Analysis of energy expenditure at different ambient temperatures in mice lacking DGAT1

HUBERT C. CHEN,1–3 ZULEIKA LADHA,1 STEVEN J. SMITH,1,2 AND ROBERT V. FARESE, JR.1–3
1 Gladstone Institute of Cardiovascular Disease, San Francisco 94103; and 2 Cardiovascular Research Institute and 3 Department of Medicine, University of California, San Francisco, California 94143

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Chen, Hubert C., Zuleika Ladha, Steven J. Smith, and Robert V. Farese, Jr. Analysis of energy expenditure at different ambient temperatures in mice lacking DGAT1. Am J Physiol Endocrinol Metab 284: E213–E218, 2003. First published September 3, 2002; 10.1152/ajpendo.00248.2002.—Mice lacking acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1), a key enzyme in triglyceride synthesis, have increased energy expenditure and thermoregulation in DGAT1-deficient [Dgat1(+/−)] mice. Dgat1(+/−) mice had increased energy expenditure irrespective of changes in the ambient temperature. Although core temperature was normal, surface temperature was increased in Dgat1(+/−) mice, most likely reflecting an active mechanism to dissipate heat from increased thermogenesis. Dgat1(+/−) mice had increased food intake at baseline, and this hyperphagia could result in increased heat loss and contribute to their increased energy expenditure. To investigate these questions, we studied the effects of different ambient temperatures on energy expenditure, food intake, and thermoregulation in Dgat1(+/−) mice. Our findings offer insights into the mechanisms of obesity resistance in mice that lack acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1). DGAT1 is one of two known enzymes that catalyze the final step in mammalian triglyceride synthesis (1, 2). DGAT1-deficient [Dgat1(−/−)] mice have reduced adiposity and are resistant to diet-induced obesity (17). Energy expenditure on either a chow or a high-fat diet, as measured by indirect calorimetry, is ~15% higher than in wild-type [Dgat1(+/+) mice (17). Part of this increase may be because of increased leptin sensitivity in Dgat1(−/−) mice (6). In addition, Dgat1(−/−) mice fed a high-fat diet have a twofold increase in locomotor activity (17).

Several aspects of the DGAT1-deficiency phenotype intrigued us. For example, despite their increased sensitivity to leptin, Dgat1(+/−) mice eat more than Dgat1(+/+) mice (6, 5). In addition, Dgat1(+/−) mice have abnormal fur lipid composition, which results in impaired water repulsion and prolonged hypothermia after immersion in water (7). Because fur lipids may have an important role in insulating rodents from a cold environment (19), we considered that altered fur lipid composition in Dgat1(+/−) mice could result in increased heat loss and contribute to their increased energy expenditure. To investigate these questions, we studied the effects of different ambient temperatures on energy expenditure, food intake, and thermoregulation in Dgat1(−/−) mice. Our results suggest a model in which DGAT1 deficiency constitutively activates thermogenesis regardless of changes in the ambient temperature. This increase in thermogenesis, in turn, results in enhanced heat loss to the environment and compensatory hyperphagia.

MATERIALS AND METHODS

Mice. Dgat1(+/−) mice (98% C57BL/6 and 2% 129/SvJae background) were generated previously (17). Dgat1(+/+) mice (in C57BL/6 background) were from the Jackson Laboratory (Bar Harbor, ME). Age-matched 8- to 12-wk-old female mice were used for experiments unless noted otherwise. Mice were housed at 20°C in a pathogen-free barrier facility (12:12-h light-dark cycle) and fed rodent chow (Ralston Pu...
rina, St. Louis, MO). For experiments at 4°C, mice were housed in a walk-in refrigerator for 8 h unless noted otherwise. For experiments at 32°C, an electric heating pad was placed underneath the mouse cages, and mice were acclimatized to the new ambient temperature for 24 h before the start of experiments. For high-fat diet experiments, mice were fed a Western-style diet containing 21% fat by weight (Harlan Teklad Laboratory, Madison, WI). The studies were approved by the Committee on Animal Research of the University of California, San Francisco.

Real-time PCR. Real-time PCR for uncoupling protein 1 (UCP1) was performed as described (7) with primers 5′/H11032-CAC-CTTCCCGCTGGACACT-3′ and 5′/H11032-GTGATGGTCCCTAGGACCTTTA-3′/H11032 and probe 5′/H11032-CAAAGTCCGCCTTCAGATCCA-AGGTGA-3′/H11032.

Temperature measurements. Body temperatures were measured with a digital thermometer (model 4600; Yellow Springs Instruments, Yellow Springs, OH). Surface temperatures were measured by placing the probe in the interscapular region for 30 s. Core temperatures were measured rectally.

Measurement of metabolic parameters. Inguinal, reproductive, mesenteric, and perirenal fat pads were used to determine total fat pad content. Plasma free fatty acid and triglyceride levels were measured as described (17). Plasma glucose concentrations were measured with a glucometer (Accu-chek; Roche Diagnostics, Indianapolis, IN).

For glycogen measurements, tissues were treated with potassium hydroxide, followed by saturated sodium sulfate and ethanol to isolate glycogen granules. The samples were then boiled in hydrochloric acid and neutralized with potassium hydroxide and triethanolamine. Glucose concentrations were measured with a colorimetric kit (Sigma Chemical, St. Louis, MO). The quadriceps muscles from the lower extremities were used for skeletal muscle measurements.

**Statistical analysis.** Data are expressed as means ± SD. Measurements were compared with the two-tailed t-test or Mann-Whitney rank-sum test. For metabolic parameters involving three groups of mice, results were compared with ANOVA, followed by a post hoc Tukey-Kramer test. Correlation was determined by linear regression.

**RESULTS**

**Increased energy expenditure and resistance to diet-induced obesity in Dgat1(-/-) mice housed at different ambient temperatures.** To determine whether changes in the ambient temperature affected the increased en-

![Fig. 1. Increased fasting-induced weight loss in Dgat1-deficient (-/-) mice at different ambient temperatures; n = 5 mice/group.](image1)

![Fig. 2. Resistance to obesity in Dgat1(-/-) mice fed a high-fat diet at different ambient temperatures. A: change in body weight. B: total fat pad content (n = 4–5 mice/group for A and B).](image2)

![Fig. 3. Effect of different ambient temperatures on uncoupling protein 1 (UCP1) mRNA expression in Dgat1(-/-) mice (n = 5/group).](image3)

![Fig. 4. Increased heat dissipation in Dgat1(-/-) mice. A: core and surface body temperatures in Dgat1(-/-) mice. For experiments at 4°C, mice were placed in a cold room for 4 h (n = 5 mice/group). B: core temperature in Dgat1(-/-) carcasses. Male mice were cervically dislocated and placed in a cold room (n = 6/group).](image4)
ergy expenditure in Dgat1(−/−) mice, we measured their weight loss after an 8-h fast at 4, 20, and 32°C (thermoregulatory). Because energy intake is eliminated, the amount of fasting-induced weight loss provides a simple approximation of energy expenditure. As shown previously (5), Dgat1(−/−) mice lost more weight than Dgat1(+/+) mice after an 8-h fast at 20°C (Fig. 1). Exposure to 4°C resulted in further weight loss in both Dgat1(−/−) and Dgat1(+/+) mice, reflecting an increase in energy expenditure because of increased heat loss in both groups of mice. When housed at 32°C to minimize heat loss to the environment, the magnitude of fasting-induced weight loss was reduced in both Dgat1(−/−) and Dgat1(+/+) mice. However, Dgat1(−/−) mice still lost more weight than Dgat1(+/+) mice after fasting at either 4 or 32°C. Dgat1(−/−) mice fed a high-fat diet also remained resistant to weight gain (Fig. 2A) and had ~50% less adipose tissue than Dgat1(+/+) mice (Fig. 2B) at 32°C. These results suggest that Dgat1(−/−) mice have increased energy expenditure across a range of ambient temperatures, including at thermoregulatory. They provide evidence that the increased energy expenditure in Dgat1(−/−) mice does not simply result from increased heat loss to the environment. Rather, thermogenesis appears to be constitutively activated in Dgat1(−/−) mice regardless of changes in the ambient temperature.

Modulation of increased UCP1 expression in Dgat1(−/−) mice by changes in the ambient temperature. In rodents, thermogenesis is primarily mediated by UCP1, a brown adipocyte protein that disrupts the mitochondrial proton gradient, resulting in the generation of heat instead of ATP (8; also reviewed in Ref. 13). We have shown that UCP1 expression is increased in Dgat1(−/−) mice (5, 6). Consistent with these findings, UCP1 expression was twofold higher in Dgat1(−/−) mice than in Dgat1(+/+) mice at 20°C (Fig. 3). At 32°C, UCP1 expression was decreased in both Dgat1(−/−) and Dgat1(+/+) mice, although it remained significantly higher in Dgat1(−/−) mice. At 4°C, UCP1 expression was increased in both Dgat1(−/−) and Dgat1(+/+) mice. However, because the magnitude of increase was greater in Dgat1(+/+) mice, UCP1 expression levels were now similar in Dgat1(−/−) and Dgat1(+/+) mice. These results suggest that thermogenesis, as reflected by UCP1 expression, is increased in Dgat1(−/−) mice at 20 and 32°C. The difference is not observed at 4°C, perhaps because UCP1 is expressed maximally in both Dgat1(−/−) and Dgat1(+/+) mice.

Increased surface body temperatures in Dgat1(−/−) mice. To determine the effects of increased energy expenditure on thermoregulation in Dgat1(−/−) mice, we measured the core and surface body temperatures of Dgat1(−/−) and Dgat1(+/+) mice. Consistent with previous findings (7), Dgat1(−/−) and Dgat1(+/+) mice had similar core temperatures at both 20 and 4°C (Fig. 4A). However, Dgat1(−/−) mice had higher surface temperatures than Dgat1(+/+) mice. To determine whether this difference resulted from active heat dissipation or impaired insulation, we placed Dgat1(−/−) and Dgat1(+/+) carcasses at 4°C and found that they had similar rates of decrease in core temperature (Fig. 4B). These findings suggest that the increased surface temperature in Dgat1(−/−) mice results from an active process to dissipate heat rather than from impaired insulation resulting from altered fur lipid composition.

Modulation of hyperphagia in Dgat1(−/−) mice by changes in the ambient temperature. We have shown that Dgat1(−/−) mice eat more than Dgat1(+/+) mice at room temperature (5, 6). To determine whether changes in the ambient temperature modulate food intake in Dgat1(−/−) mice, we measured the daily food consumption of Dgat1(−/−) and Dgat1(+/+) mice housed at different temperatures. At 20°C, Dgat1(−/−) mice ate ~15% more than Dgat1(+/+) mice (Fig. 5). Both Dgat1(−/−) and Dgat1(+/+) mice ate more in
response to cold exposure, but the increase was greater in Dgat1(−/−) mice. As a result, Dgat1(−/−) mice ate ~30% more than Dgat1(+/+) mice at 4°C. When housed at 32°C, both Dgat1(−/−) and Dgat1(+/+) mice reduced their food intake, and the difference between Dgat1(−/−) and Dgat1(+/+) mice was minimal. Thus the increased food intake in Dgat1(−/−) mice was most pronounced at cold temperatures.

Hypothermia in Dgat1(−/−) mice fasted at 4°C. To further explore the effects of ambient temperature on energy expenditure and food intake, we fasted Dgat1(−/−) mice at 4°C for 8 h and measured their core temperatures. Dgat1(−/−) mice maintained relatively normal core temperatures when fed ad libitum but developed significant hypothermia when fasted (Fig. 6). Dgat1(−/−) mice also had slightly lower core temperatures than Dgat1(+/+) mice after fasting for 8 h at 20°C (36.5 ± 0.2 vs. 37.1 ± 0.3°C, n = 5, P < 0.05). These findings indicate that food intake is essential for Dgat1(−/−) mice to maintain a normal body temperature during exposure to cold.

One possible explanation for the hypothermia in fasted Dgat1(−/−) mice was that, because of their increased energy expenditure and decreased body fat content, Dgat1(−/−) mice were exhausting their fuel reserves for thermogenesis. To test this hypothesis, we restricted the daily food intake of a group of Dgat1(+/+) mice to control for the difference in body fat. After 3 days of a low-calorie diet (~70% of normal), these Dgat1(+/+) mice had a mean total fat pad content similar to that of age-matched Dgat1(−/−) mice (2.1 ± 0.3 vs. 2.2 ± 0.2% total body wt). When fasted for 8 h at 4°C, calorie-restricted Dgat1(+/+) mice also developed hypothermia, although not as severely as Dgat1(−/−) mice. These results indicate that decreased body fat content is unlikely to fully account for the profound hypothermia in fasted Dgat1(−/−) mice. In addition, fat pads were easily identifiable in Dgat1(−/−) and calorie-restricted Dgat1(+/+) mice after fasting, and their plasma free fatty acid and triglyceride levels were similar to those of fasted Dgat1(+/+) mice (Table 1). These results provide additional evidence that the depletion of lipids is unlikely to be a major reason for the hypothermia in Dgat1(−/−) mice fasted at 4°C.

Decreased plasma glucose levels in Dgat1(−/−) mice fasted at 4°C. In contrast to the results for plasma lipids, plasma glucose levels were significantly lower in Dgat1(−/−) mice than in Dgat1(+/+) mice after fasting (Table 1). Calorie-restricted Dgat1(+/+) mice also had decreased plasma glucose levels. In addition, the severity of hypothermia in Dgat1(−/−) mice correlated with the degree of hypoglycemia (Fig. 7). These findings suggest that the depletion of glucose may contribute to the hypothermia in Dgat1(−/−) mice fasted at 4°C.

Reduction of fasting-induced hypothermia in Dgat1(−/−) mice after oral feeding of glucose. To further explore the relationship between plasma glucose levels and thermoregulation, we fasted Dgat1(−/−) mice at 4°C for 4 h and fed them either 0.2 kcal glucose or 0.2 kcal corn oil. Oral feeding of glucose restored normal plasma glucose levels and prevented the development of profound hypothermia in Dgat1(−/−) mice after an additional 4 h of fasting (Fig. 8). Oral feeding of corn oil also increased plasma glucose levels and reduced the severity of hypothermia in Dgat1(−/−) mice, although not to the same extent as glucose feeding.

Increased glycogen content in Dgat1(−/−) mice. Because glycogenolysis plays an important role in maintaining euglycemia during fasting states, we hypothesized that Dgat1(−/−) mice have decreased tissue glycogen content. In fed mice housed at room temperature, liver glycogen content trended lower in Dgat1(−/−) mice than in Dgat1(+/+) mice (Fig. 9), although the difference was not significant (P = 0.06). However, skeletal muscle glycogen content was significantly reduced in fed Dgat1(−/−) mice. After 4 h of fasting at 4°C, liver glycogen content was decreased in both Dgat1(−/−) and Dgat1(+/+) mice but was ~50% lower in Dgat1(−/−) mice. Similar findings were observed in the skeletal muscle.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>After Fast</th>
<th>Calorie-Restricted Dgat1(+/+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat pad content, % body wt</td>
<td>3.8 ± 0.1</td>
<td>2.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Plasma free fatty acid, mmol/l</td>
<td>0.06 ± 0.03</td>
<td>0.05 ± 0.03</td>
<td>0.37 ± 0.08</td>
</tr>
<tr>
<td>Plasma triglycerides, mg/dl</td>
<td>34.4 ± 4.3</td>
<td>42.8 ± 9.1</td>
<td>17.8 ± 0.9</td>
</tr>
<tr>
<td>Plasma glucose, * mg/dl</td>
<td>230 ± 36</td>
<td>227 ± 14</td>
<td>95 ± 19</td>
</tr>
</tbody>
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Values are means ± SD; n = 5–7 mice/group, except for plasma glucose measurements where n = 8–12/group. *P < 0.01 vs. fasted Dgat1(+/+). †P < 0.05 vs. fasted Dgat1(−/−).

Fig. 7. Correlation of hypothermia and hypoglycemia in Dgat1(−/−) mice after an 8-h fast at 4°C. Each point represents an individual mouse.
DISCUSSION

In this study, we show that Dgat1(-/-) mice had increased energy expenditure irrespective of changes in the ambient temperature. We also show that surface body temperature was increased in Dgat1(-/-) mice, possibly reflecting an active mechanism to dissipate heat from increased thermogenesis. Upon exposure to cold, Dgat1(-/-) mice had more pronounced hyperphagia, most likely to compensate for increased utilization of fuel substrates for thermogenesis. This increased utilization may explain why Dgat1(-/-) mice became hypoglycemic and hypothermic more quickly than Dgat1(+/+) mice when fasted in a cold environment.

Energy expenditure of small mammals (such as mice) differs considerably from that of large mammals (such as humans). For example, because of their high surface area-to-volume ratio, mice can lose a substantial amount of heat to the environment, and this heat loss can play an important role in determining their rate of energy expenditure (reviewed in Ref. 16). Our results, however, suggest that increased heat loss to the environment is not a primary mechanism for the increased energy expenditure in Dgat1(-/-) mice. Instead, we speculate that the increased heat loss reflects an active, compensatory process (e.g., increased vasodilatation in the skin) to prevent hyperthermia in a state of increased thermogenesis. The mechanism for this constitutive activation of thermogenesis in Dgat1(-/-) mice may relate to their increased sensitivity to leptin, an adipocyte-derived hormone that enhances UCP1 expression (8; also reviewed in Refs. 11 and 13).

Our findings also shed light on the observation of increased food intake in Dgat1(-/-) mice (5, 6). The hyperphagia in Dgat1(-/-) mice was minimal at thermoneutrality and accentuated by exposure to cold. This suggests that the hyperphagia in Dgat1(-/-) mice reflects a compensatory process for an increased demand on fuel substrates. These results help to explain why Dgat1(-/-) mice eat more despite an increased sensitivity to leptin (6), which decreases food intake (reviewed in Ref. 11). Apparently, the need to replenish fuel substrates is a more powerful appetite stimulus in Dgat1(-/-) mice than the inhibitory effects of increased leptin sensitivity on food intake.

Our results indicate that glucose plays an important role in maintaining normal energy homeostasis in cold-exposed Dgat1(-/-) mice. The severity of hypothermia in fasted mice correlated with plasma glucose concentrations rather than with plasma lipid concentrations. Moreover, oral feeding of glucose restored normal plasma levels and prevented the development of profound hypothermia in Dgat1(-/-) mice. Oral feeding of triglycerides (corn oil) with a comparable caloric content had a less significant effect in ameliorating the hypoglycemia and hypothermia in Dgat1(-/-) mice, most likely because only a relatively small portion of the corn oil can be converted directly into glucose (via...
glycerol and gluconeogenesis). These results provide evidence that the depletion of glucose, and not calories in general, triggers the drop in body temperature in Dgat1(-/-) mice.

Our findings are consistent with the clinical observation that hypoglycemia is a predisposing factor for hypothermia in humans (15). At least two possible mechanisms may account for the hypoglycemia-induced hypothermia in Dgat1(-/-) mice. One possibility is that hypoglycemia per se induces hypothermia, perhaps through a mechanism involving the central nervous system. Another possibility may relate to the adage “fat burns in the flame of carbohydrates” (18). In the setting of hypoglycemia, fatty acids may not be fully oxidized, because oxaloacetate, a major component of the citric acid cycle, is shunted to gluconeogenesis. As a result, acetyl-CoA derived from the breakdown of fatty acids cannot enter the citric acid cycle by binding with oxaloacetate. In this scenario, cellular energy metabolism is impaired, even if lipid substrates are still readily available.

Interestingly, perilipin-deficient mice, another murine model of increased energy expenditure, obesity resistance, and hyperphagia, also develop profound hypothermia when fasted in a cold environment (14). It would be of interest to determine whether increased depletion of fuel substrates also accounts for the fasting-induced hypothermia in perilipin-deficient mice. It would also be of interest to examine whether other murine models of increased energy expenditure and hyperphagia, such as mice lacking protein kinase A-RIIβ (9) or protein tyrosine phosphatase-1B (10), have a similar mechanism for their increased food consumption.

In summary, our findings offer insights into the mechanisms of hyperphagia and increased energy expenditure in a murine model of obesity resistance. We show that the increased energy expenditure in Dgat1(-/-) mice is not dependent on changes in the ambient temperature. Instead, Dgat1(-/-) mice appear to have a constitutive activation of thermogenesis, which results in increased heat dissipation to the environment. This is associated with a dependency upon exogenous fuel sources to maintain normal body temperature during exposure to cold. Similar mechanisms may be active in other murine models of obesity resistance and increased energy expenditure.

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