Hepatic triglyceride contents are genetically determined in mice: results of a strain survey

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Hepatic triglyceride contents are genetically determined in mice: results of a strain survey. Am J Physiol Gastrointest Liver Physiol 288: G1179–G1189, 2005. First published December 9, 2004; doi:10.1152/ajpgi.00411.2004.—To assess whether genetic factor(s) determine liver triglyceride (TG) levels, a 10-mouse strain survey of liver TG contents was performed. Hepatic TG contents were highest in BALB/cByJ, medium in C57BL/6J, and lowest in SWR/J in both genders. Ninety and seventy-six percent of variance in hepatic TG in males and females, respectively, was due to strain (genetic) effects. To understand the physiological/biochemical basis for differences in hepatic TG among the three strains, studies were performed in males of the BALB/cByJ, C57BL/6J, and SWR/J strains. In vivo hepatic fatty acid (FA) synthesis rates and hepatic TG secretion rates ranked BALB/cByJ ≈ C57BL/6J > SWR/J. Hepatic 1-14C-labeled palmitate oxidation rates and plasma β-hydroxybutyrate concentrations ranked in reverse order: SWR/J > BALB/cByJ ≈ C57BL/6J. After 14 h of fasting, plasma-free FA and hepatic TG contents rose most in BALB/cByJ and least in SWR/J. β-Hydroxybutyrate concentrations rose least in BALB/cByJ and most in SWR/J. Adaptation to fasting was most effective in SWR/J and least in BALB/cByJ, perhaps because BALB/cByJ are known to be deficient in SCAD, a short-chain FA oxidizing enzyme. To assess the role of insulin action, glucose tolerance test (GTT) was performed. GTT-glucose levels ranked C57BL/6J > BALB/cByJ ≈ SWR/J. Thus strain-dependent (genetic) factors play a major role in setting hepatic TG levels in mice. Processes such as FA production and hepatic export in VLDL on the one hand and FA oxidation on the other, explain some of the strain-related differences in hepatic TG contents. Additional factor(s) in the development of fatty liver in BALB/cByJ remain to be demonstrated.

fatty liver; genetics of hepatic steatosis; lipogenesis; fatty acid oxidation; very low-density lipoprotein

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) is highly prevalent (9, 20). Although it resembles alcoholic fatty liver histopathologically, NAFLD, by definition, develops in the absence of alcohol abuse (9). The majority of patients with NAFLD are affected with various aspects of the metabolic syndrome, i.e., obesity, hypertension, insulin resistance, Type 2 diabetes mellitus, and hyperlipidemia (4). In the setting of insulin resistance, hepatic lipogenesis may be stimulated sufficiently to overcome the adaptive capacities of the liver to secrete more VLDL and result in the accumulation of triglycerides (TG) in the liver (33). On the other hand, insulin resistance may be the consequence of chronic lipid accumulation in insulin-sensitive tissues. For example, muscle- and liver-specific overexpression of lipoprotein lipase causes TG accumulation in muscle and liver as well as impairment of insulin signaling and action in the respective tissues (17). A strong relationship between fatty liver and insulin resistance also is established in patients (26, 27).

NAFLD poses a major health problem because it may lead to steatohepatitis, cirrhosis, and liver failure. The pathophysiological mechanisms by which steatosis may progress to steatohepatitis, fibrosis, and cirrhosis remain unclear, but steatosis appears to be an essential precursor (10). Thus controlling hepatic TG levels may be important in reducing the risk of developing chronic liver disease.

Fatty liver is due to an imbalance between the availability of hepatic TG for export and the disposal system of the liver (1, 20, 36). Both genetic and environmental factors are likely to be important. The major sources of hepatic fatty acids (FA) for TG assembly are those synthesized de novo in the liver and free FAs (FFA) derived from plasma. Genes involved in de novo lipogenesis are controlled by sterol regulatory element-binding protein (SREBP)-1c (40), a mediator of both nutritional stimulus and insulin action in hepatic lipogenesis (16). Mice overexpressing SREBP-1a develop severe fatty liver due to overproduction of cholesterol and FAs (32). Elevated levels of FFA in plasma, such as those in fasting, increase delivery of FFA to the liver, which may cause excessive hepatic TG accumulation despite an accompanying increased FA oxidation (15).

The major disposal routes for hepatic FAs and TG are composed of the export of hepatic TG via VLDL (39) and the oxidation of component FAs in mitochondria, peroxisomes, and microsomes (41). Here too, both genetic and environmental factors are important. For example, the impaired capacity for FA β-oxidation on fasting in peroxisome proliferator-agonist receptor (PPAR)-α null mice results in the accumulation of hepatic TG (18). A defective VLDL transport system that impairs hepatic lipid-exporting capacity also may result in fatty liver. This is seen in abetalipoproteinemia, caused by mutations in microsomal triglyceride transfer protein that is essential in the assembly of chylomicrons and VLDL particles (37), and in familial hypobetalipoproteinemia due to truncation-specifying mutations in apolipoprotein (apo)B, the indispensable structural protein in the formation and secretion of VLDL (6, 31, 35).

Thus the major genes for developing fatty liver, identified to date, are selected genes in the FA synthetic and oxidative pathways and in the VLDL export system. However, modifier genes (22, 23) as yet not identified may be contributing to the development of steatosis. This is suggested by the finding that engineered apoB gene mutation-bearing mice (e.g., apoB+/−/38.9 and apoB+/−/27.6 heterozygotes) produced on mixed C57BL/
6JX129/SVJ genetic backgrounds manifest significantly elevated hepatic TG on average, but interindividual variation is high (7, 8, 31), probably reflecting the heterogeneity of the genetic backgrounds. Indeed, although groups of these mice on average contain 50% C57BL/6J and 50% 129/SVJ genes, the genetic complements of individual animals may deviate materially from the 50/50 mean (P. Yue, X. Lin, and G. Schonfeld, unpublished observation). Interindividual variation of hepatic TG levels should be significantly smaller in congenic strains bearing apoB truncation-producing mutations than in the apoB mutation-bearing mice of 50/50% C57BL/6J and 129/SVJ mixed background.

To identify potentially useful parental strains for the production of congenic mice, we performed a mouse-strain survey of liver TG levels in 10 inbred strains, the results of which form the bulk of this presentation. Significant strain-related differences in hepatic TG contents were found. To ascertain which physiological, biochemical processes may play roles in determining the differences in liver TG contents among the selected strains, experiments, including hepatic mRNA expression profiling, were performed in the strains with the highest [BALB/cByJ (BALB/c)], middle [C57BL/6J (C57BL)], and lowest [SWR/J (SWR)] hepatic TG contents.

MATERIALS AND METHODS

Mice and Diet

Ten inbred strains of mice (6 wk old), AKR/J (AKR), BALB/c, C3H/HeJ (C3H), C57BL, C57BL/6ByJ (C57BLBy), DBA/2J (DBA), 129/SVJ, NZB/B1NJ (NZB), PL/J (PL), and SWR, of both genders were purchased from Jackson Laboratory (Bar Harbor, ME). AKR males were not studied because only AKR females were available from the supplier. Mice were housed in a room maintained at 24°C with a 12:12-h light-dark cycle (6:00 AM to 6:00 PM). All mice were given Purina mouse chow 5053 containing 4.5% fat, 20.0% protein, and 54.8% carbohydrate (LabDiet). Food was removed in the beginning of the light cycle, and mice were fasted for 4–6 h, except in the fasting experiment where times are indicated. Mice were 11–12 wk of age when killed. All animal procedures were performed in accordance with guidelines of Washington University’s Animal Studies Committee and approved by the IACUC of Washington University.

Analytical Procedures

Body fat (%) was assessed on anesthetized living mice by dual-energy X-ray absorptiometry with the use of a small animal densitometer (Lunar) (3). Plasma samples were assayed for triglycerides, total cholesterol, and free cholesterol as described (7, 19). Lipids were extracted from liver and assayed for TG (7). Hepatic TG levels were somehow lower in the three strains compared with their counterparts during the survey carried out at 4–6 h of fasting, probably due to the use of a different TG analysis kit from the supplier, which systematically did yield lower hepatic TG levels when used on the surveyed livers (data not shown). The same kit was used for all of the fasting experiment. Cellular protein contents were determined as described (7). Hepatic TG contents were expressed as milligrams of lipid per gram of protein. Plasma FFA were analyzed by an enzymatic method (Wako). Blood glucose was determined using a B-glucose analyzer (Hemocue, Ångelholm, Sweden). The Core Laboratory of the General Clinical Research Center at Washington University analyzed plasma β-hydroxybutyrate (BHB) concentration. Mice were weighed and killed. Blood was obtained from the inferior vena cava. Livers were excised, washed in cold PBS, weighed, cut into pieces that were then frozen in liquid nitrogen, and stored at −80°C until further analysis.

Glucose Tolerance Tests

After mice were fasted for 5 h, mice received an intraperitoneal injection of 10% d-glucose (0.75 g/kg body wt) for glucose tolerance. Blood (~10 μl) was drawn from the tail vein at 0, 30, 60, and 120 min and assayed for glucose.

In Vivo Measurement of FA Synthesis

Mice were injected with [1-14C]acetate (0.25 Ci/ml) at 25 μCi/g body wt and killed after 1.0 h. Liver (300 mg) was excised and digested in potassium hydroxide. After extraction of the nonsaponifiable lipids with petroleum ether, the sample solution containing the saponified lipids was acidified with sulfuric acid and FAs were extracted with petroleum ether as described (11). The radioactivity in total FAs was determined by scintillation counting. The FA synthesis rates are reported as disintegrations per hour per milligram of liver.

Determination of In Vivo Hepatic TG Secretion Rates

Hepatic production of VLDL-TG was measured after injection (intravenous) of Triton WR1339 (500 mg/kg body wt) on mice fasted for 4 h (7). Tail vein blood samples were taken at the specified times after injection for TG measurement and measured as described above.

Determination of FA Oxidation in Primary Cultures of Hepatocytes

Primary mouse hepatocytes were isolated as described (7). Cells (1.0 × 10⁶) were cultured in a flask for 2 h in 4 ml DMEM containing 10% FBS, and medium was then changed to DMEM containing 5% FBS. After an overnight culture, cells were washed three times with PBS. To determine FA oxidation, 14C-labeled palmitate (0.25 μCi/ml) was added to the medium. At the end of 2-h incubation, a portion of the cell medium (250 μl) was removed to determine acid-soluble products (ASP) as described (13). A septum and center well were then fitted into the flask. Sodium hydroxide (2 N, 0.25 ml) was injected onto the filter paper placed in the center well to capture CO2. Hydrochloric acid (6 N, 2 ml) was injected into the medium to release 14CO2. Both ASP and 14CO2 were counted. Two separate flasks treated in the same way without the 14C-labeled palmitate were used for assay of cellular protein. Total FA oxidation activity was obtained by adding the counts of 14CO2 and ASP and expressed as DPM per hour per microgram of cellular protein.

Short-chain Acyl-CoA Dehydrogenase Mutation Assay

A PCR assay was used to distinguish between the wild-type and mutant alleles for the structural gene of short-chain acyl-CoA dehydrogenase (SCAD), which employs oligonucleotide primers that flank a 278-bp deletion in the mutant allele to produce PCR products of 870 and 592 bp for the wild-type and mutant alleles for SCAD, respectively (38).

Responses of Liver TG Levels to Fasting

To determine whether there was a differential susceptibility of the fasting raising effect on liver TG levels, male mice of BALB/c, C57BL, and SWR were killed at 0, 6, and 14 h of fasting. Liver TG contents, plasma FFA, and BHB concentrations were determined as described in Analytical Procedures. A separate kit (Thermo Electron, Melbourne, Australia) was used to determine hepatic TG levels, because Wako discontinued its TG kit used in this study. The latter kit, although yielding precise results, deviated systematically downward from the Wako kit, yielding somewhat lower values for the fasting study (see Results).

mRNA Expression Profiling

Dual-channel microarray analysis was performed on total liver RNA pooled from each strain (male, n = 5 for each strain). Extracted RNA was further purified using RNeasy spin columns (Qiagen,
Valencia, CA) following the manufacturer’s protocol. Purified RNA was quantitated by ultraviolet absorbance at 260 and 280 nm and assessed qualitatively using Bioanalyzer 2100 (Agilent, Palo Alto, CA). Three comparison pairs (BALB/c vs. C57BL, BALB/c vs. SWR, C57BL vs. SWR) were set up comparing against each pair of two strains of mice. In each pair, 5 µg of purified total RNA were converted to cDNA that was labeled either with Cy3 or Cy5, which were hybridized to mouse Oligo Array (Sigma 65-mer Probe Set) containing 21,676 transcripts. In “dye-flip” experiments with the same pair of comparison, the dye labeling was reversed. Oligonucleotide array analysis was performed by the Genomic Core Facility of Digestive Diseases Research Core Center at Washington University in St. Louis, MO. All protocols were performed as recommended by Genisphere (Hatfield, PA).

Array images were scanned on an Axon scanner. Array data were extracted and analyzed using GenePix Pro 4.1 software from Axon Instruments. Further analysis was performed by using BRB-Array Tools (version 3.1, http://linus.nci.nih.gov/BRB-ArrayTools.html) according to the instructions provided. Differential gene expression between two of the three strains (BALB/c, SWR, and C57BL) was done by using class comparison expression analysis.

Quantitative Real-Time RT-PCR

Total RNA from male BALB/c, C57BL, and SWR each was pooled and digested with RNase-free DNase, followed by purification with RNeasy Mini Kit (Qiagen). mRNAs of interest were quantified by fluorescence RT-PCR with an Applied Biosystems GeneAmp 5700 sequence-detection system using SYBR Green dye binding to the PCR product (5). Primer Express 2.0 software was used to design amplimers for both genes of interest and GAPDH, the latter of which was used for normalization in RT-PCR.

Statistics

All statistic analyses were conducted using SAS (version 9, SAS Institute, Cary, NC). Data are expressed as means ± SD. ANOVA (PROC GLM) followed by either Duncan’s multiple tests or t-tests were performed for comparisons between treatments as appropriate with an overall a-level of 0.01 or 0.05 as indicated. Pearson’s correlation was performed using SAS Proc CORR using data from all 10 strains. The total variance (Vtotal) in the general linear model was partitioned into variance due to “environment” (Venv) and variance due to strain or genetic effects (Vstrain), where Vstrain/Vtotal gave an estimate of Vtotal explained by strain effect, an indication of heritability (12).

RESULTS

Hepatic TG Levels

Liver TG levels, averaged on both genders, varied over a sixfold range among the strains, from 49.7 ± 5.5 mg/g protein (SWR) to 316.5 ± 25.8 (BALB/c; Fig. 1, A and B). Liver TG contents showed similar strain-related differences across both genders. The presence of the SCAD mutation was confirmed in...
Table 1. Strain survey: body and liver weights and body fat

<table>
<thead>
<tr>
<th>Strain</th>
<th>Male BW, g</th>
<th>Female BW, g</th>
<th>Male Body Fat, %</th>
<th>Female Body Fat, %</th>
<th>Male LW, g</th>
<th>Female LW, g</th>
<th>Male LB, %</th>
<th>Female LB, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H</td>
<td>25.3 ± 2.7a</td>
<td>20.9 ± 1.7bc</td>
<td>12.2 ± 1.4a</td>
<td>16.4 ± 3.8abc</td>
<td>1.49 ± 0.5a</td>
<td>1.08 ± 0.8b</td>
<td>5.3 ± 0.2a</td>
<td>5.2 ± 0.2a</td>
</tr>
<tr>
<td>C57BL</td>
<td>27.4 ± 0.7a</td>
<td>18.4 ± 0.8de</td>
<td>13.5 ± 2.2c</td>
<td>15.4 ± 2.6bcd</td>
<td>1.20 ± 0.06bc</td>
<td>0.80 ± 0.08cd</td>
<td>4.2 ± 0.1b</td>
<td>4.3 ± 0.3cd</td>
</tr>
<tr>
<td>C57BLBY</td>
<td>27.6 ± 3.2a</td>
<td>19.0 ± 2.2ed</td>
<td>13.7 ± 0.6e</td>
<td>13.0 ± 0.9ed</td>
<td>1.31 ± 0.20ab</td>
<td>0.94 ± 0.12bc</td>
<td>4.7 ± 0.2b</td>
<td>5.0 ± 0.2ab</td>
</tr>
<tr>
<td>129/SVJ</td>
<td>25.0 ± 1.8a</td>
<td>20.3 ± 2.0cd</td>
<td>13.7 ± 0.8e</td>
<td>14.5 ± 1.7bcd</td>
<td>0.98 ± 0.10ad</td>
<td>0.75 ± 0.15a</td>
<td>3.9 ± 0.2r</td>
<td>3.6 ± 0.4r</td>
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<tr>
<td>AKR</td>
<td>NA</td>
<td>26.8 ± 1.7c</td>
<td>15.2 ± 3.1abc</td>
<td>12.3 ± 1.1ab</td>
<td>NA</td>
<td>13.2 ± 0.13a</td>
<td>NA</td>
<td>4.9 ± 0.3bc</td>
</tr>
<tr>
<td>NZB</td>
<td>26.5 ± 3.3a</td>
<td>22.3 ± 0.4b</td>
<td>15.3 ± 2.1abc</td>
<td>12.3 ± 1.1ab</td>
<td>NA</td>
<td>1.00 ± 0.03b</td>
<td>3.8 ± 0.3c</td>
<td>4.5 ± 0.2bc</td>
</tr>
<tr>
<td>PL</td>
<td>24.1 ± 2.0ab</td>
<td>15.7 ± 0.6f</td>
<td>20.9 ± 3.1abc</td>
<td>18.1 ± 1.8ab</td>
<td>0.95 ± 0.07bd</td>
<td>0.67 ± 0.08bd</td>
<td>3.9 ± 0.2r</td>
<td>4.3 ± 0.5cd</td>
</tr>
<tr>
<td>DBA</td>
<td>24.6 ± 1.1ab</td>
<td>18.3 ± 0.8ae</td>
<td>18.5 ± 4.5ab</td>
<td>12.1 ± 1.4d</td>
<td>1.07 ± 0.04cd</td>
<td>0.77 ± 0.06ad</td>
<td>4.4 ± 0.1b</td>
<td>4.2 ± 0.4cd</td>
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<tr>
<td>C3H</td>
<td>26.0 ± 0.7a</td>
<td>19.7 ± 1.0ed</td>
<td>14.3 ± 1.0f</td>
<td>12.5 ± 1.6ed</td>
<td>1.17 ± 0.06bc</td>
<td>0.94 ± 0.11bc</td>
<td>4.5 ± 0.2b</td>
<td>4.8 ± 0.4abc</td>
</tr>
<tr>
<td>SWR</td>
<td>20.8 ± 2.8b</td>
<td>16.5 ± 0.4f</td>
<td>12.2 ± 1.9g</td>
<td>12.8 ± 1.4ed</td>
<td>1.13 ± 0.07bcd</td>
<td>0.64 ± 0.03cd</td>
<td>4.7 ± 0.2b</td>
<td>3.9 ± 0.13de</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 5 males and 5 females for each strain). Duncan’s multiple tests were used to compare the differences of means with an overall α = 0.01. Values in the same column with different superscripts are different (P < 0.01). BW, body wt; LW, liver wt; LB, liver-to-body wt ratio; NA, not applicable.

Table 2. Strain survey: plasma lipid concentrations

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasma TG, mg/dl</th>
<th>Plasma TC, mg/dl</th>
<th>Plasma FC, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>C3H</td>
<td>173.8 ± 12.4a</td>
<td>171.3 ± 33.9ab</td>
<td>88.4 ± 10.0f</td>
</tr>
<tr>
<td>C57BL</td>
<td>88.1 ± 4.2de</td>
<td>61.1 ± 17.5f</td>
<td>84.7 ± 4.3e</td>
</tr>
<tr>
<td>C57BLBY</td>
<td>59.2 ± 16.6e</td>
<td>125.2 ± 20.0d</td>
<td>70.6 ± 5.0d</td>
</tr>
<tr>
<td>129/SVJ</td>
<td>153.9 ± 16.3b</td>
<td>152.3 ± 59.8f</td>
<td>108.5 ± 9.8b</td>
</tr>
<tr>
<td>AKR</td>
<td>113.8 ± 8.4cd</td>
<td>69.0 ± 16.3f</td>
<td>153.1 ± 4.7a</td>
</tr>
<tr>
<td>NZB</td>
<td>71.6 ± 11.5a</td>
<td>203.8 ± 22.7f</td>
<td>81.3 ± 2.9bc</td>
</tr>
<tr>
<td>PL</td>
<td>125.5 ± 29.9bcd</td>
<td>113.8 ± 19.4d</td>
<td>76.4 ± 2.4cd</td>
</tr>
<tr>
<td>DBA</td>
<td>139.0 ± 32.7abc</td>
<td>177.2 ± 48.0b</td>
<td>114.2 ± 4.3b</td>
</tr>
<tr>
<td>C3H</td>
<td>95.9 ± 13.6e</td>
<td>174.3 ± 14.6a</td>
<td>83.5 ± 6.3f</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 5 males and 5 females for each strain). Duncan’s multiple tests were used to compare the differences of means with an overall α = 0.01. Values in the same column with different superscripts are different (P < 0.01). TG, triglycerides; TC, total cholesterol; FC, free cholesterol.
correlations existed between hepatic TG levels and plasma BHB concentrations (Table 4). Thus univariate analysis did not identify any consistent covariates of hepatic TG except for the liver-to-body weight ratio.

We next examined how much the differences among strains were due to “environmental” effects versus strain (genetic) effects, using variance component analysis. On two-way ANOVA to assess the effects of strains and gender, there were significant differences in plasma lipids and plasma BHB and glucose concentrations among 10 strains (Tables 1–3). An overall gender effect was observed for plasma TG levels in the mouse strains. When the total liver TG variance was partitioned into Venv and Vstrain, significant strain effect was observed in both genders \( (P < 0.001) \). Interstrain variation explained \( \sim 90\% \) of total hepatic TG variation in males and \( \sim 76\% \) of total variation in females.

Physiological/Biochemical Studies on Male BALB/c, C57BL, and SWR

Hepatic lipogenesis, TG secretion, and FA oxidation. In vivo rates of hepatic lipogenesis, measured by \([1-14\text{C}]\)-acetate incorporation were similar in BALB/c and C57BL, but smaller in SWR (Fig. 2A). Rates of liver TG secretion, quantified by Triton WR-1339 injection, were similar in BALB/c and C57BL and lower in SWR (Fig. 2B). FA β-oxidation rates, determined in primary hepatic cell cultures, were similar in BALB/c and C57BL and higher in SWR (Fig. 2C). These data suggest that SWR livers synthesize FAs at a lesser rate and oxidize them at a faster rate than the other two strains, leaving less TG for hepatic accumulation. Clearly, low levels of hepatic TG in SWR are not due to enhanced export from the liver.

Responses to fasting: liver TG levels, plasma FFA, and BHB concentrations. Mean hepatic TG contents in the fed state were BALB/c>C57BL>SWR (same rank order as noted during the 10-strain survey). Hepatic TG contents rose significantly with fasting in BALB/c and C57BL at 6 and 14 h, but in SWR, only at 14 h (Table 5). Mean fasting-induced rises (Δ14–0 h) were \(-80 \text{ mg/g} \) in SWR, \(-200 \text{ mg/g} \) in C57BL, and \(-230 \text{ mg/g} \) in BALB/c \( (P < 0.01 \) for the overall trend). Rises also were largest for BALB/c, at 6 h, i.e., the livers of BALB/c and C57 BL were less able to “adapt” to fasting than SWR.

In the fed state, FFA concentrations were highest in SWR and rose in all strains with fasting, reaching the highest level in BALB/c at 14 h. The rise in FFA levels reflects the stimulatory effect of fasting on lipolytic rates in adipose tissue, balanced by rates of uptake by liver, muscle, and other tissues \( (25) \). It is not clear whether the largest fasting-induced rise in BALB/c is due to more rapid lipolysis, slower rate of removal from plasma, or slower rate of hepatic oxidation perhaps due to the deficiency of SCAD.

In the fed state, BHB levels were highest in SWR. With fasting, levels rose in all strains, but rises were greatest in SWR and least in BALB/c. These data are compatible with a greater capacity by livers of SWR to adapt to fasting-induced FFA mobilization by most effectively enhancing FA oxidation among the three strains, thus limiting hepatic TG accumulation.

Glucose tolerance. Responses to the glucose tolerance test were similar between male and female; thus results were combined for both genders. Glucose levels were highest in C57BL in response to glucose loading (Fig. 3). Levels were similar in BALB/c and SWR.

Hepatic gene expression profiling. A 2.5-fold or greater difference in gene expression was considered a notable change. Expressed transcripts were analyzed in pairwise comparison between two strains of mice. Eight transcripts were overexpressed, and 14 were underexpressed in C57BL relative to BALB/c (Table 6). Ten were overexpressed, and 13 were underexpressed in C57BL relative to SWR (Table 7). Fifteen genes were overexpressed, and 12 were underexpressed in SWR relative to BALB/c (Table 8). When one strain was compared with two others, three transcripts [metallothionein 1 (MT1), MHC class II H2-IA-α gene (q haplotype), and hemoglobin-α, adult chain 1] were upregulated, and four [selenium binding protein 1 (SELENBP1), hemolytic complement, G0/G1 switch gene 2, and adult male kidney cDNA, RIKEN full-length enriched] were downregulated in SWR compared with both C57BL and BALB/c. Three transcripts [stearyl-coenzyme A desaturase 1 (SCD1), serum amyloid A1, and MHC class I H-2K1-k pseudogene] were more expressed, and three transcripts [metallothionein 1 (MT1), MHC class II H2-IA-α gene (q haplotype), and hemoglobin-α, adult chain 1] were upregulated, and four [selenium binding protein 1 (SELENBP1), hemolytic complement, G0/G1 switch gene 2, and adult male kidney cDNA, RIKEN full-length enriched] were downregulated in SWR compared with both C57BL and BALB/c. Three transcripts [stearylcoenzyme A desaturase 1 (SCD1), serum amyloid A1, and MHC class I H-2K1-k pseudogene] were more expressed, and one [RIKEN cdNA 4933406L18 gene (4933406L18Rik)] was less expressed in C57BL compared with both SWR and BALB/c.

Table 3. Strain survey: plasma BHB and glucose concentrations

<table>
<thead>
<tr>
<th>Strain</th>
<th>Blood BHB, mM</th>
<th>Glucose, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>0.87±0.13</td>
<td>132.2±17.6d</td>
</tr>
<tr>
<td>C57BL</td>
<td>0.88±0.19</td>
<td>129.4±8.6e</td>
</tr>
<tr>
<td>C57BLBY</td>
<td>0.94±0.16</td>
<td>173.8±9.9abc</td>
</tr>
<tr>
<td>129/SVJ</td>
<td>1.03±0.31</td>
<td>154.2±22.2cd</td>
</tr>
</tbody>
</table>

Values are means ± SD \( (n = 5 \) males and 5 females for each strain). Duncan’s multiple tests were used to compare the differences of means with an overall \( \alpha = 0.01 \). Values in the same column with different superscripts are different \( (P < 0.01) \). BHB, β-hydroxybutyrate.

Table 4. Strain survey: correlation between hepatic TG and metabolic parameters

<table>
<thead>
<tr>
<th>Hepatic TG</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF</td>
<td>-0.268</td>
<td>0.246</td>
</tr>
<tr>
<td>P</td>
<td>0.0791</td>
<td>0.0501</td>
</tr>
<tr>
<td>LB</td>
<td>0.568</td>
<td>0.469</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BHB</td>
<td>0.096</td>
<td>0.121</td>
</tr>
<tr>
<td>P</td>
<td>0.5455</td>
<td>0.3403</td>
</tr>
<tr>
<td>PFG</td>
<td>0.418</td>
<td>-0.075</td>
</tr>
<tr>
<td>P</td>
<td>0.0065</td>
<td>0.5534</td>
</tr>
<tr>
<td>PTC</td>
<td>-0.158</td>
<td>-0.196</td>
</tr>
<tr>
<td>P</td>
<td>0.3242</td>
<td>0.1196</td>
</tr>
<tr>
<td>PFC</td>
<td>-0.459</td>
<td>-0.502</td>
</tr>
<tr>
<td>P</td>
<td>0.0025</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

BF, body fat; PTG, plasma total triglycerides; PTC, plasma total cholesterol; PFC, plasma-free cholesterol. Pearson’s correlation was performed using SAS Proc CORR on all mice \( (n = 5 \) males and 5 females for each strain).
BALB/c. Four transcripts (SELENBP1, phosphatidylethanolamine binding protein, amino levulinate synthase, and 18-day embryo cDNA, RIKEN full-length enriched) were overexpressed in BALB/c relative to both SWR and C57BL, and three [0-day neonate head cDNA, RIKEN full-length enriched, clone MGC:19436 IMAGE:3495833, and growth arrest specific 5] were underexpressed. It is worth noting that SCD-1 was 3.2- to 3.6-fold overexpressed in C57BL relative to both BALB/c and SWR. This is compatible with the relative glucose intolerance exhibited by this strain (see Fig. 3) that may lead to increased synthesis of FAs.

To confirm that the large-scale analysis had correctly identified differentially expressed transcripts, four transcripts were selected for validation by RT-PCR. Results were similar to those obtained from gene-expression profiling (Table 9). Primer pairs used also are listed in Table 9.

**DISCUSSION**

Although, as mentioned above, genetic manipulation of genes involving the FA biosynthetic, oxidation, and VLDL-TG exporting pathways has produced fatty livers (18, 30, 32), here we provide evidence of a significant genetic contribution to hepatic TG contents in inbred mice. The 10 mouse strains were of similar age and were maintained under currently accepted identical environmental conditions and diet. The highest liver TG levels were obtained in both male and female BALB/c, whereas the lowest ones were in SWR, showing six- to sevenfold difference between the two extremes (Fig. 1). ANOVA analysis indicated that strain-related effects per se accounted for the majority of the interstrain variation of hepatic TG level. The genetic bases for the interstrain differences in hepatic TG are not clear, with the possible exception of BALB/c, which were SCAD deficient (see below). However, significant strain-related effects on hepatic TG were seen even when BALB/c were left out of the ANOVA calculation.

In an attempt to identify metabolic covariates of liver TG, we performed a series of correlation analyses. Only liver-to-body weight ratios were significantly and positively correlated with liver TG in both genders. The other parameters of body weight, body fatness and plasma metabolites such as glucose, BHB, plasma TG were not consistently correlated across strains and genders (Table 4). Thus the parameters of adiposity or insulin action that correlate with liver fat in humans (31, 34) appear not to be correlated in mice, for reasons that are not clear. The negative correlation of hepatic TG with plasma-free cholesterol remains unexplained.

**Table 5. Effects of fasting on hepatic TG, plasma FFA, and BHB levels in 3 strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Hepatic TG, mg/g protein</th>
<th>FFA, mM</th>
<th>BHB, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>6 h</td>
<td>14 h</td>
</tr>
<tr>
<td>BALB/c</td>
<td>159.3±20.1^A</td>
<td>247.3±33.5^B</td>
<td>391.5±36.1^B</td>
</tr>
<tr>
<td>C57BL</td>
<td>53.2±5.7^B</td>
<td>116.0±32.0^B</td>
<td>247.1±11.1^A</td>
</tr>
<tr>
<td>SWR</td>
<td>32.6±4.6^C</td>
<td>56.7±13.8^C</td>
<td>113.6±21.2^C</td>
</tr>
</tbody>
</table>

Values are means ± SD. Male mice were killed after being fasted for 0 (BALB/c, n = 6; C57BL, n = 6; and SWR, n = 3), 6 (BALB/c, n = 3; C57BL, n = 3) and 14 h (BALB/c, n = 3; C57BL, n = 3; and SWR, n = 3). FFA, free fatty acids, Duncan’s multiple tests were used to compare the differences of means with an overall α = 0.05. Lower-case superscripts refer to differences across the rows, i.e. related to increasing duration of fasting within the same strain. Upper case superscripts refer to columns, i.e. same duration of fasting across the 3 strains. Different superscript letters indicate significant differences (P < 0.05).
To understand some of the physiological/biochemical processes underlying the strain-related differences in hepatic TG, we studied mice representing the highest (BALB/c), middle range (C57BL), and the lowest (SWR) levels of hepatic TG. Males were used because they were more readily available, and we saw similar strain differences in hepatic TG in both genders. Glucose tolerance, reflecting insulin action, was studied in the 4- to 6-h fasted state. Processes contributing to hepatic TG levels such as hepatic FA synthesis and oxidation and hepatic TG transport were studied (7, 9, 15, 20) in the fed state. Finally, responses of hepatic TG to 6 and 14 h of fasting were compared with the fed state. We attempt to explain the differences among the three strains in terms of the relative rates of the above processes we studied.

SWR showed significantly lower rates of hepatic lipogenesis (Fig. 2A) and TG secretion (Fig. 2B) and higher rates of FA oxidation (Fig. 2C) than both the BALB/c and C57BL strains, whereas these parameters were similar in BALB/c and C57BL. These data suggest that SWR may maintain lower hepatic TG levels than the other two strains by presenting smaller TG loads to the VLDL export system. Clearly, because hepatic TG export was low, liver TG levels were not low because export was increased. SWR livers also have more efficient capacities to resist the hepatic TG-raising effects of fasting (Table 5).

Table 6. Differential gene expression: C57BL vs. BALB/c

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fold Difference</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcripts upregulated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune response</td>
<td>0.0004651</td>
<td>mRNA for Qa-2 antigen.</td>
</tr>
<tr>
<td></td>
<td>9.98</td>
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</tr>
<tr>
<td></td>
<td>0.001183</td>
<td>MHC class I H-2K1-k pseudogene</td>
</tr>
<tr>
<td>Acute phase response</td>
<td>0.0004773</td>
<td>serum amyloid A 1 (SAA1)</td>
</tr>
<tr>
<td></td>
<td>3.60</td>
<td></td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td>0.0014558</td>
<td>stearoyl-Coenzyme A desaturase 1 (SCD1)</td>
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<tr>
<td></td>
<td>3.62</td>
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<tr>
<td>Unknown cDNA</td>
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<td></td>
<td>10.42</td>
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<tr>
<td></td>
<td>0.0038124</td>
<td>0 day neonate head cDNA, RIKEN full-length enriched</td>
</tr>
<tr>
<td></td>
<td>8.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.61e-05</td>
<td>growth arrest specific 5 (GAS5)</td>
</tr>
<tr>
<td></td>
<td>7.85</td>
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</tr>
<tr>
<td>Transcripts downregulated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute phase response</td>
<td>0.0008274</td>
<td>selenium binding protein 1 (SELENBP1)</td>
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<tr>
<td></td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0001338</td>
<td>selenium binding protein 2 (SELEBP2)</td>
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<tr>
<td></td>
<td>0.30</td>
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</tr>
<tr>
<td></td>
<td>0.0018503</td>
<td>glutathione S-transferase, theta 2 (GSTT2)</td>
</tr>
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<td></td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Immune response</td>
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<td>Q5 class I MHC gene</td>
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<td></td>
<td>0.34</td>
<td></td>
</tr>
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<td>Lipid metabolism</td>
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<td>phosphatidylethanolamine binding protein (PBP)</td>
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<td>alpha/beta hydrolase-1 (LOC57742)</td>
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<td></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Transcriptional factors</td>
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<td>CCAAT/enhancer binding protein (C/EBP), beta</td>
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<td></td>
<td>0.28</td>
<td></td>
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<tr>
<td>Heme synthesis</td>
<td>0.0010088</td>
<td>amino levulinate synthase (ALAS-H)</td>
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<tr>
<td>Steroid binding</td>
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<td>corticosteroid binding globulin (CBG)</td>
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<td>0.33</td>
<td></td>
</tr>
<tr>
<td>cDNA or unknown</td>
<td>0.00017946</td>
<td>10, 11 days embryo cDNA, RIKEN full-length enriched</td>
</tr>
<tr>
<td></td>
<td>0.0001338</td>
<td>18 days embryo cDNA, RIKEN full-length enriched</td>
</tr>
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<td></td>
<td>0.00018503</td>
<td>RIKEN cDNA 4933406L 18 gene (4933406L 18Rik)</td>
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<tr>
<td></td>
<td>0.28</td>
<td>10-day-old male pancreas cDNA, RIKEN full-length</td>
</tr>
<tr>
<td></td>
<td>0.29</td>
<td>11 days embryo cDNA, RIKEN full-length enriched</td>
</tr>
</tbody>
</table>

Hepatic gene expression profiling and analysis were performed from pooled total liver RNA (n = 5 each strain, male), as described in MATERIALS AND METHODS. A 2.5-fold difference or greater was considered a notable change. GB acc, GenBank accession number.
They appear to accomplish this by keeping plasma FFA levels low, which may indicate relatively low rates of lipolysis in adipose tissue and less delivery of FFA substrate to hepatic TG production, and BHB levels high, which may indicate efficient rates of ketogenesis. Both processes would tend to reduce the amounts of hepatic TG loads available for export via the VLDL system during fasting, compared with BALB/c and C57BL strains.

Glucose levels were highest in C57BL in the survey (Table 3) and during the glucose tolerance test (Fig. 3). The relatively high expression level of the SCD-1 gene in C57BL (Tables 6 and 7) is compatible with the relatively high rates of hepatic FA synthesis and the relative glucose intolerance of C57BL. These data suggest that although livers of C57BL may produce higher amounts of hepatic TG (certainly relative to SWR), the presence of adequate VLDL-TG export rates and FA oxidation rates was able to restrain hepatic TG contents. It is possible that the accumulation of hepatic TG over time, in C57BL, may further contribute to the increasing glucose intolerance with age (4, 26), but high levels of hepatic TG do not explain the absence of glucose intolerance in BALB/c. It is possible that not all causes of fatty liver produce insulin resistance.

SCAD is a mitochondrial enzyme that catalyzes the first reaction in the β-oxidation of short-chain FAs. BALB/c mice bear a spontaneous deletion in SCAD gene and develop fatty liver after 18 h of fasting (28, 29). However, it is worth noting that in the fed state, whereas BALB/c had considerably higher hepatic TG levels than C57BL, the two strains had similar rates of hepatic FA synthesis, hepatic TG secretion, and similar concentrations of plasma FFA and BHB. Similar rates of long-chain FA β-oxidation, as measured by using 14C-labeled palmitate as the substrate, were also found between C57BL and BALB/c. This raises questions as to whether the SCAD mutation is sufficient to explain the higher hepatic TG contents in BALB/c in the fed state.

It is possible that static measurements of plasma concentrations do not adequately reflect the deficiency in the action of SCAD. Plasma levels of FFA are determined by relative rates of lipolysis in adipose tissue (input) and rates of uptake by the liver, muscle, and other organs (output) (25). Plasma BHB concentrations are determined by relative rates of ketogenesis in liver (input) and ketone body uptake by brain and other organs (output) (21). It is impossible from the present experiments to determine whether the similar plasma levels of the two metabolites in fed BALB/c and C57BL reflect similar rates in input or output. It is conceivable that the rates of input and output differ in the two strains, but the balance between the two rates resulted in similar plasma levels. For example, if rates of

### Table 7. Differential gene expression: C57BL vs. SWR

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid metabolism</td>
<td>0.0004591</td>
<td>3.26</td>
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<tr>
<td>Immune response</td>
<td>0.0033534</td>
<td>2.57</td>
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<tr>
<td></td>
<td>0.0001976</td>
<td>2.63</td>
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<tr>
<td></td>
<td>0.0008779</td>
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<td></td>
<td>0.0003951</td>
<td>3.27</td>
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<tr>
<td></td>
<td>0.0008195</td>
<td>4.05</td>
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<tr>
<td>Acute phase response</td>
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<td>3.27</td>
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<tr>
<td></td>
<td>0.0007113</td>
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<td>cDNA</td>
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<tr>
<td></td>
<td>0.00222096</td>
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<tr>
<td>Signal transduction</td>
<td>2.68e-05</td>
<td>0.13</td>
</tr>
<tr>
<td>Transcriptional factors</td>
<td>0.0028646</td>
<td>0.28</td>
</tr>
<tr>
<td>Nucleic acid binding</td>
<td>0.0009634</td>
<td>0.32</td>
</tr>
<tr>
<td>Immune response</td>
<td>0.0040761</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>0.0001713</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>0.0008195</td>
<td>4.05</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td>0.0019176</td>
<td>2.63</td>
</tr>
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<td>cDNA</td>
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<td>0.0014501</td>
<td>0.37</td>
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<td>0.00043909</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>0.0030628</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Hepatic gene expression profiling and analysis were performed from pooled total liver RNA (n = 5 each strain, male), as described in MATERIALS AND METHODS. A 2.5-fold difference or greater was considered a notable change.
Lipolysis and tissue uptake are both higher in BALB/c, FFA levels in plasma could remain similar to C57BL levels, but the higher hepatic uptake of FFA in BALB/c could result in higher levels of hepatic TG, not explainable by the SCAD defect alone.

The fasting experiment appeared to support an FA β-oxidation defect in BALB/c mice (the least rise in plasma BHB levels in response to fasting). However, this reflected more of a defect in the fasting situation, consistent with Table 8.

### Table 8. Differential gene expression: SWR vs. BALB/c

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P Value</th>
<th>Fold Difference</th>
<th>GB acc</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid metabolism</td>
<td>0.0009032</td>
<td>2.654</td>
<td>NM_010634</td>
<td>Fatty acid binding protein 5, epidermal (FABP5)</td>
</tr>
<tr>
<td>Immune response</td>
<td>0.0055601</td>
<td>3.04</td>
<td>K01925</td>
<td>MHC class II H2-IA-alpha gene (q haplotype)</td>
</tr>
<tr>
<td></td>
<td>0.0020473</td>
<td>2.535</td>
<td>M19689</td>
<td>MHC class I H-2 (q-haplotype) classic transplantation</td>
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<tr>
<td>Signal transduction</td>
<td>0.0019972</td>
<td>2.539</td>
<td>NM_008218</td>
<td>hemoglobin alpha, adult chain 1 (HBA-A1)</td>
</tr>
<tr>
<td>2.1e-06</td>
<td>19.496</td>
<td>0.013602</td>
<td></td>
<td>Metallothionein 1 (MT1)</td>
</tr>
<tr>
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<td>0.0002479</td>
<td>3.518</td>
<td>J03953</td>
<td>glutathione transferase GT9.3</td>
</tr>
<tr>
<td>DNA binding</td>
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<td>3.358</td>
<td>AF171080</td>
<td>histone macroH2A1.2 variant mRNA</td>
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<td>Amino acid metabolism</td>
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<td>NM_010324</td>
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</tr>
<tr>
<td>cDNA or unknown</td>
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<td>0 day neonate head cDNA, RIKEN full-length enriched</td>
</tr>
<tr>
<td></td>
<td>0.0055816</td>
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<tr>
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<td>2.33e-05</td>
<td>7.764</td>
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<td>0.0009285</td>
<td>3.505</td>
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<td>RIKEN cDNA 0610010I23 gene (0610010I23Rik)</td>
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<tr>
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<td>0.0010505</td>
<td>2.944</td>
<td>AJ278735</td>
<td>Hypothetical protein (ORF1), 1975 BP</td>
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<tr>
<td></td>
<td>7.89e-05</td>
<td>4.522</td>
<td>NM_013525</td>
<td>growth arrest specific 5 (GAS5)</td>
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<td></td>
<td>0.0010782</td>
<td>3.036</td>
<td>AJ279951</td>
<td>Hypothetical protein, clone mv10</td>
</tr>
</tbody>
</table>

Hepatic gene expression profiling and analysis were performed from pooled total liver RNA (n = 5 each strain, male), as described in MATERIALS AND METHODS. A 2.5-fold difference or greater was considered a notable change.

The fasting experiment appeared to support an FA β-oxidation defect in BALB/c mice (the least rise in plasma BHB levels in response to fasting). However, this reflected more of a defect in the fasting situation, consistent with Table 9.

### Table 9. mRNA levels by real-time PCR analysis

<table>
<thead>
<tr>
<th>Genes</th>
<th>Accession Number</th>
<th>Primers (5’ to 3’)</th>
<th>BALB/c</th>
<th>C57BL</th>
<th>SWR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stearoyl-CoA desaturase 1: SCD1</td>
<td>NM_009127</td>
<td>Forward primer, GTTGCTCCATCCATTGC; Backward primer, AACCATGGGAAAGCAAGATTT</td>
<td>1.0</td>
<td>5.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Aldehyde oxidase 1: AOX1</td>
<td>NM_009676</td>
<td>Forward primer, CACGCTGAGCTGCTTTT; Backward primer, CACGCTGAGCTGCTTTT</td>
<td>1.0</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Metallothionein 1: MT1</td>
<td>NM_013602</td>
<td>Forward primer, CCTCAAGATGAACTGAAA; Backward primer, GAGAGACGCTGGCTTGT</td>
<td>1.0</td>
<td>2.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Cytochrome P450, 2c29: CYP2C29</td>
<td>NM_007815</td>
<td>Forward primer, AAGAACATCCACCAATCC; Backward primer, TTACACCCTCTCACAAC</td>
<td>1.0</td>
<td>0.1</td>
<td>1.1</td>
</tr>
<tr>
<td>GAPDH</td>
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<td>Forward primer, TGGCCTCTCTGGTTCT; Backward primer, AGGGCCAGTCAGATC</td>
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<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Liver total RNA was isolated and pooled from 5 male mice from each strain. Quantitative real-time PCR was performed from the pooled total RNA as described in MATERIALS AND METHODS.
the development of fatty liver on 18 h of fasting in this strain (29).

One way to settle these issues would be to examine another BALB/c strain with intact SCAD function. We have studied another BALB/c strain available from Jackson Laboratories: the BALB/cJ strain. Hepatic TG contents of male BALB/cJ in fact are lower, resembling those of SWR. However, the two BALB/c strains also differ in at least 43 of ~300 microsatellite markers genotyped across the genome (http://www.cider.jhml.edu/mouse/mouse.html). Thus, at this time, it is not possible unequivocally to ascribe the excess hepatic TG in the fed state to the SCAD deficiency alone. It remains to be investigated whether any other factor(s) besides SCAD mutation causes fatty liver in this mouse strain.

We performed a gene expression survey expecting to find additional explanations for the interstrain differences in hepatic TG contents. Most of the differences in hepatic gene expression remain to be explained. However, as noted, the relative overexpression of the SCD-1 transcript in C57BL was useful in explaining the contribution of presumed increased palmitate desaturation to oleate to the higher hepatic TG levels in C57BL (Tables 6 and 7) (24). It is not clear whether high hepatic FA synthesis with lower SCD1 mRNA level in BALB/c results from a lower oxidation rate of newly synthesized FA. MT1 has been linked to the regulation of energy balance in mice, because mice deficient in both MT1 and MT2 were obese (2). SWR had the highest level of expression in MT1 when compared with both C57BL and BALB/c, consistent with the lowest body weight of SWR in the three strains. Mouse hepatic SELENBP1 was decreased in response to peroxisome proliferators such as ciperoxib (14). The lowest expression of hepatic SELENBP1 in SWR relative to both C57BL and BALB/c may be related to the highest hepatic FA oxidation in SWR. Another consideration is that differential gene expression may be the consequence, rather than the cause of differences in liver TG levels. For example, hepatic TG accumulation may raise the level of reactive oxygen products in liver (1) stimulating alterations in the expression levels of mRNAs of acute phase reactant molecules.

In conclusion, results of these studies have demonstrated a significant genetic contribution to hepatic TG contents in inbred mice. They also helped identify potential parental strains for construction of congenic strains bearing apoB truncations. Breeding is in progress.

REFERENCES


