The role of ApoCI, LPL and CETP in plasma lipoprotein metabolism - studies in mice

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3 Apolipoprotein CI Causes Hypertriglyceridemia Independent of the Very-Low-Density Lipoprotein Receptor and Apolipoprotein CIII in Mice

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Published in Biochim Biophys Acta 2006;1761(2):213-220

Objective - We have recently shown that the predominant hypertriglyceridemia in human apolipoprotein C1 (*APOC1*) transgenic mice is mainly explained by apoCI-mediated inhibition of the lipoprotein lipase (LPL)-dependent triglyceride (TG)-hydrolysis pathway. Since the very-low-density lipoprotein receptor (VLDLr) and apoCIII are potent modifiers of LPL activity, our current aim was to study whether the lipolysis-inhibiting action of apoCI would be dependent on the presence of the VLDLr and apoCIII *in vivo*.

Methods and Results - Hereto, we employed liver-specific expression of human apoCI by using a novel recombinant adenovirus (Ad*APOC1*). In wild-type mice, moderate apoCI expression leading to plasma human apoCI levels of 12-33 mg/dl dose-dependently and specifically increased plasma TG (up to 6.6-fold, $P<0.001$), yielding the same hypertriglyceridemic phenotype as observed in human *APOC1* transgenic mice. Ad*APOC1* still increased plasma TG in *vldlr*$^{-/-}$ mice (4.1-fold, $P<0.001$) and in *apoc3*$^{-/-}$ mice (6.8-fold, $P<0.001$) that were also deficient for the low-density lipoprotein receptor (LDLr) and LDLr-related protein (LRP) or apoE, respectively. Thus, irrespective of receptor-mediated remnant clearance by the liver, liver-specific expression of human apoCI causes hypertriglyceridemia in the absence of the VLDLr and apoCIII.

Conclusion - We conclude that apoCI is a powerful and direct inhibitor of LPL activity independent of the VLDLr and apoCIII.

Human apolipoprotein CI (apoCI), encoded by the *APOC1* gene, is primarily expressed in the liver and secreted into the circulation.[1-4] To study the function of apoCI, mice have been generated that express human apoCI.[5-7] These mice have a hyperlipidemic phenotype, with the most pronounced effect on triglycerides (TG) in VLDL. Initially, it has been suggested that apoCI exerts its hyperlipidemic effect by interfering with the apoE-mediated hepatic remnant clearance via the low-density lipoprotein (LDL) receptor (LDLr)[8] and LDLr-related protein (LRP).[9] However, *APOC1* mice deficient for apoE still show severe hypertriglyceridemia,[10,11] indicating that apoCI predominantly affects a lipid clearance route other than receptor recognition. Indeed, we and others showed that apoCI directly and dose-dependently inhibits lipoprotein lipase (LPL)-mediated TG hydrolysis *in vitro* with a 60% efficiency compared with the main endogenous LPL inhibitor apoCIII,[10,12] and we have recently demonstrated that the apoCI-induced hypertriglyceridemia is mainly explained by impaired LPL-mediated lipolytic conversion of TG-rich lipoproteins.[10]

The VLDL receptor (VLDLr) is required for normal LPL regulation *in vivo* as disruption of the VLDLr results in hypertriglyceridemia associated with reduced LPL activity.[13,14] Therefore, we wondered whether the effect of apoCI on LPL is dependent on the VLDLr. Both *vldlr*$^{-/-}$ and *APOC1* mice are protected from diet-induced obesity on a wild-type as well as an *ob/ob* background[13,15] because of defective TG hydrolysis leading to reduced delivery of VLDL-derived FFA into adipose tissue. Since enrichment of VLDL with apoCI inhibits its binding to the VLDLr *in vitro*,[16] this mechanism may be responsible for the TG-raising effect of apoCI *in vivo*. Likewise, LPL activity is also highly dependent on the presence of the main endogenous LPL inhibitor apoCIII,[17,18] but a potential interaction between apoCI and apoCIII in lipolysis is unknown. We have observed by direct comparison that apoCIII is a more potent LPL inhibitor than apoCI *in vitro*,[10] but the effect of apoCI independent of apoCIII has not yet been assessed *in vivo*.

Taken together, the hypertriglyceridemic effect of apoCI is mainly explained by the inhibition of LPL-mediated TG clearance. However, it is unknown whether this effect results from direct inhibition of LPL or indirectly via interactions with the VLDLr and/or apoCIII. Therefore, the aim of this study was to investigate whether apoCI inhibits LPL-mediated lipolytic conversion *in vivo* through interaction with the VLDLr or apoCIII. Hereto, we expressed human apoCI using an adenovirus in mice that lack the VLDLr or apoCIII. Our results show that apoCI is a powerful inhibitor of LPL activity *in vivo* independent of the VLDLr and apoCIII.

Materials and methods

Transgenic Animals

MX1Cre^{+}*lrp*$^{lox/lox}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$,[19] *apoe*$^{-/-}$, *apoe*$^{-/-}$*apoc3*$^{-/-}$[18] and wild-type mice (C57Bl/6 background), 3-4 months of age, were used in the experiments. Mice were obtained from our breeding colonies at the Institutional Animal Facility and housed under standard conditions in conventional cages and were fed regular chow *ad libitum*. LRP deficiency was induced in MX1Cre^{+}*lrp*$^{lox/lox}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice by intraperitoneal injection of polyi-

nosinic:polycytidylic ribonucleic acid (pI:pC, Sigma, St Louis, MO, USA), which results in the complete absence of LRP protein in liver membrane extracts.[19] Experiments were performed after 4 h of fasting at 12:00 pm with food withdrawn at 8:00 am. Female mice were used unless indicated otherwise. All experiments have been approved by the Institutional Ethical Committee on Animal Care and Experimentation.

Construction of Recombinant Adenoviral Vector Expressing Human ApoCI
A recombinant, replication-deficient adenoviral vector expressing human *APOC1* (Ad*APOC1*) under control of a cytomegalovirus promoter was constructed by the method of He *et al*.[20] Briefly, the N-terminal *Kpn*I-*Hin*dIII (2140 bp) fragment of human *APOC1* genomic DNA was transposed into the corresponding sites of the pAdTrack-CMV vector. Subsequently, the C-terminal *Hin*dIII-*Hin*dIII (2403 bp) fragment of human genomic *APOC1* DNA was cloned into the resulting plasmid. The identity of the resulting construct was verified by sequence analysis, and homologous recombination with the adenoviral backbone vector pAdEasy-1 took place in BJ5183 cells. Recombinant plasmids were transfected into the adenovirus packaging cell line PER.C6[21] and amplified. *E. coli* strain BJ5183 and the pAdTrack-CMV and pAdEasy-1 vectors were a kind gift of Dr. Vogelstein (Johns Hopkins School of Medicine, Baltimore, MD, USA). Recombinant adenovirus was purified twice via caesium chloride gradient centrifugation and dialyzed against 25 mM Tris, 137 mM NaCl, 5 mM KCl, 0.73 mM NaH_2PO_4, 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2$, pH 7.45, followed by dialysis against the same buffer supplemented with sucrose (50 g/l). For storage, aliquots of 150 µl virus were frozen at -80°C. Routinely, virus titers of the stocks varied from $1x10^{10}$ to $1x10^{11}$ pfu (plaque forming units)/ml.

Administration of Adenoviral Vectors to Mice
At least 3 days before adenovirus injection into mice, basal serum lipid values were measured (t=0). At day 0, mice were injected into the tail vein with either Ad*APOC1* or a recombinant virus expressing β-galactosidase (AdLacZ) as a control, both diluted with PBS to a total volume of 200 µl. To prevent sequestration of low doses of virus by Kupffer cells and to achieve a more linear dose-response relationship of *APOC1* expression, mice were pre-injected with $0.5x10^9$ pfu AdLacZ at 3 h before injection.[22]

Plasma Lipid and Lipoprotein Analysis
In all experiments blood was collected from the tail vein into chilled paraoxon (Sigma, St. Louis, MO, USA)-coated capillary tubes to prevent ongoing *in vitro* lipolysis.[23] The tubes were placed on ice, centrifuged at 4°C, and the obtained plasma was assayed for total cholesterol (TC), TG, and free fatty acids (FFA) using the commercially available enzymatic kits 236691, 11488872 (Roche Molecular Biochemicals, Indianapolis, IN, U.S.A.), and NEFA-C (Wako Chemicals, Neuss, Germany), respectively. For determination of the plasma lipoprotein distribution by FPLC, 50 µl of pooled plasma per group was injected onto a Superose 6 column (Äkta System; Amersham Pharmacia Biotech, Piscataway, NJ, USA), and eluted at a flow rate of 50 µl/min with PBS, 1 mM EDTA ,

pH 7.4. Fractions of 50 µl were collected and assayed for TC and TG as described above. Human apoCI was quantified by ELISA exactly as described.[10]

Hepatic VLDL-TG Production

Mice were fasted for 4 h and anesthetized by *i.p.* administration of 6.2 mg/kg of Acepromazine (Pfizer Animal Health, Capelle a/d IJssel, The Netherlands), 0.3 mg/kg of Fentanyl Bipharma (Pharma Hameln, Hameln, Germany), and 6.2 mg/kg of Midazolam (Roche, Mijdrecht, The Netherlands). Subsequently, mice were injected via the tail vein with Triton WR-1339 (500 mg/kg of body weight) to block TG-hydrolysis and hepatic lipoprotein uptake.[24] Blood samples were drawn at 1, 30, 60, 90, and 120 min after administration, and plasma TG levels were measured as described above. The VLDL-TG production rates were calculated from the linear increase in TG in time (mM TG/min).

Hepatic mRNA Expression

Total RNA was isolated from liver samples using RNA Insta-Pure reagent (Eurogentec, Seraing, Belgium), treated with DNAseI, and reverse-transcribed using random primers. cDNA levels were determined by real-time PCR on an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using SYBR-Green technology. PCR primers (forward: 5'-GAGGACAAGGCTCGGGAACT-3'; reverse: 5'-TGAAAACCACTCCCGCATCT-3') were designed using Primer Express 1.5 Software with the manufacturer's default settings and obtained from Isogen Bioscience (Maarssen, The Netherlands). The expression levels of human *APOC1* were determined relative to murine HPRT.

Statistical Analysis

Data were analyzed using the unpaired Student's t test unless indicated otherwise. *P*-values less than 0.05 were considered statistically significant.

Results

Characterization of the Effect of AdAPOC1 on Plasma Lipid Levels in Wild-Type Mice

First, we addressed the effects of Ad*APOC1* on lipid metabolism in wild-type mice. Figure 1 illustrates the time-course changes for human apoCI and lipid levels in plasma up to 14 days after injection of a low dose ($0.5x10^9$ pfu) of Ad*APOC1*. Plasma human apoCI protein was detected already at day 3, reached peak concentrations 5 days after injection (18±9 mg/dl), and was disappeared from the plasma at day 14 (Fig. 1A). Plasma TC levels were increased only from day 5 (170±19% of initial value; $P<0.01$), and returned to basal levels at day 10. However, apoCI expression had the most profound effect on plasma TG levels. TG were already increased at day 3, reached peak levels at day 5 (335±117% of initial value; $P<0.05$) and stayed significantly elevated up to day 10 (Fig. 1B).

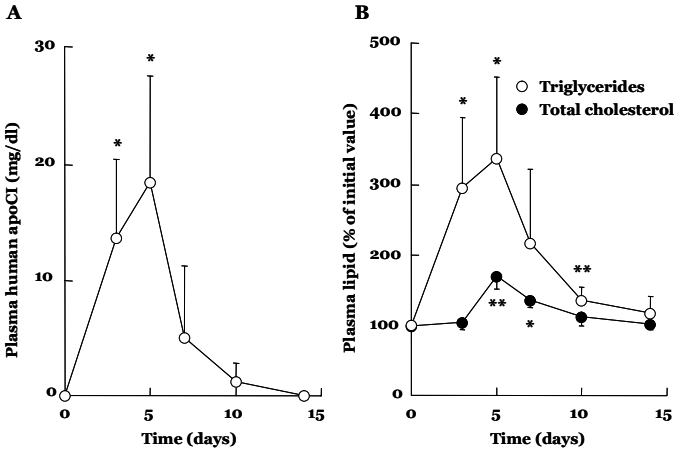

Figure 1. Effect of Ad*APOC1* on time-course changes of human apoCI, TG, and TC plasma levels in wild-type mice. Mice were injected with Ad*APOC1* ($0.5x10^9$ pfu). Before injection and at indicated times after injection, fasted plasma was collected from the individual mice and assayed for (A) human apoCI protein, (B) total cholesterol (closed circles) and triglycerides (open circles). Initial values before injection are set at 100% and values are expressed as percentage of the value before injection ± S.D. (n=4). Asterisks indicate statistically significant differences with the initial value (*P<0.05; **P<0.01).

Subsequently, we evaluated the virus dose-dependent effects of Ad*APOC1* in wild-type mice (Fig. 2). Injection of 0, 0.3, 1.0 and $3.3x10^9$ pfu of Ad*APOC1* dose-dependently increased human *APOC1* mRNA expression in the liver at 5 days after injection (Fig. 2A). Concomitantly, plasma human apoCI protein levels were dose-dependently increased up to 33±11 mg/dl at the highest dose (Table 1). Table 1 summarizes the plasma lipid levels in the wild-type mice before and 5 days after administration of Ad*APOC1*. Virus administration *per se* resulted in a slight increase in TG at day 5 (0.53±0.10 vs. 0.22±0.03 mM). In mice that were given Ad*APOC1* TG dose-dependently increased up to 6.6-fold at the highest dose, which reached statistical significance for $1.0x10^9$ and $3.3x10^9$ pfu of virus. The elevated TG levels were mainly confined to the VLDL and IDL/LDL fractions (Fig. 2B). Ad*APOC1* also dose-dependently increased TC, however to a lower extent as compared to TG (up to 1.8-fold at the highest dose), which was also due to an increase in both VLDL and IDL/LDL (not shown). In addition to TG and TC, FFA plasma levels were also increased 1.9-fold (P<0.001) at the highest dose. Thus, injection of Ad*APOC1* into wild-type mice leads to a major increase in TG levels accompanied by a mild increase in TC and FFA levels. To address the mechanism(s) underlying the hypertriglyceridemia in these mice, we determined whether the hepatic VLDL-TG production after Triton WR-1339 administration was affected. As shown in Table 2, no differences were observed between the groups of mice that received Ad*APOC1* and mice that received control virus. In addition, as the mice were 4 h fasted in all experiments, the observed effect of Ad*APOC1* on plasma TG levels cannot be caused by an effect on intestinal chylomicron-TG production. Taken together, the hypertriglyceridemic effect of Ad*APOC1* is caused by impaired TG clearance.

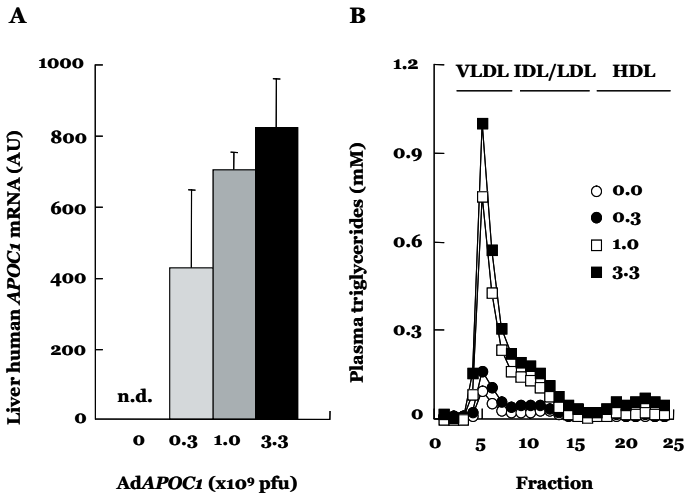

Figure 2. Effect of Ad*APOC1* on hepatic *APOC1* expression and plasma TG in wild-type mice. Mice were injected with either AdLacZ ($3.3x10^9$ pfu) or Ad*APOC1* ($0.3\text{-}3.3x10^9$ pfu). After 5 days the liver was collected for mRNA isolation and human *APOC1* mRNA measurement (A), and plasma was isolated to determine TG (B). Values are expressed as means ± S.D. (n=5).

Table 1. Dose-dependent effect of Ad*APOC1* administration on plasma lipid levels in wild-type mice

Condition	Human apoCI (mg/dl)	TG (mM)	TC (mM)	FFA (mM)
Before adenovirus				
	-	0.22±0.03	1.8±0.2	0.49±0.10
After adenovirus				
Control	n.d.	0.53±0.10	2.2±0.2	0.54±0.03
$0.3x10^9$	12±5	0.79±0.33	2.7±0.2**	0.76±0.04
$1.0x10^9$	23±8	2.36±0.78***	3.4±0.9*	0.75±0.20
$3.3x10^9$	33±11	3.52±0.11***	4.0±0.7***	1.00±0.13***

n.d., not detectable; TG, triglycerides; TC, total cholesterol. Plasma was obtained from fasted wild-type mice before and 5 days after administration of either AdLacZ or Ad*APOC1*. Plasma lipid levels were measured and values are expressed as means ± S.D. (n=5). Statistical differences were assessed between control virus and Ad*APOC1* receiving mice 5 days after injection. *$P<0.05$. **$P<0.01$. ***$P<0.05$.

Table 2. Effect of Ad*APOC1* administration on hepatic VLDL-TG production in wild-type mice

Condition	VLDL-TG production (mM TG/min)
Control	0.082±0.023
$0.3x10^9$	0.080±0.015
$1.0x10^9$	0.094±0.042
$3.3x10^9$	0.068±0.007

Five days after administration of either AdLacZ or Ad*APOC1*, mice were fasted for 4 h, anesthetized, and injected with Triton WR-1339 (500 mg/kg). Plasma triglyceride (TG)-levels were determined at 1, 30, 60, 90 and 120 min after injection and from the linear increase in TG in time, the VLDL-TG production rate was calculated. Values are expressed as means ± S.D. (n=5).

Effect of AdAPOC1 on Plasma Lipid Levels in VLDLr-Deficient mice

Besides interfering with the peripheral TG hydrolysis by LPL, apoCI has been postulated to inhibit the hepatic uptake of TG-rich lipoproteins via the LRP and LDLr. To investigate the VLDLr-dependency of the apoCI-inhibited TG hydrolysis without potential concomitant effects of apoCI on hepatic receptor recognition, the effect of Ad*APOC1* was compared in *lrp*$^{-}$*ldlr*$^{-/-}$ double knockout mice versus *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ triple knockout mice. At a low viral dose ($0.5x10^9$ pfu), Ad*APOC1* resulted in moderate levels of human apoCI protein in *lrp*$^{-}$*ldlr*$^{-/-}$ and *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice (17±10 and 45±25 mg/dl, respectively) (Table 3). Similar as on a wild-type background, *APOC1* expression in both *lrp*$^{-}$*ldlr*$^{-/-}$ and *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice resulted in a more pronounced relative increase of TG than of TC as compared to mice that received control virus, mainly in VLDL and IDL/LDL (Fig. 3). However, the effect of Ad*APOC1* on plasma lipid levels was remarkably higher in *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice as compared to *lrp*$^{-}$*ldlr*$^{-/-}$ mice. Specifically, Ad*APOC1* resulted in 2-fold higher plasma TG levels in *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice as compared to *lrp*$^{-}$*ldlr*$^{-/-}$ mice (14.1±4.3 vs. 7.2±1.5 mM, $P<0.05$). Thus, apoCI does not require the presence of the VLDLr for inhibiting the LPL-dependent TG hydrolysis *in vivo*. On the contrary, the TG-raising effect of apoCI is even enhanced in the absence of the VLDLr.

Effect of AdAPOC1 on Plasma Lipid Levels in ApoCIII-Deficient Mice

ApoCIII[16,17] and apoE[25,26] are both inhibitors of LPL *in vivo*, albeit that apoCIII is the most prominent endogenous inhibitor of LPL activity.[27] To investigate whether the

Table 3. Effect of Ad*APOC1* administration on plasma lipid levels in *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice

Genotype	Virus	Human apoCI (mg/dl)	TG (mM)	TC (mM)	FFA (mM)
Before adenovirus					
Lrp^{-}*ldlr*$^{-/-}$	-	-	2.3±0.8	18±3	0.6±0.1
Lrp^{-}*ldlr*$^{-/-}$*vldlr*$^{-/-}$	-	-	3.2±0.7	22±5	0.6±0.1
After adenovirus					
Lrp^{-}*ldlr*$^{-/-}$	AdLacZ	n.d.	5.0±1.0	18±3	1.4±0.4
Lrp^{-}*ldlr*$^{-/-}$	Ad*APOC1*	17±10	7.2±1.5*	21±1	1.6±0.4
Lrp^{-}*ldlr*$^{-/-}$*vldlr*$^{-/-}$	AdLacZ	n.d.	6.2±1.8	24±3	1.8±0.1
Lrp^{-}*ldlr*$^{-/-}$*vldlr*$^{-/-}$	Ad*APOC1*	45±25	14.1±4.3**	31±3**	2.1±0.3*

n.d., not detectable; TC, total cholesterol; TG, triglycerides. Plasma was obtained from fasted *lrp*$^{-}$*ldlr*$^{-/-}$ and *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice before and 5 days after administration of either AdLacZ or Ad*APOC1* and levels of human apoCI and lipids were measured. Values are expressed as means ± S.D (n=5). Statistical differences were assessed between control virus and Ad*APOC1* receiving mice 5 days after injection. *P<0.05. **P<0.01.

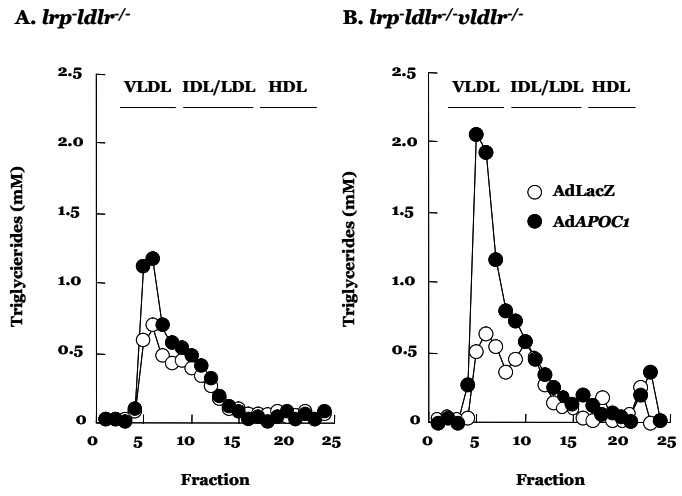

Figure 3. Effect of Ad*APOC1* on plasma TG distribution in *lrp*$^{-}$*ldlr*$^{-/-}$ and *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ mice. *Lrp*$^{-}$*ldlr*$^{-/-}$ (A) and *lrp*$^{-}$*ldlr*$^{-/-}$*vldlr*$^{-/-}$ (B) mice were injected with 0.5x10^{9} pfu AdLacZ (open circles) or Ad*APOC1* (closed circles). After 5 days, fasted plasma was collected, pooled per group (n=5), and subjected to FPLC to separate lipoproteins. Fractions were assayed for TG.

effect of human apoCI on LPL-dependent TG hydrolysis *in vivo* depends on the interaction between apoCI and apoCIII, Ad*APOC1* was administered to apoE-knockout mice with or without concomitant apoCIII deficiency. Both *apoe*$^{-/-}$ and *apoe*$^{-/-}$*apoc3*$^{-/-}$ mice received a low adenoviral dose (0.5x10^{9} pfu), which resulted in human apoCI plasma levels of 45±4 and 52±10 mg/dl, respectively. In *apoe*$^{-/-}$ mice, plasma TG was increased by 5.4-fold (P<0.001), whereas TC and FFA were not affected (Table 4). Again, the TG-raising effect was specific for VLDL (Fig. 4). In the absence of apoCIII, a 6.8-fold (P<0.001) increase in VLDL and IDL/LDL TG was found, in addition to a 2.0-fold (P<0.001) increase in TC levels. Thus, expression of human apoCI leads to hypertriglyceridemia even in the absence of the major LPL inhibitor apoCIII, which indicates that apoCI is an independent and direct modulator of LPL activity *in vivo*.

Table 4. Effect of Ad*APOC1* administration on plasma lipid levels in *apoe*$^{-/-}$ and *apoe*$^{-/-}$*apoc3*$^{-/-}$ mice

Genotype	Virus	Human apoCI (mg/dl)	TG (mM)	TC (mM)	FFA (mM)
Before adenovirus					
Apoe$^{-/-}$	-	-	1.0±0.3	13±3	0.8±0.1
Apoe$^{-/-}$*apoc3*$^{-/-}$	-	-	0.6±0.2	8±2	0.5±0.1
After adenovirus					
Apoe$^{-/-}$	AdLacZ	n.d.	1.2±0.1	15±2	1.0±0.1
Apoe$^{-/-}$	Ad*APOC1*	45±4	6.3±1.3**	17±2	1.0±0.2
Apoe$^{-/-}$*apoc3*$^{-/-}$	AdLacZ	n.d.	0.6±0.1	8±1	0.6±0.1
Apoe$^{-/-}$*apoc3*$^{-/-}$	Ad*APOC1*	52±10	4.0±1.2***	17±3***	0.8±0.1*

n.d., not detectable; TC, total cholesterol; TG, triglycerides. Plasma was obtained from fasted *apoe*$^{-/-}$ and *apoe*$^{-/-}$*apoc3*$^{-/-}$ mice before and 5 days after administration of either AdLacZ or Ad*APOC1*. Plasma human apoCI and plasma lipid levels were measured. Values are expressed as means ± S.D. (n=4). Statistical differences were assessed between control virus and Ad*APOC1* receiving mice 5 days after injection. *P<0.05. **P<0.01. ***P<0.001.

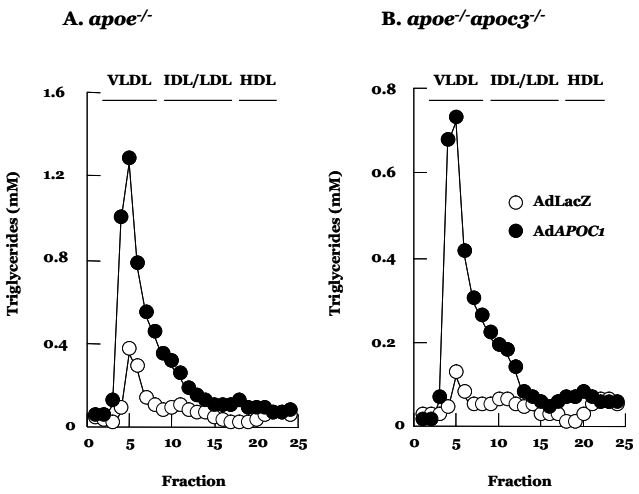

Figure 4. Effect of Ad*APOC1* on plasma TG distribution in *apoe*$^{-/-}$ and *apoe*$^{-/-}$*apoc3*$^{-/-}$ mice. *Apoe*$^{-/-}$ (A) and *apoe*$^{-/-}$*apoc3*$^{-/-}$ (B) mice were injected with 0.5x10^{9} pfu AdLacZ (open circles) or Ad*APOC1* (closed circles). After 5 days, fasted plasma was collected, pooled per group (n=5), and subjected to FPLC to separate lipoproteins. Fractions were assayed for TG.

Discussion

Increased expression of apoCI in mice has been shown to result in combined hyperlipidemia, with a pronounced increase in TG levels in addition to elevated TC levels.[5-7,10] We have recently shown that the combined hyperlipidemia in human *APOC1* transgenic mice is the consequence of impaired LPL-mediated hydrolysis of VLDL-TG.[10] However, it was still unclear whether apoCI impairs the lipolytic conversion and clearance of lipoproteins *in vivo* by inhibiting LPL activity directly or indirectly via effects on the VLDLr and/or apoCIII, which are both modulators of LPL activity. We generated a novel adenoviral vector encoding human apoCI (Ad*APOC1*) as this vector allows us to study the interactions of human apoCI with the VLDLr and apoCIII without the need of elaborate crossbreeding of transgenic mouse lines. Upon administration of Ad*APOC1* to wild-type mice, apoCI dose-dependently increased plasma lipid levels, with a preferential increase of TG as compared to TC, which is specific for VLDL and its remnants (*i.e.* IDL and LDL). Together with the observation that Ad*APOC1* raises TG levels at an earlier stage (day 3) than TC levels (day 5), these data underscore our previous findings that apoCI primarily interferes with the LPL-dependent processing of VLDL rather than with the hepatic uptake of its core remnants.[10] The apoCI-induced hypertriglyceridemia was still apparent upon deficiency of the LDLr and LRP (involved in hepatic remnant clearance), or VLDLr and apoCIII (involved in peripheral LPL-dependent TG hydrolysis), indicating that apoCI is indeed a powerful and direct modulator of LPL activity. Since the hepatic uptake of TG-rich lipoproteins is highly dependent on processing by LPL, thereby generating remnant particles with reduced size with an altered apolipoprotein composition, apoCI will have a secondary inhibiting effect on hepatic remnant uptake. In addition, the hepatic remnant uptake may be directly affected by apoCI.

At similar plasma human apoCI levels, the plasma lipid phenotype of Ad*APOC1*-treated mice is virtually identical to that of *APOC1* transgenic mice.[6,7,10] Since intravenous injection of recombinant adenoviruses leads to nearly exclusive infection of the liver, this indicates that liver-derived apoCI can be fully responsible for the hyperlipidemia in *APOC1* transgenic mice. We did not observe any effect of hepatic *APOC1* expression on hepatic VLDL-TG production, which is in line with our recent observations in *APOC1* transgenic mice.[10] Furthermore, we can rule out that the hypertriglyceridemic effect of *APOC1* expression is caused by an effect on TG influx into plasma by modulating intestinal lipid absorption as we used 4 h fasted mice to exclude potential influx of chylomicrons from the intestine. In addition, we have recently shown that intestinal TG absorption in transgenic *APOC1* mice is not affected as compared to wild-type mice.[10] Therefore, our present experiments with Ad*APOC1* confirm our previous conclusion that the hypertriglyceridemia in *APOC1* transgenic mice is truly caused by a defect in peripheral lipolysis,[10] thereby preventing subsequent hepatic remnant uptake.

ApoCI has been shown to inhibit the binding of VLDL to the VLDLr *in vitro* and *in vivo*.[16] Since the VLDLr is commonly assumed to be required for normal LPL functioning *in vivo*, apoCI might indeed act by disrupting the binding of VLDL to the VLDLr, thereby inhibiting the LPL-mediated TG hydrolysis. We thus evaluated the effect of hepatic human apoCI expression in VLDLr-deficient mice. These mice were also deficient for the LDLr and hepatic LRP to exclude a potentially additional effect of apoCI on receptor-mediated hepatic remnant clearance. Hepatic apoCI expression in LRP and LDLr double-deficient mice increased plasma VLDL-TG and VLDL-cholesterol. Remarkably, the additional absence of the VLDLr resulted in an aggravated combined hyperlipidemia, associated with higher plasma human apoCI levels, while the hepatic apoCI expression levels were similar in $lrp^{-}ldlr^{-/-}$ and $lrp^{-}ldlr^{-/-}vldlr^{-/-}$ mice. This suggests that, although apoCI reduces the binding affinity of VLDL for the VLDLr,[16] the VLDLr can still facilitate the lipolysis of apoCI-enriched VLDL by bringing VLDL in close proximity of LPL at the endothelial surface. The VLDLr could also be involved in the clearance of apoCI, leading to accumulation of plasma apoCI in the absence of the VLDLr, with a concomitant increase in combined hyperlipidemia. Regardless of the precise mechanism, the TG levels in LRP, LDLr and VLDLr triple-knockout mice increased 7.9 mM after Ad*APOC1* administration, illustrating that the inhibition of LPL-mediated TG hydrolysis by apoCI can indeed occur independently of the VLDLr *in vivo*.

It is commonly known that apoCIII is the most prominent physiological inhibitor of LPL activity.[12,18,28,29] Indeed, human *APOC3* transgenic mice show marked hypertriglyceridemia and hypercholesterolemia, with severely elevated VLDL levels.[30] Reciprocally, *apoc3*-deficient mice on an $apoe^{-/-}$ background show decreased VLDL-TG levels.[18,31] Therefore, we also questioned whether the capacity of apoCI to inhibit LPL proceeds via an interaction with apoCIII. Since we and others have shown that apoE is also an inhibitor of LPL *in vivo*,[25,26] we determined the effect of apoCI expression in apoE-knockout and apoE/apoCIII double-knockout mice. Injection of Ad*APOC1* into $apoe^{-/-}$ mice resulted in aggravated hyperlipidemia, which confirms previous findings that apoCI can act independently of apoE.[10,11] Importantly, apoCI expression in $apoe^{-/-}$ $apoc3^{-/-}$ mice led to a similar increase in TG as observed in $apoe^{-/-}$ only mice, establish-

ing that the LPL-inhibiting effect of apoCI occurs without interaction of apoCI with endogenous apoCIII.

In conclusion, we have demonstrated that low to moderate liver-specific expression of human apoCI in mice results in dose-dependent hypertriglyceridemia even in the absence of the VLDLr or apoCIII, presumably by direct inhibition of LPL activity, as we have recently shown.[10] It is evident that LPL activity strongly determines plasma TG levels, as overexpression of LPL in mice markedly reduces VLDL-TG levels,[32,33] whereas heterozygous deficiency of LPL results in accumulation of VLDL-TG.[34] Furthermore, LPL is an important factor for the influx of fatty acids into adipose tissue. We have shown that an increased LPL function leads to aggravated obesity on high fat diet,[17] whereas a decreased LPL function results in protection against diet-induced obesity.[13,15] Therefore, our findings may have important implications for the treatment of hypertriglyceridemia and obesity.

Acknowledgements

This work was performed in the framework of the Leiden Center for Cardiovascular Research LUMC-TNO, and supported by the Leiden University Medical Center (Gisela Thier Fellowship to P.C.N.R.), the Netherlands Organization for Scientific Research (NWO RIDE grant 014-90-001, NWO grant 908-02-097, and NWO VIDI grant 917.36.351), and the Netherlands Heart Foundation (NHS grant 2003B136).

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