Hepatic secretion of apoB-100 is impaired in hypobetalipoproteinemic mice with an apoB-38.9-specifying allele

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Abstract **Apolipoprotein B (apoB) truncation-specifying mutations cause familial hypobetalipoproteinemia (FHBL). Lipoprotein kinetics studies have shown that production rates of apoB-100 are reduced by 70–80% in heterozygous FHBL humans, instead of the expected 50%. To develop suitable mouse models to study the underlying mechanism, apoB-38.9-only (*Apob*$^{38.9/38.9}$) mice were crossbred with Apobec-1 knockout (*Apobec-1*$^{-/-}$) mice or apoB-100-only (*Apob*$^{100/100}$) mice to produce two lines of apoB-38.9 heterozygous mice that produce only apoB-38.9 and apoB-100, namely *Apobec-1*$^{-/-}$*/Apob*$^{38.9/+}$ and *Apob*$^{38.9/100}$ mice. In vivo rates of apoB-100 secretion were measured using [^{35}S]Met/Cys to label proteins and Triton WR-1339 to block apoB-100 VLDL lipolysis/uptake. Rates of secretion were reduced by 80%, rather than the expected 50%, in both *Apobec-1*$^{-/-}$*/Apob*$^{38.9/+}$ and *Apob*$^{38.9/100}$ mice compared with those of the respective *Apobec-1*$^{-/-}$*/Apob*$^{+/+}$ and *Apob*$^{100/100}$ control mice. Continuous labeling and pulse-chase experiments in primary hepatocyte cultures revealed that rates of apoB-100 synthesis by *Apobec-1*$^{-/-}$*/Apob*$^{38.9/+}$ and *Apob*$^{38.9/100}$ hepatocytes were reduced to the expected 50% of those of the respective controls, but the efficiency of secretion of apoB-100 was significantly lower in apoB-38.9 heterozygous hepatocytes. The greater-than-expected decreases in apoB-100 production rates of FHBL heterozygous humans appear to be attributable to a defect in secretion rather than in the synthesis of apoB-100 from the unaffected apoB allele.**—Chen, Z., R. L. Fitzgerald, G. Li, N. O. Davidson, and G. Schonfeld. **Hepatic secretion of apolipoprotein B-100 is impaired in hypobetalipoproteinemic mice with an apolipoprotein B-38.9-specifying allele. *J. Lipid Res.* 2004.** 45: **155–163.**

Supplementary key words VLDL secretion • gene targeting • animal model

Mutations in the apolipoprotein B (apoB) gene (*Apob*) that lead to carboxyl-terminal truncation of apoB-100 cause familial hypobetalipoproteinemia (FHBL) in humans (1–3), an autosomal codominant disorder characterized by low levels (<5th percentile) of plasma apoB and LDL cholesterol (1–3). In these subjects, the truncated apoB variants usually circulate in the plasma at very low levels as a result of their enhanced catabolism (4–7) and/or reduced secretion rates (8, 9). One might expect humans heterozygous for FHBL attributable to apoB truncation-specifying mutations to have half-normal plasma levels of apoB-100, because they possess one normal apoB-100 allele. However, this is not usually the case. Instead, the typical plasma concentrations of apoB-100 are only 20–30% of normal (1, 3). Likewise, lipoprotein kinetic studies of heterozygous FHBL humans indicate that production rates of apoB-100, the sole protein product of the unaffected apoB allele in human livers, are reduced by 70–80%, instead of the expected 50% (10–12). Thus, the presence of a premature-termination codon-specifying mutation in one apoB allele may unexpectedly impair the synthesis and/or secretion of apoB-100, the gene product of the unaffected apoB-100 allele. FHBL patients are usually asymptomatic (1–3), posing ethical barriers to obtaining liver biopsies for direct biochemical studies to determine rates of apoB-100 synthesis and secretion.

In recent years, we (13, 14) and other investigators (15–17) have developed several lines of *Apob*-modified mice to model the FHBL condition. These mice provided new insights into the FHBL syndrome and the structure-function relationship of apoB-100 (13–16). However, apoB-48, instead of apoB-100, is the major protein product of the normal mouse apoB allele as a result of the presence of apoB mRNA-editing machinery in the mouse liver (18, 19). Thus, the currently available FHBL mouse models

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are not suitable for studying the effect of apoB-truncation mutations on the synthesis and secretion of apoB-100 from the normal apoB allele in the liver.

In this study, we bred our previously generated apoB-38.9-only mice (*Apob*$^{38.9/38.9}$) (13) with apoB mRNA-editing catalytic enzyme-1 (*Apobec-1*) knockout mice (*Apobec-1*$^{-/-}$) (20) and with apoB-100-only mice (*Apob*$^{100/100}$) (21) to generate two different lines of apoB-38.9 heterozygous mice in which only apoB-38.9 and apoB-100 are produced by the liver. These mice provided us an opportunity to determine directly the effects of the apoB-38.9 mutation on the synthesis and secretion of apoB-100 and to explore the underlying mechanisms at the cellular level.

METHODS

Materials

[^{35}S]Promix (530 MBq/ml), an L-[^{35}S]Met and L-[^{35}S]Cys metabolic labeling solution, was from Amersham Bioscience Corp. (Piscataway, NJ). Triton WR-1339, rabbit anti-human albumin antisera, *N*-acetyl-L-leucyl-L-leucyl-L-norleucinal (ALLN), (2S,3S)-*trans*-epoxysuccinyl-L-leucylamido-3-methylbutane ethyl ester (EST), and heparin (from porcine intestinal mucosa) were from Sigma Chemical Co. (St. Louis, MO). Glutathione-*S*-transferase (GST)-LDL receptor-associated protein (RAP) fusion protein (GST-RAP) was produced and purified as described (7).

Mice

The generation of the apoB-38.9-producing mouse has been described (13). This mouse carries a single nucleotide deletion in its *Apob*, leading to the formation of a premature stop codon at residue 1,767. The apoB-100-only mouse was purchased from Jackson Laboratories (Bar Harbor, ME). This mouse was generated by Farese et al. (21). It carries a targeted missense mutation at codon 2,153 that prevents the formation of apoB-48 resulting from apoB mRNA editing. Both lines of mice have a mixed genetic background, with 50% C57BL/6 and 50% 129/SvJ (13, 21). The mice doubly heterozygous for the apoB-38.9- and apoB-100-specifying alleles (*Apob*$^{100/38.9}$) were generated by crossbreeding of *Apob*$^{38.9/38.9}$ and *Apob*$^{100/100}$ mice. The *Apob*$^{100/38.9}$ and *Apob*$^{100/100}$ mice obtained from offspring of the intercross of *Apob*$^{100/38.9}$ mice were used in this study. All of these offspring had a mixed genetic background, with 50% C57BL/6 and 50% 129/SvJ. The *Apob*$^{100/38.9}$ mice were also crossed with LDL-receptor knockout (*Ldlr*$^{-/-}$) and apoE knockout (*Apoe*$^{-/-}$) mice (Jackson Laboratories) to produce *Apob*$^{100/100}$/*Ldlr*$^{-/-}$, *Apob*$^{100/38.9}$/*Ldlr*$^{-/-}$, *Apob*$^{100/100}$/*Apoe*$^{-/-}$, and *Apob*$^{100/38.9}$/*Apoe*$^{-/-}$ mice. The *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mice had a C57BL/6 genetic background; thus, the resulting *Apob*$^{100/100}$/*Ldlr*$^{-/-}$, *Apob*$^{100/38.9}$/*Ldlr*$^{-/-}$, *Apob*$^{100/100}$/*Apoe*$^{-/-}$, and *Apob*$^{100/38.9}$/*Apoe*$^{-/-}$ mice were predicted to have a mixed genetic background, with 75% C57BL/6 and 25% 129/SvJ.

The *Apobec-1*$^{-/-}$ mouse was generated by Hirano et al. (20). It has a C57BL/6 genetic background. Thus, offspring (*Apobec-1*$^{-/+}$/*Apob*$^{38.9/+}$) produced from crossbreeding of *Apob*$^{38.9/38.9}$ with *Apobec-1*$^{-/-}$ mice had a mixed genetic background, with 75% C57BL/6 and 25% 129/SvJ. *Apobec-1*$^{-/-}$/*Apob*$^{+/+}$ and *Apobec-1*$^{-/-}$/*Apob*$^{38.9/+}$ mice generated from intercrossing of *Apobec-1*$^{-/+}$/*Apob*$^{38.9/+}$ mice were used for this study.

Only offspring from intercross breeding were used in this study, and in most cases, littermates were used to minimize variation in the strain percentage.

All mice were weaned at 3 weeks of age, housed in a specific-pathogen-free barrier facility with a 12 h light/dark cycle, and fed a regular mouse chow diet (Ralston Purina, St. Louis, MO).

Fast-protein liquid chromatography fractionation and Western blot analysis of plasma apoB

For Western blot analysis, mouse plasma was subjected to electrophoresis on 3–12% gradient SDS-PAGE gels under reducing conditions and electrotransferred onto Immobilon-P (Millipore Corp., Bedford, MA). Western blot analyses were carried out using rabbit antisera raised against a GST fusion protein containing amino acids 26 to 289 of mouse apoB (13, 14) and an enhanced chemiluminescence (ECL) Western blot detection kit (Amersham Pharmacia Biotech, Inc.). The ECL signals were quantified by analyzing the density of the protein bands on Kodak X-Omat film using SigmaGel computer software (SPPS Science Corp., Chicago, IL).

Fast-protein liquid chromatography (FPLC) fractionation of plasma lipoproteins was carried out as described (13, 14) using 200 μl of plasma obtained from *Apob*$^{100/100}$/*Ldlr*$^{-/-}$ or *Apob*$^{100/38.9}$/*Ldlr*$^{-/-}$ mice. Cholesterol contents in each fraction were determined enzymatically, and apoB contents were determined by Western blot analysis.

In vivo study

To determine the in vivo secretion rates of apoB and triglycerides, mice (12 weeks old) were fasted for 4 h and administered Triton WR-1339 (Sigma) (500 mg/kg body weight) and [^{35}S]Promix (300 μCi/g body weight) via intravenous injection. Blood samples (~80 μl each) were taken using heparinized capillary tubes at 0 h and at 0.5, 1, 2, and 3 h thereafter. VLDL was isolated from 20 μl of plasma in 200 μl of EDTA-saline-KBr (d = 1.009) using 250 μl Polyallomer Centrifuge tubes (Beckman) and a 42.2 Ti rotor (Beckman). Aliquots of VLDL and infranatant were electrophoresed on SDS-PAGE gels. ^{35}S activity in apoB-100 of VLDL samples and albumin of infranatant were quantified on a GS-525 PhosphorImager system using a low β-screen (Bio-Rad). The relative amount of [^{35}S]apoB-100 in each plasma sample was expressed as the ratio of [^{35}S]apoB-100 to [^{35}S]albumin.

Triglyceride concentrations in the plasma samples were also determined. The values obtained at the 0 time point were subtracted from the values obtained at later times.

Hepatocyte culture study

Primary hepatocytes were isolated from mouse liver by perfusion of a collagenase solution via the portal vein (13, 14). Viability of the cell was ~80% as determined by Trypan exclusion. Cells were plated onto six-well plates (0.6×10^6 cells/well) coated with poly-D-lysine (Sigma) and incubated at 37°C under 5% CO_2 in 10% FBS/DMEM. After 1 h of attachment, cell monolayers were washed twice and incubated in 10% FBS/DMEM until use. All experiments involving cultured hepatocytes were commenced at 7–8 h after the cells were cultured. After this initial culture period, cells were washed three times with PBS and incubated in Met- and Cys-free DMEM for 30 min to deplete the cellular pool of Met and Cys. Thereafter, the medium was replaced with 1 ml of Met- and Cys-free DMEM containing 200 μCi of [^{35}S]Promix with or without oleic acid (OA) (0.5 mM; to give anOA/BSA ratio of 3.6) (14), and cells were labeled for the specified time period to determine the rates of apoB secretion.

For pulse-chase experiments, the cells were pulsed with [^{35}S]Promix for 45 min as described above. Thereafter, they were washed twice with PBS and incubated in 1 ml of DMEM containing 10 mM Met and 3 mM Cys for the specified time period with or without the specified additives.

Immunoprecipitation and quantification of ^{35}S-labeled apoB

Immunoprecipitation of the labeled apoB in cell lysate and cultured media was carried out using rabbit anti-mouse apoB antisera as described (13, 14). Rabbit anti-human albumin antisera (Sigma) were also included in the immunoprecipitation mixture to immunoprecipitate mouse albumin simultaneously. The immunoprecipitated proteins (apoB and albumin) were resolved on a 3–12% gradient gel under reducing conditions. The gels were dried, and the radioactivity associated with apoB and albumin was quantified on a GS-525 PhosphorImager system using a low β-screen (Bio-Rad). Our initial studies found no difference in the rates of synthesis and secretion of albumin between the *Apobec-1*$^{-/-}$*/Apob*$^{+/+}$ and *Apobec-1*$^{-/-}$*/Apob*$^{+/38.9}$ and between the *Apob*$^{100/100}$ and *Apob*$^{100/38.9}$ hepatocytes. Thus, the ^{35}S-labeled albumin signal was used to correct the [^{35}S]apoB-100 signal in each lane.

Miscellaneous procedures

Liver triglyceride contents were determined as described (13, 14). Cellular protein contents were determined using a modified Lowry method (22). Plasma triglyceride concentrations were determined using an enzymatic kit (WAKO Chemicals USA, Inc., Richmond, VA). Student's *t*-test and ANOVA analysis were performed to determine the significance of the differences.

RESULTS

Low plasma apoB-100 in *Apobec-1*$^{-/-}$*/Apob*$^{+/38.9}$ and *Apob*$^{100/38.9}$ mice

As expected, apoB-100 was the sole apoB in *Apobec-1*$^{-/-}$*/Apob*$^{+/+}$ and *Apob*$^{100/100}$ mouse plasma, whereas both apoB-100 and apoB-38.9 were present in *Apobec-1*$^{-/-}$*/Apob*$^{+/38.9}$ and *Apob*$^{100/38.9}$ mouse plasma (**Fig. 1**). The *Apobec-1*$^{-/-}$*/Apob*$^{+/+}$, *Apobec-1*$^{-/-}$*/Apob*$^{+/38.9}$, *Apob*$^{100/100}$, and *Apob*$^{100/38.9}$ mice all appeared healthy and gained weight at normal rates.

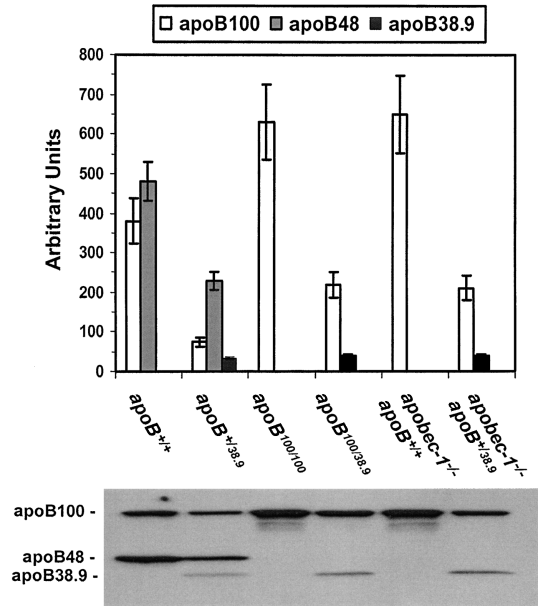

Fig. 1. Plasma concentrations of apolipoprotein B (apoB) in mice with various apoB genotypes. Two microliters of plasma pooled from four fasted mice in each genotype were separated on a 3–12% SDS-PAGE gel. Western blotting was carried out using monospecific rabbit anti-mouse apoB polyclonal antibodies as described in Methods. Each data point represents the mean and SEM of four determinations.

Compared with those of their respective *Apobec-1*$^{-/-}$*/Apob*$^{+/+}$ and *Apob*$^{100/100}$ littermates, plasma apoB-100 levels of the *Apobec-1*$^{-/-}$*/Apob*$^{+/38.9}$ and *Apob*$^{100/38.9}$ mice were reduced by 70% (Fig. 1). In *Apobec-1*$^{-/-}$*/Apob*$^{+/38.9}$ and *Apob*$^{100/38.9}$ mice, plasma apoB-38.9 concentrations were approximately one-sixth of those of apoB-100 (Fig. 1).

To determine whether the low levels of plasma apoB-100 in *Apobec-1*$^{-/-}$*/Apob*$^{+/38.9}$ and *Apob*$^{100/38.9}$ mice were caused by enhanced clearance or reduced secretion rates of apoB-100-containing lipoproteins, we crossed the *Apob*$^{100/38.9}$ mice with *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ mice, thereby blocking apoE-mediated plasma VLDL remnant and LDL receptor-mediated LDL catabolism, respectively. As shown in **Fig. 2**, apoB-100 levels in *Apob*$^{100/38.9}$*/Apoe*$^{-/-}$ mice amounted to only 15–20% of those of *Apob*$^{100/100}$*/Apoe*$^{-/-}$ mice (Fig. 2A), similar to the results comparing *Apob*$^{100/38.9}$*/Apoe*$^{+/+}$ to *Apob*$^{100/100}$*/Apoe*$^{+/+}$ mice. This suggests that apoE in the apoB-38.9 mice did not affect either the secretion of VLDL from the liver or its catabolism. Disruption of LDL receptor function yielded similar results (Fig. 2B). FPLC analysis showed that plasma LDL cholesterol concentrations in *Apob*$^{100/38.9}$*/Ldlr*$^{-/-}$ mice were also ~25% of those the *Apob*$^{100/100}$*/Ldlr*$^{-/-}$ mice (**Fig. 3**). This suggested that LDL receptor-mediated uptake and catabolism of VLDL did not account for the apparent low rates of VLDL secretion in the apoB-38.9 mice. Together, these results provided physiological evidence indicating that low rates of hepatic apoB-100 VLDL secretion may be the major mechanism for the lower than expected levels of plasma apoB-100 in the *Apobec-1*$^{-/-}$*/Apob*$^{+/38.9}$ and *Apob*$^{100/38.9}$ mice.

In vivo rates of hepatic apoB-100 secretion

To directly determine hepatic secretion rates of apoB-100, mice were fasted for 4 h and injected with Triton WR-1339 and [^{35}S]Met/Cys simultaneously. ^{35}S-labeled apoB-100 and albumin in plasma were quantified in plasma samples obtained at 0.5, 1, 2, and 3 h after injection. Levels of the labeled apoB-100 and albumin reached a plateau at 2 h. All of the ^{35}S-labeled apoB-100 floated at $d < 1.009$. Ratios of [^{35}S]apoB-100 to [^{35}S]albumin in *Apobec-1*$^{-/-}$*/Apob*$^{+/38.9}$ mice were only 15–20% of those of *Apobec-1*$^{-/-}$*/Apob*$^{+/+}$ mice at each time point (**Fig. 4A**). The ratios of [^{35}S]apoB-100 to [^{35}S]albumin in *Apob*$^{100/38.9}$ mice were reduced by a similar extent compared with those of *Apob*$^{100/100}$ mice (Fig. 4A). Thus, rates of apoB-100 VLDL secretion by both the *Apobec-1*$^{-/-}$*/Apob*$^{+/38.9}$ and *Apob*$^{100/38.9}$ mice were reduced by an extent similar to that of humans heterozygous for a truncation-specifying apoB allele.

Along with the dramatically reduced secretion rates of apoB-100 in *Apobec-1*$^{-/-}$*/Apob*$^{+/38.9}$ and *Apob*$^{100/38.9}$ mice, hepatic triglyceride secretion rates were reduced by ~50% compared with those of *Apobec-1*$^{-/-}$*/Apob*$^{+/+}$ and *Apob*$^{100/100}$ mice, respectively (Fig. 4B), which was similar to the level in humans (12).

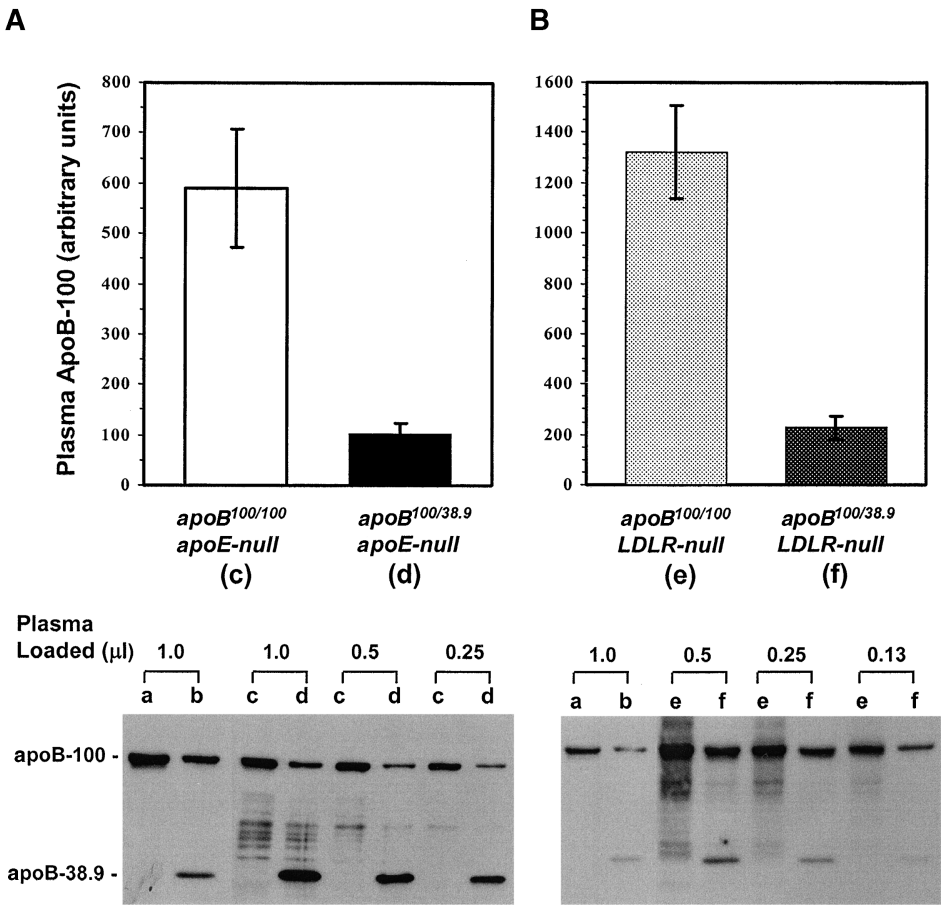

Fig. 2. Disruption of apoE (A) or the LDL receptor gene (B) did not diminish the larger than expected difference in plasma apoB-100 levels between *Apob*$^{100/100}$ and *Apob*$^{100/38.9}$ mice. Plasma samples were obtained from six fasted mice for each compound genotype and pooled. After dilution, the indicated amounts of pooled plasma were applied to 3–12% SDS-PAGE for Western blot analysis. Lanes are as follows: a, *Apob*$^{100/100}$ control; b, *Apob*$^{100/38.9}$ control; c, *Apob*$^{100/100}$/*Apoe*$^{-/-}$; d, *Apob*$^{100/38.9}$/*Apoe*$^{-/-}$; e, *Apob*$^{100/100}$/*Ldlr*$^{-/-}$; f, *Apob*$^{100/38.9}$/*Ldlr*$^{-/-}$. Each data point represents the mean and SEM of three determinations.

To assess the potential difference in the characteristics of apoB-100 VLDL particles secreted by *Apob*$^{100/100}$ and *Apob*$^{100/38.9}$ mice, VLDLs were isolated from the plasma of *Apob*$^{100/100}$ and *Apob*$^{100/38.9}$ mice at 2 h after injection with Triton WR-1339. Western blot analysis confirmed that VLDL from the *Apob*$^{100/38.9}$ mice contained almost no apoB-38.9 (data not shown). Chemical analyses revealed that the VLDL produced by the *Apob*$^{100/38.9}$ mice had a significantly higher triglyceride content than did the VLDL of *Apob*$^{100/100}$ mice (20.1 ± 1.46 vs. 14.9 ± 1.27 mg/mg VLDL protein; $P < 0.001$, n = 3 mice). There was no significant difference in cholesterol contents in VLDL particles between the *Apob*$^{100/38.9}$ and *Apob*$^{100/100}$ mice (4.65 ± 0.42 vs. 4.10 ± 0.41 mg/mg VLDL protein; $P > 0.05$, n = 3 mice).

Synthesis and secretion of apoB-100 by cultured hepatocytes

To determine the mechanism responsible for the dramatic reductions in secretion rates of apoB-100 by livers of *Apobec-1*$^{-/-}$/*Apob*$^{+/38.9}$ and *Apob*$^{100/38.9}$ mice, we determined the rates of intracellular accumulation and secretion of newly synthesized apoB-100 by cultured hepatocytes. On continuous metabolic labeling in the absence of OA, the rates of accumulation of [^{35}S]apoB-100 in the cell and in the medium of *Apob*$^{100/38.9}$ hepatocytes were approximately the expected 50% of those of the *Apob*$^{100/100}$ hepatocytes (**Fig. 5**). However, supplementation of the labeling media with 0.5 mM OA, although significantly stimulating the secretion of apoB-100 by *Apob*$^{100/100}$ hepatocytes, did not affect apoB-100 secretion by the *Apob*$^{100/38.9}$ hepatocytes (Fig. 5). Thus, in the presence of OA, the rate of secretion of apoB-100 by *Apob*$^{100/38.9}$ hepatocytes was only 25% of that of *Apob*$^{100/100}$ hepatocytes (Fig. 5). The total amounts of [^{35}S]apoB-100 accumulating in the cells and media at each time point were not affected by OA. Together, these data suggest that the secretion, but not the synthesis, of apoB-100 from the apoB-100-specifying allele may be impaired in the *Apob*$^{100/38.9}$ hepatocytes.

The experiments described above were also repeated using hepatocytes isolated from *Apobec-1*$^{-/-}$/*Apob*$^{+/38.9}$ and *Apobec-1*$^{-/-}$/*Apob*$^{+/+}$ mice, and similar observations were made (data not shown).

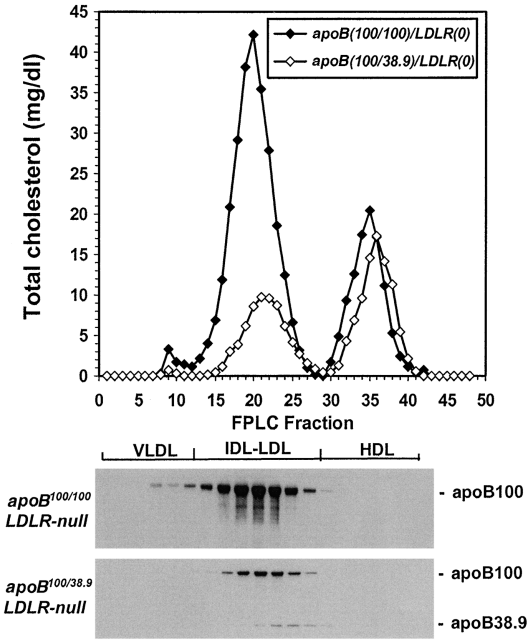

Fig. 3. ApoB and cholesterol distribution among fast-protein liquid chromatography (FPLC) fractions of pooled plasma of *Apob*$^{100/100}$/*Ldlr*$^{-/-}$ or *Apob*$^{100/38.9}$/*Ldlr*$^{-/-}$ mice. FPLC was carried out using 200 μl of pooled plasma samples. IDL, intermediate density lipoprotein.

Reduced secretion efficiency of apoB-100 in *Apob*$^{100/38.9}$ hepatocytes

Pulse-chase experiments were carried out to examine the kinetics and efficiency of secretion of the newly synthesized apoB-100 in the *Apob*$^{100/38.9}$ hepatocytes. In the absence of OA, there were no differences between *Apob*$^{100/38.9}$ and *Apob*$^{100/100}$ hepatocytes during the chase period in rates of secretion, intracellular decay, and total recovery of [^{35}S]apoB-100 during the pulse period (**Fig. 6**). However, in the presence of OA, a significantly greater percentage of initially ^{35}S-labeled apoB-100 synthesized during the pulse period was secreted into the media after a 2 h chase by *Apob*$^{100/100}$ hepatocytes than by *Apob*$^{100/38.9}$ hepatocytes (62 vs. 41%; $P < 0.02$, n = 3). Rates of intracellular decay of apoB-100 were similar in hepatocytes from the two genotypes (Fig. 6). Consequently, the total recovery of [^{35}S]apoB-100 was significantly lower in the *Apob*$^{100/38.9}$ than in the *Apob*$^{100/100}$ hepatocytes (Fig. 6), suggesting greater proportional degradation of apoB-100 in the *Apob*$^{100/38.9}$ hepatocytes.

To determine whether the decrease in total recovery of the labeled apoB-100 from the *Apob*$^{100/38.9}$ hepatocytes was attributable to reuptake of the newly secreted apoB-100, heparin or GST-RAP was used to block the potential cellular reuptake of VLDL. Neither of these agents affected the recovery of labeled apoB-100 in either the *Apob*$^{100/38.9}$ or the *Apob*$^{100/100}$ hepatocytes (**Fig. 7**). Likewise, the proteasomal inhibitors ALLN and EST were also ineffective (Fig. 7).

Fatty livers

Liver triglyceride contents in *Apobec-1*$^{-/-}$/*Apob*$^{+/38.9}$ and *Apob*$^{100/38.9}$ mice were 1.9- and 2.5-fold higher than those in *Apobec-1*$^{-/-}$/*Apob*$^{+/+}$ and *Apob*$^{100/100}$ mice, respectively (**Fig. 8**).

DISCUSSION

ApoB truncation-specifying mutations cause FHBL in humans and mice. We (10, 12) and others (11) have previously shown that production rates of apoB-100, the product of the normal apoB allele, are reduced by 70% to 80%

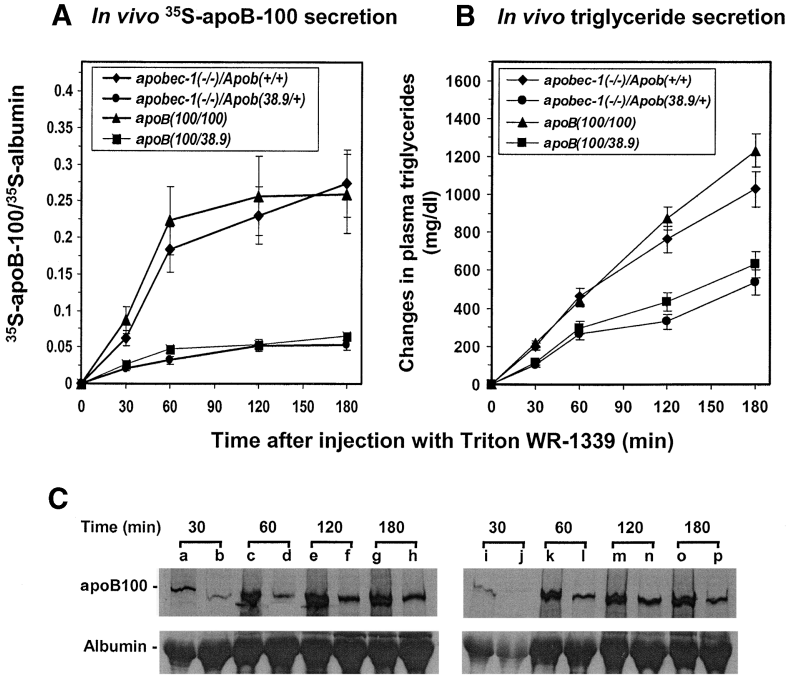

Fig. 4. Hepatic secretion of ^{35}S-labeled apoB-100 (A) and triglycerides (B) in vivo. After fasting for 4 h, mice were injected simultaneously with [^{35}S]Promix and Triton WR-1339. Plasma samples were taken at the indicated time points. VLDL was isolated from each sample using a 42.2 rotor. Aliquots of VLDL and infranatant equivalent to 5 μl of the original plasma were subjected to SDS-PAGE. ^{35}S radioactivity in apoB-100 and albumin was quantified using a PhosphorImager. Each data point represents the mean and SD of three mice. A: Relative rates of apoB-100 secretion expressed as ratios of [^{35}S]apoB-100 to [^{35}S]albumin. B: Changes in plasma triglyceride levels at each time point. C: Autoradiograms showing [^{35}S]apoB-100 and [^{35}S]albumin of combined plasma samples obtained from *Apobec-1*$^{-/-}$/*Apob*$^{+/+}$ (lanes a, c, e, g), *Apobec-1*$^{-/-}$/*Apob*$^{38.9/+}$ (lanes b, d, f, h), *Apob*$^{100/100}$ (lanes i, k, m, o), or *Apob*$^{100/38.9}$ (lanes j, l, n, p) mice.

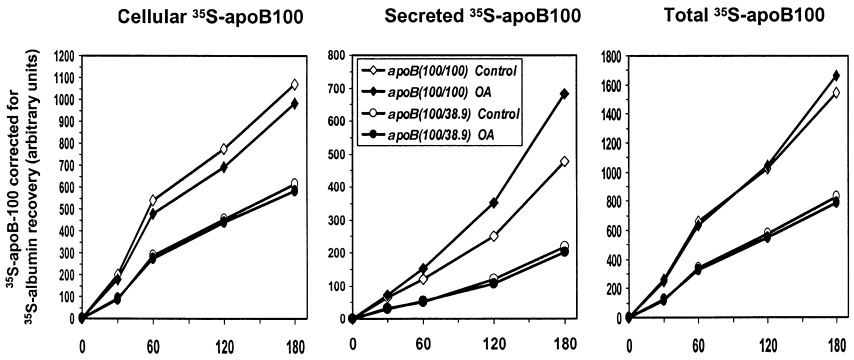

Fig. 5. Synthesis and secretion of ^{35}S-labeled apoB-100 by cultured hepatocytes. Hepatocytes isolated from *Apob*$^{100/100}$ and *Apob*$^{100/38.9}$ mice were labeled with [^{35}S]Promix in the presence or absence of 0.5 mM oleic acid (OA) for the specified time period. ^{35}S-labeled apoB protein and albumin in cell lysates and media were subjected to immunoprecipitation with dual antibodies and separated by SDS-PAGE. ^{35}S radioactivity in the apoB-100 and albumin bands was quantified using a PhosphorImager. [^{35}S]apoB-100 data were normalized to [^{35}S]albumin data. Each data point represents the mean of two independent experiments.

in heterozygous FHBL humans, instead of the expected 50%. In the present study, we confirmed and extended these observations using our newly created FHBL mouse models and provide insights into the underlying mechanism.

Two lines of apoB-38 heterozygous mice that produced only apoB-38.9 and apoB-100 were created in this study. The first line is deficient in Apobec-1 and thus incapable of editing apoB RNA; the second line is doubly heterozygous for an engineered apoB-100-only allele and the apoB-38.9-specifying allele. The apoB-38.9 mutation caused a 70% reduction in plasma apoB-100 in these mice. Plasma apoB-100 levels in the *Apob*$^{100/38.9}$*/ApoE-null* and *Apob*$^{100/38.9}$*/LDL-receptor-null* mice were 80–85% lower than those of their respective controls, i.e., *Apob*$^{100/100}$*/ApoE-null* and *Apob*$^{100/100}$*/LDL-receptor-null* mice, indicating that the low plasma levels of apoB-100 in the *Apob*$^{100/38.9}$ mice result mainly from a lower than expected rate of apoB-100 secretion. Indeed, by using [^{35}S]Met/Cys to label newly synthesized proteins and Triton WR-1339 to block the lipolysis/uptake of apoB-100 VLDL (23–26), we demonstrated that apoB-100 secretion rates in both of the apoB-38.9 heterozygotes were reduced to 20% of those of their respective *Apobec-1*$^{-/-}$*/Apob*$^{+/+}$ or *Apob*$^{100/100}$ littermates. To the best of our knowledge, the present study is the first that has used appropriate FHBL mouse models to confirm the previously reported lower than expected production rates of apoB-100 observed in FHBL heterozygous humans based on metabolic kinetic modeling (9–12).

Like apoB-100 secretion, hepatic triglyceride secretion rates were also reduced in these apoB-38.9 heterozygotes, but only to 50% of control levels. This may be attributable to the fact that the apoB-38.9 protein retains some capacity for transporting triglycerides, albeit in reduced amounts (13, 14). In addition, our data on the lipid composition of newly secreted apoB-100 VLDL particles in the *Apob*$^{100/38.9}$ mice strongly suggested that increased amounts of triglycerides were packed into the apoB-100 VLDL particles.

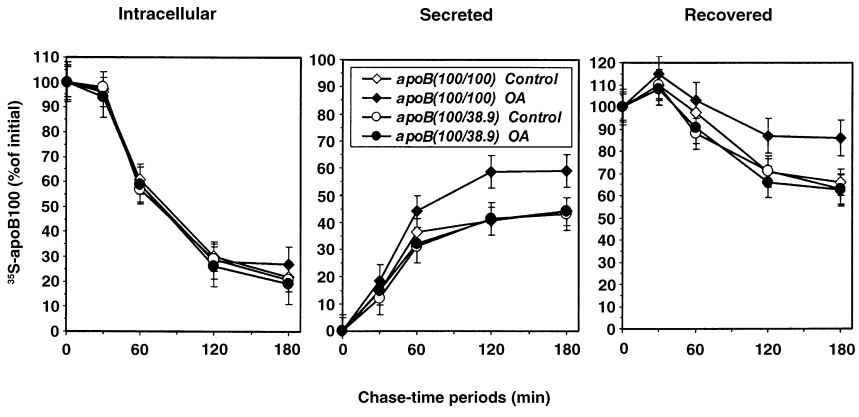

Fig. 6. Pulse-chase analysis of apoB-100 in *Apob*$^{100/100}$ and *Apob*$^{100/38.9}$ hepatocytes. Cells were labeled with [^{35}S]Promix in OA-free media for 45 min and chased for the indicated time periods in the presence or absence of 0.5 mM OA. Immunoprecipitation of apoB and albumin was carried out and ^{35}S radioactivity in apoB-100 and albumin was quantified as described in the legend to Fig. 5. Each data point represents the mean and SD of three independent experiments.

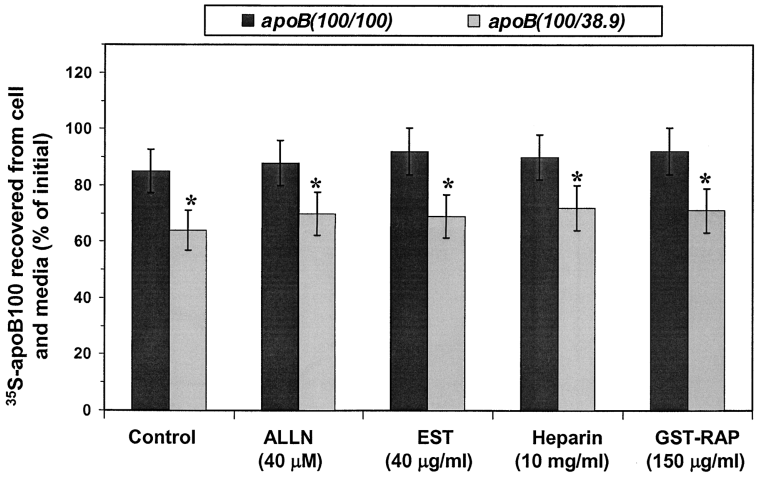

Fig. 7. Effects of protease inhibitors and apoB lipoprotein receptor blockers on the total recovery of labeled apoB-100. *Apob*$^{100/100}$ and *Apob*$^{100/38.9}$ hepatocytes were labeled with [^{35}S]Promix for 45 min and chased for 2.5 h in media containing 0.5 mM OA plus the specified inhibitors. Except for *N*-acetyl-L-leucyl-L-leucyl-L-norleucinal (ALLN), which was subjected to preincubation for 1 h, all other additives were used only in the chase period. Each bar represents the mean and SD of triplicate incubations. Asterisks indicate significant ($P < 0.01$) differences from the corresponding value of *Apob*$^{100/100}$ hepatocytes. EST, (2S,3S)-*trans*-epoxysuccinyl-L-leucylamido-3-methylbutane ethyl ester; GST-RAP, glutathione-*S*-transferase-LDL receptor-associated protein fusion protein.

Similar to these mice, VLDL triglyceride secretion rates in the apoB-2 human heterozygotes were 40% of those of control subjects, albeit there was an 80% reduction in apoB-100 production rates (12). Thus, the apoB-100 produced by the unaffected apoB allele appears to recruit more triglycerides to compensate for the overall decreased triglyceride-exporting capacity of the liver. Nevertheless, this compensatory mechanism is apparently insufficient, because fatty livers developed in heterozygous FHBL humans (27–30) as well as in our *Apobec-1*$^{-/-}$/*Apob*$^{+/38.9}$ and *Apob*$^{100/38.9}$ mice.

A greater than expected decrease in the synthesis of apoB-100 from the apoB-100 allele could have contributed to the >50% reduction in apoB-100 secretion rates from our mice and FHBL heterozygous humans. However, we (13, 14) and others (15, 16) have previously shown that the presence of a truncated apoB allele in the mouse liver does not affect the steady-state levels of mRNA transcripts of the unaffected apoB allele. Moreover, in the present study, our continuous labeling experiments demonstrated that the rates of accumulation of ^{35}S-labeled apoB-100 synthesized by the *Apob*$^{100/38.9}$ or *Apobec-1*$^{-/-}$/*Apob*$^{+/38.9}$ hepatocytes were approximately the expected 50% compared with those in the hepatocytes derived from *Apob*$^{100/100}$ or *Apobec-1*$^{-/-}$/*Apob*$^{+/+}$ mice, respectively. Similarly, in our pulse-chase experiments, the amounts of ^{35}S-labeled apoB-100 synthesized by the *Apob*$^{100/38.9}$ hepatocytes during the pulse period were ~50% of the amounts synthesized by the *Apob*$^{100/100}$ hepatocytes (data not shown). Together, these results suggest that the reduced secretion rates of apoB-100 from the unaffected apoB allele of FHBL heterozygous humans and mice probably did not result from a larger than expected decrease in apoB-100 synthetic rates. Rather, they were more likely attributable to impaired secretion of apoB-100 by the hepatocytes. This conclusion was supported by the results of our continuous labeling and pulse-chase experiments. In the presence of OA in the culture media, which is more likely to reflect the situation in vivo, apoB-100 secretion rates in *Apob*$^{100/38.9}$ hepatocytes amounted to only 30% of those of the controls. Furthermore, the efficiency of secretion of newly synthesized apoB-100 was significantly lower in *Apob*$^{100/38.9}$ hepatocytes than in *Apob*$^{100/100}$ hepatocytes. In the absence of OA, the rates of secretion of apoB-100 in *Apobec-1*$^{-/-}$/*Apob*$^{+/38.9}$ or *Apob*$^{100/38.9}$ hepatocytes were ~50% of those of the hepatocytes from *Apobec-1*$^{-/-}$/*Apob*$^{+/+}$ or *Apob*$^{100/100}$ mice. The addition of 0.5 mM OA in the culture media enhanced apoB-100 secretion in *Apobec-1*$^{-/-}$/*Apob*$^{+/+}$ or *Apob*$^{100/100}$ hepatocytes but not in the *Apobec-1*$^{-/-}$/*Apob*$^{+/38.9}$ or *Apob*$^{100/38.9}$ hepatocytes. It is not clear why the larger than expected differences in apoB-100 secretion between *Apobec-1*$^{-/-}$/*Apob*$^{+/38.9}$ or *Apob*$^{100/38.9}$ hepatocytes and their corresponding controls did not emerge in the absence of exogenous OA. However, it is important to note that because of the presence of fatty liver in the apoB-38.9 mice, cellular triglyceride levels of the cultured *Apobec-1*$^{-/-}$/*Apob*$^{+/38.9}$ or *Apob*$^{100/38.9}$ hepatocytes were 1.5- to 2.0-fold higher than those of the respective controls. Apparently, the status of lipid availability for VLDL formation may be very different between these cells. It is possible that even in the absence of OA in the culture media, the availability of apoB-100 was a limiting factor for VLDL production in the *Apobec-1*$^{-/-}$/*Apob*$^{+/38.9}$

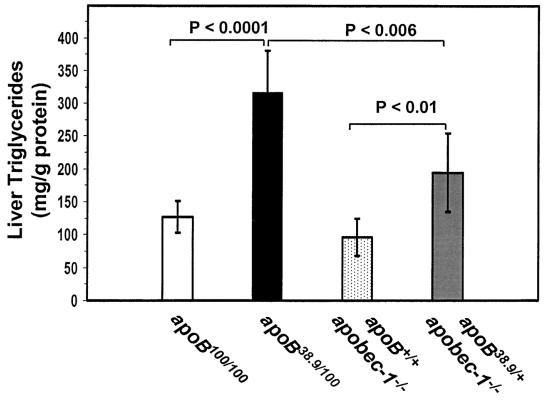

Fig. 8. Increased liver triglyceride levels in *Apob*$^{100/38.9}$ and *Apobec-1*$^{-/-}$/*Apob*$^{38.9/+}$ mice under ad libitum feeding. Triglyceride concentrations were measured in six *Apob*$^{100/100}$, nine *Apob*$^{100/38.9}$, five *Apobec-1*$^{-/-}$/*Apob*$^{+/+}$, and five *Apobec-1*$^{-/-}$/*Apob*$^{38.9/+}$ mice.

and *Apob*$^{100/38.9}$ hepatocytes; thus, VLDL secretion in these hepatocytes was no longer responsive to another increase in cellular triglyceride availability resulting from OA supplementation to the culture media. It is unlikely that the apparently reduced secretion of apoB-100 by the *Apob*$^{100/38.9}$ or *Apobec-1*$^{-/-}$/*Apob*$^{+/38.9}$ hepatocytes was attributable to enhanced reuptake of the newly secreted apoB-100, as heparin and RAP did not diminish the difference in total recovery of newly synthesized apoB-100 between *Apob*$^{100/38.9}$ and *Apob*$^{100/100}$ hepatocytes.

The assembly and secretion of apoB-100 VLDL is a complex process during which a significant amount of apoB-100 is subjected to presecretory degradation (31, 32). The proteasomal pathway plays an important role in the intracellular degradation of apoB-100 in hepatoma cells (31, 32). In primary hepatocytes, nonproteasome-mediated degradation pathways, such as the post-endoplasmic reticulum protein degradation pathway (32–34), may play a more important role in the presecretory degradation of apoB-100. A larger fraction of the apoB-100 was subjected to intracellular degradation in the *Apob*$^{100/38.9}$ hepatocytes than in the *Apob*$^{100/100}$ hepatocytes. It is not known whether this is the cause or the consequence of the reduced efficiency of apoB-100 secretion in these cells. The LDL receptor has been implicated in mediating the presecretory degradation of apoB (35, 36). However, our results from the *Apob*$^{100/38.9}$/*LDL-receptor-null* mice indicated that even in the absence of the LDL receptor, apoB-100 secretion in the *Apob*$^{100/38.9}$ mice was still much lower than 50% of that in the *Apob*$^{100/100}$ mice, suggesting that the LDL receptor pathway is not responsible for the enhanced presecretory degradation of apoB-100 in our apoB-38.9 mice.

Another potential explanation for the reduced secretion efficiency of apoB-100 in apoB-38.9 heterozygous hepatocytes is that apoB-38.9 may directly interfere with the assembly and/or secretion of apoB-100 VLDL. However, numerous in vitro studies have shown that overexpression of apoB-100 or of truncated apoB variants in rat (37–39) or human (40, 41) hepatoma cells does not affect the secretion rates of the endogenous apoB-100. Nor does overexpression of apoB-17 in liver using recombinant adenoviruses affect the levels of apoB-48 and apoB-100 in mouse plasma (41). Nevertheless, more in vivo studies are needed on the effects of additional apoB truncation-specifying mutations on the secretion of apoB-100.

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