Overexpression of apoC-III produces lesser hypertriglyceridemia in apoB-48-only gene-targeted mice than in apoB-100-only mice

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Abstract **The adaptive value of apolipoprotein B-48 (apoB-48), the truncated form of apoB produced by the intestine, in lipid metabolism remains unclear. We crossed human apoC-III transgenic mice with mice expressing either apoB-48 only (*apoB*$^{48/48}$) or apoB-100 only (*apoB*$^{100/100}$). Cholesterol levels were higher in *apoB*$^{48/48}$ mice than in *apoB*$^{100/100}$ mice but triglyceride levels were similar. Lipid levels were increased by the apoC-III transgene. However, triglyceride levels were significantly higher in *apoB*$^{100/100}$C-III than in *apoB*$^{48/48}$C-III mice (895 ± 395 mg/dl vs. 690 ± 252 mg/dl; $P < 0.01$), whereas cholesterol levels were higher in the *apoB*$^{48/48}$C-III mice than in *apoB*$^{100/100}$C-III (144 ± 35 mg/dl vs. 94 ± 30 mg/dl; $P < 0.00001$). Triglyceride clearance from VLDL was impaired to a greater extent in *apoB*$^{100/100}$C-III vs. *apoB*$^{100/100}$ mice than in *apoB*$^{48/48}$C-III vs. *apoB*$^{48/48}$ mice. Triglyceride secretion rates were no different in apoC-III transgenic mice than in their nontransgenic littermates. ApoB-48 triglyceride-rich lipoproteins were more resistant to the triglyceride-increasing effects of apoC-III but appeared more sensitive to the remnant clearance inhibition. Our findings support a coordinated role for apoB-48 in facilitating the delivery of dietary triglycerides to the periphery. Consistent with such a mechanism, glucose levels were significantly higher in *apoB*$^{48/48}$ mice vs. *apoB*$^{100/100}$ mice, perhaps on the basis of metabolic competition.**—Conde-Knape, K., K. Okada, R. Ramakrishnan, and N. S. Shachter. **Overexpression of apoC-III produces lesser hypertriglyceridemia in apoB-48-only gene-targeted mice than in apoB-100-only mice. *J. Lipid Res.* 2004.** 45: **2235–2244.**

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Apolipoprotein B-48 (apoB-48) is the principal structural protein of intestinal lipoproteins. In humans, apoB-100 plays this role for liver-derived lipoproteins. However, in mice, approximately two-thirds of liver-derived lipoproteins are also based on apoB-48. ApoB-100 and apoB-48 are the translated protein products of the same gene, present in humans on chromosome 2. ApoB-48 is produced by editing of the apoB mRNA, with insertion of a stop codon and synthesis of a truncated protein with ~48% the length of apoB-100 (1). A homeostatic rationale for the maintenance of two forms of apoB is unclear. ApoB metabolism in humans has recently been comprehensively reviewed (2). Postlipolysis remnants of intestinally derived lipoproteins associate with apoE and are rapidly removed from the circulation. In contrast, VLDL (apoB-100) remnants in part escape this fate and circulate for prolonged periods as the cholesterol reservoir LDL. Therefore, the importance of apoB-48 has been presumed to relate to this rapid clearance of postprandial lipoproteins and that of apoB-100 to the ability to form LDL. However, labeling studies have not found evidence of faster clearance of apoB in apoB-48 vs. apoB-100 triglyceride (TG)-rich lipoproteins (3–7). In contrast, the metabolic fates of lipids associated with apoB-48 or apoB-100 TG-rich lipoproteins have not been compared in humans because of the exchangeability of lipids across lipoprotein species, which has constrained the performance of such studies. The study of gene-targeted mice of otherwise similar genetic backgrounds is a general methodology that can enhance our understanding of the effects of genetic isoforms of a protein uncontaminated by both intraindividual (the presence of both isoforms) and interindividual heterogeneity.

To study the question of the adaptive value of apoB-48 in postprandial TG metabolism, we crossed the human apoC-III transgenic mouse, a model of hypertriglyceridemia, with mice harboring a gene-targeted apoB allele that expressed apoB-48 only (*apoB*$^{48/48}$) in both liver and intestine or with mice that only expressed the full-length form of apoB, apoB-100 (*apoB*$^{100/100}$), in both sites. ApoC-III is a 79 amino acid protein with a molecular mass of 8.8 kDa that is encoded by a gene in the chromosome 11 apolipo-

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protein gene cluster. Three plasma isoforms differ by the linkage of zero, one, or two molecules of sialic acid to the threonine residue at position 74 (8). ApoC-III delays the clearance of TG-rich lipoproteins by interfering with both the receptor-mediated uptake of lipolytically modified lipoproteins (remnant clearance) and with lipolysis, by decreasing the affinity of TG-rich lipoproteins for glycosaminoglycan-bound lipases and by the biochemical inhibition of lipases (9–12). The importance of apoC-III in increasing TGs has now been validated by clinical correlation (13–15), the study of the deficiency state (16), genetic association studies (17), and both transgenic (18–20) and gene-knockout (21) mice. We hypothesized that the presence of increased apoC-III in these mouse models would exaggerate phenotypic differences between apoB-48 and apoB-100 that relate to the clearance of TG-rich lipoproteins. We studied four genotypes: *apoB*$^{48/48}$ homozygotes, *apoB*$^{48/48}$ homozygotes transgenic for human apoC-III (*apoB*$^{48/48}$C-III), *apoB*$^{100/100}$ homozygotes, and *apoB*$^{100/100}$ homozygotes transgenic for human apoC-III (*apoB*$^{100/100}$C-III).

MATERIALS AND METHODS

Animals

Human apoC-III transgenic mice (CIII) backcrossed for at least eight generations to the C57BL/6 background were used to generate the transgenic/gene-targeted mice (18). Gene-targeted mice secreting either apoB-48 only (*apoB*$^{48/48}$) or apoB-100 only (*apoB*$^{100/100}$) in a mixed C57BL/6 129S F2 genetic background, generated as described, were obtained from Dr. Stephen G. Young (Gladstone Institute, San Francisco, CA) and were maintained in this background (22, 23). CIII mice were crossed with the *apoB*$^{48/48}$ or the *apoB*$^{100/100}$ mice to generate mice homozygous for the *apoB*$^{48/48}$ or *apoB*$^{100/100}$ gene-targeted alleles and heterozygous for the human apoC-III transgene (*apoB*$^{48/48}$C-III or *apoB*$^{100/100}$C-III). Animals were housed in an approved animal care facility with a period of light from 7:00 AM to 7:00 PM. Mice were fed a standard mouse chow diet containing 4.5% fat (10% of calories) and 0.02% cholesterol. Access to food and water was ad libitum except where indicated. Fasting blood was drawn in the afternoon 6 h after food removal. Nonfasting blood was drawn at 9:00 AM. Animals were anesthetized with methoxyflurane for retro-orbital plexus phlebotomy and femoral vein intravenous injections.

Analysis of plasma lipids

Cholesterol, TGs, and glucose were determined in fasting and nonfasting plasma samples using enzymatic kits from Sigma-Aldrich (St. Louis, MO). HDL cholesterol was determined after the precipitation of apoB-containing lipoproteins from plasma using an HDL-cholesterol reagent from Sigma-Aldrich.

Lipoprotein composition

Gel filtration chromatography was performed on 200 μl of pooled plasma obtained from male mice (at least six mice per group) using two Superose 6 columns in series [fast-protein liquid chromatography (FPLC); Pharmacia LKB Biotechnology, Piscataway, NJ]. Forty-five 0.5 ml fractions were collected and assayed for cholesterol and TGs as described above. In addition, at least three distinct pools of plasma from each genotype, each from at least six male mice, were used to isolate lipoprotein fractions. VLDL ($d < 1.006$ g/ml), intermediate density lipoprotein (IDL)+LDL ($d = 1.006$–1.063 g/ml), and HDL ($d = 1.063$–1.21 g/ml) were separated by sequential density ultracentrifugation (24). Cholesterol, TG, free cholesterol (FC), phospholipids (PLs), and protein concentrations were determined for all fractions. FC and PLs were determined using kits from Wako Chemicals (Richmond, VA), and protein was determined using the BCA protein assay (Pierce Chemical, Rockford, IL).

Fat tolerance testing

Male animals were gavaged with 0.4 ml of peanut oil at 12 noon after the removal of food at 8:00 AM. Plasma TGs were determined at baseline and then hourly for 5 h.

VLDL remnant clearance studies

Six male *apoB*$^{48/48}$ mice and six male *apoB*$^{100/100}$ mice were fed the high-fat, high-cholesterol, 0.5% cholic acid diet (Research Diets) described by Paigen et al. (25) for 1 week. The animals were killed and $d < 1.006$ lipoproteins were isolated from fasting plasma. VLDL apoproteins were labeled with ^{125}I using the Iodobeads technique (Pierce Chemical). After labeling, VLDL was fractionated by SDS-PAGE and exposed to film to determine the incorporation of radioactivity into apoB. The apoB label was present in a major band of molecular weight (MW) consistent with apoB-48 in the apoB-48 preparation and in a major band of MW consistent with apoB-100 in the apoB-100 preparation, confirming the specificity of the labeling.

Clearance studies were performed in male mice. One million counts per minute of the dialyzed apoB-48 preparation was injected into six *apoB*$^{48/48}$ and six *apoB*$^{48/48}$C-III mice fed chow. Similarly, 10^6 cpm of the dialyzed apoB-100 preparation was injected into six *apoB*$^{100/100}$ and six *apoB*$^{100/100}$C-III mice fed chow. Tracer apoB in plasma was determined at 30 s and 5, 10, 20, 40, 80, and 120 min after injection by SDS-PAGE of whole plasma followed by autoradiography and γ-counting of the identified apoB bands, which were excised from the gels. The rate of clearance of post-lipolysis lipoprotein remnant particles was modeled by the disappearance of tracer apoB from this preparation, as described, assuming the value obtained at 30 s to be 100% of the injected dose (26).

TG and apoB production rates

TG and apoB production rates were determined in male mice by inhibiting the plasma catabolism of VLDL with the injection of Triton WR-1339 and simultaneously radiolabeling apoB with [^{35}S]methionine, as described (27).

VLDL TG clearance

ApoB$^{48/48}$ and *apoB*$^{100/100}$ mice were used to obtain VLDL labeled on its TG constituent. Six mice from each group were injected with 75 μCi of [^{3}H]oleate via the tail vein. Forty-five minutes after radioisotope injection, blood was collected for the isolation of VLDL. VLDL was isolated by ultracentrifugation. Radioactivity incorporation into VLDL TG was assessed by thin-layer chromatography. Approximately 85% of the tritium label was incorporated into TG for both VLDL preparations.

Five male animals of mean age 12 months were studied for each genotype. Counts in whole plasma were determined at 30 s and at 5, 10, 20, 40, 80, and 120 min after injection. The rate of clearance of TG was modeled by the disappearance of TG from plasma, as described, assuming the value obtained at 30 s to be 100% of the injected dose (26).

Statistical analysis

Statistical analysis was done by ANOVA with prespecified pairwise comparisons of interest. Two-tailed *P* values of 0.05 or less were considered statistically significant.

TABLE 1. Fed-state and fasted-state plasma lipids and glucose in *apoB*$^{48/48}$, *apoB*$^{48/48}$C-III, *apoB*$^{100/100}$, and *apoB*$^{100/100}$C-III male mice

Mouse Type (N)	Fed State			Fasted State		
	CHOL	TG	GLU	CHOL	TG	GLU
apoB$^{48/48}$ (31)	81 ± 18	106 ± 35	138 ± 28	85 ± 21	82 ± 39	180 ± 32
apoB$^{48/48}$C-III (27)	157 ± 34	802 ± 231	144 ± 28	150 ± 30	536 ± 166	193 ± 19
P: *apoB*$^{48/48}$ vs. *apoB*$^{48/48}$C-III	<0.00001	<0.00001	NS	<0.00001	<0.00001	NS
apoB$^{100/100}$ (10)	60 ± 15	128 ± 33	115 ± 38	60 ± 13	70 ± 18	140 ± 28
apoB$^{100/100}$C-III (19)	109 ± 21	1,054 ± 394	124 ± 33	107 ± 27	727 ± 215	144 ± 32
P: *apoB*$^{100/100}$ vs. *apoB*$^{100/100}$C-III	<0.00001	<0.00001	NS	<0.00001	<0.00001	NS
P: *apoB*$^{48/48}$ vs. *apoB*$^{100/100}$	0.01	NS	0.04	0.001	NS	0.002
P: *apoB*$^{48/48}$C-III vs. *apoB*$^{100/100}$C-III	<0.00001	0.005	0.03	0.0005	0.01	0.0001

apoB$^{48/48}$, apolipoprotein B (apoB) allele that expressed apoB-48 only; *apoB*$^{48/48}$C-III, *apoB*$^{48/48}$ homozygotes transgenic for human apoC-III; CHOL, cholesterol; GLU, glucose; TG, triglyceride. Values shown are means ± SD (mg/dl). The number of mice (N) in each group is shown in parentheses.

RESULTS

Plasma lipids

We crossed gene-targeted mice that expressed either apoB-48 only or apoB-100 only with a model of hypertriglyceridemia, transgenic mice overexpressing human apoC-III. Plasma lipid levels in the morning (fed state) and in the afternoon (fasted state) are presented for male mice in **Table 1**. In the fed state, overexpression of apoC-III in the *apoB*$^{48/48}$ background resulted in a 94% increase in cholesterol levels compared with *apoB*$^{48/48}$ mice (157 ± 34 mg/dl vs. 81 ± 18 mg/dl; $P < 0.00001$). Overexpression of apoC-III in the *apoB*$^{100/100}$ background resulted in an 82% increase in cholesterol compared with *apoB*$^{100/100}$ mice (109 ± 21 mg/dl vs. 60 ± 15 mg/dl; $P < 0.00001$). The higher cholesterol levels in *apoB*$^{48/48}$ mice vs. levels in *apoB*$^{100/100}$ mice, both for nontransgenic ($P = 0.01$) and apoC-III transgenic ($P < 0.00001$) mice, were significantly different. Similarly significant differences were observed in the fasted state.

In contrast, *apoB*$^{100/100}$C-III mice showed higher relative TG levels, compared with nontransgenic littermates in the fed state, than did *apoB*$^{48/48}$C-III mice [802 ± 231 mg/dl (*apoB*$^{48/48}$C-III) vs. 106 ± 35 mg/dl (*apoB*$^{48/48}$); 1,054 ± 394 mg/dl (*apoB*$^{100/100}$C-III) vs. 128 ± 33 mg/dl (*apoB*$^{100/100}$)]. Although the fed-state TG levels of *apoB*$^{100/100}$ and *apoB*$^{48/48}$ mice were not significantly different, *apoB*$^{100/100}$C-III mice had 31% higher fed-state TG levels than did *apoB*$^{48/48}$C-III mice ($P = 0.005$). Findings in the fasted state were similar.

Overexpression of apoC-III either in the *apoB*$^{48/48}$ background or in the *apoB*$^{100/100}$ background had no significant effect on glucose levels compared with nontransgenic littermates. However, in the fed state, glucose levels were 20% higher in *apoB*$^{48/48}$ than in *apoB*$^{100/100}$ mice (138 ± 28 mg/dl vs. 115 ± 38 mg/dl; $P = 0.04$) and levels were 16% higher in *apoB*$^{48/48}$C-III vs. *apoB*$^{100/100}$C-III mice (144 ± 28 mg/dl vs. 124 ± 33 mg/dl; $P = 0.03$). In the fasted state, *apoB*$^{48/48}$ mice had 29% higher glucose levels compared with *apoB*$^{100/100}$ mice (180 ± 32 mg/dl vs. 140 ± 28 mg/dl; $P = 0.002$) and 34% higher levels in *apoB*$^{48/48}$C-III vs. *apoB*$^{100/100}$C-III mice (193 ± 19 mg/dl vs. 144 ± 32 mg/dl; $P = 0.0001$).

Analysis of the females (**Table 2**) indicated that absolute TG and cholesterol levels were overall lower and that any differences in TG were lesser and not significant for both *apoB*$^{48/48}$ vs. *apoB*$^{100/100}$ and for *apoB*$^{48/48}$C-III vs. *apoB*$^{100/100}$C-III mice both in the fed state and in the fasted state. Differences in cholesterol levels were also lesser in females but remained significantly higher in both the fed and fasted states in *apoB*$^{48/48}$C-III vs. *apoB*$^{100/100}$C-III females. Glucose levels were similar in the males and females, other than lower glucose levels in the *apoB*$^{100/100}$C-III females, and were significantly higher in *apoB*$^{48/48}$ vs. *apoB*$^{100/100}$ mice and in *apoB*$^{48/48}$C-III than in *apoB*$^{100/100}$C-III mice. Because

TABLE 2. Fed-state and fasted-state plasma lipids and glucose in *apoB*$^{48/48}$, *apoB*$^{48/48}$C-III, *apoB*$^{100/100}$, and *apoB*$^{100/100}$C-III female mice

Mouse Type (N)	Fed State			Fasted State		
	CHOL	TG	GLU	CHOL	TG	GLU
apoB$^{48/48}$ (20)	66 ± 9	76 ± 30	141 ± 31	78 ± 12	66 ± 60	175 ± 22
apoB$^{48/48}$C-III (14)	118 ± 16	475 ± 116	142 ± 33	125 ± 19	373 ± 56	188 ± 22
P: *apoB*$^{48/48}$ vs. *apoB*$^{48/48}$C-III	<0.00001	<0.00001	NS	0.0003	<0.00001	NS
apoB$^{100/100}$ (23)	51 ± 10	87 ± 30	123 ± 36	51 ± 10	68 ± 22	141 ± 30
apoB$^{100/100}$C-III (11)	68 ± 25	577 ± 237	101 ± 24	69 ± 26	315 ± 137	125 ± 30
P: *apoB*$^{100/100}$ vs. *apoB*$^{100/100}$C-III	0.007	<0.00001	NS	0.006	<0.00001	NS
P: *apoB*$^{48/48}$ vs. *apoB*$^{100/100}$	<0.00001	NS	NS	<0.00001	NS	0.01
P: *apoB*$^{48/48}$C-III vs. *apoB*$^{100/100}$C-III	<0.00001	NS	0.002	0.0002	NS	<0.0005

Values shown are means ± SD (mg/dl). The number of mice (N) in each group is shown in parentheses.

TABLE 3. VLDL composition in *apoB*$^{48/48}$, *apoB*$^{48/48}$C-III, *apoB*$^{100/100}$, and *apoB*$^{100/100}$C-III mice in absolute mass and, in parentheses, in percentage of total mass

Mouse Type	FC	CE	TG	PL	PRO	(CE+TG)/(FC+PL+PRO)
apoB$^{48/48}$ (N = 9)	1.20 ± 1.95 (1.71 ± 1.46)	2.59 ± 2.48 (4.82 ± 1.62)	28.19 ± 18.49 (56.09 ± 2.85)	4.67 ± 3.72 (8.83 ± 1.47)	13.11 ± 5.22 (28.55 ± 4.83)	1.57 ± 0.17
apoB$^{48/48}$C- III (N = 4)	23.07 ± 5.86) (4.19 ± 0.76	14.65 ± 5.99 (2.69.±1.17)	342.42 ± 51.54 (62.46 ± 1.99)	70.10 ± 8.17 (12.84 ± 0.69)	97.99 ± 20.37 (17.82 ± 2.12)	1.89 ± 0.26
P: *apoB*$^{48/48}$ vs. *apoB*$^{48/48}$C-III	<0.0001 (NS)	<0.0001 (NS)	<0.0001 (0.002)	<0.0001 (NS)	<0.0001 (0.001)	NS
apoB$^{100/100}$ (N = 6)	0.86 ± 1.35 (2.20 ± 3.40)	0.40 ± 3.16 (0.66 ± 7.18)	22.02 ± 5.50 (61.20 ± 3.45)	3.35 ± 2.66 66 (9.07 ± 6.39)	9.40 ± 1.85 (26.87 ± 5.87)	1.70 ± 0.49
apoB$^{100/100}$C-III (N = 3)	20.84 ± 4.68) (4.72 ± 0.41	9.22 ± 3.27 (2.06 ± 0.40)	290.18 ± 48.34 (66.26 ± 1.96)	51.42 ± 10.88 (11.68 ± 0.90)	66.64 ± 8.77 (15.29 ± 1.15)	2.16 ± 0.19
P: *apoB*$^{100/100}$ vs. *apoB*$^{100/100}$C-III	<0.0001 (NS)	0.003 (NS)	<0.0001 (0.02)	<0.0001 (NS)	<0.0001 (0.002)	0.05
P: *apoB*$^{48/48}$ vs. *apoB*$^{100/100}$	NS (NS)	NS (NS)	NS (0.003)	NS (NS)	NS (NS)	NS
P: *apoB*$^{48/48}$C-III vs. *apoB*$^{100/100}$C-III	NS (NS)	NS (NS)	0.03 (NS)	0.0004 (NS)	0.0004 (NS)	NS

CE, cholesteryl ester; FC, free cholesterol; PL, phospholipid; PRO, protein. Values shown are means ± SD (mg/dl for absolute mass and % for total mass). N reflects the number of nonoverlapping plasma pools that were used for the isolation of lipoproteins. (CE+TG)/(FC+PL+PRO) indicates the ratio of core lipid to surface lipid constituents, which is an index of lipoprotein size. A higher number indicates larger lipoprotein particles.

of the more pronounced lipid phenotype in male mice, all subsequent experiments were performed in males.

Plasma lipoprotein fractions

VLDL composition is shown in **Table 3**. VLDL from *apoB*$^{48/48}$C-III mice had an increase in the absolute amounts of all lipoprotein constituents [FC, cholesteryl ester (CE), TG, PL, and protein] compared with *apoB*$^{48/48}$ mice, as would be expected. When compared on a relative basis, there was a significant increase in TG with decreased protein in *apoB*$^{48/48}$C-III vs. *apoB*$^{48/48}$ mice. VLDL from *apoB*$^{100/100}$C-III mice were also increased, showing an increase in the absolute amounts of all lipoprotein constituents compared with *apoB*$^{100/100}$ mice. On a relative basis, TG was higher and protein was lower in *apoB*$^{100/100}$C-III vs. *apoB*$^{100/100}$ mice. On a relative basis, VLDL from *apoB*$^{100/100}$ were not strikingly different from *apoB*$^{48/48}$ VLDL but did have statistically significantly more TG. On a relative basis, VLDL from *apoB*$^{100/100}$ C-III mice were not different from *apoB*$^{48/48}$C-III VLDL. The estimated core-to-surface constituent ratios in all groups were similar, suggesting that all of the VLDL preparations were in the same lipoprotein-particle size ranges.

The IDL+LDL fraction (**Table 4**) from *apoB*$^{48/48}$C-III mice had ~3-fold more of all constituents on an absolute basis vs. *apoB*$^{48/48}$ mice, but there were no significant differences on a relative basis. In contrast, the IDL+LDL fraction from *apoB*$^{100/100}$C-III mice was significantly enriched only in TG in absolute mass; this increase was present also on a relative basis compared with *apoB*$^{100/100}$ mice. Comparison of IDL+LDL fractions between *apoB*$^{48/48}$ and *apoB*$^{100/100}$ mice indicated that *apoB*$^{100/100}$ IDL+LDL had greater absolute amounts of CE, TG, and protein compared

TABLE 4. IDL+LDL composition in *apoB*$^{48/48}$, *apoB*$^{48/48}$C-III, *apoB*$^{100/100}$, and *apoB*$^{100/100}$C-III mice in absolute mass and, in parentheses, in percentage of total mass

Mouse Type	FC	CE	TG	PL	PRO	(CE+TG)/(FC+PL+PRO)
apoB$^{48/48}$ (N = 9)	2.04 ± 1.74 (6.50 ± 4.97)	4.74 ± 3.75 (16.25 ± 11.31)	5.90 ± 1.11) (19.92 ± 2.82	6.07 ± 4.35 (19.79 ± 11.98)	11.05 ± 1.27 (37.54 ± 4.81)	0.62 ± 0.30
apoB$^{48/48}$C-III (N = 4)	5.37 ± 1.92 (5.92 ± 0.83)	14.20 ± 4.27 (16.22 ± 5.31)	21.09 ± 8.14) (24.08 ± 10.09	18.03 ± 6.71 (19.82 ± 2.97)	30.47 ± 8.90 (33.95 ± 4.19)	0.70 ± 0.22
P: *apoB*$^{48/48}$ vs. *apoB*$^{48/48}$C-III	0.001 (NS)	0.0001 (NS)	<0.0001 (NS)	0.0001 (NS)	<0.0001 (NS)	NS
apoB$^{100/100}$ (N = 6)	2.74 ± 0.36 (5.78 ± 0.85)	9.88 ± 2.25 (20.56 ± 3.09)	11.24 ± 1.78 (23.62 ± 3.39)	7.20 ± 1.00 (15.08 ± 1.57)	16.68 ± 1.64 (34.97 ± 1.32)	0.79 ± 0.07
apoB$^{100/100}$C-III (N = 3)	2.78 ± 0.36 (4.59 ± 0.13)	8.31 ± 0.87 (13.83 ± 1.45)	20.52 ± 3.56 (33.85 ± 2.96)	9.10 ± 1.01 (15.09 ± 0.49)	19.62 ± 1.00 (32.64 ± 1.75)	0.91 ± 0.06
P: *apoB*$^{100/100}$ vs . *apoB*$^{100/100}$C-III	NS (NS)	NS (NS)	0.002 (<0.01)	NS (NS)	NS (NS)	NS
P: *apoB*$^{48/48}$ vs. *apoB*$^{100/100}$	NS (NS)	0.008 (NS)	0.01 (NS)	NS (NS)	0.01 (NS)	NS
P: *apoB*$^{48/48}$C-III vs. *apoB*$^{100/100}$C-III	0.03 (NS)	0.03 (NS)	NS (0.02)	0.01 (NS)	0.002 (NS)	NS

IDL, intermediate density lipoprotein. Values shown are means ± SD (mg/dl for absolute mass and % for total mass). N reflects the number of nonoverlapping plasma pools that were used for the isolation of lipoproteins.

TABLE 5. HDL composition in *apoB*$^{48/48}$, *apoB*$^{48/48}$C-III, *apoB*$^{100/100}$, and *apoB*$^{100/100}$C-III mice in absolute mass and, in parentheses, in percentage of total mass

Mouse Type	FC	CE	TG	PL	PRO	(CE+TG)/(FC+PL+PRO)
apoB$^{48/48}$ (N = 9)	2.88 ± 0.77 (1.94 ± 0.33)	60.04 ± 8.72 (40.90 ± 1.04)	2.97 ± 0.41 (2.05 ± 0.29)	19.86 ± 4.60 (13.41 ± 1.52)	61.13 ± 8.43 (41.70 ± 1.57)	0.75 ± 0.04
apoB$^{48/48}$C-III (N = 4)	0.73 ± 0.72 (0.52 ± 0.48)	52.51 ± 14.56 (36.16 ± 2.70)	5.13 ± 2.62 (3.43 ± 0.86)	21.82 ± 8.97 (14.80 ± 2.26)	65.05 ± 16.82 (45.08 ± 1.83)	0.66 ± 0.06
P: *apoB*$^{48/48}$ vs. *apoB*$^{48/48}$C-III	<0.0001 (<0.0001)	NS (<0.01)	0.006 (0.001)	NS (NS)	NS (<0.01)	0.02
apoB$^{100/100}$ (N = 6)	0.37 ± 0.41 (0.35 ± 0.40)	36.65 ± 7.99 (36.85 ± 4.14)	2.81 ± 0.45 (2.94 ± 0.84)	13.38 ± 1.41 (13.72 ± 1.66)	45.19 ± 4.07 (46.14 ± 2.16)	0.67 ± 0.09
apoB$^{100/100}$C-III (N = 3)	0.01 ± 0. 01 (0.01 ± 0.01)	39.11 ± 5.21 (32.99 ± 2.88)	4.27 ± 0.69 (3.60 ± 0.39)	19.41 ± 0.51 (16.45 ± 0.94)	55.54 ± 4.37 (46.95 ± 2.06)	0.58 ± 0.06
P: *apoB*$^{100/100}$ vs. *apoB*$^{100/100}$C-III	NS (NS)	NS (NS)	NS (NS)	NS (0.03)	NS (NS)	NS
P: *apoB*$^{48/48}$ vs. *apoB*$^{100/100}$	<0.0001 (<0.0001)	0.0002 (0.01)	NS (0.01)	0.02 (NS)	0.004 (0.0002)	0.02
P: *apoB*$^{48/48}$C-III vs. *apoB*$^{100/100}$C-III	NS (NS)	NS (NS)	NS (NS)	NS (NS)	NS (NS)	NS

Values shown are means ± SD (mg/dl for absolute mass and % for total mass). N reflects the number of nonoverlapping plasma pools that were used for the isolation of lipoproteins.

with *apoB*$^{48/48}$ IDL+LDL. However, there was no compositional difference on a relative basis. Comparison of *apoB*$^{48/48}$ C-III vs. *apoB*$^{100/100}$C-III IDL+LDL indicated greater absolute amounts of all constituents except for TG in *apoB*$^{48/48}$ C-III IDL+LDL. In contrast, on a relative basis, the *apoB*$^{100/100}$C-III IDL+LDL was significantly more TG enriched. The core-to-surface ratios were not significantly different between any fractions. It appears that apoC-III overexpression is associated with a tripling of IDL+LDL of essentially unaltered composition in the *apoB*$^{48/48}$ context but with the development of TG-enriched particles, but not of more IDL+LDL, in the *apoB*$^{100/100}$ context.

HDL levels (**Table 5**) from *apoB*$^{48/48}$C-III mice were about the same compared with *apoB*$^{48/48}$ mice. On a relative basis, *apoB*$^{48/48}$C-III HDL had less FC and CE but more TG and protein compared with *apoB*$^{48/48}$ HDL. The core-to-surface ratio was lower for HDL from *apoB*$^{48/48}$C-III mice, indicating smaller particles. In contrast, HDL levels from *apoB*$^{100/100}$C-III and *apoB*$^{100/100}$ mice were about the same and their relative composition was similar. Comparison of *apoB*$^{48/48}$ and *apoB*$^{100/100}$ mice showed more HDL in the *apoB*$^{48/48}$ mice, based on FC, CE, PL, and protein. On a relative basis, *apoB*$^{48/48}$ HDL had more FC and CE but less protein and, perhaps, TG. Core-to-surface ratios were significantly higher in *apoB*$^{48/48}$ vs. *apoB*$^{100/100}$ HDL, indicating larger particles. This ratio was not significantly different in *apoB*$^{48/48}$C-III vs. *apoB*$^{100/100}$C-III HDL, and the amount and composition of the particles were also similar.

Gel filtration chromatography of plasma from *apoB*$^{48/48}$ C-III and *apoB*$^{48/48}$ mice showed increased cholesterol in both the VLDL and IDL+LDL lipoprotein peaks of *apoB*$^{48/48}$C-III mice compared with *apoB*$^{48/48}$ mice (**Fig. 1A**). In addition, VLDL from *apoB*$^{48/48}$C-III mice showed increased TG (Fig. 1B). These results were similar to those obtained after the isolation of these lipoproteins via ultracentrifugation. *ApoB*$^{100/100}$C-III plasma had more cholesterol and TG in VLDL than did *apoB*$^{100/100}$ plasma (**Fig. 2A**, **B**). As had been seen by ultracentrifugation, there was little increase in IDL+LDL in *apoB*$^{100/100}$C-III plasma.

VLDL remnant clearance

To investigate the mechanism underlying the increase in cholesterol observed in *apoB*$^{48/48}$C-III compared with *apoB*$^{100/100}$C-III mice, we examined the clearance of remnant-like lipoproteins obtained either from *apoB*$^{48/48}$ or *apoB*$^{100/100}$ mice that had been fed a very high-cholesterol,

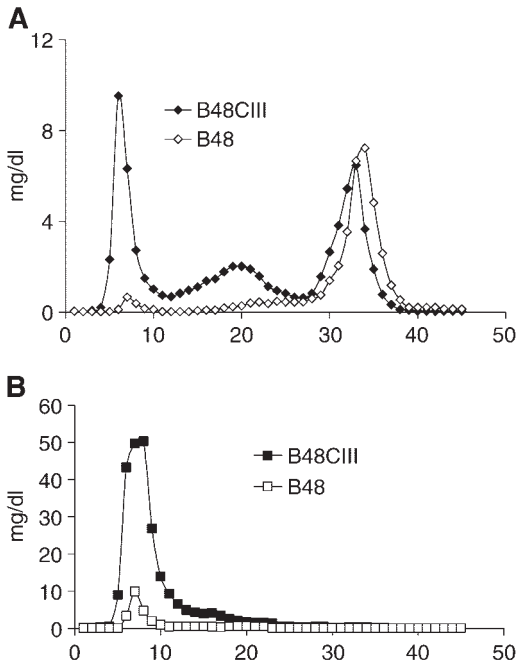

Fig. 1. Fast-protein liquid chromatography (FPLC) profiles of plasma from mice harboring a gene-targeted apolipoprotein B (apoB) allele that expressed apoB-48 only (*apoB*$^{48/48}$) and *apoB*$^{48/48}$ homozygotes transgenic for human apoC-III (*apoB*$^{48/48}$C-III). A: Cholesterol distribution in plasma pools run through gel filtration chromatography columns. Closed diamonds represent data from plasma of *apoB*$^{48/48}$C-III mice. Open diamonds represent data from *apoB*$^{48/48}$ mice. B: Triglyceride (TG) distribution in plasma pools run through gel filtration chromatography columns. At least six mice from each group were used for each pool. Closed squares represent data from plasma of *apoB*$^{48/48}$C-III mice. Open squares represent data from *apoB*$^{48/48}$ mice.

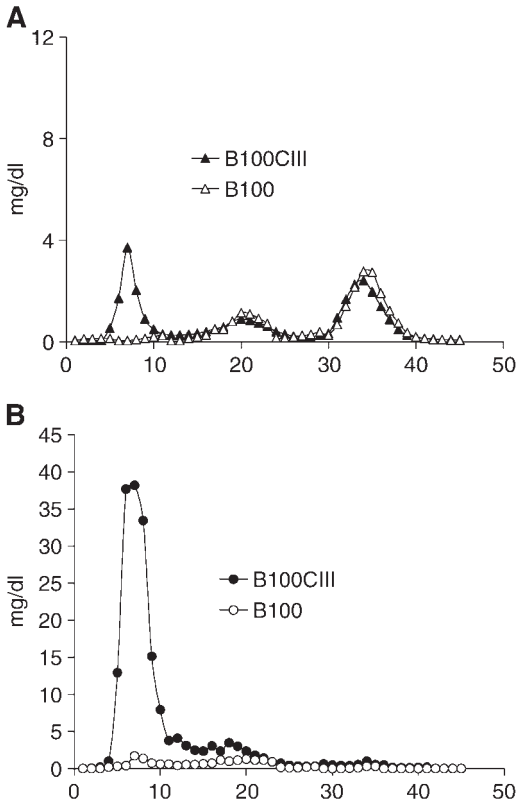

Fig. 2. FPLC profiles of plasma from *apoB*$^{100/100}$C-III and *apoB*$^{100/100}$ mice. A: Cholesterol distribution in plasma pools run through gel filtration chromatography columns. Closed triangles represent data from plasma of *apoB*$^{100/100}$C-III mice. Open triangles represent data from *apoB*$^{100/100}$ mice. B: TG distribution in plasma pools run through gel filtration chromatography columns. At least six mice from each group were used for each pool. Closed circles represent data from plasma of *apoB*$^{100/100}$C-III mice. Open circles represent data from *apoB*$^{100/100}$ mice.

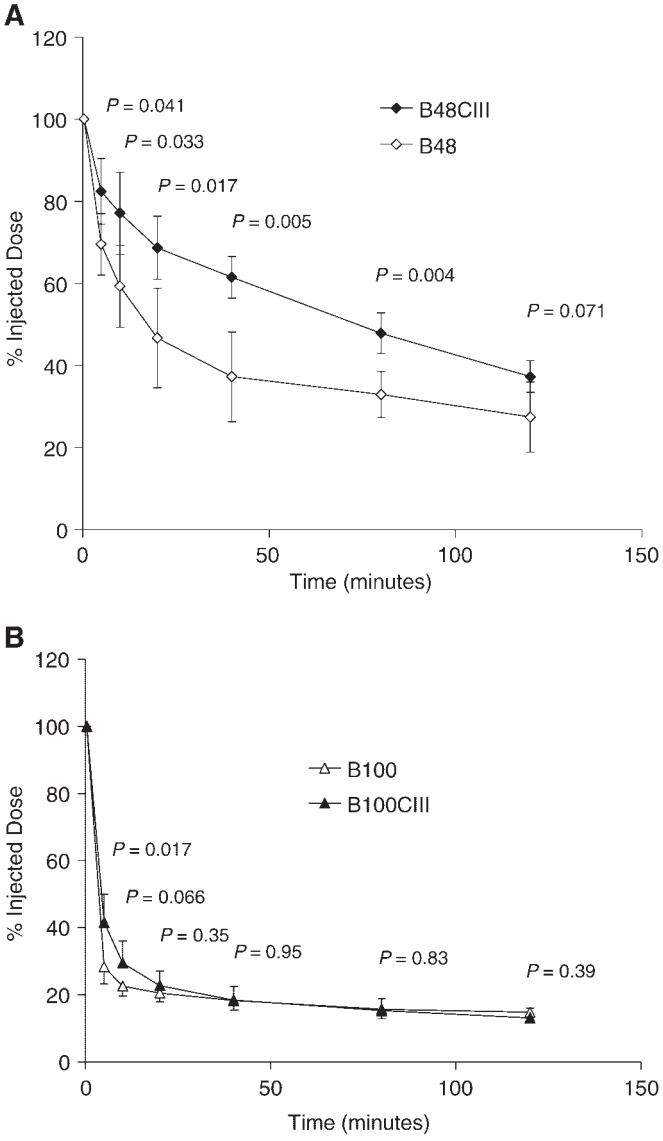

Fig. 3. Clearance of remnant-like VLDL particles in *apoB*$^{48/48}$C-III, *apoB*$^{48/48}$, *apoB*$^{100/100}$C-III, and *apoB*$^{100/100}$ mice. VLDLs were obtained from *apoB*$^{48/48}$ or *apoB*$^{100/100}$ mice fed the high-fat, high-cholesterol, cholic acid-containing diet described by Paigen et al. (25) for 1 week. VLDLs were labeled with ^{125}I and used as a tracer. *ApoB*$^{48/48}$-VLDL was injected into *apoB*$^{48/48}$C-III and *apoB*$^{48/48}$ mice. *ApoB*$^{100/100}$-VLDL was injected in *apoB*$^{100/100}$C-III and *apoB*$^{100/100}$ mice. A: VLDL clearance in *apoB*$^{48/48}$C-III and *apoB*$^{48/48}$ mice. Closed diamonds represent data from *apoB*$^{48/48}$C-III mice. Open diamonds represent data from *apoB*$^{48/48}$ mice. B: VLDL clearance in *apoB*$^{100/100}$C-III and *apoB*$^{100/100}$ mice. Closed triangles represent data from *apoB*$^{100/100}$C-III mice. Open triangles represent data from *apoB*$^{100/100}$ mice. The error bars represent the percentage of injected dose of tracer from all mice studied (as means ± standard deviation) and is shown for all sampled time points.

cholic acid-containing diet for 1 week. These particles are TG-depleted and cholesterol-enriched and are a model for postlipolysis remnant lipoproteins. As shown in **Fig. 3A**, pooled data from *apoB*$^{48/48}$C-III mice showed higher levels of labeled VLDL apoB that were significantly different at all time points compared with *apoB*$^{48/48}$ mice. In contrast, *apoB*$^{100/100}$C-III mice showed higher levels of labeled *apoB*$^{100/100}$-VLDL apoB only at the 5 min time point compared with *apoB*$^{100/100}$ mice (Fig. 3B). Least-squares curves were fitted to the individual data for each animal, and the mean percentage of apoB cleared from 0.5 to 20 min was calculated. Consistent with the appearance of the pooled data, 53.3 ± 13.12% of tracer apoB present at 0.5 min was cleared by 20 min for *apoB*$^{48/48}$ mice vs. 29.44 ± 9.51% for *apoB*$^{48/48}$C-III mice ($P < 0.001$). Results were 72.46 ± 5.83% for *apoB*$^{100/100}$ vs. 63.00 ± 19.40% for *apoB*$^{100/100}$C-III ($P =$ NS). However, fractional catabolic rates (FCRs) were also calculated for the individual animals (pools/hour) and were 5.86 ± 55% for *apoB*$^{48/48}$ mice, 4.46 ± 84% for *apoB*$^{48/48}$C-III, 23.22 ± 47% for *apoB*$^{100/100}$, and 8.40 ± 67% for *apoB*$^{100/100}$C-III. The decreases in calculated apoB FCRs with apoC-III overexpression were not in keeping with the percentage cleared calculation, and statistical comparisons of the FCR values revealed that the decrease was significantly different only in the *apoB*$^{100/100}$ vs. *apoB*$^{100/100}$C-III comparison ($P < 0.01$) but not in the *apoB*$^{48/48}$ vs. *apoB*$^{48/48}$C-III comparison.

VLDL TG production studies

To further investigate the mechanism responsible for the observed greater increase in TG in *apoB*$^{100/100}$C-III mice com-

pared with *apoB*$^{48/48}$C-III mice, we examined TG production rates via the injection of the lipolysis/clearance inhibitor Triton WR-1339. As shown in **Fig. 4A**, **B** for pooled data, there was no difference observed in the increase of TG over time between *apoB*$^{100/100}$C-III and *apoB*$^{100/100}$ mice or between *apoB*$^{48/48}$C-III and *apoB*$^{48/48}$ mice. TG secretion rates calculated for the individual animals were 4.54 ± 1.66 mg/dl/min for *apoB*$^{48/48}$, 4.35 ± 0.54 mg/dl/min for *apoB*$^{48/48}$C-III, 3.69 ± 0.95 mg/dl/min for *apoB*$^{100/100}$, and 4.80 ± 1.19 mg/dl/min for *apoB*$^{100/100}$C-III. There were no significant differences in TG secretion rates between any of the groups.

Fat tolerance testing

To determine whether a specific impairment in the clearance of intestinal lipoproteins was responsible for the higher levels of TG in the *apoB*$^{100/100}$C-III than in the *apoB*$^{48/48}$C-III mice, fat tolerance testing was performed. As shown in **Fig. 5**, there was impairment of postprandial TG clearance related to overexpression of apoC-III. However, this impairment was present equally in both groups and was independent of *apoB*$^{48/48}$ or *apoB*$^{100/100}$ background.

VLDL TG clearance studies

The possibility was evaluated of the presence of a greater effect of apoC-III in the *apoB*$^{100/100}$ context on the clearance of TG present in infused VLDL. In plots of pooled data, *apoB*$^{48/48}$C-III mice had impaired TG clearance compared with *apoB*$^{48/48}$ mice, as would be expected (**Fig. 6A**). However, the relative impairment observed in *apoB*$^{100/100}$ C-III mice vs. *apoB*$^{100/100}$ mice appeared greater (Fig. 6B). VLDL TG FCR values calculated for the individual animals (pools/hour) were 5.61 ± 2.10 for *apoB*$^{48/48}$ mice, 3.15 ± 3.04 for *apoB*$^{48/48}$C-III, 6.17 ± 2.41 for *apoB*$^{100/100}$, and 0.50 ± 0.08 for *apoB*$^{100/100}$C-III mice. Statistical comparisons of these FCR values revealed that the *apoB*$^{48/48}$ and *apoB*$^{48/48}$C-III values were not significantly different, whereas the *apoB*$^{100/100}$ and *apoB*$^{100/100}$C-III values were highly statistically different at $P < 0.001$.

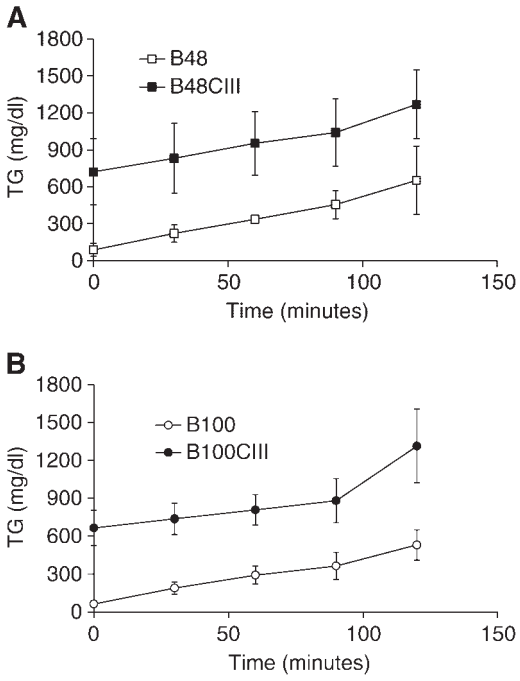

Fig. 4. TG secretion rates in *apoB*$^{48/48}$C-III, *apoB*$^{48/48}$, *apoB*$^{100/100}$C-III, and *apoB*$^{100/100}$ mice. Mice were injected with Triton WR-1339 and [^{35}S]Promix. Blood samples were collected at different time points, and TG levels in plasma were determined. A: Plasma TG levels over time in *apoB*$^{48/48}$C-III and *apoB*$^{48/48}$ mice. Closed squares represent data from *apoB*$^{48/48}$C-III mice. Open squares represent data from *apoB*$^{48/48}$ mice. B: Plasma TG levels over time in *apoB*$^{100/100}$C-III and *apoB*$^{100/100}$ mice. Closed circles represent data from *apoB*$^{100/100}$C-III mice. Open circles represent data from *apoB*$^{100/100}$ mice. The error bars represent the percentage of injected dose of tracer from all mice studied (as means ± standard deviation) and is shown for all sampled time points.

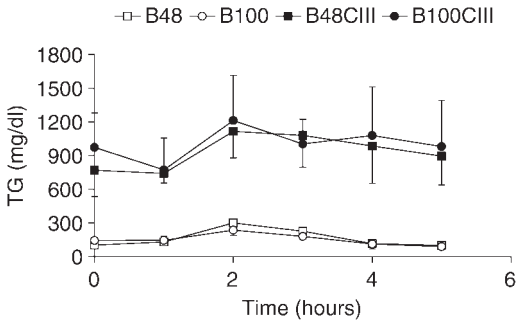

Fig. 5. Fat tolerance test in *apoB*$^{48/48}$, *apoB*$^{100/100}$, *apoB*$^{48/48}$C-III, and *apoB*$^{100/100}$C-III mice. Mice were gavaged with 400 μl of peanut oil. Blood samples were collected at the labeled time points, and TG levels in plasma were determined. Open squares represent data from *apoB*$^{48/48}$ mice. Open circles represent data from *apoB*$^{100/100}$ mice. Closed squares represent data from *apoB*$^{48/48}$C-III mice. Closed circles represent data from *apoB*$^{100/100}$C-III mice. The error bars represent the percentage of injected dose of tracer from all mice studied (as means ± standard deviation) and is shown for all sampled time points.

DISCUSSION

In the current investigation, we have applied the method of metabolic characterization of genetically modified mice to the study of the effects of apoB-48 and apoB-100 on plasma cholesterol and TG metabolism. ApoC-III, which impairs both lipolysis and remnant lipoprotein clearance (28), was introduced as a variable via a human apoC-III transgene to highlight the relevant differences between apoB-48 and apoB-100. The apoC-III transgenic mice were extensively backcrossed to the C57BL/6 inbred strain. However, the *apoB*$^{48/48}$ and *apoB*$^{100/100}$ gene-targeted mice were in a mixed, but simple and comparable, genetic background (C57BL/6 129S F2 mongrel for both). Although we acknowledge the limitation imposed by this, we do not think it likely that this fact would explain the large, consistent, and biologically plausible differences between our groups. Cholesterol levels were higher in *apoB*$^{48/48}$ mice and increased to a greater extent in these mice with introduction of the apoC-III transgene than was observed in the *apoB*$^{100/100}$ context. FPLC and ultracentrifugation revealed that the increased cholesterol in *apoB*$^{48/48}$C-III mice was

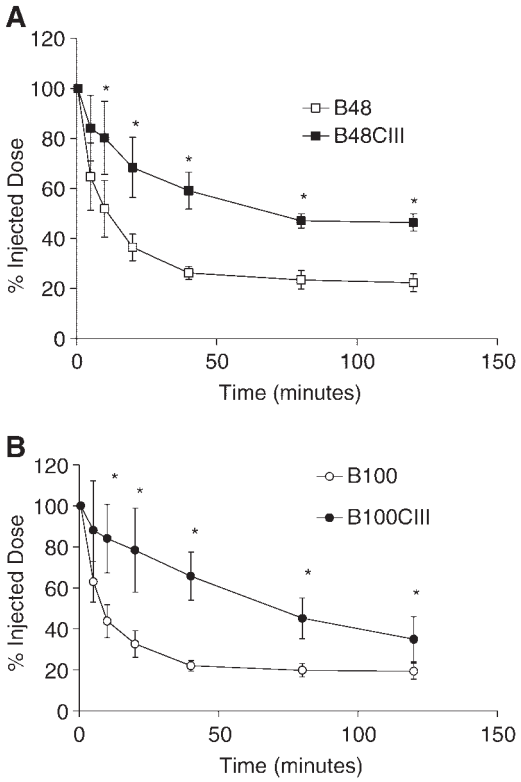

Fig. 6. Clearance of VLDL-TG in *apoB*$^{48/48}$C-III, *apoB*$^{48/48}$, *apoB*$^{100/100}$ C-III, and *apoB*$^{100/100}$ mice. VLDL labeled on its TG moiety was obtained 45 min after injecting either *apoB*$^{48/48}$ or *apoB*$^{100/100}$ mice with [^{3}H]oleate. The isolated labeled VLDL was then used as a tracer. *ApoB*$^{48/48}$ VLDL was injected into *apoB*$^{48/48}$C-III and *apoB*$^{48/48}$ mice. *ApoB*$^{100/100}$ VLDL was injected into *apoB*$^{100/100}$C-III and *apoB*$^{100/100}$ mice. A: VLDL-TG clearance in *apoB*$^{48/48}$C-III and *apoB*$^{48/48}$ mice. Closed squares represent data from *apoB*$^{48/48}$C-III mice. Open squares represent data from *apoB*$^{48/48}$ mice. B: VLDL-TG clearance in *apoB*$^{100/100}$ C-III and *apoB*$^{100/100}$ mice. Closed circles represent data from *apoB*$^{100/100}$ C-III mice. Open circles represent data from *apoB*$^{100/100}$ mice. The error bars represent the percentage of post-injection level of tracer from all mice studied (as means ± standard deviation) and is shown for all sampled time points.

found both in VLDL and in IDL+LDL. The modestly increased cholesterol in *apoB*$^{100/100}$C-III mice (vs. *apoB*$^{100/100}$) was found only in VLDL. In a likely mechanism for the increased cholesterol in *apoB*$^{48/48}$C-III mice, pooled data from kinetic studies indicated a marked effect of the apoC-III transgene to delay the uptake of cholesterol-enriched apoB-48 β-VLDL, whereas there was very little effect of the transgene in *apoB*$^{100/100}$ mice injected with apoB-100 β-VLDL. This difference was not evident in the FCR values calculated from the individual animals. FCR values are determined primarily by tracer disappearance rates during the rapid early phase of lipoprotein clearance, even when comparatively little tracer is cleared during this period. However, the apoB fractional clearance values from individual animals were derived from the same curves and were quite different in the *apoB*$^{48/48}$C-III mice vs. *apoB*$^{48/48}$ mice, whereas they were similar and statistically indistinguishable in *apoB*$^{100/100}$ C-III mice vs. *apoB*$^{100/100}$ mice. These observations, and the plasma lipoprotein data, may be more reflective of the biologically relevant differences between our groups.

In contrast, TG levels were about the same in *apoB*$^{48/48}$ and *apoB*$^{100/100}$ mice and increased more in the *apoB*$^{100/100}$ context with the introduction of the apoC-III transgene. In both *apoB*$^{48/48}$C-III and *apoB*$^{100/100}$C-III mice, the increased TG was only present in VLDL. The differential effect on TG levels also appeared to be produced principally at the level of clearance: the apoC-III transgene produced a markedly greater effect to decrease the FCR of VLDL TG in the *apoB*$^{100/100}$ context than in the *apoB*$^{48/48}$ context. This difference was also evident in the pooled data. The presence of the transgene produced no difference in VLDL TG production in either the *apoB*$^{48/48}$ or the *apoB*$^{100/100}$ context. The transgene also produced obvious worsening in tolerance (relative increase in TG levels) of an oral fat load, but there was no difference between *apoB*$^{48/48}$C-III and *apoB*$^{100/100}$C-III mice in that regard. We speculate that the oral fat tolerance test was not able to discriminate a difference in TG clearance between the *apoB*$^{48/48}$C-III and *apoB*$^{100/100}$C-III models because of the importance of the extent of increased TG from hepatic VLDL in determining plasma TG levels in the postprandial setting (29). Véniant et al. (23) observed that, compared with *apoB*$^{+/+}$ apoE0 mice, the body weights of *apoB*$^{100/100}$apoE0 mice were slightly lighter and the *apoB*$^{48/48}$apoE0 mice were heavier. Although such a difference would not explain higher TG levels in the *apoB*$^{100/100}$ context, it is worth noting that there were no differences in body weight between any of the four genotypes that we studied (data not shown).

The adaptive value and conservation of apoB-48 would thus be explained by the metabolic efficiency produced by its facilitation of peripheral lipolysis with the prevention of a need for the subsequent reexport of dietary TG by the liver. The rapid clearance of postprandial lipid appears to be explainable solely by that efficient lipolysis, without any positive contribution of apoB-48 lipoprotein structure to the efficiency of apoE-mediated lipoprotein particulate ("remnant") uptake. In contrast, the adaptive value and conservation of apoB-100 would be explained by its more efficient particulate uptake, a consequence of the relatively greater importance, in the case of apoB-100, of the adjunctive role of mediator of core lipid delivery via receptor endocytosis.

Studies showing increased action of lipoprotein lipase on apoB-48-containing vs. apoB-100-containing TG-rich lipoproteins have been reported, consistent with our observations (30, 31). In addition, significantly higher TG levels in *apoB*$^{100/100}$ vs. *apoB*$^{48/48}$ mice were also described in the original description of these mice, and a trend toward higher cholesterol levels in *apoB*$^{48/48}$ mice also appears to be present (22). Glucose levels were not reported. An unanticipated result of our study was the significantly higher glucose levels in the *apoB*$^{48/48}$ mice than in the *apoB*$^{100/100}$ mice. This difference was seen in both males and females despite the more robust lipid phenotype in the male mice, as has been commonly observed (32, 33). Further work is planned to address the basis of this observation. However, this difference may be secondary to the differences in lipid metabo-

lism that we observed. ApoB-48 TG-rich lipoproteins appear more resistant to the TG-increasing effects of apoC-III but more sensitive to the remnant clearance inhibition. In combination, this may permit apoB-48 to facilitate more complete peripheral lipolysis and deliver a greater fraction of the TG in the apoB-48 TG-rich lipoproteins to muscle. In our model, this may have led to greater metabolic competition and lesser glucose uptake by this tissue as a consequence (34, 35). In contrast, apoB-100-containing TG-rich lipoproteins would deliver a greater fraction of their TG load to the liver via remnant clearance, in part as a result of the direct interaction of lipoprotein receptors with the apoB-100 receptor binding domain. In humans, this would lead to no net delivery of TG to the liver, because essentially all VLDL originate there, but would lead to significant net delivery of cholesterol, accumulated in VLDL via the action of CE transfer protein (36). Expression of this specific apoC-III transgene has previously been shown to have no effect on plasma glucose in otherwise normal animals, as we also have observed (37).

Any mechanism that would impair remnant lipoprotein uptake and increase the partitioning of TG from TG-rich lipoproteins to skeletal muscle might have similar consequences. Indeed, overexpression of apoC-I, an inhibitor of remnant lipoprotein clearance, has been shown to produce hyperglycemia (38), as we recently confirmed in a modestly overexpressing liver-specific transgenic mouse model that did not exhibit the lipoatrophy that had been observed in the prior report (39). Diabetes has been observed to be associated with impaired fat tolerance, manifested in part by increased postprandial apoB-48 remnant lipoproteins (40, 41). It may be that this observation, in part, is reflective of an independent mechanism that is contributory to diabetes and is not solely its consequence.

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