Mice with deletion of the mitochondrial glycerol-3-phosphate dehydrogenase gene exhibit a thrifty phenotype: effect of gender

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Alfadda, Assim, Rosangela A. DosSantos, Zaruhi Stepanyan, Husnia Marrif, and J. Enrique Silva. Mice with deletion of the mitochondrial glycerol-3-phosphate dehydrogenase (mGPD; EC 1.1.99.5) in energy balance and intermediary metabolism, we studied transgenic mice not expressing mGPD (mGPD/H11002). These mice had ~14% lower blood glucose; ~50% higher serum glucose; ~80% higher serum triglycerides; and at thermoneutrality, their energy expenditure (QO2) was 15% lower than in wild-type (WT) mice. Glycerol-3-phosphate levels and lactate-to-pyruvate ratios were threefold elevated in muscle, but not in liver, of mGPD/H11002 mice. WT and mGPD/H11002 mice were then challenged with a high-fat diet, fasting, or food restriction. The high-fat diet caused more weight gain and adiposity in mGPD/H11002 challenged with a high-fat diet, fasting, or food restriction. Such a phenotype is restricted to females. Lower T4 -to-T3 conversion in these challenges. These results show that the lack of mGPD causes significant alterations of intermediary metabolism, which are more pronounced in muscle than liver and lead to a thrifty phenotype that is more marked in females than males. Lower T4 -to-T3 conversion in muscle, the G3P shuttle is much more important than the aspartate-malate shuttle (26). The preferential use of G3P in muscle, the G3P shuttle is much more important than the aspartate-malate shuttle (26). The preferential use of G3P in muscle, the G3P shuttle is much more important than the aspartate-malate shuttle (26). The preferential use of G3P in muscle, the G3P shuttle is much more important than the aspartate-malate shuttle (26). The preferential use of G3P in muscle, the G3P shuttle is much more important than the aspartate-malate shuttle (26). The preferential use of G3P in muscle, the G3P shuttle is much more important than the aspartate-malate shuttle (26). 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malate-aspartate shuttle. The importance of the G3P shuttle for muscle is evidenced by the increase in DHAP and decrease in G3P observed in muscle but not in liver of mice with cGPD deficiency (30, 31) and is further demonstrated here.

To investigate a role for mGPD in thermogenesis and intermediary metabolism, we have been studying a transgenic C57Bl/6J mouse with targeted disruption of the mGPD gene (10). We found that these animals do have a mild reduction in energy expenditure, as judged by food intake and indirect calorimetry, as well as a significant thermogenic defect (8). To pursue the hypothesis that the lack of the mGPD gene could be associated with a thrifter phenotype, we challenged mGPD−/− mice with a high-fat diet and food restriction and demonstrate that this is likely the case, albeit in a gender-dependent manner.

MATERIALS AND METHODS

Animals. All studies reported here were approved by the McGill University Animal Research Committee. Mice with targeted disruption of the mGPD gene were those described by Eto et al. (10). Additional details of the engineering of these mice were recently reported (8). Heterozygous founder couples were shipped from Japan to our laboratory after at least five backcrossings into the C57Bl background. Wild-type (WT; mGPD+/+) and homozygous mGPD null mice (mGPD−/−) were obtained by crossing heterozygous males (mGPD+/−) either with mGPD+/+ or mGPD−/− females. Mice were housed at a maximum of five per cage. They were fed ad libitum, and were reared and kept at 22 ± 1°C on a 12:12-h light/dark cycle, unless noted otherwise. Genotype was determined by PCR (10) in DNA obtained from the tail tip around the time of weaning. Mice were subsequently segregated by genotype and gender for future breeding and/or experiments. The lack of mGPD activity in the mGPD−/− genotype was confirmed by direct assay of the enzyme in isolated mitochondria, as described elsewhere (13). All experiments described here are comparisons between homozygous mGPD+/+ and mGPD−/− and were performed in 4- to 5-mo-old mice. Some results obtained in males were previously reported (8) and are not presented here. Animals selected for an experiment were matched by age within 15 days. Similar to Brown et al. (3), we found that the weight of mGPD−/− mice is lower than that of WT, although the difference in our mice was less marked. Between 3 and 5 mo of age, the difference is 5–10%, so age matching did not result in significant weight differences within experiments.

Diets, food intake, and food restriction. Mice were fed the standard mouse chow 5001 from Agribands Purina Canada, St-Hubert, QC, Canada (3.3 kcal/g, with 13, 60, and 27% of the calories from fat, carbohydrate, and proteins, respectively) or placed on a high-fat, low-carbohydrate diet (containing 5.2 kcal/g, with 60% of the calories from fat, 20% from protein, and 20% from carbohydrate; diet D12492 from Research Diets, New Brunswick, NJ). Food intake was measured by using standard metabolic cages and was expressed in kilocalories per day per 100 gram body weight.

In the fasting experiments, food was removed between 8 and 9 AM. Water was kept ad libitum. Baseline biochemical measurements were made in the postfed state, 5–6 h after removing food, and then repeated the next day, after completing 30 h of fast. For food restriction, individual ad libitum food intakes were monitored for 4 days and then food was restricted to 50–70% of the average daily intake, as indicated.

Energy expenditure. This was measured by indirect calorimetry in an open-circuit system (Oxymax System, Columbus Instruments, Columbus, OH), as described previously (8). An exponent of 0.7 was used for expression of gases exchange by weight, and QO<sub>2</sub> was expressed in ml·h<sup>−1</sup>·100 g<sup>−0.7</sup> (16). Measurements were made around the thermoneutrality temperature, 30°C, unless otherwise indicated, in a system installed in a walk-in environmental chamber that maintains ambient temperature within 0.1°C (SureTemp, Raleigh, NC). Around thermoneutrality, the contribution of BAT facultative thermogenesis to energy expenditure as well as the energy cost of maintaining body temperature are minimal, so that measurements at this ambient temperature are the closest approximation to obligatory thermogenesis or the heat resulting from basal metabolic rate (16).

Core body temperature. Core body temperature was measured with a flexible rectal probe YSI 423 (Yellow Springs Instrument, Dayton, OH) that was connected to a high-precision thermometer (YSI Precision 4000A Thermometer). Animals were not anesthetized or sedated and were restrained for no longer than 30 s for the measurement. A small rubber ring placed 2.5 cm from the tip of the probe ensured uniformity in the depth the probe was introduced in the rectum.

Blood and tissue sampling. Blood was obtained either from the tail tips or from the inferior vena cava, under light isoflurane anesthesia, when mice were killed by exsanguination. Unless indicated otherwise, “basal or basal” blood and tissue samples were obtained ~6 h after removing the food, which was done at ~8:00 AM. Blood was allowed to coagulate at room temperature, and cleanly collected serum was stored at −20°C until analyzed. Tissues were snap frozen in liquid nitrogen and kept at −80°C until analyzed for enzyme activities, DNA, protein, G3P, lactate, pyruvate, or mRNAs. mGPD activity was measured in isolated mitochondria, as described (13).

Biochemical assays. Enzymatic assay kits were used for measurement of serum triglycerides (Infinity Triglyceride Reagent, Sigma Diagnostics, St. Louis, MO), serum-free glycerol (Glycerol Colorimeter method, Randox, Mississauga, ON, Canada), and serum free fatty acids (NEFA C, Wako Chemicals, Richmond, VA). Because triglycerides are measured by the release of glycerol, free glycerol was routinely measured and subtracted from the total glycerol measured in the triglycerides assay. Blood glucose was measured with a glucometer (ADVANTAGE, Roche Diagnostics, Indianapolis, IN). Total T<sub>4</sub> and T<sub>3</sub> levels were measured by RIA using commercial kits (DPC, Diagnostic Products, Los Angeles, CA) with the modifications described elsewhere to adapt it for use with mouse serum (38). Tissue levels of G3P, of lactate and pyruvate were measured by enzymatic analysis, as described (22–24). Briefly, tissue aliquots were homogenized in 5 vol of ice-cold 0.6 M HClO<sub>4</sub> and centrifuged at 2,000 g. The supernatant was removed and neutralized with 5 M K<sub>2</sub>CO<sub>3</sub> and G3P measured from the NADH generated in the presence of an excess bovine cytoplasmic glycerol-3-phosphate dehydrogenase (Sigma) (22), whereas lactate and pyruvate were measured by following in a spectrophotometer the formation or disappearance of NADH, respectively, catalyzed by an excess of commercial lactate dehydrogenase (Sigma) (23, 24).

Triglyceride production rates. Triglyceride production rates were measured as described (28), using Tyloxapol (Triton WR-1339) to prevent the digestion of triglyceride-rich particles by lipoprotein lipase. Measurements were initiated 6 h after food removal. After the obtainment of a basal blood sample, the drug was injected intravenously and blood was obtained 1 and 2 h later. Because there is no removal of triglyceride from the blood during the period, the slope of the increase in triglyceride serum concentration is linear with influx of triglyceride into circulation, which in the postfed steady state is a reflection of the production rate. This latter is calculated from the slope of the triglyceride concentration increase after Tyloxapol multiplied by the volume of distribution of triglyceride, which is approximately equal to plasma volume [0.09 ml/g (mice)] (28).

RNA isolation and PCR analysis. Tissue RNA was extracted with acidic guanidinium-phenol-chloroform (5), was quantified by absorbance at 260 nm and was stored in DPC-treated water at −80°C until further analysis. Integrity of RNA was routinely verified by agarose gel electrophoresis. The mRNAs for mGPD and uncoupling protein (UCP)-3 were quantified by competitive RT-PCR (reverse transcription-polymerase chain reaction), using primers, competitors, and con-
ditions described elsewhere (8). Results are expressed in attomoles of mRNA per microgram of total RNA.

Other tissue measurements. DNA and tissue protein content were measured by standard methods (14, 29).

Statistical analysis. Results are expressed as means ± SE. Experiments involving several treatment or time groups were analyzed by ANOVA followed by post hoc tests for multiple comparisons (Newman-Keuls). Two-way ANOVA was used to compare the effects of treatments on two experimental groups (e.g., diet on 2 genotypes). Individual means were then compared by the Bonferroni test, if across experimental groups, or the Newman-Keuls test if within one experimental group.

RESULTS

Baseline measurements. The lack of the mGPD gene in females was associated with a small reduction in energy expenditure (QO2) and with changes in the concentrations of several metabolites in blood and tissues, the most important of which are displayed in Table 1. The mean of pooled QO2 results was 13% lower in mGPD−/− mice, which is similar to the difference found in males when measured at thermoneutrality (8). Note that the difference is small for the dispersion of the data so that in individual experiments with four to six mice the differences were not always significant. Mice with mGPD deficiency also had a small but significant reduction in blood glucose of ~14%. Serum FFA tended to be elevated in mGPD−/− mice but the increase did not reach statistical significance. Serum triglyceride concentration in mGPD−/− mice was 84% greater than in the WT genotype (P < 0.0001). This difference is most likely due to reduced utilization of triglyceride in the mGPD−/− mice, as the production rates were not significantly different. Serum glycerol concentration was also frankly elevated in mGPD−/− mice (~45%, P = 0.001). G3P concentration was not elevated in the liver but in skeletal muscle was more than three times higher in mGPD−/− than in WT mice. Lastly, the lactate-to-pyruvate ratio was reduced by 63% in liver and almost doubled in muscle of mGPD−/− mice with regard to WT mice. These differences reflected the marked effect of the mGPD−/− genotype on pyruvate, which was significantly reduced in skeletal muscle but increased in the liver of these mice.

Altogether, these data show that the lack of mGPD has an important effect in intermediary metabolism, which differs markedly in liver and skeletal muscle. These results are similar to those obtained in males and reported elsewhere (8). Specific comparisons between males and females will be made below.

Effects of a high-fat, low carbohydrate diet on mGPD−/− female mice. To gain more insight into the role of mGPD in intermediary metabolism and energy balance, we challenged mGPD−/− mice with the high-fat diet described in METHODS. Energy intake declined slightly over the period of observation and was not differentially affected by the genotype (Fig. 1A). In rats fed high-fat diets, particularly cafeteria diets, increased food intake and increased energy expenditure are routinely observed (reviewed in Ref. 40), but this is not the case with mice fed high-fat diets, in which frequently there is no associated increased food intake or energy expenditure, particularly C57Bl mice (39, 40). Interestingly, energy expenditure was not significantly changed by the diet in either genotype (Fig. 1B).

Despite the lack of a significant effect of the high-fat diet on food intake or energy expenditure, weight increased in both groups. Most importantly, the weight gain was promptly more pronounced in the mGPD−/− mice (P < 0.001). At the end of 10 wk, these mice gained 6.95 ± 0.61 g, nearly twice the weight gained by WT mice, 3.81 ± 0.64 g (P < 0.001).

The weights of the major fat depots are shown on Table 2. Because there were changes in total body weight, results are expressed as percent of body weight to reflect changes in adiposity. On regular chow, the genotype had no differential effect on either the distribution of fat among the depots or on the total weight of these depots. The high-fat diet was associated with an increase in weight in all depots, with the increment being less pronounced in the dorsal fat than in the others in both genotypes. The genotype did not affect the relative size of the various depots in the high-fat diet-fed mice, but the diet-associated fat accumulation was greater in the mGPD−/− mice, and globally these depots accounted for 18% of body weight in mGPD−/− as opposed to 11% in WT mice (P < 0.01). The weight of the four depots in mGPD−/− and WT mice fed the HF diet was 5.3 and 2.7 g, compared with 1.08 and 0.87 g on the controls on regular chow. Thus these depots

Table 1. QO2 and selected serum and tissue concentrations of metabolites in mGPD−/− mice and WT controls

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>WT</th>
<th>mGPD−/−</th>
<th>%Difference (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QO2, mol·h−1·100 g−0.7</td>
<td>129.4±6.2 (16)</td>
<td>111.8±3.9 (12)</td>
<td>−13 (0.0362)</td>
</tr>
<tr>
<td>Blood glucose, mM</td>
<td>7.5±0.3 (12)</td>
<td>6.4±0.2 (9)</td>
<td>−14 (0.022)</td>
</tr>
<tr>
<td>Serum FFA, mM</td>
<td>0.81±0.04 (12)</td>
<td>0.92±0.05 (9)</td>
<td>14 (0.091)</td>
</tr>
<tr>
<td>Serum triglycerides, mM</td>
<td>0.24±0.02 (12)</td>
<td>0.44±0.04 (9)</td>
<td>84 (&lt;0.0001)</td>
</tr>
<tr>
<td>Triglycerides production, μmol·h−1·100−1·g</td>
<td>10.8±1.12 (5)</td>
<td>11.7±0.70 (5)</td>
<td>8.7 (0.498)</td>
</tr>
<tr>
<td>Serum glycerol, mM</td>
<td>0.39±0.02 (11)</td>
<td>0.57±0.04 (8)</td>
<td>45 (0.001)</td>
</tr>
<tr>
<td>Liver G3P, μmol/g</td>
<td>0.71±0.04 (6)</td>
<td>0.69±0.03 (5)</td>
<td>−3 (0.692)</td>
</tr>
<tr>
<td>Skeletal muscle G3P, μmol/g</td>
<td>0.37±0.07 (6)</td>
<td>1.16±0.17 (6)</td>
<td>212 (0.0013)</td>
</tr>
<tr>
<td>Liver</td>
<td>Lactate, μmol/g</td>
<td>36.9±2.5 (6)</td>
<td>33.5±3.0 (6)</td>
</tr>
<tr>
<td></td>
<td>Pyruvate, μmol/g</td>
<td>0.80±0.10 (6)</td>
<td>1.88±0.14 (6)</td>
</tr>
<tr>
<td></td>
<td>Lactate/pyruvate</td>
<td>49.8±6.6 (6)</td>
<td>18.6±2.3 (6)</td>
</tr>
<tr>
<td></td>
<td>Skeletal muscle</td>
<td>Lactate, μmol/g</td>
<td>162.1±8.4 (6)</td>
</tr>
<tr>
<td></td>
<td>Pyruvate, μmol/g</td>
<td>9.76±0.94 (6)</td>
<td>6.01±0.52 (6)</td>
</tr>
<tr>
<td></td>
<td>Lactate pyruvate</td>
<td>157.7±1.16 (6)</td>
<td>29.2±3.2 (6)</td>
</tr>
</tbody>
</table>

Values are means ± SE (n in parentheses). QO2, energy expenditure; mGPD−/−, mitochondrial glycerol-3-phosphate dehydrogenase-deficient mice; WT, wild type; FFA, free fatty acids; G3P, glycerol-3-phosphate.
account for (5.3–1.08)/6.95 or 61% of the total weight gain in mGPD−/− mice (6.95 g) vs. (2.7 – 0.87)/3.81 or 48% of the total body weight gain in WT mice (3.81 g). Because these depots are just part of total body fat, these results indicate that the difference in weight between WT and mGPD−/− mice on HF diet is largely due to increased adiposity. The HF diet had no major impact on fatty acids or triglyceride blood levels. FFA tended to be reduced and triglycerides to be increased by the diet but without reaching significance or being differentially affected by the genotype (data not shown). In WT mice, blood glucose was modestly but significantly increased (from 7.5 ± 0.34 to 9.6 ± 0.89 mM, P < 0.01), widening the gap between the two genotypes.

Effects of a 30-h fasting or food restriction on weight and thermogenesis in mGPD−/− females. The above results suggest that mGPD−/− female mice have a thrifty phenotype, prone to gain more weight when fed a high-fat diet. Such phenotypes are usually associated with a better ability to defend body weight when submitted to food restriction. To test whether this was the case in the mGPD−/− mice, we submitted them to a 30-h fasting or to partial food restriction.

A 30-h fasting was associated with a significant weight loss and reduction in core body temperature, but the magnitude of the effect was dependent on the genotype (Fig. 2). Thus mGPD−/− mice lost ~60% less than in WT (P = 0.0002). In contrast, the mGPD−/− mice became significantly more hypothermic, ~0.6°C less than WT mice (P = 0.03). This was most likely due to reduced thermogenesis, because QO2 was reduced more in these mice than in the WT controls (difference 30.52 ± 13.67 vs. −15.95 ± 8.621 ml·h−1·100 g−0.7), although this difference did not reach statistical significance.

Fasting had the expected effects on blood glucose, FFA and β-OH-butyrate serum levels (Table 3). Blood glucose levels were reduced, whereas those of FFA and β-OH-butyrate increased. However, the genotype had no significant differential effect on these levels. Thus the difference in blood glucose between WT and mGPD−/− mice remained ~20%, and FFA and β-OH-butyrate increased to levels not significantly different in both genotypes.

In a subsequent experiment, food was restricted relative to the ad libitum baseline intake under the usual vivarium conditions described in METHODS. When food was restricted to 50% of the basal intake, animals rapidly lost weight (Fig. 3), particularly the WT mice. By the end of the third day, WT, but not the mGPD−/−, went into a state of torpor, so food supply

<table>
<thead>
<tr>
<th>Diet</th>
<th>Genotype</th>
<th>Dorsal</th>
<th>SC</th>
<th>Pelvic</th>
<th>RP</th>
<th>TWANOVA</th>
<th>Total Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular chow</td>
<td>WT</td>
<td>0.77±0.08</td>
<td>1.29±0.07</td>
<td>1.35±0.22</td>
<td>0.53±0.04</td>
<td>Deposite P &lt; 0.001</td>
<td>3.94±0.36</td>
</tr>
<tr>
<td></td>
<td>mGPD−/−</td>
<td>0.84±0.06</td>
<td>1.49±0.27</td>
<td>1.65±0.30</td>
<td>0.66±0.11</td>
<td>Glyte, NS</td>
<td>4.63±0.65</td>
</tr>
<tr>
<td>High-fat fed</td>
<td>WT</td>
<td>1.69±0.26</td>
<td>5.26±1.36</td>
<td>4.42±1.09</td>
<td>1.91±0.52</td>
<td>Deposite P &lt; 0.001</td>
<td>10.98±3.05</td>
</tr>
<tr>
<td></td>
<td>mGPD−/−</td>
<td>2.19±0.19</td>
<td>7.39±0.45</td>
<td>5.29±0.42</td>
<td>3.04±0.29</td>
<td>Glyte, P &lt; 0.02</td>
<td>17.92±1.11</td>
</tr>
</tbody>
</table>

Values are means ± SE in % body weight. SC, subcutaneous fat, largely abdominal and inguinal; RP, retroperitoneal fat, largely perirenal. TWANOVA, 2-way ANOVA; Depot, fat depot effect; Glyte, genotype effect. *P value in the Bonferroni’s test for the comparison of total fat; NS, not significant.
was increased to 70% of the ad libitum intake and animals were moved to a 30°C room. As illustrated in Fig. 3A, this stopped further weight loss, but at all times the weight of WT mice remained lower than that of mGPD−/− mice. QO2 was measured at 30°C before the commencement of the experiment and then at the end of the caloric restriction, whereas the animals were still at 30°C. As shown in Fig. 3B, food restriction was associated with a significant reduction in QO2 in both genotypes, but this was significantly greater in the mGPD−/− mice. The 30°C environment was chosen to avoid the confounding contribution of BAT thermogenesis, as this becomes nil in animals acclimated at thermoneutrality (16), which we recently showed occurs also in these mice (8). Body temperature was also measured at the beginning of the experiment and then at the end, 2 h after returning the animals to the vivarium at 21°C. As shown in Fig. 3C, food restriction was associated with a significant drop in core temperature in both genotypes, mice. The 30°C environment was chosen to avoid the confounding contribution of BAT thermogenesis, as this becomes nil in animals acclimated at thermoneutrality (16), which we recently showed occurs also in these mice (8). Body temperature was also measured at the beginning of the experiment and then at the end, 2 h after returning the animals to the vivarium at 21°C. As shown in Fig. 3C, food restriction was associated with a significant drop in core temperature in both genotypes.

Table 3. Effect of 30-h fasting on blood glucose, FFA, and β-OH-butyrate levels in normal and mGPD−/− mice

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>mGPD−/−</th>
<th>%Change (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mM)</td>
<td>Fed</td>
<td>9.2±0.09</td>
<td>7.3±0.19</td>
</tr>
<tr>
<td></td>
<td>Fasted</td>
<td>7.1±0.16</td>
<td>5.9±0.3</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Serum FFA (μM)</td>
<td>Fed</td>
<td>669±28</td>
<td>477±12</td>
</tr>
<tr>
<td></td>
<td>Fasted</td>
<td>975±25</td>
<td>888±88</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>β-OH-butyrate (μM)</td>
<td>Fed</td>
<td>216±27</td>
<td>201±17</td>
</tr>
<tr>
<td></td>
<td>Fasted</td>
<td>300±27</td>
<td>432±98</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 4–6).
but significantly more in the mGPD−/− than in the WT mice. The fall in temperature was similar to that observed in the fasting state, i.e., ~1°C in the WT and 1.8°C in the mGPD-deficient mice. Food restriction caused a reduction in blood glucose and an increase in glycerol and FFA, but these effects were not differentially affected by the genotype, as it occurred in the short-term fasting (data not shown).

Males do not show the same thrifty phenotypes as females. Although mGPD−/− males have a reduction in obligatory thermogenesis similar to that of females at thermoneutrality (8), the responses to high-fat diet, fasting, or food restriction were not significantly different from those of appropriately matched WT controls. As shown in Fig. 4A, both mGPD−/− and WT males gain about the same weight with the high-fat diet. Likewise, the weight loss with a 30-h fast (Fig. 4B) or 50% food restriction at ~21°C for 4 days (Fig. 4C) was not significantly affected by the genotype. In this latter experiment, the animals were placed at 30°C immediately after the third weight measurement, keeping the same level of feeding, and their core temperature was measured ~48 h later in this warm environment and then 2 h after placing them at ~20°C. As shown in Fig. 4D, temperature decreased with food restriction, but was not further reduced when animals were placed in the cooler room. Overall, the temperature reduction was less than in females (Fig. 3) submitted to similar experimental conditions and, most importantly, the difference between the two genotypes was not significant in contrast to the mGPD−/− females temperature fall being nearly double that of the WT females (Fig. 3).

To gain insight into the bases for these differences, we compared systematically potentially relevant consequences of the lack of mGPD−/− in males and females. Blood glucose and triglycerides were equally affected in males, whereas FFA levels were not affected by the genotype in either sex (data not shown). Changes in serum glycerol and tissue G3P (Table 1) were not different from those previously found in males (8). We further looked at mGPD activity in different tissues of C57Bl males and females WT to see whether there was a difference that indicated more reliance of females than males on the enzyme. As shown in Table 4, this was not the case. Essentially, we found the same hierarchy of mGPD activity previously reported for mice (21). Except in liver, where mGPD activity was significantly higher by a margin of ~30%, differences between males and females in other tissues were insignificant. Greater mGPD activity in liver of female than male rats was described earlier (6). We then considered the possibility that females rely more on mGPD to respond to the challenge of a high-fat diet. An increase has been reported in the G3P shuttle activity in the liver of rats fed a high-fat diet with a concomitant reduction in the malate-aspartate shuttle (17). There is no information regarding the response of mGPD to high-fat diets in mice. Having found a good correlation between mGPD activity and mGPD mRNA, confirming previous reports (21), we examined comparatively the response of mGPD mRNA to a high-fat diet in normal males and females. Results are shown in Fig. 5. Although the diet did not significantly change male liver mGPD mRNA levels, in females there was an impressive fivefold increase with levels, which were three to four times higher than in males on either chow or high-fat diet. Muscle mGPD mRNA levels were higher than in liver, as expected from the activity level (Table 4), but as in liver, they were increased by the high-fat diet in females (P < 0.05), but not in males, so that mGPD−/− female levels were double those of the males.

As reported previously by us, mGPD−/− male mice have higher levels of serum T4 and T3, which is ambient temperature dependent, as acclimating these mice to thermoneutrality reduces the levels of both hormones (8). As shown in Fig. 6A, serum T4 concentrations were higher in both WT and mGPD−/− females than in males, although as in males, the
concentration was higher in the mGPD−/− genotype than in the WT counterpart. In contrast to T4, serum T3 concentrations were not higher in females than in males, and the greater level of T3 observed in mGPD−/− than in WT males was not observed in females. Indeed, the serum concentration of T3 in mGPD−/− females was significantly lower than in the males with this genotype (Fig. 6B). Another difference found previously between WT and mGPD−/− males was the expression of UCP3 in skeletal muscle, which was approximately twofold greater in mGPD−/− males (8). This difference was ambient temperature- and thyroid hormone-dependent, as it was partially obliterated when mice were acclimated at 32°C and completely abolished when mice were in addition rendered hypothyroid (8). We confirmed that muscle UCP3 mRNA is about double in mGPD−/− males than in the WT counterpart (Fig. 6C), but we failed to find such difference in females, in which, additionally, the abundance of this mRNA was globally less than in males.

**DISCUSSION**

We previously showed that male mGPD−/− mice have a thermogenic defect that is compensated by increased stimulation of BAT and higher concentrations of thyroid hormones in circulation (8). Although mGPD−/− female and male mice have a very similar phenotype under standard conditions, when

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**Table 4. Mitochondrial G3P dehydrogenase activity in various tissues of males and females C57B1 WT mice**

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT</td>
<td>0.521 ± 0.066 (4)</td>
<td>0.431 ± 0.097 (4)</td>
<td>0.47</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.303 ± 0.012 (4)</td>
<td>0.279 ± 0.063 (4)</td>
<td>0.72</td>
</tr>
<tr>
<td>Brain</td>
<td>0.153 ± 0.009 (8)</td>
<td>0.159 ± 0.007 (8)</td>
<td>0.59</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.137 ± 0.009 (8)</td>
<td>0.125 ± 0.008 (8)</td>
<td>0.30</td>
</tr>
<tr>
<td>Liver</td>
<td>0.065 ± 0.006 (8)</td>
<td>0.088 ± 0.007 (8)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Values are means ± SE (n in parentheses) in ΔOD₅₀₀/min⁻¹·mg protein⁻¹. BAT, brown adipose tissue.

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**Fig. 5. Effect of high-fat diet on expression of mGPD in normal male and female mice.** Level of mRNA is expressed in attomoles per microgram total RNA (see METHODS). Data have been submitted to ANOVA followed by Newman-Keuls test for comparison between groups.

**Fig. 6. Effects of mGPD−/− genotype on serum T4 (A), serum T3 (B), and skeletal muscle uncoupling protein (UCP)-3 mRNA (C) in male and female mice.** This is expressed as attomoles per microgram of total muscle RNA. Data have been submitted to ANOVA followed by Newman-Keuls test for comparison between groups.
challenged with a high-fat diet, fasting, or food restriction, females, but not males, exhibit a thrifty phenotype.

Male and female mGPD−/− mice have a comparable reduction in energy expenditure that is associated with lower blood glucose [also found by Brown et al. (3) in another mGPD-deficient transgenic mouse], elevated plasma levels of glycerol and triglycerides, and normal FFA. Because of the lack of mGPD, the regeneration of DHAP is globally impaired and there is an excess of G3P. Interestingly, this latter was increased by a factor of nearly three in skeletal muscle, but not in the liver. The finding of elevated plasma triglyceride suggested the possibility that the liver could use the excess G3P to synthesize more triglyceride in mGPD−/− mice, explaining the unchanged hepatic levels of G3P in these animals. As shown in Table 1, this was not the case. The higher triglyceride levels are the result of reduced utilization but its mechanism remains speculative. A strong possibility is that triglyceride consumption is reduced in muscle because FFA oxidation is inhibited by the high NADH-to-NAD ratio in the muscle of mGPD−/− mice. This ratio is well reflected by the lactate-to-pyruvate ratio (26), and this was clearly elevated in skeletal muscle (Table 1). An elevated ratio of NADH to NAD inhibits not only FFA β-oxidation but also the Krebs cycle oxidations (26), so that the impairment of G3P and NADH oxidation in the muscle of mGPD−/− mice can cause an overall reduction in oxidative metabolism, contributing to explain the lower thermogenesis.

Two lines of evidence support a more important role of the G3P shuttle in muscle than in liver. For one thing, both mGPD and cGPD activities are normally four- to fivefold higher in muscle (Table 4 and Ref. 30), indicating proportionally more active fluxes. Second, the malate-aspartate NADH shuttle is very active in the liver, whereas its activity is minimal in skeletal muscle (26). We suggest that the liver is more effective disposing of the excess of G3P in mGPD−/− mice because it can be oxidized by cGPD, whereas the more active malate-aspartate shuttle in this organ oxidizes the resulting NADH. Being of minor importance in muscle, this compensation is not expected to occur in this tissue. Such differences between muscle and liver are supported by ongoing in vitro studies in the laboratory (St-Laurent S, Stepanyan Z, and Silva JE, unpublished observations) that show that G3P causes a four- to sixfold increase in respiration in muscle but does not stimulate liver respiration, whereas aminoxyacetate (an inhibitor of the malate-aspartate shuttle) reduces respiration in liver but not in muscle. Thus G3P is an important fuel for muscle, but normally not for liver. If oxidized in muscle, 1 mol of G3P generates 2 mol of ATP, with more energy dissipated as heat. In the absence of mGPD, G3P is oxidized in liver, ultimately via the malate-aspartate shuttle, generating 3 ATPs instead of 2, capturing more energy in ATP, and dissipating less as heat. Moreover, ATP in liver is used locally to support synthetic process instead of supporting muscle activity. As a result, global energy expenditure, muscle activity, and heat production are reduced.

The elevated lactate-to-pyruvate ratio in muscle, in contrast to its reduction in liver reflects again the better protection of liver from the lack of mGPD. Most of the muscle-produced lactate is normally exported to liver where it is largely used in gluconeogenesis, in the so-called Cori cycle (26). The excess of NADH in the mGPD−/− muscle favors the reduction of pyruvate into lactate that is then transported to the liver in larger amounts. Here, the lactate dehydrogenase can oxidize it back to pyruvate with the resulting NADH, which is then oxidized by the malate-aspartate shuttle. This explains both the lower concentration of pyruvate and higher lactate-to-pyruvate ratio in muscle in contrast to the increased pyruvate concentration and reduced lactate-to-pyruvate ratio in liver.

Because there are no marked differences in muscle G3P and lactate/pyruvate between males and females, the gender differences in response to high-fat diet, fasting, or starvation must result from the participation of other factors. It has been reported that female C57Bl mice drop their body temperature more than males when fasted (9), but the reasons were not identified. When food was restricted in the present studies, female mice reduced thermogenesis more effectively than males (compare Fig. 2B with 4D), but then in fasting and food restriction experiments, mGPD−/− female mice QO2 and body temperature fell more than in the WT controls. In contrast, mGPD-deficient males did not show the same propensity to reduce QO2 as females and preserved their body temperature as well as the WT males. A likely explanation for this gender difference is the failure of mGPD−/− females to increase serum T3 levels to the same level as mGPD−/− males. As shown in Fig. 6A, serum T4 levels were higher in mGPD−/− males or females than in their WT counterpart and the increase in T4 was more pronounced in both mGPD−/− and WT females than in the corresponding male controls, yet in females the levels of T3 were not affected by the genotype and were significantly lower than in mGPD−/− males (Fig. 6B). As a result, the T3/T4 concentration ratio was about half in mGPD−/− mice than in the male counterpart. We learned from mice genetically deficient in Type I 5′-deiodinase (32) or Type II 5′-deiodinase (36), that reduced extrathyroidal T4-to-T3 conversion is well reflected in this ratio. Therefore, a lower capacity of females to convert T4 into the more active T3 may explain their propensity to reduce thermogenesis when food restricted, but the causal mechanisms leading to these gender- and genotype-associated differences remain to be investigated. Another notorious difference between mGPD−/− males and females was the failure of these latter to increase UCP3 expression. In view of the absence of gross thermogenic defects in transgenic UCP3-deficient mice (15), it is unlikely that a twofold increase in UCP3 mRNA of mGPD−/− males over WT controls explains their better maintained thermogenesis. Because UCP3 mRNA levels are highly responsive to thyroid hormone, it is more likely that the lower levels in mGPD−/− females reflect the reduced availability of T3 in these mice’s skeletal muscle.

The weight gain in mice fed the high-fat diet as well as the excess weight accumulated by the mGPD−/− females over the WT controls suggests a positive energy balance. However, neither food intake was increased nor was energy expenditure reduced in high-fat diet-fed animals, nor was there a difference in these between mGPD−/− and WT mice. One possible explanation would be that the methods were not sensitive enough. Over 10 wk on high-fat diet, mGPD−/− mice gained ≈3.2 g more than the WT. Assuming that is all fat, this represents the storage of ≈27 kcal over 70 days, that is, 0.38 kcal/day (note in Fig. 1 that the increase in weight is approximately linear with time). The average respiratory quotient in these mice was 0.75, with no significant difference between
Such normal females, such response was not observed in males. Associated with increases in mGPD in liver and muscle of the diet in rats. As shown in Fig. 5, whereas the high-fat diet was compared the responses in males and females, but they suggest a difference between the gender-dependent importance of the G3P shuttle in muscle, which relies largely on the G3P shuttle for its energy needs. We hypothesize that an elevated NADH-to-NAD ratio in muscle and possibly other tissues will cause additional impairment of oxidative metabolism. This ratio normally constitutes a signal of abundance to slow down oxidation of FFA and the Krebs cycle and to divert energy to storage (26). When mGPD is lacking, the signal is inappropriately “on.” Finally, it is inescapable how evocative are the findings in mGPD-deficient mice of the readiness with which some patients gain weight as well as the difficulties these same individuals have to lose weight when they restrict their food intake.

Given the impaired energy utilization in the muscle of mGPD−/− mice, such shifting of energy from oxidation to storage is possible at the expense of two forms of energy expenditure not accurately accounted for, namely nonexercise physical activity (NEPA) and dissipation as heat. NEPA involves grooming, displacing in the cage, moving food around, etc., and it is a significant factor in total energy expenditure (reviewed in Ref. 27). The way we measured it, QO2 is a better reflection of resting energy expenditure or basal metabolic rate. Although mice were not restrained or sedated for the measurement of QO2, this was measured in the middle of the day, optimizing the conditions not to disturb the animals, which remained reasonably quiet during the measurements (8). Rodents are known to be more active at night, so we could have missed a reduction in NEPA. In another mGPD-deficient model, Brown et al. (3) reported no reduction in physical activity, but the study was performed in animals much older than ours (9–11 mo) and on regular chow. In addition, for reasons given above, muscle heat production in mGPD−/− mice is reduced and such deficiency can be exaggerated by the high-fat diet owing to reduced glucose availability and impaired FFA oxidation. Thus the greater energy storage in high-fat diet-fed mGPD−/− mice can be explained by reduced dissipation of energy as heat, less NEPA, or a combination of both, without a detectable change in total energy turnover.

The lack of difference in weight gain between WT and mGPD−/− males when fed a high-fat diet, in contrast to clear differences between WT and mGPD−/− females, may be related to the gender-dependent importance of the G3P shuttle in the response to such diet. It has been reported that liver mitochondria from rats fed a high-fat diet show increased respiration with G3P, whereas the response to other substrates is reduced (17). Moreover, muscle fat oxidation normally increases in rats fed high-fat diets (18). Such studies did not compare the responses in males and females, but they suggest that mGPD may be important to respond to an energy-dense diet in rats. As shown in Fig. 5, whereas the high-fat diet was associated with increases in mGPD in liver and muscle of normal females, such response was not observed in males. Such findings suggest that female mice rely more than males on mGPD to respond to a high-fat diet. This would make females more vulnerable to the lack of mGPD. It is tempting to speculate that males rely rather on UCP3, a fairly well-established role of which may be to facilitate the oxidation of fatty acids (see Ref. 35 for recent review). Fatty acids and high-fat diets can induce UCP3 (12, 37) and it has been reported that the response of UCP3 mRNA to another form of energy dense diet, cafeteria diet, is more vigorous in male rats than in females (34). This observation, together with the data presented here, suggests that the mechanisms to respond to energy-dense diets can be gender dependent, probably via sex hormones, and that the efficacy of the responses is different, explaining the greater tendency of the female gender to gain more weight when exposed to such diets.

In summary, the data presented here show the deletion of mGPD in mice has clear metabolic consequences, under both the standard ecological conditions of the vivarium and when challenged either with unbalanced diets, fasting, or starvation. The results are consistent with the major problem of impaired channeling of reducing equivalents into the mitochondria in muscle, which relies largely on the G3P shuttle for its energy needs. We hypothesize that an elevated NADH-to-NAD ratio in muscle and possibly other tissues will cause additional impairment of oxidative metabolism. This ratio normally constitutes a signal of abundance to slow down oxidation of FFA and the Krebs cycle and to divert energy to storage (26). When mGPD is lacking, the signal is inappropriately “on.” Finally, it is inescapable how evocative are the findings in mGPD-deficient mice of the readiness with which some patients gain weight as well as the difficulties these same individuals have to lose weight when they restrict their food intake.

GRANTS

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