Intravenous Injection of Apolipoprotein A-V Reconstituted High-Density Lipoprotein Decreases Hypertriglyceridemia in apoav−/− Mice and Requires Glycosylphosphatidylinositol-Anchored High-Density Lipoprotein–Binding Protein 1

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Objective—Apolipoprotein A-V (apoA-V), a minor protein associated with lipoproteins, has a major effect on triacylglycerol (TG) metabolism. We investigated whether apoA-V complexed with phospholipid in the form of a reconstituted high-density lipoprotein (rHDL) has potential utility as a therapeutic agent for treatment of hypertriglyceridemia (HTG) when delivered intravenously.

Methods and Results—Intravenous injection studies were performed in genetically engineered mouse models of severe HTG, including apoav−/− and gpihbp1−/− mice. Administration of apoA-V rHDL to hypertriglyceridemic apoav−/− mice resulted in a 60% reduction in plasma TG concentration after 4 hours. This decline can be attributed to enhanced catabolism/clearance of very-low-density lipoprotein (VLDL), where VLDL TG and cholesterol were reduced ≈60%. ApoA-V that associated with VLDL after injection was also rapidly cleared. Site-specific mutations in the heparin-binding region of apoA-V (amino acids 186 to 227) attenuated apoA-V rHDL TG-lowering activity by 50%, suggesting that this sequence element is required for optimal TG-lowering activity in vivo. Unlike apoav−/− mice, injection of apoA-V rHDL into gpihbp1−/− mice had no effect on plasma TG levels, and apoA-V remained associated with plasma VLDL.

Conclusion—Intravenously injected apoA-V rHDL significantly lowers plasma TG in an apoA-V deficient mouse model. Its intravenous administration may have therapeutic benefit in human subjects with severe HTG, especially in cases involving apoA-V variants associated with HTG. (Arterioscler Thromb Vasc Biol. 2010;30:2504-2509.)

Key Words: apolipoproteins ▪ hyperlipoproteinemia ▪ lipids ▪ lipoproteins

Epidemiological studies have revealed that increased plasma triacylglycerol (TG) is an independent risk factor for coronary heart disease.1,2 Furthermore, hypertriglyceridemia (HTG) is a hallmark of the metabolic syndrome and is often accompanied by obesity and insulin resistance.3 Given that the metabolic syndrome confers increased risk for development of both type 2 diabetes and cardiovascular disease,4 maintenance of plasma TG homeostasis is highly desirable.

Following its discovery in 2001,5 apoA-V (apoA-V) emerged as an important TG modulator.7 In humans, APOAV is located in the APOAI/CIII/AIV/AV gene cluster on the long arm of chromosome 11. ApoA-V is expressed exclusively by liver tissue and, in plasma, is associated with high-density lipoprotein (HDL) and VLDL.8,9 Unlike other exchangeable apolipoproteins, the plasma concentration of apoA-V in humans (∼250 ng/mL)8 and mice (∼24 ng/mL)10 is extremely low. Despite this, the contribution of apoA-V to chylomicron and VLDL metabolism is readily appreciated from genetic engineering studies in mice.5 Apoav−/− mice manifested a 4-fold increase in plasma TG, whereas the concentration in APOAV transgenic mice is 1/3 that in wild-type (WT) control littersmates. Furthermore, studies in humans revealed an association between truncation mutations in apoA-V and severe HTG.11–13 These data strongly suggest that apoA-V plays an important physiological role in plasma TG metabolism.

Previous in vivo studies demonstrated that HTG in apoA-V–deficient mice is attributable to decreased chylomicron and VLDL lipolysis and remnant removal.14,15 On the other hand, overexpression of apoA-V in mice via adenovirus-mediated gene transfer led to a decrease in plasma TG.16–18 In vitro studies with apoA-V suggest that its TG-lowering activity may be explained by an ability to increase the efficiency of lipoprotein lipase (LPL)–mediated TG hydrolysis,19 as well as an ability to increase remnant clearance by binding to members of the low-density lipoprotein (LDL) receptor family.20,21

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Lipolysis is a key step in clearance of TG-rich lipoproteins that takes place on the luminal surface of capillaries of heart, skeletal muscle, and adipose tissues. LPL, synthesized in muscle and adipocytes, is translocated to capillary endothelial cells. Recent studies have shown that glycosylphosphatidylinositol-anchored high-density lipoprotein–binding protein 1 (GPIHB1) binds the positively charged, heparin-binding domain of LPL via its Ly6 domain and a negatively charged region in its amino terminus. In the absence of GPIHB1, lipolysis is substantially diminished and plasma TG levels are markedly elevated. It has been postulated that GPIHB1 serves as a platform that supports lipolytic activity. Interestingly, apoA-V also binds to GPIHB1, most likely via a positively charged sequence motif located between residues 186 and 227.23 ApoA-V also binds to heparin in vitro, and its presence on chylomicrons significantly lowered plasma TG concentrations in apoA-V–containing reconstituted high-density lipoprotein (rHDL) mice.9 Compared with control mice in apoA-V transgenic mice,9 mice on apoA-V rHDL showed a 25% reduction in plasma TG concentration after 4 hours. Similar to apoA-V transgenic mice, rHDL treatment lowered plasma TG level and reduced heparin and GPIHBP1 binding.20,23 Based on these findings, it is conceivable that apoA-V promotes attachment of TG-rich particles to endothelial cell surface heparan sulfate proteoglycans or GPIHB1 and that such interactions enhance lipolysis.

In this report, we evaluated the potential utility of apoA-V as a TG-lowering therapeutic agent. Intravenous injection of apoA-V–containing reconstituted high-density lipoprotein (rHDL) significantly lowered plasma TG concentrations in apoav−/− mice yet had no effect in gpihb1−/− mice. Mutation of positively charged amino acids in the heparin-binding region of apoA-V attenuated its TG-lowering capacity. Taken together, the data provide new mechanistic insight into the coordinate activities of LPL, GPIHB1, and apoA-V in plasma TG homeostasis and suggest that intravenous administration of apoA-V may have therapeutic benefit in human subjects with severe HTG.

Methods

Materials

Primary antibodies included polyclonal goat anti-human apoA-V,24 polyclonal goat anti-apoB (International Immunology), polyclonal goat anti-mouse apoA-I (Abcam), and polyclonal rabbit anti-mouse apoE (Biodesign International). Bis-Tris 4% to 20% NuPAGE gradient gels were from Invitrogen. Enzymatic assay kits for TG and cholesterol were from Wako Chemicals. Heparin was from Baxter. Gradient gels were from Invitrogen. Enzymatic assay kits for TG and cholesterol were from Wako Chemicals. Heparin was from Baxter. The fluorescent lipase substrate, 1,2-dimyristoylphosphatidyl choline (DMPC) for injection24; briefly, DMPC vesicles were generated by extrusion through a 0.05-μm membrane and subsequently complexed with recombinant apoA-V protein by sonication to form rHDL. Previous electron microscopy studies revealed that apoA-V rHDL consists of discoidal particles of ≈14 nm diameter; by native polyacrylamide gel electrophoresis analysis, complexes were 12 to 17 nm in diameter.24 In the present experiments, the size of apoA-V rHDL particles was confirmed on native gels. The mean protein:phospholipid (wt:wt) ratio in apoA-V rHDL was 1:6.3±0.6 (n=4). Controls used DMPC vesicles without protein. Mice were fasted 4 hours, and blood samples were obtained by submandibular vein bleeds before injection (t=0) and at 1, 2, and 4 hours postinjection. ApoA-V rHDL was injected by tail vein so as to achieve a plasma concentration of 12.5 μg/mL (the average plasma concentration in apoA-V transgenic mice).20 Mice were anesthetized with isoflurane. Plasma samples were rapidly separated and stored at −80°C.

Isolation of Plasma Lipoproteins

Lipoproteins from pooled plasma were separated by fast protein liquid chromatography (FPLC) with a Supersose 6HR 10/30 column (Pharmacia LKB Biotechnology). Elution profiles were monitored at 280 nm, and 0.5-mL fractions were collected.

Measurement of Lipid Concentrations

Cholesterol and TG in plasma samples or FPLC fractions were determined by colorimetric assays (Wako).

Immunoblotting

Plasma (1 μL) or concentrated FPLC fractions were electrophoresed on 4% to 20% Bis-Tris gradient gels. The size-separated proteins were transferred to polyvinylidene difluoride membranes, and immunoblots were processed as described.24 In one experiment, clearance of apoA-V with time was determined by administering 12.5 μg/mL apoA-V rHDL and sampling plasma at 1 minute postinjection for baseline plasma apoA-V levels and at 1, 4, and 8 hours postinjection. Following electrophoresis on 4% to 20% gels and Western blotting, relative changes in plasma apoA-V compared with baseline were determined by densitometry using the NIH ImageJ program.

Measurement of Postheparin LPL Activity

Apoav−/− mice were injected via the tail vein with 50 μL of heparin (50 U) 2 or 4 hours after injection with apoA-V rHDL or DMPC vesicle alone. Before and 15 minutes after heparin injection, blood samples were collected, and plasma was separated. LPL activities in the plasma samples were determined with a fluorometric assay as described.23 Briefly, the lipase activity in the plasma sample was measured as the rate of fluorescence generated from hydrolysis of the lipase substrate DGGR. Two minutes after mixing plasma sample with DGGR, fluorescence intensity was monitored for 5 minutes, and lipase activity was calculated as relative fluorescence units generated per minute. LPL activity was determined by subtracting preheparin activity from postheparin activity.26

Results

Effect of Injected apoA-V on Plasma TG Concentration in apoav−/− Mice

To evaluate the effect of parenteral administration of apoA-V on plasma TG concentration, apoav−/− mice were injected with apoA-V rHDL to achieve a plasma concentration of 12.5 μg/mL, which is the average concentration of apoA-V in apoAV transgenic mice.9 Compared with control mice injected with DMPC vesicles alone, apoA-V rHDL induced a 25% reduction in TG after 1 hour and a 60% reduction at 4 hours (Figure 1A). In controls, there was a slight reduction (≈20%) in plasma TG concentration after 4 hours. Similar to changes in TG, apoA-V rHDL administration also decreased plasma cholesterol levels (Figure 1B). Consistent with the
decline in TG, the total amount of apoB-100 protein in plasma also decreased after 4 hours along with apoA-V (Figure 1C). Unlike apoB-100 and apoA-V, there was no change in plasma levels of apoB-48.

In a separate experiment to determine changes in TG and apoA-V over a more extended period of time, mice were injected with 12.5 μg/mL apoA-V rHDL (open circles) or DMPC vesicles (filled circles). Plasma samples were collected before injection and at 1, 2, and 4 hours postinjection and analyzed for TG (A) and cholesterol (B); the mean TG concentration for the mice used in the study was 18.1±1.3 mg/mL. Values are presented as percentage of initial concentration and are expressed as mean±SEM (>12 mice/group). Student t test results versus the respective controls were as follows: *P<0.05, **P<0.001. C, apoB and apoA-V levels in the plasma samples (1 μL) of individual mice were determined by Western blot. Results are representative of >12 mice.

In a separate experiment to determine changes in TG and apoA-V over a more extended period of time, mice were injected with 12.5 μg/mL apoA-V rHDL, and plasma was sampled after 1 minute to establish baseline values; subsequent plasma samples were obtained at 1, 4, and 8 hours postinjection. As noted in Supplemental Figure I (available online at http://atvb.ahajournals.org), TG continued to decline over the 8-hour period following apoA-V injection and was reduced approximately 87% at 8 hours. The DMPC control also showed a decline in TG by 8 hours but was significantly higher (P<0.01) than that of apoA-V treated mice. Using NIH ImageJ for evaluating relative intensity as a measure of apoA-V change over time, we found that 71.2±8.1%, 15.1±5.0%, and 3.0±0.7% apoA-V (Supplemental Figure II) remained in the plasma at 1, 4, and 8 hours, respectively, suggesting that apoA-V is rapidly cleared from the plasma and parallels the reduction of TG.

The above data suggest that apoA-V injection can promote VLDL clearance in apoav−/− mice. To examine this issue further, plasma lipoprotein and apolipoprotein profiles were determined in apoav−/− mice before (time 0) and 4 hours after apoA-V rHDL injection. ApoA-V rHDL administration induced a major (~60%) reduction in VLDL TG and cholesterol, indicating that the decrease in plasma TG and cholesterol observed earlier reflects enhanced clearance of VLDL (Figure 2A and 2B). The fact that no concomitant increase in LDL or HDL cholesterol occurred indicates that remnant particles derived from VLDL do not accumulate in the plasma. The effect of apoA-V rHDL administration on the distribution of plasma lipoproteins among lipoproteins was determined in apoav−/− mice before (time 0) and 4 hours after apoA-V rHDL injection. ApoA-V rHDL administration induced a major (~60%) reduction in VLDL TG and cholesterol, indicating that the decrease in plasma TG and cholesterol observed earlier reflects enhanced clearance of VLDL (Figure 2A and 2B). The fact that no concomitant increase in LDL or HDL cholesterol occurred indicates that remnant particles derived from VLDL do not accumulate in the plasma. The effect of apoA-V rHDL administration on the distribution of plasma lipoproteins among lipoproteins was then determined (Figure 2C). Similar to results obtained for plasma apoB-100, the amount of this protein in the VLDL fraction declined dramatically following apoA-V rHDL injection. By contrast, LDL apoB-100 was largely unaffected. After apoA-V rHDL injection, VLDL apoE levels decreased with a corresponding increase in HDL apoE content. VLDL apoA-I levels also decreased following apoA-V rHDL injection. Taken together, the data indicate VLDL clearance in apoav−/− mice increases following injection of apoA-V rHDL.
The distribution of exogenously administered apoA-V among lipoproteins was determined at 1 and 4 hours postinjection (Figure 2C). Whereas the preponderance of apoA-V was found associated with VLDL at 1 hour, after 4 hours, VLDL was nearly devoid of apoA-V. These data support the premise that injected apoA-V exchanges onto VLDL particles, where it functions to facilitate their catabolism and clearance.

**Dose-Response of apoA-V rHDL on TG-Lowering Activity**

To determine the effect of apoA-V dose on its TG-lowering activity, apoav<sup>−/−</sup> mice were injected with different amounts of apoA-V rHDL to reach plasma concentrations of 6.25, 12.5, or 25 μg/mL (time 0). Across this dose range, no differences in TG-lowering activity were observed, as shown in Supplemental Figure III. Thus, it may be concluded that the TG-lowering activity of apoA-V is saturated at plasma concentrations at or above 6.25 μg/mL, consistent with the exceptionally low concentration of apoA-V in plasma under physiological conditions.

**Effect of apoA-V rHDL on Postheparin LPL Activity**

Previous studies indicate that postheparin LPL activity in apoav<sup>−/−</sup> mouse plasma is low compared with WT mice. In addition, human carriers of an APOAV Gln139X mutation linked to severe HTG have reduced LPL activity. To evaluate whether exogenously administered apoA-V-mediated TG lowering is related to increased postheparin LPL activity, apoav<sup>−/−</sup> mice were injected with apoA-V rHDL followed by heparin injection 2 or 4 hours later. LPL activity measurements revealed no significant difference between apoA-V rHDL-injected and DMPC vesicle-injected mice (Supplemental Figure IV).

**Site-Specific Mutations in apoA-V Attenuate Its TG-Lowering Activity**

apoA-V contains a sequence element (amino acids 186 to 227) that lacks negatively charged residues and is enriched in positively charged amino acids. We have previously shown that this region is involved in apoA-V binding to heparin, LDL receptor family members, and GPIHBP1. Replacement of positively charged residues in this region of apoA-V with neutral or negatively charged amino acids (Mut-apoA-V) decreased its binding, in vitro, to heparin, LDL receptor–related protein and GPIHBP1. To examine effects on TG-lowering activity in vivo, Mut-apoA-V rHDL was injected into apoav<sup>−/−</sup> mice. Compared with WT apoA-V rHDL, the TG-lowering activity of Mut-apoA-V rHDL was attenuated by ~50% (Figure 3A), consistent with defective binding to heparin or GPIHBP1. The decreased TG-lowering activity of Mut-apoA-V was not due to its inability to bind to VLDL because, as seen in Figure 3B, comparable amounts of WT and Mut-apoA-V associated with VLDL. Unlike WT apoA-V, however, a higher proportion of Mut-apoA-V remained associated with VLDL after 4 hours, consistent with delayed clearance of these particles. Taken together, the data indicate that the positively charged sequence element in apoA-V is required for optimal manifestation of its TG-lowering activity in vivo.

**Effect of apoA-V rHDL Injection on Plasma TG Concentrations in gpihbp1<sup>−/−</sup> Mice**

Endothelial cell–bound GPIHBP1 plays a critical role in plasma TG homeostasis. Indeed, gpihbp1<sup>−/−</sup> mice have extremely high plasma TG concentrations and diminished lipolysis. To determine whether parenteral administration of apoA-V rHDL can lower plasma TG in gpihbp1<sup>−/−</sup> mice, injection studies were performed. Following administration of apoA-V rHDL, no significant changes in plasma TG concentration were observed, compared with control littermates injected with DMPC vesicles alone (Figure 4A). It is noteworthy that although apoA-V was found primarily associated with VLDL as early as 1 hour after injection into gpihbp1<sup>−/−</sup> mice (Figure 4B), apoA-V levels did not decrease as a function of time, as was the case in apoav<sup>−/−</sup> mice (compare Figure 2C). Taken together, the data suggest that GPIHBP1 is required for manifestation of the TG-lowering activity of apoA-V in vivo, as well as clearance of this apolipoprotein from the circulation.

**Discussion**

Apolipoproteins have remarkable properties in that they function as ligands for cell-surface receptors, modulators of lipid metabolic enzymes, and acceptors of cell lipids (eg, cholesterol). Some apolipoproteins, such as apoA-I, have been shown to have potential as therapeutic agents. Recombinant human apoA-I was used for treatment of atheromas in patients with acute coronary syndromes. In this case, a rare variant of human apoA-I, termed apoA-I<sub>Milano</sub>, was complexed with phospholipids, form-
Elevated plasma TG suggests that apoA-V therapy may be beneficial. ApoA-V variants in the general population are correlated with TG concentration and are expressed as mean ± SEM (n = 5 mice/group). A test indicated no significant difference between groups for each time point (P > 0.05). B, Plasma samples at 1 and 4 hours postinjection were pooled and subjected to FPLC; subsequently, apoA-V levels in each lipoprotein class were determined by Western blot.

Figure 4. Effect of apoA-V rHDL injection on TG and apoA-V metabolism in gphbp1−/− mice. gphbp1−/− mice were injected with 12.5 µg/mL apoA-V rHDL (open circles) or DMPC vesicles (filled circles). A. Plasma samples collected before injection and at 1, 2, and 4 hours postinjection were analyzed for TG, where the average TG concentration for mice at time 0 was 15.4 ± 0.8 mg/mL. Values are presented as percentage of the initial TG concentration and are expressed as mean ± SEM. B. Plasma samples at 1 and 4 hours postinjection were pooled and subjected to FPLC; subsequently, apoA-V levels in each lipoprotein class were determined by Western blot.

The apoA-V dose used in the current study was based on the reported average plasma concentration in APOAV transgenic mice, whereas mouse apoA-V was reported to be 24 ng/mL in WT C57BL/6 mice. Clearly, the very low levels of endogenous apoA-V in WT mice can efficiently clear newly formed TG-rich particles so that they do not accumulate in the plasma compartment. In the present study, 6.5 µg/mL apoA-V was just as effective in lowering TG in apoAV−/− mice as the 12.5 µg/mL dose. This suggests that apoA-V present in APOAV transgenic mice is functioning under saturation conditions and that concentrations lower than 6.25 µg/mL are likely sufficient to lower TG. A major difference between WT and apoAV−/− mice is that in the former case TG levels are low, whereas in the latter, TG is extremely elevated. It is likely that elevated plasma apoA-V in the form of exogenously delivered protein may be beneficial in clearance of TG in apoAV−/− mice, where TG accumulation is exaggerated and endogenous apoA-V is lacking. In the latter case, there would be no replenishment of apoA-V as it is cleared from the circulation together with TG. In WT mice, on the other hand, where there is a constant production of apoA-V to offset clearance of the protein along with TG, the low level of apoA-V is sufficient to maintain low levels of TG.

GPIHBP1 is an endothelial cell protein that is required for the lipolytic processing of TG-rich lipoproteins in plasma. In the absence of GPIHBP1, lipolysis of TG-rich particles is virtually abolished, leading to severe HTG in gphbp1−/− mice. Injection of apoA-V failed to lower plasma TG levels in gphbp1−/− mice, and apoA-V clearance was minimal, suggesting that these processes require interaction with GPIHBP1. To our knowledge, this is the first in vivo evidence suggesting that GPIHBP1 and apoA-V are functional partners in facilitating TG lipolysis.

In studies with Mut-apoA-V rHDL, we showed that mutation of key positively charged amino acids in the putative heparin-binding domain of apoA-V attenuates the TG-lowering effect of apoA-V in apoAV−/− mice. The clearance rate of the mutant protein was also slower than WT apoA-V. The decreased TG-lowering activity of the mutant was not due to a change in lipoprotein-binding ability because Mut-apoA-V, like WT apoA-V, was also found on VLDL at 1 and 4 hours postinjection. Taken together, the data suggest that the heparin-binding region of apoA-V plays an important role in its capacity to lower TG.

Previously, we proposed a mechanism whereby apoA-V could facilitate VLDL metabolism. Briefly, under conditions of increased TG, apoA-V exchanges from HDL onto VLDL, which in turn interacts with hepatic lipase and GPIHBP1 on the surface of endothelial cells (where LDL also binds). Coordination among apoA-V, LPL, and GPIHBP1 results in accelerated TG hydrolysis. Our current in vivo studies support this mechanism by showing that (1) apoA-V was able to rapidly exchange from rHDL onto VLDL, (2) the interaction between apoA-V and GPIHBP1 is critical for its TG-lowering function, and (3) the positively charged heparin-binding sequence element (residues 186 to 227) of apoA-V is required for this process. In addition, we also discovered that clearance of apoA-V from the circulation is minimal in the absence of GPIHBP1. Even though we cannot conclude that

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In summary, we show that intravenous delivery of apoA-V has a profound TG-lowering effect in apoav−/− mice. Given that the effective dose is exceptionally low, parenteral administration of apoA-V may have potential therapeutic value for treating severe HTG in humans.

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Disclosures
None.

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Intravenous Injection of Apolipoprotein A-V Reconstituted High-Density Lipoprotein Decreases Hypertriglyceridemia in apoav−/− Mice and Requires Glycosylphosphatidylinositol-Anchored High-Density Lipoprotein–Binding Protein 1

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Supplement Material

**Figure I. Effect of injected apoA-V rHDL on plasma TG over an 8 h period in apoav−/− mice.** An independent study was carried out with 5 mice per group to determine whether injection of apoA-V rHDL further reduces plasma TG concentration at 8 h. Mice (4 h fasted) were injected with apoA-V rHDL or DMPC and blood samples obtained after 1 min to establish baseline (initial) values and at 1, 4, and 8 h post-injection; mice had free access to water throughout this time period. The mean TG concentration for mice used in the study was 15.4±5.2 µg/ml. Values are presented as percentage of initial TG concentrations and expressed as mean ± SEM. As noted in this figure, TG continues to decline between 4 and 8 h such that there is an 87% decrease by 8 h. Although TG declined in DMPC treated mice between 4 and 8 h, TG reduction by apoA-V rHDL was significantly more effective; Student’s t-test versus respective controls: * p < 0.01, ** p < 0.001.

**Figure II. Changes in plasma apoA-V levels over time after injection of apoA-V rHDL.** The same mice used in Figure I were used to determine changes in plasma apoA-V levels 1, 4, and 8 h after injection of apoA-V rHDL. The initial apoA-V level was determined from blood samples drawn after 1 min (see Methods for details). Data are shown as percent of initial apoA-V. * p <0.05; ** p 0.001

**Figure III. Effect of apoA-V dose on TG-lowering ability.** Apoav−/− mice were injected at t = 0 with different doses (25 µg/ml, open triangles; 12.5 µg/ml, open circles; 6.25 µg/ml, open squares) of apoA-V rHDL as indicated (>8 mice/group). Plasma samples collected before (t = 0) and at 1, 2, and 4 h post-injection were analyzed for
TG; initial TG, 19.4 ± 2.0 mg/ml. Values are presented as percentage of the initial TG level at t = 0 and expressed as mean ± SEM.

**Figure IV. Effect of apoA-V rHDL injection on postheparin LPL activity.** *Apoav−/−* mice were injected with 12.5 µg/ml apoA-V rHDL (open bars) or DMPC vesicles (filled bars) followed by heparin injections (50 U/mouse) at 2 or 4 h. Plasma samples were collected 15 min postheparin. LPL activity was determined as described in the Methods and is presented as Relative Fluorescence Unit (RFU) per minute (mean ± SEM). Student’s t-test indicated no significant differences between groups at either time points (p > 0.05, n = 6 mice/group).
Figure 1

% Initial TG vs. Time (h)

- Solid line with circles represents a group marked with an asterisk (*).
- Dotted line with squares represents a group marked with an asterisk and a double asterisk (**).

The graph shows the decrease in % Initial TG over time, with significant differences indicated by the asterisks.
Figure II

% Initial apoA-V

0 10 20 30 40 50 60 70 80 90 100

1h 4h 8h

* **
Figure III

- Graph showing % Initial TG over Time (h) from 0 to 4 hours.