Leptin action is modified by an interaction between dietary fat content and ambient temperature

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Submitted 12 May 2004; accepted in final form 19 July 2004

Haltiner, Andrea L., Tiffany D. Mitchell, and Ruth B. S. Harris. Leptin action is modified by an interaction between dietary fat content and ambient temperature. Am J Physiol Integr Comp Physiol 287: R1250–R1255, 2004. First published July 22, 2004; doi:10.1152/ajpregu.00313.2004.—Mice adapted to a high-fat diet are reported to be leptin resistant; however, we previously reported that mice fed a high-fat (HF) diet and housed at 23°C remained sensitive to peripheral leptin and specifically lost body fat. This study tested whether leptin action was impaired by a combination of elevated environmental temperature and a HF diet. Male C57BL/6 mice were adapted to low-fat (LF) or HF diet from 10 days of age and were housed at 27°C from 28 days of age. From 35 days of age, baseline food intake and body weight were recorded for 1 wk and then mice on each diet were infused with 10 µg leptin/day or PBS from an intraperitoneal miniosmotic pump for 13 days. HF-fed mice had a higher energy intake than LF-fed mice and were heavier but not fatter. Serum leptin was lower in PBS-infused HF- than LF-fed mice. Leptin significantly inhibited energy intake of both LF-fed and HF-fed mice, and this was associated with a significant increase in hypothalamic long-form leptin receptors with no change in short-form leptin receptor or brown fat uncoupling protein-1 mRNA expression. Leptin significantly inhibited weight gain in both LF- and HF-fed mice but reduced the percentage of body fat mass only in LF-fed mice. The percentage of lean and fat tissue in HF-fed mice did not change, implying that overall growth had been inhibited. These results suggest that dietary fat modifies the mechanisms responsible for leptin-induced changes in body fat content and that those in HF-fed mice are sensitive to environmental temperature.

WHEN THE ADIPOCYTE-DERIVED hormone leptin was first identified, it was hypothesized to be a negative feedback signal in the regulation of energy balance (28). It is clear that leptin replacement in leptin-deficient ob/ob mice results in a rapid weight loss and that central or peripheral leptin administration to normal weight experimental animals causes a loss of body fat (13), with or without a significant reduction in food intake (5). In contrast, leptin action is inhibited in obese animals (13). In humans, the majority of obese individuals also have high endogenous concentrations of leptin (9), but this does not prevent, or reverse, the accumulation of excess body fat. If leptin is involved in the regulation of body weight or body composition, then leptin action must be impaired early in the process of weight gain; otherwise, body fat would not accumulate. This means that factors other than obesity can override leptin action.

In previous animal studies (15), we have reported that leptin does not reduce body fat in older (15 wk old) male mice fed a high-fat (HF) diet for 5 wk. This “leptin resistance” appears to be independent of the development of obesity. In contrast, young (5 wk old) or older (15 wk old) male mice fed a HF diet from 10 days of age, before they are weaned, remain responsive to peripheral infusions of leptin even if they are fatter than their low-fat (LF)-fed counterparts (5), and female mice fed an HF diet for 8 wk lose body fat when leptin is infused peripherally (16). Therefore, there is not a simple relation between diet composition or adiposity and leptin action.

We also have found that leptin action is dependent on the housing conditions of mice (5). Young, 5-wk-old, mice that are fed a HF diet from 10 days of age and are housed individually on grid floors lose body fat in response to leptin, whereas body composition does not change in those that are group housed in cages with bedding. Others have reported that central injections of leptin stimulate sympathetic outflow to peripheral tissues (11), activate uncoupling protein-1 (UCP-1) in brown adipose tissue (8), and stimulate energy expenditure in rodents (27), supporting the concept that an increase in thermogenesis could contribute to the reduction in body fat mass of leptin-treated animals. We suggested that group housing provided an environment in which the need for thermogenesis to maintain body temperature was minimized and that this environment inhibited a leptin-induced stimulation of thermogenesis that would normally contribute to the loss of fat in leptin-treated mice (5). This study was designed to determine whether housing animals in a warm environment exaggerated the inhibitory effects of dietary fat on leptin action. If this was found to be the case, then it could potentially explain why leptin resistance develops before obesity because most individuals use clothing and housing to maintain a warm environmental temperature. On the basis of the group housing study, we hypothesized that a warm environment would not influence weight loss in LF-fed, leptin-treated C57BL/6 mice but would inhibit the loss of fat in HF-fed mice. Young mice were fed a LF- or HF diet from 10 days of age and were then housed in a room at 27°C that should minimize the need for thermogenesis. The response to peripheral infusions of leptin was tested when the mice were 6 wk old.

METHODS

C57BL/6 mice were obtained from a breeding colony maintained at the University of Georgia. The colony was housed in a room maintained at 22.7°C (73°F) with lights on for 12 h each day from 7:00 AM. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Georgia and were conducted according to APS Guiding Principles in the Care and Use of Animals (1). Litters in the colony were randomly assigned to either a HF diet (45% kcal fat, Diet 12451, Research Diets, New Brunswick, NJ).
NJ) or a LF diet (10% kcal fat, Diet 12450B, Research Diets) when the pups were 10 days of age and still nursing with the dams. Pups were weaned at 28 days of age, and the males were maintained on their respective HF or LF diets and group housed in shoebox cages.

At 28 days of age, 38 male mice were moved to a room maintained at 27.2 ± 0.4°C (81.1 ± 0.1°F) and housed individually in cages with grid floors to facilitate measurement of food intake. Baseline food intakes, corrected for spillage and body weights, were recorded daily for 7 days starting when the mice were 35 days old, and then the mice within each dietary group were divided into two weight-matched subgroups. Each mouse was anesthetized with isoflurane, and fitted with an intraperitoneal Alzet miniosmotic pump (model 1002; Durect; Cupertino, CA) delivering either sterile PBS or 10 μg of recombinant murine leptin per day (R&D Systems, Minneapolis, MN). Daily measurements of body weight and food intake continued for 13 days. On the third day of infusion, the mice were fed deprived from 7:00 AM to 12:00 PM. A small sample of blood was collected from the tail and then the mice returned to ad libitum feeding. The blood was used to measure serum leptin (Murine Leptin RIA kit, Linco Research, St. Louis, MO), free fatty acids (FFA; NEFA C kit; WAKO, Richmond, VA), and triglycerides (L-type triglyceride kit; WAKO Chemical). On day 13 of infusion, the mice were food deprived for 2 h and then decapitated. Trunk blood was collected for measurement of serum FFA (NEFA C kit; WAKO, Richmond, VA), post hoc Duncan’s multiple-range test. Differences were considered significant at P < 0.05. Analysis was performed using Statistica (StatSoft, Tulsa, OK).

RESULTS

Mice fed HF diet from 10 days of age were significantly heavier at the start of the experiment than the mice that had been fed LF diet (Fig. 1A). All of the mice initially lost weight in response to the surgery required for pump placement, but the loss was reversed within 3 days. There was a significant difference in the weights of leptin-treated and control HF mice from the day 2 of infusion to the end of the experiment, the weights of LF-fed mice were different from days 5–12 of infusion [Fig. 1: Diet: not significant (NS), Leptin: P < 0.001, Day: P < 0.001, Leptin × Day: P < 0.05]. This difference in weight was associated with a reduction in energy intake of the leptin-treated mice that was significant for both LF- and HF-fed mice when cumulative intake during the period of infusion was considered (Fig. 1B: Diet: P < 0.001, Leptin: P < 0.001, Interaction: NS). Both control and leptin-treated HF mice ate more than their LF-fed counterparts.

At the end of the experiment, there was a variable response to leptin in the different fat pads. Both diet and leptin had a significant effect on the weights of inguinal, retroperitoneal and epididymal depots (Fig. 2A: Diet: P < 0.001, Leptin: P < 0.001, Interaction, NS). These fat depots were larger in control HF-fed than control LF-fed mice, and leptin significantly reduced the size of each pad independent of diet composition. In contrast, neither diet nor leptin had any significant effect on the size of the mesenteric fat depot, and the perirenal fat depot was reduced by leptin only in the LF-fed mice (Diet: P < 0.01, Leptin: P < 0.007, Interaction: P < 0.02). Although leptin reduced the size of some of the fat depots in the HF-fed mice, the percentage of total carcass fat was the same for the two groups of HF-fed mice but was significantly lower in leptin-infused than PBS-infused LF-fed mice (Table 1: Diet: P < 0.001, Leptin: P < 0.001, Interaction: P < 0.05). Leptin did not reduce lean tissue in LF-fed mice, so it represented a significantly greater proportion of carcass weight in leptin-treated than PBS-treated animals. In contrast, there was no
effect of leptin on the percentage of carcass lean tissue in HF-fed mice, implying that leptin had caused a uniform inhibition of growth of both lean and fat tissue (Table 1: Diet: $P < 0.008$, Leptin: $P < 0.0005$, Interaction: $P < 0.01$).

Leptin infusion caused a significant increase in serum leptin concentrations of both LF- and HF-fed mice after 3 days of infusion. Leptin was similar in the two leptin-treated groups, but it was lower in HF-fed controls than LF-fed controls (Table 1: Diet: NS, Leptin: $P < 0.003$, Interaction: NS). Serum FFA and triglycerides were not different between groups 3 days of leptin infusion (Table 1). At the end of the experiment, both FFA and triglycerides were higher in LF-fed than HF-fed mice (Diet: $P < 0.04$, Leptin: NS, Interaction: NS) There were no differences in the level of IBAT UCP-1 mRNA expression between the different groups of mice (Fig. 3). Leptin infusion caused a significant reduction in the weight of IBAT when all mice were considered (Table 1: Diet: NS, Leptin: $P < 0.03$, Interaction: NS), but posthoc analysis did not show a significant effect for mice in any dietary group. In contrast, leptin infusion caused a substantial increase in the hypothalamic expression of ObRb (Fig. 4: Diet: NS, Leptin: $P < 0.004$, Interaction: NS) with no effect on the mRNA expression of ObRa. Neither diet nor leptin had any effect on the expression of either ObRa or ObRb in white adipose tissue (data not shown). Abundance of mRNA for ObRa was ~20-fold lower and ObRb was ~1,000-fold lower in white fat than in hypothalamic tissue.

**DISCUSSION**

This study shows that diet composition modifies the effect of peripheral leptin administration on body composition when animals are housed in a warm environment. Both LF-fed and HF-fed leptin-treated mice weighed less than their controls during the experimental period, implying that both dietary groups remained responsive to leptin. Because the mice in this study were young, the reduced weight of leptin-treated mice compared with controls represented an inhibition of growth, rather than a loss of weight, and the composition of the diet influenced the tissue composition of the weight difference. Leptin reduced the percentage of body fat and increased percent lean tissue if the mice were fed LF diet, but there was no proportional change in body composition if the mice were fed HF diet. Because leptin did cause a significant inhibition of growth in the HF-fed mice, maintenance of the same proportional body composition indicates that there was a uniform inhibition of growth of lean and fat tissue. In contrast, leptin appeared to specifically inhibit accumulation of body fat in the LF-fed mice. The difference in the effects of leptin in LF-fed versus HF-fed mice was due to diet composition, not development of obesity, because percent carcass fat was not different between LF- and HF-fed control groups. The HF-fed mice were larger than the LF-fed mice, but they were not fatter. We did not measure tissue metabolism in this study; therefore, the reason for the different effects of leptin on nutrient partitioning cannot be identified. In addition, the nature of the differences in tissue weight was not evaluated, and they may represent a true failure to grow or a failure to store energy as lipid in white fat and as glycogen in muscle and liver. Further studies are needed to elucidate why increasing dietary fat changes the mechanisms by which leptin reduces body weight.

In a previous study (5), using the same experimental design but with mice housed at 23°C, we found that HF-fed leptin-treated C57BL/6 mice from the same colony as was used for this study had a reduced body fat mass compared with their PBS-treated controls (PBS: 10.7 ± 0.9%, Leptin: 7.7 ± 1.0% carcass fat). Therefore, the switch in leptin effect from a specific depletion of fat to an overall inhibition of growth in
this study suggests that a warm environment modifies leptin action in a manner that influences the composition of tissue loss in HF-fed but not LF-fed mice. This assumption also is supported by the observation that HF-fed NIH Swiss mice that are group housed at 23°C do not lose body fat in response to peripheral infusions of leptin, whereas the same mice housed individually in cages with grid floors lose body fat when they are infused with leptin (5). Others have reported that central leptin infusions activate peripheral sympathetic nerves (11) and stimulate UCP-1 expression in brown and white adipose tissue (8). Therefore, it is reasonable to assume that increased sympathetic output to brown and white fat is at least partially responsible for the change in body composition of leptin-treated mice. Direct support for this has been provided by Dobbins et al. (10), who reported that there was a reduced thermogenic response to central infusions of leptin in rats that had been chemically sympathectomized, compared with their intact controls. It has not been determined whether peripherally administered leptin causes a central activation of sympathetic flow to peripheral tissues. If peripherally administered leptin action is modified by ambient temperature, then this would provide a mechanism to explain the increased body fat loss seen in LF-fed mice housed at 23°C. This is consistent with the observation that leptin is a powerful stimulus of energy expenditure in humans (12, 13). The results of this study are also consistent with the hypothesis that increased sympathetic output to brown and white fat is at least partially responsible for the increased thermogenic response to central leptin infusions in peripherally adrenalectomized rats (7). If peripherally administered leptin actions are modified by ambient temperature, then this would provide a mechanism to explain the increased body fat loss seen in LF-fed mice housed at 23°C. This is consistent with the observation that leptin is a powerful stimulus of energy expenditure in humans (12, 13). The results of this study are also consistent with the hypothesis that increased sympathetic output to brown and white fat is at least partially responsible for the increased thermogenic response to central leptin infusions in peripherally adrenalectomized rats (7).

Table 1. Serum and carcass measures

<table>
<thead>
<tr>
<th></th>
<th>Low Fat</th>
<th>Leptin</th>
<th>High Fat</th>
<th>Leptin</th>
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<tr>
<td><strong>Day 3 serum</strong></td>
<td></td>
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</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>4.2±0.5*</td>
<td>5.3±0.6*</td>
<td>2.7±0.3†</td>
<td>5.0±0.7*</td>
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<tr>
<td>FFA, nmol/l</td>
<td>1.09±0.11</td>
<td>1.01±0.10</td>
<td>0.90±0.06</td>
<td>0.99±0.15</td>
</tr>
<tr>
<td>Triglycerides, ng/dl</td>
<td>29±4</td>
<td>43±6</td>
<td>36±4</td>
<td>35±7</td>
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<tr>
<td><strong>Day 13 measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFA, nmol/l</td>
<td>1.22±0.08*</td>
<td>1.16±0.09*</td>
<td>0.93±0.04†</td>
<td>1.04±0.08†</td>
</tr>
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<td>Triglycerides, ng/dl</td>
<td>70±11*</td>
<td>65±3†</td>
<td>50±3†</td>
<td>56±6†</td>
</tr>
<tr>
<td>IBAT weight, mg</td>
<td>81±5*†</td>
<td>62±6†</td>
<td>84±6††</td>
<td>72±8††</td>
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<tr>
<td>Carcass weight, g</td>
<td>18.7±0.5*</td>
<td>18.2±0.3*†</td>
<td>21.2±0.6†</td>
<td>20.2±0.2††</td>
</tr>
<tr>
<td>Carcass fat, %</td>
<td>10.8±0.5*</td>
<td>6.3±0.9†</td>
<td>11.0±0.3*†</td>
<td>10.1±0.8*†</td>
</tr>
<tr>
<td>Carcass fat, g</td>
<td>2.01±0.07*</td>
<td>1.17±0.17†</td>
<td>2.33±0.09*</td>
<td>2.05±0.18*</td>
</tr>
<tr>
<td>Lean tissue, %</td>
<td>89.2±0.5*</td>
<td>93.7±0.8†</td>
<td>89.0±0.4*</td>
<td>89.9±0.8*†</td>
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<tr>
<td>Lean tissue, g</td>
<td>15.9±0.5*</td>
<td>16.3±0.2*†</td>
<td>18.1±0.5†</td>
<td>17.3±0.2††</td>
</tr>
</tbody>
</table>

Values are means ± SE for groups of 9 or 10 mice. FFA, free fatty acid; IBAT, intrascapular brown adipose depot. * Values for a specific parameter that do not share a common superscript are significantly different at P < 0.05, determined by two-way ANOVA and a post hoc Duncan’s multiple-range test.
tin crosses the blood-brain barrier, then there also is the potential for an increase in thermogenesis and for promotion of lipolysis, both of which could contribute to the decrease in the size of body fat stores (7). When animals are housed in a warm environment, the opportunity to stimulate thermogenesis would be limited by mechanisms responsible for maintenance of body temperature. Because we did not run animals housed at 23°C in parallel with those housed at 27°C we cannot conclusively attribute the differences in body composition of LF- and HF-fed mice to an interaction between diet and environmental temperature. In the environmental conditions used in this study, however, feeding the mice a HF diet shifted the effect of leptin on nutrient partitioning from specifically depleting body fat to causing a general inhibition of growth.

Although the thermogenic effects of leptin have been attributed to stimulation of the sympathetic nervous system, it is possible that energy wasting also is mediated by other mechanisms such as futile cycles. The mice used in the study were C57BL/6, a strain that is reported to be very susceptible to diet-induced obesity because of a low thermogenic and lipolytic response to adrenergic agonists (7). Therefore, if the specific loss of fat in leptin-treated mice is due to sympathetic stimulation of white fat lipolysis and thermogenesis (10), we inadvertently selected a strain of mice that is least likely to respond to an environment in which adrenergic responses are minimized. The only indirect measure of sympathetic function that was made in this study was UCP-1 mRNA expression in brown fat, which would be expected to increase with increased sympathetic activity. We found no effect of either diet or leptin on UCP-1 mRNA expression. This does not, however, exclude the possibility that there is tissue-selective activation of the sympathetic nerves. Differential regulation of sympathetic output to different organs and tissues has been demonstrated by Hausberg et al. (17), who found that baroreceptor activation, caused by an elevation of blood pressure, inhibited leptin-induced activation of renal sympathetic nerves but did not inhibit the leptin-induced increase in sympathetic activity in brown fat.

In this study, we found an unexpected reduction in circulating concentrations of leptin in HF-fed controls compared with LF-fed controls. Because we did not measure leptin mRNA expression, it is not clear whether this was due a decrease in leptin production or an increase in leptin clearance. The decrease was surprising considering that the percent body fat was the same for the LF- and HF-fed mice. Others have reported that HF-fed mice and rats are resistant to leptin (18, 20, 26), and the resistance of obese animals and humans to peripheral leptin has been attributed to a failure to transport leptin across the blood-brain barrier (3, 6). In previous studies (5, 15, 16), we have been unable to prevent loss of body fat in response to physiological doses of leptin administered peripherally in mice fed a diet containing 45% kcal fat. In this study the HF-fed mice were not “resistant” to leptin, as evidenced by the inhibition of food intake and weight gain. In addition, the amount of leptin transported into the brain should have been similar for both the LF- and HF-fed mice as the circulating concentrations of leptin in the two groups of leptin-treated mice were the same, as were the levels of mRNA expression for hypothalamic short-form receptors, which may function as leptin transporters (25). Banks et al. (2) reported that increased concentrations of triglycerides inhibited leptin transport across the blood-brain barrier. In this study, we found that serum triglyceride and FFA concentrations were higher in LF- than HF-fed mice, therefore, it is unlikely that blood lipids selectively inhibited leptin transport in HF-fed mice. We also found that leptin treatment increased hypothalamic expression of ObRb in both LF- and HF-fed rats, an additional indication that dietary fat did not modify the hypothalamic response to leptin treatment. The reason for the increase in leptin receptor expression with leptin treatment is not clear but also has been documented by Peiser et al. (23), who found a 62% increase in hypothalamic ObRb protein in the hypothalamus of HF-fed rats, compared with LF-fed rats, in the absence of any difference in receptor mRNA expression. We did not measure receptor protein levels in this study.

In summary, we found that housing mice in a warm environment did not prevent leptin from specifically reducing body fat in LF-fed mice, but it resulted in an overall inhibition of growth in the HF-fed mice such that the percentage of body fat and percent lean tissue did not change. The reason for the change in leptin action caused by the combination of an increase in dietary fat and environmental temperature is unknown, but it may be related to room temperature inhibiting leptin-induced thermogenesis. The HF-fed mice were not leptin resistant because leptin inhibited food intake and weight gain. Therefore, the mechanisms responsible for a reduction in body fat in LF- and HF-fed mice housed at 23°C must be different, and this study shows that those in the HF-fed mice are more sensitive to modification by environmental conditions than those in the LF-fed mice.

**GRANTS**

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant RO1-DK-53903 (to R. B. S. Harris). A. L. Haltiner was the recipient of a Summer Scholarship from the University of Georgia Center for Undergraduate Research Opportunities.

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