A mouse model of insulin resistance and its associated dyslipidemia was generated by crossing mice expressing human apolipoprotein B (apoB) with mice lacking only brown adipose tissue (BATless). On a high fat diet, male apoB/BATless mice became obese, hypercholesterolemic, hypertriglycerideremic, and hyperinsulinemic compared with control apoB mice. Fast performance liquid chromatography revealed increased triglyceride concentrations in intermediate density lipoprotein/low density lipoprotein (LDL) and reduced high density lipoprotein cholesterol concentrations. Inhibition of lipolysis by the drug, tetrathydrofiprostatin, demonstrated that very low density lipoprotein-sized particles were initially secreted. Metabolic studies employing Triton WR-1339 and either \([3H]glycerol\) or \([3H]palmitate\) showed that the hypertriglyceridemia in apoB/BATless mice was due to the increased synthesis and secretion of triglyceride. Furthermore, lipoprotein lipase and hepatic lipase activities were not defective. ApoB was also secreted at increased rates in the apoB/BATless mice. Similar levels of apoB mRNA in apoB and apoB/BATless mice indicated that apoB secretion was regulated post-transcriptionally. LDL receptor mRNA was increased in the apoB/BATless mice, indicating that the observed increase in apoB-lipoprotein secretion was not due to their decreased reuptake. Finally, mRNA levels of the large subunit of microsomal triglyceride transfer protein, a required component for very low density lipoprotein assembly, were not different between apoB and apoB/BATless mice. This rodent model should prove useful in exploring mechanisms underlying the regulation of apoB secretion in the context of insulin resistance.

Increased secretion of apolipoprotein B (apoB) has been associated with the hyperlipidemia that presents in a number of physiological disorders, including familial combined hyperlipidemia (1–4) and the dyslipidemia associated with insulin resistance (5, 6). As such, understanding the processes that regulate the secretion of apoB has been the subject of extensive clinical and molecular investigation.

Cell culture experiments have provided convincing data that the primary regulation of apoB secretion occurs post-translationally and that the intracellular degradation of apoB is an important determinant of the amount of the protein that is ultimately secreted (7–9). In several cell systems, the availability of intracellular triglyceride has been shown to affect the rates of apoB degradation and thus secretion. Specifically, in HepG2 cells, incubation with oleate led to the increased secretion of apoB into the medium by preventing the intracellular degradation of the protein (10). This stimulatory effect of oleate on apoB secretion has also been demonstrated in McARH-7777 cells, a rat hepatoma cell line (11); perfused livers from fasted rats (12); and livers from rats fed a high carbohydrate diet (13). In contrast, apoB secretion rates were unaltered by oleate availability in primary rat hepatocytes (14) and in perfused livers (12, 15) from fed rats.

We sought to create a mouse model that would allow for the in vivo investigation of mechanisms underlying the increased apoB secretion observed in humans with insulin resistance. Mice have distinctly different lipid profiles compared with humans with most cholesterol carried in HDL (16). Transgenic mice expressing human apoB100 display an accumulation of lipid in LDL (17) and have been used to investigate the regulation of apoB secretion by genetic (18) as well as dietary factors (19). Imposing insulin resistance on these mice was achieved by crossing them with mice selectively lacking brown adipose tissue as a result of genetic ablation (20). As a murine model of obesity and insulin resistance, mice lacking BAT exhibit peripheral insulin resistance, i.e. they have fewer insulin receptors and dampened insulin receptor kinase activity in fat and muscle as well as decreased expression of the glucose transporter, GLUT-4, in fat (21, 22). In this report, we show that imposing insulin resistance onto a mouse with human-like levels of apoB100 leads to increased synthesis of TG and post-transcriptional increases in the secretion of apoB100.
Experimental Procedures

Animals—Congenic human apoB transgenic mice of the C57BL/6J background were generated as described (18). BATless mice (20) were purchased from the Jackson Laboratory (Bar Harbor, ME). These mice were created in the FVB/N strain by using the promoter from a BAT-specific protein, uncoupling protein-1, to drive the expression of the A chain of diphtheria toxin. BATless mice were identified by PCR using sequences from the diphtheria toxin gene. The breeding of heterozygous apoB mice with heterozygous BATless mice resulted in the generation of F1 mice with four possible genotypes: those that expressed no transgene (wild type controls), those that expressed either apoB or diphtheria toxin, and those that had both transgenes (apoB/BATless).

All mice were maintained on a 12-h light/dark cycle (light cycle was 7 a.m. to 7 p.m.) and were weaned onto a Western-type diet (number 88137; Teklad Premier Laboratory Diets, Madison, WI) containing 21% (w/w) fat (polysaturated/saturated = 0.07), 0.15% (w/w) cholesterol, and 19.5% casein. The Western-type diet was free of sodium cholate.

Glucose and Insulin Tolerance Tests—Glucose tolerance tests were performed after a 4-h fast. Blood samples were obtained from the retroorbital plexus, plasma was isolated at 4 °C, and samples were immediately frozen at −70 °C. The ages of animals for any given experiment are specified under “Results.”

Lipid, Apolipoprotein, Glucose, and Insulin Determinations—Total plasma TG and cholesterol concentrations were measured using commercial kits (Roche Molecular Biochemicals) on a Hitachi automated spectrophotometer (model 705). Plasma human apoB levels were measured using a Beckman Array 360 nephelometer with modifications made for the assessment of mouse plasma (23). The antibody used in this assay does not measure mouse apoB. Glucose levels were measured using an enzymatic kit (number 315-100; Sigma-Aldrich). FFA levels were measured by colorimetry using a commercial kit (number 994-75409) from Wako Chemicals (Richmond, VA). Plasma insulin concentrations were measured by radioimmunoassay using a commercial kit (number SRI-13K) obtained from Linco Research (St. Charles, MO).

Glucose and Insulin Tolerance Tests—Glucose tolerance tests were conducted after an overnight fast. After a base-line blood collection, mice (n = 5–6 in each group) were injected intra-peritoneally with 15% glucose in a 0.9 NaCl solution (1 g of glucose/kg of body weight). Blood samples were obtained from the retro-orbital plexus, plasma was isolated at 4 °C, and samples were immediately frozen at −70 °C. The ages of animals for any given experiment are specified under “Results.”

Inhibition of Lipase Activities by Tetrahydrolipostatin Injection—Post-transcriptional Regulation of apoB in Vivo

To measure liver stores of TG, total liver lipids were extracted according to method modified from that of Polch et al. (29). Briefly, snap frozen liver tissues (~150 mg) were homogenized and extracted twice with a chloroform:methanol (2:1 v/v) solution. The organic layer was dried under nitrogen gas and resolubilized in chloroform. An aliquot of this Polch extraction was resuspended in an aqueous solution containing ~2% Triton X-100 (30) for the determination of TG mass. [14C]Triton was added to each sample before lipid extraction to account for the percentage of recovery, and final TG concentrations were adjusted accordingly. A second aliquot of the resolubilized sample was subjected to thin layer chromatography with hexane(diethyl ether:acetic acid (70:20:1) as the solvent to determine tritium counts associated with TG. Liver TG specific activities (dpm/mg) were calculated by dividing the counts associated with TG by TG mass. The rate of secretion of newly synthesized TG from the liver was estimated by dividing the [3H]TG dpm (either from [3H]glycerol or [3H]palmitate) that accumulated in plasma between 30 and 90 min after Triton by hepatic [3H]TG specific activity at 90 min.

Northern Blot Analysis—Total cellular RNA samples were isolated from the livers using guanidinium thiocyanate. RNA (10 μg) was separated on 6% formaldehyde, 0.8% agarose gels and then transferred to a nylon membrane. Hybridizations were carried out as described previously (31). A human cDNA probe containing 6 kilobases of human apoB sequences in exon 28 (31) was used to detect human apoB mRNA. A mouse β-actin riboprobe (Ambion Co., Austin, TX) was used in each experiment to normalize for variations in total RNA used. For quantification, autoradiograms were scanned with a densitometer.

RNA Probe Preparation and RNA Protection Assays—The probe used for mouse LDL receptor has been described previously (32). The RNA probe for mouse MTP was generated by amplification of the target gene using a PCR transfection kit (number SRI-13K) obtained from Linco Research (St. Charles, MO). The probe was labeled using an [32P]ATP transcription kit (Ambion Co., Austin, TX) and [32P]ATP (800 Ci/mmol).

Results.

"AAT TCC ATT GAA CCA GAA ATA TCA-3."

Base substitutions made in the MTP sequence to engineer an AAT TCC ATT GAA CCA GAA ATA TCA-3."

The probe was then used in an in vitro transcription kit obtained from Promega (Madison, WI) and [32P]ATP (800 Ci/mmol).

Either mouse β-actin or cyclophilin (Ambion Co., Austin, TX) was used in each experiment to normalize for variations in total RNA used. RNase protection assays were carried out as described previously (32). Briefly, total cellular RNA (10 μg) was used to perform a probe in hybridization buffer (30 μl) and incubated at 45 °C overnight. The next day, 20 units of RNase T2 (Life Technologies, Inc.) was added to the mix. After incubation at 37 °C for 2 h, RNase was removed by phenol extraction, and protected RNA fragments were ethanol-precipitated and resuspended in 5 μl of loading buffer (95% formamide, 0.05% xylene cyanol, 0.05% bromphenol blue, 20 μl EDTA). Protected frag-

2 P. Siri, H. N. Ginsberg, and L.-S. Huang, unpublished data.
mice (\textit{H11005} i.e. \textit{p/\textit{H11021}}) counter. Protected RNA fragments were cut and counted in a liquid scintillation counter.

22 weeks, apoB/BATless and BATless mice averaged 17–20 fasting the mice from 8 a.m. to 4 p.m. were stable throughout this period. ApoB and WT mice were tested by Student’s \textit{t} test. The response to glucose and insulin challenges was measured by determining the areas under the curve, and the insulin resistance was assessed by Student’s \textit{s} test. The response to glucose and insulin challenges was measured by determining the areas under the curve, and the significant differences between the apoB and apoB/BATless mice were tested by Student’s \textit{t} test.

RESULTS

Male apoB/BATless Mice Are Obese, Insulin-resistant, and Dyslipidemic—To generate an insulin-resistant mouse model with human-like dyslipidemia, we crossed transgenic mice overexpressing human apoB100 with mice selectively lacking brown adipose tissue by genetic ablation. This cross led to the generation of mice with either (WT), either (apoB or BATless), or both transgenes (apoB/BATless). Based on data indicating that female apoB/BATless mice were not insulin-resistant, only results from male mice are presented. On the Western type diet, all four groups of mice gained weight steadily (n = 17–27 in each group) (Fig. 1). By 10 weeks of age and at each time point thereafter, BATless and apoB/BATless mice were significantly heavier than apoB and WT mice (p < 0.0001). At 22 weeks, apoB/BATless and BATless mice averaged ~60 g. ApoB and WT mice were ~45 g.

Plasma cholesterol and TG concentrations obtained after fasting the mice from 8 a.m. to 4 p.m. were stable throughout the experimental period. Representative data at 19 weeks are shown in Fig. 2 (n = 9–15 in each group). WT and BATless mice had similar cholesterol levels at 176 ± 21 and 152 ± 24 mg/dl, respectively. ApoB mice showed the expected response to the Western type diet (33) with an increase in plasma cholesterol levels to 321 ± 35 mg/dl (p < 0.001 versus WT and BATless). In apoB/BATless mice, cholesterol concentrations were even greater (466 ± 98 mg/dl) (p < 0.001 versus the other three genotypes). A similar pattern was observed when we measured fasting plasma TG concentrations (Fig. 2B). ApoB mice had elevated TG levels relative to both WT and BATless mice (137 ± 27 mg/dl \textit{versus} 67 ± 30 and 34 ± 9 mg/dl, respectively; p < 0.001 for both comparisons). ApoB/BATless mice had even higher plasma TG levels (271 ± 61 mg/dl) (p < 0.001 versus the other three genotypes).

Fasting plasma glucose, insulin, and FFA levels were also determined (n = 9–14 in each group) (Table I). Plasma glucose levels were not consistently different among the four groups of mice, whereas plasma insulin levels were significantly higher in mice in which BAT was ablated. The hyperinsulinemia observed in the BATless and apoB/BATless mice relative to apoB and WT mice was indicative of insulin resistance. To confirm insulin resistance in our model of interest, apoB/BATless mice were subjected to glucose and insulin tolerance tests with apoB mice as controls. ApoB/BATless mice were significantly impaired in their ability to clear exogenously administered glucose compared with apoB mice (Fig. 3A). Insulin tolerance tests further revealed a greater resistance to insulin in apoB/BATless mice compared with apoB mice (Fig. 3B). Plasma FFA concentrations did not vary significantly among the four groups after an 8-h fast. Because fatty acids become a more important fuel source during starvation, we extended the fasting period to an overnight 16-h fast in an attempt to induce an increase in fatty acid concentrations and detect potential differences that could not be seen with the daytime 8-h fast. Although we observed the expected increase in plasma concentrations of FFA with a longer fast, there were no differences in FFA concentrations according to genotype.

ApoB/BATless Mice Display a Human-like Dyslipidemia—The sizes and relative concentrations of lipoprotein particles secreted by apoB/BATless and apoB mice were determined by FPLC (n = 4–6 in each group). Compared with the apoB mice, apoB/BATless mice had a more heterogeneous distribution of cholesterol in the IDL/LDL region, with more cholesterol in a size range that was consistent with the presence of smaller LDL (Fig. 4A). Interestingly, apoB/BATless mice also showed a significant reduction of HDL cholesterol relative to apoB mice.

![FIG. 1. ApoB/BATless and BATless mice gained more weight than apoB and WT mice.](http://www.jbc.org/)

![FIG. 2. ApoB/BATless and apoB mice were hyperlipidemic compared with BATless and WT mice.](http://www.jbc.org/)

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*Plotted are the mean body weights of mice from WT, BATless, apoB, and apoB/BATless backgrounds. WT and BATless mice were subjected to glucose and insulin tolerance tests with apoB mice as controls. ApoB/BATless mice were significantly impaired in their ability to clear exogenously administered glucose compared with apoB mice (Fig. 3A). Insulin tolerance tests further revealed a greater resistance to insulin in apoB/BATless mice compared with apoB mice (Fig. 3B). Plasma FFA concentrations did not vary significantly among the four groups after an 8-h fast. Because fatty acids become a more important fuel source during starvation, we extended the fasting period to an overnight 16-h fast in an attempt to induce an increase in fatty acid concentrations and detect potential differences that could not be seen with the daytime 8-h fast. Although we observed the expected increase in plasma concentrations of FFA with a longer fast, there were no differences in FFA concentrations according to genotype.

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*ApoB/BATless Mice Display a Human-like Dyslipidemia*—The sizes and relative concentrations of lipoprotein particles secreted by apoB/BATless and apoB mice were determined by FPLC (n = 4–6 in each group). Compared with the apoB mice, apoB/BATless mice had a more heterogeneous distribution of cholesterol in the IDL/LDL region, with more cholesterol in a size range that was consistent with the presence of smaller LDL (Fig. 4A). Interestingly, apoB/BATless mice also showed a significant reduction of HDL cholesterol relative to apoB mice.
The distribution of TG among lipoprotein subfractions was similar in both groups of mice (Fig. 4B), with quantitatively much more TG seen in the IDL/LDL region in apoB/BATless mice, a finding consistent with the differences observed in plasma TG levels.

Despite the increased IDL/LDL TG in apoB/BATless mice as compared with apoB mice, VLDL-TG was not present. Because rapid rates of in vivo lipoprotein TG lipolysis in mice (34) may have limited our ability to detect nascent VLDL-TG by FPLC, we conducted experiments to determine whether VLDL-sized particles were initially secreted in the apoB/BATless mice.

THL inhibits LPL and HL activities by binding to their active sites (35). We injected apoB/BATless mice (n/H110052) intravenously with THL and collected blood samples after 2 h. Plasma TG levels were increased to 596 and 627 mg/dl in these two mice. Fig. 5 shows the altered lipid distribution in pooled plasma from the apoB/BATless mice after the administration of THL. A peak of TG and cholesterol in the VLDL size range was clearly demonstrated (compare with Fig. 4). The lipid levels of apoB/BATless mice (n/H110052) injected with Me2SO alone did not change, and the FPLC profiles showed no redistribution of either TG or cholesterol (data not shown). These results indicated that under normal conditions, nascent VLDL were initially secreted in apoB/BATless mice, but these particles were subjected to rapid lipolysis, resulting in the accumulation of TG in IDL/LDL particles.

The Hypertriglyceridemia Observed in apoB/BATless Mice Is Due to the Increased Synthesis and Secretion of VLDL Triglycerides—To better understand the basis of the increased plasma TG levels in apoB/BATless mice, TG secretion rates were determined at 22 weeks using Triton WR 1339, a surfactant that

**TABLE I**

Plasma glucose, insulin, and fatty acid concentrations in male mice

| Glucose (8-h fast) | Insulin (8-h fast) | FFA (8-h fast) | FFA (16-h fast) |
|-------------------|-------------------|---------------|---------------|
| mg/dl             | ng/ml             | mEq/liter     | mEq/liter     |
| WT                | 214 ± 104         | 2.07 ± 1.80   | 0.46 ± 0.12   | 0.56 ± 0.11 |
| BATless           | 225 ± 55          | 8.22 ± 9.04a  | 0.34 ± 0.09   | 0.54 ± 0.13 |
| ApoB              | 246 ± 94          | 1.90 ± 2.07   | 0.46 ± 0.11   | 0.58 ± 0.06 |
| ApoB/BATless      | 224 ± 69          | 4.37 ± 2.04a  | 0.46 ± 0.15   | 0.59 ± 0.15 |

a Subsequent t tests showed significantly higher insulin levels in BATless and apoB/BATless mice compared to apoB and WT mice.
coats lipoprotein particles and inhibits their lipolysis. With lipoprotein catabolism suppressed, the increase in plasma TG over time is indicative of the rate at which TG is being secreted from the liver (26). Fig. 6 shows the increase in TG concentrations over 90 min in apoB versus apoB/BATless mice after the intravenous administration of Triton (n = 21–22 in each group). The increase in TG between 30 and 90 min was found to be twice as high in apoB/BATless versus apoB mice (382 ± 76 versus 191 ± 103 mg/dl; p < 0.001). The calculation of TG secretion as a rate showed that apoB/BATless mice secreted 2.5 times the TG secreted by apoB mice (12.3 ± 2.6 versus 4.9 ± 2.9 mg/h for apoB/BATless and apoB mice, respectively; p < 0.001).

Similar turnover studies in BATless and WT mice (n = 6 in each group) revealed TG secretion rates comparable with apoB/BATless and apoB mice, respectively (10.2 ± 4.6 and 4.9 ± 1.5 mg/hour for BATless and WT, respectively). These observations were noteworthy given the significantly lower plasma levels of TG in WT and BATless mice compared with their littersmates carrying the human apoB transgene (Fig. 2B).

To determine whether the greater TG secretion rate in apoB/BATless mice was due to increased de novo synthesis of TG, additional turnover experiments using either [3H]glycerol or [3H]palmitate in addition to Triton WR 1339 were conducted. Because increased liver TG stores in apoB/BATless versus apoB mice could dilute the pool of labeled, newly synthesized TG available for secretion, we also measured liver TG concentrations and specific activities of [3H]TG in apoB/BATless and apoB mice at the end of the Triton secretion studies.

The results of experiments in 22–26-week-old male mice (n = 6–7 per group) using [3H]glycerol as the tracer for newly synthesized TG are presented in Table II. Significantly more intracellular TG was found in the livers of apoB/BATless compared with apoB mice (411 ± 95 versus 218 ± 138 µg/mg cellular protein). This increased “cold” pool of triglyceride resulted in a lower specific activity of [3H]TG in apoB/BATless mice, therefore, could not be taken as a direct reflection of VLDL secretion rates, because the pools from which the VLDL was derived were not labeled equivalently in the two groups of mice. To calculate VLDL-TG secretion rates, the appearance of labeled TG in plasma VLDL in apoB/BATless versus apoB mice was derived were not labeled equivalently in the two groups of mice. To calculate VLDL-TG secretion rates, the appearance of labeled TG in plasma VLDL in apoB/BATless versus apoB mice was measured for total cholesterol (A) or triglyceride (B) concentrations and expressed as µg/fraction.

**Fig. 5.** THL inhibition of lipolysis revealed VLDL-sized particles in apoB/BATless mice. Male apoB/BATless mice (n = 2) fasted for 4 h were injected intravenously with the lipase inhibitor, THL, and blood samples were taken 2 h after injection. Pooled plasma samples (200 µl) were subjected to FPLC analysis. Each FPLC fraction was measured for total cholesterol (A) or triglyceride (B) concentrations and expressed as µg/fraction.

**Fig. 6.** ApoB/BATless mice had increased in vivo triglyceride secretion rates. Male apoB (n = 22) and apoB/BATless mice (n = 21) were given Triton WR1339 intravenously after a 4-h fast. Mice were bled prior to injection and at 30, 60, and 90 min post-injection. Plasma samples from each time point (x axis) were measured for TG levels, and these data are plotted on the y axis. The data from male apoB mice are shown using solid circles. The data from male apoB/BATless mice are given by the open circles.

**ApoB/BATless Mice Have Increased in Vivo apoB Secretion**

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**References:**

1. Post-transcriptional Regulation of apoB in Vivo

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**Fig. 5.** THL inhibition of lipolysis revealed VLDL-sized particles in apoB/BATless mice. Male apoB/BATless mice (n = 2) fasted for 4 h were injected intravenously with the lipase inhibitor, THL, and blood samples were taken 2 h after injection. Pooled plasma samples (200 µl) were subjected to FPLC analysis. Each FPLC fraction was measured for total cholesterol (A) or triglyceride (B) concentrations and expressed as µg/fraction.

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Liver triglyceride concentrations and specific activities and VLDL-TG secretion rates of newly synthesized TG in apoB and apoB/BATless mice

Means and standard deviations from 22–26-week-old male mice are presented. Liver TG specific activities were calculated by dividing the counts associated with liver TG by liver TG mass. Plasma VLDL-TG values represent the difference in labeled VLDL-TG in plasma between 30 and 90 min. VLDL-TG secretion rates of newly synthesized TG were calculated by dividing plasma VLDL-TG (90–30 minutes) by the specific activity of the liver at 90 min.

|          | Liver weight | Intraobserver TG/ | Liver TG specific | Plasma VLDL-TG | VLDL-TG secretion rate |
|----------|--------------|-------------------|-------------------|----------------|------------------------|
|          | g            | cellular protein   |                   | dpm/mg          | dpm/100 μl/100 g      | mg/100 μl/h            |
| ApoB     | 6            | 1.83 ± 0.77        | 218 ± 138         | 24,313 ± 9,549  | 10,826 ± 2,754         | 0.98 ± 0.35            |
| ApoB/BATless | 7     | 4.37 ± 0.63×       | 411 ± 95°         | 13,197 ± 4,122° | 12,184 ± 5,483         | 1.80 ± 0.84×            |

× Significant differences (p < 0.05) relative to apoB mice by Student’s t test.

Rates—Elevated TG concentrations may be due either to the secretion of more apoB-containing lipoproteins or to the secretion of the same number of apoB-lipoproteins carrying more TG per particle. To differentiate between these possibilities, plasma levels of apoB and rates of apoB secretion were determined. Fasting plasma human apoB levels in apoB/BATless mice (n = 12) were 60% greater compared with apoB mice (n = 14) (251 ± 42 mg/dl versus 157 ± 40 mg/dl, respectively; p < 0.05). Using Triton WR 1339 and 35S-methionine to label newly synthesized proteins, VLDL-apoB100 and apoB48 was shown to accumulate in plasma at significantly higher rates in apoB/BATless mice compared with apoB mice (Fig. 7). It should be noted that this method does not discriminate between mouse and human apoB but is compatible with the marked increases in the plasma levels of human apoB demonstrated by the immunonephelometric method specific to human apoB proteins.

Experiments using isolated VLDL samples and whole plasma showed similar results, indicating again that the apoB/BATless mice had increased rates of VLDL secretion. The results of quantitative densitometry from studies using whole plasma are given in Table III. At the 2-h time point, there was approximately three to four times more labeled apoB100 in plasma from apoB/BATless mice compared with apoB mice (7.93 ± 3.96 versus 1.97 ± 1.17 pixels, respectively; p = 0.03). Apo B Secretion in apoB/BATless Mice Is Regulated Post-transcriptionally—Greater rates of apoB secretion could have resulted from increased transcription of the apoB gene and subsequent increases in apoB synthesis or, alternatively, decreased intracellular degradation of constitutively synthesized apoB (8, 36). To further define the mechanism underlying the increased apoB secretion in apoB/BATless mice, we compared hepatic human apoB mRNA levels in apoB/BATless and apoB mice by Northern blot analysis. Representative samples of human apoB mRNA levels are shown in Fig. 8. The levels of human apoB mRNA in male apoB/BATless mice were similar to male apoB control mice (82 ± 17%, n = 6 versus 100 ± 29%, n = 8). These data indicate that the increased secretion of apoB in apoB/BATless mice was regulated at the post-transcriptional level.

The net secretion of apoB into plasma may be affected by the reuptake of newly secreted lipoprotein particles still associated with the space of Disse (37). To test whether the observed difference in the secretion rate of apoB into plasma between apoB andapoB/BATless mice was mediated by differences in hepatic LDL receptor activity, message levels of the LDL receptor were measured. The latter has been shown to correlate well with receptor activity (38). LDL receptor mRNA levels were significantly increased in apoB/BATless versus apoB mice (Fig. 9). This result indicates that the increased rates of appearance of plasma apoB in apoB/BATless mice compared with apoB controls were due to increases in secretion rather than reduced lipoprotein particle reuptake.

Finally, to determine whether the critical lipid transfer protein, MTP, was involved in the regulation of apoB secretion in this model, liver MTP mRNA levels were measured by RNase protection assay (Fig. 9). No differences in MTP message were detected between apoB/BATless and apoB mice.

DISCUSSION

Recent murine models of lipodystrophy have presented evidence that the absence or near absence of total fat can lead to insulin resistance and hepatic steatosis, metabolic derangements that have usually been associated with excess fat (39–41). The common link between these lipodystrophic mouse models and murine models of obesity has been proposed to be an inability of adipose tissue to function normally as a site for fat storage, which leads to a shift in the lipogenic burden to the liver (42). These lipodystrophic mouse models support the concept that the role of fat in overall energy homeostasis is more complicated than that of an inert storage depot.

Interestingly, the selective loss of brown fat has also been demonstrated to lead to an insulin-resistant and obese phenotype (20), albeit through a different mechanism. Mice with genetic ablation of BAT presumably become obese because of the loss of this thermogenic tissue and the subsequent development of increased energy efficiency. We chose to use the latter model with selective ablation of thermogenic adipocytes to create a novel murine model in which the regulation of apoB could be studied in the context of obesity and insulin resistance.

Crossing mice expressing human apoB100 with mice lacking brown adipose tissue led to the generation of four possible phenotypes, i.e. WT, apoB, BATless, and apoB/BATless. BATless and apoB/BATless mice were significantly hyperinsulineemic compared with apoB and WT mice. Insulin resistance in apoB/BATless mice relative to apoB mice was confirmed by both glucose and insulin tolerance tests. Interestingly, insulin inhibits VLDL secretion acutely in cultured hepatocytes and in normal humans, despite increases in TG synthesis (43–47). However, insulin-resistant patients do not exhibit this insulin-induced inhibition of apoB secretion (44, 48). Resistance to the insulin-induced inhibition of apoB secretion has also been doc-
ability of the BATless mice to maintain normal levels of plasma TG despite very high secretion rates is indicative of very efficient clearance of their circulating native apoB-lipoproteins. Based on these observations, one might speculate that the final VLDL and LDL levels in humans with insulin resistance and increased VLDL secretion will be impacted significantly by either the affinity of their apoB for LDL receptors (i.e. defective apoB) or, more commonly, the number of LDL receptors present.

The apparent gene-gene interaction (i.e. human apoB and toxigene ablation of BAT) was not limited to effects on lipid parameters. Plasma apoB levels were also increased in apoB/BATless mice versus apoB mice, suggesting that more particles were being secreted by the livers of apoB/BATless mice. When we characterized the TG distribution in lipoprotein subfractions by FPLC in our model, we were surprised to find most of the TG in IDL and LDL. Limitation of TG-rich particles to these lipoprotein classes might have been due to the high rate of apoB synthesis inherent in our apoB transgenic mice, resulting in the secretion of smaller apoB-lipoproteins. Alternatively, because lipolysis is very efficient in mice, the TG in any VLDL-sized particles initially secreted might have been subject to rapid hydrolysis, leading to the rapid conversion of VLDL to IDL and/or LDL. Indeed, when we inhibited LPL activity in apoB/BATless mice with the intravenous injection of the competitive antagonist, THL (35), we were able to demonstrate an accumulation of VLDL-TG and VLDL-cholesterol. The apoB/BATless mouse therefore appears to be a valid model for the obesity, the Zucker fatty rat (50). This resistance to regulation of apoB synthesis by insulin was also implied in a hamster model in which hypertriglyceridemia was associated with increased apoB secretion rates (51). Our results are consistent with these previous models of chronic hyperinsulinemia and insulin resistance.

Lipid concentrations in apoB mice were significantly increased compared with BATless and WT animals. Even greater plasma TG and cholesterol concentrations were observed in apoB/BATless mice versus apoB mice, suggesting a synergistic relationship between the expression of human apoB and the obese and insulin-resistant BATless phenotype. The hyperlipidemia in apoB and apoB/BATless mice may be explained in part by the decreased affinity of the mouse LDL receptor for human apoB100 (52). Human apoB100 appears to be cleared less efficiently from plasma than mouse apoB. In fact, we observed comparable TG secretion rates in BATless and apoB/BATless mice, even though plasma levels of TG in BATless mice were one-fourth the levels observed in apoB/BATless mice and not different from the levels observed in WT mice. The

![Image](http://www.jbc.org/)

**FIG. 8.** Human apoB mRNA levels were similar in apoB and apoB/BATless mice. Total cellular RNA samples isolated from the livers of male apoB/BATless and apoB mice were subjected to Northern blot analysis. Representative samples of mRNA levels for male apoB and apoB/BATless mice are shown. The blots were hybridized with a human apoB100 cDNA probe (top panel) for human apoB mRNA levels or a mouse β-actin probe (bottom panel) for normalization of sample loading.

**TABLE III**

**In vivo apoB secretion in apoB and apoB/BATless mice**

In each experiment, two male mice of each genotype were assayed. Autoradiograms were scanned with a densitometer and analyzed using an imaging program (NIH Image 1.57). The data presented are taken from the 120-min time point and are expressed in the arbitrary unit, pixel. The nonparametric Wilcoxon rank test was used to compare apoB100 and apoB48 secretion in apoB versus apoB/BATless mice.

| Experiment number | ApoB100 | ApoB/BATless | ApoB | ApoB/BATless |
|-------------------|---------|-------------|------|-------------|
| 1                 | 1.08    | 2.22        | 0.85 | 2.53        |
| 2                 | 0.50    | 5.47        | 1.73 | 7.49        |
| 3                 | 2.90    | 7.15        | 0.98 | 3.00        |
| 4                 | 2.47    | 8.05        | 1.33 | 5.70        |
| 5                 | 1.34    | 12.23       | 4.39 | 13.88       |
| 6                 | 3.50    | 12.46       | 2.58 | 5.19        |
| Mean              | 1.97    | 7.93        | 1.98 | 6.30        |
| S.D.              | 1.17    | 3.96        | 1.34 | 4.14        |
| p value           | 0.03    | 0.03        |      |             |
assembly and secretion of VLDL is regulated post-transcriptionally in these mice, and they support data generated from cultured liver cells that show the primary regulatory step in the determination of apoB secretion to be at the post-translational level (7–9).

Based on tissue culture experiments, it has been proposed that the rate of VLDL secretion may also be modulated by the level of LDL receptor activity, because increased LDL receptor activity can lead to increased reuptake of newly secreted lipoprotein particles before their secretion into plasma (37). Recently, reuptake mediated by the LDL receptor was shown to be a significant determinant of the net output of VLDL in a hyperlipidemic mouse model (53). Mice with hepatic overexpression of SREBP-1a, a transcription factor known to stimulate cholesterol and fatty acid biosynthesis (54), had lipid-engorged livers but normal plasma levels of TG and cholesterol. These SREBP-1a transgenic mice had very high levels of LDL receptors, and it was only when they were crossed with animals with a targeted disruption of the LDL receptor gene that plasma lipid levels were raised (55). This result indicated that the LDL receptor could play an important role in the net secretion of apoB-containing lipoproteins into plasma. Another study in primary hepatocytes from LDL receptor knockout mice (56) corroborated the findings from the SREBP-1a/LDL receptor knockout mice. Hepatocytes from LDL receptor knockout mice were found to secrete apoB at twice the rate of control hepatocytes. In contrast, when we measured hepatic LDL receptor mRNA levels in our animals, we found a modest but significant increase in LDL receptor mRNA in apoB/BATless versus apoB mice. The 2–3-fold increase in apoB secretion rates in the apoB/BATless mouse could not, therefore, be explained by decreased LDL receptor activity.

In cultured liver cells, the post-translational regulation of apoB secretion actually begins co-translationally when a “decision” is made that determines whether apoB is ubiquinated and degraded by the proteasome (57, 58) or completes translocation and assemblies into a lipoprotein particle (59). A critical player in this early post-translational regulation is the heterodimer, MTP, which binds to the amino terminus of apoB and is required for lipoprotein assembly (60). Genetic deficiency of the large subunit of MTP has been shown to be the basis for the lack of plasma apoB in patients with abetalipoproteinemia (61). In mice with adenovirus-induced hepatic overexpression of MTP, increased secretion of VLDL-TG and VLDL-apoB was observed (62). On the other hand, MTP knockout mice showed a marked reduction in plasma cholesterol, particularly LDL cholesterol, and apoB (63) because of the significantly impaired hepatic secretion of apoB-containing lipoproteins (64). In Watanabe-heritable hyperlipidemic rabbits, a model for human hypercholesterolemia (65), treatment with MTP inhibitors led to the decreased assembly and secretion of apoB-containing lipoproteins. Importantly, MTP mRNA and protein levels were both increased in a hamster model of insulin resistance and increased VLDL secretion (51). However, we did not observe differences in mRNA levels of MTP in apoB/BATless versus apoB mice as determined by RNase protection assay. This makes it unlikely that MTP played an important role in the regulation of apoB secretion in our model.

The apoB/BATless mouse exhibited hepatomegaly as well as liver steatosis. Although increased hepatic lipid availability presumably drives net VLDL output in this model, the mechanisms leading to the increased hepatic lipid stores remain unclear. One cause of increased hepatic lipid stores may be increases in plasma flux of FFA to the liver that act as additional substrate. Consistent elevations in plasma-free fatty acid concentrations, however, were not observed in apoB/BATless mice versus the other three genotypes. Although plasma FFA concentrations in apoB/BATless versus apoB mice were significantly elevated at 13 weeks (0.52 ± 0.09 versus 0.39 ± 0.12 meq/liter for apoB/BATless and apoB mice, respectively; p = 0.03), no differences were seen at 19 weeks (Table I). Prolongation of the fasting period from 8 to 16 h also did not induce differences in fatty acid concentrations between genotypes (Table I). As a static measurement, plasma concentrations of free fatty acids may not accurately represent the dynamic situation. Whether apoB/BATless mice have increased FFA flux relative to apoB mice remains to be determined.

Of interest, FPLC analysis of lipid distribution also revealed a reduction in HDL cholesterol specific to apoB/BATless mice. In contrast to humans where cholesterol is mostly contained in LDL, wild type mice carry the majority of their cholesterol in HDL. The overexpression of human apoB in mice leads to an elevation of lipid in LDL, so that transgenic apoB mice assume a more atherogenic lipid profile (66). That a reduction of HDL cholesterol was seen in apoB/BATless mice compared with apoB mice suggests a role for insulin resistance in the determination of HDL cholesterol concentrations in this model. In support of this notion, female apoB/BATless mice who were not insulin-resistant did not show a similar reduction in HDL cholesterol.2 Additionally, reduced HDL concentrations in apoB/BATless mice are noteworthy because mice lack cholesterol ester transfer protein (67), a protein known to mediate the exchange of VLDL-TG for HDL cholesterol in humans. This suggests that alternative mechanisms for the regulation of HDL cholesterol may be important in insulin-resistant states. Recently, peroxisome proliferator-activated receptor α and γ agonists have been shown to be involved in the up-regulation of ATP-binding cassette A1 gene expression (68, 69). In addition, insulin-resistant rhesus monkeys treated with a peroxisome proliferator-activated receptor δ agonist (70) exhibited marked increases in HDL cholesterol. These data support the hypothesis that reverse cholesterol transport may be impaired in the apoB/BATless mouse.

The apoB/BATless mouse presents an interesting model for future study. We have shown that the regulation of apoB secretion in this mouse occurs post-transcriptionally and that this regulation is not associated with changes in mRNA expression of the LDL receptor or MTP. The apoB/BATless mouse is also unique in that it presents with many of the abnormalities of what has come to be known as the insulin resistance or metabolic syndrome (71), that is, obesity, insulin resistance, hypertriglyceridemia, reduced HDL cholesterol levels, and smaller, more dense LDL. Exploitation of this rodent model should allow us to explore mechanisms involved in the regulation of both apoB secretion per se and the impact of insulin resistance.

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