Apolipoprotein C-III deficiency accelerates triglyceride hydrolysis by lipoprotein lipase in wild-type and apoE knockout mice

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Abstract **Previous studies with hypertriglyceridemic *APOC3* transgenic mice have suggested that apolipoprotein C-III (apoC-III) may inhibit either the apoE-mediated hepatic uptake of TG-rich lipoproteins and/or the lipoprotein lipase (LPL)-mediated hydrolysis of TG. Accordingly, *apoC3* knockout (*apoC3*$^{-/-}$) mice are hypotriglyceridemic. In the present study, we attempted to elucidate the mechanism(s) underlying these phenomena by intercrossing *apoC3*$^{-/-}$ mice with *apoE*$^{-/-}$ mice to study the effects of apoC-III deficiency against a hyperlipidemic background. Similar to *apoE*$^{+/+}$ *apoC3*$^{-/-}$ mice, *apoE*$^{-/-}$*apoC3*$^{-/-}$ mice exhibited a marked reduction in VLDL cholesterol and TG, indicating that the mechanism(s) by which apoC-III deficiency exerts its lipid-lowering effect act independent of apoE. On both backgrounds, *apoC3*$^{-/-}$ mice showed normal intestinal lipid absorption and hepatic VLDL TG secretion. However, turnover studies showed that TG-labeled emulsion particles were cleared much more rapidly in *apoC3*$^{-/-}$ mice, whereas the clearance of VLDL apoB, as a marker for whole particle uptake by the liver, was not affected. Furthermore, it was shown that cholesteryl oleate-labeled particles were also cleared faster in *apoC3*$^{-/-}$ mice. Thus the mechanisms underlying the hypolipidemia in *apoC3*$^{-/-}$ mice involve both a more efficient hydrolysis of VLDL TG as well as an enhanced selective clearance of VLDL cholesteryl esters from plasma. In summary, our studies of *apoC3*$^{-/-}$ mice support the concept that apoC-III is an effective inhibitor of VLDL TG hydrolysis and reveal a potential regulating role for apoC-III with respect to the selective uptake of cholesteryl esters.**—Jong, M. C., P. C. N. Rensen, V. E. H. Dahlmans, H. van der Boom, T. J. C. van Berkel, and L. M. Havekes. **Apolipoprotein C-III deficiency accelerates triglyceride hydrolysis by lipoprotein lipase in wild-type and apoE knockout mice.** ***J. Lipid Res.*** **2001.** 42: **1578–1585.**

Supplementary key words hypercholesterolemia • transgenic mice • triglyceride metabolism

Apolipoprotein C-III (apoC-III) is the most abundant C apolipoprotein in human plasma, where it is present as an 8.8-kDa mature protein on chylomicrons, VLDL, and HDL. ApoC-III is synthesized in the liver and in minor quantities by the intestine (1). Several lines of evidence have implicated apoC-III as contributing to the development of hypertriglyceridemia in the human population. A positive correlation has been observed between plasma apoC-III levels and elevated levels of plasma TG (2, 3) and VLDL TG (4). In addition, complete apoC-III deficiency has been reported in several families, with one family showing an increased fractional catabolic rate of VLDL and an unusually efficient conversion of VLDL to IDL and LDL (5). However, in all cases, apoC-III deficiency was associated with apoA-I deficiency, making it difficult to estimate the exact contribution of lack of apoC-III to changes in VLDL TG metabolism.

Work in vitro has shown that apoC-III effectively inhibits the LPL-mediated hydrolysis of VLDL TG (6, 7), and at higher concentrations also inhibits hepatic lipase (HL) activity (8). In addition, it has been suggested that apoC-III interferes with the apoE and apoB-mediated binding of lipoproteins to hepatic lipoprotein receptors (9–11). The observation that transgenic mice overexpressing human apoC-III develop hypertriglyceridemia (12) suggests that a direct inhibiting effect of apoC-III on either VLDL TG hydrolysis or the binding of TG-rich lipoproteins to hepatic receptors holds true for the in vivo situation as well. In vivo turnover studies showed an impaired clearance of VLDL TG from the circulation of *APOC3* transgenic mice

Abbreviations: apo, apolipoprotein; CE, cholesteryl ester; HL, hepatic lipase; HSPG, heparan sulfate proteoglycan; LDLR, low density lipoprotein receptor; LRP, LDLR-related protein; PL, phospholipid.

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(13, 14), which could be overcome when *APOC3* transgenic mice were cross-bred with *APOE* mice overexpressing human apoE (13). From these results it was concluded that apoC-III interferes with the apoE-mediated clearance of TG-rich lipoprotein particles. However, in more recent studies in which *APOC3* transgenic mice were cross-bred with *apoE*$^{-/-}$ mice, it was shown that the amount of apoC-III on the VLDL particle itself causes hypertriglyceridemia, independent of the amount of apoE on the lipoprotein particle (15).

To directly investigate the effect of apoC-III deficiency on lipid metabolism, mice lacking apoC-III were generated by gene targeting. *ApoC3*$^{-/-}$ mice exhibited reduced plasma cholesterol and TG levels and were protected from postprandial hypertriglyceridemia (16). Although these data point to a regulatory role for apoC-III in TG metabolism, the exact mechanisms underlying the reduced plasma lipid levels in *apoC3*$^{-/-}$ mice were not investigated. In the present study we performed in vivo turnover experiments in *apoC3*$^{-/-}$ mice to characterize the effect of apoC-III deficiency on VLDL metabolism. Because wild-type mice have little VLDL circulating in plasma, and to exclude effects of endogenous apoE on VLDL metabolism, we also investigated the effects of apoC-III deficiency on lipid metabolism in hyperlipidemic *apoE*$^{-/-}$ mice. It was found that apoC-III deficiency reduces plasma VLDL cholesterol and TG levels and protects against post-fat load hypertriglyceridemia, independent of the presence or absence of apoE. These strong effects on VLDL metabolism in the *apoC3*$^{-/-}$ mice appeared to be due to an increased TG hydrolysis rate in the circulation as well as an enhanced selective uptake of cholesteryl esters, whereas intestinal lipid absorption and hepatic VLDL TG production were not affected.

MATERIALS AND METHODS

Animals

ApoC3$^{-/-}$ mice (C57BL/6J background) were obtained from the Jackson Laboratories (Bar Harbor, ME) and intercrossed with *apoE*$^{-/-}$ knockout mice (C57BL/6J background) that have previously been generated in our laboratory (17). Wild-type and *apoE*$^{-/-}$ littermates were used as controls in the respective experiments. All mice were males between the age of 2 and 4 months, and housed under standard conditions with free access to water and food. 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 paraoxonized capillary tubes as described previously (18). The tubes were placed in ice and centrifuged at 4°C, and the obtained plasma was immediately assayed for cholesterol, TG (without free glycerol), and FFA, using available enzymatic kits 236691 (Roche Molecular Biochemicals, Indianapolis, IN), 337-B (GPO-Trinder; Sigma, St. Louis, MO), and NEFA-C (Wako Chemicals, Neuss, Germany), respectively. The VLDL fractions (d < 1.006 g/ml) were isolated from pooled sera of at least eight mice per group. Isolation was achieved by sequential ultracentrifugation at the respective density at 40,000 rpm in a Ti-50 fixed-angle rotor (Beckman Instruments, Geneva, Switzerland) for 18 h at 5°C, followed by dialysis at 4°C overnight against PBS, pH 7.4. Protein concentrations in the VLDL fractions were determined as previously described (19), with BSA as a standard. TG, free cholesterol, and phospholipid content of the VLDL fractions (d < 1.006 g/ml) were measured with enzymatic assay kits (701904 and 310328; Roche Molecular Biochemicals) and an analytical kit B from Wako Chemicals, respectively. For fast protein liquid chromatography (FPLC) fractionation of lipoproteins, 50 μl of pooled plasma from at least eight mice per group was injected onto a Superose 6 column (3.2 × 30 mm, SMART system; Amersham Pharmacia Biotech, Piscataway, NJ), and eluted at a constant flow rate of 50 μl/min with PBS (pH 7.4, containing 1 mM EDTA). Fractions of 50 μl were collected and assayed for total cholesterol and TG levels as described above.

Postprandial TG response

Mice were fasted overnight. After taking a basal blood sample by tail bleeding at $t = 0$, animals received an intragastric load of 400 μl of olive oil. Additional blood samples were drawn 1, 2, 3, 4, 5, and 6 h after olive oil administration. Plasma TG levels were measured at the different time points as described above.

Intestinal TG absorption

Mice were fasted overnight and injected intravenously with 500 mg of Triton WR1339 (Sigma) per kg body weight as a 15-g/dl solution in sterile saline. Subsequently, mice were given an intragastric load of glycerol [^{3}H]trioleate (100 μCi; Amersham, Arlington Heights, IL) in 100 μl of olive oil. Blood samples were drawn 30, 60, 90, 120, and 180 min thereafter. Lipids were extracted from plasma according to the method of Bligh and Dyer (20), and the amount of radioactivity in the TG fraction was determined after separation of the TG from the other lipid components by TLC on silica gel 60 plates (E. Merck, Darmstadt, Germany) by using hexane–diethyl ether–acetic acid 83:16:1 (v/v/v) as resolving solution. [^{14}C]trioleate (Amersham) was used as internal standard, and the proportion of radioactivity in the plasma TG fraction was calculated in relation to the body mass of the mice.

Hepatic VLDL TG production

Mice were fasted for 4 h and anesthetized by intraperitoneal injection of Hypnorm (0.5 ml/kg; Janssen Pharmaceutica, Tilburg, The Netherlands) and midazolam (12.5 mg/kg; Roche Netherlands, Mijdrecht, The Netherlands). Body temperature was maintained by the use of heat lamps. A basal blood sample was taken ($t = 0$) and subsequently mice were intravenously injected with 500 mg of Triton WR1339 (Sigma) per kg body weight as a 15-g/dl solution in sterile saline. Turnover studies showed that plasma VLDL clearance is completely inhibited under these conditions (results not shown). Additional blood samples were collected at the indicated times after Triton injection and plasma TG concentrations were measured enzymatically as described above. Plasma TG concentrations were related to the body mass of the mice and the hepatic TG production rate was calculated from the slope of the curve and expressed as micromoles per kilogram body weight. Alternatively, hepatic VLDL TG production was assessed by quantification of glycerol esterification. Hereto, [1(3)-^{3}H]glycerol (40 μCi; Amersham) was injected intravenously 15 min after Triton treatment, and blood samples were collected at the indicated times. Lipids were extracted from plasma and separated by TLC as described above. TG-associated ^{3}H activity was measured, and expressed as

TABLE 1. Fasting plasma lipid levels in *apoC3* knockout mice in the presence and absence of apoE

Presence of ApoE			Absence of ApoE		
Mice	TC	TG	Mice	TC	TG
	mM			*mM*	
$apoE^{+/+}apoC3^{+/+}$	2.5 ± 0.4	0.42 ± 0.07	$apoE^{-/-}apoC3^{+/+}$	16.1 ± 2.4	0.74 ± 0.32
$apoE^{+/+}apoC3^{-/-}$	1.1 ± 0.2[a]	0.11 ± 0.04[a]	$apoE^{-/-}apoC3^{-/-}$	10.3 ± 1.6[a]	0.11 ± 0.03[a]

Total cholesterol (TC) and triglyceride (TG) levels were measured in the plasma of 4-h fasted wild-type and knockout mice (25–30 g) fed a normal chow diet. Values are expressed as the means ± SD of at least 10 mice per group.

[a] $P < 0.05$, indicating the difference between $apoC3^{-/-}$ and wild-type mice (left column; in the presence of apoE) or between $apoE^{-/-}apoC3^{-/-}$ and $apoE^{-/-}$ mice (right column; in the absence of apoE), using the nonparametric Mann-Whitney test.

microcuries per kilogram body weight. [1-^{14}C]Palmitic acid (Amersham) was used as internal standard.

Preparation and removal of VLDL-like TG-rich emulsions

The preparation and characterization of emulsions have previously been described (21). Briefly, emulsions (~45 nm) were prepared by sonication from 100 mg of total lipid at a triolein: egg yolk phosphatidylcholine:lysophosphatidylcholine:cholesteryl oleate:cholesterol weight ratio of 70:22.7:2.3:3.0:2.0. For the experiments, 200 μCi of glycerol [^{3}H]trioleate and 25 μCi of [^{14}C]cholesteryl oleate (Amersham) were added to 100 mg of total lipid. The lipid composition of the emulsions was determined as described above. Particle size (~45 nm) and homogeneity (polydispersity, 0.2) of the emulsions were assayed by photon correlation spectroscopy, using a Malvern 4700 C system (Malvern Instruments, Malvern, UK). Emulsions were stored at 20°C under argon and were used within 5 days. To study the in vivo clearance of the labeled emulsions, mice fasted for 4 h were anesthetized with Hypnorm and midazolam as described above. Subsequently, mice were injected intravenously with the ^{3}H- and ^{14}C-labeled emulsions (150 μg of TG). At the indicated times, blood samples (50 μl) were taken, and radioactivity in duplicate plasma samples of 10 μl was counted after combustion in a Packard (Downers Grove, IL) TriCarb 306 sample oxidizer (recovery >97%). The total plasma volumes of the mice were calculated from the equation: V (ml) = 0.04706 × body weight (g), as determined from ^{125}I-labeled BSA clearance studies of mice weighing 23–35 g as previously described (21).

Labeling and removal of ^{125}I-labeled VLDL apoB

Blood was collected from 10 $apoE^{-/-}$ mice and 15 $apoE^{-/-}apoC3^{-/-}$ mice that were fasted for 4 h. Serum was pooled and VLDL ($d < 1.006$ g/ml) was obtained by ultracentrifugation as described above. VLDL was labeled with ^{125}I by the ICl method (22). The specific radioactivity of ^{125}I-labeled VLDL ranged from 500 to 1,000 cpm/ng of protein. The fraction of ^{125}I label present in apoB was determined by propan-2-ol precipitation (23, 24) and ranged from 50% to 65% of total label. After iodination the VLDL samples were dialyzed extensively against PBS, pH 7.4, stored at 4°C, and used within 1 week. Mice were fasted for 4 h and were injected in the tail vein with ^{125}I-labeled autologous VLDL (10 μg of tracer in 200 μl of 0.9% NaCl containing BSA at 2 mg/ml). Blood samples of approximately 25 μl were collected from the tail vein 2.5, 5, 10, 30, 60, and 90 min after injection of VLDL. The plasma content of ^{125}I-labeled apoB was determined by selective ^{125}I-apoB precipitation using propan-2-ol. Under these conditions of precipitation, apoB was found exclusively in the pellet, and no other apolipoproteins were present. The data were modeled by using a biexponential decay curve.

RESULTS

Characterization of mice

Table 1 summarizes the plasma lipid levels in fasted wild-type and $apoC3^{-/-}$ mice fed a regular mouse chow diet. In accordance with previously published data (16), $apoC3^{-/-}$ mice exhibit significantly reduced plasma cholesterol and TG levels compared with wild-type mice. The reduction in plasma cholesterol levels observed in the $apoC3^{-/-}$ mice was reflected by a lowering of VLDL- and IDL/LDL-sized particles (**Fig. 1A**). The reduction in plasma TG levels in the absence of apoC-III was primarily due to a decrease in VLDL-sized particles (results not shown). Because wild-type mice are HDL animals and have little VLDL circulating in plasma, and to exclude effects of endogenous apoE on VLDL metabolism, we also investigated the effects of apoC-III deficiency on VLDL metabolism in hyperlipidemic $apoE^{-/-}$ mice. Therefore, $apoC3^{-/-}$ mice were cross-bred with $apoE^{-/-}$ mice. Similar to that observed in the presence of apoE, introduction of apoC-III deficiency significantly lowered plasma cholesterol and TG levels in $apoE^{-/-}$ mice (Table 1). As shown

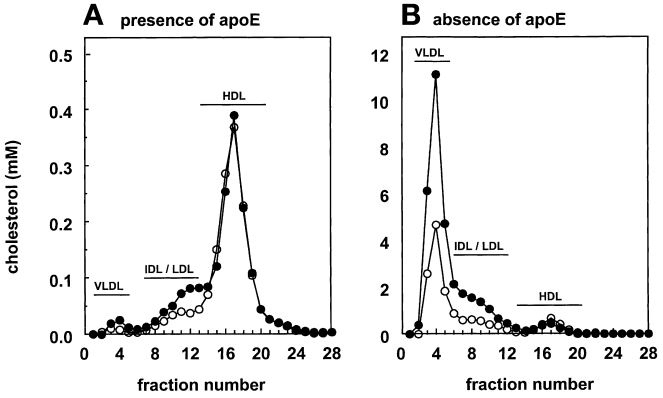

Fig. 1. Lipoprotein cholesterol profiles in wild-type and *apoC3* knockout mice in the presence (A) and absence (B) of apoE. (A) Plasma of wild-type (solid circles) and $apoC3^{-/-}$ (open circles) mice and (B) plasma of $apoE^{-/-}$ (solid circles) and $apoE^{-/-}apoC3^{-/-}$ (open circles) mice were pooled and separated on the basis of size, using FPLC (see Materials and Methods). The total cholesterol content of each fraction was measured enzymatically. Note the difference in scale of the y axis of (A) and (B).

TABLE 2. VLDL lipid composition in *apoC3* knockout mice in the presence and absence of apoE

Presence of ApoE					Absence of ApoE				
VLDL	TG	FC	CE	PL	VLDL	TG	FC	CE	PL
	μmol/mg VLDL protein					*μmol/mg VLDL protein*			
$apoE^{+/+}apoC3^{+/+}$	4.0	0.5	1.6	1.0	$apoE^{-/-}apoC3^{+/+}$	2.5	12.5	19.6	5.0
$apoE^{+/+}apoC3^{-/-}$	1.2	0.5	0.1	0.2	$apoE^{-/-}apoC3^{-/-}$	1.3	6.5	14.0	4.0

VLDL (d < 1.006 g/ml) was isolated from pooled serum of fasted mice by ultracentrifugation and the triglyceride (TG), free cholesterol (FC), cholesteryl ester (CE), and phospholipid (PL) content was measured as described in Materials and Methods.

in Fig. 1B, the strong reduction in plasma cholesterol observed in the double knockout $apoE^{-/-}apoC3^{-/-}$ mice was due to a decrease in the VLDL- and IDL/LDL-sized fractions. The decrease in plasma TG was mainly confined to the VLDL-sized fractions (results not shown).

To investigate the effects of apoC-III deficiency on lipoprotein lipid composition, VLDL (d < 1.006 g/ml) was isolated from pooled sera of fasted mice fed a regular mouse chow diet. As shown in **Table 2**, VLDL from $apoC3^{-/-}$ mice contained less TG, cholesteryl ester, and phospholipids when compared with VLDL from wild-type mice. Furthermore, we also observed that the TG, cholesteryl ester, and free cholesterol content was decreased in VLDL of $apoE^{-/-}apoC3^{-/-}$ mice as compared with VLDL from $apoE^{-/-}$ mice (Table 2).

In vivo chylomicron and VLDL metabolism

To investigate the mechanism(s) underlying the marked reduction in plasma TG levels of $apoC3^{-/-}$ mice, chylomicron and VLDL TG metabolism was studied in vivo. First, mice received an intragastric olive oil load after which plasma TG levels were determined over a period of 6 h. Wild-type mice showed a postprandial increase in plasma TG, peaking 2 h after olive oil administration (**Fig. 2A**). The postprandial response of plasma TG was completely absent in the $apoC3^{-/-}$ mice. In the absence of apoE, mice showed a gradual increase in plasma TG after an intragastric olive oil load (Fig. 2B). Similar to $apoC3^{-/-}$ mice, the double knockout $apoE^{-/-}apoC3^{-/-}$ mice showed no increase in plasma TG after having received olive oil.

The low plasma TG levels and total absence of a postprandial TG response in the $apoC3^{-/-}$ mice can be due either to *i*) impaired intestinal lipid absorption, *ii*) decreased hepatic TG production, or *iii*) enhanced lipolysis and/or clearance of TG from the circulation. To differentiate between these possibilities, we first injected mice with Triton WR1339 and subsequently gave them an intragastric load of olive oil containing [^{3}H]trioleate. As shown in **Fig. 3A**, labeled TG appeared gradually in the plasma of $apoC3^{-/-}$ and wild-type mice, without significant differences between both groups of mice. In addition, the appearance of [^{3}H]trioleate in plasma of $apoE^{-/-}apoC3^{-/-}$

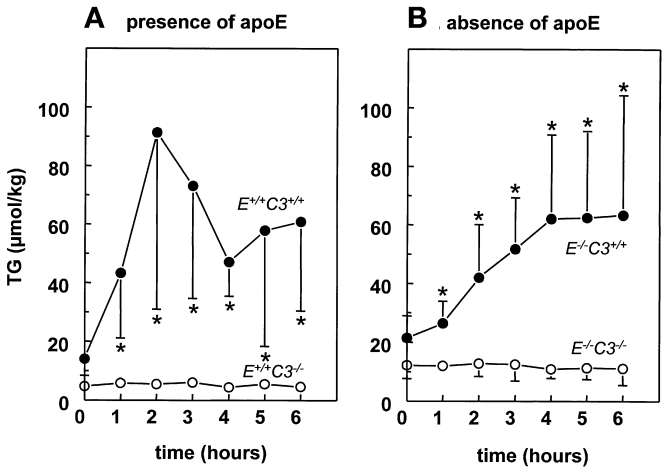

Fig. 2. Fat-loading test in wild-type and *apoC3* knockout mice in the presence (A) and absence (B) of apoE. (A) Wild-type (solid circles) and $apoC3^{-/-}$ (open circles) mice, and (B) $apoE^{-/-}$ (solid circles) and $apoE^{-/-}apoC3^{-/-}$ (open circles) mice were fasted overnight and received an intragastric fat load (400 μl of olive oil) at time point 0. Subsequently, plasma TG levels were determined at the respective time points and values are depicted as means ± SD of six mice per group. * $P < 0.05$.

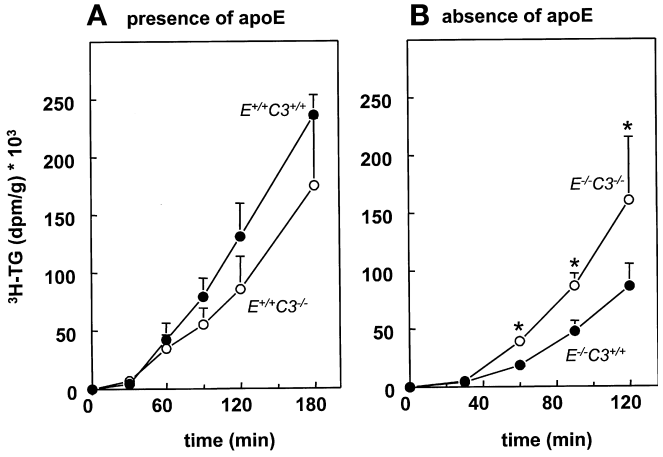

Fig. 3. Intestinal lipid absorption in wild-type and *apoC3* knockout mice in the presence (A) and absence (B) of apoE. (A) Wild-type (solid circles) and $apoC3^{-/-}$ (open circles) mice, and (B) $apoE^{-/-}$ (solid circles) and $apoE^{-/-}apoC3^{-/-}$ (open circles) mice were fasted overnight and received an intravenous injection of Triton WR1339. Subsequently, at time point 0 mice were administered an intragastric fat load (400 μl of olive oil) containing [^{3}H]TG. The appearance of ^{3}H label in the plasma TG fraction was measured over time as described in Materials and Methods. Values are depicted as means ± SD of six mice per group. Note the difference in scale of the x axis of (A) and (B). * $P < 0.05$

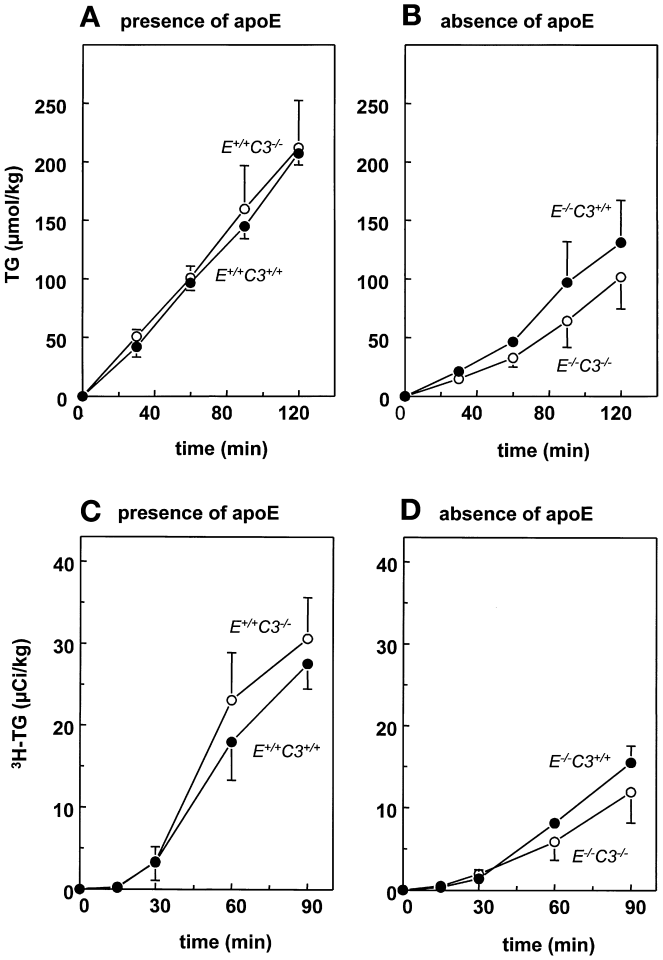

Fig. 4. Hepatic VLDL TG production in wild-type and *apoC3* knockout mice in the presence (A and C) and absence (B and D) of apoE. Triton WR1339 (500 mg/kg body weight) was injected into 4-h fasted (A and C) wild-type (solid circles) and $apoC3^{-/-}$ (open circles) mice, and (B and D) $apoE^{-/-}$ (solid circles) and $apoE^{-/-}apoC3^{-/-}$ (open circles) mice. A and B: Plasma TG levels were determined at the respective time points and corrected for the TG level at the time of Triton injection (0 min). C and D: [^{3}H]Glycerol was injected after Triton WR1339 treatment, and plasma [^{3}H]TG levels were determined in time. The values shown are the means ± SD of five or six mice per group.

mice was significantly increased compared with that in $apoE^{-/-}apoC3^{+/+}$ mice (Fig. 3B). These results indicate that it is unlikely that the low plasma lipid levels and protection from postprandial hypertriglyceridemia in $apoC3^{-/-}$ mice are due to impaired intestinal lipid absorption.

To determine whether apoC-III deficiency affects the production of TG by the liver, mice were injected with Triton WR1339 and the accumulation of endogenous VLDL TG in plasma (**Fig. 4A** and **B**) or the appearance of [^{3}H]TG in plasma after intravenous injection of [^{3}H]glycerol (Fig. 4C and D) was monitored over time. Figure 4A and C show that the increase in TG over time was similar for control and $apoC3^{-/-}$ mice. In accordance with previous published data (25), the increase in plasma TG after Triton WR1339 injection in $apoE^{-/-}$ mice was less pronounced as compared with the wild-type and $apoC3^{-/-}$ mice (Fig. 4B and D). However, no significant differences were observed between $apoE^{-/-}$ and $apoE^{-/-}apoC3^{-/-}$ mice in this respect, indicating that apoC-III deficiency does not affect the hepatic TG production rate.

We next investigated whether the low plasma TG levels and total absence of a postprandial TG response in the $apoC3^{-/-}$ mice were due to enhanced TG clearance. As shown in Tables 1 and 2, in the absence of apoC-III mice have extremely low TG concentrations in plasma and VLDL. Thus, in order to study TG clearance, mice were injected with ^{3}H-labeled VLDL-sized TG-rich emulsions. The metabolic behavior of these TG-rich emulsions with respect to plasma clearance (21) and hepatic uptake (26) has been shown to fully mimic that of VLDL-sized lipoproteins. As shown in **Fig. 5A**, TG were more rapidly cleared from the circulation in the absence of apoC-III. Similar effects of apoC-III deficiency were observed on either an $apoE^{+/+}$ and $apoE^{-/-}$ background, indicating that the effect of apoC-III is independent of the presence of apoE. To examine whether this enhanced clearance of TG in the $apoC3^{-/-}$ mice was due to enhanced TG lipolysis or enhanced hepatic particle uptake, VLDL apoB turnover studies were performed as a marker for whole particle clearance. VLDL was isolated from the respective mice by ultracentrifugation, labeled and injected (autologous in-

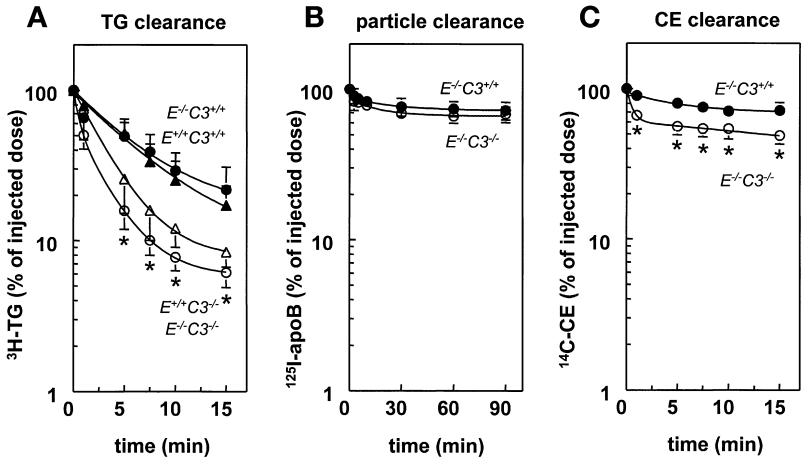

Fig. 5. In vivo metabolism of [^{3}H]TG, ^{125}I-labeled apoB, and [^{14}C]cholesteryl oleate (CE) in mice. $apoE^{+/+}apoC3^{+/+}$ (solid triangles), $apoE^{+/+}apoC3^{-/-}$ (open triangles), $apoE^{-/-}apoC3^{+/+}$ (solid circles), and/or $apoE^{-/-}apoC3^{-/-}$ (open circles) mice were either injected with glycerol [^{3}H]trioleate-labeled emulsions (A), ^{125}I-labeled VLDL ($d < 1.006$ g/ml) (B), or [^{14}C]cholesteryl oleate-labeled emulsions (C). The plasma decay of the respective labels was determined at the indicated time points (see Materials and Methods). The values represent means ± SD of six mice per group. * $P < 0.05$ (between a $apoE^{-/-}apoC3^{+/+}$ and $apoE^{-/-}apoC3^{-/-}$ mice).

jections) into the tail vein of *apoE*$^{-/-}$ and *apoE*$^{-/-}$*apoC3*$^{-/-}$ mice. As shown in Fig. 5B, ^{125}I-labeled VLDL apoB was cleared at a similar rate in both groups of mice. Thus, enhanced TG clearance in the *apoE*$^{-/-}$*apoC3*$^{-/-}$ mice compared with the *apoE*$^{-/-}$ mice is primarily due to enhanced lipolysis of TG, rather than to enhanced whole particle uptake.

As depicted in Tables 1 and 2, *apoC3*$^{-/-}$ mice also exhibited decreased plasma cholesterol levels due to a lower VLDL cholesterol content, both on the wild-type and *apoE*$^{-/-}$ background. To investigate further the mechanisms underlying the cholesterol-lowering effect of the absence of apoC-III, cholesteryl ester clearance was monitored over time in *apoE*$^{-/-}$ and *apoE*$^{-/-}$*apoC3*$^{-/-}$ mice. Figure 5C shows that the clearance from the circulation of particles labeled with [^{14}C]cholesteryl oleate was more rapid in *apoE*$^{-/-}$*apoC3*$^{-/-}$ mice compared with *apoE*$^{-/-}$ mice, indicating that the absence of apoC-III may lead to an enhanced selective uptake of VLDL cholesteryl esters.

DISCUSSION

It has been proposed that the hypertriglyceridemia observed in human *APOC3* transgenic mice is due either to the interference of apoC-III with apoE-mediated hepatic uptake of VLDL particles (13, 14), or to an inhibitory action of apoC-III on the LPL-mediated hydrolysis of VLDL TG (15). In line with these studies, it has been reported that mice deficient for apoC-III exhibit low plasma TG levels and are protected from elevated levels of plasma TG after an oral fat load (16). However, the exact mechanisms underlying the low TG levels in apoC-III-deficient mice remained to be elucidated. In the current study we performed in vivo VLDL turnover studies in *apoC3*$^{-/-}$ mice and *apoE*$^{-/-}$*apoC3*$^{-/-}$ double knockout mice. We found that the marked reduction in plasma cholesterol and TG levels on apoC-III deficiency, both after 4 h of fasting as well as after a fat load, occurs independent of apoE (Table 1 and Fig. 2). These results thus indicate that in the absence of apoE as an important ligand for particle clearance via liver receptors, apoC-III deficiency is still able to exert its lipid-lowering effect. These findings are in accordance with data published by Ebara et al. (15), showing that overexpression of human apoC-III by itself causes a marked hypertriglyceridemia in wild-type mice, as well as in *apoE*$^{-/-}$ mice.

Previously, it has been reported that deletion of the mouse *apoC3* gene lowers the intestinal expression of its neighboring genes *apoA1* and *apoA4* by ~50% (16). A reduction in intestinal expression of apoA-I and apoA-IV may therefore diminish intestinal lipid absorption in *apoC3*$^{-/-}$ mice. However, no significant reduction in intestinal lipid absorption was observed on apoC-III deficiency. Interestingly, apoC-III deficiency on an *apoC3*$^{-/-}$ background resulted in enhanced lipid absorption, but the mechanism underlying this effect remains to be clarified. We have also performed the same experiment with *apoC1*$^{-/-}$ mice, which show a disturbed postprandial response similar to that of *apoC3*$^{-/-}$ mice. In these mice, we were able to detect a strong (5.9-fold) inhibiting effect of the absence of apoC-I on intestinal lipid absorption as compared with wild-type mice, which was highly significant ($P < 0.0001$) using the same group size (M. C. Jong, P. C. N. Rensen, V. E. H. Dahlmans, H. van der Boom, T. J. C. van Berkel, and L. M. Havekes, unpublished observations). These combined data thus show that, whereas the attenuated postprandial response in *apoC1*$^{-/-}$ mice can be explained by decreased intestinal lipid uptake, it is unlikely that fat malabsorption can explain the hypotriglyceridemia observed in apoC-III-deficient mice.

Previously, Maeda et al. (16) suggested that increased whole particle clearance may underlie the hypotriglyceridemia found in *apoC3*$^{-/-}$ mice. Because autologous VLDL of *apoC3*$^{-/-}$ mice contained little TG (Table 2), TG clearance studies were performed with VLDL-sized TG-rich emulsions. We previously showed that these TG-rich emulsions rapidly acquire apolipoproteins on injection into the circulation, and are processed by LPL and cleared by hepatic lipoprotein receptors like native chylomicron and VLDL particles (27, 28). In addition, the size of these emulsions was similar to that previously reported for native VLDL particles synthesized by *apoE*$^{-/-}$ hepatocytes (25), indicating that these emulsions closely mimic VLDL kinetics in *apoE*$^{-/-}$ mice in vivo. We found that the clearance of [^{3}H]TG in VLDL-like emulsions, as a marker for VLDL TG clearance, was markedly enhanced in *apoC3*$^{-/-}$ mice, whereas the clearance of VLDL apoB, as a marker for whole particle uptake by the liver, was not affected. Such a specific increase in VLDL TG clearance on apoC-III deficiency suggests enhanced in vivo VLDL TG lipolysis, which is in line with previous in vitro work (6, 7, 29). The observation that significantly increased TG hydrolysis is observed in *apoE*$^{-/-}$*apoC3*$^{-/-}$ mice as compared with *apoE*$^{+/+}$*apoC3*$^{-/-}$ mice at time points 7.5, 10, and 15 min ($P < 0.05$) can be explained by our previous findings that apoE is also an inhibitor of LPL (28). However, the fact that larger differences are observed between TG hydrolysis in *apoE*$^{+/+}$*apoC3*$^{+/+}$ and *apoE*$^{+/+}$*apoC3*$^{-/-}$ mice (detecting the effect of apoC-III on lipolysis) than between *apoE*$^{+/+}$*apoC3*$^{+/+}$ and *apoE*$^{-/-}$*apoC3*$^{+/+}$ (detecting the effect of apoE on lipolysis) indicates that the LPL-inhibitory effect of apoC-III may be greater even than that of apoE. The mechanisms by which apoC-III inhibits LPL activity have been proposed to involve either a direct interaction between apoC-III and LPL (6, 7), or a decreased binding of the apoC-III-enriched VLDL particle to cell surface glycosaminoglycans, which renders the particle less accessible to LPL (29).

The findings that the clearance of VLDL apoB was similar between *apoE*$^{-/-}$*apoC3*$^{-/-}$ mice and *apoE*$^{-/-}$ mice (Fig. 5B), despite the significant reduction in total cholesterol and cholesteryl ester in the apoB-containing lipoprotein particles of *apoE*$^{-/-}$*apoC3*$^{-/-}$ mice (Table 2 and Fig. 1), suggest enhanced selective cholesterol clearance on apoC-III deficiency. This was confirmed by metabolic studies showing that the clearance of [^{14}C]cholesteryl oleate from VLDL-like emulsions was increased in the *apoE*$^{-/-}$*apoC3*$^{-/-}$

double knockout mice compared with *apoE*$^{-/-}$*apoC3*$^{+/+}$ mice (Fig. 5C). Previous studies have also reported on the selective uptake of cholesteryl esters from remnant lipoproteins in *apoE*$^{-/-}$ mice (30). Furthermore, overexpression of hepatic lipase (HL) in *apoE*$^{-/-}$ mice enhanced the selective uptake of cholesteryl esters by the liver (30). In vitro studies suggest that this enhanced HL-dependent selective uptake of cholesteryl esters is most likely mediated by a member of the scavenger receptor family, SR-BI, because HL was shown to stimulate cholesteryl ester uptake via Cla-1, the human analog of mouse SR-BI (31). In line with these findings, it has been reported that adenovirus-mediated expression of Cla-1 in *apoE*$^{-/-}$ mice specifically lowers cholesterol in the apoB-containing particles, without significant changes in plasma apoB levels (32). Because in addition to LPL apoC-III also inhibits HL activity (8), it is tempting to speculate that the absence of apoC-III may facilitate the HL-mediated selective clearance of cholesteryl esters from remnant lipoproteins in *apoE*$^{-/-}$ mice. Further analyses are underway to investigate a direct interaction between apoC-III and SR-BI with respect to the binding and (HL-mediated) selective uptake of cholesteryl esters from apoB-containing lipoprotein particles.

Our present findings on the enhanced LPL-mediated lipolysis of VLDL TG in *apoC3*$^{-/-}$ mice, independent of the absence or presence of apoE, strongly suggest that the hypertriglyceridemia in mice on overexpression of human apoC-III (13, 14) is primarily due to an inhibition of the LPL-mediated VLDL lipolysis. In contrast to these findings, it has been proposed that apoC-III primarily interferes with the apoE-mediated hepatic uptake of the whole particle, because hypertriglyceridemia in *APOC3* transgenic mice is corrected by overexpression of apoE (13, 14, 33). However, it can be envisioned that the poorly lipolysed TG-rich lipoprotein particle in *APOC3* transgenic mice may accumulate in plasma because of their lower binding affinity for hepatic receptors as a consequence of their low apoE content or large size. Subsequently, addition of apoE to the lipoprotein particle may be able to overcome this reduction in lipoprotein binding to the receptors.

Altogether, it can be concluded that increased amounts of apoC-III and total absence of apoC-III on the lipoprotein particle have opposite effects on VLDL TG metabolism in vivo. Such a conclusion could not be drawn from studies regarding apoC-I, because transgenic mice overexpressing human apoC-I exhibit elevated levels of plasma cholesterol and TG (34, 35), whereas *apoC1*$^{-/-}$ mice have normal plasma lipid levels when fed a regular chow diet and, remarkably, develop hypercholesterolemia when fed a high fat diet (36). The hyperlipidemia in *APOC1* transgenic mice is primarily due to an impaired uptake of TG-rich lipoproteins via hepatic receptors (27,34), particularly the LRP (34), rather than an impaired LPL-mediated lipolysis of VLDL as evidenced for apoC-III in the present study. These findings thus point to a distinct role for apoC-I and apoC-III in lipoprotein metabolism.

In summary, mice deficient for apoC-III are hypolipidemic primarily due to an increased hydrolysis of VLDL TG and a selective clearance of VLDL cholesteryl esters from plasma. These studies support the concept that apoC-III is an effective modulator of VLDL metabolism and as such may contribute to hyperlipidemia in the human population.

This work was supported by the Netherlands Hearth Foundation (97067 and D99.024), and by the Netherlands Organization for Scientific Research (903-39-194).

Manuscript received 6 December 2000 and in revised form 30 April 2001.

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