>
<tr>
<td>HF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HuB</td>
<td>9</td>
<td>453 ± 119</td>
<td>79 ± 16</td>
</tr>
<tr>
<td>VLDLr0/HuB</td>
<td>8</td>
<td>420 ± 104</td>
<td>105 ± 33</td>
</tr>
</tbody>
</table>

Blood was taken from 16 h fasted mice.

Values are expressed as means ± SD. <sup>A</sup><em>P</em> < 0.05 versus wild type; <sup>B</sup><em>P</em> < 0.01 versus wild type; <sup>C</sup><em>P</em> < 0.01 versus LpL1; <sup>D</sup><em>P</em> < 0.01 versus VLDLr0; <sup>E</sup><em>P</em> < 0.01 versus HuB.

HF, high fat/ cholic acid diet.
Table 2 Plasma glucose and FFA levels in VLDLr0 mice.

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>FFA</th>
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<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Wild Type</td>
<td>6</td>
<td>81 ± 13</td>
</tr>
<tr>
<td>VLDLr0</td>
<td>6</td>
<td>78 ± 19</td>
</tr>
<tr>
<td>LpL1</td>
<td>11</td>
<td>83 ± 16</td>
</tr>
<tr>
<td>VLDLr0/LpL1</td>
<td>12</td>
<td>88 ± 25</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. Plasma glucose and FFA levels did not differ between the four genotypes.
Table 3. Plasma lipoproteins in VLDLr0/LpL1 and VLDLr0/HuB mice

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>VLDL</td>
<td>IDL/LDL</td>
<td>HDL</td>
<td>VLDL</td>
<td>IDL/LDL</td>
<td></td>
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<tr>
<td>chow</td>
<td></td>
<td>mg/dl</td>
<td>mg/dl</td>
<td>mg/dl</td>
<td>mg/dl</td>
<td>mg/dl</td>
<td>mg/dl</td>
</tr>
<tr>
<td>LpL1</td>
<td></td>
<td>9</td>
<td>22 ± 9</td>
<td>22 ± 11</td>
<td>76 ± 18</td>
<td>152 ± 56</td>
<td>58 ± 24</td>
</tr>
<tr>
<td>VLDLr0/LpL1</td>
<td></td>
<td>13</td>
<td>39 ± 9^A</td>
<td>20 ± 6</td>
<td>77 ± 13</td>
<td>382 ± 111^A</td>
<td>60 ± 15</td>
</tr>
<tr>
<td>chow</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HuB</td>
<td></td>
<td>5</td>
<td>12 ± 3</td>
<td>33 ± 10</td>
<td>75 ± 9</td>
<td>84 ± 20</td>
<td>67 ± 16</td>
</tr>
<tr>
<td>VLDLr0/HuB</td>
<td></td>
<td>9</td>
<td>27 ± 8^B</td>
<td>26 ± 8</td>
<td>64 ± 11</td>
<td>258 ± 106^B</td>
<td>65 ± 10</td>
</tr>
<tr>
<td>HF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HuB</td>
<td></td>
<td>9</td>
<td>125 ± 48</td>
<td>249 ± 92</td>
<td>74 ± 12</td>
<td>37 ± 7</td>
<td>29 ± 10</td>
</tr>
<tr>
<td>VLDLr0/HuB</td>
<td></td>
<td>8</td>
<td>179 ± 73</td>
<td>163 ± 55^B</td>
<td>64 ± 11</td>
<td>65 ± 23^B</td>
<td>28 ± 10</td>
</tr>
</tbody>
</table>

Lipoproteins were isolated by sequential ultracentrifugation individually. Values are expressed as means ± SD.

^A P < 0.01 versus LpL1; ^B P ≤ 0.01 versus HuB.

HF, high fat/cholic acid diet.
Figure 2

<table>
<thead>
<tr>
<th>VLDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
</tr>
<tr>
<td>LpL1</td>
</tr>
<tr>
<td>VLDLr0/LpL1</td>
</tr>
</tbody>
</table>
Figure 4

A

LpL activity (μmol FFA/ml/hr)

Wild VLDLr0 LpL1 VLDLr0/LpL1

B

LpL mass (% of wild type)

Wild VLDLr0 LpL1 VLDLr0/LpL1

C

LpL activity (μmol FFA/ml/hr)

HuB VLDLr0/HuB

D

LpL mass (% of HuB)

HuB VLDLr0/HuB
Figure 5

A

LpL activity (μmol FFA/g tissue/hr)

- Heart
- Muscle
- Adipose

B

LpL mass (ng/g tissue)

- LpL1
- VLDLr0/LpL1

C

[Image of LpL and GAPDH gel bands]

LpL/GAPDH 1.5 1.5
Figure 6

A

LpL activity (μmol FFA/ml/hr)

Wild type  VLDLr0

B

% increase of plasma TG

Wild type  VLDLr0

A
VLDDL receptor deficient mice have reduced lipoprotein lipase activity: Possible causes of hypertriglyceridemia and reduced body mass with VLDL receptor deficiency
Hiroaki Yagyu, E. Peer Lutz, Yuko Kako, Steven Marks, Yunying Hu, Sungshin Y. Choi, Andrè Bensadoun and Ira J. Goldberg

J. Biol. Chem. published online January 14, 2002

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