Endogenous apoC-I increases hyperlipidemia in apoE-knockout mice by stimulating VLDL production and inhibiting LPL

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Abstract Previous studies have shown that overexpression of human apolipoprotein C-I (apoC-I) results in moderate hypercholesterolemia and severe hypertriglyceridemia in mice in the presence and absence of apoE. We assessed whether physiological endogenous apoC-I levels are sufficient to modulate plasma lipid levels independently of effects of apoE on lipid metabolism by comparing apolipoprotein E gene-deficient/apolipoprotein C-I gene-deficient (*apoe*$^{-/-}$*apoc1*$^{-/-}$), *apoe*$^{-/-}$*apoc1*$^{+/-}$, and *apoe*$^{-/-}$*apoc1*$^{+/+}$ mice. The presence of the apoC-I gene-dose-dependently increased plasma cholesterol (+45%; $P < 0.001$) and triglycerides (TGs) (+137%; $P < 0.001$), both specific for VLDL. Whereas apoC-I did not affect intestinal [^{3}H]TG absorption, it increased the production rate of hepatic VLDL-TG (+35%; $P < 0.05$) and VLDL-[^{35}S]apoB (+39%; $P < 0.01$). In addition, apoC-I increased the postprandial TG response to an intragastric olive oil load (+120%; $P < 0.05$) and decreased the uptake of [^{3}H]TG-derived FFAs from intravenously administered VLDL-like emulsion particles by gonadal and perirenal white adipose tissue (WAT) (−34% and −25%, respectively; $P < 0.05$). As LPL is the main enzyme involved in the clearance of TG-derived FFAs by WAT, and total postheparin plasma LPL levels were unaffected, these data demonstrate that endogenous apoC-I suffices to attenuate the lipolytic activity of LPL. Thus, we conclude that endogenous plasma apoC-I increases VLDL-total cholesterol and VLDL-TG dose-dependently in *apoe*$^{-/-}$ mice, resulting from increased VLDL particle production and LPL inhibition.—Westerterp, M., W. de Haan, J. F. P. Berbée, L. M. Havekes, and P. C. N. Rensen. **Endogenous apoC-I increases hyperlipidemia in apoE-knockout mice by stimulating VLDL production and inhibiting LPL. *J. Lipid Res.* 2006.** 47: **1203–1211.**

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Hypertriglyceridemia is a common finding in the general population. Although it can be caused by many factors, including dietary habits, alcohol intake, decreased physical activity, medication, and various diseases, it is clear that a relatively large number of individuals have a genetic tendency to develop hypertriglyceridemia.

Interestingly, a polymorphism in the promoter region of apolipoprotein C-I (apoC-I), the *HpaI* polymorphism, has been identified that is associated with increased plasma triglyceride (TG) levels (1). Although this promoter polymorphism leads to 50% increased apoC-I transcription in vitro (2), linkage disequilibrium with apoE polymorphisms hampers interpretation of the effect of apoC-I on plasma TG levels. In fact, it has been suggested that apoC-I protein levels are influenced by the apoE genotype rather than the *HpaI* polymorphism (3).

To gain better understanding about the role of human apoC-I in lipoprotein metabolism, mice expressing only the human apoC-I gene have been generated. *APOC1* transgenic mice show highly increased plasma TG levels and mildly increased total cholesterol (TC) and FFA levels (4–6). This hyperlipidemic phenotype was initially explained by the inhibition of apoE-mediated hepatic remnant clearance (7). However, we (8) and others (9) recently showed that human apoC-I expression still resulted in massive hypertriglyceridemia on an apolipoprotein E-deficient (*apoe*$^{-/-}$) background. In fact, we have demonstrated that apoC-I is a potent inhibitor of the lipolytic activity of LPL in vitro and in vivo, which contributes to a great extent to the hyperlipidemia observed in *APOC1* mice (8). Perhaps related to the LPL inhibition, *APOC1* mice are also protected against obesity

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Abbreviations: apoC-I, apolipoprotein C-I; *apoe*$^{-/-}$, apolipoprotein E gene-deficient; C_t, threshold cycle number; LDLr, low density lipoprotein receptor; TC, total cholesterol; TG, triglyceride; TO, triolein; WAT, white adipose tissue.

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development on the leptin-deficient *ob/ob* background (10). Collectively, *APOC1* expression in mice is consistently associated with hypertriglyceridemia and protects against obesity.

Although human apoC-I expression leads to a clear and consistent hypertriglyceridemic effect in several mouse models, it is not clear whether physiological expression of apoC-I is already functional in affecting TG levels. The aim of this study was to elucidate the role of endogenous apoC-I in plasma lipid metabolism irrespective of the expression of apoE, using $apoe^{-/-}apoc1^{-/-}$, $apoe^{-/-}apoc1^{+/-}$, and $apoe^{-/-}apoc1^{+/+}$ littermate mice. The $apoe^{-/-}$ background enables us to study the effect of endogenous apoC-I on lipid metabolism in the absence of apoE, which has been shown to interfere with VLDL production (11), VLDL lipolysis (12), and remnant clearance (13). Our results show that physiological apoC-I expression on an $apoe^{-/-}$ background gene-dose-dependently increases VLDL-specific plasma TG and TC levels. From subsequent mechanistic studies, we conclude that endogenous apoC-I has a physiologically important function in stimulating VLDL particle production and attenuating the lipolytic activity of LPL.

EXPERIMENTAL PROCEDURES

Animals

$Apoe^{-/-}apoc1^{-/-}$ mice were generated as described previously (14) and were back-crossed at least eight times to the C57Bl/6 background. $apoe^{-/-}apoc1^{-/-}$ mice were crossed with $apoe^{-/-}apoc1^{+/+}$ mice to generate $apoe^{-/-}apoc1^{+/-}$ mice. These mice were intercrossed to obtain $apoe^{-/-}apoc1^{-/-}$, $apoe^{-/-}apoc1^{+/-}$, and $apoe^{-/-}apoc1^{+/+}$ littermates, the males of which were used in all experiments. Plasma lipid analysis was performed in 12 week old $apoe^{-/-}apoc1^{-/-}$, $apoe^{-/-}apoc1^{+/-}$, and $apoe^{-/-}apoc1^{+/+}$ mice, and subsequent experiments were performed with 12–20 week old $apoe^{-/-}apoc1^{-/-}$ and $apoe^{-/-}apoc1^{+/+}$ mice. Mice were housed under standard conditions with a 12 h light cycle (7:00 AM–7:00 PM) and were fed ad libitum with regular chow. Experiments were performed after 4 h of fasting at 1:00 PM, with food withdrawal at 9:00 AM, unless stated otherwise.

Analysis of gene expression by real-time quantitative PCR

Fasted $apoe^{-/-}apoc1^{-/-}$, $apoe^{-/-}apoc1^{+/-}$, and $apoe^{-/-}apoc1^{-/-}$ mice were euthanized by cervical dislocation, and isolated livers were snap-frozen. Total RNA was isolated according to Chomczynski and Sacchi (15), treated with DNase (DNase I), and reverse-transcribed (RevertAid) according to the protocols supplied by the manufacturers. Quantitative gene expression analysis using SYBR Green technology (Eurogentec) was performed as described (16). The *apoc1*, *apoc2*, and *apoc3* mouse PCR primers were validated for identical efficiencies. Hypoxanthine-guanine phosphoribosyltransferase and β-actin were used as standard housekeeping genes. Relative gene expression was calculated by subtracting the threshold cycle number (C_t) of the target gene from the average C_t of hypoxanthine-guanine phosphoribosyltransferase and β-actin ($C_{t\ \mathrm{housekeeping}}$) and raising 2 to the power of this difference. The average C_t of two housekeeping genes was used to exclude the possibility that changes in the relative expression of *apoc* genes were caused by variations in the expression of the separate housekeeping genes.

ApoC-I ELISA

Plasma murine apoC-I concentrations were determined using a human apoC-I sandwich ELISA, which shows cross-reaction with murine apoC-I at relatively low plasma dilutions (1:20). 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, 1:20) 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 $apoe^{-/-}apoc1^{-/-}$ mice spiked with human apoC-I (Labconsult, Brussels, Belgium) was used as a standard.

Plasma lipid and lipoprotein analysis

Blood was collected by tail bleeding into chilled paraoxon (Sigma, St. Louis, MO)-coated capillary tubes to prevent ongoing in vitro lipolysis (17), unless indicated otherwise. The tubes were placed on ice and centrifuged at 4°C, and the obtained plasma was snap-frozen in liquid nitrogen and stored at −20°C. Plasma was assayed for TC, TG, and FFA using commercially available enzymatic kits 236691, 1488872 (Roche Molecular Biochemicals, Indianapolis, IN), and NEFA-C (Wako Chemicals, Neuss, Germany), respectively. For determination of the lipid distribution over plasma lipoproteins by fast-performance liquid chromatography, 50 μl of pooled plasma from 10 mice per group was injected onto a Superose 6 HR 10/30 column (Äkta System; Amersham Pharmacia Biotech, Piscataway, NJ) and eluted at a constant flow rate of 50 μl/min PBS, 1 mM EDTA (Sigma), pH 7.4. Fractions of 50 μl were collected and assayed for TC and TG as described above.

Intestinal lipid absorption

To measure intestinal lipid absorption, overnight fasted mice received an intravenous injection of Triton WR-1339 (0.5 g/kg body weight, 10% solution in PBS; Sigma) to block plasma lipoprotein clearance (18). Subsequently, mice received an intragastric load of [^{3}H]triolein ([^{3}H]TO) (12 μCi; Amersham Biosciences) in 200 μl of olive oil (Carbonell). Blood samples (50 μl) were drawn before gavage (time 0) and 0.5, 1, 2, 3, and 4 h after gavage. Plasma TG was measured as described above, and plasma ^{3}H activity was counted in 2 ml of Hionic Fluor (Packard). To verify that the radioactivity was incorporated into TG, lipids were extracted according to the method of Bligh and Dyer (19) and separated by thin-layer chromatography on Kieselgel 60 F_{254} plates using hexane-diethylether-acetic acid (83:16:1, v/v/v) as eluent. The ^{3}H activities in extracted TG and other lipid fractions were counted.

Hepatic VLDL particle production

Mice were fasted and anesthetized with an intraperitoneal injection of acepromazine (6.25 μg/g; Neurotanq; Alvasan International BV, Weesp, The Netherlands), dormicum (6.25 μg/g; Roche Netherlands, Mijdrecht, The Netherlands), and fentanyl (0.31 μg/g; Janssen-Cilag BV, Tilburg, The Netherlands). Mice received an intravenous injection of Tran^{35}S label (150 μCi/mouse; Amersham) to label newly produced apoB, followed, 30 min later, by an intravenous injection of Triton WR-1339 (0.5 mg/g, 10% solution in PBS). Blood samples were drawn before (time 0) and 15, 30, 60, and 90 min after injection. Plasma was assayed for TG as described above. After the last sampling, mice were euthanized by cervical dislocation and exsanguinated via the retro-orbital plexus. VLDL was quantitatively isolated from plasma after density gradient ultracentrifugation at d < 1.006 g/ml by aspiration (20). VLDL-apoB was selectively precipitated with 2-propanol (21) and counted for incorporated ^{35}S. VLDL-TG and cholesterol were measured as described above, and phosphatidylcholine was measured using the commercially available kit 990-54009 (Wako Chemicals) according to the manufacturer's instructions. Protein content was measured according to Lowry et al. (22).

Postprandial TG response

To determine the effect of endogenous apoC-I on the postprandial TG response, overnight fasted mice received an intragastric load of 200 μl of olive oil. Blood samples of 35 μl were drawn as described above just before gavage (time 0) and 1, 2, 4, and 8 h after gavage. Obtained plasma samples were assayed for TG as described above.

Total plasma and tissue LPL levels

To determine total plasma LPL activity levels, fasted mice were injected via the tail vein with heparin (0.1 U/g; Leo Pharmaceutical Products BV, Weesp, The Netherlands) and blood was collected after 10 min. The plasma thereof was snap-frozen and stored at −80°C until analysis. *Apoe*$^{-/-}$ *apoc1*$^{-/-}$ and *apoe*$^{-/-}$ *apoc1*$^{+/+}$ mice in the fed state were euthanized, and liver, heart, hind limb muscle, and white adipose tissue (WAT; i.e., gonadal, perirenal, and intestinal) were collected. The organ samples were cut into small pieces and added to 1 ml of DMEM supplemented with 2% BSA. Heparin (2 units) was added, and LPL was released by shaking for 60 min at 37°C. After centrifugation (10 min at 13,000 rpm), supernatants were removed, snap-frozen, and stored at −80°C until analysis. Total LPL activity of all samples was determined as modified from Zechner (23). Ten microliters of post-heparin plasma or supernatant of the respective tissue was incubated for 30 min at 37°C in 260 μl of LPL substrate mixture {3.5 mg/ml TO, 1.9 μCi/ml [^{3}H]TO, 0.078% Triton X-100, 15% (v/v) heat-inactivated (1 h, 56°C) human serum, and 15 mg/ml FFA-free BSA in 0.076 M Tris-HCl, pH 8.6}. Samples were incubated in the presence and absence of 1 M NaCl, which inhibits LPL activity completely, to determine both LPL and HL activities. After incubation, 50 μl of the reaction mixture was added to 3.25 ml of heptane-methanol-chloroform (100:128:137, 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 generated [^{3}H]oleate, 500 μl of the aqueous phase obtained after vigorous mixing and centrifugation (15 min, 3,600 rpm) was counted for ^{3}H activity. TG hydrolase activity was expressed as the amount of [^{3}H]oleate released per hour per milliliter of plasma. The fraction of TG hydrolase activity not inhibited by 1 M NaCl was considered as HL activity. LPL activity was calculated as the fraction of total TG hydrolase activity inhibited by 1 M NaCl.

VLDL lipolysis in vitro

Apoe$^{-/-}$ *apoc1*$^{-/-}$ and *apoe*$^{-/-}$ *apoc1*$^{+/+}$ mice were euthanized by cervical dislocation, and blood was drawn from the retro-orbital vein. Sera were collected after centrifugation at 4°C and pooled from four mice per group. 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 as described above. VLDL (0.3 mM TG) was incubated at 37°C with 0.3 μg/ml LPL (Sigma) in 0.1 M Tris-HCl and 60 mg/ml FFA-free BSA, pH 8.5. Samples were taken just after the addition of LPL (time 0) and at 15, 30, 60, and 90 min. LPL activity was inhibited by the addition of NaCl (1 M final concentration), and samples were assayed for FFA as described above.

Generation of VLDL-like emulsion particles

VLDL-like TG-rich emulsion particles were prepared and characterized as described previously (24, 25). One hundred milligrams of lipid at a weight ratio of egg yolk phosphatidylcholine/TO/lysophosphatidylcholine/cholesteryl oleate/cholesterol of 22.7:70:2.3:3.0:2.0, supplemented with 200 μCi of [^{3}H]TO, was sonicated at 10 microns output using a Soniprep 150 (MSE Scientific Instruments, Crawley, UK). An emulsion fraction containing 80 nm emulsion particles was obtained by consecutive density gradient ultracentrifugation steps and used for subsequent experiments. The TG content of the emulsions was determined as described above. Emulsions were stored at 4°C under argon and were used within 7 days.

In vivo clearance of VLDL-like emulsions

To study the in vivo clearance of the VLDL-like emulsion particles, mice were anesthetized as described above. The abdomens were opened, and 200 μl of [^{3}H]TO-labeled emulsion particles was administered via the vena cava inferior at a dose of 150 μg of TG per mouse. Blood samples (50 μl) were taken from the vena cava inferior at 1, 2, 5, 10, and 15 min after the injection, and serum ^{3}H activity was counted. Plasma volumes (ml) were calculated as 0.04706 × body weight (g) as determined from ^{125}I-BSA clearance studies, as described previously (26). After taking the last blood sample, liver, heart, spleen, hind limb muscle, and WAT (i.e., gonadal, perirenal, and intestinal) were harvested. Organs were dissolved overnight at 60°C in 500 μl of Solvable™ (Perkin-Elmer, Boston MA), and ^{3}H activity was counted (25).

Statistical analysis

The Mann-Whitney nonparametric test for two independent samples was used to define differences between data sets from experimental groups. The criterion for significance was set at $P < 0.05$. Statistical analyses were performed using SPSS 11.5 (SPSS, Inc., Chicago, IL).

RESULTS

Expression levels of endogenous apoC-I

The relative mRNA expression levels of *apoc1* in *apoe*$^{-/-}$ *apoc1*$^{-/-}$, *apoe*$^{-/-}$ *apoc1*$^{+/-}$, and *apoe*$^{-/-}$ *apoc1*$^{+/+}$ littermates were measured in the liver, which is the main source of *apoc1* mRNA expression (27), and apoC-I protein levels were determined in plasma (**Table 1**). These

TABLE 1. Expression levels of endogenous apoC-I

Genotype	Liver *apoc1* mRNA Expression	Plasma ApoC-I Level
	% of apoe$^{-/-}$ *apoc1*$^{+/+}$ *mice*	
apoe$^{-/-}$ *apoc1*$^{-/-}$	n.d.	n.d.
apoe$^{-/-}$ *apoc1*$^{+/-}$	56 ± 1[a]	50 ± 8[a]
apoe$^{-/-}$ *apoc1*$^{+/+}$	100 ± 29	100 ± 11

apoC-I, apolipoprotein C-I; *apoe*$^{-/-}$, apolipoprotein E gene-deficient; n.d., not detected. Livers and plasma were obtained from 12 week old, 4 h fasted *apoe*$^{-/-}$ *apoc1*$^{-/-}$ (n = 3), *apoe*$^{-/-}$ *apoc1*$^{+/-}$ (n = 3), and *apoe*$^{-/-}$ *apoc1*$^{+/+}$ (n = 3) male mice. *Apoc1* mRNA expression was measured using Taqman analysis. Plasma levels of mouse apoC-I protein were measured using a human apoC-I ELISA that cross-reacts with murine apoC-I. Values are expressed relative to those found in *apoe*$^{-/-}$ *apoc1*$^{+/+}$ mice and are represented as means ± SD.

[a] Significant difference compared with *apoe*$^{-/-}$ *apoc1*$^{+/+}$ mice ($P < 0.05$).

TABLE 2. Effect of endogenous apoC-I on fasted plasma lipid levels

Genotype	Weight	FFA	TC	TG
	g	*mM*		
apoe$^{-/-}$*apoc1*$^{-/-}$	30.2 ± 2.6	0.43 ± 0.12	3.97 ± 0.46	0.54 ± 0.15
apoe$^{-/-}$*apoc1*$^{+/-}$	30.1 ± 2.3	0.45 ± 0.09 (n.s.)	5.05 ± 0.84[a] (+27%)	0.69 ± 0.17[b] (+28%)
apoe$^{-/-}$*apoc1*$^{+/+}$	30.2 ± 1.9	0.45 ± 0.10 (n.s.)	5.75 ± 0.71[c] (+45%)	1.28 ± 0.47[c] (+137%)

n.s., not significant; TC, total cholesterol; TG, triglyceride. Plasma was obtained from 12 week old, 4 h fasted *apoe*$^{-/-}$*apoc1*$^{-/-}$ (n = 11), *apoe*$^{-/-}$*apoc1*$^{+/-}$ (n = 19), and *apoe*$^{-/-}$*apoc1*$^{+/+}$ (n = 10) male mice. Plasma FFA, TC, and TG levels were measured, and values are represented as means ± SD. Values in parentheses represent percentage differences relative to *apoe*$^{-/-}$*apoc1*$^{-/-}$ mice.

[a] Significant difference compared with *apoe*$^{-/-}$*apoc1*$^{-/-}$ mice ($P < 0.01$).

[b] Significant difference compared with *apoe*$^{-/-}$*apoc1*$^{-/-}$ mice ($P < 0.05$).

[c] Significant difference compared with *apoe*$^{-/-}$*apoc1*$^{-/-}$ mice ($P < 0.001$).

data indicate that the lack of one *apoc1* allele reduced both the hepatic *apoc1* mRNA and plasma apoC-I protein levels by ~50%, whereas complete deficiency for *apoc1* led to nondetectable levels (Table 1). In contrast, *apoc1* deficiency did not affect hepatic mRNA levels of *apoc2* and *apoc3* (results not shown).

Effect of endogenous apoC-I on plasma lipid levels

To determine whether physiological apoC-I expression on an apoE-deficient background affects plasma lipid levels, plasma samples from *apoe*$^{-/-}$*apoc1*$^{-/-}$, *apoe*$^{-/-}$*apoc1*$^{+/-}$, and *apoe*$^{-/-}$*apoc1*$^{+/+}$ littermates were assayed for FFA, TC, and TG (**Table 2**). Whereas endogenous apoC-I did not affect plasma FFA levels, apoC-I did cause a gene-dose-dependent increase in plasma TC and TG levels. Plasma TC levels were increased by 27% ($P < 0.01$) in the presence of a single allele of *apoc1* and were further increased by 45% ($P < 0.001$) in the presence of both *apoc1* alleles. The most predominant effect of apoC-I, however, was observed on plasma TG levels, which were increased by 28% ($P < 0.05$) and 137% ($P < 0.001$) in the presence of one and two alleles of *apoc1*, respectively. Lipoprotein fractionation by fast-performance liquid chromatography showed that the *apoc1* gene-dose-dependent increase in TG and TC was confined mainly to the VLDL fraction and the LDL/intermediate density lipoprotein (IDL) fraction, whereas HDL was hardly affected (**Fig. 1**).

Effect of endogenous apoC-I on intestinal lipid absorption

First, we determined whether an increased intestinal lipid absorption may underlie the observed increase in plasma TG. Fasted mice received an intravenous injection with Triton WR-1339 followed by an intragastric gavage of [^{3}H]TO in 200 μl of olive oil. Plasma TG and ^{3}H activity were assayed over a 4 h period (**Fig. 2**). After Triton injection and olive oil bolus, plasma TG increased dramatically without significant differences between *apoe*$^{-/-}$*apoc1*$^{-/-}$ and *apoe*$^{-/-}$*apoc1*$^{+/+}$ littermates (results not shown). Additionally, both groups showed no differences with respect to the time-dependent appearance of plasma ^{3}H activity (Fig. 2). Extraction and separation of the various lipid fractions from plasma obtained at 4 h after gavage revealed that 93% of ^{3}H activity was incorporated into TG (results not shown). Collectively, these results indicate that endogenous apoC-I does not influence intestinal lipid uptake.

Effect of endogenous apoC-I on hepatic VLDL production

Because the increase in plasma TG in the presence of apoC-I is apparently not caused by increased intestinal TG absorption, we investigated whether the increased TG can be explained by increased hepatic VLDL production. Anesthetized mice were injected intravenously with Tran^{35}S label, followed, 30 min later, by an intravenous injection of Triton WR-1339 to block lipolysis. Plasma lipids were measured over a period of 90 min, after which VLDL was isolated and characterized. Endogenous apoC-I significantly increased VLDL-TG production by 35% ($P < 0.05$) (**Fig. 3A**). Characterization of VLDL isolated 110 min after Triton injection showed that apoC-I expression also significantly increased VLDL-[^{35}S]apoB production (39%; $P < 0.01$). TG/apoB ratios were not significantly different, indicating that *apoe*$^{-/-}$*apoc1*$^{+/+}$ mice produced

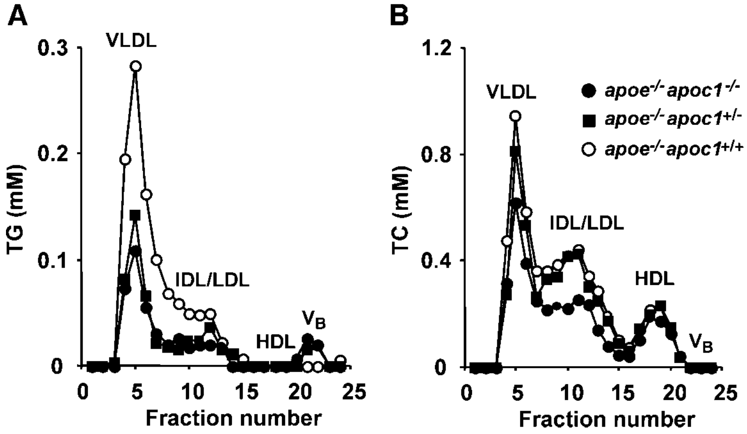

Fig. 1. Effect of endogenous apolipoprotein C-I (apoC-I) on the distribution of lipids among lipoproteins. Plasma from 4 h fasted apolipoprotein E gene-deficient/apolipoprotein C-I gene-deficient (*apoe*$^{-/-}$*apoc1*$^{-/-}$; black circles; n = 11), *apoe*$^{-/-}$*apoc1*$^{+/-}$ (black squares; n = 19), and *apoe*$^{-/-}$*apoc1*$^{+/+}$ (white circles; n = 10) mice was pooled per genotype. Lipoproteins were size-fractionated by fast-performance liquid chromatography on a Superose 6 column, and the individual fractions were assayed for triglyceride (TG; A) and total cholesterol (TC; B). IDL, intermediate density lipoprotein; V$_B$, total bed volume.

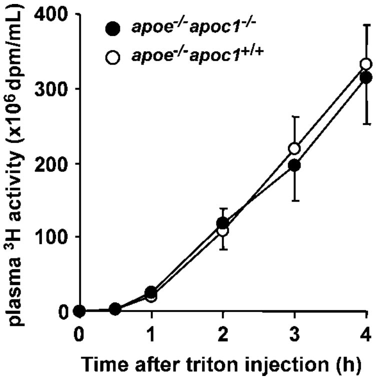

Fig. 2. Effect of endogenous apoC-I on intestinal lipid absorption. *apoe*$^{-/-}$*apoc1*$^{-/-}$ (black circles; n = 5) and *apoe*$^{-/-}$*apoc1*$^{+/+}$ (white circles; n = 5) mice were fasted overnight and injected intravenously with Triton WR-1339 (0.5 mg/g). Subsequently, mice received an intragastric load of 12 μCi of [^{3}H]triolein ([^{3}H]TO) in olive oil (200 μl). Blood samples were drawn just before gavage (time 0) and at the indicated times after gavage. Plasma ^{3}H activity was measured. Values are depicted as means ± SEM.

similarly sized VLDL particles compared with *apoe*$^{-/-}$*apoc1*$^{-/-}$ littermates. Further characterization of VLDL showed no changes in composition regarding cholesterol and phosphatidylcholine content (results not shown). Together, these results suggest that endogenous apoC-I stimulates VLDL particle production, leaving VLDL composition unaffected. Therefore, the increased production of VLDL particles may contribute to the observed increase in plasma TG in the presence of endogenous apoC-I.

Effect of endogenous apoC-I on postprandial TG response

To evaluate whether endogenous apoC-I may also result in reduced TG clearance, we first determined the effect of endogenous apoC-I on the postprandial TG response. Overnight fasted mice received an intragastric olive oil bolus (200 μl), and plasma TG levels were determined over an 8 h period. Both groups showed a postprandial increase of plasma TG peaking at 4 h after gavage. Based on the area under the curve between 0 and 8 h, apoC-I expression led to a 120% ($P < 0.05$) increase in the total postprandial TG response (**Fig. 4**). Because intestinal lipid absorption was not affected, these results indicate that expression of endogenous apoC-I results in reduced TG clearance.

Effect of endogenous apoC-I on LPL levels

As LPL is the most important enzyme in plasma TG hydrolysis, we determined whether endogenous apoC-I reduces levels of active LPL, thereby contributing to hypertriglyceridemia. ApoC-I expression did not affect the activities of either LPL or HL in postheparin plasma (**Fig. 5**). Likewise, tissue-specific LPL and HL activities in liver, heart, hind limb muscle, and gonadal, perirenal, and intestinal WAT were not different between *apoe*$^{-/-}$*apoc1*$^{-/-}$ and *apoe*$^{-/-}$*apoc1*$^{+/+}$ mice (results not shown). Therefore, the increase in plasma TG and postprandial TG response in *apoe*$^{-/-}$*apoc1*$^{+/+}$ mice cannot be explained by decreased plasma levels of LPL or HL.

Effect of endogenous apoC-I on VLDL lipolysis in vitro

As apoC-I expression does not affect total plasma LPL levels, we questioned whether apoC-I is able to directly inhibit lipolytic LPL activity posttranscriptionally in vitro. VLDL was isolated from *apoe*$^{-/-}$*apoc1*$^{-/-}$ and *apoe*$^{-/-}$*apoc1*$^{+/+}$ littermates. Incubation of VLDL with LPL led to a 28% ($P < 0.05$) decrease in lipolysis in the presence of apoC-I (**Fig. 6**), indicating that VLDL-associated apoC-I decreased LPL function in vitro.

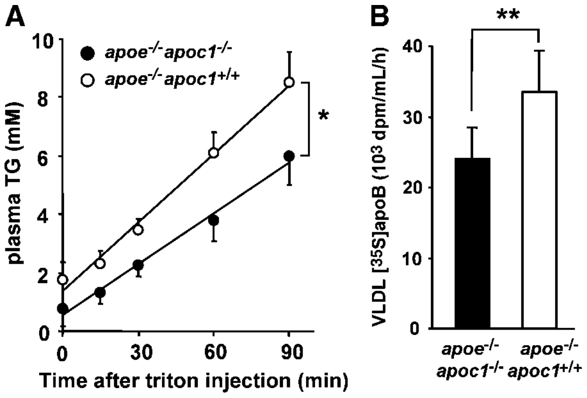

Fig. 3. Effect of endogenous apoC-I on hepatic VLDL production. A: Fasted *apoe*$^{-/-}$*apoc1*$^{-/-}$ (black circles; n = 6) and *apoe*$^{-/-}$*apoc1*$^{+/+}$ (white circles; n = 6) mice received consecutive intravenous injections of Tran^{35}S to label protein and Triton WR-1339 to block lipolysis. Blood samples were drawn just before Triton injection (time 0) and at the indicated times after Triton injection. Plasma TG levels were determined, and the TG production rates were calculated by linear regression analysis. B: After the last sampling, mice were exsanguinated and VLDL was isolated and assayed for [^{35}S]apoB. Values are depicted as means ± SD. * $P < 0.05$, ** $P < 0.01$.

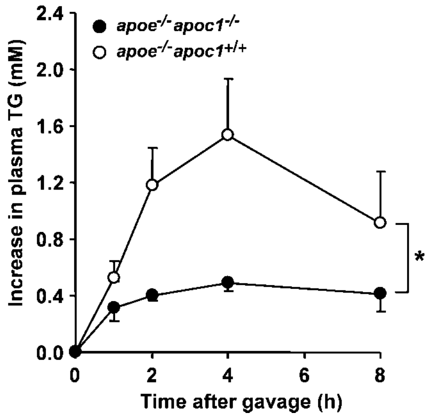

Fig. 4. Effect of endogenous apoC-I on the postprandial TG response. Overnight fasted *apoe*$^{-/-}$*apoc1*$^{-/-}$ (black circles; n = 6) and *apoe*$^{-/-}$*apoc1*$^{+/+}$ (white circles; n = 6) mice received an intragastric olive oil load (200 μl). Plasma samples were taken just before gavage (time 0) and at the indicated times after gavage. Plasma TG levels were measured and were corrected for the TG value at time 0. Values are depicted as means ± SEM. * Significant difference with respect to area under the curve between 0 and 8 h ($P < 0.05$).

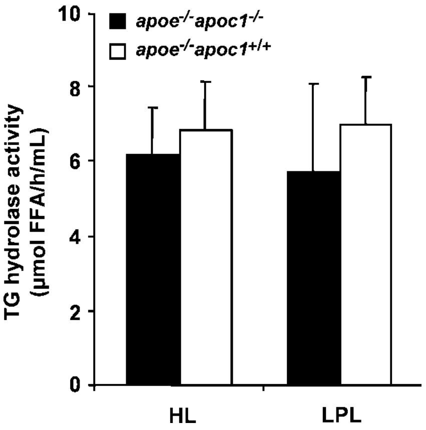

Fig. 5. Effect of endogenous apoC-I on plasma HL and LPL levels in vivo. Four hour fasted *apoe*$^{-/-}$*apoc1*$^{-/-}$ (n = 10) and *apoe*$^{-/-}$*apoc1*$^{+/+}$ (n = 9) mice were injected intravenously with heparin (0.1 U/g), and plasma was collected at 10 min after injection. To determine total plasma lipase levels, plasma was incubated with a substrate mixture containing [^{3}H]TO in the presence or absence of 1 M NaCl, which inhibits LPL. Generated [^{3}H]oleate was extracted and quantified to estimate TG hydrolase activity. Values represent means ± SD.

Effect of endogenous apoC-I on the uptake of TG-derived FFAs by peripheral tissues

We investigated the relevance of the in vitro data regarding the inhibitory effect of apoC-I on LPL-mediated lipolysis for TG clearance in vivo. Fed, anesthetized *apoe*$^{-/-}$*apoc1*$^{-/-}$ and *apoe*$^{-/-}$*apoc1*$^{+/+}$ littermates received an intravenous injection of [^{3}H]TO-labeled, TG-rich, VLDL-like emulsion particles, which have been shown to mimic VLDL with respect to in vivo kinetics (12, 25). Plasma was assayed for ^{3}H activity during a 15 min period after injection, and subsequently, organs were isolated and ^{3}H uptake was measured. The serum decay of the emulsion particles was mildly reduced in *apoe*$^{-/-}$*apoc1*$^{+/+}$ mice ($t_{1/2}$=6.8 min) compared with *apoe*$^{-/-}$*apoc1*$^{-/-}$ littermates ($t_{1/2}$=5.8 min), although statistical significance was not reached. However, apoC-I expression reduced the uptake of [^{3}H]TO-derived activity by gonadal and perirenal WAT (34% and 25%, respectively; $P < 0.05$). Likewise, intestinal WAT and skeletal muscle also showed a tendency for decreased uptake of ^{3}H activity. The uptake of ^{3}H activity was not different in liver, heart, and spleen (**Fig. 7**). In the fed state, LPL is expressed mainly in WAT and to a lower extent in skeletal muscle, and it plays an important role in the hydrolysis of TG into FFAs that are subsequently taken up by these tissues. These results thus indicate that apoC-I expression also decreases LPL function in vivo.

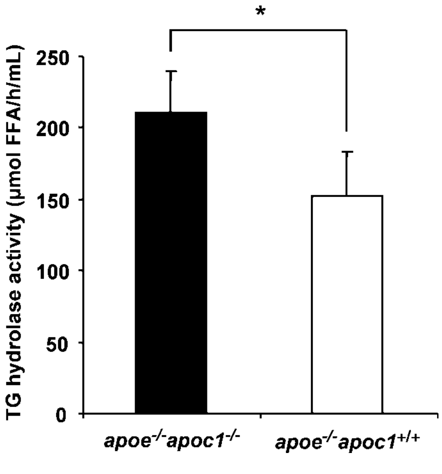

Fig. 6. Effect of endogenous apoC-I on VLDL-TG lipolysis in vitro. Four hour fasted *apoe*$^{-/-}$*apoc1*$^{-/-}$ (n = 4) and *apoe*$^{-/-}$*apoc1*$^{+/+}$ (n = 4) mice were euthanized and bled via the retro-orbital vein. Sera were pooled, and VLDL was isolated by ultracentrifugation. VLDL (0.3 mM TG) was incubated with LPL. The released FFAs were determined in time, and TG hydrolase activities were calculated. Values represent means ± SD. * $P < 0.05$.

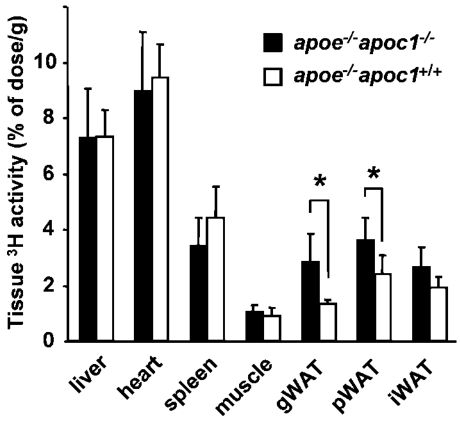

Fig. 7. Effect of endogenous apoC-I on serum decay and organ distribution of VLDL-like emulsion particles. Fed *apoe*$^{-/-}$*apoc1*$^{-/-}$ (black circles; n = 6) and *apoe*$^{-/-}$*apoc1*$^{+/+}$ (white circles; n = 5) mice received an intravenous bolus of [^{3}H]TO-labeled VLDL-like emulsion particles (150 µg of TG per mouse). At 15 min after injection, mice were euthanized, organs were isolated, and the uptake of ^{3}H label by the organs was measured. Values are depicted as means ± SD. * $P < 0.05$. gWAT, pWAT, and iWAT denote gonadal, perirenal, and intestinal white adipose tissue, respectively.

DISCUSSION

It has been established that overexpression of human apoC-I in C57Bl/6 mice gene-dose-dependently induces combined hyperlipidemia, with a prominent increase in plasma TG (4–6). Although this effect was initially explained by attenuation of the apoE-mediated remnant clearance (7), our recent insights indicate that the hypertriglyceridemic effect is caused mainly by the apoC-I-induced impairment of the LPL-mediated VLDL-TG clearance (8). However, it is not clear yet whether the expression level of endogenous apoC-I suffices to affect plasma TG metabolism. As plasma TG metabolism is to a great extent affected by apoE, which induces VLDL-TG production (11), inhibits VLDL-TG lipolysis (12), and mediates remnant clearance (13), we studied the effect of endogenous apoC-I on plasma TG metabolism in the absence of the effects of apoE. On the *apoe*$^{-/-}$ background, endogenous apoC-I indeed gene-dose-dependently increased plasma TG and TC levels, with a more pronounced effect on TG (+137%; $P < 0.001$) than on TC (+45%; $P < 0.05$) in the presence of both *apoc1* alleles. The increases of both TG and TC were confined to the VLDL and IDL/LDL fractions. This combined lipid-increasing effect was attributed to increased VLDL particle production (+39%; $P < 0.05$) and decreased local LPL activity in vivo, independently of total and tissue-specific LPL levels.

Although we previously observed no effects of human apoC-I overexpression on VLDL production in wild-type and $apoe^{-/-}$ mice (8), we now demonstrate that endogenous apoC-I increased VLDL particle production in $apoe^{-/-}$ mice. Apparently, a physiological concentration of apoC-I is necessary for efficient VLDL particle production, although higher hepatic apoC-I expression cannot further enhance the effect. Moreover, modulation of VLDL particle production by endogenous apoC-I may be specific for the $apoe^{-/-}$ background, as we previously reported that VLDL production is not affected by apoC-I deficiency on the wild-type background (28). A potential increasing effect of endogenous apoC-I on VLDL production in wild-type mice may have been overruled by the presence of endogenous apoE, because endogenous apoE already induces VLDL production (29, 30). Thus, in $apoe^{-/-}apoc1^{-/-}$ mice, VLDL particle production is attenuated by the absence of endogenous apoC-I on top of the inhibiting effect caused by the absence of endogenous apoE. In addition to apoC-I and apoE, other apolipoproteins have also been reported to affect VLDL-TG production (31, 32). VLDL-TG production is stimulated by apoA-II (31) and attenuated by apoA-V (32), yet apoC-III does not affect VLDL-TG production (26).

The mechanism underlying the stimulating effect of apoC-I on VLDL particle production remains to be established. ApoA-II and apoA-V have been shown to selectively modulate VLDL-TG production, whereas apoE is believed to modulate VLDL particle production (29), although others have shown that apoE may selectively affect the production of VLDL-TG (30). It has been reported that the production of VLDL-TG may be regulated by the number of particles secreted as a result of apoB output (33). ApoE has been suggested to enhance VLDL particle production by inhibiting the degradation of apoB in the hepatocyte (29). As the low density lipoprotein receptor (LDLr) has been demonstrated to be involved in the intracellular degradation of apoB, it was suggested that apoE could inhibit apoB degradation by competing with the binding of apoB to the LDLr (34). Although those results were not confirmed in vivo in $apoe^{-/-}ldlr^{-/-}$ mice, in which the effect of apoE on hepatic VLDL production was independent of the LDLr (35), the same line of reasoning could still be true for apoC-I. We demonstrated previously that apoC-I-enriched lipoproteins bind to the LDLr in vitro (7). Accordingly, endogenous apoC-I may also enhance VLDL particle production by reducing LDLr-mediated apoB degradation.

In addition to inducing VLDL particle production, the lipid-increasing effect of endogenous apoC-I also appears to result from decreased LPL activity. Endogenous apoC-I did not affect total levels of postheparin LPL and HL. However, the expression of endogenous apoC-I greatly increased the postprandial TG response, indicative of reduced TG clearance. As the hepatic mRNA expression levels of the LPL cofactor *apoc2* or the LPL inhibitor *apoc3* did not differ between $apoe^{-/-}apoc1^{-/-}$ and $apoe^{-/-}apoc1^{+/+}$ mice, it is unlikely that effects of apoC-I expression on LPL activity are caused by indirect effects on apoC-II and apoC-III. In fact, VLDL-associated apoC-I decreased the lipolysis of VLDL-TG in vitro and the uptake of TG-derived ^{3}H activity (representing liberated FFAs) from intravenously injected VLDL-like emulsion particles by WAT in vivo. As LPL catalyzes TG hydrolysis into glycerol and FFAs in the vasculature of WAT and is the gatekeeper of tissue FFA uptake, these results indicate that LPL is less active upon the expression of apoC-I in $apoe^{-/-}$ mice. These data corroborate our recent observations that the predominantly hypertriglyceridemic phenotype of human apoC-I-overexpressing mice is attributable to LPL inhibition (8). Furthermore, human apoC-I was able to dose-dependently inhibit LPL activity in vitro, and enrichment of TG-rich, VLDL-like emulsion particles with apoC-I before intravenous injection in mice reduced TG clearance (8). Collectively, these data show that both endogenous murine apoC-I and exogenous human apoC-I can inhibit LPL in mice and that the expression level of apoC-I is predictive of the plasma TG level.

To date, apoC-III has been considered the most prominent endogenous LPL inhibitor. Because it is now clear that both human apoC-I and endogenous murine apoC-I inhibit LPL, the question arises whether apoC-I or apoC-III is most potent and selective regarding LPL inhibition. Such data may be retrieved from comparison of the phenotypes of mice overexpressing or lacking these proteins. Both *APOC1* mice (7) and *APOC3* mice (36) show combined hyperlipidemia with prominent hypertriglyceridemia. The phenotypes of both mice were initially explained by inhibition of apoE-dependent uptake of TG-rich lipoprotein remnants by the liver (7, 36). Nevertheless, both $apoe^{-/-}APOC1$ mice and $apoe^{-/-}APOC3$ mice are still severely hypertriglyceridemic (8, 37), indicating that the effects on hypertriglyceridemia can be independent of the expression of apoE. Likewise, both the expression of endogenous apoC-III (26) and that of endogenous apoC-I (this study) add to the hyperlipidemia observed on the $apoe^{-/-}$ background. ApoC-I and apoC-III thus appear to be equally selective regarding LPL inhibition. However, several lines of evidence obtained in mouse models indicate that the LPL-inhibiting potency of apoC-III is somewhat higher than that of apoC-I: *1*) apoC-III is 2-fold more effective than apoC-I with respect to inhibiting LPL activity in vitro (8); *2*) *APOC3* mice show ∼2-fold higher TG levels than *APOC1* mice, whereas the plasma levels of the apolipoprotein are similar (i.e., 40–50 mg/dl) (8, 38); *3*) the presence of endogenous apoC-III on a wild-type background increases TG levels by 180% (26), whereas endogenous apoC-I does not markedly affect TG levels on this background (28, 39); and *4*) the presence of endogenous apoC-III in $apoe^{-/-}$ mice shows a more pronounced TG increase (570%) (26) than the presence of apoC-I in $apoe^{-/-}$ mice (137%) (this study) compared with $apoe^{-/-}$ littermates.

The molecular mechanism underlying the LPL-inhibitory properties of apoC-I remains to be elucidated. It is possible that apoC-I interacts with the crucial cofactor for LPL, apoC-II, through displacement from VLDL particles or masking its presence, as reported previously for apoE (40, 41). Likewise, apoC-I may also interact with apoA-V, which we recently demonstrated to be a potent stimulator of apoC-II-dependent lipolysis (32). In addition, apoC-I may also interact directly with LPL, thereby inhibiting its lipolytic function. Our previous studies have shown that *APOC1* mice exhibit increased FFA (8), which may add to LPL inhibition as a result of product inhibition. However, this cannot be an explanation for the reduced LPL activity in the presence of apoC-I in $apoe^{-/-}$ mice, as FFA levels were not affected. Furthermore, we have shown that apoC-I can inhibit the apoE-dependent binding of VLDL to the VLDL receptor (7), and apoC-I may thus interfere with the VLDL receptor-mediated facilitation of LPL-mediated TG hydrolysis by docking VLDL to the endothelium in the vicinity of LPL (42). Again, such a mechanism seems unlikely to contribute to the effects of apoC-I on TG levels, because these effects are also observed on an apoE-deficient background. Therefore, a direct interaction between apoC-I and LPL, or a more indirect interaction with one of its stimulators, is most probable as a mechanistic explanation for LPL inhibition.

In conclusion, we found that the physiological expression level of apoC-I gene-dose-dependently increases hyperlipidemia in $apoe^{-/-}$ mice by increasing VLDL-TG and VLDL-TC as a consequence of increased VLDL particle production and reduced LPL activity.

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