Effect of food availability and leptin on the physiology and hypothalamic gene expression of the golden spiny mouse: a desert rodent that does not hoard food

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Gutman R, Hacman-Keren R, Choshniak I, Kronfeld-Schor N. Effect of food availability and leptin on the physiology and hypothalamic gene expression of the golden spiny mouse: a desert rodent that does not hoard food. Am J Physiol Regul Integr Comp Physiol 295: R2015–R2023, 2008. First published October 8, 2008; doi:10.1152/ajpregu.00105.2008.—Food availability and quality in desert habitats are spatially and temporally unpredictable, and animals face periods of food shortage. The golden spiny mouse (Acomys russatus) is an omnivorous desert rodent that does not hoard food, requiring it to withstand such periods by physiological means alone. In response to food restriction, plasma leptin concentrations, core body temperature, and energy expenditure of the spiny mouse decrease significantly after 24 h, and most spiny mice are able to maintain their body mass to ∼85% of ad libitum for a prolonged period of time. Both 1-day food deprivation and long-term food restriction had a significant effect on body mass and plasma leptin concentrations, which decreased significantly with a high correlation, as well as on the orexigenic agouti-related protein, which increased significantly as a result of the 24-h food deprivation; and on neuropeptide Y (NPY), in which the increase was more pronounced under long-term food restriction. Food restriction and food deprivation had no effect, however, on the anorexigenic pro-opiomelanocortin and cocaine and amphetamine-related transcript. Leptin administration to food-restricted golden spiny mice did not affect food intake or the rate of decrease in body mass, indicating that it cannot overcome the drive to eat when food is scarce. However, it did result in a significant decrease in NPY levels, and the spiny mice spent less time at low body temperatures compared with PBS-treated golden spiny mice. These results show that in food-restricted golden spiny mice, leptin affects thermogenesis, but not food consumption, and suggest that the thermoregulatory effects of leptin are mediated by NPY.

torpid; thermogenesis; Acomys russatus; desert; hypothalamic neuuropeptides

BECAUSE OF THE WAY IN WHICH THE NEW SURGE of research into body mass regulation started (i.e., the discovery that absence of leptin is the cause of obesity in ob/ob mice; see Ref. 61), and the high and growing prevalence of obesity worldwide, much focus is currently being placed on the role of body mass-regulating mechanisms in fighting obesity. From an evolutionary perspective, however, it is more likely that such mechanisms evolved under a strong selection pressure to allow survival and reproduction under conditions of low food availability (2, 52). Understanding the response to food restriction is therefore of extreme importance and may also shed light on the mechanisms that protect the body from (a sometimes desired) weight loss, but less so from weight gain (60).

Usually, during food shortage, hunger increases (if possible, resulting in increased food consumption) and energy expenditure decreases, ultimately maintaining body fat at a constant level (or at least minimizing body fat loss). During the last decade, major progress has been made in understanding the processes that lead to this response. It was found that peripheral signals of the energetic state, with leptin concentration as the main indicator, provide the brain with information that translates to neuropeptide expression levels that, in turn, control the response to the energetic state (even though there are many examples of disassociation between plasma leptin concentrations and body mass, body fat, and energetic state, e.g., Refs. 33–35). One of the major sites of leptin activity in the brain is the arcuate nucleus of the hypothalamus, where orexigenic neuropeptides such as neuropeptide Y (NPY) and agouti-related protein (AgRP), and the anorexigenic pro-opiomelanocortin (POMC) and cocaine and amphetamine-related transcript (CART) expression levels change in response to information from the periphery [reviewed by Schwartz et al. (52)].

Previous research has shown that food restriction results in a significant decrease in plasma leptin concentrations (e.g., Refs. 1, 2, 15, 59). As a result, hypothalamic expression of NPY and AgRP is up-regulated, while that of POMC and CART is down-regulated, but to a lesser extent (52). Nevertheless, preventing a decrease in plasma leptin concentrations by means of leptin administration during fasting or food restriction did not induce a further decrease in body mass (1, 2, 14, 19, 59), even though it blunts or even prevents the expected decrease in energy expenditure (10, 19, 28, 43, 46, 50, 59).

The effect of leptin administration on energy expenditure during food restriction appears to result mainly from its effect on thermogenesis. It was found that exogenous leptin administration to fasted ob/ob mice (18) and to food-restricted mice under moderate cold conditions (10) inhibits torpor. Intracerebroventricular NPY or NPY Y1 receptor agonist treatment induces torpor-like hyperthermia in cold-acclimated Siberian hamsters (47, 48), and NPY Y1 receptor antagonist prevents this NPY-induced torpor like hyperthermia (9). In food-deprived stripe-faced Dunnarts (Smynthopsis macroura), it halved the duration of torpor bouts and raised the daily minimum body temperature and metabolic rate, resulting in a net increase in total daily energy expenditure (19). In rat pups, leptin treatment
increased body temperature during daily torpor (56); and in Siberian hamsters, it eliminated torpor in a significant proportion of treated hamsters (16). All these results indicate that leptin plays a significant role in thermogenesis and the use of torpor. At the hypothalamic control level inconsistent results were reported: injecting leptin to mice after 24 h of food deprivation did not affect NPY, AgRP, or POMC mRNA expression levels (57), whereas 48 h of fasting-induced changes in NPY mRNA expression levels were prevented by leptin administration (2, 59). Seventy hours of leptin infusion to fasted rats prevented the rise in NPY and fall in POMC and CART mRNA expression levels (1).

Food availability and quality in desert habitats are spatially and temporally unpredictable, and animals often face periods of food shortage. To increase the available energy during such periods, some desert mammals use seed caches (30), whereas others store energy as body fat (39), which they use during the periods of food shortage. The efficiency of hoarding food or storing fat is influenced by several parameters, including the species body mass: since fat storing capacity increases linearly with body mass, while resting metabolic rate scales according to mass$^{0.75}$ (31), food shortage endurance time is expected to increase in a linear fashion with body mass. Nevertheless, many small mammals use fat as an energy reserve (e.g., 8, 33, 36, 44, 49), usually increasing the efficiency of using fat reserves dramatically by the use of torpor or hibernation, which reduces the rate of energy expenditure (19, 23a), thereby increasing endurance time considerably. Hence, the problem of food shortage is more pronounced in small mammals, such as rodents, due to their relatively high specific metabolic rate (energy demand per body mass unit; Ref. 31), and even more so in desert rodents that do not create food caches. These rodents have to cope with food shortage periods by physiological means alone. One such example is the golden spiny mouse (Acomys russatus, Muridae) that inhabits rocky deserts in Jordan, Sinai (Egypt), and southern Israel (40). This rodent does not dig burrows but inhabits rock crevasses, and does not hoard food (54), possibly because its diet is comprised mainly of arthropods (32), which cannot be stored. Unexpectedly for their small body size (38), when food is plentiful, golden spiny mice store energy as body fat (unpublished data). Food restriction in these mice results in a very rapid response aimed at maintaining energy balance: plasma leptin concentrations [also reported in Syrian hamsters; see Schneider et al. (51)], core body temperature, and energy expenditure decrease significantly after 24 h of 50% food restriction (21). After an initial decrease, most (about 75%) golden spiny mice are able to remain at −85% of their ad libitum body mass for a prolonged period of time (12, 21, 22, 42). During the long-term food restriction metabolic rates, activity levels and body temperature decrease significantly (12, 21, 22, 42), and using the criteria of body temperature lower than the minimum normothermic body temperature (32.7°C in Ref. 12) for the use of torpor in golden spiny mice, the golden spiny mice enter daily torpor (12, 21). The rapid response to food restriction and the ability to withstand long-term food restriction makes the golden spiny mouse an interesting and unique animal for the study of the response to short- and long-term food restriction, and the role played by leptin in these responses.

The current study was designed to answer two questions: 1) How does short-term food deprivation and long-term food restriction influence mRNA expression levels of energy balance-related hypothalamic neuropeptides in golden spiny mice? and 2) What is the effect of leptin on body mass, body temperature, and mRNA expression levels of energy balance-related hypothalamic neuropeptides in food-restricted golden spiny mice?

We first describe the effect of a 1-day food deprivation and long-term 50% food restriction on mRNA expression levels of energy balance-related hypothalamic neuropeptides. We then show the effect of leptin infusion on body mass, body temperature, and food intake, and mRNA expression levels of energy balance-related hypothalamic neuropeptides in golden spiny mice subjected to long-term food-restriction.

MATERIALS AND METHODS

Animals and Housing

A breeding colony of golden spiny mice, originally trapped near the Dead Sea, Israel, is kept at the I. Meier Segals Garden for Zoological Research at Tel Aviv University (permit number 2003/16295). All procedures were conducted in accordance with the Institutional Animal Ethics Committee (L-02-45).

Male golden spiny mice were housed individually in standard plastic cages (21 × 31 × 13 cm) under a 12:12-h light-dark cycle. Room temperature was regulated at 30 ± 1°C, which is approximately (55) or just below (12) the low critical temperature of the thermal neutral zone of the golden spiny mouse. We chose to work at an ambient temperature within the thermal neutral zone of the species to keep the golden spiny mice from investing energy in thermoregulation during ad libitum feeding. Moreover, near the Dead Sea during the summer, the average maximal temperature is 38°C, and the average minimal temperature is 28°C (29), and we have recently documented that in their natural habitat, golden spiny mice use torpor more frequently during summer (O. Levy, T. Dayan, and N. Kronfeld-Schor, unpublished data). Water and standard rodent pellets (Koffolk serial no. 19510: protein, 21%; fat, 4%; carbohydrates, 75%) were provided ad libitum. This diet was provided during at least 3 wk acclimation period to the experimental conditions, and during experimental periods as a standard diet, although in the breeding colony, the golden spiny mice receive mixed food (standard rodent pellets and fruit, vegetables, and animal protein from mealworms, fly larvae, and eggs). The diet was changed for three reasons. 1) Most experiments published so far on golden spiny mice physiology used this standard diet, including Gutman et al. (21, 22) and Ehrhardt et al. (12), which provide the framework for the current experiment. Hence, we used the standard diet to allow comparison of the results to these experiments. 2) Using a standard diet allowed the replication of the experiment, in our and in other laboratories. 3) The mice do not lose weight when switched to this diet.

Body mass of the golden spiny mice at the beginning of the experiments was stable and was averaged 61 ± 1.6, which is higher than body mass of golden spiny mice in nature (yearly average: 44 ± 0.6 and 41 ± 1 for males and females, respectively; see Ref. 53). Under ad libitum food availability oxygen consumption, core body temperature, heart rate, and activity peak right after onset of the dark period (21, 22). Therefore, during the food restriction period, a weighed daily individual portion (50% of the individual average ad libitum daily consumption, measured during the 10-day baseline period) was given −10 min after onset of the dark period. The effects

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of meal feeding on activity, energy metabolism, and body temperature are discussed elsewhere (21, 22).

**Experimental Protocol**

**Effect of short-term food deprivation and long-term food restriction on mRNA expression levels of hypothalamic neuropeptides.** Animals were killed under ad libitum feeding (n = 15), following 24-h food deprivation (n = 8), and 17 days of 50% food restriction (n = 10), using anesthesia followed by decapitation. Since results of diurnal profile of hypothalamic energy balance gene expression in other species are inconsistent (13), and time points selected for such studies are usually early or in the middle of the light phase (13), we chose to kill the animals in similar hours, between 1000 and 1200. Blood was collected and centrifuged, and plasma was frozen at −70°C for the analysis of leptin. Brains were removed and hypothalami excised, rapidly frozen in liquid nitrogen, and stored at −70°C for measurement of expression levels of selected neuropeptides. Throughout the entire experiment, body mass and food intake (at ad libitum diet regime) were measured daily. Trunk blood for leptin analysis was collected, brains were removed, and hypothalami were excised, rapidly frozen in liquid nitrogen, and stored at −70°C for measurement of expression levels of selected neuropeptides. Throughout the entire experiment, body mass and food intake (at ad libitum diet regime) were measured on alternate days. To the results of the body mass and plasma leptin concentrations of this experiment, we added the results of the control animals from the next experiment, which were food restricted for 23 days (n = 6), which was performed under the same conditions.

**Effect of leptin administration on the response of golden spiny mice to food restriction.** At least 4 wk before the experiment, 12 golden spiny mice were implanted with body temperature transmitters. The experiment started after the recovery period, with 10 days of baseline measurements of body mass and food intake. Six pairs of weight-matched golden spiny mice were randomly assigned to two groups, and implanted with osmotic minipumps filled with PBS or leptin (body mass: PBS, 69.1 ± 4.29 g; leptin, 69.0 ± 1.64 g). Pumps were replaced with freshly filled pumps after 14 days. Four days after implantation of the pumps (recovery period), blood from the infra-orbital sinus was collected for leptin level analysis, and food was restricted to 50% of the individual ad libitum consumption for the following 23 days. At the end of the food restriction period animals were killed between 1000 and 1200 using anesthesia followed by decapitation. Trunk blood for leptin analysis was collected, brains were removed, and hypothalami were excised, rapidly frozen in liquid nitrogen, and stored at −70°C for measurement of expression levels of selected neuropeptides. Throughout the entire experiment, body mass and food intake (at ad libitum diet regime) were measured on alternate days, while core body temperature was measured continuously. Plasma leptin concentrations were measured 4 days after implantation (before the beginning of food restriction) and at the end of the experiment.

**Body mass and food intake.** Body mass was measured using electronic scales [Sekel (± 0.01 g)]. To measure individual food intake, golden spiny mice were given weighed [Sekel (± 0.01 g)] standard rodent pellets. Food spillage was collected every other day, dried to a constant mass at 60°C, and weighed.

**Telemetry.** Core body temperature was monitored continuously, using transmitters (Model No. TA10ETA-F20, 3.5 g, Data Science International telemetry system) implanted in the animal abdominal cavity. For full description of implantation procedures, see Gutman et al. (21). Data were collected at 3-min intervals throughout the experiment. Daily average and minimum (lowest continuous 3-min intervals) core body temperature were calculated.

**Leptin infusion.** For continuous delivery of recombinant mouse leptin (R&D), osmotic mini-pumps (Alzet, model 2002) were implanted subcutaneously, and Golden spiny mice were anesthetized with isofluorane in medical grade oxygen, using an anesthetic machine (Ohmeda), skin was sutured with absorbable surgical sutures, using a cutting needle (5–0, Dexon), and the incision was treated with topical antibiotic (silver sulfadiazine 1%; Silverol Cream). Antibiotics (Baytril 5%, Bayer, 24 mg/kg) were injected intramuscularly before implantation, as a prophylactic measure. The pumps delivered leptin at ~800 ng leptin/h. This dose was chosen since it is twice the optimal concentration used by Halaas et al. (23) and Ioffe et al. (27), who studied laboratory mice weighing one-half to one-third the weight of our golden spiny mice. Control golden spiny mice were infused with sterile PBS.

**Plasma leptin concentrations.** Plasma leptin concentration was measured using a commercial multispecies leptin RIA kit (Linco). The use of RIA for golden spiny mice was previously validated by Gutman et al. (21).

**Real-time PCR quantification of mRNA.** Total RNA was extracted from the frozen hypothalami using RNeasy Lipid Tissue Mini Kit (Qiagen) according to the manufacturer’s instructions. The mRNA (1.5 µg) was reverse-transcribed using Oligo(dT) primer and M-MLV reverse-transcriptase (Promega). The hypothalamic neuropeptides levels were determined by real-time PCR using the ABI Prism 7000 thermocycler (Applied Biosystems) following the manufacturer’s instructions. Triplicate single-strand cDNA from each animal served as a template in a PCR consisting of master mix, SYBR Green I fluorescent dye (Applied Biosystems) and gene-specific primers, which were designed based on the entire sequence of golden spiny mouse AgRP (accession no. Eu477388), POMC (accession no. Eu477390), NPY (accession no. Eu477389), and CART (accession no. Eu477391) (Table 1). The copy number in the samples was determined by comparing CT (the threshold cycle at which product is first detectable) values with those of recombinant standards containing the cDNA inserts. β actin gene was used as a reference gene.

**Statistical analysis.** Statistical analysis was performed using STATISTICA 7.1 (StatsSoft). Linear correlation was performed to analyze the correlation between the decrease in body mass and plasma leptin levels during food deprivation and restriction. One-way ANOVA followed by Fisher’s least significant difference (LSD) post hoc test was used to detect the effect of time on body mass, plasma leptin concentrations, and mRNA expression levels. Wherever the data were not normally distributed, a Kruskal-Wallis ANOVA by ranks was performed.

Repeated-measures ANOVA (with time as the repeated measure) were used to detect and compare the effect of leptin or PBS administration on different parameters measured in the golden spiny mouse held under 50% food restriction. The within factor was the days on the diet or leptin infusion, and the between factor (where appropriate) was leptin infusion. Significant ANOVAs were followed by Fisher’s least significant difference (LSD) post hoc test. Two-way ANOVA with temperature (in 0.1 increments) and treatment as variables, and number of observations at each temperature as the dependent variable was used to detect the effect of food restriction and leptin/PBS treatment on body temperature frequency distribution, followed by Fisher’s LSD post hoc test.

Student’s t-tests, following arc-sin transformations were used to compare the difference in neuropeptide expression levels between treatments. Significance level was set at P < 0.05.

**Table 1. Forward and reverse primers for RT-PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
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<tr>
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<td>5’ggctcactggaacatc3’</td>
<td>5’ttcgctccagctcagttg3’</td>
</tr>
<tr>
<td>POMC</td>
<td>5’ggtaagggtgacaccccagc3’</td>
<td>5’gcacccacctccttccttg3’</td>
</tr>
<tr>
<td>NPY</td>
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<td>5’aggctcagctaccacacc3’</td>
</tr>
<tr>
<td>CART</td>
<td>5’tgcctctcgcggatcaccac3’</td>
<td>5’atgaggctgcgagcaggt3’</td>
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<tr>
<td>β-actin</td>
<td>5’atgctcttggtcctggt3’</td>
<td>5’atgctcttggtcctggt3’</td>
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Primers were designed based on partial sequence of golden spiny mice agouti-related protein (AgRP) (accession no. Eu477388), pro-opiomelanocortin (POMC) (accession no. Eu477390), neuropeptide Y (NPY) (accession no. Eu477389), and cocaine- and amphetamine-regulated transcript peptide (CART) (accession no. Eu477391).
RESULTS

The Effect of Short- and Long-Term Food Restriction on mRNA Expression Levels of Hypothalamic Neuropeptides

Body mass decreased significantly ($P < 0.001, F = 34, df = 3$) as a result of food deprivation and food restriction, with all groups significantly different ($P < 0.001, df = 27, Fig. 1A$). Plasma leptin concentrations also decreased as a result of food deprivation and food restriction ($P < 0.05, F = 3.45, df = 3$), but the decrease was significant ($P < 0.01, df = 28$) only after 23 days of food restriction (Fig. 1B). Nevertheless, there was a significant correlation ($r^2 = 0.97, P < 0.05$) between plasma leptin concentration and the decrease in body mass (Fig. 2). The expression levels of AgRP increased significantly as a result of food deprivation and food restriction ($P < 0.001, F = 9.83, df = 2$); the increase was significant after 1 day of food deprivation ($P < 0.005, df = 31$) and remained high after 17 days of food restriction (Fig. 3). The expression levels of NPY increased significantly ($P < 0.001, F = 13.4, df = 2$) after the 1 day of food deprivation and were even higher after 17 days of food restriction ($P < 0.005, df = 29$, Fig. 3). Expression levels of CART and POMC did not change significantly after 1 day of food deprivation or long-term food restriction; however, both 1-day food deprivation and long-term food restriction resulted in an increase in the variation of POMC expression levels (CART: $P = 0.53, \chi^2 = 1.25, df = 2$, POMC: $P = 0.08, \chi^2 = 4.85, df = 2$, Fig. 3).

Effect of Leptin Administration on the Response of Golden Spiny Mice to Food Restriction

Plasma leptin concentrations and leptin infusion. There was a significant and different effect of treatment on plasma leptin concentrations (treatment: $P < 0.01, F = 18.4, df = 1$; days: $P < 0.05, F = 8.86, df = 1$; interaction: $P < 0.001, F = 31.27, df = 1$). Plasma leptin concentrations of PBS-infused golden spiny mice decreased significantly during food restriction (from $7.6 \pm 1.49$ to $2.9 \pm 1.43, P < 0.01, df = 18$). Leptin infusion prevented the decrease in plasma leptin concentrations during food restriction and even caused an increase (from $8.1 \pm 0.70$ to $23.5 \pm 3.7$, post hoc, $P < 0.001$).

Food Intake and Body Mass

There was no difference in average daily food intake between the groups before treatment (leptin vs. PBS) under ad libitum food supply. During 50% food restriction, all golden spiny mice consumed their entire rations of food. Leptin infusion during food restriction did not lead to a significantly different rate of body mass change between leptin-infused and PBS-infused golden spiny mice on 50% food restriction (Fig. 4).

Core Body Temperature

Throughout the entire experiment there was no significant difference between treatments in average and minimal core body temperature, either under the ad libitum food availability or food restriction diet regime (Fig. 5, A and B). In both treatments, average core body temperature decreased significantly as a result of food restriction ($P < 0.001, F = 17.27, df = 35$, Fig. 5A). The decrease in average body temperature resulted mainly from a significant decrease in the minimum body temperature and a significant increase in the time spent at a lower body temperature ($P < 0.001, F = 18.98, df = 35$, and $P < 0.001, F = 55.95, df = 35$, respectively, Fig. 5, B and C). An immediate plateau of body temperature was observed following recovery from the second infusion. The frequency distribution of body temperatures was significantly affected by diet but not leptin administration (diet effect: $P < 0.001, F = 649, df = 1$; treatment effect: $P = 0.35, F = 0.85, df = 1$; diet×treatment interaction: $P > 0.05, F = 0.947, df = 102$) (Fig. 6). Summing the time spent at body temperatures lower
than the ad libitum minima revealed that the leptin-treated, food-restricted golden spiny mice spent 33% less time ($P < 0.01$, $t = 2.26$, df = 9) at lower minimal temperature during food restriction than PBS-treated, food-restricted golden spiny mice (Fig. 7).

Hypothalamic neuropeptides expression levels. Both AgRP and NPY mRNA expression levels were lower in the leptin-infused compared with the PBS-infused group (AgRP: $P < 0.01$, $t = -3.5$; NPY: $P < 0.01$, $t = -3.6$). No differences in POMC or CART expression levels were found between the two groups (POMC: $P = 0.27$, $t = 1.6$, CART: $P = 0.11$, $t = 1.7$; Fig. 8).

**DISCUSSION**

Both 1-day food deprivation and long-term food restriction had a significant effect on body mass, which decreased significantly and showed a high correlation with plasma leptin concentrations. mRNA expression levels changed significantly when food was scarce and were significantly different between ad libitum fed, 1-day food-deprived and long-term food-restricted golden spiny mice. The response to food shortage was manifested by the orexigenic NPY and AgRP but not by the anorexigenic POMC and CART: both AgRP and NPY mRNA levels increased significantly as a result of the 24-h food deprivation, with the increase in NPY mRNA levels being more pronounced under long-term food restriction. No change in CART or POMC was observed. A similar response to food restriction was found in fasted rats, in which the response to long-term fasting was mediated by the orexigenic rather than the anorexigenic system (3). In that study, however, AgRP increased only after long-term food restriction, while the response in NPY expression levels was similar to our results. In another study in rats (4), acute food deprivation and chronic food restriction had differential effect on hypothalamic peptide gene expression: both resulted in increased NPY and decreased POMC expression, but only acute deprivation resulted in increased AgRP expression. In a study by Mercer et al. (41) on Siberian hamsters maintained under short photoperiod, 3-wk food restriction resulted in a significant increase in AgRP and NPY and a nonsignificant influence on POMC and CART expression.

We have previously shown (21) that food restriction (50% food restriction, given in one portion, as in the current study) in golden spiny mice results in a very rapid response aimed at maintaining energy balance: plasma leptin concentrations [also reported in Syrian hamsters; e.g., Schneider at al. (51)], core body temperature, and energy expenditure decrease significantly after 24 h of 50% food restriction (21). After an initial decrease, most (about 75%) golden spiny mice are able to remain at $\sim 85\%$ of their ad libitum body mass for a prolonged period. This rapid response is likely mediated by the orexigenic system, which is activated by food deprivation and maintains energy balance until body mass is restored to the ad libitum level.
period of time (12, 21, 22, 42). During the long-term food restriction, metabolic rates, activity levels, and body temperature decrease significantly (12, 21, 22, 42), and using the criteria of body temperature lower than the minimum normothermic body temperature [32.7°C as used by Ehrhardt et al. (12)] for the use of torpor in golden spiny mice, we and others have observed that the golden spiny mice enter daily torpor (12, 21, 22).

Leptin administration did not affect food intake or the rate of decrease in body mass of food-restricted golden spiny mice, as also previously documented in other species (e.g., 1, 2, 14, 19, 59), indicating that leptin cannot overcome the drive to eat when food is scarce. An immediate plateau of body temperature was observed following recovery from the second infusion. A similar plateauing of body temperature was observed by Gutman et al. (21), who studied the effect of food restriction in golden spiny mice without minipump implantations. Furthermore, there was no effect on average body temperature or minimal body temperature, as reported for several other species (e.g., 16). During food restriction, however, leptin-treated golden spiny mice spent 33% less time at body temperatures lower than the ad libitum fed minima, during food restriction (average ± SE, **P < 0.01).
that were lower than their ad libitum fed minimal body temperature. Several studies have shown an effect of leptin on thermoregulation; however, the effect is complex and differs among species and experiments. In Siberian hamsters, leptin administration decreased the proportion of individuals that entered torpor, but not the depth or duration of torpor bouts (16). In the marsupial Sminthopsis macroura, it halved the duration of torpor bouts and raised the daily minimum body temperature and metabolic rate, resulting in a net increase in total daily energy expenditure (19). In rat pups, it increased body temperature during daily torpor (56), and in fasted ob/ob mice (18) and food-restricted mice under moderate cold conditions (10), it inhibited torpor; and it was shown that sympathetically mediated depression of circulating leptin during fasting is a requisite for the initiation of a torpor bout (58). Our current results support the notion that leptin, through its central receptor, permits or inhibits thermogenesis rather than simply stimulating it. It is possible that its effect would have been more pronounced had we used a lower ambient temperature (below the thermoneutral zone), as reported in other studies (10, 56). In seminatural field enclosures in their natural habitat during winter, golden spiny mice thermoregulate at ambient temperatures higher than 24°C and abandoned thermoregulation when ambient temperatures are between 19 and 24°C, with body temperature passively following ambient temperature, decreasing by 1°C for each 1°C decrease in ambient temperature, down to a body temperature of 31°C. Below the ambient temperature of 19°C, the golden spiny mice return to thermoregulating, defending their body temperature at a new set point of 31°C. During summer, when ambient temperatures decrease to below 38°C, body temperatures start to drop, with a slope of 0.67, until they reach 35°C at an ambient temperature of 29°C. Below that ambient temperature, the golden spiny mice again start thermoregulating (O. Levy, T. Dayan, and N. Kronfeld-Schor, unpublished data). In Sminthopsis macroura, it was suggested that leptin administration increased the torpor body temperature set-point (19). In the current study, ambient temperature was 30 ± 1°C, which is approximately (55) or just below (12) the low critical temperature of the thermoneutral zone of the golden spiny mouse, thus making it impossible to determine the defended body temperature of the golden spiny mouse, or whether leptin affected it. Nevertheless, while the lowest body temperature recorded under ad libitum feeding was 34.5°C, during food restriction, it was 32°C (below the torpor criteria of 32.7°C; Ref. 12).

Leptin was shown to increase sympathetic outflow to brown adipose tissue (BAT) of ob/ob mice (6), to increase uncoupling protein (UCP) expression in both ad libitum fed and food-restricted rats (50), and to increase mitochondrial 3-H-GDP binding in 48-h fasted rats BAT (56a). Leptin has also been shown to prevent the decrease in thyroid hormone induced by fasting (2, 56a), which may partially prevent BAT deactivation, since T3 is an important regulator of BAT activity (7). These reports suggest that the effect of leptin on thermoregulation may be mediated by its effect on BAT and UCP expression and activation and that the drop in plasma leptin concentrations during fasting or food restriction is involved in a reduction of BAT activity, which may influence thermoregulation and eventually energy expenditure. Nevertheless, the fact that leptin had a similar effect on thermoregulation in the marsupial Sminthopsis macroura, which presumably lacks BAT (45), may suggest that the presence of functional BAT is not necessary for the leptin-mediated effects on energy expenditure (19) and that additional mechanisms mediate that effect.

The effect of leptin on thermoregulation may be mediated by NPY and/or POMC. NPY plays a role in regulating energy homeostasis, food intake, thermogenesis, and social behavior in a variety of organisms. Decreased leptin concentrations lead to increased NPY expression levels and increased food intake. When centrally applied, NPY stimulates food intake and suppresses the sympathetic nerve activity to brown adipose tissue, eventually suppressing thermogenesis (11). Intracerebroventricular NPY or NPY Y1 receptor agonist treatment induce torpor-like hypothermia in cold-acclimated Siberian hamsters (47, 48), and NPY Y1 receptor antagonist prevents this NPY-induced torpor-like hypothermia (9). Therefore, it is reasonable to assume that the increase in NPY expression under food restriction, also observed in the current study, at least partly mediates the decrease in body temperature during fasting and food restriction. In a study that compared the response of three inbred mouse strains to food restriction, one strain exhibited upregulated expression of the NPY Y1 receptor, which may have caused an antithermogenic effect leading to the relatively large decrease in body temperature observed in that strain (20). Leptin is known to inhibit hypothalamic NPY expression levels (1). In the current study, during food deprivation and 17 days of food restriction, leptin concentrations did not statistically decrease, but tended to be lower, and decreased significantly after 23 days of food restriction, while NPY levels increased significantly after 17 days of food restriction. Furthermore, when we administered leptin to food-restricted golden spiny mice NPY levels decreased, and the golden spiny mice spent more time at low body temperatures compared with PBS-treated golden spiny mice.

In previous studies, POMC and CART were shown to be either downregulated or not affected during food restriction (e.g., 1, 3, 41). POMC is the precursor of the hormones of the melanocortin (MC) system, which controls several functions, including energy balance. The melanocortin system comprises several receptors (MC-R), which have both an agonist that is derived from the POMC gene, α-MSH, and inverse agonists,
agouti and AgRP (17). Increased leptin concentrations are known to increase POMC expression, resulting in an increase in α-MSH. Activation of the MC system by central infusion of α-MSH or its synthetic analog results in hypophagia, activation of the hypothalamo-pituitary axis, and an increase in energy expenditure (26), most likely due to the stimulatory effects of MC4-R on thermogenesis (5), with a consequent loss of weight. In the current study, however, both food deprivation and food restriction resulted in a nonsignificant effect on POMC expression levels, and therefore it is impossible to determine whether the thermoregulatory response to food restriction (a significant decrease in average core body temperature, which was achieved by a significant decrease in minimum body temperature, and a significant increase in the time spent at a lower body temperature) was mediated by MC4-R.

In summary, our results support the notion that when food availability is low, the drop in leptin concentrations suppresses energy investment in thermogenesis by changing the frequency (this study) and/or depth of torpor-like decrease in body temperature (10), and that under these conditions, exogenous leptin cannot overcome the drive to eat. They also suggest that the thermoregulatory effects of leptin are mediated by NPY expression in the hypothalamus.

Perspectives and Significance

The energy homeostasis system constitutes one of the most important systems enabling an individual to maintain a normal healthy lifespan. During the course of evolution and in much of the world today, many mammalian species, including humans, faced periods of food shortage, while an overabundant food supply was presumably an uncommon threat to survival. Therefore, it is only reasonable to assume that body mass regulatory systems developed under a strong selection pressure to defend against deficits of body fat and allow survival and reproduction in environments with limited access to food. Understanding the response to food restriction is therefore of extreme importance, will contribute to our understanding of species abundance and distribution, and may also shed light on the mechanisms that protect the human body from (a sometimes desired) weight loss, but less so from weight gain.

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