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Intravenous injection of apoA-V reconstituted HDL decreases hypertriglyceridemia in *apoav-/-* mice and requires GPIHBP1

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Abstract

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 HDL (rHDL) has potential utility as a therapeutic agent for treatment of hypertriglyceridemia when delivered intravenously.

Methods and Results—Intravenous injection studies were performed in genetically engineered mouse models of severe hypertriglyceridemia 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 h. This decline can be attributed to enhanced catabolism/clearance of VLDL where VLDL TG and cholesterol were reduced ~60%. ApoA-V which associated with VLDL after injection was also rapidly cleared. Site-specific mutations in the heparin-binding region of apoA-V (amino acids 186-227) attenuated apoA-V rHDL TG-lowering activity by 50% suggesting 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.

Keywords

apoav-/- *mice*; *gpihbp1*-/- mice; very low density lipoproteins; apoA-V heparin binding mutant; lipoprotein lipase

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.

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Following its discovery in 2001 [5, 6], apolipoprotein (apo) A-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 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, while the concentration in *APOAV* transgenic mice is one-third that in wild-type control littermates. Furthermore, studies in humans revealed an association between truncation mutations in apoA-V and severe HTG.[11-13]. These data strongly suggest 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 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]

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 (GPIHBP1) binds the positively charged, heparin-binding domain of LPL [22, 23] *via* its Ly6 domain and a negatively charged region in its amino terminus. In the absence of GPIHBP1, lipolysis is substantially diminished and plasma TG levels are markedly elevated. It has been postulated that GPIHBP1 serves as a platform that supports lipolytic activity. Interestingly, apoA-V also binds to GPIHBP1, 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 and VLDL confers heparin-binding capability.[19] Mutations in the positively charged sequence element of apoA-V result in 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 (HSPG) or GPIHBP1 and that such interactions enhance lipolysis.

In this report, we evaluate the potential utility of apoA-V as a TG-lowering therapeutic agent. Intravenous injection of apoA-V–containing reconstituted HDL (rHDL) significantly lowered plasma TG concentrations in *apoav*−/− mice yet had no effect in *gpihbp1*−/− 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, GPIHBP1, 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 4–20% NuPAGE 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-*O*-dilauryl-rac-glycero-3-glutaric acid-(6′-methylresorufin) ester (DGGR), was from Sigma.

Animals

Previously described [9, 22] male mice (*apoav*−/− and *gpihbp1*−/−), 2–4 months of age were used in these studies. Research was conducted in conformity with the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and was approved by Animal Use Committees at Children's Hospital Oakland Research Institute and University of California, Los Angeles.

Preparation and injection apoA-V rHDL

Human wild type apoA-V recombinant protein was prepared as described [24]; the apoA-V mutant with changes in its positively charged sequence element (residues 186-227; R210E/K211Q/K215Q/K217E) was previously described.[23] Since apoA-V is not soluble at pH 7.4, the protein was complexed with dimyristoylphosphatidyl choline (DMPC) for injection [24]; 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 are discoidal particles ~14 nm diameter; by native polyacrylamide gel electrophoresis analysis, complexes were 12 - 17 nm 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 h and blood samples were obtained by submandibular vein bleeds before, t = 0, and at 1, 2 and 4 h post-injection. 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 *APOAV* transgenic mice [9]). 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 Superose 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 4–20% Bis-Tris gradient gels. The size-separated proteins were transferred to PVDF membranes, and immunoblots processed as described.[24] In one experiment, clearance of apoA-V with time was determined by administrating 12.5 μg/ml apoA-V rHDL and sampling plasma at 1 min post-injection for baseline plasma apoA-V levels and at 1, 4, and 8 h post-injection. Following electrophoresis on 4–20% gels and western blotting, relative changes in plasma apoA-V compared to baseline were determined by densitometry using the NIH ImageJ program.

Measurement of postheparin LPL activity

Apoav−/− mice were injected *via* tail vein with 50 μl heparin (50 units) 2 or 4 h after injection with apoA-V rHDL or DMPC vesicles alone. Before and 15 min after heparin injection, blood samples were collected and plasma separated. LPL activities in the plasma samples were determined with a fluorometric assay as described.[25] Briefly, the lipase activity in the plasma

sample was measured as the rate of fluorescence generated from hydrolysis of the lipase substrate DGGR. Two min after mixing plasma sample with DGGR, fluorescent intensity was monitored for 5 min, and lipase activity was calculated as relative fluorescent units generated per min (RFU/min). 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, apoA-V rHDL was injected into *apoav−/−* mice to achieve a plasma concentration of 12.5 μg/ml, which is the average concentration of apoA-V in *APOAV* transgenic mice.[9] Compared to control mice injected with DMPC vesicles alone, apoA-V rHDL induced a 25% reduction in TG after 1 h and a 60% reduction at 4 h (Figure 1A). In controls, there was a slight reduction (~20%) in plasma TG concentration after 4 h. 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 h 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 (n=5) were injected with 12.5 μg/ml apoA-V rHDL and plasma sampled after 1 min to establish baseline values; subsequent plasma samples were obtained at 1, 4, and 8 h post-injection. As noted in supplement Figure I, TG continues to decline over the 8 h period following apoA-V injection and is reduced approximately 87% at 8 h. The DMPC control also shows a decline in TG by 8 h but is 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 (supplement Figure II) remained in the plasma at 1h, 4 h and 8 h, 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 h 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 B). 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 apolipoproteins among different lipoprotein fractions 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 h and 4 h post-injection (Figure 2C). Whereas the preponderance of apoA-V was found associated with VLDL at 1 h, after 4 h, 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*−/− 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 supplement 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 postheparin LPL activity in *apoav*−/− mouse plasma is low compared to wild-type mice.[14] Additionally, human carriers of an *APOAV* Q139X mutation linked to severe HTG have reduced LPL activity [11]. To evaluate if exogenously administered apoA-V-mediated TG lowering is related to increased postheparin LPL activity, *apoav*−/− mice were injected with apoA-V rHDL followed by heparin injection 2 or 4 h later. LPL activity measurements revealed no significant difference between apoA-V rHDL–injected and DMPC vesicle-injected mice (supplement Figure IV).

Site-specific mutations in apoA-V attenuate its TG-lowering activity

ApoA-V contains a sequence element (amino acids 186–227) that lacks negatively charged residues and is enriched in positively charged amino acids.[19] We have previously shown that this region is involved in apoA-V binding to heparin, LDL receptor family members, and GPIHBP1.[19,20,23] Replacement of positively charged residues in this region of apoA-V with neutral and/or negatively charged amino acids (Mut-apoA-V) decreased its binding, *in vitro*, to heparin, low density lipoprotein receptor-related protein and GPIHBP1. To examine effects on TG-lowering activity *in vivo*, Mut-apoA-V rHDL was injected into *apoav*−/− mice. Compared to 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 and/or GPIHBP1. The decreased TG-lowering activity of Mut-apoA-V was not due to its inability to bind to VLDL since, 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 h, 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*−/− mice

Endothelial cell bound GPIHBP1 plays a critical role in plasma TG homeostasis [22]. Indeed, *gpihbp1*−/− mice have extremely high plasma TG concentrations and diminished lipolysis. To determine if parenteral administration of apoA-V rHDL can lower plasma TG in *gpihbp1*−/− 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 h after injection into *gpihbp1*-/- mice (Figure 4B), apoA-V levels did not decrease as a function of time as was the case in *apoav*−/− mice (compare Figure 2C). Taken together, the data suggest that GPIHBP1 is required for manifestation of apoA-V's TG-lowering activity *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.[27] In this case, a rare variant of human apoA-I, termed apoA-I_{Milano}, was complexed with phospholipids, forming rHDL that were infused into patients; treatment resulted in significant reduction in atheroma burden.

Studies of apoA-V indicate that it is a potentially useful candidate for *in vivo* therapeutic applications. The severe HTG observed in human subjects harboring truncation mutations [11, 13], as well as the strong correlation between coding and noncoding *APOAV* SNPs and elevated plasma TG [28-30] suggest apoA-V therapy may be beneficial. ApoA-V variants in the general population are quite prevalent. Indeed, a recent analysis of multiple studies on TG elevation in cardiovascular disease suggest that the -1131T>C allele, known to be correlated with TG concentrations, has an allele frequency of 8% [31]; furthermore, Pullinger at al. [30] showed that the c.553G>T minor allele associated with elevated TG in unrelated Chinese-Americans has a frequency of 4.5%. Like apoA-I, apoA-V readily forms rHDL that can be injected intravenously. In the current study, we demonstrate that injected apoA-V rHDL has the capacity to significantly reduce plasma TG concentrations in hypertriglyceridemic *Apoav*−/− mice.

Although it is present in plasma at exceeding low concentrations, apoA-V is an important modulator of plasma TG.[5, 7] *Apoav*−/− mice exhibit elevated TG while *APOAV* transgenic mice have reduced plasma TG.[5,9] In addition to increased TG concentrations, diminished lipolysis of VLDL and a reduced rate of remnant uptake were observed in *apoav*−/− mice.[14] VLDL particles from *apoav*−/− mice are a poor substrate for LPL and have low binding to LDLR family members. In the current study, we show that parenteral delivery of apoA-V into *apoav*−/− mice lowered plasma TG concentrations, and also reduced VLDL TG, cholesterol, and apoB-100 levels. These data indicate apoA-V injection improves VLDL catabolism in *apoav*−/− mice.

The apoA-V dose employed 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 wild-type (WT) C57BL/6 mice[10]. 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 *apoa5*-/- 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.[22] In the absence of GPIHBP1, lipolysis of TG-rich particles is virtually abolished, leading to severe HTG in *gpihbp1*−/− mice. Injection of apoA-V failed to lower plasma TG levels in *gpihbp1*−/− mice while 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 show 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 h post-injection. 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.[7] Briefly, under conditions of increased TG, apoA-V exchanges from HDL onto VLDL, which in turn interacts with HSPG and GPIHBP1 on the surface of endothelial cells (where LPL also binds). Coordination between apoA-V, LPL, and GPIHBP1 results in accelerated TG hydrolysis. Our current *in vivo* studies support this mechanism by showing: (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; (3) the positively charged heparin binding sequence element (residues 186-227) 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 apoA-V is directly cleared through GPIHBP1, it is likely that apoA-V's heparin-binding region is essential for its interaction with GPIHBP1, which in turn, is essential for TG lipolysis.

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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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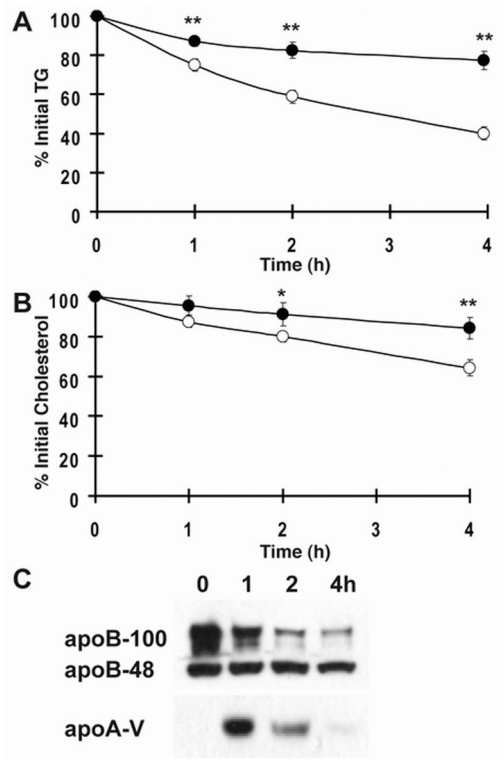

Figure 1.

Effect of apoA-V rHDL injection on plasma lipids and apolipoprotein levels in *apoav*−/− mice. *Apoav*−/− mice were injected with 12.5 μg/ml apoA-V rHDL (open circles) or DMPC vesicles (filled circles). Plasma samples were collected before and at 1, 2, and 4 h post-injection and analyzed for (A) TG and (B) cholesterol; 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 TG concentrations and expressed as mean ± SEM (>12 mice/group). Student's *t*-test *versus* respective controls: $*p < 0.05$; $**p < 0.001$. (C) ApoB and apoA-V levels in the plasma samples (1μl) of individual mice were determined by western blot. Results are representative of >12 mice.

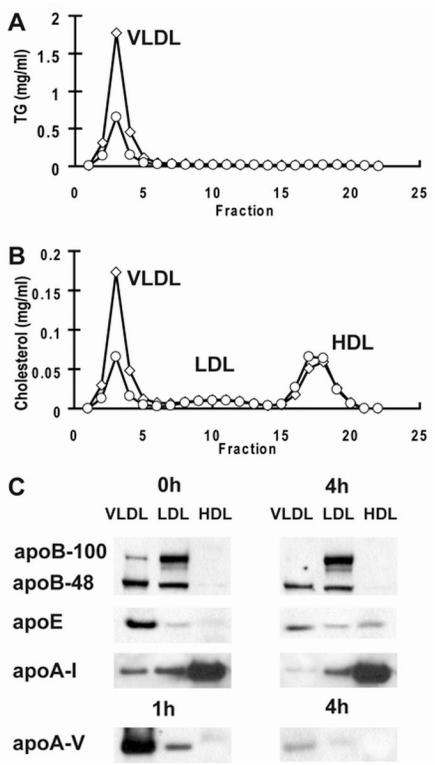

Figure 2.

Effect of apoA-V rHDL injection on the distribution of plasma lipids and apolipoproteins among lipoproteins. *Apoav*−/− mice ($n = 12$; mean initial TG concentration, 14.6 ± 1.9 mg/ml) were injected with 12.5 μg/ml apoA-V rHDL at t = 0. Plasma samples were collected before (open triangles) and 4 h post-injection (open circles), pooled and subjected to FPLC as described in the *Materials and Methods*. (A) TG and (B) cholesterol concentrations in each fraction were determined. (C) Fractions representing VLDL, LDL, and HDL were pooled and apolipoprotein levels in each lipoprotein class were determined by western blot. Results are representative of two independent experiments.

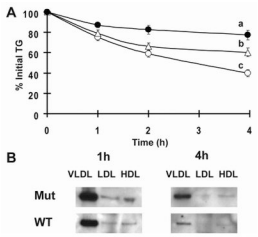

Figure 3.

Effect of mutations within the heparin binding region of apoA-V on TG-lowering ability. *Apoav* −/− mice were injected with 12.5 μg/ml apoA-V rHDL (open circles), Mut-apoA-V rHDL (open triangles), or DMPC vesicles (filled circles) (>7 mice/group); mean starting TG concentration was 19.8 ± 1.4 mg/ml. (A) Plasma samples collected before (t = 0) and at 1, 2, and 4 h post-injection were analyzed for TG. Values are presented as percentage of the initial TG concentration and expressed as mean ± SEM. One-way analysis of variance was used to test for significance followed by *post hoc* analysis (Tukey-Kramer HSD) to examine differences between groups. There was a significant difference between group a, b, and c, $p < 0.05$ at 4 h. (B) Plasma samples at 1 and 4 h post-injection were pooled and subjected to FPLC. ApoA-V level in each lipoprotein class was determined by western blot. Results are representative of two independent experiments.

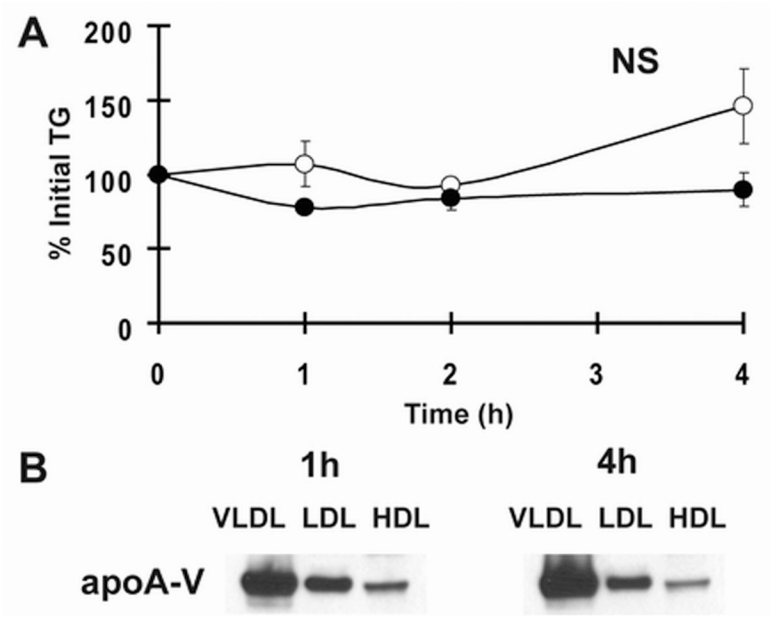

Figure 4.

Effect of apoA-V rHDL injection on TG and apoA-V metabolism in *gpihbp1*−/− mice. *Gpihbp1*−/− mice were injected with 12.5 μg/ml apoA-V rHDL (open circles) or DMPC vesicles (filled circles). (A) Plasma samples collected before and at 1, 2, and 4 h post-injection 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 expressed as mean ± SEM (n = 5 mice/group). Student's t-test indicated no significant difference between groups for each time point ($p > 0.05$). (B) Plasma samples at 1 and 4 h post-injection were pooled and subjected to FPLC; subsequently, apoA-V levels in each lipoprotein class were determined by western blot.