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Phenotypic analysis of mice expressing exclusively apolipoprotein B48 or apolipoprotein B100

(cholesterol/triacylglycerols/lipoproteins/gene targeting/homologous recombination)

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ABSTRACT **Apolipoprotein (apo)-B is found in two forms in mammals: apo-B100, which is made in the liver and the yolk sac, and apo-B48, a truncated protein made in the intestine. To provide models for understanding the physiologic purpose for the two forms of apo-B, we used targeted mutagenesis of the apo-B gene to generate mice that synthesize exclusively apo-B48 (apo-B48-only mice) and mice that synthesize exclusively apo-B100 (apo-B100-only mice). Both the apo-B48-only mice and apo-B100-only mice developed normally, were healthy, and were fertile. Thus, apo-B48 synthesis was sufficient for normal embryonic development, and the synthesis of apo-B100 in the intestines of adult mice caused no readily apparent adverse effects on intestinal function or nutrition. Compared with wild-type mice fed a chow diet, the levels of low density lipoprotein (LDL)-cholesterol and very low density lipoprotein- and LDL-triacylglycerols were lower in apo-B48-only mice and higher in the apo-B100-only mice. In the setting of apo-E-deficiency, the apo-B100-only mutation lowered cholesterol levels, consistent with the fact that apo-B100-lipoproteins can be cleared from the plasma via the LDL receptor, whereas apo-B48-lipoproteins lacking apo-E cannot. The apo-B48-only and apo-B100-only mice should prove to be valuable models for experiments designed to understand the purpose for the two forms of apo-B in mammalian metabolism.**

The B apolipoproteins (apo), apo-B48 and apo-B100, are structural proteins required for the assembly of triacylglycerol-rich lipoproteins and are constituents of all classes of lipoproteins that are considered atherogenic (1). Both apo-B48 and apo-B100 are products of a single gene (2). Apo-B100 is synthesized by the liver, where it is necessary for the formation of very low density lipoproteins (VLDL), and by the yolk sac during development, where it appears to play a role in the nutrition of the embryo (3, 4). Apo-B48, which contains the amino-terminal 2152 amino acids of apo-B100, is synthesized as a result of an apo-B mRNA-editing catalytic enzyme peptide-1 (apobec-1) process that converts apo-B codon 2153 (CAA, glutamine) to a stop codon (UAA) (5). Apo-B mRNA editing is extensive in the intestines of all mammals tested (6), and thus, apo-B48 is the principal structural protein of intestinally-derived chylomicrons. Although apo-B mRNA editing does not occur in the human liver, the mRNA editing activity, and thus apo-B48 formation, does occur in the livers of some species, including mice and rats (6).

Although progress has been made in understanding distinct metabolic differences between apo-B48- and apo-B100-containing lipoproteins (7), the physiologic purpose for the two different forms of apo-B in mammals has remained obscure. The fact that apo-B48 synthesis in the intestine has been preserved throughout mammalian evolution suggests that apo-B48 may be optimal for chylomicron formation and intestinal fat absorption; whether apo-B100 would suffice for this purpose has been hitherto unclear. Similarly, why apo-B100 synthesis has been evolutionarily conserved in the liver is unclear. It is not clear why apo-B48 alone would not be suitable for hepatic lipoprotein metabolism. Additionally, it is unclear whether apo-B48 production in the yolk sac would support normal mouse embryonic development, given the studies of Homanics *et al.* (8) that showed neurologic developmental abnormalities in mice producing a truncated apo-B, apo-B70.

To understand why two forms of apo-B exist in mammals, and to determine whether either apo-B100 or apo-B48 is critical for survival of embryos or adult mice, we used gene-targeting techniques to generate mice that synthesize exclusively either apo-B100 (apo-B100-only mice, or *apo-B*$^{100/100}$ mice) or apo-B48 (apo-B48-only mice, or *apo-B*$^{48/48}$ mice). In this study, we describe the initial phenotypic characterization of these mice, both in the presence and absence of apo-E.

MATERIALS AND METHODS

Apo-B Gene Targeting. To produce *apo-B*$^{48/48}$ and *apo-B*$^{100/100}$ mice, we used "hit and run" (9) gene targeting in mouse embryonic stem (ES) cells to introduce stop and nonstop mutations, respectively, into the apo-B48 editing codon (codon 2153) in the mouse apo-B gene, as described in Fig. 1*A* below. The genetic background of the mice described here was ≈50% C57BL/6 and ≈50% 129/Sv. The *apo-B*$^{48/48}$ and *apo-B*$^{100/100}$ mice were bred with apo-E deficient mice (*apo-E*$^{-/-}$) (10) to obtain mice with the following three genotypes: *apo-B*$^{+/+}$*apo-E*$^{-/-}$, *apo-B*$^{48/48}$*apo-E*$^{-/-}$, and *apo-B*$^{100/100}$*apo-E*$^{-/-}$.

Analysis of RNA. Total cellular RNA was prepared from liver and duodenum. RNase protection assays of apo-B mRNA levels were performed using a 245-bp riboprobe from the 5′ portion of

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Abbreviations: FPLC, fast phase liquid chromatography; apobec-1, apolipoprotein B mRNA-editing catalytic enzyme peptide-1; LDL, low density lipoprotein; VLDL, very low density lipoprotein; apo, apolipoprotein; ES, embryonic stem.

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exon 26 of the apo-B gene, and a 316-bp probe for mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Analysis of the Plasma Lipoproteins. Lipid and lipoprotein levels were measured on fresh plasma from females of wild-type, *apo-B*$^{48/48}$, and *apo-B*$^{100/100}$ mice (n = 12 in each group), both on a chow diet and on a synthetic high-fat diet (3). All animals were 12–15 weeks old and were on a diet for 8 weeks before blood samples were obtained. Plasma lipid levels were also measured in chow-fed, female *apo-B*$^{+/+}$*apo-E*$^{-/-}$, *apo-B*$^{48/48}$*apo-E*$^{-/-}$, and *apo-B*$^{100/100}$*apo-E*$^{-/-}$ mice (n = 21 in each group). Plasma α-tocopherol levels were measured as described (11). Comparisons between groups were evaluated using a one-way analysis of variance (ANOVA). To determine the size and abundance of the apo-B species, plasma samples (or lipoprotein samples isolated by ultracentrifugation) were analyzed on SDS/polyacrylamide gels. Gels were either stained with silver or used for immunoblotting, using a ^{125}I-labeled rabbit antibody to mouse apo-B (12) or a rabbit antiserum specific for rat apo-E and apo-AI (provided by K. Weisgraber, Gladstone Laboratories). Immunoblots were

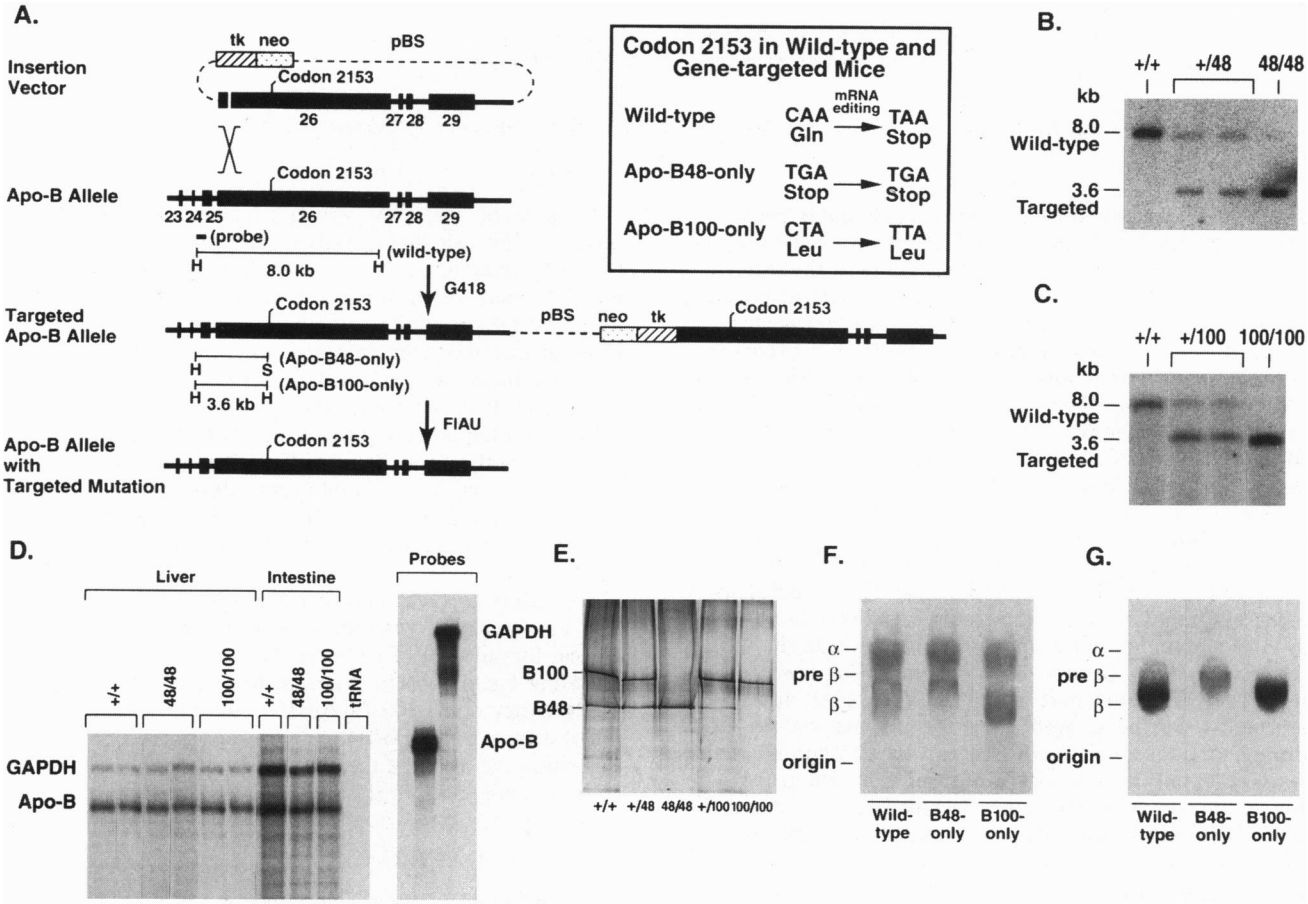

FIG. 1. (*A*) Gene-targeting strategy for the generation of *apo-B*$^{48/48}$ and *apo-B*$^{100/100}$ mice. Vectors were generated from mouse apo-B plasmid clones 15A5 and 15A6SE (strain B10.A). Clone 15A5 starts 55 bp past the beginning of exon 26 and extends to the *Sac*I site 6540 bp into exon 26. Clone 15A6SE starts at the same *Sac*I site and extends to an *Eco*RI site located ≈1.2 kb downstream from the apo-B gene's stop translation codon. For the apo-B48-only vector, a TGA stop codon was introduced into the apo-B48-editing codon (codon 2153) of 15A5 by site-directed mutagenesis, using the oligonucleotide 5′-ATCTTTAATATACTAGTCAAACTATATCGCGTATGTCTCAAGTTGAGAGAG-3′, which also introduces an *Spe*I site downstream from the stop codon. The mutant 15A5 clone was ligated into the *Sac*I site of 15A6SE to create a single contiguous clone containing 10.5 kb of the mouse apo-B gene. Next, a 3.7-kb *Xho*I–*Sal*I fragment from pLF1 [including both a neomycin (*neo*) (from pKT1Neo3PA2, containing the RNA polymerase II promoter) and a thymidine kinase gene (from pIC19R/MC1-TK)] was cloned into a *Sal*I site in the vector. To construct the apo-B100-only targeting vector, a CTA ("nonstop") mutation (13) was introduced into codon 2153, along with a novel *Hin*dIII site, using the oligonucleotide 5′-AATATACTGATCAAATAGTATCGCGTATGTCTCAAGTTGAGAAAGCTTTTCA-TTG-3′. After ligating the *Sac*I fragment from the mutant 15A5 into 15A6SE, the vector was completed by ligating a *neo* (an *Sal*I–*Xho*I fragment from pKT1Neo3PA2) into the polylinker *Sal*I site. Targeting vectors were linearized at a unique *Sau*I site within the apo-B gene (located 609 bp into exon 26 and 1567 bp 5′ of the apo-B48-editing codon) and were electroporated into AB1 (14) or JL1.1 mouse ES cells. Genomic DNA from G418^r ES cell clones was digested with *Spe*I and *Hin*dIII (for the apo-B48-only vector) or *Hin*dIII (for the apo-B100-only vector) and analyzed by Southern blots using an ≈700-bp *Hin*dIII–*Xba*I fragment spanning from intron 24 to exon 25. Targeted apo-B48-only clones or apo-B100-only clones were identified by a novel 3.6-kb *Hin*dIII–*Spe*I fragment or a novel 3.6-kb *Hin*dIII fragment, respectively. The targeting frequency for each vector was ≈1 in 50. One apo-B48-only clone, 17–8, was subjected to counterselection with 0.2 μM of 1-[2-fluoro-β-D-arabinofuranosyl]-5-iodouracil (the "run" step). This step yielded a clone, 17–8F, which, by intrachromosomal recombination, had retained the targeted mutation, yet had deleted the irrelevant sequences 3′ to the gene (the *neo,* the thymidine kinase, the plasmid, and the duplicated exon 26–exon 29 sequences). Chimeras from 17–8F transmitted the mutation to their offspring. For the apo-B100-only experiments, germ-line transmitting chimeras were generated following the "hit" step. H, *Hin*dIII; S, *Spe*I. (*B*) Southern blot identification of heterozygous and homozygous apo-B48-only mice. (*C*) Southern blot identification of heterozygous and homozygous apo-B100-only mice. (*D*) RNase protection assay of liver and intestinal RNA from *apo-B*$^{+/+}$, *apo-B*$^{48/48}$, and *apo-B*$^{100/100}$ mice, using both GAPDH and apo-B probes. The ratio of radioactivity in the apo-B band, relative to the radioactivity in the GAPDH band, was identical in all groups of mice (3.1 for the liver RNA and 1.0 for the intestinal RNA). (*E*) SDS/polyacrylamide gel of the d < 1.21 g/ml lipoprotein fractions from a wild-type mouse, and heterozygous and homozygous apo-B48-only and apo-B100-only mice. (*F*) Lipid-stained agarose gel of the plasma of wild-type and homozygous apo-B48-only and apo-B100-only mice. (*G*) Western blot of the agarose gel using an antiserum to mouse apo-B.

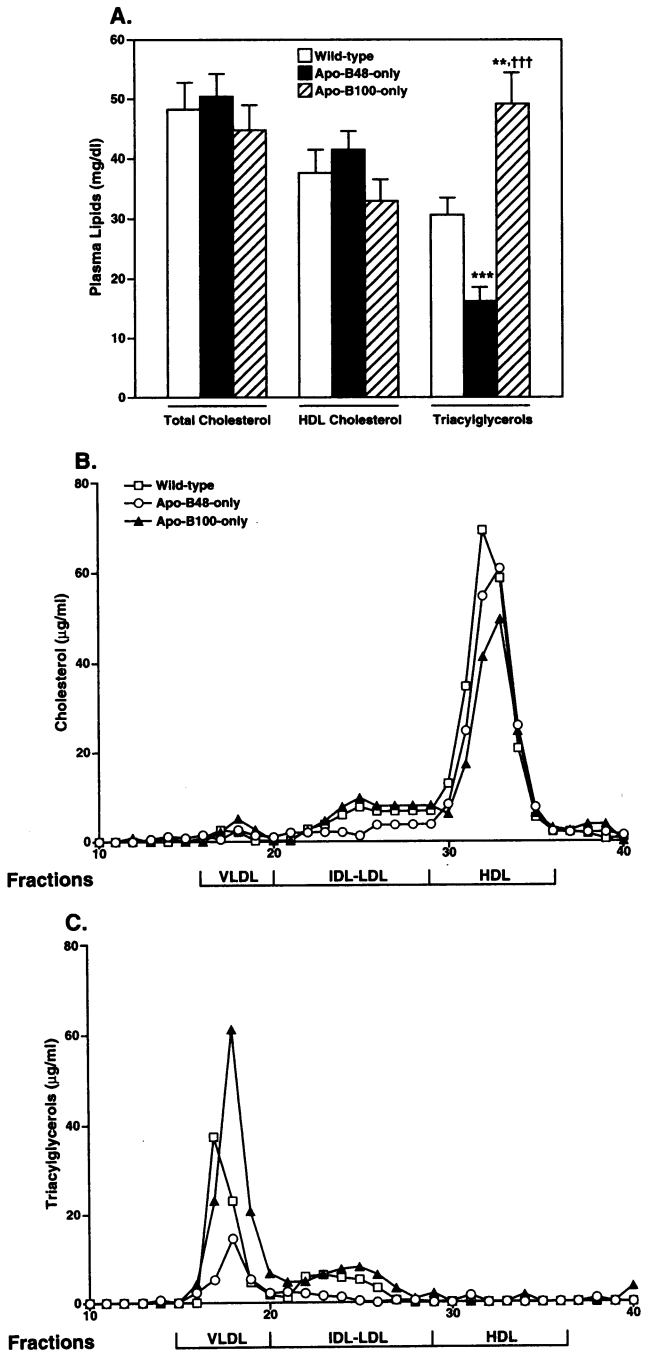

FIG. 2. (*A*) Comparison of the lipids and lipoproteins in wild-type, apo-B48-only, and apo-B100-only mice on a chow diet. Bars represent means, and lines are standard errors of the mean. (**, $P < 0.01$ versus wild-type mice; ***, $P < 0.001$ versus wild-type mice; †††, $P < 0.001$ versus apo-B48-only mice) (*B*) FPLC distribution of cholesterol within the plasma lipoproteins. (*C*) FPLC distribution of triacylglycerols within the plasma lipoproteins.

quantified with a phosphorimager (Fuji Medical Systems). Plasma lipoprotein electrophoresis was performed on 1% agarose gels (12); the gels were either stained for neutral lipids with Fat Red 7B (Sigma) or used for Western blots with the antibody to mouse apo-B. Fractionation of plasma by fast phase liquid chromatography (FPLC) was performed as described (12).

Pathology. Samples of liver and intestine from 2-month-old female mice were fixed and embedded in paraffin. Sections (10 µm thick) were stained with hematoxylin and eosin and examined by light microscopy.

RESULTS

To generate *apo-B*$^{48/48}$ and *apo-B*$^{100/100}$ mice, we used the gene-targeting strategy illustrated in Fig. 1*A*. Targeted ES cell clones were used to generate chimeric mice, which transmitted the mutations to their offspring (Fig. 1 *B* and *C*). For both mutations, intercrosses of heterozygotes yielded the expected 25% homozygotes. Both the *apo-B*$^{48/48}$ and *apo-B*$^{100/100}$ mice developed normally, were fertile and healthy, and grew normally, both on a chow diet and a high-fat diet. RNase protection assays demonstrated that the apo-B mRNA levels in the *apo-B*$^{48/48}$ and the *apo-B*$^{100/100}$ mice were identical to those observed in wild-type mice (Fig. 1*D*). The lipoproteins of the *apo-B*$^{48/48}$ mice contained apo-B48, but no apo-B100, whereas the lipoproteins of the *apo-B*$^{100/100}$ mice contained apo-B100, but no apo-B48 (Fig. 1*E*). As might have been predicted from studies of humans with truncated apo-B proteins (15), the apo-B-containing lipoproteins in the *apo-B*$^{48/48}$ mice displayed nearly exclusively pre-β-mobility on agarose gel electrophoresis, whereas the apo-B-containing lipoproteins in the plasma of the *apo-B*$^{100/100}$ mice showed nearly exclusively β-mobility. The plasma of the wild-type mice contained both pre-β- and β-migrating lipoproteins (Fig. 1 *F* and *G*). As judged by immunoblotting, the concentration of apo-B48 in the plasma of four chow-fed *apo-B*$^{48/48}$ mice was 40.0 ± 8.7% greater than the concentration of apo-B48 in four *apo-B*$^{+/+}$ mice, whereas the concentration of apo-B100 in the plasma of four *apo-B*$^{100/100}$ mice was 35.5 ± 12.3% greater than the concentration of apo-B100 in four *apo-B*$^{+/+}$ mice. Compared with the wild-type animals, neither the *apo-B*$^{48/48}$ nor the *apo-B*$^{100/100}$ mice had any alterations in the plasma levels of apo-E or apo-AI (data not shown).

The plasma levels of α-tocopherol (nmol/ml; $n = 4$ in each group) were not significantly different in the *apo-B*$^{100/100}$ (4.4 ± 0.3), *apo-B*$^{+/+}$ (3.9 ± 0.6), and *apo-B*$^{48/48}$ mice (2.8 ± 0.7). Histologic examination of the intestines of the mice revealed no abnormalities, either on the chow or the high-fat diet (data not shown). On the chow diet, the livers of all of the mice appeared normal, but on the high-fat diet, the hepatocytes of all of the mice contained large amounts of cytosolic fat.

On a chow diet, no significant differences were observed in total or high density lipoprotein cholesterol levels for any of the groups (Fig. 2*A*). The *apo-B*$^{100/100}$ mice had 59% higher plasma levels of triacylglycerols compared with the *apo-B*$^{+/+}$ mice ($P = 0.007$), whereas the *apo-B*$^{48/48}$ mice had 48% lower triacylglycerol levels compared with the *apo-B*$^{+/+}$ mice ($P < 0.001$) (Fig. 2*A*).

The distribution of lipids within the lipoproteins of *apo-B*$^{48/48}$ and *apo-B*$^{100/100}$ mice on a chow diet, as assessed by FPLC fractionation of plasma, revealed distinct differences that were unequivocal and highly reproducible. In four FPLC experiments (performed on samples from different groups of six female animals over an 18-month span), the *apo-B*$^{48/48}$ animals invariably had lower levels of cholesterol in the low density lipoprotein (LDL) fractions. The apo-B100-only animals had higher levels of cholesterol in these fractions (Fig. 2*B*). The apo-B100-only mutation increased levels of triacylglycerol in both the VLDL and LDL fractions, whereas the apo-B48-only mutation reduced the level of triacylglycerols in the VLDL fraction (Fig. 2*C*). Immunoblotting of FPLC fractions from the *apo-B*$^{48/48}$ and *apo-B*$^{100/100}$ mice revealed that >90% of both apo-B species were found in LDL-sized lipoproteins, although the apo-B48-containing lipoproteins were distinctly smaller (fractions 27–28) than the apo-B100-only lipoproteins (fractions 25–26) (data not shown).

Analysis by FPLC of cholesterol distribution in the plasma of the *apo-B*$^{48/48}$, *apo-B*$^{100/100}$, and *apo-B*$^{+/+}$ mice revealed that all three groups developed striking increases in the levels of VLDL cholesterol when fed a high-fat diet; however, the VLDL cholesterol levels were lowest in the *apo-B*$^{100/100}$ mice

(Fig. 3). This finding was supported by the lower total cholesterol levels in the apo-B100-only mice compared with the *apo-B*$^{+/+}$ mice (142 ± 10 versus 200 ± 22 mg/dl, $P < 0.02$). The lower levels of cholesterol in the *apo-B*$^{100/100}$ mice may relate, at least in part, to the fact that the plasma levels of apo-B48 and apo-B100 responded differently when the mice were placed on a high-fat diet. When the wild-type mice were fed a high-fat diet, the intensity of the apo-B100 band was reduced by 60% (± 12%) and the intensity of the apo-B48 band was increased by 128% (± 18%), compared with the intensities of these bands in wild-type mice fed a chow diet. Similarly, the intensity of the apo-B100 band in the *apo-B*$^{100/100}$ mice on the high-fat diet was reduced by 36% (± 5%) and the intensity of the apo-B48 band in the *apo-B*$^{48/48}$ mice on the high-fat diet was increased by 113% (± 24%), compared with the intensities of the bands from chow-fed *apo-B*$^{100/100}$ or *apo-B*$^{48/48}$ mice, respectively.

We hypothesized that the differences in LDL-cholesterol levels between the *apo-B*$^{100/100}$ and *apo-B*$^{48/48}$ mice might be dependent on apo-E expression. To test this, we crossed the two targeted apo-B mutations into the background of apo-E deficiency (10). The *apo-B*$^{100/100}$*apo-E*$^{-/-}$ mice had significantly *lower* cholesterol levels compared with the *apo-B*$^{+/+}$*apo-E*$^{-/-}$ mice or the *apo-B*$^{48/48}$*apo-E*$^{-/-}$ mice (Fig. 4*A*). The lower cholesterol levels in the *apo-B*$^{100/100}$*apo-E*$^{-/-}$ mice were largely due to lower cholesterol levels in the intermediate density lipoprotein–LDL fractions (Fig. 4*B*). Despite lower cholesterol levels, the *apo-B*$^{100/100}$*apo-E*$^{-/-}$ mice had significantly *higher* triacylglycerol levels than the *apo-B*$^{+/+}$*apo-E*$^{-/-}$ or the *apo-B*$^{48/48}$*apo-E*$^{-/-}$ mice (Fig. 4*A*). The increased amounts of triacylglycerols in the *apo-B*$^{100/100}$*apo-E*$^{-/-}$ mice was due to increased amounts of triacylglycerols in the VLDL and LDL fractions (data not shown). Compared with *apo-B*$^{+/+}$ mice, *apo-B*$^{+/+}$*apo-E*$^{-/-}$ mice exhibited an 81% decrease in plasma apo-B100 levels and a 582% increase in plasma apo-B48 levels. Compared with *apo-B*$^{48/48}$ mice, *apo-B*$^{48/48}$*apo-E*$^{-/-}$ mice exhibited a 848% increase in plasma apo-B48 levels. Compared with *apo-B*$^{100/100}$ mice, *apo-B*$^{100/100}$*apo-E*$^{-/-}$ mice exhibited a 62% increase in plasma apo-B100 levels.

DISCUSSION

Although gene targeting in ES cells has typically been used to produce mice with null alleles, it can also be used to introduce subtle mutations into mice (9, 16), making it possible to perform "site-directed mutagenesis" in a whole animal. A few examples of the successful use of this approach have been reported (17, 18). In this study we used such an approach to introduce point mutations into the apo-B48-editing codon of the mouse apo-B gene to generate mice that synthesize exclusively one of the two forms of apo-B found in mammals, apo-B48 and apo-B100. To generate the apo-B48-only mice, the apo-B48-editing codon was replaced with a stop codon; to generate the apo-B100-only mice, the same codon was replaced with a CTA codon, which prevents the formation of a stop codon by mRNA editing.

Several important findings are evident by the generation of these mice. First, although all mammals studied to date produce apo-B48 in their intestine (6), it appears that apo-B48 production is not essential for intestinal function. The *apo-B*$^{100/100}$ mice were healthy and showed no histologic evidence of significant fat accumulation in the intestine, even on a high-fat diet. The plasma levels of α-tocopherol, a fat-soluble vitamin, were similar in the *apo-B*$^{48/48}$, *apo-B*$^{+/+}$, and *apo-B*$^{100/100}$ mice. In addition, retinol absorption tests performed to date have not uncovered a fat absorption defect in the *apo-B*$^{100/100}$ mice (C.M.C. and S.G.Y., unpublished data). Although systematic ultrastructural studies on the intestines of these animals have not been completed, large, chylomicron-sized particles can be visualized in the villus enterocytes of both the *apo-B*$^{100/100}$ and *apo-B*$^{48/48}$ mice (R. Hamilton and J. S. Wong, personal communication).

A second conclusion from our studies is that apo-B48 synthesis by the yolk sac is sufficient for supporting normal mouse embryonic development. The vitality of the *apo-B*$^{48/48}$ mice stands in contrast to the embryonic lethality and developmental abnormalities observed in the homozygous apo-B70 mice (8). We suspect that the different developmental phenotypes in the two models probably relate to differences in apo-B mRNA levels. In the apo-B70 mice (8), the gene-targeting vector interrupted exon 26 of the gene, and the apo-B transcripts from the targeted allele were structurally abnormal (terminating within plasmid sequences downstream from exon 26) and reduced in quantity, both in liver and intestine. It is likely that apo-B mRNA levels were also reduced in the yolk sac, leading to the developmental defects (3, 4). In contrast, the vectors used in our experiments did not interrupt the apo-B gene, and the apo-B mRNA levels were normal in both the liver and the intestine. These data indicate that a truncated

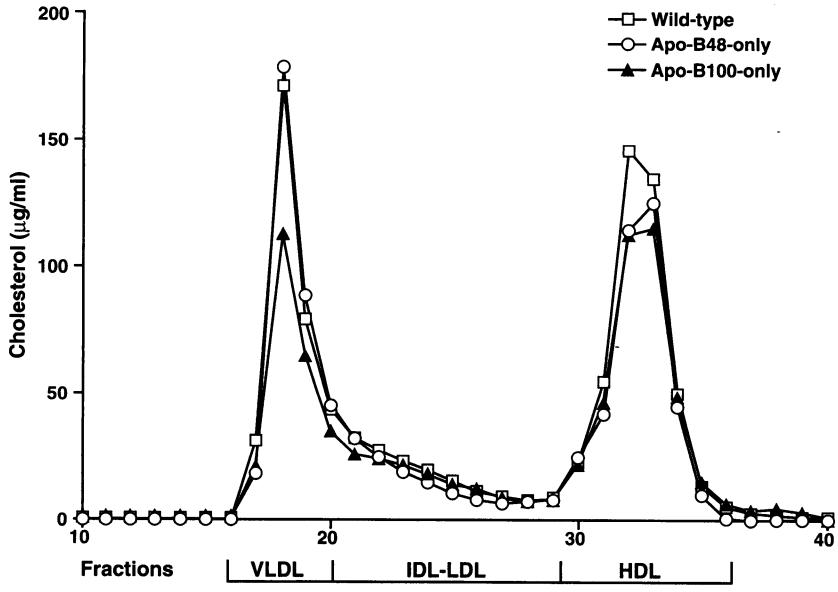

FIG. 3. FPLC distribution of cholesterol within the plasma lipoproteins on a high-fat diet.

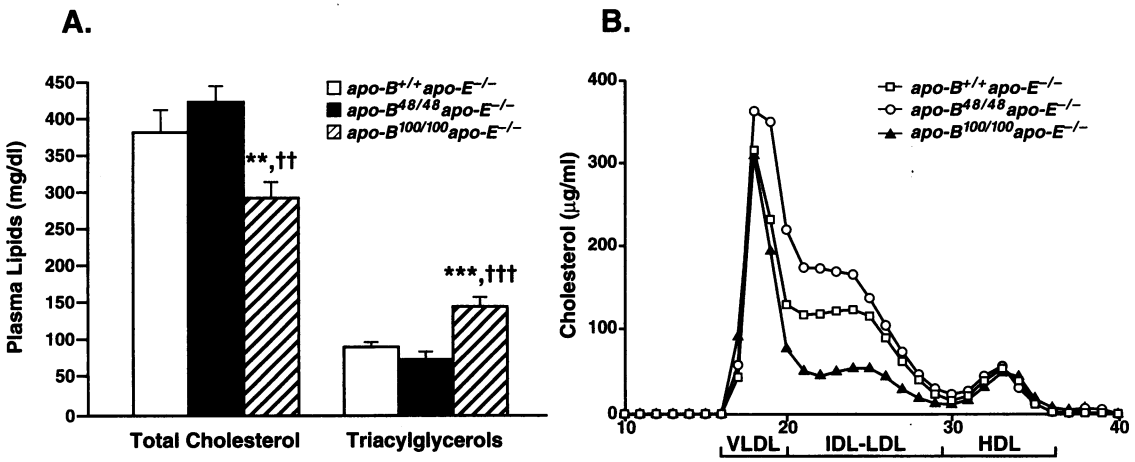

FIG. 4. (*A*) Plasma cholesterol and triacylglycerol levels in the setting of apo-E deficiency. Bars represent means, and lines are standard errors of the mean. (**, $P < 0.01$ versus *apo-B*$^{+/+}$*apo-E*$^{-/-}$ mice; ***, $P < 0.001$ versus *apo-B*$^{+/+}$*apo-E*$^{-/-}$ mice; ††, $P < 0.01$ versus *apo-B*$^{48/48}$*apo-E*$^{-/-}$ mice; †††, $P < 0.001$ versus *apo-B*$^{48/48}$*apo-E*$^{-/-}$ mice). (*B*) Distribution of cholesterol within the plasma lipoproteins in the setting of apo-E deficiency.

apo-B as short as apo-B48 suffices for embryonic development, provided that the level of apo-B gene expression is normal.

The FPLC characterization of the lipids and lipoproteins in chow-fed *apo-B*$^{48/48}$ and *apo-B*$^{100/100}$ mice revealed distinct differences that were highly reproducible. The *apo-B*$^{48/48}$ mice had lower levels of LDL cholesterol and smaller-sized LDL particles than did *apo-B*$^{+/+}$ or *apo-B*$^{100/100}$ mice. The low plasma levels of LDL cholesterol and smaller-sized LDL particles in the *apo-B*$^{48/48}$ mice are similar to what is observed in humans with apo-B gene mutations causing the synthesis of a truncated apo-B (15). The smaller-sized LDL is also consistent with studies of the lipoproteins of mice in which the amount of apo-B48 expression was increased by adenovirus-mediated overexpression of apobec-1 (19). The lower LDL cholesterol levels in the *apo-B*$^{48/48}$ mice may relate to the fact that apo-B48-containing lipoproteins accommodate more apo-E, a high-affinity ligand for the LDL receptor. Along these lines, Milne *et al.* (20) isolated the apo-B48-VLDL and the apo-B100-VLDL from the plasma of type III hyperlipoproteinemia patients, and found that the apo-B48-VLDL contained more apo-E.

The lower levels of LDL cholesterol in *apo-B*$^{48/48}$ mice and higher levels of LDL cholesterol in chow fed *apo-B*$^{100/100}$ mice occurred only in the chow-fed mice expressing apo-E. In the setting of apo-E deficiency, the opposite relationship was observed. The total and LDL cholesterol levels were significantly *lower* in the *apo-B*$^{100/100}$*apo-E*$^{-/-}$ mice than in the *apo-B*$^{48/48}$*apo-E*$^{-/-}$ or *apo-B*$^{+/+}$*apo-E*$^{-/-}$ mice. The lower cholesterol levels in the *apo-B*$^{100/100}$*apo-E*$^{-/-}$ mice are probably due to the fact that apo-B100 (unlike apo-B48) can bind to the LDL receptor and mediate the removal of lipoproteins from the plasma. In support of this finding, apo-E deficiency resulted in an enormous increase in apo-B48 levels in the apo-B48-only mice, but resulted in only a very small increase in apo-B100 levels in the apo-B100-only mice.

When fed a high-fat diet the *apo-B*$^{100/100}$ mice had less VLDL cholesterol than the other animals, a finding that was supported by the lower total plasma cholesterol levels in the *apo-B*$^{100/100}$ mice. This finding might be explained, at least in part, by the observation that placing mice on the high-fat diet resulted in decreased apo-B100 levels in the *apo-B*$^{100/100}$ mice, but resulted in increased apo-B48 levels in the *apo-B*$^{48/48}$ mice. The lower apo-B100 levels on the high-fat diet may relate to the fact that apo-B100 can function as a ligand for the LDL receptor. The apo-B100 role as a ligand may be important in this metabolic setting, where the amount of apo-E on the surface of VLDL may be limiting in terms of mediating the clearance of lipoproteins from the plasma (21).

The differences in triacylglycerol levels in the *apo-B*$^{48/48}$ and *apo-B*$^{100/100}$ mice is one of the most intriguing aspects of this study. On the chow diet, fasted *apo-B*$^{100/100}$ mice had higher levels of VLDL and LDL triacylglycerols than the *apo-B*$^{+/+}$ or *apo-B*$^{48/48}$ mice. In addition, triacylglycerol levels from fasted *apo-B*$^{100/100}$*apo-E*$^{-/-}$ mice were nearly twice as high as those in the *apo-B*$^{48/48}$*apo-E*$^{-/-}$ or *apo-B*$^{+/+}$*apo-E*$^{-/-}$ mice, despite the fact that the *apo-B*$^{100/100}$*apo-E*$^{-/-}$ mice had significantly lower cholesterol levels. The higher triacylglycerol levels in the *apo-B*$^{100/100}$ mice, both in the presence and absence of apo-E expression, suggests that triacylglycerols within the core of apo-B100-containing lipoproteins may not be as accessible for lipolytic processing, as compared with the triacylglycerols within apo-B48-containing lipoproteins. Future *in vivo* and *in vitro* experimental studies are needed to address this.

The apo-B48-only and apo-B100-only mice described herein should prove to be valuable models for future metabolic and lipoprotein assembly experiments aimed at understanding functional differences between the two apo-B species. In addition, we have recently generated homozygous apobec-1 knockout mice (22), whose plasma contains apo-B100, but no apo-B48. Further genetic, dietary, hormonal, and drug manipulations of each of these mouse models should yield fresh insights into lipoprotein metabolism and ultimately will lead to a clear understanding of the physiologic purpose for two forms of apo-B in mammalian metabolism. Additionally, these models should provide insights into the relative atherogenicity of lipoproteins containing apo-B48 and apo-B100.

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