Sexually different actions of leptin in proopiomelanocortin neurons to regulate glucose homeostasis

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Leptin, a circulating adipocyte-derived signal secreted in abundance in adipose tissue, exerts its effects on the regulation of energy balance and glucose homeostasis through activation of the long form of the leptin receptor; energy balance; glucose tolerance; insulin sensitivity; fat distribution

Obesity is associated with adverse metabolic effects, including non-insulin-dependent diabetes mellitus, hyperlipidemia, certain cancers, and cardiovascular disease (16, 21). In addition to the detrimental effects of excess fat, where that fat is located is critical and directly related to health risks of obesity. Specifically, excess fat in the central region of the body carries a greater risk than does fat distributed subcutaneously (7, 19, 42, 50). Men and women differ greatly with respect to where their fat is distributed. On average, women carry more fat subcutaneously whereas men carry more fat viscerally (31, 34, 50). Hence, there are sex-based differences with regard to obesity associated health risks, with males being more likely to develop obesity-related disturbance of glucose homeostasis (17, 32).

Leptin, a circulating adipocyte-derived signal secreted in direct proportion to body fat, and especially to subcutaneous fat, exerts its effects on the regulation of energy balance and glucose homeostasis through activation of the long form of the leptin receptor and subsequent activation of the JAK-STAT3 pathway in specific hypothalamic neurons (3, 9, 23, 49).

Although leptin receptors are widely expressed in the brain (24, 27, 37, 43), previous studies have identified the arcuate nucleus of the hypothalamus (ARC) as a critical site to mediate leptin’s anorectic action (25, 45). The ARC contains two distinct populations of leptin-responsive neurons: proopiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART) neurons and agouti-related peptide (AgRP)/neuropeptide Y (NPY) neurons. Within the ARC, leptin directly inhibits orexigenic AgRP/NPY neurons and activates anorexigenic POMC/CART neurons (15, 22, 44), leading to coordinated control of energy homeostasis (45, 46).

A requirement for normal leptin action is illustrated by the syndrome that results from loss-of-function mutations in genes encoding leptin or the leptin receptor in rodents or humans (8, 10, 13, 26, 38). For example, rodents that lack functional leptin receptors are obese, hyperphagic, hypoactive, hyperglycemic, and hyperinsulinemic, revealing leptin’s role in regulating body fat, energy balance, and glucose homeostasis (8, 10, 33). Studies utilizing specific loss-of-function and return-of-function approaches have yielded important information on the functional significance of specific populations of leptin receptors. For example, mice lacking leptin receptors selectively in POMC neurons display hyperleptinemia and modest obesity (1), implying that leptin receptor-expressing POMC neurons are required for normal body weight homeostasis. Furthermore, ARC-selective restoration of leptin receptors in mice with a leptin receptor-null background causes a modest decrease in body weight but strikingly improved glucose homeostasis and normalized locomotor activity (14), implying that, in addition to their profound effects on food intake and body weight, leptin receptors in the ARC are important for glucose homeostasis and locomotor activity.

Leptin is secreted mainly from subcutaneous adipose tissue, and the level of circulating leptin correlates better with subcutaneous than with visceral fat (20, 35). Consistent with differential fat distribution, plasma leptin is higher in women than in men with the same body mass index (40). An important implication is that leptin signaling within the brain may differ in males and females, with females relying on leptin signaling to a greater extent than males due to differential fat distribution and leptin signaling. Consistent with this, female rats are more sensitive to the central anorectic effects of leptin than their male counterparts (11, 12).

In the present experiments, we asked whether energy and glucose homeostasis, as well as body fat distribution, are...
impacted to a greater extent in female than in male mice when leptin signaling in POMC neurons is uniquely disturbed. We used a Cre/loxP mouse model with targeted disruption of the leptin receptor in POMC neurons as described by Balthasar et al. (1). Lack of leptin receptors in POMC neurons of these Pomc-Cre,Lepr<sup>flox/flox</sup> mice results in increased accumulation of total body fat in both males and females relative to their Lepr<sup>flox/flox</sup> counterparts (1). In the present experiments we evaluated energy balance, body fat distribution, and glucose homeostasis in male and female Pomc-Cre,Lepr<sup>flox/flox</sup> mice.

METHODS

Animals

Pomc-Cre,Lepr<sup>flox/flox</sup> mice were obtained by crossing Pomc-Cre mice (provided by Drs. Bradford B. Lowell and Joel K. Elmquist, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA) with Lepr<sup>flox/flox</sup> mice (provided by Dr. Streamson C. Chua, Jr., Albert Einstein University, New York, NY). All mice were maintained and bred in our colony at the University of Cincinnati. After weaning at 3 wk of age, mice were housed individually in microisolator cages in pathogen-free, temperature- and humidity-controlled rooms with a 12:12-h light-dark cycle with lights on at 0500 and off at 1700. RT-PCR analysis was used to genotype the mice.

Two cohorts of mice were used in the current study. Mice from the first cohort were maintained on pelleted chow (Harlan Teklad, Madison, WI; rodent diet 8604, 12.8% kcal% fat, 31.6% kcal% protein, 55.6% kcal% carbohydrates) until 16 wk of age, when they were changed to a high-fat butter oil-based diet (HFD; Research Diets, New Brunswick, NJ; D12451, 45% kcal% fat, 20% kcal% protein, 35% kcal% carbohydrates). Mice from the second cohort were maintained on pelleted chow throughout. All mice had ad libitum access to water. All animal procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Experimental Design

Experiment 1: energy balance of chow-fed mice. While on the chow diet, male and female Lepr<sup>flox/flox</sup> and Pomc-Cre,Lepr<sup>flox/flox</sup> mice from the first cohort were used to assess locomotor activity at 12 wk of age and energy expenditure using indirect calorimetry at 14 wk of age.

Experiment 2: body fat distribution of chow- and HFD-fed mice. Fat distribution was determined in the mice from the first cohort after they had been on the HFD for over 8 wk with age-matched, chow-fed mice in the second cohort.

Experiment 3: glucose homeostasis of chow- and HFD-fed mice. Intraperitoneal glucose tolerance tests (ipGTT) were performed at 15 wk of age and insulin tolerance tests (ipITT) at 16 wk of age in the mice from the first cohort while being fed chow. These mice were then placed on the HFD, and ipGTTs were conducted at 22 wk of age and ipITTs at 23 wk of age.

Energy Expenditure

Heat production, oxygen consumption ($\dot{V}O_2$), and locomotor activity were measured in males and in females at diestrus because the females in these experiments had 6-day mean estrous cycles with diestrus lasting over 2 days. We opted to assess mice during diestrus to reduce variance resulting from different phases of the cycles. Estrous cyclicity was monitored by daily vaginal lavage. Vaginal smears were performed between 1000 and 1100 each day in the middle of the light cycle and were stained with a DipQuick staining kit (Jorgensen Laboratories, Loveland, CO) for determination of the phase of the estrous cycle in the females on the basis of the pattern of cell types of smear samples (4).

Heat production and $\dot{V}O_2$ were measured using an Oxymax System (Accuscan Instruments, Columbus, OH). Briefly, mice were placed in individual metabolic chambers at the end of the light cycle and remained in the chambers for 24 h with ad libitum access to water and food. Whole animal heat production and $\dot{V}O_2$ were determined as kilocalories per hour and milligrams per kilogram per hour, respectively, and were subsequently adjusted to kilograms of lean body mass. Locomotor activity was measured using a running wheel appa-

![Fig. 1. Weekly body mass, total body fat, and lean tissue of age-matched, chow- or high-fat diet (HFD)-fed mice. Body mass (A), fat tissue mass (B), and lean tissue mass (C) of mice. ](image-url)
ratus (Lafayette Instrument, Lafayette, IN). Briefly, mice were housed in cages (23.62 cm × 35.3 cm × 19.56 cm) fitted with an anodized aluminum wheel (12.7 cm in diameter) for 24 h. Wheel revolutions were recorded, and cumulative distance was calculated using Animal Wheel Monitor Software (Lafayette Instrument).

Body Composition and Body Fat Distribution

A mouse-specific nuclear magnetic resonance (NMR) Echo MRI whole body composition analyzer (EchoMedical Systems, Houston, TX) was used to assess body fat and lean mass (48) in conscious mice, providing longitudinal data. After the mice were euthanized, the amounts of subcutaneous and visceral fat were assessed separately by dividing the carcass into two portions and using the pelting method (11). In this procedure, the skin with any attached fat and fat on the outer surface of any skeletal muscle was removed from the carcass. This pelt portion and the remaining carcass portion, including all muscle, skeleton, organs, and intramyocellular and internal fat, were then assessed separately using the NMR.

ipGTTs and ipITTs

ipGTTs and ipITTs were performed according to previously established procedures (47). Mice were fasted for 16 h, and all blood samples were obtained from the tip of the tail vein. For the ipGTT, after a baseline blood sample was taken (0 min), 1.5 g/kg body wt (for chow-fed cohort) or 0.75 g/kg body wt (for HFD-fed cohort) of 20% D-glucose (Phoenix Pharmaceutical St. Joseph, MO) was injected intraperitoneally. A lower dose of glucose was administered to the HFD-fed mice to ensure that the resulting glucose levels were in the sensitivity range of the glucometers; i.e., 20–500 mg/dl. For the ipITT, 1 U/kg body wt insulin (for both chow- and HFD-fed cohorts; Novolin, Novo Nordisk, Princeton, NJ) was injected intraperitoneally. Subsequent blood samples were taken at 15, 30, 45, 60, and 120 min after

Table 1. Body masses and fat distribution of chow- or HFD-fed male and female mice

<table>
<thead>
<tr>
<th>Cohort 1: HFD-fed</th>
<th>Male</th>
<th>Female</th>
<th>Cohort 2: Chow fed</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass</td>
<td>29.73 ± 1.24</td>
<td>19.65 ± 0.95</td>
<td>Body mass</td>
<td>27.90 ± 0.87</td>
<td>21.55 ± 0.50</td>
</tr>
<tr>
<td>Total fat</td>
<td>4.61 ± 0.76</td>
<td>1.43 ± 0.38</td>
<td>Total fat</td>
<td>2.49 ± 0.19</td>
<td>0.97 ± 0.28</td>
</tr>
<tr>
<td>Visceral fat</td>
<td>3.12 ± 0.44</td>
<td>0.72 ± 0.23</td>
<td>Visceral fat</td>
<td>2.22 ± 0.06</td>
<td>0.79 ± 0.17</td>
</tr>
<tr>
<td>Subcutaneous fat</td>
<td>1.42 ± 0.26</td>
<td>0.47 ± 0.13</td>
<td>Subcutaneous fat</td>
<td>0.28 ± 0.14</td>
<td>0.18 ± 0.12</td>
</tr>
</tbody>
</table>

Values are mean ± SE in grams; n = 10 from each group. HFD, high-fat diet between 16 and 24 wk old. *Statistically significant difference between genotypes within sex (P < 0.05).

![Fig. 2](https://www.ajpendo.org)
glucose administration and at 15, 30, 45, and 60 min after insulin administration. Glucose was measured on duplicate samples using FreeStyle glucometers and test strips (FreeStyle, Alameda, CA). During the ipGTT, an additional blood sample was taken from the tail vein 13–15 min after the glucose administration for measurement of plasma insulin using the rat insulin enzyme-linked immunosorbent assay kits (Crystal Chem, Downers Grove, IL). The coefficients of variation of intra-assay and interassay were 5.5 and 6.1%, respectively. To evaluate glucose tolerance, calculations of the area under the glucose curves (AUC) were made on the basis of the glucose baseline levels at 0 min, and glucose clearance rates were calculated between the 15-min peak level and the 30-min value following glucose administration. To assess insulin sensitivity, the slope of the glucose decrease between 0 and 60 min was calculated.

**Statistical Analysis**

Data are expressed as means ± SE. Comparisons among multiple groups were made using appropriate one-way or two-way analysis of variance. Post hoc tests of individual groups were made using Tukey’s tests (SigmaStat 3.1, San Rafael, CA). Significance was set at *P* < 0.05. Exact probabilities and test values were omitted for simplicity and clarity of the presentation of the results.

**RESULTS**

Male and female Lepr<sup>flox/flox</sup> or Pomp-Cre, Lepr<sup>flox/flox</sup> mice gained body weight and adiposity after being switched to the HFD, with Pomp-Cre, Lepr<sup>flox/flox</sup> mice accumulating more body fat than Lepr<sup>flox/flox</sup> controls (Fig. 1, A and B). Body weights and total body fat masses were significantly greater in Pomp-Cre, Lepr<sup>flox/flox</sup> mice compared with their same-sex counterparts (Fig. 1 and Table 1), whereas total lean body masses were not different (Fig. 1C).

**Experiment 1: Energy Balance of Chow-Fed Mice**

Consistent with a previous report (1), daily food intake was not changed (data not shown); thus, Pomp-Cre, Lepr<sup>flox/flox</sup> mice have greater weight gain with no alteration in feeding. In contrast to the previous report of energy expenditure at 8–10 wk of age (1), total heat production was greater over a 24-h period in 14-wk-old chow-fed Pomp-Cre, Lepr<sup>flox/flox</sup> males compared with Lepr<sup>flox/flox</sup> control males. This was true for absolute 24-h total heat production (Fig. 2A) as well as after adjustment for lean body mass (Fig. 2B). The opposite was observed in females, with less heat production in Pomp-Cre, Lepr<sup>flox/flox</sup> females than controls (Fig. 2, A and B). VO<sub>2</sub> had a similar trend as heat production, although absolute VO<sub>2</sub>...
of male mice was not different between Pomc-Cre,Lepr<sup>floxflox</sup> and their controls (Fig. 2, C and D).

Control Lepr<sup>floxflox</sup> females ran a significantly longer distance than Pomc-Cre,Lepr<sup>floxflox</sup> and Lepr<sup>flox</sup>flox males (Fig. 3). Lepr<sup>floxflox</sup> females had a tendency to run farther than Pomc-Cre,Lepr<sup>floxflox</sup> females, suggesting a slight decrease of locomotor activity and thus energy conservation of Pomc-Cre,Lepr<sup>floxflox</sup> females (Fig. 3). The difference in the cumulative running distance, however, did not reach statistical significance between genotypes of the same sex.

**Experiment 2: Body Fat Distribution of Chow- and HFD-Fed Mice**

Female Pomc-Cre,Lepr<sup>floxflox</sup> mice accumulated more body fat than Pomc-Cre,Lepr<sup>flox</sup>flox males (Table 1). The difference between males and females was exacerbated on the HFD. After being placed on the HFD, Pomc-Cre,Lepr<sup>floxflox</sup> females accumulated nearly seven times the total body fat of controls, whereas male Pomc-Cre,Lepr<sup>floxflox</sup> mice only doubled their body fat relative to their male controls (Table 1). The difference in total fat mass between female Pomc-Cre,Lepr<sup>floxflox</sup> and Lepr<sup>flox</sup>flox mice was greater than the difference between male Pomc-Cre,Lepr<sup>floxflox</sup> and Lepr<sup>flox</sup>flox mice under the same dietary conditions (Table 1). Specifically, when fed the Chow diet, total fat mass was 2.12 g greater in female Pomc-Cre,Lepr<sup>floxflox</sup> mice than in female Lepr<sup>flox</sup>flox mice, whereas total fat mass was 1.67 g greater in male Pomc-Cre,Lepr<sup>floxflox</sup> mice than in male Lepr<sup>flox</sup>flox controls; when fed the HFD, total fat mass was 8.06 g greater in female Pomc-Cre,Lepr<sup>floxflox</sup> mice than in Lepr<sup>flox</sup>flox females, whereas total fat mass was 6.33 g greater in male Pomc-Cre,Lepr<sup>floxflox</sup> mice than in male Lepr<sup>flox</sup>flox controls.

In addition to the differences in total body fat accumulation between male and female mice, there were also important differences in where that body fat was accumulated. To compare the amount of fat added in the subcutaneous and visceral compartments in male and female mice by disruption of leptin signaling in POMC neurons, we used the following calculations: % increase in visceral fat = (visceral fat of each Pomc-Cre,Lepr<sup>floxflox</sup> mouse − average visceral fat of Lepr<sup>flox</sup>flox mice)/(total body fat of each Pomc-Cre,Lepr<sup>floxflox</sup> mouse − average total body fat of Lepr<sup>flox</sup>flox mice) × 100%; % increase in subcutaneous fat = (subcutaneous fat of each Pomc-Cre,Lepr<sup>floxflox</sup> mouse − average subcutaneous fat of Lepr<sup>flox</sup>flox mice)/(total fat of each Pomc-Cre,Lepr<sup>floxflox</sup> mouse − average total body fat of Lepr<sup>flox</sup>flox mice) × 100%. Using these calculations, female Pomc-Cre,Lepr<sup>floxflox</sup> mice had a greater increase in visceral fat and a smaller increase in subcutaneous fat than Pomc-Cre,Lepr<sup>flox</sup>flox males (Table 2). Male Pomc-Cre,Lepr<sup>floxflox</sup> mice accumulated similar percentages of fat at both visceral and subcutaneous depots, whereas female Pomc-Cre,Lepr<sup>floxflox</sup> mice accumulated a greater amount of their additional body fat in the form of visceral fat when they were fed either Chow or HFD (Table 2). Female Pomc-Cre,Lepr<sup>floxflox</sup> mice accumulated more visceral fat than males on either Chow or HFD, suggesting that the lack of functional leptin signaling in the POMC neurons affected female fat distribution more than that of males by shifting fat accumulation to the visceral compartment.

**Experiment 3: Glucose Homeostasis of Chow- and HFD-Fed Mice**

At 15 wks of age, chow-fed male Pomc-Cre,Lepr<sup>floxflox</sup> mice had higher fasting glucose and higher glucose levels 45 and 60 min following glucose administration than Lepr<sup>flox</sup>flox males (Fig. 4A). In addition, glucose clearance between the 15-min peak level and 30-min post-glucose injection was significantly lower in male Pomc-Cre,Lepr<sup>floxflox</sup> than in male Lepr<sup>flox</sup>flox controls (Table 3), suggesting impaired glucose clearance by Pomc-Cre,Lepr<sup>floxflox</sup> males following a glucose challenge. The 15-min insulin levels during ipGTT were not different between chow-fed male Pomc-Cre,Lepr<sup>floxflox</sup> and Lepr<sup>floxflox</sup> mice (male Lepr<sup>flox</sup>flox, 0.65 ± 0.11, male Pomc-Cre,Lepr<sup>floxflox</sup>, 1.01 ± 0.22; Fig. 4A). Chow-fed females had comparable fasting glucose levels and comparable glucose levels at all time points, and their 15- to 30-min glucose clearance constants were also comparable (Fig. 4B and Table 3). However, the 15-min insulin level during ipGTT of female Pomc-Cre,Lepr<sup>floxflox</sup> mice was significantly higher than that of female Lepr<sup>flox</sup>flox mice (female Lepr<sup>flox</sup>flox, 0.83 ± 0.17, female Pomc-Cre,Lepr<sup>floxflox</sup>, 1.36 ± 0.21; Fig. 4B), implying that the female Pomc-Cre,Lepr<sup>floxflox</sup> mice increased their insulin secretion to maintain normal glucose tolerance.

In the ipiTTs, although glucose disappearance rates following insulin administrations were similar in males between 0 and 60 min (male Lepr<sup>flox</sup>flox, 2.07 ± 0.17, male Pomc-Cre,Lepr<sup>floxflox</sup>, 1.56 ± 0.28; Fig. 5A), chow-fed male Pomc-Cre,Lepr<sup>floxflox</sup> mice had higher fasting glucose levels and higher glucose levels at all time points after insulin administration (Fig. 5A), suggesting that although hyperglycemia occurred, the animals were comparably insulin sensitive to chow-fed Lepr<sup>flox</sup>flox males. Chow-fed female Pomc-Cre,Lepr<sup>floxflox</sup> mice, on the other hand, had comparable glucose levels at all

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**Table 3. Baseline glucose, glucose disappearance rates, and glucose AUCs of chow- or HFD-fed male or female Lepr<sup>flox</sup>flox or Pomc-Cre,Lepr<sup>floxflox</sup> mice during ipGTT**

<table>
<thead>
<tr>
<th></th>
<th>Baseline Glucose, mg/dl</th>
<th>15- to 30-min Glucose Rd, mg/dl</th>
<th>0- to 120-min AUC, mg·dl&lt;sup&gt;−1&lt;/sup&gt;·min</th>
<th>Baseline Glucose, mg/dl</th>
<th>15- to 30-min Glucose Rd, mg/dl</th>
<th>0- to 120-min AUC, mg·dl&lt;sup&gt;−1&lt;/sup&gt;·min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chow fed</strong></td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>128.3 ± 6.2</td>
<td>1.84 ± 0.61</td>
<td>8,729.25 ± 1,385.01</td>
<td>153.3 ± 10.2†</td>
<td>0.65 ± 0.35†</td>
<td>1,190.25 ± 2,200.66</td>
</tr>
<tr>
<td>Female</td>
<td>102.3 ± 5.4*</td>
<td>2.87 ± 0.47</td>
<td>5,691.00 ± 782.51</td>
<td>95.8 ± 6.4*</td>
<td>2.56 ± 0.57*</td>
<td>6,438.00 ± 886.61*</td>
</tr>
<tr>
<td><strong>HFD fed</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Male</td>
<td>145.0 ± 7.3</td>
<td>0.81 ± 0.27</td>
<td>11,313.75 ± 2,119.84</td>
<td>140.8 ± 6.2</td>
<td>0.12 ± 0.21</td>
<td>15,020.83 ± 2,194.01</td>
</tr>
<tr>
<td>Female</td>
<td>95.8 ± 5.5*</td>
<td>2.07 ± 0.34*</td>
<td>3,878.75 ± 1,008.17*</td>
<td>109.7 ± 8.5*</td>
<td>2.89 ± 0.33*</td>
<td>6,081.38 ± 1,297.75*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 from each group. AUC, area under the curve; ipGTT, intraperitoneal glucose tolerance test; Rd, disappearance rate.
†Statistically significant difference between genotypes within sex (P < 0.05). *Statistically significant difference between sexes within genotype (P < 0.05).
time points following insulin injections (Fig. 5) and similar glucose disappearance rates as their female Lepr flox/flox counterparts (female Lepr flox/flox: 1.41 ± 0.38, female POMC-Cre, Lepr flox/flox: 1.80 ± 0.20; Fig. 5B).

After the mice consumed the HFD, glucose levels across ipGTT were comparable between male Lepr flox/flox and POMC-Cre,Lepr flox/flox mice (Fig. 6A). In addition, their average 15- to 30-min glucose clearance constants were not significantly different (Table 3). Although glucose excursion curves were similar between male Lepr flox/flox and POMC-Cre,Lepr flox/flox mice, the 15-min insulin level during ipGTTs of male POMC-Cre,Lepr flox/flox mice was significantly higher than that of Lepr flox/flox males (male Lepr flox/flox: 1.62 ± 0.22, male POMC-Cre,Lepr flox/flox: 3.03 ± 0.31; Fig. 6A), suggesting that HFD-fed POMC-Cre,Lepr flox/flox males secreted more insulin to achieve similar glucose levels than Lepr flox/flox males; thus POMC-Cre,Lepr flox/flox males were relatively insulin insensitive compared with male Lepr flox/flox controls. This was confirmed by ipITT, which revealed significantly higher glucose levels at 30, 45, and 60 min following insulin injections in POMC-Cre,Lepr flox/flox males than in Lepr flox/flox males (Fig. 7A). ipITT also indicated significantly lower glucose disappearance rates for male POMC-Cre,Lepr flox/flox mice than for Lepr flox/flox males (male Lepr flox/flox: 1.52 ± 0.27, male POMC-Cre,Lepr flox/flox: 0.54 ± 0.20; Fig. 7A), implying a relative insulin resistance in POMC-Cre,Lepr flox/flox males compared with their Lepr flox/flox male counterparts in the context of the HFD.

Similarly to the chow-fed female POMC-Cre,Lepr flox/flox mice, HFD-fed female POMC-Cre,Lepr flox/flox also had a significantly higher level of 15-min insulin levels during ipGTT than Lepr flox/flox females (female Lepr flox/flox: 0.79 ± 0.11, female POMC-Cre,Lepr flox/flox: 2.17 ± 0.18; Fig. 6B), suggesting insulin insensitivity of POMC-Cre,Lepr flox/flox females compared with female Lepr flox/flox controls. However,
A wealth of data has demonstrated that POMC neurons are important for regulation of glucose homeostasis by responding to signals, including leptin. Leptin stimulates melanocortin POMC neurons within the ARC by acting through its specific receptors (41) and regulates energy homeostasis through diverse effects on food intake and metabolism (45, 46), a model implicating leptin signaling in the ARC POMC neurons as a general integrator of glucose and energy homeostasis. Dysregulation of this leptin-POMC regulatory system, via either the ability of POMC neurons to sense and integrate coordinated responses to changing levels of leptin or defective leptin signaling, causes pathophysiology of energy balance and thus predisposes to obesity, diabetes, and other components of metabolic syndrome.

Mutations of the leptin receptor in mice (db/db) or in rats (fa/fa) cause disruption in energy balance, progressive insulin resistance, hyperinsulinemia, and hyperglycemia (10, 30). Studies using db/db mice or fa/fa rats, however, give no insight into the role played by specific populations of leptin receptors. Given that leptin receptors are found in POMC neurons where they regulate both gene expression and neuronal firing, ablation of leptin receptors specifically in POMC neurons may affect energy balance and glucose homeostasis. The present study focused on male and female Pomc-Lepr flox/flox mice that have a targeted disruption of leptin action selectively in POMC neurons and compared them with Lepr flox/flox mice, in which leptin receptor signaling should be unaffected. This allows for the determination of the physiological role of leptin signaling in the POMC neurons.

The current data point to important sex differences in the role of leptin action in POMC neurons on energy balance and glucose homeostasis. Lack of leptin receptors in POMC neurons led to conservation of energy expenditure in females but not in males. Chow-fed Pomc-Lepr flox/flox females accumulated greater adiposity by decreasing heat production and VO_2 than control females. Pomc-Lepr flox/flox males, on the other hand, had greater energy expenditure than male Lepr flox/flox mice when heat production and VO_2 were measured using indirect calorimetry at 14 wk of age, a time when adiposity and body weights were relatively stable and the mice were not continuously growing. Energy expenditure is proportional to body weight; thus the greater energy expenditure of Pomc-Lepr flox/flox males could be a possible outcome of greater adiposity and body weights of male Pomc-Lepr flox/flox mice than Lepr flox/flox males. Consistent with this sex difference in energy expenditure, female Pomc-Lepr flox/flox mice accumulated more total and visceral adiposity than males. Lack of leptin receptors in POMC neurons resulted in glucose intolerance and insulin resistance in males. Chow- or HFD-fed Pomc-Lepr flox/flox females, however, had glucose tolerance curves no different from their controls during ipGTT. Consistent with this important sex difference, female Pomc-Lepr flox/flox mice on the HFD had normal glucose disappearance after administration of peripheral insulin, whereas male Pomc-Lepr flox/flox on the HFD had an impaired glucose response to insulin.

The outcome is surprising in two ways. First, it might be expected that leptin would exert a more powerful effect on glucose homeostasis in females, since leptin more potently reduces food intake after central administration in female compared with male rats (11, 12). Second, the effect of disrupting leptin signaling in the POMC neurons had different effects on body fat distribution. Consistent with a previous report (1), both male and female Pomc-Lepr flox/flox mice had higher body fat content than their appropriate controls on either chow or HFD. However, female Pomc-Lepr flox/flox
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mice accumulated a larger percentage of visceral fat and a smaller percentage of subcutaneous fat than Pomc-Cre, Lepr flox/flox males when fed either chow or HFD. The higher amount of visceral fat might have been expected to produce both glucose intolerance and relative insulin resistance in the female Pomc-Cre, Lepr flox/flox mice. Despite this body fat distribution, it is clear that neither glucose nor insulin tolerance were impaired in the female Pomc-Cre, Lepr flox/flox mice.

The female steroid hormone estrogen may contribute to the sex differences in the action of leptin in POMC neurons. First, estrogen influences synaptic plasticity by changing the axosomatic synapses in the ARC (39), increases the number of excitatory inputs to POMC neurons, and enhances POMC tone in the ARC (28). In addition, enhanced POMC tone in the ARC is a leptin-independent effect that also occurs in leptin-deficient (ob/ob) and leptin receptor-deficient (db/db) mice (28). Thus, estrogen modulates POMC neuronal activity in the ARC. Female Pomc-Cre, Lepr flox/flox and Lepr flox/flox mice have similar circulating levels of estrogen (H. Shi and R. J. Seeley, unpublished finding), which may influence glucose tolerance of Pomc-Cre, Lepr flox/flox females independently of dysfunctional leptin signaling in the POMC neurons.

Second, estrogen modulates leptin signaling and leptin receptor expression. This has been tested in intact and ovariec-tomized female rats. In intact female rats, estrogen treatment downregulates the leptin receptors in the hypothalamus (6). Estrogen levels also regulate the expression of the leptin receptors during the estrus cycle. Specifically, leptin receptor expression is lowest in proestrus, when estrogen level is the highest, and leptin receptor expression in the ARC is highest during metestrous, when estrogen level declines (6). Because circulating leptin levels do not change during the estrous cycle, estrogen regulates leptin receptor expression independently of leptin levels. Ovariectomy, which leads to estrogen insufficiency, or treatment with estradiol or raloxifene (a selective estrogen receptor modulator), which leads to increased estrogen level or enhanced estrogen action, modulates leptin receptor expression in the hypothalamus. Specifically, expression of leptin receptor in the ARC is increased in ovariec-tomized rats (5, 36), and these effects were reversed by either estradiol or raloxifene treatment (5, 36), suggesting that central leptin receptor expression is modified by estrogen’s action. The potential sites that would allow for the regulation of estrogen by leptin receptor are unknown but could include nuclei that coexpress the estrogen and leptin receptors, such as the ARC, as well as extra-ARC regions, including the medial preoptic area, the parvicellular paraventricular nucleus, and the ventromedial hypothalamic nucleus (18). Certainly the ARC is not the only CNS regulator of glucose homeostasis. Consequently, Pomc-Cre, Lepr flox/flox females with similar circulating estrogen levels as Lepr flox/flox females may have compensated leptin signaling on non-POMC neurons that work to maintain relatively normal glucose tolerance.

It is interesting to note that chow- and HFD-fed female Pomc-Cre, Lepr flox/flox mice displayed significantly elevated levels of insulin during an ipGTT compared with their controls. Although the female Pomc-Cre, Lepr flox/flox mice and controls had identical glucose tolerance curves, the Pomc-Cre, Lepr flox/flox females required greater insulin secretion to lower plasma glucose, suggestive of insulin resistance. The increase in insulin in the female Pomc-Cre, Lepr flox/flox mice compared with the controls is consistent with the disproportionate increase in visceral adipose tissue, as circulating insulin is closely associated with visceral adiposity. These data are consistent with a role for leptin action in POMC neurons in the regulation of glucose homeostasis and body weight gain. Opposite to these findings, but conceptually consistent with our current data, is the inhibitory role of the suppressor of cytokine signaling-3 (SOCS-3) in POMC neurons within the ARC on glucose regulation and body weight (29). Mice lacking SOCS-3 within POMC neurons in the ARC are functionally more sensitive to leptin’s actions since the endogenous suppressor is not present. These mice show enhanced glucose tolerance on both chow and HFD compared with their wild-type or SOCS-3-intact controls. Interestingly, these mice also showed resistance to the obesogenic effects of the HFD that was attributed to increased energy expenditure. Food intake in mice with SOCS-3 deficiency in POMC neurons was identical to that of wild-type controls (29).

A major theme of contemporary research is to use genetic mouse models to assess the role of specific populations of leptin receptors to regulate energy balance and glucose homeostasis. The present results indicate that leptin receptors in POMC neurons influence energy balance and glucose homeostasis. In females, leptin receptors in POMC neurons have a greater influence over energy balance and fat distribution than glucose homeostasis, whereas in males leptin action in the POMC neurons has a greater influence on glucose homeostasis than on fat distribution. Such results contribute to a growing literature indicating an important sexual difference involved in the circuitry that regulates crucial metabolic processes. Current outcomes highlight the possibility that treatment strategies for metabolic disorders may need to be very different for males and females, and the crucial need for detailed comparisons between males and females in metabolic research.

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REFERENCES


