Changes in environmental temperature influence leptin responsiveness in low- and high-fat-fed mice

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Harris RB, Mitchell TD, Kelso EW, Flatt WP. Changes in environmental temperature influence leptin responsiveness in low- and high-fat-fed mice. Am J Physiol Regul Integr Comp Physiol 293: R106–R115, 2007. First published April 18, 2007; doi:10.1152/ajpregu.00848.2006.—Loss of body fat in leptin-treated animals has been attributed to reduced energy intake, increased thermogenesis, and preferential fatty acid oxidation. Leptin does not decrease food intake or body fat in leptin-resistant high-fat (HF)-fed mice, possibly due to a failure of leptin to activate hypothalamic receptors. We measured energy expenditure of male C57BL/6 mice adapted to low-fat (LF) or HF diet and infused them for 13 days with PBS or 10 μg leptin/day from an intraperitoneal miniosmotic pump to test whether leptin resistance prevented leptin-induced decreases in energy expenditure and fatty acid oxidation. There was no effect of low-dose leptin infusions on either of these measures in LF-fed or HF-fed mice, even though LF-fed mice lost body fat. Experiment 2 tested leptin responsiveness in LF-fed and HF-fed mice housed at different temperatures (18°C, 23°C, 27°C), assuming that the cold would increase and the hot environment would inhibit food intake and thermogenesis, which could potentially interfere with leptin action. LF-fed mice housed at 23°C were the only mice that lost body fat during leptin infusion, suggesting that an ability to modify energy expenditure is essential to the maintenance of leptin responsiveness. HF-fed mice in cold or warm environments did not respond to leptin. HF-fed mice in the hot environment were fatter than other HF-fed mice, and, surprisingly, leptin caused a further increase in body fat, demonstrating that the mice were not totally leptin resistant and that partial leptin resistance in a hot environment favors positive energy balance and fat deposition. Leptin injections have been shown to increase norepinephrine turnover in brown adipose tissue (BAT) of leptin-deficient ob/ob mice (11), and central infusion of leptin or large peripheral doses of leptin have been found to increase BAT uncoupling protein-1 (UCP-1) mRNA expression (12, 44), an indicator of nonshivering thermogenesis in rodents. In previous studies, in which rats and mice have been infused peripherally with physiological doses of leptin, we have been unable to detect significant changes in BAT UCP-1 expression (22, 36), but it is possible that the low doses of leptin, which maintained circulating leptin within physiological concentrations, caused only subtle increases in energy expenditure that did not require a significant stimulation of UCP-1 mRNA expression.

Although exogenous leptin administered to normal weight animals in experimental conditions can reduced body fat mass, many humans have elevated levels of body fat and high circulating concentrations of endogenous leptin and obviously are resistant to leptin (13). Similarly, studies have shown that animals fed high-energy, palatable diets develop resistance to peripheral and central leptin administration (16, 21, 30, 39). The studies examining leptin resistance have used measures of food intake (30), body weight (21), activation of leptin receptor signaling proteins (16), and elevated serum leptin concentrations (19) as indexes of leptin responsiveness, but it has not been determined whether leptin resistance also is associated with a failure of leptin to stimulate energy expenditure. The objectives of the studies described here were to test whether energy expenditure was increased in mice given chronic peripheral infusions of physiological doses of leptin and whether these potential changes in expenditure were abolished in mice that had become leptin resistant on a high-fat (HF) diet. The first study used indirect calorimetry to measure energy expenditure of mice fed a low-fat (LF) diet or made leptin resistant on HF diets, and there was no significant effect of leptin on daily energy expenditure of mice in either dietary treatment. Therefore, we subsequently used thermal stress to modify energy expenditure in the mice to determine whether this changed leptin responsiveness in either LF-fed or HF-fed mice. Our laboratory had previously established that young, growing mice fed an HF diet for 10 days remain leptin responsive in that peripheral infusions of leptin reduce body fat mass, but do not change lean body mass (6). If, however, young mice are housed in a hot environment (28°C), then leptin inhibits overall growth in HF-fed mice. In contrast, young LF-fed mice housed in the hot environment show the normal leptin response of selective loss of body fat (22), indicating that the metabolic response to leptin is influenced by an interaction between diet composition and environmental temperature. In the second study described here, 15-wk-old...
mice either were fed a LF diet or were made leptin resistant by being fed a HF diet from 10 to 15 wk of age (24). They were then housed at different environmental temperatures to determine whether thermal stress, which would modify both energy intake and energy expenditure, also changed leptin responsiveness of the mice on the different diets. The results show that lowering or raising the environmental temperature prevented leptin from reducing body fat mass in LF-fed animals. Leptin had no effect on body composition of the leptin-resistant HF-fed mice housed in warm or cold environments, but, surprisingly, the combination of leptin resistance and an increase in environmental temperature resulted in a leptin-induced increase in body fat content of the HF-fed mice.

METHODS

Experiment 1: energy expenditure of leptin-treated mice fed LF and HF diets. The objective of this experiment was to determine whether HF-fed mice were resistant to the effects of leptin on energy expenditure. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Georgia and followed the guiding principles of the American Physiological Society (3). In a previous study, our laboratory established that feeding male mice a HF diet for 5 wk starting at 9 wk of age would induce leptin resistance (24). For this study, 36 male C57BL/6 mice were obtained from a breeding colony maintained at the University of Georgia. Because energy expenditure could be measured on only 12 mice at a time, the mice were tested in three cohorts, with each treatment represented equally within each cohort. The mice were weaned at 4 wk of age and group housed two to four per cage in a room maintained at 23°C with lights on for 12 h a day from 7:00 AM. At 9 wk of age, each cage of mice was randomly assigned to either HF (45% kcal fat, Diet 12451, Research Diets, New Brunswick, NJ) or LF diet (10% kcal fat, Diet 12450B, Research Diets). At 13 wk of age, the mice were separated into individual cages with grid floors, and at 15 wk of age the mice were housed in individual cages in the indirect calorimeter, which has been described in detail previously (20). Oxygen consumption and carbon dioxide production were measured on each cage at intervals of 19.5 min for 24 h/day, except for a period of ~1 h during which body weights and food intakes were recorded and the gas analyzers were calibrated. Energy expenditure was calculated using the Brouwer equation (7), without a correction for nitrogen, and the precision of measurement of energy expenditure (kcal/h) has been calculated to be ±7.5% (~0.003 kcal/mouse·h⁻¹·h⁻¹ in this experiment).

The mice were housed in the calorimeter for 3 days for baseline measures of energy expenditure and RER. On the fourth day, the LF-fed and HF-fed mice were divided into two weight-matched groups, and each mouse was fitted with an intraperitoneal Alzet miniosmotic pump (model 1002: Direc, Cupertino, CA) while under isoflurane anesthesia. The pumps delivered either 10 µg leptin/day (mouse recombinant leptin, R&D Systems, Minneapolis, MN) or an equivalent volume of 0.01 M phosphate-buffered saline (PBS). The mice remained in the calorimeter until the morning of day 5 of infusion so that we could obtain measurements of energy expenditure during the early stages of leptin infusion. The mice were then removed from the calorimetry cages and housed in the same room in cages with grid floors to enable continued measures of daily food intake. Calorimetry measures were resumed for days 9–13 of infusion to determine whether there was any adaptation to leptin infusion. On the morning of day 13, food was removed from the cages at 8:00 AM. Starting at 10:00 AM, mice were decapitated, and trunk blood was collected for measurement of serum leptin and adiponectin concentrations (mouse leptin and mouse adiponectin RIA kits; Linco Research, St. Charles, MO). Epididymal, retroperitoneal, mesenteric, and intrascapular BAT (IBAT) were dissected and weighed, and then the tissues were returned to the carcass for determination of carcass composition, as described previously (23).

Experiment 2: leptin response in mice fed LF or HF diets and housed at different environmental temperatures. The previous experiment did not show any effect of leptin on energy expenditure of LF-fed or HF-fed mice, although energy intake was inhibited during the first 4 days of infusion, and weight gain and body fat content decreased in leptin-infused LF-fed animals. Because it was possible that leptin did increase expenditure of the LF-fed mice, but that the energy deficit needed to produce the measured reduction in body fat mass (~1 kcal/day) was too small to be detected by calorimetry measurements, in this experiment we tested whether disruption of energy balance by housing mice fed LF or HF diet in hot or cold environments influenced the response of either dietary treatment group to leptin. We anticipated that mice housed in the cold would have elevated energy expenditure and be hyperphagic compared with mice housed at their normal room temperature, whereas mice housed in a hot environment would limit thermogenesis to prevent the development of hyperthermia and would have reduced energy intake to balance the low-energy expenditure. These disturbances of energy balance caused by the environment could then potentially interfere with the normal modifications to energy balance produced by leptin.

Because of the large number of animals included in this experiment, it was completed using three cohorts of mice, with treatment groups represented equally within each cohort. One hundred and thirty male C57BL/6 mice were obtained from a breeding colony maintained at the University of Georgia and were housed and adapted to LF or HF diet, as described for experiment 1. At 14 wk of age, the mice were separated into individual cages and housed on grids so that food intake, corrected for spillage, could be measured. At 15 wk of age, the mice within each dietary group were separated into three weight-matched groups. One group on each diet remained in the room at 23°C (warm), one was moved to a room maintained at 18°C (cold), and the other was moved to a room maintained at 27°C (hot). The mice were adapted to the environment for 2 days, and then baseline measures of food intake and body weight were recorded for 1 wk. At the end of the baseline period, the mice within each diet/temperature group were divided into two weight-matched subgroups, and each mouse was fitted with an intraperitoneal Alzet miniosmotic pump while under isoflurane anesthesia. One subgroup was infused with PBS, and the other with 10 µg leptin/day. Daily measures of food intake and body weight continued to the end of the experiment.

After 3 days of infusion, the mice were food deprived from 7:00 AM to 2:00 PM, and a small sample of blood was collected by tail-bleeding for measurement of serum leptin concentration (Mouse Leptin RIA; Linco Research, St. Louis, MO). After 13 days of infusion, food was removed from the cages at 7:00 AM, and the mice were killed by decapitation between 10:00 AM and 12:00 PM. Trunk blood was collected for measurement of serum leptin, and adiponectin, epididymal, retroperitoneal, perirenal, and mesenteric white fat depots were dissected and weighed. IBAT was dissected, weighed, and snap frozen for measurement of UCP-1 mRNA expression by Northern blot, as described previously (25). The carcass, less gut content, was analyzed for composition.

Statistical analysis. Measures of change in body weight, energy expenditure, and energy intake were compared by repeated-measures analysis of variance, with time as the repeated measure. Independent variables for experiment 1 were diet and infusion. Independent variables for experiment 2 were diet, infusion, and environmental temperature. If there was a significant effect, then Duncan’s multiple-range test was used for post hoc comparisons between specific groups. Single end-point measures were compared by two- or three-way analysis of variance and post hoc Duncan’s multiple-range test (Statistica, Stat Soft, Tulsa, OK).
RESULTS

Experiment 1. LF-fed and HF-fed mice weighed the same at the start of the experiment (LF-fed = 28.0 ± 0.7 g, HF-fed = 29.3 ± 0.6 g). During the baseline period, there were no significant differences in energy intake of the mice fed LF or HF diet (Fig. 1A). Although the mice consumed different amounts of energy during the different time intervals of the experiment [diet: nonsignificant (NS), infusion: NS, time: P < 0.0001], there was no significant effect of diet or infusion on energy intakes of the mice, either at the beginning (day 1–3 diet: NS, infusion: P < 0.06, interaction: P < 0.08) or at the end of the infusion (days 11–13). The anesthesia and surgery required for pump placement inhibited food intake of all mice for 2 days, which is reflected in the intake calculated for days 1–3 of infusion. By the end of the infusion period, the intakes of the mice remained lower than at baseline. LF-fed mice lost weight during days 1–13 of leptin infusion, but there was no significant effect of leptin on weight gain of HF-fed mice (Fig. 1B, diet: P < 0.07, infusion: NS, diet × infusion: P < 0.04).

Neither diet nor leptin infusion had any effect on energy expenditure of the mice during any period of the study. This was the case when expenditure was expressed either per mouse or per metabolic body size (Fig. 2, A and B). RER was always lower in mice fed HF than LF diet. Also, during the days immediately following pump placement, the RER of all mice was lower than during the baseline period, but RER increased by the end of the infusion period (Fig. 2C: diet: P < 0.001, infusion: NS, time interval: P < 0.001, diet × time: P < 0.004, diet × infusion × time: P < 0.03). The hourly expenditures of

Fig. 1. A: 3-day energy intake of low-fat (LF)- and high-fat (HF)-fed mice in experiment 1 at different stages of the experiment. There were no differences in energy intake of any groups during the baseline period or at the end of the experimental period. B: weight change of mice in experiment 1 during 12 days of infusion. Data are means ± SE for groups of 8 or 9 mice. mse, Mouse. A,B,C,D: Statistically significant differences between treatment groups (P < 0.05).

Fig. 2. A: 24-h heat production expressed per mouse averaged over 3 days at different times during experiment 1. B: 24-h energy expenditure expressed per unit metabolic body size averaged over 3 days at different times during experiment 1. C: respiratory exchange ratio (RER) of mice averaged over 3 days at different times during experiment 1. Data are means ± SE for groups of 8 or 9 mice. A,B,C,D: Statistically significant differences between treatment groups (P < 0.05).
the mice, plotted as a mean for the average expenditure of each mouse on days 1–3 of infusion, are shown in Fig. 3. These data show that the calorimetry measures were sensitive enough to detect changes in expenditure during the day, but also show that variability between individual animals (variance = 12 – 23% of the mean) was much greater than limits set by the precision of measurement (~7.5%).

At the end of the study, there were no significant differences in the carcass weights or lean body mass of the mice, but the leptin-infused LF-fed mice had less carcass fat than their PBS-infused controls (Table 1). There was no effect of leptin on the body composition of HF-fed mice. The reduction in carcass fat of the leptin-treated LF-fed mice was reflected in a reduction in weight of the epididymal and retroperitoneal fat depots, but not the mesenteric or IBAT fat depots. Serum leptin concentrations of LF-fed-leptin-infused mice at the end of the study were higher than those in the PBS-infused mice, but the difference did not reach statistical significance (Table 1; \( P < 0.07 \)). There was no effect of either diet composition or leptin infusion on the serum adiponectin concentrations measured at the end of the experiment (Table 1).

**Experiment 2.** At the start of the experiment, HF-fed mice were significantly heavier than LF-fed mice, although the difference in weight was small (28 ± 0.3 vs. 26 ± 0.3 g, \( P < 0.001 \)). Leptin infusion, environmental temperature, and diet all had significant effects on serum leptin measured 2 days after the start of infusion (Fig. 4A: environment: \( P < 0.0001 \), diet: \( P < 0.0003 \), leptin: \( P < 0.04 \), environment \( \times \) diet: \( P < 0.0001 \)). As would be expected, mice housed in the cold had a higher energy intake than those in the warm or hot environments (Fig. 5A: diet: NS, environment: \( P < 0.0001 \), infusion: \( P < 0.02 \), diet \( \times \) environment: \( P < 0.01 \), diet \( \times \) environment \( \times \) infusion: \( P < 0.03 \)), but there was no effect of diet composition or leptin on the energy intake of mice housed in either the hot or the cold environment. In contrast, leptin

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### Table 1. Carcass composition and serum hormone concentrations of mice in experiment 1

<table>
<thead>
<tr>
<th>Carcass Composition</th>
<th>LF PBS</th>
<th>LF Leptin</th>
<th>HF PBS</th>
<th>HF Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>27±1</td>
<td>24±1</td>
<td>27±1</td>
<td>27±1</td>
</tr>
<tr>
<td>Fat, g</td>
<td>5.1±0.2a</td>
<td>4.1±0.2b</td>
<td>5.8±0.2c</td>
<td>6.2±0.2c</td>
</tr>
<tr>
<td>Lean tissue, g</td>
<td>20.5±0.6</td>
<td>19.4±0.6</td>
<td>20.1±0.5</td>
<td>20.2±0.4</td>
</tr>
<tr>
<td>Epididymal fat, mg</td>
<td>893±83abc</td>
<td>662±52</td>
<td>1,130±128a</td>
<td>1,150±134a</td>
</tr>
<tr>
<td>Retroperitoneal fat, mg</td>
<td>261±25abc</td>
<td>190±18b</td>
<td>354±34ae</td>
<td>360±34f</td>
</tr>
<tr>
<td>Mesenteric fat, mg</td>
<td>380±27</td>
<td>301±30</td>
<td>346±36</td>
<td>335±37</td>
</tr>
<tr>
<td>IBAT, mg</td>
<td>221±20</td>
<td>177±17</td>
<td>176±20</td>
<td>200±15</td>
</tr>
<tr>
<td>Serum leptin, ng/ml</td>
<td>8±2</td>
<td>12±3</td>
<td>10±2</td>
<td>10±2</td>
</tr>
<tr>
<td>Serum adiponectin, ng/ml</td>
<td>11±1</td>
<td>12±2</td>
<td>13±2</td>
<td>14±2</td>
</tr>
</tbody>
</table>

Values are means ± SE for groups of 8 or 9 mice. LF, low-fat fed; HF, high-fat fed; IBAT, intrascapular brown adipose tissue. All measurements were made at the end of the study when the mice had been infused with PBS or 10 µg leptin/day for 13 days. a,b,cAny values for a specific parameter that do not share a common superscript are significantly different at \( P < 0.05 \).

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**Fig. 3.** Hourly heat production per mouse in LF-fed (A) and HF-fed (B) mice measured during days 1–3 of infusion in experiment 1. Data are means ± SE for 8 or 9 mice. The average expenditure was calculated for each mouse over the 3 days, and the mean represents the average for mice within a treatment group.

**Fig. 4.** Serum leptin, measured on day 2 of infusion (A), and serum adiponectin concentration, measured at the end of experiment 2 (B). Data are means ± SE for groups of 8–12 mice. a,b,cSignificant differences between treatment groups (\( P < 0.05 \)).
significantly reduced energy intake of LF-fed, but not HF-fed, mice housed in the warm environment. The leptin-infused LF-fed mice had the same energy intake as the HF-fed mice. All of the mice lost between 1 and 2 g of weight in response to the surgical placement of the pump, but started to regain weight within 2 days. When weight change from the day after pump placement to the end of the study was considered for all animals, there was no effect of diet or leptin on weight gain of mice housed in either the cold or the hot environment, but both HF diet and leptin infusion inhibited weight gain of mice housed in the warm environment (Fig. 5B: diet: P < 0.002, environment: P < 0.03, infusion: P < 0.01, diet × environment: P < 0.04). Efficiency of energy utilization (gain in body weight per kilocalories consumed) during these 12 days was lowest in HF-fed mice housed in a warm environment and highest in mice housed in the hot environment and in LF-fed, PBS-infused mice housed in a warm environment (Fig. 5C: diet: P < 0.002, environment: P < 0.001, infusion: P < 0.02). Despite the low efficiency of energy utilization and failure to gain weight during the experimental period, the HF-fed mice housed in the warm environment were fatter than any of the LF-fed mice and fatter than HF-fed mice housed in the cold.

Carcass analysis, measured at the end of the study, indicated that neither weight change nor efficiency of energy utilization were representative of body composition of the mice. Lean body mass (protein + water) of the mice was not influenced by either leptin or diet, although the mice housed in the hot environment tended to have smaller amounts of lean tissue than those housed in the warm or cold environments (Fig. 6A: diet: NS, environment: P < 0.02, infusion: NS). In contrast, there were some large effects of diet and temperature on body fat content of the mice (Fig. 6C: diet: P < 0.0001, environment: P < 0.001, infusion: NS, diet × environment: P < 0.001, temperature × infusion: P < 0.06). The HF-fed mice housed in the hot environment were substantially fatter than any other group of animals, and, surprisingly, leptin caused a further increase in body fat. In contrast, there were no differences in body fat content of the HF-fed mice housed in cold and warm environments, and leptin had no effect on body fat content in either of these groups of animals. PBS-infused LF-fed mice housed in the cold had less body fat than those housed in warm or hot environments, but there was no effect of temperature on body fat content of leptin-infused LF-fed mice. Leptin reduced body fat only in the LF-fed mice housed in a warm environment. Body composition expressed as a percentage of carcass weight followed the same pattern of response as carcass composition expressed as actual weights of different tissues (Table 2). The weights of individual fat depots (Table 3) and the weight of dissected fat (epididymal, retroperitoneal, perirenal, and mesenteric fat) followed the same pattern as total carcass fat content (Fig. 6B). There was a significant effect (P < 0.0001) of diet and of environmental temperature and an interaction between diet and environment (P < 0.01) for all of the white fat depots weighed. In LF-fed rats housed in the warm environment, leptin reduced the size of perirenal, mesenteric, and IBAT. In HF-fed mice housed in a hot environment, leptin significantly increased epididymal fat, but not any of the other depots that were measured. There was no effect of diet on IBAT weight, except for the leptin-infused mice housed in a warm environment (diet: NS, environment: P < 0.001, infusion: NS, environment × infusion: P < 0.03), and there was no effect of diet, environmental temperature, or leptin infusion on IBAT UCP-1 mRNA expression in any group (data not shown). Adiponectin, measured at the end of the study, was much higher in LF-fed leptin-infused mice housed in a warm environment than in any other group (Fig. 4B: diet: NS, environment: P < 0.006, infusion: 0.004, environment × diet: P < 0.003, environment × infusion: P < 0.04, diet × infusion: P < 0.05). Serum leptin, measured at the end of the study,
showed essentially the same pattern of concentration as at day 2 of infusion (data not shown).

DISCUSSION

The initial objective of the studies described here was to determine whether mice adapted to a HF diet and resistant to the effects of leptin on food intake and body fat content, demonstrated the leptin-induced stimulation of energy expenditure that has been reported for chow-fed rats (44) and mice (28). The results of experiment 1, however, did not show any effect of leptin on energy expenditure of either LF-fed or HF-fed mice. Even though the energy expenditure of the LF-fed mice did not change, the mice were obviously leptin responsive, demonstrating a transient inhibition of food intake during the first days of leptin infusion and a reduced body fat mass at the end of the experiment, compared with PBS-infused controls. In contrast, the HF-fed mice met the criteria for leptin resistance, because leptin infusion did not produce any change in food intake or body composition.

Other investigators have reported that leptin stimulates sympathetic outflow to IBAT (26), increases IBAT UCP-1 mRNA expression (42, 45), and increases whole animal energy expenditure (44, 47). There are a number of differences between those studies and experiment 1 described here. Previously, our laboratory has found no effect of either peripheral infusions or central injections of leptin on IBAT UCP-1 mRNA expression or norepinephrine turnover, which is an indirect measure of sympathetic activity in that tissue in rats (36). Thus it is unlikely that the species used to test for an effect of leptin on energy expenditure was the reason for finding no effect in experiment 1. Another difference between experiment 1 and studies that have reported an effect of leptin on whole animal energy expenditure (44, 47) is that we used lower doses of leptin than were used in these other experiments. It has been suggested that the regulation of different physiological end points is determined by hypothalamic leptin concentration (29), and Ahima et al. (2) reported that physiological doses of leptin decrease hypothalamic neuropeptide Y mRNA expression, but do not alter proopiomelanocortin, corticotropin-releasing factor, or cocaine- and amphetamine-regulated transcript mRNA expression in fed animals. Others have suggested that leptin-induced changes in thermogenesis are mediated by the melanocortin system (43). Taking these pieces of information together, it may not be surprising that low doses of leptin had no effect on expenditure of fed mice. We did not measure energy expenditure of fasted mice, but we may have found an effect of leptin in these conditions, because leptin has been shown to prevent a decrease in energy expenditure of fasted mice (15, 21), even when it did not stimulate expenditure in fed mice (21).

The indirect calorimetry measurements in experiment 1 allowed an evaluation of RER of the mice, which gives an indication of the type of nutrient being oxidized for energy. In vivo and in vitro studies have shown that leptin increases fatty acid oxidation in liver (27) and muscle (33, 34) tissue, a response associated with phosphorylation of acetyl-CoA carboxylase (27, 33). In addition, calorimetry studies have shown a decrease in RER of rats or mice injected intracerebroventricularly with leptin (20, 28, 47) or peripherally infused with leptin (5), indicative of an increase in whole body oxidation of fatty acids or amino acids (32). In experiment 1 described here, there was a small decrease in RER in the LF-fed mice during the first 4 days of leptin treatment, when it fell from ~1.2 before infusion to ~1.0 during the infusion. Because an RER > 1.0 is representative of a state of net energy deposition and an RER of 1.0 is representative of high rates of carbohydrate oxidation (32), it is possible that the change seen in the LF-fed mice in experiment 1 represented an inhibition of weight gain rather than a shift in substrate oxidation. Whole
animal RER is determined by the relative activity of different metabolic pathways. The stoichiometry of the overall reaction for lipid synthesis from glucose does not use oxygen; however, because of the requirement to produce acetyl-CoA as an intermediate (18), this experiment may have been associated with a subtle decrease in fatty acid oxidation and tissue lipid mobilization, but this difference in body fat could also have been achieved through a change in nutrient partitioning that inhibited deposition of body fat.

Although the results from experiment 1 did not answer the question that we initially set out to answer, they did suggest that the changes in RER and energy expenditure that have been reported in other studies may only be relevant to specific experimental conditions, or a specific physiological state, such as fasting. The difference in body fat content of leptin- and PBS-infused LF-fed mice was only 1 g (9 kcal), which represented a significant portion of total body fat but would have required only a small disturbance of energy balance, and the change in 24-h energy expenditure required for this difference in carcass energy over 13 days could easily have been too small to detect in the calorimeter, given the between-mouse variability. Therefore, the objective of the second experiment was to place the animals in environments that compromised their ability to adjust thermogenesis. We reasoned that housing the mice in a hot environment would limit their capacity to increase energy expenditure to prevent hyperthermia. In contrast, in the cold environment, energy expenditure would already be stimulated to compensate for increased heat loss. The temperature changes were moderate as the hot temperature of 27°C was below thermoneutrality (~30°C), a temperature at which body temperature is maintained only by controlling metabolic pathways.

Table 2. Carcass composition as percent carcass weight of mice in experiment 2

<table>
<thead>
<tr>
<th>Carcass Component</th>
<th>Cold LF PBS</th>
<th>Cold LF Leptin</th>
<th>Cold HF PBS</th>
<th>Cold HF Leptin</th>
<th>Warm LF PBS</th>
<th>Warm LF Leptin</th>
<th>Warm HF PBS</th>
<th>Warm HF Leptin</th>
<th>Hot LF PBS</th>
<th>Hot LF Leptin</th>
<th>Hot HF PBS</th>
<th>Hot HF Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>23.3 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.3 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.8 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.2 ± 0.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>24.9 ± 0.7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>23.0 ± 0.5&lt;sup&gt;f&lt;/sup&gt;</td>
<td>24.7 ± 0.7&lt;sup&gt;g&lt;/sup&gt;</td>
<td>24.2 ± 0.7&lt;sup&gt;h&lt;/sup&gt;</td>
<td>23.0 ± 0.5&lt;sup&gt;i&lt;/sup&gt;</td>
<td>23.1 ± 0.5&lt;sup&gt;j&lt;/sup&gt;</td>
<td>25.4 ± 0.6&lt;sup&gt;k&lt;/sup&gt;</td>
<td>25.8 ± 0.5&lt;sup&gt;l&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat, %</td>
<td>9.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.2 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.0 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.0 ± 0.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.6 ± 0.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.8 ± 0.8&lt;sup&gt;f&lt;/sup&gt;</td>
<td>13.2 ± 0.8&lt;sup&gt;g&lt;/sup&gt;</td>
<td>11.2 ± 1.3&lt;sup&gt;h&lt;/sup&gt;</td>
<td>12.0 ± 0.7&lt;sup&gt;i&lt;/sup&gt;</td>
<td>10.5 ± 1.0&lt;sup&gt;j&lt;/sup&gt;</td>
<td>16.8 ± 1.2&lt;sup&gt;k&lt;/sup&gt;</td>
<td>19.3 ± 1.1&lt;sup&gt;l&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water, %</td>
<td>63.2 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.2 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.2 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60.5 ± 0.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>62.2 ± 0.8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>63.4 ± 0.9&lt;sup&gt;f&lt;/sup&gt;</td>
<td>59.1 ± 0.8&lt;sup&gt;g&lt;/sup&gt;</td>
<td>61.0 ± 1.1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>61.5 ± 0.7&lt;sup&gt;i&lt;/sup&gt;</td>
<td>62.0 ± 0.7&lt;sup&gt;j&lt;/sup&gt;</td>
<td>57.2 ± 0.8&lt;sup&gt;k&lt;/sup&gt;</td>
<td>55.6 ± 0.8&lt;sup&gt;l&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash, %</td>
<td>4.6 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.0 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.1 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.5 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.5 ± 0.1&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.1 ± 0.1&lt;sup&gt;g&lt;/sup&gt;</td>
<td>4.3 ± 0.1&lt;sup&gt;h&lt;/sup&gt;</td>
<td>4.2 ± 0.1&lt;sup&gt;i&lt;/sup&gt;</td>
<td>4.3 ± 0.1&lt;sup&gt;j&lt;/sup&gt;</td>
<td>3.7 ± 0.1&lt;sup&gt;k&lt;/sup&gt;</td>
<td>3.7 ± 0.1&lt;sup&gt;l&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE for groups of 8–12 mice per group. a,b,c,d,e Values for a carcass component that do not share a common superscript are significantly different at P < 0.05.

Table 3. Fat pad weights of mice in experiment 2

<table>
<thead>
<tr>
<th>Fat Pad</th>
<th>Epididymal</th>
<th>Perirenal</th>
<th>Retroperitoneal</th>
<th>Mesenteric</th>
<th>IBAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold LF PBS</td>
<td>399 ± 35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63 ± 5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>98 ± 11&lt;sup&gt;n&lt;/sup&gt;</td>
<td>175 ± 9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>143 ± 7&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cold LF Leptin</td>
<td>366 ± 34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60 ± 5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>88 ± 10&lt;sup&gt;n&lt;/sup&gt;</td>
<td>160 ± 11&lt;sup&gt;h&lt;/sup&gt;</td>
<td>138 ± 8&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cold HF PBS</td>
<td>606 ± 37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72 ± 5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>170 ± 11&lt;sup&gt;e&lt;/sup&gt;</td>
<td>174 ± 10&lt;sup&gt;e&lt;/sup&gt;</td>
<td>152 ± 10&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cold HF Leptin</td>
<td>644 ± 50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80 ± 5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>186 ± 16&lt;sup&gt;e&lt;/sup&gt;</td>
<td>177 ± 13&lt;sup&gt;e&lt;/sup&gt;</td>
<td>152 ± 8&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Warm LF PBS</td>
<td>536 ± 79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101 ± 14&lt;sup&gt;f&lt;/sup&gt;</td>
<td>127 ± 21&lt;sup&gt;e&lt;/sup&gt;</td>
<td>231 ± 32&lt;sup&gt;e&lt;/sup&gt;</td>
<td>159 ± 20&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Warm LF Leptin</td>
<td>410 ± 57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58 ± 8&lt;sup&gt;g&lt;/sup&gt;</td>
<td>83 ± 19&lt;sup&gt;e&lt;/sup&gt;</td>
<td>164 ± 23&lt;sup&gt;e&lt;/sup&gt;</td>
<td>104 ± 9&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Warm HF PBS</td>
<td>770 ± 81&lt;sup&gt;n&lt;/sup&gt;</td>
<td>86 ± 8&lt;sup&gt;h&lt;/sup&gt;</td>
<td>225 ± 25&lt;sup&gt;n&lt;/sup&gt;</td>
<td>198 ± 21&lt;sup&gt;e&lt;/sup&gt;</td>
<td>134 ± 13&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Warm HF Leptin</td>
<td>708 ± 134&lt;sup&gt;n&lt;/sup&gt;</td>
<td>82 ± 11&lt;sup&gt;n&lt;/sup&gt;</td>
<td>167 ± 36&lt;sup&gt;h&lt;/sup&gt;</td>
<td>168 ± 18&lt;sup&gt;h&lt;/sup&gt;</td>
<td>123 ± 10&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hot LF PBS</td>
<td>522 ± 57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66 ± 6&lt;sup&gt;n&lt;/sup&gt;</td>
<td>131 ± 15&lt;sup&gt;n&lt;/sup&gt;</td>
<td>160 ± 11&lt;sup&gt;n&lt;/sup&gt;</td>
<td>102 ± 8&lt;sup&gt;n&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hot LF Leptin</td>
<td>497 ± 61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77 ± 6&lt;sup&gt;n&lt;/sup&gt;</td>
<td>132 ± 20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>190 ± 21&lt;sup&gt;e&lt;/sup&gt;</td>
<td>123 ± 10&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hot HF PBS</td>
<td>937 ± 99&lt;sup&gt;d&lt;/sup&gt;</td>
<td>115 ± 9&lt;sup&gt;n&lt;/sup&gt;</td>
<td>267 ± 22&lt;sup&gt;h&lt;/sup&gt;</td>
<td>276 ± 28&lt;sup&gt;h&lt;/sup&gt;</td>
<td>121 ± 9&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hot HF Leptin</td>
<td>1,094 ± 104&lt;sup&gt;d&lt;/sup&gt;</td>
<td>117 ± 11&lt;sup&gt;n&lt;/sup&gt;</td>
<td>309 ± 32&lt;sup&gt;h&lt;/sup&gt;</td>
<td>272 ± 36&lt;sup&gt;h&lt;/sup&gt;</td>
<td>119 ± 9&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE in mg for groups of 8–12 mice per group. a,b,c,d,e Values for a specific fat depot that do not share a common superscript are significantly different at P < 0.05.
sensible heat loss (41), and the cold temperature was not low enough to cause weight loss.

As would be expected, energy intake was inversely related to environmental temperature. The increase in energy intake of HF-fed mice housed in the cold was enough to compensate for the increased requirement for heat production, because their body composition was similar to that of HF-fed mice in the warm environment. The LF-fed mice increased their energy intake to the same level as that of the HF-fed mice, but they did not achieve energy balance because efficiency of energy utilization was reduced, and there was a small loss of body fat compared with PBS-infused, LF-fed mice housed in a hot or warm environment. The HF-fed mice housed in the hot environment were fatter than their counterparts housed at other temperatures, indicating that their inhibition of food intake did not fully compensate for the suppression of heat production. LF-fed mice housed in the warm were the only treatment group that ate less in response to leptin infusion, and this also was the only group in which leptin inhibited body weight gain, efficiency of energy utilization, and body fat content. Thus even moderate changes in environmental temperature abolished leptin responsiveness of LF-fed mice, which suggests that mechanisms that maintain homeostasis override those activated by administration of low doses of leptin.

The HF-fed mice in a warm or cold environment met the criteria for leptin resistance, because neither food intake nor body composition was changed by leptin administration. The HF-fed mice in the hot environment had ∼30% more fat than those in the warm environment, and, surprisingly, the increase in body fat was exaggerated by leptin. Although this change in body fat was in the opposite direction than would be expected for leptin treatment, it demonstrates that the HF-fed mice were not totally leptin resistant. The LF-fed mice would be expected to be leptin responsive, but leptin did not increase body fat content of these mice in the hot environment; therefore, the increase in body fat content of HF-fed mice was specific to the combination of diet-induced “leptin resistance” and environmental temperature. Fat deposition must occur only when there is the combination of leptin resistance in pathways that normally inhibit food intake and decrease body fat, and there is also a limited ability to increase energy expenditure. The increase in body fat content of the HF-fed mice did not influence serum adiponectin or leptin concentrations, but housing in a cold environment decreased serum leptin concentrations of HF-fed mice. This observation is consistent with reports that cold exposure inhibits leptin secretion from white fat due to direct effects of the cold on adipose tissue (48) or increased sympathetic activity in cold-exposed animals (38).

The LF-fed mice that were leptin responsive in the warm environment did not lose fat in response to leptin when they were housed in a hot environment, which implies that environmental temperature restrained the shifts in energy balance that facilitate a leptin-induced reduction in fat mass. In addition, unlike the HF-fed mice in the hot environment, the PBS-infused, LF-fed mice did not gain fat compared with those housed in a warm environment, suggesting that leptin responsiveness at least prevented a gain in body fat. In a previous study with young (35-day-old) mice, we found that leptin inhibited accretion of body fat in LF-fed mice. The difference in response between that study and experiment 2 described here must be related to leptin influencing nutrient partitioning in the mice that are in a state of rapid growth (22) compared with older mice that are gaining weight slowly.

Central leptin resistance in HF-fed rats has been attributed to a failure of leptin to cross the blood-brain barrier (4) and activate hypothalamic leptin signaling pathways (31). Thus, in HF-fed mice, peripherally infused leptin would have access to peripheral leptin receptors, but may not have activated hypothalamic receptors. When the mice also were housed in a hot environment, energy expenditure was limited, and under this condition the selective activation of leptin receptors located outside of the blood-brain barrier resulted in an increase in body fat mass. This possibility is supported by observations in chronically decerebrate rats, in which the forebrain is surgically isolated from the brain stem. A 2-wk infusion of leptin in decerebrate rats resulted in a significant increase in body fat content, even though their food intake was fixed by tube feeding (37). The effect of leptin in HF-fed mice is dependent on both the presence of leptin resistance and an environmental restraint on energy intake and expenditure (i.e., hot ambient temperature). It does not result simply from the mice consuming a HF diet, because our laboratory has previously shown that there is an overall inhibition of growth in leptin-infused young mice housed in a room at 28°C and fed HF diet for only 10 days (22). These mice would have been considered leptin responsive, because they decreased food intake and lost weight during the leptin infusion, whether they were housed in a hot (22) or a warm environment (6). Leptin may also have been effective in the young mice, because they were in a phase of rapid growth, whereas the mice in this study were gaining weight slowly. Therefore, the increase in body fat content of the leptin-treated HF-fed mice housed in the hot environment in experiment 2 described here was dependent on there being some degree of leptin resistance and could not simply be explained by an interaction between diet, leptin administration, and temperature. We did not define the mechanism of response in this study, and the increase in body fat of HF-fed, leptin-infused mice in the hot environment has to have resulted from a decrease in energy expenditure, an increase in energy intake, or a change in nutrient partitioning to favor fat deposition. We did not find a measurable increase in food intake of the mice, but the amount of energy required to account for the increase in fat that was observed may have been too small to detect with 24-h measures of food intake. Leptin has been reported to inhibit activity of leptin-deficient mice (35), and restoration of leptin receptors to the arcuate nucleus increases locomotor activity of receptor-deficient mice (14). Similarly, a decrease in motor activity is a typical thermoregulatory response of mice housed in a hot environment (40). Therefore, it is possible that the mice deposited more energy as body fat due to a decrease in physical activity.

Overall, the results of the experiments described here suggest that physiological doses of leptin administered as continuous peripheral infusions do not produce a measurable increase in energy expenditure or a dramatic change in RER, which would be indicative of increased rates of fatty acid oxidation. The results of the second study demonstrate that relatively small changes in environmental temperature abolish leptin responsiveness in adult LF-fed mice, possibly due to the environmental restraint on heat production, leaving open the possibility that leptin produces small changes in energy expenditure that contribute to the loss of fat in leptin-responsive
mice. Of most interest is the observation that adult, leptin-resistant, HF-fed mice housed in a hot environment gained body fat when they were treated with leptin; this response is similar to that found in leptin-infused chronically deacrebrate rats (37). These results demonstrate that the HF-fed mice in a hot environment are not totally leptin resistant, and it is possible that the failure of leptin to activate central leptin receptors facilitated a leptin-induced change in nutrient utilization, mediated by leptin receptors in the periphery or caudal brain stem, to allow an increase in body fat mass. This could only occur if forebrain leptin receptors normally inhibited this specific peripheral response to leptin.

REFERENCES