Temperature-dependent feeding: lack of role for leptin and defect in brown adipose tissue-ablated obese mice

ANNA MELNYK AND JEAN HIMMS-HAGEN
Department of Biochemistry, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5

Melynk, Anna, and Jean Himms-Hagen. Temperature-dependent feeding: lack of role for leptin and defect in brown adipose tissue-ablated obese mice. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1131–R1135, 1998.—The objective was to characterize the ability of control and transgenic brown adipose tissue (BAT)-ablated uncoupling protein diphtheria toxin A chain (UCP-DTA) mice to adjust food intake in relation to changes in environmental temperature and to assess the involvement of leptin in this adjustment. We measured serum leptin in mice from a previous study of UCP-DTA mice raised at thermoneutrality (35°C) or at the usual rearing temperature (24°C) from weaning [Melnyk, A., M.-E. Harper, and J. Himms-Hagen. Am. J. Physiol. 272 (Regulatory Integrative Comp. Physiol. 41): R1088–R1093, 1997] and extended the study by acclimating control and obese UCP-DTA mice at 18 wk of age to cold (14°C) for up to 14 days. Leptin levels did not change in control mice at 14°C; however, food intake increased threefold within 1 day and remained at this level. Serum leptin level was elevated in UCP-DTA mice at 24°C compared with control mice at 24°C; this elevated level decreased within 1 day at 14°C and was not different from the level in control mice by 14 days. Food intake of UCP-DTA mice that were hyperphagic at 24°C did not change during 7 days at 14°C, then increased slowly. Similar low leptin levels were present in control mice at 24 or 35°C and in UCP-DTA mice at 35°C. Food intake of control mice raised at 24°C was two times that of control mice raised at 35°C. UCP-DTA mice raised at 35°C ate the same low amount as control mice raised at 35°C. UCP-DTA mice at 24°C were hyperphagic relative to control mice at 24°C yet had elevated leptin levels in their serum. Two principal conclusions are drawn. First, adjustment of food intake over a fourfold range by control mice acclimated to temperatures from 35 down to 14°C is independent of changes in serum leptin levels. Second, this adjustment of food intake in relation to temperature is defective in the UCP-DTA mouse; the defect leads to hyperphagia at 24°C and a failure to increase food intake as rapidly as control mice when exposed to 14°C. Because lack of UCP-1-mediated thermogenesis in BAT of knockout mice is known not to induce hyperphagia, we propose that deficiency of UCP-1-expressing brown adipocytes in BAT of UCP-DTA mice results in lack of a satiety factor, secreted by these cells in BAT of control mice in inverse relationship to sympathetic nervous system activity.

brown adipocyte; white adipose tissue; cold acclimation; thermogenesis; uncoupling protein-1; obesity; transgenic brown adipose tissue-ablated mice

PARTIAL ABLATION OF BROWN ADIPOCYTES in a transgenic mouse was achieved by using regulatory elements of the uncoupling protein-1 (UCP-1) gene, expressed uniquely in brown adipocytes, to drive expression of diphtheria toxin A chain (DTA) (21). UCP-DTA mice were predicted to become obese because of a deficit in energy expenditure for thermogenesis in their brown adipose tissue (BAT). They did indeed become obese, but they also became hyperphagic; this consequence was not predicted by any function of brown adipocytes known at the time and suggested a role for BAT in the control of food intake (24). Obesity of UCP-DTA mice is characterized by the insulin resistance, hyperglycemia, hyperinsulinemia, and hyperlipidemia seen in non-insulin-dependent diabetes mellitus (15, 17) and is aggravated by a high-fat diet (17). Obesity of UCP-DTA mice is also associated with an increased expression of leptin in their white adipose tissue (WAT) and with an increased concentration of leptin in their blood (12, 13, 16). Their hyperphagia is not improved by administration of additional leptin (16).

We have shown that both the obesity and hyperphagia of the UCP-DTA mouse are temperature sensitive, occurring only when these mice are raised from weaning at an environmental temperature below thermoneutrality (which is 33–35°C for mice) (23). At thermoneutrality, sympathetic nervous system activity in BAT is at a minimum (27), as is stimulation of expression of UCP (2, 23) and of thermogenesis in brown adipocytes. It is probable that stimulation of DTA expression via the UCP-1 promoter is also at a minimum in brown adipocytes of mice at thermoneutrality; therefore, some brown adipocytes might survive at thermoneutrality that would otherwise die at a lower temperature.

Initially we interpreted the prevention of obesity and hyperphagia resulting from raising UCP-DTA mice at thermoneutrality as due to abolition of any deficit in BAT thermogenesis at this temperature (23); brown adipocytes were expected to be thermogenically inactive in both control and UCP-DTA mice at 35°C, with any potential deficit felt only at lower environmental temperatures. This interpretation has now been revised in light of a recent report of a transgenic mouse with targeted disruption of the UCP-1 gene in its brown adipocytes (10). This transgenic mouse totally lacks UCP-1 in its brown adipocytes when living at the usual animal facility temperature of 24°C, and its capacity for a thermogenic response to injection of a β3-adrenergceptor agonist is severely reduced (10), to a greater extent than the reduction in this response in the UCP-DTA mouse (21). Yet the UCP-1 knockout mouse is neither hyperphagic nor obese, in contrast to the hyperphagia and obesity of the UCP-DTA mouse and despite the similar lack of UCP-1-mediated thermogenesis they presumably both experience. Moreover, the UCP-1 knockout mouse is extremely cold intolerant (10), whereas the UCP-DTA mouse can survive for at least 2 days at 4°C (21).

The initial objective of the present experiments was to characterize further the ability of the obese UCP-DTA mouse to adapt to mild cold (14°C). Because we knew that food intake of mice can vary over a fourfold
range from thermoneutrality down to 14°C (7, 25), we also measured serum leptin in mice during acclimation to cold and in samples from our previous study of UCP-DTA mice raised at thermoneutrality (23) to see whether there was any correlation of leptin levels with the wide variation in food intake over the temperature range from thermoneutrality (35°C) down to cold (14°C).

METHODS

A colony of UCP-DTA mice was established with six female UCP-DTA mice (provided by Drs. J. S. Flier and B. B. Lowell, Beth Israel Hospital and Harvard Medical School, Boston, MA) and six male FVB/N mice (from Taconic, Germantown, NY). Breeding pairs were housed at 24°C with free access to food (Agway R-M-H 4020 chow, 3.5 kcal/g, 14.5% of energy from fat) and water and lights on 12 h/day (0600 to 1800). At 21 days of age, mice were weaned and housed in groups of littermates of the same sex. Female mice from the colony were separated into single cages at 17 wk of age. These mice were allowed free access to chow and water for 1 wk before the start of the experiment. At 18 wk of age, mice were either killed (time 0) or they were put into a cold room kept at 14°C for up to 14 days. Groups of six to eight mice were killed at 1, 2, 7, and 14 days. Body weights and rectal temperatures were measured after various times in the cold (between 1130 and 1500). Food intake was evaluated over the course of the experiment. Serum samples from mice raised from weaning to 8 wk of age at either 24 or 35°C in the previous study (23) were also used; food intake and inguinal WAT weights of these mice are repeated in this paper to allow easier comparison with leptin levels. In the previous study (23), inguinal WAT weights were found to be an accurate index of carcass fat. Mice were killed by cervical dislocation and exsanguination. Inguinal WAT depots were removed, cleaned, and weighed. Leptin was assayed in serum using a commercial kit for mouse leptin (Linco Research Labs (St. Louis, MO) from Cedarlane Laboratories).

RESULTS

Temperatures and body weights. When UCP-DTA mice are housed at 24°C, their body temperatures are significantly lower than those of control mice (Fig. 1).

When they are exposed to mild cold (14°C), both groups experience a slight decline in body temperature (Fig. 1). UCP-DTA mice at 14°C have a significantly lower body temperature than control mice for the first week of acclimation (Fig. 1); they could not, however, be described as hypothermic, maintaining a temperature consistently between 37.0 and 37.5°C. After 14 days of acclimation to cold, both groups of mice are able to thermoregulate and maintain their body temperature at the level at which they started.

Initial body weights of all mice were 25.3 ± 0.38 g (control mice, n = 31) and 33.1 ± 0.87 g (UCP-DTA mice, n = 32) (P < 0.0001, transgenic vs. control mice). Weight gain continued in control mice during the period of acclimation, whereas weight gain of UCP-DTA mice ceased. After 14 days at 14°C, the weights of UCP-DTA mice and control mice were not significantly different (control mice 27.9 ± 1.2 g, n = 7; UCP-DTA mice 31.7 ± 2.4 g, n = 6).

Food intake and serum leptin concentration. Food intake of control mice at 14°C increased by 200% within 1 day of exposure to cold, then remained at this elevated level until 14 days (Fig. 2). Before the exposure to cold, food intake of UCP-DTA mice was almost double that of control mice. Food intake of UCP-DTA mice did not, however, change during the first 4 days of exposure to cold (Fig. 2); thus they were hypophagic compared with control mice during this period. By 7 days, food intake of the UCP-DTA mice had increased by 50%, reaching the same high level as in the control mice.

The concentration of leptin in serum of control mice was low and remained unchanged throughout the 14 days of acclimation to cold (Fig. 3). The markedly elevated level of serum leptin in UCP-DTA mice on day 0, 4.6 times that of the level in control mice, decreased rapidly within 1 day of exposure to cold. Thereafter it decreased further, reaching a level not significantly different from that of control mice by day 14. The decrease in leptin level in UCP-DTA mice paralleled that of their inguinal WAT weight (Fig. 4), which likewise reached a level not significantly different from that of control mice by day 14 (Fig. 4).

In control mice raised at thermoneutrality, serum leptin was low and was the same as in control mice
raised at 24°C (Table 1). Fat stores, indexed by the weight of their inguinal WAT depot (23), were identical in these two groups of mice (Table 1). Serum leptin in UCP-DTA mice raised at 24°C was 3.5 times that of control mice raised at this temperature until 8 wk of age. In contrast, serum leptin in UCP-DTA mice raised at 35°C was low and similar to that of control mice at either 24 or 35°C (Table 1). Serum leptin levels correlated well with body fat. Food intake of control and UCP-DTA mice raised at 35°C was the same and about one-half of that of control mice raised at 24°C (Table 1). However, UCP-DTA mice at 24°C were hyperphagic, eating 35% more than control mice at this same temperature despite the elevated level of leptin in their blood.

**DISCUSSION**

There are two principal conclusions about control of food intake that arise from the present study of mice acclimated to a cold environment (14°C) and from the previous study of mice raised in a thermoneutral environment (35°C) (23). The first conclusion is that mice can adjust their food intake over a fourfold range within these temperature limits, conserving the same energy reserves over the entire range, presumably balancing almost exactly their varying energy intake with their varying energy expenditure. This energy balance is achieved in the absence of any changes in serum leptin concentration. Leptin levels reflect body fat stores and are not related to either energy intake or energy expenditure at the temperatures studied. Thus the major adjustment in food intake that occurs over this large range of environmental temperatures in the mouse is independent of changes in serum leptin levels.

Reinterpretation of results of previous studies of food intake in genetically obese ob/ob and db/db mice acclimated to different temperatures (7), in light of what is now known about the nature of the genetic defects in these mice (5, 6, 20, 28), supports this conclusion about the leptin-independent nature of this adjustment of food intake. Both mutant strains are hyperphagic when acclimated to various temperatures between 33 and 10°C (data from Ref. 7 replotted in Fig. 5). The hyperphagia due to lack of leptin (ob/ob mice) or to lack of leptin action (db/db mice) is of similar absolute magnitude at 33 and 22°C, somewhat less as the temperature of acclimation decreases further and intake increases. More important, however, is that both ob/ob and db/db mice are able to adjust their food intake upwards as the temperature of acclimation decreases; thus this adjustment can occur in the complete absence of leptin or of leptin action. A similar conclusion can be reached from the pattern of food intake in fa/fa rats, which also lack leptin action (6),

### Table 1. Food intake, weights of inguinal WAT depots, and serum leptin concentrations in control and UCP-DTA mice

<table>
<thead>
<tr>
<th></th>
<th>Control Mice, °C</th>
<th>UCP-DTA Mice, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Food intake, g/day</td>
<td>5.36 ± 0.26</td>
<td>2.80 ± 0.12*</td>
</tr>
<tr>
<td>Serum leptin, ng/ml</td>
<td>2.22 ± 0.23</td>
<td>2.39 ± 0.29</td>
</tr>
<tr>
<td>Inguinal WAT weight, mg</td>
<td>260.8 ± 32.0</td>
<td>297.9 ± 14.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of mice. Mice were raised at 24 or 35°C from time of weaning to 8 wk of age [values for food intake and inguinal white adipose tissue (WAT) weights are from Figs. 1 and 2 of Ref. 23]. UCP-DTA, uncoupling protein diphtheria toxin A. *Significantly different from mice of same genotype at 24°C; †significantly different from control mice at same temperature; P < 0.05.
adapted to temperatures from 30°C (thermoneutrality for the rat) down to 5°C (4, 11, 18).

The second principal conclusion about control of food intake in mice is that the adjustment of food intake in relation to temperature of acclimation outlined above is defective in the UCP-DTA mouse. This mouse eats the same low amount as a control mouse at 35°C yet becomes hyperphagic at 24°C (Fig. 6). Once acclimated to 24°C, this mouse does not immediately raise its food intake in response to cold (14°C), as does the control mouse, but eventually eats the same large amount as control mice in the cold; this amount is appropriate for normal energy balance at this temperature in both control and UCP-DTA mice. Again, leptin levels reflect body fat stores in UCP-DTA mice and are not directly related to either energy intake or energy expenditure. In this pattern of adjustment of food intake with temperature, the UCP-DTA mouse resembles neither the control mouse of the FVB/N strain (Fig. 6) nor the strains of mutant mice suffering from lack of leptin action (ob/ob or db/db mice) (Fig. 5). A hypothesis to explain what part of a temperature-sensitive control mechanism for food intake might be defective in the UCP-DTA mouse is presented in Perspectives. The only change in food intake in the UCP-DTA mouse that is associated with a change in leptin level is the delayed increase in food intake seen during the second week at 14°C. This increase followed a cold-induced reduction in the elevated leptin level that occurred several days before and suggests that some leptin-mediated constraint on food intake might have been present in this mouse when it was hyperphagic at 24°C; this constraint disappeared when the leptin concentration decreased. The UCP-DTA mouse is not able to decrease its food intake any further in response to administration of additional leptin (16). Thus food intake of the UCP-DTA mouse at 24°C, with an elevated leptin level in its blood, is presumably already maximally responsive to its high leptin level of 14 ng/ml; the mouse eats more when this level decreases at 14°C to a level not significantly different from that in control mice.

Suppression of food intake that is independent of changes in leptin level can also occur in mice in response to acute stimulation of the β3-adrenoceptor by a selective agonist (22). In this case, suppression is probably mediated by the increase in heat production, which can also serve as a satiety signal when not balanced by an increase in heat loss (19). A similar explanation probably accounts for the leptin-independent reversal of hyperphagia in the fa/ fa rat during chronic treatment with a β3-adrenoceptor agonist (14).

Perspectives

Why is the UCP-DTA mouse with partial ablation of its BAT (21) hyperphagic at 24°C but not at thermoneutrality? Comparison with the UCP-1-knockout mouse (10), which lacks UCP-1-mediated thermogenesis but is not hyperphagic, suggests that the loss of the normal adjustment of food intake with respect to temperature in the UCP-DTA mouse cannot be presumed to be due to loss of UCP-1-mediated thermogenesis but must be related to the loss of UCP-1-expressing brown adipocytes. Considering the close inverse relationship that acclimation temperature (35 down to 14°C) has with food intake, sympathetic nervous system activity in BAT (27), UCP concentration in BAT mitochondria (2), and energy expenditure at these different temperatures (26), it is plausible that the signal that informs the mouse how much to eat at these different temperatures to match its energy expenditure might originate in the tissue that is so exquisitely sensitive to changes in the thermal environment, namely, BAT. We suggest therefore that brown adipocytes that express UCP-1 generate a signal, possibly a satiety factor, in inverse relationship to environmental temperature. The generation of this signal would thus be maximal at thermoneutrality, progressively suppressed by norepinephrine as temperature of acclimation decreases and sympathetic nervous system activity increases, and minimal in a cold environment in which sympathetic nervous system activity reaches a maximum level. UCP-DTA mice are predicted to secrete this factor normally at thermoneutrality; when unstimulated, UCP-1-expressing brown adipocytes survive, so that their food intake is normal and low at this temperature. UCP-DTA mice are predicted to lose the ability to secrete the factor at 24°C when these cells die, becoming hyperphagic. A rapid increase in food intake at a lower temperature (14°C) is not possible because the postulated factor is already absent. Their hyperphagia eventually increases to the level seen in control mice at 14°C because of decreased leptin levels, secondary to the negative energy balance and loss of body fat stores they experience for the first few days at this temperature.

There is precedence for a satiety factor that is secreted by BAT and very sensitive to suppression by sympathetic nervous system activity in adult life; this factor is leptin itself (8, 9). We therefore propose the existence of a second satiety factor secreted by UCP-1-expressing brown adipocytes in an inverse relationship
to their heat production, perhaps a cytokine like leptin, and acting entirely independently of leptin on a different type of receptor. This factor would be responsible for the ability of ob/ob and db/db mice to adjust their food intake in relation to environmental temperature without leptin or without leptin’s action. Leptin can be regarded as a signal of the adequacy of the fat stores in WAT for a variety of purposes, including reproduction; leptin’s main function, indicated by a decrease in its concentration, is to permit adaptations for starvation to occur and to promote hunger (1). Laboratory mice continue to reproduce at low environmental temperatures (3), and it would be inappropriate for the cold-induced increase in their food intake to be under the control of any decrease in leptin concentration. The postulated satiety factor from UCP-1-expressing brown adipocytes is a signal of the need for food intake to support thermoregulatory heat production while still achieving energy balance and adequate fat stores appropriate for reproduction. The main function of this postulated factor, mediated by a decrease in its concentration, is to promote an increase in food intake to match increasing energy expenditure as environmental temperature decreases.

We are grateful to Drs. J. S. Flier and B. B. Lowell of the Beth Israel Deaconess Medical Center and Harvard Medical School for supplying the UCP-DTA mice used to start the colony (supported by National Institute of Diabetes and Digestive and Kidney Diseases grant DK-46930; J. S. Flier, principal investigator). This research was supported by a grant from the Medical Research Council of Canada. A. Melnyk was supported by an Ontario Graduate Scholarship.

Address for reprint requests: J. Himms-Hagen, Dept. of Biochemistry, Univ. of Ottawa, 451 Smyth Road, Ottawa, ON, Canada K1H 8M5.

Received 3 October 1997; accepted in final form 8 January 1998.

REFERENCES


17. Hamann, A., J. S. Flier, and B. B. Lowell. Decreased brown fat markedly enhances susceptibility to diet-induced obesity, diabeti,


