Reduced intestinal fat absorptive capacity but enhanced susceptibility to diet-induced fatty liver in mice heterozygous for ApoB38.9 truncation

Xiaobo Lin, Pin Yue, Yan Xie, Nicholas O. Davidson, Nobuhiro Sakata, Richard E. Ostlund, Jr., Zhouji Chen, and Gustav Schonfeld

Department of Medicine, Washington University School of Medicine, St. Louis, Missouri

Submitted 13 July 2004; accepted in final form 10 March 2005

Lin, Xiaobo, Pin Yue, Yan Xie, Nicholas O. Davidson, Nobuhiro Sakata, Richard E. Ostlund, Jr., Zhouji Chen, and Gustav Schonfeld. Reduced intestinal fat absorptive capacity but enhanced susceptibility to diet-induced fatty liver in mice heterozygous for ApoB38.9 truncation. Am J Physiol Gastrointest Liver Physiol 289: G146–G152, 2005. First published March 24, 2005; doi:10.1152/ajpgi.00309.2004.—Fatty liver is prevalent in apolipoprotein B (apoB)-defective familial hypobetalipoproteinemia (FHBL). Similar to humans, mouse models of FHBL produced by gene targeting (apoB38.9/H11001) manifest low plasma cholesterol and increased hepatic triglycerides (TG) even on a chow diet due to impaired hepatic very low density lipoprotein (VLDL)-TG secretory capacity. Because apoB truncations shorter than apoB48 are expressed in the intestine, we examined whether FHBL mice may have limited capacity for intestinal TG absorption. In addition, we investigated whether FHBL mice are more susceptible to diet-induced hepatic TG accumulation. Fat absorption capacity was impaired in apoB38.9 mice in a gene-dose-dependent manner. Relative fractional fat absorption coefficients for apoB38.9, apoB48, and apoB89/38.9 were 1.00, 0.96, and 0.71, respectively.apoB38.9 mice were 1.00, 0.96, and 0.71, respectively. To raise hepatic TG, we fed high-fat (HF) and low-fat (LF) pellets. Hepatic TG level was observed in rank order: HF > LF > chow. On both LF and HF, liver TG level was higher in the apoB38.9 than in apoB48. Hepatic TG secretion remained impaired in the apoB38.9/H11001 on the HF diet. Thus the FHBL mice are more susceptible to diet-induced fatty liver despite relatively reduced intestinal TG absorption capacity on a HF diet.

Address for reprint requests and other correspondence: Gustav Schonfeld, 660 S. Euclid, Campus Box 8046, St. Louis, MO 63110 (E-mail: gschonfe@wustl.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
tion of various lipogenic enzyme genes (18, 34). Because of these complexities, it is difficult in these FHBL mice to predict the quantitative magnitude of response of liver TG to diets that tend to raise liver TG content.

Diet high in fat, in particular saturated fat, may cause increases in hepatic TG, resulting from the delivery of excess fatty acids to the liver, even in livers with normal VLDL export (26). Diets high in carbohydrates, particularly simple sugars, enhance hepatic lipogenesis resulting in increased levels of hepatic TG and hypertriglyceridemia (32). The VLDL system adapts to increased needs for export by 1) producing more VLDL particles and 2) by packaging more TG molecules into each VLDL particle (17, 40), resulting in enhanced hepatic TG export. The liver may also increase the oxidation of fatty acids.

In the present study, we have examined two hypotheses: 1) that the capacities of the apoB38.9 mice to absorb dietary fat were impaired, 2) that the apoB<sup>+</sup>/apoB<sup>+/−</sup> mice would accumulate more hepatic TG in response to liver TG-raising diets than the apoB<sup>+</sup>/apoB<sup>+/−</sup>. The results support our hypotheses and provide a rationale for testing diet responsiveness in humans with apoB-defective FHBL, in an attempt to control hepatic fat accumulation.

MATERIALS AND METHODS

Mice. ApoB<sup>+/−</sup> mice were generated using gene targeting in embryonic stem cells (9). All mice have a mixed genetic background of ~50% 129/SVJ and 50% C57BL/6J. Mice were housed in a pathogen-free barrier facility with a 12:12-h light-dark cycle (6:00 AM to 6:00 PM). Food was removed in the beginning of the light cycle, and mice were fasted for 4 h before death, except in the in vivo intestinal TG secretion and intestinal mucosal TG determination experiments, in which food was removed on the day before the experiment for an overnight fasting. All animal procedures were performed in accordance with guidelines of Washington University’s Animal Studies Committee.

Fat balance study. Mice were kept on the chow or high-fat (HF) diet. Three weeks after mice started receiving the experimental diets, food intake was recorded and feces were collected for 3 days. Lipids were extracted from ~1.0 g of dried chow, HF diet, or fecal powder by the Folch method (14). Fat absorption coefficients were determined gravimetrically and expressed relative to that of the apoB<sup>+/+</sup> control.

Determination of intestinal TG secretion rate in vivo and intestinal mucosal TG level after a bolus challenge. Mice were maintained on the regular chow diet. After a 15-h fast, age-matched mice of apoB<sup>+/+</sup>, apoB<sup>+/−</sup>, and apoB<sup>−/−</sup> were injected intravenously with Triton WR-1339 (Tylopaol; Sigma) in saline (500 mg/kg body wt) (41). Thirty minutes after Tylopaol administration, the mice received an intragastric bolus of 0.5 ml lipid emulsion (20% Intralipid and corn oil, 4:1 vol/vol). Blood was collected at 0, 1, 2, and 3 h and subjected to centrifugation at 4,000 rpm for 20 min. Total TG content was measured enzymatically (Wako Chemicals).

In a separate experiment, 15-h-fasted mice were given by gavage 0.5 ml of the above lipid emulsion. Two hours later, mice were killed. The proximal one-half of the small intestine was cut open, washed in cold PBS four times, and the mucosa was scraped. Lipids were extracted from the mucosa and dried (6). The dried extracts were dissolved in 1.0% Triton X-100 in chloroform, dried under a stream of N<sub>2</sub>, and redissolved in H<sub>2</sub>O for the determination of TG using enzymatic kits (WAKO Chemicals). Cellular protein content was determined as described previously (9). The intestinal mucosal TG content is expressed as milligrams of TG per gram of protein.

Dietary protocols. Three diets were fed (Table 1). The standard chow diet (LabDiet) contains 12% energy from fat. The HF and low-fat relatively high in carbohydrate (LF) diets (TestDiet, Rich-
**Table 2. Age, body wt, and BWG**

<table>
<thead>
<tr>
<th>Mice and Diets</th>
<th>Age-0 wk, wk</th>
<th>BW-0 wk, g</th>
<th>BW-10 wk, g</th>
<th>BWG, g/wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>apob&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chow (13)</td>
<td>14.6±1.3</td>
<td>22.9±3.2</td>
<td>26.2±3.6</td>
<td>3.3±1.3</td>
</tr>
<tr>
<td>LF (14)</td>
<td>13.7±1.4</td>
<td>25.0±4.5</td>
<td>28.0±4.9</td>
<td>3.0±1.5</td>
</tr>
<tr>
<td>HF (14)</td>
<td>16.4±0.7</td>
<td>24.7±3.3</td>
<td>29.4±4.9</td>
<td>4.7±2.9</td>
</tr>
<tr>
<td>apob&lt;sup&gt;+/38.9&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chow (10)</td>
<td>16.4±1.3</td>
<td>26.0±4.5</td>
<td>28.9±5.1</td>
<td>2.9±1.7</td>
</tr>
<tr>
<td>LF (11)</td>
<td>14.5±1.5</td>
<td>24.3±3.7</td>
<td>27.1±4.3</td>
<td>2.7±1.4</td>
</tr>
<tr>
<td>HF (11)</td>
<td>16.4±0.7</td>
<td>23.8±4.3</td>
<td>27.4±4.7</td>
<td>3.6±1.9</td>
</tr>
</tbody>
</table>

Values are means (SD) (nos. of mice in parenthesis). Mice were fed the normal chow diet for 2 wk before switching to a HF [apob<sup>+/+,</sup> 7 males and 7 females; apob<sup>+/+38.9</sup>, 6 and 5] or LF [apob<sup>+/+,</sup> 7 and 7; apob<sup>+/+38.9</sup>, 6 and 5]. Twenty-three mice remained on the chow diet [apob<sup>+/+,</sup> 6 and 7; apob<sup>+/+38.9</sup>, 6 and 4] for the chow group. Food and water were available at all times. Results were averaged from both genders.

Intestinal TG secretion rates and intestinal mucosal TG levels. The increase over the baseline value in serum TG after gavage of the lipid emulsion was in the following order at 2 and 3 h: apob<sup>+/+</sup> > apob<sup>+/38.9</sup> > apob<sup>+/38.9/38.9</sup>. At 1 h, the increase in serum TG was significantly higher in the apob<sup>+/+</sup> than those in the apob<sup>+/+38.9</sup> or apob<sup>+/38.9/38.9</sup> (Fig. 2A). In a separate experiment, apob<sup>+/+38.9</sup> and apob<sup>+/+38.9</sup> mice were fasted for 15 h and then injected with Triton WR-1339 but without gavage of lipid emulsion. Plasma TG levels, mainly reflecting hepatic origin, were similar at 2 h after Triton injection (450 ± 70 mg/dl for apob<sup>+/+38.9</sup> and 465 ± 63.6 for apob<sup>+/+38.9</sup>). Similar plasma TG levels between these two genotypes were also observed for the rest of experimental time points (data not shown). When these TG levels were subtracted from analogous TG levels of animals given an oral fat load, the differences reflected the contributions of intestinal absorption. These differences remained statistically significant between the two genotypes at the respective time points. These results indicate that intestinal lipoprotein secretion, in response to an acute bolus challenge, is impaired in the setting of this apoB truncation.

The mucosal TG level measured after administration of the lipid emulsion (no Triton WR-01399 given) was significantly higher in the apob<sup>+/38.9</sup> than in the apob<sup>+/+</sup> (Fig. 2B). The increase in the mucosal TG level in the apob<sup>+/+38.9</sup> mice suggests normal uptake and/or digestion of dietary TG in the heterozygotes, with a delay in the TG secretory step.

**RESULTS**

**Dietary intakes and body weights.** In the chronic feeding study, mean starting ages and body weights of the comparison groups were similar (Table 2). Food intakes also were similar (means ~4 g/day). Rates of weight gain were alike among the six dietary genotypic groups (Table 2).

**Fat balance.** Relative fractional fat absorption was similar among the apob<sup>+/+</sup>, apob<sup>+/+38.9</sup>, and apob<sup>+/38.9/38.9</sup> mice when fed the chow (Fig. 1A). However, relative fractional fat absorption was significantly lower in the apob<sup>+/+38.9</sup> and apob<sup>+/38.9/38.9</sup> mice compared with their wild-type controls when fed the HF diet (Fig. 1B). A further 25% reduction in relative fractional fat absorption was seen in apob<sup>+/+38.9</sup> compared with apob<sup>+/+38.9</sup> on the HF diet.

![Fig. 1. Fat balance study in apolipoprotein B (apoB)38.9 mice and controls on chow (A) or high-fat diets (B).](http://ajpgi.physiology.org/DownloadedfromG148 FAT ABSORPTION AND FATTY LIVER IN APOB<sup>+/38.9</sup> MICE)
This suggests that livers of the apob<sup>+/+38.9</sup> mice were unable to compensate sufficiently for the increased need to secrete more TG on an exogenous dietary fat load.

**Effects of dietary perturbations on hepatic FAS and SCD-1 mRNA levels.** The mRNA levels of two enzymes involved in fatty acid synthesis, FAS and SCD-1, were increased by the LF diet in the apob<sup>+/+</sup> and apob<sup>+/+38.9</sup>, relative to the chow diet (Fig. 4). The LF-induced increases in both FAS and SCD-1 were significantly higher in the apob<sup>+/+</sup> relative to apob<sup>+/+38.9</sup>. The HF diet significantly reduced mRNA levels of both transcripts only in the apob<sup>+/+</sup> but not in the apob<sup>+/+38.9</sup>.

**DISCUSSION**

**Impaired intestinal lipid absorption.** ApoB variants shorter than apoB48 (i.e., apoB27.6 and apoB38.9) are produced and secreted from the liver (9, 11). In addition, truncations shorter than apoB48 also are produced in the intestine (19). The fat-balance study, reflecting intestinal fat digestion and enterocyte uptake of dietary TG, demonstrated similar fat absorption coefficients among apoB38.9 heterozygotes, homozygotes, and wild-type controls, when all were fed the chow diet (Fig. 1A). This suggests that the capacity for intraluminal fat digestion and fat uptake into enterocytes may be sufficient even in the homozygotes, when low amounts of fat are ingested from the chow. However, when fed the HF diet, both the apob<sup>+/+38.9</sup> and apob<sup>38.9/38.9</sup> mice showed reduced fractional fat absorption (Fig. 1B), albeit the reduction was minimal in the heterozygotes.

By contrast, intestinal TG secretion rates, measured in Triton WR-1339 pretreated mice gavaged with a lipid emulsion and reflecting the rates of chylomicron secretion by enterocytes and their entry into the circulation, were significantly decreased in the heterozygotes and even more so in the homozygotes (Fig. 2A). In addition, more intestinal mucosal TG was accumulated in the apob<sup>+/+38.9</sup> than in the controls in response to an oral fat overload (Fig. 2B). These findings suggest near-normal digestion of dietary TG and uptake into the enterocytes of the heterozygotes but impaired intestinal lipoprotein secretion in response to the acute bolus challenge. Both enterocyte uptake and secretion of dietary, intestinal TG appeared to be impaired in the homozygotes, resembling the physiological impairment seen in abetalipoproteinemia (5). One adaptation to the defective intestinal fat absorption may involve the recruitment of additional segments of the small intestine. To the extent that this occurred, it was clearly insufficient to compensate for the apoB defect in the apoB38.9-defective animals.

Vitamin A oral fat-loading test shows normal fat absorption in FHBL subjects heterozygous for apoB mutations smaller than apoB48 (4). However, the FHBL group consisted of subjects with various apoB truncations. Larger variability in fat absorption indexes in the heterozygotes, probably due to subtle differences among the various lengths of truncated apoB, weakened statistical power in that study. In fact, the apoB31 heterozygote vomited on two occasions 12–14 h after eating the test meal, suggesting possible reduced dietary fat tolerance (4). In light of results from the present study, further investigation on fat absorption in FHBL subjects with well-defined apoB truncations is warranted.

**Diet effects on hepatic TG accumulation.** In the most common variety of fatty liver, obesity and insulin resistance somehow stimulate the hepatic synthesis of fatty acids sufficiently to overcome the adaptive capacities of the liver, resulting in the accumulation of hepatic TG (35). By contrast, fatty liver in the apoB-defective FHBL results from the limited capacities for lipid transport of the truncated apoB (9, 11) and from the secretion of less-than-expected amounts of apoB100 (25–30% normal rates rather than the expected 50% based on 1 functioning apoB100 allele) (1, 10, 13, 39). We tested the hypo-
esis that the apoB defects render the livers of FHBL mice more susceptible to dietary perturbation than the livers of controls. Direct testing was necessary because it would have been difficult to predict the results, because the liver seems to adapt to increased loads of TG. For example, the apoB mutation-induced accumulations of liver TG are accompanied by lower rates of endogenous hepatic fatty acid synthesis, mediated by the downregulation of hepatic FAS and SCD-1 (20). The apoB

\[
\text{apoB}^{+/38.9}
\]

mice accumulated additional hepatic TG on the LF diet when compared with the chow-fed group (Table 3). The apoB

\[
\text{apoB}^{+/+}
\]

mice accumulated even more hepatic TG on the HF diet compared with the chow-fed group (Table 3). In addition, the apoB-defective mice achieved higher hepatic TG levels than the

\[
\text{apoB}^{+/+}
\]

, and the diet-induced rises (ΔTG) were greater in the apoB38.9 mice than in the controls (Table 3).

The mRNA levels of hepatic FAS and SCD-1 were increased on LF but reduced on HF diet in the

\[
\text{apoB}^{+/+}
\]

 (Fig. 4), demonstrating that the two diets raised hepatic TG by different mechanisms. The HF diet did not significantly reduce the

\[
\text{apoB}^{+/+}
\]

, and the diet-induced rises (ΔTG) were greater in the apoB38.9 mice than in the controls (Table 3).

Table 3. Diet-induced differences in liver-triglycerides by genotype

<table>
<thead>
<tr>
<th>TG</th>
<th>Chow</th>
<th>LF</th>
<th>HF</th>
<th>ΔTG by genotypes</th>
<th>LF-chow</th>
<th>P</th>
<th>HF-chow</th>
<th>P</th>
</tr>
</thead>
</table>
| apoB

\[
\text{apoB}^{+/+}
\]

 | 87.9 ± 34.6 | 131 ± 34.1 | 146 ± 46.8 | 43.2 | <0.05 | 58.1 | <0.01 |
| apoB

\[
\text{apoB}^{+/+}
\]

 | 66.6 ± 25.0 | 81.2 ± 20.7 | 89.8 ± 29.1 | 14.6 | NS    | 23.2 | <0.05 |
| ΔTG by genotypes |        |         |        | 28.6 |        | 34.9 |        |

Values are means (SD) for the hepatic triglyceride (TG) concentrations (mg/g protein). Two-sample t-test was performed on between-diet-mean-difference from all mice on all diets [chow - apoB

\[
\text{apoB}^{+/+}
\]

., n = 13 (6 males and 7 females); chow - apoB

\[
\text{apoB}^{+/+}
\]

, n = 10 (6 and 4); LF - apoB

\[
\text{apoB}^{+/+}
\]

, n = 11 (6 and 5); HF - apoB

\[
\text{apoB}^{+/+}
\]

, n = 11 (6 males and 7 females). Results were averaged from both males and females because there was no gender effect. NS, not significant.

Fig. 3. Hepatic TG production in apoB38.9 mice and controls on the high-fat diet. Mice were fed the high-fat diet [apoB

\[
\text{apoB}^{+/+}
\]

, n = 6 (3 males and 3 females); apoB

\[
\text{apoB}^{+/+}
\]

, n = 4 (2 males and 2 females)] for 5 wk. Hepatic TG production was determined as described in MATERIALS AND METHODS. Values are means (SD). Significant differences in the TG levels between the genotypes are indicated by the letters a and b (P < 0.05).

Fig. 4. Effects of diets on hepatic mRNA levels of fatty acid synthase (FAS) and stearoyl-CoA desaturase 1 (SCD-1). Levels of mRNAs for hepatic FAS and SCD-1 were determined by quantitative real-time PCR from individual mice fed the chow diet (apoB

\[
\text{apoB}^{+/+}
\]

, 6 males and 7 females; apoB

\[
\text{apoB}^{+/+}
\]

, 6 males and 4 females), low-fat (LF) (apoB

\[
\text{apoB}^{+/+}
\]

, 7 males and 7 females; apoB

\[
\text{apoB}^{+/+}
\]

, 6 males and 3 females), or high-fat (HF) diet (apoB

\[
\text{apoB}^{+/+}
\]

, 7 males and 7 females; apoB

\[
\text{apoB}^{+/+}
\]

, 6 males and 5 females). Each value (arbitrary, relative to apoB

\[
\text{apoB}^{+/+}
\]

 fed the chow) represents means ± SD of individual determinations. The levels of statistical differences relative to the chow-fed group within each genotype or between LF-fed apoB

\[
\text{apoB}^{+/+}
\]

 and apoB

\[
\text{apoB}^{+/+}
\]

 are indicated by *P < 0.05 and **P < 0.01.
mRNA levels of either transcript in the apob\(^{+/8.9}\), probably due to their initial reduced expression levels on the chow diet. The LF diet was still able to increase hepatic FAS and SCD-1 mRNA levels in the apob\(^{+/8.9}\) mice, albeit to a lesser extent than those in the apob\(^{+/+}\), indicating insufficient adaptation to the LF diet in the apob\(^{+/8.9}\) mice. Recently, SCD-1 deficiency has been linked to increased fatty acid oxidation (27) and lower hepatic TG levels in mice (25). However, to the extent that hepatic fatty acid β-oxidation is reflected by plasma β-hydroxybutyrate concentrations, the greater susceptibility of the apob\(^{+/8.9}\) mice to accumulate more hepatic TG (compared with the apob\(^{+/+}\)) was not accounted for by differences in fatty acid oxidation, because plasma β-hydroxybutyrate did not differ across diets or genotypes (not shown).

Hepatic TG production in the apob\(^{+/8.9}\) remained impaired under the HF diet compared with the wild-type controls (Fig. 3), similar to that reported on the chow diet (9). Mice heterozygous for apoB27.6, a protein with even lower TG-transport capacity than apoB38.9 (11), developed more severe fatty liver under the same HF diet (data not shown). Thus results of the present study confirmed our hypothesis of increased susceptibility of FHBL mice with defective hepatic VLDL-TG transport capacity to develop more severe fatty liver under an increased need to secrete more hepatic TG.

In conclusion, mice heterozygous for apoB38.9 truncation with impaired hepatic TG secretion are more susceptible to diet-induced fatty liver despite reduced fat absorption capacity from the intestine. These feeding studies may have relevance for humans with apoB-defective FHBL in attempts to control the fat contents of their livers, but the clinical trials remain to be performed.

**ACKNOWLEDGMENTS**

We thank Tom Kitchens for excellent technical assistance. Present address for Nobuhiro Sakata: Division of Medical Informatics, Shinshu University School of Medicine, Shinshu University Hospital, 3–1–1 Asashi, Matsumoto, Nagano 390–8621, Japan.

**GRANTS**

This work was supported by grants from The Alan and Edith Wolf charitable fund and by National Institutes of Health (NIH) R37-HL-424460 and R01-HL-59513. Support from the following sources is greatly appreciated: the General Clinical Research Center (NIH Grant SM01RR-00036), the Diabetes Research Training Center (5P60DK-20579), the Clinical Nutrition Research Unit (DK-56341), the Digestion Disease Research Care Center (1P30DK-52574), HL-38180, and DK-56260 (to N. Davidson), R01-HL-50420 (to R. Ostlund), and R01-HL-52139 (to Z. Chen).

**REFERENCES**


