Apolipoprotein AV Accelerates Plasma Hydrolysis of Triglyceride-rich Lipoproteins by Interaction with Proteoglycan-bound Lipoprotein Lipase*

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Apolipoprotein A5 (APOA5) is associated with differences in triglyceride levels and familial combined hyperlipidemia. In genetically engineered mice, apoAV plasma levels are inversely correlated with plasma triglycerides. To elucidate the mechanism by which apoAV influences plasma triglycerides, metabolic studies and in vitro assays resembling physiological conditions were performed. In human APOA5 transgenic mice (hAPOA5tr), catabolism of chylomicrons and very low density lipoprotein (VLDL) was accelerated due to a faster plasma hydrolysis of triglycerides by lipoprotein lipase (LPL). Hepatic VLDL and intestinal chylomicron production were not affected. The functional interplay between apoAV and LPL was further investigated by cross-breeding a human LPL transgene with the apoA5 knock-out and the hAPOA5tr to an lpl-deficient background. Increased LPL activity completely normalized hypertriglyceridemia of apoA5-deficient mice; however, overexpression of human apoAV modulated triglyceride levels only slightly when LPL was reduced. To reflect the physiological situation in which LPL is bound to cell surface proteoglycans, we examined hydrolysis in the presence or absence of proteoglycans. Without proteoglycans, apoAV derived either from triglyceride-rich lipoproteins, hAPOA5tr, high density lipoprotein, or a recombinant source did not alter the LPL hydrolysis rate. In the presence of proteoglycans, however, apoAV led to a significant and dose-dependent increase in LPL-mediated hydrolysis of VLDL triglycerides. These results were confirmed in cell culture using a proteoglycan-deficient cell line. A direct interaction between LPL and apoAV was found by ligand blotting. It is proposed, that apoAV reduces triglyceride levels by guiding VLDL and chylomicrons to proteoglycan-bound LPL for lipolysis.

Plasma triglyceride (TG) levels are an important independent risk factor for cardiovascular disease susceptibility with both genetic and environmental determinants (1). Among the genetic factors, the contribution of variations at the APOA1/C3A4/A5 locus to the determination of TG levels has been well defined (2, 3). APOA5, the newest member of the apolipoprotein family, is located at this locus 27 kb distal (3) to APOA4. It was discovered 3 years ago independently by comparative sequencing (4) and by differential display as a liver regeneration protein (5). Single nucleotide polymorphisms in the APOA5 gene have been associated with differences in human plasma TG levels, familial combined hyperlipidemia, and increased risk of cardiovascular disease (4, 6–8). Mice expressing a human APOA5 transgene had one-third lower plasma TG levels, whereas knock-out mice lacking apoA5 had four times higher plasma TG levels (4). These data indicate that apoAV plays a major role in the regulation of TG metabolism.

However, the molecular mechanism underlying the influence of apoAV on plasma TG is currently not understood. Because apoAV is an apolipoprotein with high lipid affinity and low elasticity, it has been proposed that apoAV may impair the second step of VLDL assembly by binding to lipids and cellular membranes and therefore reduce liver VLDL production (9). In wild-type mice with adenoviral overexpression of apoA5, a decreased production rate of VLDL TG has been described (10). On the other hand, apoAV could serve as a receptor ligand or bridge TG-rich lipoproteins to heparan sulfate proteoglycans (HSPGs), thereby enhancing the uptake of remnant particles into tissues. In addition, apoAV could accelerate plasma turnover of TG-rich particles. This could happen by a direct activation of lipoprotein lipase (LPL) or by affecting the concentration or function of other apolipoproteins, e.g. apoCIII. It was found recently that mice with transgenic or adenoviral expression of apoAV have faster VLDL-TG turnover rates and that postprandial TG response after lipid load is markedly reduced in these animals (10, 11). Both groups suggested an effect of apoAV on LPL-mediated plasma TG hydrolysis. However, the mechanism and possible involvement of other lipoproteins in this process remain unclear.

Because apoCIII and apoAV have a directly opposing impact on plasma TGs, it has been proposed that the effects of apoAV could be mediated by apoCIII because genetic modifications of mice at the apoA1/C3A4/A5 locus frequently result in changes

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The abbreviations used are: TG, triglyceride; apo, apolipoprotein; LPL, lipoprotein lipase; THL, tetrahydrolipstatin; FFA, free fatty acid; VLDL, very low density lipoprotein; HDL, high density lipoprotein; HSPG, heparan sulfate proteoglycan; PBS, phosphate-buffered saline; CHO, Chinese hamster ovary.

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of neighboring gene expression (12, 13). A possible effect of apoAV on apoCIII levels is further supported by the fact that human APOA5 transgenic (hAPOA5tr) mice had 40% decreased plasma levels of apoCIII, and apoA5-deficient mice (apoA5−/−) had 90% increased plasma levels of apoCIII (4). However, in vitro studies (10) and experiments on mice doubly deficient or transgenic for apoC3 and apoA5 (APOC3 and APOA5) suggested that the apoAV effect on TG levels is at least in part independent of apoCIII (14).

The aim of this study was to provide a model for the mechanism of apoAV action. Having ruled out effects of altered lipoprotein synthesis by metabolic studies in various genetically engineered mice, we used an assay employing lipoproteins isolated from these different genotypes and LPL, physiologically immobilized on HSPG, to demonstrate a profound apoAV-dependent difference in TG hydrolysis. Taken together, our findings prompt us to propose a model in which apoAV targets TG-rich lipoproteins to HSPG-bound LPL in the extracellular matrix, enhancing the efficiency of TG-rich lipoprotein hydrolysis.

EXPERIMENTAL PROCEDURES

Animal Breeding—The origin of the different mouse lines is as follows: apoA5-deficient (apoA5−/−) and APOA5 transgenic (hAPOA5tr) mice were as described previously (4); mice with muscle-specific transgenic expression of human LPL (hLPLtr) were from R. Zechner (Graz, Austria) (Mck-LPL, Ref. 15), and mice heterozygous for the mouse lpl gene (LPL+/−; Ref. 16) were from J. L. Breslow (New York, NY). Mice were transferred to a specific pathogen-free facility by embryo transfer at Hamburg University. They were fed a regular chow diet containing 4.5% of energy from fat with free access to food and water. Animal experiments were done in accordance with the guidelines of the Federation of American Societies for Experimental Biology and approved by the Department of Veterinary Affairs of the State of Hamburg. According to their respective backgrounds, hAPOA5tr and apoA5−/− mice were bred with FVB mice, and hLPLtr and lpl+/− mice were bred with C57BL/6 mice. To obtain apoA5−/− mice with transgenic overexpression of human LPL, the hLPLtr line was crossed two times to the apoA5-deficient background. The final cross was apoA5 heterozygote (apoA5−/+ × apoA5−/−) hLPLtr. To obtain lpl-deficient mice with transgenic overexpression of human APOA5, the hAPOA5tr line was crossed two times to the lpl+/− background. The final cross was lpl+/− × lpl+/− hAPOA5tr. Genotyping of Mutant Mice—Genotypes were determined by double PCRs from tail tip DNA. PCRs for hAPOA5tr and apoA5−/− mice lines were reported previously (4). To determine the genotype at the mouse apoA5 locus, a probe-specific PCR (Ref. 10) for the hLPL transgene was performed as described in Ref. 17.

Lipid and Lipoprotein Analysis—Blood was taken after 6 h of day-time fasting by puncture of the retro-orbital plexus. Plasma TGs and cholesterol were determined using commercial kits (Roche Diagnostics, Mannheim, Germany), which were adapted to microtitration plates. Lipoproteins were separated by sequential ultracentrifugation (18) using 60 μl of plasma from individual mice, and TGs and cholesterol were determined in the fractions. To analyze the lipoprotein profile, pooled mouse plasma was separated by fast protein liquid chromatography gel filtration on a Superose 6 column (Amersham Biosciences). Protein concentrations were analyzed by using a BCA test kit (Pierce).

Lipoprotein Production Studies—Anesthetized male mice received intravenous injection of 500 μg/kg Triton WR 1339 (tyloxapol; Sigma) using a 10% (w/v) solution. After 2 min, for hepatic VLDL production, 100 μCi of [2-3H]glycerol (Amersham Biosciences) were added to radiolabeled VLDL prior to injection.

To determine LPL activity, 1 ml of plasma from individual mice, and TGs and cholesterol were determined in the fractions. To analyze the lipoprotein profile, pooled mouse plasma was separated by fast protein liquid chromatography gel filtration on a Superose 6 column (Amersham Biosciences). Protein concentrations were analyzed by using a BCA test kit (Pierce).

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To compare hydrolysis of TG-rich particles by HSPG-bound LPL with hydrolysis rates of free LPL (liquid phase), all assays were performed in parallel with unbound LPL. For this, 96-well microtiter plates were blocked with PBS containing 1% FFA-free bovine serum albumin for 1 h at 37 °C. After washing three times with 0.1% Tween, the wells were washed again three times with 0.1%Tween, pH 8.5. VLDL was isolated from apoA5−/− or hAPOA5tr mice and adjusted to 1 mM TG concentration. Lipolysis was started by the addition of VLDL to the LPL-containing plates. In some cases, lipoproteins were incubated before hand with recombinant human apoA5, bovine LPL, or HDL isolated from apoA5−/− or hAPOA5tr mice, respectively. In some experiments, these incubated VLDL particles were re-isolated by ultracentrifugation before lipolysis. Lipolysis was stopped after 10 min by the addition of Triton X-100 (1% final concentration). FFA concentrations were measured by using a commercial NEFA C Kit (WAKO Chemicals) adapted to 96-well microtiter plates. As a control, HSPG-coated plates were incubated with LPL as described and then treated with a solution of 2.4 units/ml heparinase I and heparinase III (both from Sigma), respectively.

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produced as described above was incubated with various amounts of LPL. The reaction was stopped, and FFAs were measured as described.

Hydrolysis of VLDL on CHO Cells—CHO-K1 cells expressing HSPG and HSPG-deficient CHO-745 cells (25) were seeded onto 24-well cell culture plates (75,000 cells/well). After washing twice with ice-cold PBS, cells were incubated with and without different amounts of LPL at 4 °C for 60 min. Non-bound LPL was removed by extensive PBS washes before the in vitro hydrolysis was started. Experiments were identically performed as described above with Tris-containing buffers replaced by PBS. After the reaction, the content of every well was transferred into a reaction tube containing Triton X-100 (1% final concentration), and FFAs were determined.

Statistical Analysis—Unless otherwise stated, results are given as mean ± S.D. Statistical significance was tested using a two-tailed Student's t test or, if more than two groups were compared, with one-way analysis of variance with Tukey's Multiple Comparison Post Test. Analysis of turnover studies was carried out using the computer program Prism 4 (GraphPad Software, San Diego, CA).

RESULTS
Chylomicron and VLDL Production and Turnover Studies—To test whether apoAV-mediated reduction in TG was the result of the decreased production of TG-rich lipoproteins, intestinal production of chylomicron and hepatic production of VLDL were measured. For chylomicron production, wild-type and hAPOA5tr mice were gavaged with glycerol tri[1-14C]oleate. Plasma TG (circles) and radioactivity (triangles) indicating synthesized particles were determined at different time points. B, for hepatic VLDL production, 2 min after tyloxapol injection, radiolabeled glycerol was injected into hAPOA5tr and wild-type mice (6 mice/group). Plasma TG was measured (circles). [3H]Glycerol incorporated in newly synthesized TG was measured after lipid extraction (triangles). C, turnover study in hAPOA5tr and wild-type mice (7 mice/group) with human chylomicrons labeled with 125I-tyramine cellobiose. D, plasma clearance of murine VLDL from apoA5−/− mice labeled with either [3H-TG (circles) or 125I-tyramine cellobiose (triangles) in hAPOA5tr and wild-type mice (6 mice/group). Plasma removal of VLDL followed a one-phase exponential decay with significantly shorter half-life in hAPOA5tr compared with wild-type mice. E, to dissect apoAV and LPL activity during VLDL turnover, the experiment shown in D was repeated with (squares) and without (circles) prior injection of the LPL inhibitor THL (6 mice/group). Data are presented as percentage of the 2 min plasma radioactivity. F, VLDL from apoA5−/− mice was labeled in vivo with [3H-TG and incubated with (final concentration, 2 μg/ml after injection) and without recombinant apoAV. Turnover experiments were carried out as described in D with 5 mice/group. All data are mean ± S.D. Student's t test versus respective control: *, p < 0.05; **, p < 0.01; ***, p < 0.001.
by transgenic apoAV, plasma catabolism of these particles was measured. Experiments using \(^{125}\)I-tyramin cellobiose-labeled human chylomicrons showed that chylomicron particles were removed significantly faster from plasma in hAPOA5tr mice compared with wild-type littersmate (Fig. 1C). Organ uptake revealed a significant increase in uptake into adipose tissue and a nonsignificant trend to higher uptake into the muscle and liver of hAPOA5tr mice (data not shown). To analyze VLDL plasma clearance, apoAV-deficient VLDLs were labeled at the TG portion \(\text{in vivo}\) by injecting \(\text{[1-}^{3}\text{H]palmitic acid into apoA5-deficient mice. After isolation, VLDL apolipoproteins were labeled \(\text{in vitro}\) with \(^{125}\)I-tyramin cellobiose. In both wild-type and hAPOA5tr mice, plasma removal of VLDL TG followed a one-phase exponential decay with significantly shorter half-life in hAPOA5tr compared with wild-type mice (Fig. 1D, circles: wild-type mice, 3.8 ± 0.6 min; hAPOA5tr mice, 2.5 ± 0.8 min; \(p < 0.01\)). An organ uptake study completed after 10 min revealed that hAPOA5tr mice had a significantly increased uptake of VLDL fatty acids into muscle (\(+40\%\), heart (\(+150\%\)), and adipose tissue (\(+95\%\)). Plasma removal of \(^{125}\)I-tyramin cellobiose VLDL particles was significantly slower than TG removal but was also faster in hAPOA5tr mice than in wild-type mice (Fig. 1D, triangles: wild-type mice, 6.7 ± 2.0 min; hAPOA5tr mice, 4.2 ± 1.0 min; \(p < 0.01\)). ApoAV-mediated acceleration of removal of TG was seen earlier (greatest differences were seen after 2 and 5 min) than that of \(^{125}\)I-tyramin cellobiose particles (greatest differences were seen after 5–20 min). These data show that hAPOA5tr mice have a faster plasma hydrolysis of TG-rich particles and a more rapid organ uptake of remnant particles.

To investigate whether apoAV can accelerate VLDL turnover independently of plasma LPL hydrolysis, the LPL inhibitor THL and, after 2 min, \(\text{in vivo}\) labeled apoAV-deficient VLDL were injected into wild-type and hAPOA5tr mice. As seen above, VLDL catabolism was faster in hAPOA5tr mice compared with wild-type mice without THL (Fig. 1E, circles). However, after administration of THL, catabolism of VLDL TG as well as the differences between the genotypes was completely abolished (Fig. 1E, squares). Furthermore, to test whether the apoAV-mediated acceleration of plasma TG hydrolysis \(\text{in vivo}\) was really due to apoAV and not the result of other changes in other lipoprotein composition, VLDL from apoA5–/– mice was labeled \(\text{in vivo}\) with \(^{3}\text{H-TG}\) and incubated with PBS or 30 \(\mu\text{g/ml}\) recombinant apoAV, and these particles were then injected into apoA5–/– mice. The turnover of VLDL particles supplemented with apoAV was significantly faster compared with apoAV-deficient VLDL in these mice (Fig. 1F).

Taken together, apoAV did not influence synthesis of chylomicrons and VLDL but accelerated catabolism of both lipoproteins. ApoAV triggered these effects independently of other apolipoproteins but required active LPL.

Interaction of apoAV and LPL \(\text{in Vivo}\)—Because the effect of apoAV on the catabolism of TG-rich lipoproteins turned out to be dependent on LPL, interactions between apoAV and LPL were investigated by cross-breeding apoA5–/– mice with LPLtr mice. From the final cross (apoA5–/– × apoA5+/– LPLtr, all expected genotypes were obtained (apoA5–/–, apoA5+/–, and apoA5+/+; each without and with transgenic hLPL, respectively). LPL was able to decrease plasma TG by 69\% (Fig. 2A) and VLDL TG by 78\% (Fig. 2B) on the apoA5–/– background. This TG-lowering effect of LPL was less pronounced, although highly significant, on the apoA5+/– and apoA5+/+ (wild-type) backgrounds.

To produce LPL-deficient mice with transgenic overexpression of human apoAV, the hAPOA5tr line was crossed onto the LPL-deficient background. From the final cross (apoA5–/– × apoA5+/– hLPLtr), all apoA5–/– mice died within 24 h, regardless of apoAV expression. All other genotypes (apoA5+/–, apoA5+/– hAPOA5tr, apoA5+/+ and apoA5+/+ hAPOA5tr) were present as expected. On both the apoA5–/– and apoA5+/+ backgrounds, apoAV significantly decreased plasma TG (by 31\% and 22\%; Fig. 2C) and VLDL TG (by 29\% and 28\%; Fig. 2D).

To test the hypothesis that apoAV increases the catabolism of TG-rich lipoproteins by enhancing LPL activity \(\text{in vivo}\), pre- and post-heparin LPL activity were measured in different apoA5 genotypes. There were no changes in pre- or post-heparin LPL activity among apoA5–/–, apoA5+/–, apoA5+/+, and hAPOA5tr mice (Fig. 3A). These emulsion-based lipolytic activity measurements were validated by control experiments (Fig. 3B), in which lpl–/–, lpl+/–, and LPLtr mice showed the expected changes in post-heparin LPL activity.

Taken together, these data suggest that increased LPL activity completely compensated for apoA5 deficiency, whereas human apoAV overproduction only slightly modulated TG, even if LPL activity was decreased. This suggests that LPL plays the primary role in plasma hydrolysis, but this process can be modulated by apoAV. However, there is no evidence for a direct activation of LPL by apoAV in post-heparin plasma.
Because LPL is detached from HSPG after heparin treatment, post-heparin plasma does not resemble the physiological environment of LPL at the vascular endothelium. To analyze the potential interaction between apoAV and LPL on a matrix mimicking the endothelium, a previously described assay (24) to measure lipolytic activity in a system that was composed of free or HSPG-bound LPL was adapted. In the first set of experiments, VLDLs from hAPOA5tr and apoa5−/− mice were used to further approximate the physiological situation. These lipoproteins, which contained abundant or no apoAV, were not hydrolyzed at different rates by a broad range (three powers of 10) of unbound LPL concentrations (Fig. 4A). In contrast, LPL bound to HSPG hydrolyzed VLDL much more efficiently in the presence of apoAV (66% and 47% faster compared with apoAV-deficient VLDL at intermediate LPL concentrations, respectively; Fig. 4B). Remarkably, at high LPL concentrations, these differences disappeared. To address the question of whether these apoAV-mediated changes were a consequence of a direct physical interaction between apoAV and LPL, ligand blotting experiments were performed. In contrast to apoE, recombinant human apoAV was able to bind to immobilized LPL, and vice versa, LPL was detected on immobilized apoAV (Fig. 5).

Combining the idea of apoAV affecting lipolysis mediated by HSPG-bound LPL with the transfer of apoAV from HDL to TG-rich lipoproteins during the postprandial phase (11), apoAV-deficient VLDL was first incubated with HDL from hAPOA5tr, wild-type, and apoa5-deficient mice. Then, VLDL hydrolysis, mediated by free and HSPG-bound LPL, was determined. Again, at various concentrations of unbound LPL, VLDL particles incubated with the different HDL preparations were hydrolyzed with similar rates (Fig. 6A). In contrast, LPL bound to HSPG hydrolyzed VLDL incubated with human apoAV containing HDL up to 65% faster than VLDL incubated with apoAV-deficient HDL (Fig. 6B). VLDL incubated with wild-type HDL was hydrolyzed at intermediate rates. The changes in VLDL hydrolysis were the same when VLDL was re-isolated by ultracentrifugation prior to the lipolysis reaction.
In another set of experiments, VLDL was separated from HDL by ultracentrifugation before incubation with free LPL (C) or with LPL immobilized on HSPG (D). Data are mean ± S.D. (n = 3). One-way analysis of variance was used to test for significant changes between hydrolysis rates with HDL from different genotypes at a specific LPL concentration (a, p < 0.05; ***, p < 0.01; ****, p < 0.001). Tukey post test in B: a), at all LPL concentrations, hydrolysis rates with HDL from hAPOA5tr and wild-type differ significantly from apoA5−/− HDL, but not from each other. Tukey post test in D, at all LPL concentrations, hydrolysis rates with different HDL are significantly different from each other, except for the following: b) no difference between HDL from wild-type and apoA5−/−; and c) no difference between HDL from wild-type and hAPOA5tr. E, after hydrolysis, VLDL was re-isolated and analyzed for the presence of apolipoproteins by Western blot (Fig. 6C and D). Transfer of human apoAV from HDL to VLDL was confirmed by Western blot (Fig. 6E). Because the observed changes could also be the result of transfer of apolipoproteins other than apoAV, VLDL derived from apoA5−/− mice was associated with different amounts of recombinant apoAV. Once more, apoA5 did not influence VLDL hydrolysis mediated by free LPL either with (Fig. 7A) or without (Fig. 7C) previous re-isolation of the VLDL particles. In contrast, recombinant apoA5 led to a dose-dependent increase of VLDL hydrolysis by HSPG-bound LPL (Fig. 7B). As for the experiments employing HDL, acceleration of VLDL hydrolysis by recombinant apoA5 was also increased after re-isolation of VLDL if LPL was bound to HSPG (Fig. 7D).

To demonstrate the physiological relevance of the HSPG-based in vitro lipolysis assay, the impact of apoAV on LPL-mediated VLDL hydrolysis was measured in a cell culture system utilizing CHO cells with (CHO-K1) and without (CHO-745) HSPG. Again, apoA5 accelerated hydrolysis of apoA5-deficient VLDL only when LPL was bound to CHO cells with HSPG (1 μg LPL/ml, 71% (p < 0.05); 5 μg LPL/ml, 50% (p < 0.01)), but not on HSPG-deficient CHO cells (Fig. 8, A and B).

Further characterization of the molecular mechanism of apoAV and LPL interaction was achieved by using increasing amounts of VLDL TG in the hydrolysis assay. ApoAV increased the maximal hydrolysis rate of HSPG-bound LPL (23%; Fig. 9A), but not that of unbound LPL (data not shown). In all experiments conducted thus far, LPL was bound to HSPG before hydrolysis was started. To test whether binding of LPL to HSPG prior to hydrolysis is obligatory for the apoAV effect, experiments were performed with LPL bound to VLDL before hydrolysis. As shown in Fig. 9B, hydrolysis was also augmented by LPL supplied by VLDL; however, again, this effect was only seen if HSPG was present in the system.

In summary, our data consistently demonstrate that the hydrolysis of TG-rich lipoproteins by LPL is affected by apoAV only in the presence of HSPG. A dose-dependent correlation between apoAV and HSPG-bound LPL-mediated hydrolysis was found in vitro and in vivo.

**DISCUSSION**

The aim of this study was to elucidate the mechanism by which apoAV reduces plasma TG levels. It was found that apoAV accelerates plasma hydrolysis of TG-rich lipoproteins by proteoglycan-bound LPL without influencing their production rate. Based on its physical properties, apoAV could also interfere with VLDL or chylomicron assembly and therefore decrease production of TG-rich particles (23). This may be especially true because some similarity with microsomal TG transfer protein has been noted (9). Schaap et al. (10) reported a lower VLDL TG production in mice with adenovirus-mediated apoAV expression, proposing that apoAV may interfere with the second step of VLDL assembly. However, in hAPOA5tr mice, we did not find any inhibition of hepatic glycerol incorporation into TG, apoB (data not shown), or TG secretion (Fig. 1B). If anything, hAPOA5tr mice had a slightly higher VLDL TG production rate, probably as a result of increased hepatic lipoprotein uptake (see below). The results obtained by Schaap et al. (10) could be due to changes in liver metabolism or apoAV processing after the 10–30-fold increase in adenovirus-mediated apoAV transcription. In contrast, plasma levels of apoAV in transgenic mice are within the physiological range (14). We (data not shown) and others (10, 11) have found a pronounced reduction of postprandial TG response by transgenic or adenovirus-mediated apoAV expression. This and the production of other lipoproteins of the APOA1/C3/A4/A5 locus in the intestine raise the question...
of whether apoAV interferes with intestinal lipid absorption or chylomicron assembly. Because incorporation of oleic acid into chylomicrons after a gastric fat bolus was the same in wild-type and hAPOA5tr mice (Fig. 1A), it is assumed that apoAV does not change intestinal lipid absorption and lipoprotein production.

In contrast, the catabolism of VLDL and chylomicrons was markedly accelerated in the presence of the human hAPOA5 transgene (Fig. 1, C and D). Because this effect was observed in TG earlier than it was seen in total lipoproteins, it was proposed that apoAV increases the efficiency of plasma TG hydrolysis, resulting in a faster clearance of the respective remnants. These results are in accordance with data of other groups. Lipoprotein-free VLDL-like emulsions were metabolized more rapidly upon adenoviral apoAV expression in mice (10), and VLDL had a faster turnover in APOA5/APOC3 double transgenic mice (11). Because all turnover studies conducted thus far have been performed in mice with different plasma lipoprotein levels, activity or concentration changes of other apolipoproteins could cause these effects. This is especially possible because hAPOA5tr mice had 40% decreased apoCIII plasma levels, and apo5−/− mice had 90% increased apoCIII plasma levels (4). To prove a direct action of apoAV, apo5−/− mice had 90% increased apoCIII plasma levels (4).

To test whether VLDL-bound LPL was activated by apoAV, apo5−/− VLDL was incubated with LPL (5 μg/ml) for 30 min at 4 °C. After washing, hydrolysis reactions were carried out as reported. t test (apoAV versus PBS): **, p < 0.01.

Because active LPL was required for the apoAV effect in vivo (Fig. 1E), we investigated interactions between apoAV and LPL in vivo by breeding a human LPL transgene (27) on the apo5−/− background, and, vice versa, by breeding the hAPOA5tr onto the lpl-deficient mouse background (16).
Whereas increased LPL activity could completely normalize hypertriglyceridemia of apo(a)−/− mice, apoAV overproduction only slightly modulated plasma TG in LPL heterozygote-deficient mice. Apo(a)5 did not rescue LPL-deficient mice from neonatal death (Fig. 2). Thus, the apoAV effects on plasma TG levels depend on LPL, which plays the predominant role in the catalysis of TG-rich lipoproteins. Above a certain ratio of apoAV to LPL, all LPL molecules are maximally stimulated. Even abundant apoAV is not able to increase activity further, apoAV to LPL, all LPL molecules are maximally stimulated. Plasma TG levels depend on LPL, which plays the predominant role in the natal death (Fig. 2). Thus, the apoAV effects on plasma TG only slightly modulated plasma TG in LPL heterozygote-deficient hypertriglyceridemia of apoa5−/−.

Several investigators (28, 29) have postulated that plasma lipolysis is limited by factors other than the amount of post-heparin LPL activity itself, e.g. lipoprotein-HSPG binding. Taking into account that post-heparin plasma also does not reflect the physiological situation in which LPL is bound to cell surface HSPG, hydrolysis of VLDL isolated from hAPOA5tr and apo(a)5−/− mice by LPL associated with HSPG was measured. The presence of apoAV led to a clear increase in LPL-mediated release of free fatty acids (Fig. 4B). Because it was shown that apoAV could be transferred from HDL to TG-rich lipoproteins (Fig. 6E) (11), HDL was used as an apoAV donor. Indeed, apoAV was able to be exchanged from HDL to VLDL, and hydrolysis was increased by HDL containing apoAV if LPL was HSPG-bound (Fig. 6). The same results were achieved utilizing different concentrations of purified human recombinant apoAV (Fig. 7), proving a direct apoAV effect. In contrast to all experiments with HSPG-bound LPL, no influence of apoAV on VLDL hydrolysis by free (unbound) LPL was found in any experiment (Figs. 4, 6, and 7). Fruchtch-Najib et al. (11) have reported an increased hydrolysis of VLDL by free LPL in the presence of very high amounts of recombinant apoAV (600 µg/ml; about 1000-fold of physiological plasma concentration, Ref. 30). Similarly, Schäpf el et al. (10) showed an influence of apoAV on LPL lipolysis of TG-rich, apolipoprotein-free emulsions. The reason for these differences could be that, at high concentrations, apoAV might produce non-specific hydrophobic interactions increasing hydrolysis or stabilizing functional LPL during a long time course of up to 120 min. Under the most likely physiological conditions, however, we found an effect of apoAV only in the presence of HSPG. To show that the effects seen are not limited to HSPG of a specific origin, our findings were confirmed in a cell culture system. Again, apoAV could increase hydrolysis rates only in normal CHO cells with HSPG, but not in proteoglycan-deficient CHO cells (Fig. 8, A and B). Experiments using different TG concentrations showed that apoAV increases the maximal LPL hydrolysis rate in the presence of HSPG (Fig. 9A), but not in the absence of HSPG. Therefore, apoAV could increase substrate availability by affecting HSPG confirmation or by modifying VLDL in a way that makes TG better available to proteoglycan-bound LPL. Within the HSPG layer, apoAV could route TG-rich lipoproteins to the places of hydrolysis. This hypothesis is supported by in vitro experiments in which the effect of apoAV is abolished at high LPL concentrations (Fig. 4B). An explanation could be that guiding of apoAV becomes dispensable when HSPG is saturated with LPL. Alternatively, apoAV could act as an allosteric activator of proteoglycan-bound LPL, e.g. by supporting dimerized confirmation, by binding to an LPL activation site that is not accessible in unbound LPL, or by stabilizing LPL-HSPG complexes. ApoAV could also change VLDL confirmation, making it better hydrolysable for LPL. This is supported by kinetic data (Fig. 9A) and the fact that apoAV is able to exhibit its effects on a HSPG matrix even when LPL and apoAV are pre-assembled on VLDL before hydrolysis (Fig. 9B). However, LPL could instead be exchanged from VLDL to HSPG during initial hydrolysis because it was reported to occur in the opposite direction in vivo (31).

Taken together, our data demonstrate that apoAV accelerates plasma hydrolysis of TG-rich lipoproteins by facilitating interaction with proteoglycan-bound LPL, but not with free LPL. Therefore, it is proposed that apoAV augments the function of the natural lipolytic system as an allosteric LPL activator at the endothelial wall.

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