Increased weight gain after ovariectomy is not a consequence of leptin resistance

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Chen, Yanyun, and Mark L. Heiman. Increased weight gain after ovariectomy is not a consequence of leptin resistance. Am J Physiol Endocrinol Metab 280: E315–E322, 2001.—The positive correlation between leptin and body fat mass has caused some investigators to speculate that leptin resistance contributes to obesity. Loss of ovarian function in human and rat is associated with increased fat mass gain and increased circulating leptin levels. To study whether ovariectomy produces leptin resistance, Sprague-Dawley female rats were ovariectomized or sham operated and injected with leptin for 35 days. Ovariectomy (OVX) produced hyperphagia and increased gain in both lean and fat mass.Daily leptin injections initially decreased food intake significantly, but feeding gradually increased to a stable level by day 16 and remained at that level for the duration of study. Body composition analysis indicated that chronic injection of leptin to OVX rats dramatically decreased (P < 0.05) fat mass [30 ± 2 (SE) g, vehicle, to 3 ± 1 g, leptin]. Using indirect calorimetry, we observed that OVX did not change energy expenditure or total level of fuel utilization. Leptin administration increased fat utilization and prevented reduction in calorie expenditure that is typically associated with food restriction. Leptin treatment to OVX rats decreased plasma triglyceride, free fatty acid, and insulin concentrations, whereas glucose concentration was normal. Withdrawal of leptin triggered hyperphagia, indicating that leptin biology remained throughout the duration of the chronic treatment. The same dose of leptin produced qualitatively similar data in sham-operated rats. Thus we concluded that the loss of ovarian function in rats is not associated with a change in leptin sensitivity.

Key words: body composition; indirect calorimetry; energy expenditure; leptin sensitivity; body composition

LEPTIN IS A HORMONE synthesized and secreted predominantly by adipose tissue, but expression of leptin is also found in other tissues, including placenta, liver, and gastrointestinal tract (17). One of leptin’s functions is to regulate food intake and energy homeostasis. Mutation of the gene leads to obesity and diabetes (17). Administration of recombinant leptin to leptin-deficient [Lepob/Lepob (ob/ob)] mice reduces food intake, body mass, and fat mass as well as normalizing blood glucose and insulin concentrations (2, 4, 6, 19, 33, 41). Leptin also decreases food intake and body mass in lean rodents (7, 9, 18). Circulating leptin concentrations are lower in lean rodents than in obese rodents (22). Plasma leptin concentrations decrease with fasting (5), and leptin administration to fasted mice reverses endocrine adjustments to food restriction (1). It has been suggested that leptin provides signals to the hypothalamus, informing systems regulated by the available fuel supply that sufficient energy stores exist (16). If leptin levels are increased by exogenous administration, a message of caloric abundance is provided.

Leptin exerts its function by binding to leptin receptors, but obesity due to leptin receptor mutations is extremely rare (8, 10, 12). Leptin resistance in Leprdb/ Leprdb (db/db) mice and Leprob/Leprob Zucker rats is caused by mutations of the long form leptin receptor (8, 10, 23). In contrast, most obese animals and humans have no mutated leptin receptor and present with elevated leptin concentrations that are positively correlated to body fat mass and body mass index, suggesting leptin resistance (5). Diet-induced obese mice and age-related obese CD-1 mice have intact leptin receptors but reduced sensitivity to peripherally administered leptin (18, 34). New Zealand obese and agouti mice also have a normal leptin receptor but show no response to peripherally administered leptin (18). Thus leptin resistance is a state in which the species demonstrates a reduced response to leptin and is typically associated with compensatory elevated leptin levels.

Menopause is associated with increased body mass and body fat mass as well as a changed body fat distribution in women (3, 26). Withdrawal of ovarian hormones by ovariectomy in rodents increases daily food intake as well as increasing body mass and body fat mass and can be reversed by treatment with estradiol (27). Like most obese phenotypes just described, which are characterized by increased circulating leptin levels, loss of ovarian function in humans and rats is also associated with increased circulating leptin levels. To test the hypothesis that increased leptin levels that result in fat mass gain are a consequence of leptin resistance, we challenged ovariectomized Sprague-Dawley rats with leptin to explore leptin sensitivity after loss of ovarian function. We observed that these
castrated rats were hyperphagic without accompanying changes in fuel utilization. The intact rats and castrated rats had similar sensitivity to leptin; thus fat gain observed after ovariectomy is not a consequence of leptin resistance.

METHODS

Animals and treatment. Six-month-old virgin Sprague-Dawley female rats, ranging in mass from 250 to 270 g, were purchased from Harlan Sprague Dawley (Indianapolis, IN) and maintained on a 12:12-h light-dark cycle, with lights on at 0600. The animals were housed individually throughout the study at 24°C ambient temperature. All rats received standard rat chow (Purina 5001 chow; Ralston-Purina, St. Louis, MO) and had free access to water. Rats were randomized to four groups (n = 18/group) that had similar mean body mass. Animals were bilaterally ovarioctomized or sham operated under isoflurane anesthesia. Rats were injected with vehicle (5% dextrose in H2O) for 4 days to acclimate them to the procedures while they were recovering from surgery. Vehicle or human leptin (synthesized at Eli Lilly) was subcutaneously injected twice daily (at 0800 and 1700) at a dose of 250 μg (total daily dose of 500 μg) for 35 days. Body and food weights were measured daily at 0800. Pair-fed animals were provided with the same amount of food as voluntarily ingested the previous day by leptin-treated animals at 1700. Animals were killed by decapitation on days 7, 35, or 37. All animal use in this study was conducted in compliance with approved institutional animal care and use protocols according to NIH guidelines (NIH Publication No. 86–23, 1985).

Body composition analysis. Carcasses were frozen in dry ice and cut into 1-in. square pieces with a small band saw. The samples were then soaked in liquid nitrogen and homogenized in a Willy mill. Lipid was determined from a 2-g aliquot by Soxhlet extraction (21). The nonvolatile matter was defined as ash after a 2-g aliquot was ignited at 550°C in an electric furnace. The remaining portion represents lean body mass.

Indirect calorimetry. Twenty-four-hour energy expenditure (EE) and respiratory quotient (RQ) were measured by indirect calorimetry on days 5, 12, 19, and 26 after treatment and on the 1st day after treatment (day 36) by use of an open circuit calorimetry system (Oxymax; Columbus Instruments International, Columbus, OH). The instrument was calibrated before each experiment with standard gas mixtures containing known concentrations of CO2, N2, and O2. After the first daily injection, rats were placed in calorimeter chambers containing food and water in a room maintained under conditions identical to those just described throughout the treatment period. Gas sampled from each of 16 chambers was first dried by a condenser. The volume of oxygen consumed (V02) and carbon dioxide produced (VCO2) in an hour was measured using a paramagnetic oxygen sensor and a spectrophotometric CO2 sensor. Such measurements were obtained hourly for 23 h to allow the rats to acclimate to the chambers during the first h. RQ is the ratio of VCO2 to V02. EE was calculated as the product of the calorific value (CV) of O2 and V02 per kilogram of body mass, where CV is 3.815 + 1.232 × RQ (14). The value of total calories expended was adjusted to 24 h for the determination of daily fuel utilization. We used formulas and constants derived by Elia and Livesey (14) to calculate the proportion of protein, fat, and carbohydrate that is utilized during that 24-h period. Daily caloric intake was calculated as (mass of daily food intake in g) × (physiological fuel value of the diet in kiloJoules/g).

Circulating indicators of metabolism. Rats were decapitated at 0900, and trunk blood was collected in both heparinized and EDTA tubes on ice and centrifuged at 2,000 rpm to obtain plasma, which was stored at −20°C until assay. Blood glucose concentration was determined using the glucose oxidase method (Sensor Devices, Waukesha, WI). Free fatty acid (FFA) and triglyceride (TG) concentrations were measured using the Monarch 2000 Multianalyzer by acyl-CoA synthetase and lipoprotein lipase methods, respectively (Instrumentation Laboratories, Lexington, MA). Insulin and leptin were measured by radioimmunoassay (Coat-a-Count method; Diagnostic Products, Los Angeles, CA, and Linco Research, St. Charles, MO, respectively).

Statistics. All data are expressed as means ± SE. Statistical analyses were performed by repeated-measures ANOVA with post hoc testing using Tukey’s t-test for multiple comparisons.

RESULTS

Caloric intake and body mass gain. Ovariectomy increased caloric intake 24% in the first 18 days of study, and then intake was reduced and maintained at 15% above sham control levels throughout the study (Fig. 1A). Ovariectomy increased body mass during the first 24 days after surgery (first 20 days of the study), and OVX animals weighed 50 g more than their sham counterparts by the end of the study (Fig. 1B).

Sensitivity to acute leptin administration. Plasma rat leptin concentration was significantly increased in OVX rats on day 14 (Fig. 2A). To investigate possible leptin resistance in ovariectomy-associated hyperphagia and body mass gain, the dose response of leptin was evaluated in sham- and OVX-operated rats 10 days after surgery. Injection of leptin at doses of 250 and 500 μg/day reduced food intake 25% in OVX rats. Only the 500 μg/day dose reduced caloric intake by 25% of that of the sham animals. Injection of 62.5 μg/day had no effect in sham animals and reduced food intake 18% in OVX rats (Fig. 2B).

Sensitivity to chronic leptin treatment. To study the physiology of chronic leptin treatment, OVX rats were injected with leptin (500 μg/day) subcutaneously for 35 days. Caloric intake of OVX rats was reduced by 22% during the first 24 h of leptin treatment (Fig. 3A). This reduced food consumption reached a nadir on the 4th day that was 40% less than that consumed by the vehicle-treated OVX control group. Food intake then gradually increased to a stable level by day 16 that was 10–15% less than that of the OVX control group. Withdrawal of leptin administration was associated with a rapid rebound in caloric intake (Fig. 3A). OVX rats lost 4 g body mass during the first 5 days of leptin treatment, followed by a slower rate of mass gain than that of vehicle-treated OVX rats (Fig. 3B). Mass gain reached a plateau on the 17th day of study and remained there for the duration of treatment. Leptin-treated rats gained ~20 g during 35 days of treatment compared with ~70 g for OVX control rats. Pair-fed animals, which were ovariectomized and injected with vehicle but were restricted to eating the same amount of food that the leptin-treated animals ate, weighed the same as the leptin-treated rats during the first 10 days.
and then gained mass at a rate similar to the ad libitum-fed OVX control group. They gained ~40 g of mass during the entire treatment period (Fig. 3B).

Body composition. Castrated animals gained significantly more lean mass (27 g) than sham rats by the first 7 days and did not accrue fat mass. During the next 28 days, they gained significantly more fat (13 g) and lean mass (53 g) than did the sham-operated rats (Fig. 4). Pair-fed OVX rats tended to lose fat by day 7 and did not gain lean mass. As food consumption of that group increased during the final 28 days, both fat and lean mass tended to increase but did not reach the level of OVX ad libitum-fed rats. Leptin administration during the first 7 days of study specifically reduced body fat mass and retarded the gain in lean mass that was observed after OVX. Such specific attrition of fat mass was more evident at the end of the treatment period, when lean mass was significantly greater than that of sham-operated controls but significantly less than that of OVX vehicle-treated controls. After 35 days of leptin administration, lipid content was reduced from ~9% of total carcass to ~1% (Fig. 4A).

Moreover, complete depletion of visible peritoneal adipose tissue in leptin-treated animals was observed at postmortem examination (data not shown). Although lipid content in pair-fed OVX rats did not reach the level of OVX ad libitum-fed rats by the end of study, gain of abdominal fat was more obvious in these animals (data not shown).

Energy metabolism. Indirect calorimetry was performed for 24 h on days 5, 12, 19, and 26 as well as 1 day after treatment withdrawal (day 36) to study energy balance and fuel utilization. There was no difference in daily fuel utilization between vehicle-treated sham and vehicle-treated OVX animals in the first half of study (Fig. 5, days 5 and 12). However, starting day 19, when food intake reached a stable level, OVX rats tended to expend less energy and utilized fat fuel less than did sham controls.

Total fuel utilization (EE) of the leptin-treated group was significantly higher than that calculated for the vehicle-treated pair-fed OVX group throughout the treatment (Fig. 5). Although the leptin-treated rats consumed significantly fewer calories (Fig. 3A) than...
the ad libitum-fed group throughout the treatment period, total EE was never reduced from the level of ad libitum-fed rats as a compensatory adjustment. To pay for this energy debt, the leptin-treated rats significantly increased utilization of the calorically dense fat tissue. This increased lipid utilization was observed only on day 5 of the pair-fed group and was best observed during the 24-h monitoring of RQ. A diurnal variation in RQ, with RQ rising during the dark cycle, when the animals were more active and eating, was observed (Fig. 6). This was most dramatic for the pair-fed OVX rats during the period with the greatest level of food restriction (days 1–5). These rats received their daily aliquot of food at the time of treatment and immediately began to eat, as evidenced by the sharp increase in RQ (Fig. 6). Because the amount of food offered did not rescue the rats from a negative energy balance, RQ returned to lower levels as soon as the food was consumed, whereas the leptin-treated and ad libitum-fed rats continued to eat throughout the dark photoperiod. On day 5, when food intake was at the nadir, leptin administration maintained an RQ that was less than that of OVX ad libitum-fed rats throughout the day (Fig. 6). During the remainder of the
treatment period, the pair-fed group appeared to meet its reduced energy intake by decreasing total energy utilization. Calorimetric measurements on days 12 and 19, when food intake was returning to the control level, confirmed that leptin promotes fat utilization and prevents the decrease of total daily fuel utilization associated with food restriction. Withdrawal of leptin treatment was associated with a rebound increase in RQ and a shift in fuel utilization toward carbohydrate (Fig. 5 and Table 1), not only sparing fat oxidation but promoting lipid synthesis.

Circulating indicators of metabolism. Plasma TG and FFA concentrations were similar in vehicle-treated sham and OVX animals on days 7 and 35 (Table 2). TG was reduced 30% after 7 days when food intake was decreased (Fig. 3A) and lipid utilization was increased (Fig. 5 and Table 1) in leptin-treated and pair-fed groups. Only leptin-treated animals had significantly lower TG after 35 days of treatment. Both leptin treatment and food restriction increased plasma FFA concentrations about twofold after 7 days of treatment. Leptin treatment, but not food restriction, decreased FFA concentrations at the end of the experiment (Table 2). Plasma insulin concentration increased 30% in OVX- compared with sham-operated rats when caloric intake was greatest on the 7th day of study. This was not seen in either leptin-treated OVX rats or pair-fed OVX rats. Plasma insulin was significantly ($P < 0.05$) reduced after 35 days of leptin injection, whereas blood glucose levels remained unchanged in all groups at both time points (Table 2). Plasma rat leptin concentration was significantly increased and plateaued in OVX rats on day 14 (Fig. 2A). Human leptin administration as well as food restriction significantly decreased endogenous plasma leptin concentration on days 7 and 14. Withdrawal of leptin treatment was associated with an increase in circulating leptin (Fig. 7). One, four, and eight hours after injection, plasma human leptin concentrations were 48.9 ± 1.0, 6.0 ± 0.8, and 0.8 ± 0.1 ng/ml, respectively. Circulating human leptin was undetectable after 8 h.

**DISCUSSION**

Our results are consistent with the phenomenon that OVX in rats increases food consumption and body mass (27). It is well known that ovarian hormones play a role in the regulation of food intake and body mass. It has been noted that, during the estrus cycle, food intake tends to be lowest around the time of ovulation when estrogen is highest, and highest at the diestrus period when estrogen is lowest (42). Withdrawal of ovarian hormones in rats increases food intake and decreases motor activity (30, 36). It has been shown that neither the sympathetic nervous activity (measured as rate of norepinephrine turnover) in brown adipose tissue (BAT) nor the thermogenic activity (measured by mitochondrial GDP binding) of this tissue was suppressed after OVX (27). Data presented in this study are the first to demonstrate that the loss of ovarian hormones did not affect resting EE or fuel utilization. Thus the rapid mass gain in the first 17 days after withdrawal of ovarian hormones appears to be the result of increased caloric intake. Increased fat mass gain in the next 3 wk may result from slightly decreased EE in OVX animals (Fig. 5, days 19, 26, and 36) because of the loss of estradiol. There is evidence to suggest that estradiol can decrease adiposity by the rate of uptake and storage of circulating TGs in adipose tissue and that estradiol can increase resting oxygen consumption in OVX rats (43). Furthermore, our data indicate that the hyperphagia after OVX was not a consequence of leptin resistance.

Leptin administration is well documented to decrease food consumption, reduce body mass, and increase EE in leptin-deficient (Lep<sup>ob/ob</sup>/Lep<sup>ob/ob</sup>) mice (4, 19, 33). We previously reported that leptin administration to lean female Sprague-Dawley rats suppresses daily food intake transiently and promotes lipid oxidation until fat mass is greatly reduced (9). In the current study, ovari-intact and OVX rats had similar sensitivity to leptin. Leptin-reduced food intake and body fat in

![Graph](https://example.com/graph.png)

Fig. 6. Respiratory quotient (RQ) during day 10 of treatment. Leptin administration results in a sustained decreased RQ. Rx, time of injection and presentation of food to pair-fed rats. Values are means ± SE; $n = 5$.

**Table 1. Respiratory quotient on days 5, 12, 19, 26, and 36**

<table>
<thead>
<tr>
<th></th>
<th>Day 5</th>
<th>Day 12</th>
<th>Day 19</th>
<th>Day 26</th>
<th>Day 36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham vehicle</td>
<td>0.90 ± 0.01</td>
<td>0.90 ± 0.01</td>
<td>0.89 ± 0.01</td>
<td>0.87 ± 0.01</td>
<td>0.90 ± 0.01</td>
</tr>
<tr>
<td>OVX vehicle</td>
<td>0.89 ± 0.01</td>
<td>0.90 ± 0.01</td>
<td>0.90 ± 0.00</td>
<td>0.90 ± 0.01</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td>OVX leptin</td>
<td>0.82 ± 0.01*</td>
<td>0.87 ± 0.01*†</td>
<td>0.88 ± 0.01*</td>
<td>0.89 ± 0.01</td>
<td>0.97 ± 0.01*</td>
</tr>
<tr>
<td>OVX pair-fed</td>
<td>0.53 ± 0.00*</td>
<td>0.93 ± 0.00</td>
<td>0.92 ± 0.00</td>
<td>0.88 ± 0.00</td>
<td>0.97 ± 0.00*</td>
</tr>
</tbody>
</table>

Values are means ± SE ($n = 5$). OVX, ovariectomy. *$P < 0.05$ vs. OVX vehicle-treated ad libitum-fed group; †$P < 0.05$ vs. OVX vehicle-treated pair-fed group.
Animals overexpressing UCP3 have increased oxygen consumption and decreased circulating TG and FFA concentrations measured on day 35 that were possible consequences of leptin-stimulated lipid oxidation.

Leptin-stimulated lipid oxidation was reflected by increased FFA concentrations on day 7 and decreased circulating TG and FFA concentrations measured on day 35. Daily fuel utilization of the pair-fed group was decreased on day 5 and tended to remain depressed throughout the study (Fig. 5). This is likely a consequence of reduced feeding and a reduction in other physical activities, as well as a possible decrease in basal usage in attempt to conserve energy (24). Such reduction in expended energy was not present in the leptin-treated animals, even when energy intake was at the nadir, suggesting that leptin administration counters such physical adaptations to food restriction (1).

Leptin was reported to either increase UCP expression in BAT or prevent the decrease occurring during a pair-fed regimen (13). The calculated proportion of daily fuel utilization derived from fat and carbohydrate oxidation indicated that leptin stimulated fat oxidation and spared utilization of glucose during the first 7 days of treatment. Preventing an EE decrease and paying the energy debt with fat fuel rather than carbohydrate are two main metabolic effects of leptin that were observed. Fat utilization by the leptin-treated group remained greater throughout the study. Persistence of leptin physiology was best demonstrated by withdrawal of treatment that triggered over-eating and fat synthesis.

OVARIECTOMY rats is in agreement with the observation that estradiol does not directly mediate leptin's effect (32). However, leptin did not significantly reduce body mass of OVARIECTOMY rats in the first 7 days because of a gain in lean mass. The leptin-treated animals continued to gain mass, but at a slower rate than control rats. They continued to lose fat, as evidenced by reduced lipid content and decreased circulating TG and FFA concentrations measured on day 35 that were possible consequences of leptin-stimulated lipid oxidation.

Leptin activation of the sympathetic nervous system stimulates lipolysis, and the elevated FFA levels induce uncoupling protein 3 (UCP3) expression in muscle (44). Skeletal muscle plays a major role in regulating energy metabolism. Animals overexpressing UCP3 have increased oxygen consumption and a marked reduction in body mass and adiposity (11). We used indirect calorimetry to monitor fuel utilization during leptin treatment and food restriction (pair-fed group). Total fuel utilization is the sum of fuel used for basal processes, thermogenesis, and physical activities such as eating and fidgeting (25, 35). Daily fuel utilization of the pair-fed group was decreased on day 5 and tended to remain depressed throughout the study (Fig. 5). This is likely a consequence of reduced feeding and a reduction in other physical activities, as well as a possible decrease in basal usage in attempt to conserve energy (24). Such reduction in expended energy was not present in the leptin-treated animals, even when energy intake was at the nadir, suggesting that leptin administration counters such physical adaptations to food restriction (1). Leptin was reported to either increase UCP expression in BAT or prevent the decrease occurring during a pair-fed regimen (13). The calculated proportion of daily fuel utilization derived from fat and carbohydrate oxidation indicated that leptin stimulated fat oxidation and spared utilization of glucose during the first 7 days of treatment. Preventing an EE decrease and paying the energy debt with fat fuel rather than carbohydrate are two main metabolic effects of leptin that were observed. Fat utilization by the leptin-treated group remained greater throughout the study. Persistence of leptin physiology was best demonstrated by withdrawal of treatment that triggered over-eating and fat synthesis.

Our rats reached a steady body mass before OVARIECTOMY at 6 mo of age. The loss of ovarian hormones stimulated the rats to gain both lean and fat mass, and they reached a new steady state in ~3 wk. These data indicate that this dramatic increase was primarily a consequence of hyperphagia and not a result of reduced fuel utilization or a shift in substrate oxidation to spare fat. Leptin administration after OVARIECTOMY prevented the hyperphagia, maintained daily fuel utilization at intact ad libitum control levels, and stimulated a shift to utilize fat to meet the energy requirement. However, unlike OVARIECTOMY rats that gained both lean and fat mass, the leptin-treated OVARIECTOMY rats lost fat mass. Furthermore, lean mass gain of this group was greater than that of intact rats after 35 days of treatment despite the prevention of hyperphagia. Our data suggest that OVARIECTOMY removes feedback to a central integrating center that regulates body mass and that such input by ovar-
ian hormones is inhibitory to feeding but not to regulating metabolism of fuel. It is likely that leptin feeds back to the same center (15) as well as other nuclei that regulate metabolism, an action not dependent on estradiol (32).

Leptin supplies a signal that is proportional to the level of fat (5) to the hypothalamus and hindbrain (15). This signal decreases expression of neuropeptides such as agouti-related protein (29) and neuropeptide Y (39, 41), which are orexigenic, and increases expression of other neuropeptides that are anorexigenic, such as proopiomelanocortin (28, 40) and corticotropin-releasing hormone (38). Leptin increases expression of UCP3 in BAT, which is important for thermogenesis in rodents (37). When leptin administration is suddenly halted, energy regulatory systems that were inhibited become disinhibited, and those that were stimulated are no longer targeted with such stimuli to produce an apparent rebound that can be observed in both feeding behavior and a shift toward carbohydrate utilization in an attempt to replenish the lost lipid reserve. A compensatory hyperphagia was observed, and RQ values near unity were measured from both the food-restricted rats and the leptin-treated groups that are indicative of almost exclusive carbohydrate utilization.

Indeed, fat synthesis was predicted by indirect calorimetry for the leptin-treated and pair-fed groups. As a result, these rats began rapidly to regain the mass that was lost. Together, these data indicate that leptin biology is manifest with chronic treatment even though food intake has returned to normal.

Higher plasma leptin concentrations in O VX rats after day 7 confirm that leptin provides information of fuel supply to the hypothalamus (16). The gradual increase in leptin concentrations of leptin-treated rats after day 14 could reflect increased food intake during this gradual return to normal feeding. The greatest increase in plasma leptin was observed within 24 h of leptin withdrawal, a period when the rats were most hyperphagic.

In conclusion, OV X increased body mass by producing hyperphagia. The loss of ovarian hormones did not affect resting EE or selection of fuel utilization. Although circulating leptin levels increased after OV X, this elevation was not a consequence of leptin resistance, because sham and OV X rats similarly responded to leptin. Chronic leptin administration to OV X rats reduced fat mass by decreasing energy intake and increasing lipid oxidation. We speculate that the shift toward fat utilization not only spared lean mass but also supported gain in lean mass. Thus leptin reduced fat mass and allowed accumulation of lean mass without hyperphagia. Leptin prevented a decline in EE that typically accompanies a negative energy balance. When the animal can no longer utilize its lipid reserve, rats increase feeding to spare lean mass. Further studies are needed to understand how OV X produces hyperphagia. Leptin levels appeared to increase with the increased feeding after OV X, and although the rats responded to leptin, they gained fat and lean mass. Our data suggest that the increased circulating leptin levels are not to prevent obesity but to inform the hypothalamus that sufficient fuel is available to support growth (20).

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