Severe hypertriglyceridemia in human *APOC1* transgenic mice is caused by apoC-I-induced inhibition of LPL

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Abstract **Studies in humans and mice have shown that increased expression of apolipoprotein C-I (apoC-I) results in combined hyperlipidemia with a more pronounced effect on triglycerides (TGs) compared with total cholesterol (TC). The aim of this study was to elucidate the main reason for this effect using human apoC-I-expressing (*APOC1*) mice. Moderate plasma human apoC-I levels (i.e., 4-fold higher than human levels) caused a 12-fold increase in TG, along with a 2-fold increase in TC, mainly confined to VLDL. Crossbreeding of *APOC1* mice on an apoE-deficient background resulted in a marked 55-fold increase in TG, confirming that the apoC-I-induced hyperlipidemia cannot merely be attributed to blockade of apoE-recognizing hepatic lipoprotein receptors. The plasma half-life of [^{3}H]TG-VLDL-mimicking particles was 2-fold increased in *APOC1* mice, suggesting that apoC-I reduces the lipolytic conversion of VLDL. Although total postheparin plasma LPL activity was not lower in *APOC1* mice compared with controls, apoC-I was able to dose-dependently inhibit the LPL-mediated lipolysis of [^{3}H]TG-VLDL-mimicking particles in vitro with a 60% efficiency compared with the main endogenous LPL inhibitor apoC-III. Finally, purified apoC-I impaired the clearance of [^{3}H]TG-VLDL-mimicking particles independent of apoE-mediated hepatic uptake in lactoferrin-treated mice. Therefore, we conclude that apoC-I is a potent inhibitor of LPL-mediated TG-lipolysis.**—Berbée, J. F. P., C. C. van der Hoogt, D. Sundararaman, L. M. Havekes, and P. C. N. Rensen. **Severe hypertriglyceridemia in human *APOC1* transgenic mice is caused by apoC-I-induced inhibition of LPL. *J. Lipid Res.* 2005.** 46: **297–306.**

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The human apolipoprotein C-I (apoC-I)-encoding gene *APOC1* is part of the *APOE/APOC1/APOC2* gene cluster (1). *APOC1* is primarily expressed in the liver but also in the lung, skin, spleen, adipose tissue, and brain (2). ApoC-I is secreted as a 6.6 kDa protein into the plasma, where it is present at a relatively high concentration of ~10 mg/dl (3), and is mainly bound to chylomicrons, VLDLs, and HDLs (4). Although human studies to date have not revealed any polymorphism in the *APOC1* gene leading to functional apoC-I variants, an *HpaI* polymorphism in the promotor region has been described that leads to 57% increased expression of the *APOC1* gene (5). Interestingly, *HpaI* carriers display increased plasma triglyceride (TG) levels, which are independent of total cholesterol (TC) levels (6). To gain more insight into the function of apoC-I in lipoprotein metabolism, we and others have generated mice that either lack endogenous apoC-I (7, 8) or express the human *APOC1* gene (9, 10). Although apoC-I-deficient mice did not show a phenotype with respect to plasma lipid levels (7), *APOC1* transgenic mice showed an *APOC1* dose-dependent increase in plasma levels of TG, TC, and FFAs. The most prominent increasing effect of *APOC1* was observed on TG levels and could be attributed to severely increased levels of VLDLs (9, 11).

Early reports have postulated that apoC-I may function by modulation of the activity of plasma enzymes involved in lipid metabolism and modulation of TG-rich lipoprotein (remnant) binding and uptake by hepatic receptors. In vitro studies have shown that apoC-I may interfere with VLDL metabolism by partial activation of LCAT (12), inhibition of LPL (13), and inhibition of HL (14). Recently, Conde-Knape et al. (15) confirmed such an HL-modulating function of apoC-I in vitro and suggested that HL modulation may contribute to the hypertriglyceridemic phenotype of *APOC1* transgenic mice. Strikingly, HL-defi-

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Abbreviations: apoC-I, apolipoprotein C-I; *APOC1*, human apolipoprotein C-I-encoding gene; *apoe*$^{-/-}$, apolipoprotein E-deficient mice; CETP, cholesteryl ester transfer protein; CO, cholesteryl oleate; FC, free cholesterol; FPLC, fast-performance liquid chromatography; LDLr, low density lipoprotein receptor; LRP, low density lipoprotein receptor-related protein; TC, total cholesterol; TG, triglyceride; TO, triolein; VLDLr, very low density lipoprotein receptor.

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cient mice do not show any sign of disturbed TG metabolism (16–18). In addition, *LCAT* transgenic mice do not show increased VLDLs (19), suggesting that potential LCAT-activating properties of apoC-I do not contribute to the phenotype of *APOC1* mice.

Besides modulation of plasma enzymes, apoC-I has also been reported to interfere with the apoE-dependent hepatic uptake of lipoprotein remnants by the LDL receptor (LDLr) and LDLr-related protein (LRP). In the isolated rat liver perfusion model, it was demonstrated that addition of human apoC-I inhibits the catabolism of chylomicrons and TG-rich emulsions (20, 21). Subsequently, it was shown that apoC-I can interfere with the apoE-mediated uptake of VLDLs by the LDLr (22) and LRP (23), possibly related to apoC-I-induced masking of the receptor binding domain of apoE (22) or displacement of apoE from the lipoprotein particle (23). More recent studies from our group (9, 24) have suggested that the inhibiting properties of apoC-I toward the LRP may exceed those toward the LDLr, because apoC-I-associated hyperlipidemia was severely aggravated on an LDLr-deficient background (9). In addition, it was shown that transfection of LDLr-deficient *APOC1* mice with a recombinant adenovirus encoding the 39 kDa receptor-associated protein further increased plasma TG levels 4-fold. However, although these studies certainly suggest a modulating role of apoC-I with respect to hepatic lipoprotein recognition, it remains remarkable that complete blockade of the apoE-mediated lipoprotein clearance in apoE-deficient mice hardly affects plasma TG levels (16, 25, 26), whereas TG levels are severely increased by *APOC1* expression, indicating that apoC-I has a profound additional effect.

Because the proposed functions of apoC-I cannot explain the severe hypertriglyceridemic phenotype of *APOC1* mice, the aim of the present study was to elucidate the main mechanism underlying the apoC-I-induced combined hyperlipidemia in *APOC1* mice. We demonstrate that apoC-I is a potent inhibitor of LPL, which can explain the combined hyperlipidemia observed in *APOC1* transgenic mice in both the presence and absence of endogenous apoE.

EXPERIMENTAL PROCEDURES

Transgenic animals

Transgenic *APOC1* mice with hemizygous expression of the human *APOC1* gene were generated previously as described (7, 9) and backcrossed at least 10 times to the C57Bl/6 background. *APOC1* mice were intercrossed with apoE-deficient (*apoe*$^{-/-}$) mice (C57Bl/6 background) that were originally obtained from Jackson Laboratories (Bar Harbor, ME) to generate mice hemizygous for the *APOC1* gene on an apoE-deficient background (*apoe*$^{-/-}$*APOC1*). After initial characterization of both male and female mice, 10–12 week old male *APOC1* and *apoe*$^{-/-}$*APOC1* mice were used for subsequent experiments, with wild-type and *apoe*$^{-/-}$ littermates as controls. Mice were housed under standard conditions in conventional cages and were fed ad libitum with regular chow (Ssniff, Soest, Germany). Experiments were performed after 4 h of fasting at 1:00 PM with food withdrawn at 9:00 AM, unless stated otherwise.

Plasma lipid and lipoprotein analysis

In all experiments, blood was collected from the tail vein into chilled paraoxon (Sigma, St. Louis, MO)-coated capillary tubes to prevent ongoing in vitro lipolysis (27), unless indicated otherwise. These tubes were placed on ice and centrifuged at 4°C, and the plasma obtained was assayed for TC, TG (without free glycerol), and FFA using the commercially available enzymatic kits 236691 (Roche Molecular Biochemicals, Indianapolis, IN), 337-B (GPO-Trinder kit; Sigma), and NEFA-C (Wako Chemicals, Neuss, Germany), respectively. For determination of the plasma lipoprotein distribution by fast-performance liquid chromatography (FPLC), 50 μl of pooled plasma from 10 mice per group was injected onto a Superose 6 column (Äkta System; Amersham Pharmacia Biotech, Piscataway, NJ), and eluted at a constant flow rate of 50 μl/min with PBS, 1 mM EDTA (Sigma), pH 7.4. Fractions of 50 μl were collected and assayed for TC and TG as described above. Human apoC-I was quantified by ELISA as described below.

VLDL isolation and characterization

Fasted mice were killed by cervical dislocation, and blood was drawn from the retro-orbital vain into Microvette® CB 1000 Z capillaries (Sarstedt, Nümbrecht, Germany). Sera were collected after centrifugation at 4°C and pooled from 10 mice. VLDLs were isolated by flotation ($d < 1.006$ g/ml) after ultracentrifugation in a SW 40 Ti rotor (Beckman Instruments, Geneva, Switzerland) at 40,000 rpm during 18 h at 4°C. The VLDL fractions were assayed for TG and TC as described above and for free cholesterol (FC) and phospholipids using the commercially available analytical kits 274-47109 and 990-54009 (Wako Chemicals), respectively. Cholesteryl esters were calculated by subtracting the molar concentration of FC from the molar concentration of TC and corrected for the presence of the fatty acid. Protein was determined by the method of Lowry et al. (28), with BSA as a standard. VLDL particle size was determined by photon correlation spectroscopy using a Zetasizer 3000 HS_A (Malvern Instruments, Malvern, UK) at 25°C with a 90° angle between the laser and the detector.

Human apoC-I ELISA

Plasma human apoC-I concentrations were determined using a human apoC-I-specific sandwich ELISA. To this, a polyclonal goat anti-human apoC-I antibody (Academy Biomedical Co., Houston, TX) was coated overnight onto Costar medium binding plates (Corning, Inc., New York, NY) (dilution 1:10^4) at 4°C and incubated with diluted mouse plasma (dilution 1:10^6 to 1:10^7) or FPLC fractions (1:10^4) for 2 h at 4°C. Subsequently, HRP-conjugated polyclonal goat anti-human apoC-I antibody (dilution 1:500; Academy Biomedical Co.) was added and incubated for 2 h at room temperature, and HRP was detected by incubation with tetramethylbenzidine (Organon Teknika, Boxtel, The Netherlands) for 20 min at room temperature. Plasma from wild-type mice spiked with human apoC-I (Labconsult, Brussels, Belgium) was used as a standard.

Intestinal TG absorption

APOC1 mice and wild-type littermates were fasted overnight and injected intravenously with 500 mg of Triton WR-1339 (Sigma) per kilogram of body weight as a 10% (v/v) solution in sterile saline to block LPL-mediated TG hydrolysis (29). Subsequently, mice were given an intragastric load of glycerol tri[9,10 (n)-^{3}H]oleate (10 μCi; Amersham, Buckinghamshire, UK) in 200 μl of olive oil. Blood samples were drawn before and at the indicated times after olive oil administration by tail bleeding. Lipids were extracted from plasma according to the method of Bligh and Dyer (30), and TG was separated from the other lipid components by thin-layer chromatography on Kieselgel 60 F_{254}

plates (Merck, Darmstadt, Germany) using hexane-diethyl ether-acetic acid (83:16:1, v/v/v) as eluents. The radioactivity in the TG fraction was determined by scintillation counting (Packard Instruments, Dowers Grove, IL) according to Voshol et al. (31).

Hepatic VLDL-TG production

Mice were fasted, anesthetized by intraperitoneal injection of Domitor (0.5 mg/kg; Pfizer, New York, NY), Dormicum (5 mg/kg; Roche Netherlands, Mijdrecht, The Netherlands), and fentanyl (0.05 mg/kg; Janssen-Cilag B.V., Tilburg, The Netherlands), and injected via the tail vein with 500 mg of Triton WR-1339 per kilogram of body weight (32). Blood samples were drawn at 1, 30, 60, 90, and 120 min after administration, and plasma TG levels were measured as described above.

Preparation and in vivo clearance of VLDL-like TG-rich emulsion particles

The preparation and characterization of 80 nm protein-free VLDL-like emulsion particles have previously been described (33). Briefly, emulsion particles were prepared by sonication from 100 mg of total lipid at an egg yolk phosphatidylcholine (Lipoid, Ludwigshafen, Germany)-triolein-lysophosphatidylcholine-cholesteryl oleate-cholesterol (all from Sigma) weight ratio of 22.7:70:2.3:3.0:2.0 in the presence of either 75 μCi of [^{3}H]TO or 150 μCi of [1α,2α(n)-^{3}H]cholesteryl oleate ([^{3}H]CO; Amersham) using a Soniprep 150 (MSE Scientific Instruments, Crawley, UK) at 10 μm output. The lipid composition of the emulsions was determined as described above. Emulsions were stored at 4°C under argon and were used within 7 days. To study the in vivo serum clearance of the radiolabeled emulsions, mice were anesthetized as described above and the abdomens were opened. The emulsion (100 μg of TG), preincubated (30 min at 37°C) with or without human apoC-I (50 μg), was injected intravenously via the inferior vena cava. Where indicated, mice received a preinjection of bovine lactoferrin (70 mg/kg; Serva, Heidelberg, Germany) at 1 min before injection of the radiolabeled emulsion. Blood samples (<50 μl) were taken via the inferior vena cava at the indicated times, and the radioactivity in serum was counted as described above. The total plasma volumes of the mice were calculated from the equation V (ml) = 0.04706 × body weight (g), as determined from ^{125}I-BSA clearance studies as previously described (34).

Plasma LPL level assay

Fasted *APOC1* mice and wild-type littermates were injected via the tail vein with heparin (0.1 unit/g; Leo Pharmaceutical Products B.V., Weesp, The Netherlands), and blood was collected after 10 min. The plasma was snap-frozen and stored at −80°C until analysis of the total LPL activity as modified from Zechner (35). In short, a TG substrate mixture containing triolein (TO; 4.6 mg/ml), [^{3}H]TO (2.5 μCi/ml), essentially fatty acid-free BSA (20 mg/ml; Sigma), Triton X-100 (0.1%; Sigma), and heat-inactivated (30 min at 56°C) human serum (20%) in 0.1 M Tris-HCl, pH 8.6, was generated by six sonication periods of 1 min using a Soniprep 150 at 7 μm output, with 1 min intervals between on ice. Ten microliters of postheparin plasma was added to 0.2 ml of substrate mixture and incubated for 30 min at 37°C in the presence or absence of 1 M NaCl, which completely inhibits LPL activity, to estimate both the LPL and HL levels. The reaction was stopped by the addition of 3.25 ml of heptane-methanol-chloroform (1:1.28:1.37, v/v/v), and 1 ml of 0.1 M K_2CO_3 in saturated H_3BO_3 (pH 10.5) was added. To quantify the [^{3}H]oleate generated, 0.5 ml of the aqueous phase obtained after vigorous mixing (15 s) and centrifugation (15 min at 3,600 rpm) was counted in 4.5 ml of Ultima Gold (Packard Bioscience, Meriden, CT). The LPL activity was calculated as the fraction of total triacylglycerol hydrolase activity inhibited by 1 M NaCl and expressed as the amount of FFA released per hour per milliliter of plasma.

In vitro LPL activity assay

The effect of apolipoproteins on the TG hydrolysis of VLDL-like emulsion particles was determined as described (36). [^{3}H]TO-labeled emulsion particles (0.5 mg TG/ml) were preincubated with apoC-I, apoC-III (Labconsult), apoA-I (Calbiochem, San Diego, CA), or recombinant apoA-V (37) at the indicated TG/protein weight ratios (30 min at 37°C). Subsequently, the protein-enriched particles were incubated with LPL in the presence of essentially fatty acid-free BSA (60 mg/ml) and heat-inactivated human serum (5%) in 0.1 M Tris-HCl, pH 8.5. At the indicated times, 50 μl samples from a 400 μl total incubation volume were added to 1.5 ml of methanol-chloroform-heptane-oleic acid (1,410:1,250:1,000:1, v/v/v/v) and 0.5 ml of 0.2 N NaOH to terminate lipolysis. Generated [^{3}H]oleate was counted as described above and expressed as a percentage of the total [^{3}H]activity added.

Statistical analysis

Statistical differences with respect to in vivo serum half-lives were determined using a two-way main-effects ANOVA. All other data were analyzed using nonparametric Mann-Whitney *U* tests. $P < 0.05$ was regarded as significant.

RESULTS

Effect of *APOC1* on plasma apoC-I and lipid levels

Table 1 summarizes the plasma human apoC-I and lipid levels in fasted male *APOC1* mice and wild-type littermates on chow diet. *APOC1* mice had ∼4-fold higher plasma levels of human apoC-I compared with humans (3), and this was accompanied by severe combined hyperlipidemia. The enhancing effect of *APOC1* expression on TG (12-fold) was much more pronounced than that on TC (2.1-fold) and FFA (1.5-fold), in agreement with our previous reports (9, 11). Similar effects of human apoC-I expression were observed in females compared with males (data not shown). Lipoprotein fractionation by FPLC showed that the plasma human apoC-I was primarily distributed toward HDLs and VLDLs (**Fig. 1A**). The increase in both plasma TG and TC as a result of *APOC1* expression could be mainly attributed to highly increased levels observed in VLDLs and mildly increased levels in intermediate density lipoproteins/LDLs, whereas the neutral lipid levels of the HDL fraction were hardly affected (Fig. 1B, C).

Effect of *APOC1* in apoE-deficient mice

Although apoC-I has been postulated to inhibit the apoE-dependent hepatic uptake of TG-rich lipoprotein remnants, *apoe*$^{-/-}$ mice show only minor increases of plasma TG. To investigate the effects of *APOC1* in the absence of endogenous apoE, *APOC1* mice were intercrossed with *apoe*$^{-/-}$ mice to generate *apoe*$^{-/-}$*APOC1* mice. Plasma human apoC-I levels in *apoe*$^{-/-}$*APOC1* mice were ∼4-fold higher compared with *APOC1* mice and severely further aggravated the hyperlipidemia observed in *apoe*$^{-/-}$ littermates (Table 1). As on a wild-type background, *APOC1* expression on an *apoe*$^{-/-}$ background had a much more pronounced effect on TG (55-fold) than on TC (3.2-fold)

TABLE 1. Effect of *APOC1* on plasma lipid levels in wild-type and *apoe*$^{-/-}$ mice

Genotype	Human ApoC-I	TG	Total Cholesterol	FFA
	mg/dl		*mmol/l*	
Wild-type background				
Wild type	n.d.	0.32 ± 0.06	2.06 ± 0.17	0.79 ± 0.15
APOC1	39.7 ± 9.4	3.86 ± 0.75[a]	4.28 ± 0.57[a]	1.18 ± 0.20[a]
apoe$^{-/-}$ background				
apoe$^{-/-}$	n.d.	0.59 ± 0.20	11.0 ± 5.2	0.78 ± 0.13
apoe$^{-/-}$*APOC1*	160 ± 60	32.6 ± 8.8[a]	35.7 ± 7.1[b]	2.52 ± 0.77[a]

apoC-I, apolipoprotein C-I; *APOC1*, human apoC-I-expressing gene; *apoe*$^{-/-}$, apolipoprotein E-deficient; n.d., not detectable; TG, triglyceride. Four hour fasted plasma was obtained from 10–12-week-old male *APOC1* (n = 23), wild-type (n = 10), *apoe*$^{-/-}$*APOC1* (n = 10), and *apoe*$^{-/-}$ (n = 6) mice. Plasma human apoC-I, TG, total cholesterol, and FFA levels were measured, and values are represented as means ± SD. Statistical differences were assessed between *APOC1* and wild-type mice and between *apoe*$^{-/-}$*APOC1* and *apoe*$^{-/-}$ mice.

[a] $P < 0.001$.

[b] $P < 0.01$.

and FFA (3.2-fold). Again, similar data were observed in females compared with males (data not shown).

Effect of *APOC1* on VLDL composition

To investigate whether the effect of *APOC1* expression on plasma lipid levels was accompanied by a change in VLDL composition and/or size, VLDLs were isolated from *apoe*$^{-/-}$*APOC1* mice and their *apoe*$^{-/-}$ littermates, and their relative lipid compositions were determined (**Table 2**). The composition of VLDLs from wild-type mice could not be determined accurately because of low circulating levels (Fig. 1). VLDLs of *apoe*$^{-/-}$*APOC1* mice were predominantly enriched in TG, compared with VLDLs from *apoe*$^{-/-}$ littermates, and had a higher core lipid (TG + cholesteryl ester) to surface lipid (FC + phospholipid) ratio (2.7 vs. 2.4, respectively), indicative of larger VLDL particles. Indeed, VLDL particle size analysis confirmed that *APOC1* expression markedly increased VLDL size compared with control littermates on both the wild-type background (average size, 72.9 vs. 44.4 nm, respectively) and the *apoe*$^{-/-}$ background (average size, 64.5 vs. 50.6 nm, respectively).

Effect of *APOC1* on intestinal TG absorption and hepatic VLDL-TG production

To further address the mechanism(s) underlying the hypertriglyceridemia in *APOC1* mice, we determined whether the intestinal TG absorption and/or the hepatic VLDL-TG production rate were enhanced in these mice. First, the intestinal TG absorption was studied by intravenously injecting Triton WR-1339 to block LPL-mediated TG-hydrolysis (29), after which an intragastric load of olive oil containing [^{3}H]TO was given. As shown in **Fig. 2**, no differences were observed between *APOC1* and wild-type mice with respect to the appearance of radioactivity in plasma TG, indicating that apoC-I expression does not enhance TG absorption from the intestinal lumen.

The hepatic VLDL-TG production rate was measured by determining plasma TG levels at the indicated times after intravenous Triton WR-1339 injection (**Fig. 3**). Whereas

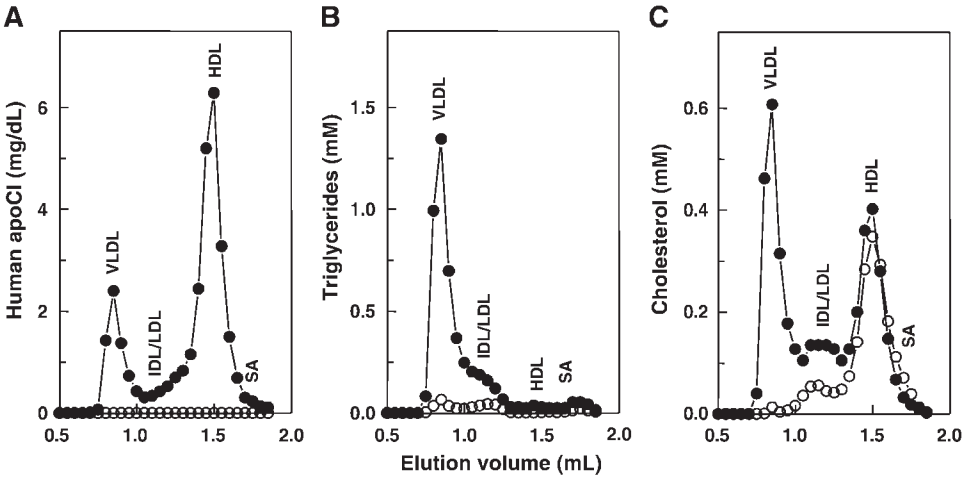

Fig. 1. Effect of the human apolipoprotein C-I (apoC-I)-expressing gene *APOC1* on fast-performance liquid chromatography (FPLC) profiles of human apoC-I and lipids. Plasma of male *APOC1* (closed circles) and wild-type (open circles) mice (n = 10) was pooled and size-fractionated by FPLC on a Superose 6 column. The individual fractions were analyzed for human apoC-I (A), triglyceride (TG; B), and total cholesterol (C). IDL, intermediate density lipoprotein; SA, Serum Albumin.

TABLE 2. Effect of *APOC1* on VLDL lipid composition

Genotype	TG	Cholesteryl Ester	Free Cholesterol	Phospholipid
		mg/mg VLDL-protein		
apoe$^{-/-}$	1.3	2.0	0.55	0.82
apoe$^{-/-}$*APOC1*	3.0	0.94	0.56	0.89

VLDL ($d < 1.006$ g/ml) was isolated from pooled plasma of fasted mice by ultracentrifugation, and the TG, cholesteryl ester, free cholesterol, phospholipid, and protein contents were measured.

the TG levels in the *APOC1* mice were higher compared with the wild-type littermates at the start of the experiment (4.9 ± 2.1 mM vs. 0.42 ± 0.08 mM, respectively; Fig. 3A), the relative increase in TG was similar for both types of mice (7.4 ± 0.9 mM/h vs. 6.6 ± 0.8 mM/h, respectively). Likewise, we found no difference in the relative increase in TG levels in *apoe*$^{-/-}$*APOC1* compared with *apoe*$^{-/-}$ mice (3.3 ± 1.4 mM/h vs. 3.1 ± 0.7 mM/h, respectively; Fig. 3B), indicating that apoC-I expression does not affect the hepatic VLDL-TG production rate either.

Effect of *APOC1* on in vivo clearance of VLDL-like emulsion particles

To investigate whether an impaired lipolytic processing of TG-rich lipoproteins may contribute to the hypertriglyceridemia observed in *APOC1* mice, mice were injected with [^{3}H]TO-labeled protein-free VLDL-like emulsion particles, which have previously been shown to mimic the metabolic behavior of TG-rich lipoproteins (33, 38). As shown in **Fig. 4**, the clearance of [^{3}H]TO was markedly decreased in *APOC1* mice compared with their wild-type littermates, as evident from a 2-fold increased serum half-life of [^{3}H]TO (7.9 ± 2.1 min vs. 4.0 ± 0.3 min, respectively; $P < 0.05$). This observation indicates that *APOC1* expression impairs TG clearance, which may result from inhibition of the LPL-mediated VLDL-TG hydrolysis.

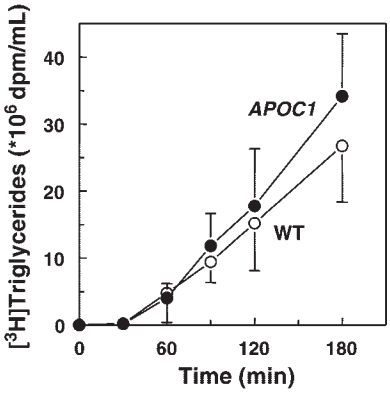

Fig. 2. Effect of *APOC1* on intestinal lipid absorption. Overnight-fasted *APOC1* (closed circles) and wild-type (WT; open circles) mice were injected intravenously with Triton WR-1339 (500 mg/kg) and subsequently given an intragastric load of [^{3}H]triolein (TO) in 200 μl of olive oil. Blood samples were drawn at the indicated times, and lipids were extracted from plasma. Lipids were separated by thin-layer chromatography, and the radioactivity in the TG fraction was determined by scintillation counting. Values are means ± SD (n = 7).

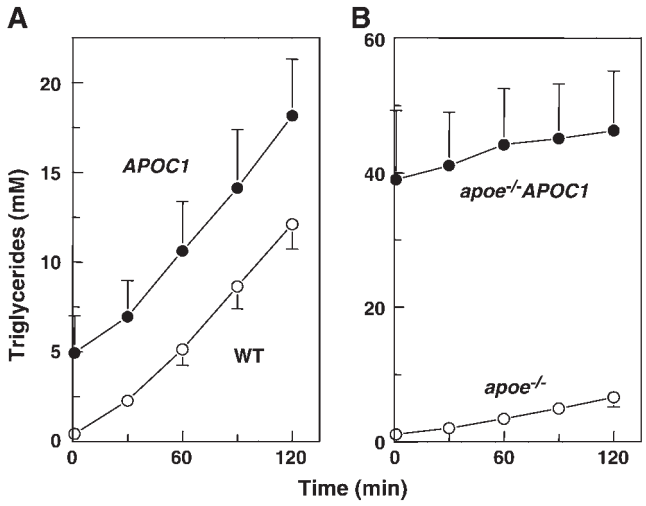

Fig. 3. Effect of *APOC1* on hepatic VLDL-TG production. Triton WR-1339 (500 mg/kg) was injected (time 0) into fasted *APOC1* (closed circles) and wild-type (WT; open circles) mice (A) and in apolipoprotein E-deficient (*apoe*$^{-/-}$) and *apoe*$^{-/-}$*APOC1* mice (B). Plasma TG levels were determined at 1, 30, 60, 90, and 120 min after injection. Values represent means ± SD (n = 6).

Effect of *APOC1* on plasma LPL levels

An impaired clearance of VLDL-TG in *APOC1* mice can be attributable to either decreased expression of LPL and/or apoC-I-induced inhibition of the activity of LPL. Therefore, we first determined plasma lipase levels in postheparin plasma by incubation with a [^{3}H]TO-containing substrate mixture (**Fig. 5**). Whereas the postheparin HL level was only slightly increased in *APOC1* mice compared with wild-type littermates (12.8 ± 1.2 μmol FFA/h/ml vs. 11.3 ± 1.0 μmol FFA/h/ml, respectively; $P < 0.05$), the postheparin LPL level was even 1.8-fold increased in *APOC1* mice compared with wild-type mice (40.7 ± 6.1 μmol FFA/h/ml vs. 22.5 ± 2.2 μmol FFA/h/ml, respectively; $P < 0.01$). Therefore, the impaired lipolytic conversion of VLDL in *APOC1* mice cannot be attributable to decreased levels of LPL.

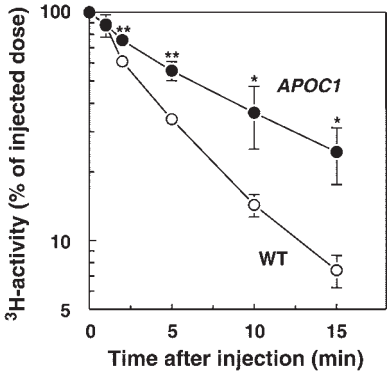

Fig. 4. Effect of *APOC1* on serum clearance of VLDL-like emulsion particles in vivo. [^{3}H]TO-labeled emulsion particles (100 μg of TG) were injected via the inferior vena cava into anesthetized *APOC1* (closed circles) and wild-type (WT; open circles) mice. Blood samples were taken at the indicated times, and ^{3}H activity was determined in serum. Values are means ± SD (n = 3). * $P < 0.05$, ** $P < 0.01$.

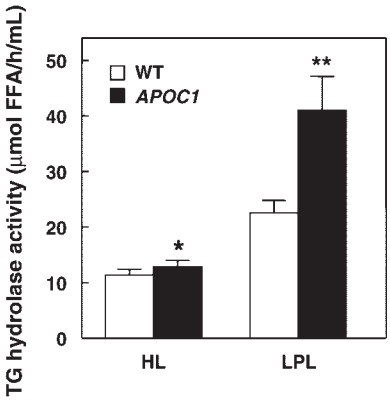

Fig. 5. Effect of *APOC1* on plasma HL and LPL levels in vivo. Fasted *APOC1* (closed bars) and wild-type (WT; open bars) mice were injected intravenously with heparin (0.1 unit/g). Plasma, collected at 10 min after injection, was incubated (30 min at 37°C) with a [^{3}H]TO-containing substrate mixture in the absence or presence of 1 M NaCl to estimate both the LPL and HL activity. Generated [^{3}H]oleate was extracted and determined as described. Values represent means ± SD (n = 8). * $P < 0.05$, ** $P < 0.01$.

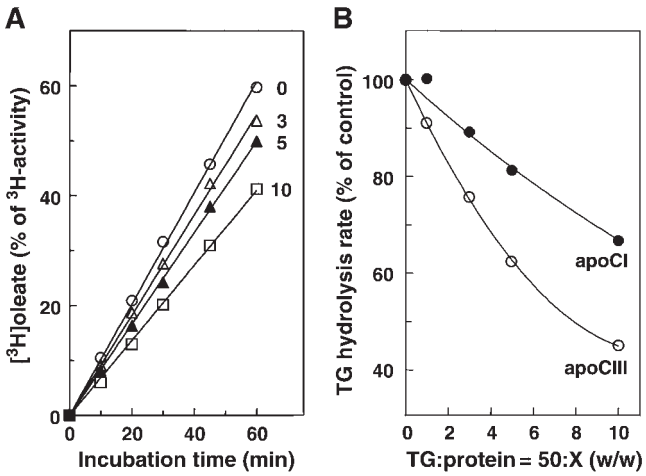

Fig. 6. Effect of apoC-I on LPL-mediated hydrolysis of VLDL-like emulsion TGs. A: [^{3}H]TO-labeled protein-free emulsion particles were preincubated (30 min at 37°C) in the absence (open circles) and presence of apoC-I at TG/apoC-I weight ratios of 50:3 (open triangles), 50:5 (closed triangles), and 50:10 (open squares). At the indicated times after addition of LPL, generated [^{3}H]oleate was extracted and quantified. B: The effect of apoC-I (closed circles) on LPL-mediated TG hydrolysis was compared with that of apoC-III (open circles) and is depicted as a percentage of the TG hydrolysis rate in the absence of protein.

Effect of apoC-I on LPL activity

To investigate whether the apoC-I-related impaired lipolytic conversion of VLDL can be attributable to a direct inhibiting effect of apoC-I on LPL activity, protein-free VLDL-like emulsion particles were enriched with increasing concentrations of purified human apoC-I and subsequently incubated with LPL. The well-established endogenous LPL inhibitor apoC-III (39, 40) was used as a control. ApoC-I and apoC-III were compared on a mass basis, as they are also present in human plasma at similar mass concentrations (i.e., 10 and 13 mg/dl) (3). ApoC-I appeared to dose-dependently inhibit the TG hydrolysis rate (**Fig. 6A**). At a TG/protein weight ratio of 50:10, apoC-I and apoC-III inhibited the triacylglycerol hydrolase activity of LPL by 33% and 55%, respectively (Fig. 6B). In contrast, apoA-I did not affect lipolysis, and apoA-V even dose-dependently increased the lipolysis rate up to 1.5-fold at a TG/apoA-V weight ratio of 50:3 (data not shown), in agreement with our previous observations (36, 37).

Effect of apoC-I enrichment of VLDL-like emulsion particles on in vivo clearance

To assess whether apoC-I can directly inhibit lipolysis in vivo, the effect of apoC-I protein was determined on the plasma decay of [^{3}H]TO-labeled protein-free VLDL-like emulsion particles in wild-type mice. To focus on the effects of apoC-I on peripheral lipolysis rather than on liver uptake, mice were preinjected with lactoferrin. Lactoferrin has previously been shown to block the interaction of chylomicrons and emulsion particles with the liver in vivo (33, 41), which we confirmed using [^{3}H]CO-labeled protein-free VLDL-like emulsion particles (results not shown). As depicted in **Fig. 7**, preincubation of emulsion particles with apoC-I markedly delayed the clearance of [^{3}H]TO, as is evident from a 1.9-fold increased serum half-life (17.6 ± 5.7 min vs. 9.2 ± 3.7 min, respectively; $P < 0.05$), indicating that apoC-I can indeed inhibit lipolytic TG conversion in vivo.

DISCUSSION

Studies in both humans (6) and mice (9, 10) have shown that increased expression of apoC-I results in combined hyperlipidemia, with a more pronounced enhancing effect on TG compared with TC. Because a variety of effects on lipid metabolism have been attributed to apoC-I, in-

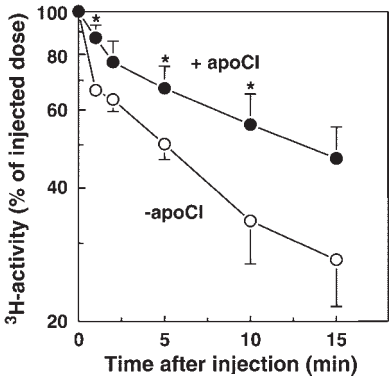

Fig. 7. Effect of human apoC-I enrichment of VLDL-like emulsion particles on their serum clearance in lactoferrin-treated mice. [^{3}H]TO-labeled emulsion particles (100 μg of TG) were preincubated without (open circles) or with (closed circles) human apoC-I (50 μg) for 30 min at 37°C and injected via the inferior vena cava into anesthetized lactoferrin-treated wild-type mice. Blood samples were taken at the indicated times, and ^{3}H activity was determined in serum. Values are means ± SEM (n = 3). * $P < 0.05$.

cluding activatory effects (e.g., LCAT) and inhibitory effects [e.g., HL, cholesteryl ester transfer protein (CETP), intestinal absorption, and apoE-dependent recognition by LRP, LDLr, and VLDL receptor (VLDLr)], the aim of the present study was to elucidate the main mechanism underlying the apoC-I-related hypertriglyceridemia using *APOC1* transgenic mice. We demonstrated that at moderate plasma human apoC-I levels [i.e., 4-fold higher than those found in humans (3)], the 12-fold increase in plasma TG levels was mainly attributable to inhibition of the lipolytic processing of VLDLs.

The effects of apoC-I on lipid metabolism were mainly confined to VLDL metabolism, leaving HDL metabolism (which crucially involves both CETP and LCAT) unaffected. Analysis of the HDL protein constituents for CETP-modulating properties showed that apoC-I is a very potent and highly selective inhibitor of CETP (42). In addition, Gautier et al. (43) have shown that cross-breeding of human *CETP* transgenic mice with apoC-I-deficient mice resulted in higher CETP activity in vivo. Although apoC-I thus appears to be a physiologically relevant inhibitor of CETP, this function of apoC-I cannot contribute to the phenotype of *APOC1* mice, because mice do not express CETP (44). Activation of LCAT should be expected to lead to increased HDL size and HDL lipids, as was observed in mice and rabbits that overexpress LCAT (19, 45). Because both the cholesterol level and the size of HDL are not affected by apoC-I expression in *APOC1* mice, potential LCAT-activating properties of apoC-I (12) do not appear to be relevant for determining HDL levels in mice.

ApoC-I expression thus predominantly affects VLDL-TG metabolism, which can result from *i*) increased intestinal TG absorption, *ii*) increased VLDL-TG production, and/or *iii*) disturbed lipolytic conversion and/or hepatic clearance of VLDL. Previously, we have reported that mice deficient for apoC-I showed a significantly lower intestinal lipid absorption compared with wild-type mice (34). However, no changes in intestinal lipid absorption were observed in *APOC1* mice compared with wild-type littermates, which can be related to a relatively low expression of human apoC-I in the intestine. Previously, we have shown that apoE-deficient mice show a decreased VLDL-TG secretion rate (26), and we confirm this observation in our present study. Although human apoC-I is highly expressed in the liver, we did not detect any effect of human apoC-I expression on hepatic VLDL-TG production rate on either a wild-type or an apoE-deficient background, which is in agreement with our previous studies showing that apoC-I deficiency did not alter the hepatic VLDL production rate (7). Apparently, expression of human apoC-I cannot compensate for the decreased VLDL-TG production in apoE-deficient mice. Collectively, the hypertriglyceridemia in *APOC1* mice is not caused by either an effect on intestinal TG absorption or hepatic TG production.

Next, we evaluated the effect of apoC-I expression on apoE-dependent VLDL uptake by the liver. ApoC-I has been shown to inhibit the apoE-mediated binding of TG-rich lipoprotein remnants by hepatic lipoprotein receptors (i.e., LDLr and LRP) (22, 23, 46), although Quarfordt, Michalopoulos, and Schirmer (21) reported that the apoC-I-mediated inhibition of the uptake of TG-rich emulsions by cultured hepatocytes was (at least partly) independent on the presence of apoE. Indeed, we have shown that *APOC1* expression in mice can interfere with the hepatic interaction of VLDLs primarily via LRP (9, 24). However, the contribution of this effect to the *APOC1*-induced severe hypertriglyceridemia can be questioned, because complete blockade of the apoE-dependent hepatic lipoprotein clearance in *apoe*$^{-/-}$ mice only mildly affects plasma TG levels (25), whereas *APOC1* expression increases plasma TG as much as 12-fold. In addition, we now show that *APOC1* expression on an *apoe*$^{-/-}$ background further dramatically increased TG levels, showing that the hypertriglyceridemic effects of apoC-I can be independent of the presence of apoE. Taken together, these data indicate that the hypertriglyceridemia observed in *APOC1* mice also cannot be explained by the inhibition of apoE-mediated hepatic remnant uptake.

Finally, we evaluated the possibility that the lipolytic conversion of VLDLs may be impaired in *APOC1* mice, because such a mechanism may explain the dramatic accumulation of plasma TGs in primarily VLDLs. In addition, decreased plasma TG hydrolysis may also explain the increased VLDL particle size observed in *APOC1*-expressing mice on both the wild-type and *apoe*$^{-/-}$ backgrounds and the observed impaired clearance of VLDL-like emulsion particles upon intravenous administration.

Recently, Conde-Knape et al. (15) described the cross-breeding of their human apoC-I-expressing mouse strain with *apoe*$^{-/-}$ mice, which resulted in a comparable, albeit more modest, hypertriglyceridemic phenotype as our *apoe*$^{-/-}$ *APOC1* mice, and they suggested that the hypertriglyceridemia in these mice was attributable to inhibition of HL-mediated TG hydrolysis. However, HL deficiency or overexpression in mice and rabbits predominantly affects plasma HDL-TC levels, with only mild effects (if any) on TG levels on both wild-type and *apoe*$^{-/-}$ backgrounds (16–18, 47). Furthermore, HL has a much lower preference for TG compared with LPL (48), and HL is known to primarily mediate the conversion of intermediate density lipoprotein to LDL and of HDL_2 to HDL_3 (49), whereas both our studies and those of Conde-Knape et al. (15) indicate that *APOC1*-expressing mice merely have a disturbed VLDL metabolism. Therefore, although a potential inhibiting effect of apoC-I on the activity of HL in vivo cannot be ruled out, and it may add to the observed hypercholesterolemia, it does not contribute to the severe hypertriglyceridemia observed in *APOC1* mice.

Thus, impairment of LPL remains the most likely mechanism to explain the hypertriglyceridemic phenotype of *APOC1* mice. Although the *APOC1* mice showed increased LPL levels in postheparin plasma, we indeed found that apoC-I is very effective in attenuating the LPL activity in vitro, with 60% efficiency on a mass basis compared with the well-known endogenous LPL inhibitor apoC-III (34, 50). Our observations confirm previous in vitro studies by Havel et al. (13), who showed that apoC-I and apoC-III

were equally effective on a mass basis with respect to inhibition of the apoC-II-stimulated LPL-mediated TG hydrolysis. In fact, the LPL inhibitory properties of apoC-I and apoC-III are specific for these apolipoproteins, because addition of the negative control apoA-I (36) had no effect on the LPL activity, and the recently identified LPL stimulator apoA-V (37) enhanced the LPL activity in this assay. Importantly, the TG/apoC-I ratios applied in the in vitro assay at which apoC-I inhibited LPL (50:3 to 5:10, w/w) were similar to those found in both *APOC1* mice (50:6) and *apoe*$^{-/-}$*APOC1* mice (50:3), indicating that the LPL inhibitory properties observed in vitro are relevant for the in vivo situation. Indeed, preincubation of VLDL-like emulsion particles with apoC-I inhibited the liver-independent serum clearance of emulsion TG, as was demonstrated in lactoferrin-treated mice. Concomitantly, the uptake of TG-derived fatty acids by white adipose tissue was 1.8-fold decreased (data not shown). Given the fact that apoC-I readily exchanges between lipoproteins, a part of the injected emulsion-associated apoC-I will presumably rapidly redistribute toward endogenous lipoproteins, which will even lead to underestimation of the inhibiting effect of apoC-I on emulsion TG clearance. In a previous study from our group in which VLDL clearance was assessed in functionally hepatectomized *APOC1* mice on a low fat/low-cholesterol diet, we also found a tendency toward a decreased VLDL-TG lipolysis rate in *APOC1* mice (i.e., 32%), although a statistically significant difference was not reached under the applied experimental conditions (9).

The phenotype of *APOC1* mice closely resembles that of human apoC-III-expressing *APOC3* mice with respect to the predominant increase of VLDL-TG levels (51). In addition, both *APOC1* mice and *APOC3* mice (52) show a modest increase in plasma cholesterol levels. In fact, the relative increase in TG compared with cholesterol induced by apoC-I expression (i.e., 5.8) is similar to that induced by apoC-III expression (i.e., 5.5) (52). Indeed, it has been established that LPL activity strongly determines plasma TG levels. Overexpression of LPL in mice markedly reduces plasma VLDL-TG levels (53, 54), whereas heterozygous deficiency of LPL results in the accumulation of plasma VLDL-TG (55). As in *APOC1* and *APOC3* mice, the effects of LPL modulation on plasma TG exceeded those on TC.

Inhibition of the lipolytic conversion of TG-rich lipoproteins in *APOC1* mice can fully account for our previous observation that *APOC1* protects against the development of obesity on a genetically obese leptin-deficient (*ob/ob*) background (56) by impeding the disposition of LPL-liberated fatty acids into adipose tissue. Likewise, we recently observed that deletion of the main endogenous LPL inhibitor apoC-III in *apoc3*$^{-/-}$ mice markedly aggravates diet-induced obesity as related to increased adipose tissue stores (our unpublished observations). Interestingly, we have reported that VLDLr-deficient (*vldlr*$^{-/-}$) mice are protected from diet-induced obesity on both wild-type and *ob/ob* backgrounds (57). Subsequently, Yagyu et al. (58) have shown that *vldlr*$^{-/-}$ mice have reduced LPL activity as related to the LPL chaperone function of the VLDLr (59), which may partially explain their resistance to obesity. In addition, the VLDLr may also be involved in LPL-mediated lipolysis by bridging of lipoproteins to the endothelial surface, thereby facilitating the LPL-particle interaction. Because we have firmly established that apoC-I strongly inhibits the interaction of VLDL with the VLDLr (24), a concurring VLDLr-inhibiting effect of apoC-I may further hamper the LPL-mediated VLDL-TG hydrolysis in vivo as observed in *APOC1* mice.

In conclusion, we have demonstrated that the hypertriglyceridemic effect of moderate human apoC-I expression in mice is the consequence of an impaired lipolytic conversion of VLDL-TG. This effect probably results from a direct inhibiting effect of apoC-I on LPL activity, although a concomitant inhibiting effect of apoC-I on VLDL binding to the VLDLr, which facilitates lipolysis, cannot be excluded. The mechanism by which apoC-I inhibits LPL activity is currently under investigation.

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