Sexually dimorphic responses to fat loss after caloric restriction or surgical lipectomy

Haifei Shi, April D. Strader, Stephen C. Woods, and Randy J. Seeley

1University of Cincinnati, Cincinnati, Ohio; and 2Southern Illinois University School of Medicine, Carbondale, Illinois

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Shi H, Strader AD, Woods SC, Seeley RJ. Sexually dimorphic responses to fat loss after caloric restriction or surgical lipectomy. Am J Physiol Endocrinol Metab 293: E316–E326, 2007. First published April 10, 2007; doi:10.1152/ajpendo.00710.2006.—White adipose tissue is the principal site for lipid accumulation. Males and females maintain distinctive white adipose tissue distribution patterns. Specifically, males tend to accumulate relatively more visceral fat, whereas females accumulate relatively more subcutaneous fat. The phenomenon of maintaining typical sex-specific fat distributions suggests sex-specific mechanisms that regulate energy balance and adiposity. We used two distinct approaches to reduce fat mass, caloric restriction (CR), and surgical fat removal (termed lipectomy) and assessed parameters involved in the regulation of energy balance. We found that male and female mice responded differentially to CR- and to lipectomy-induced fat loss. Females decreased energy expenditure during CR or after lipectomy. In contrast, males responded by eating more food during food return after CR or after lipectomy. Female CR mice conserved subcutaneous fat, whereas male CR mice lost adiposity equally in the subcutaneous and visceral depots. In addition, female mice had a reduced capability to restore visceral fat after fat loss. After CR, plasma leptin levels decreased in male but not in female mice. The failure to increase food intake after returning to ad libitum intake in females could be due to the relatively stable levels of leptin in summary, we have found sexual dimorphisms in the response to fat loss that point to important underlying differences in the strategies by which male and female mice regulate body weight.

THE WORLDWIDE PREVALENCE OF obesity with its accompanying comorbidities, including type 2 diabetes, cardiovascular disease, and cancer (7, 41, 58), continues to rise and has reached epidemic proportions (22, 59, 81). White adipose tissue (WAT) is the principal site for lipid accumulation in the body, and decreasing WAT is an important therapeutic goal because it would reduce medical complications. This is difficult to achieve, however, because total body fat is regulated by a robust and accurate process that matches energy intake to energy expenditure over time (39). Demonstrations of this include the failure of humans to maintain weight and fat loss after dieting (24, 75) and liposuction surgeries (6) and the return to baseline fat levels after caloric restriction (CR) or partial lipectomy in rodent models.

CR and lipectomy represent two ways to accomplish decreased adiposity. CR leads to a spectrum of changes, including decreased basal metabolic rate (33), increased spontaneous activity (32, 56, 65), increased lipid mobilization (26, 37), and many others that collectively lead to reduced body fat (60). In contrast, surgical removal of fat via lipectomy may result in comparably decreased body fat, but the fat loss is specific to selected fat depots depending on the removal site. Altered metabolism and behavior after lipectomy induce compensatory enlargement of nonexcised WAT pads in many species, including mice and rats (48). The fat restoration after either CR or lipectomy is consistent with the hypothesis that total body fat is highly defended and maintained.

In humans, the distribution of body fat differs between males and females, with women carrying relatively more subcutaneous fat and men having a greater percentage of visceral fat (31, 40, 76). The mechanism for maintaining sex-specific fat distribution is not well understood. Leptin is an adiposity signal (78) that has diverse actions throughout the body, including decreasing food intake and increasing energy expenditure (27). It is mainly secreted from subcutaneous adipocytes (8, 49), and the level of circulating leptin correlates better with subcutaneous fat than with total adiposity (16, 23, 38, 55, 64, 66). Consistent with differential fat distribution, plasma leptin is higher in women than in men with the same body mass index (52). We have found that female rats are more sensitive to the central anorexic effects of leptin than their male counterparts (9, 10). Thus male and female rodents may respond differentially to either generalized fat loss induced by CR or depot-specific fat loss caused by removal of subcutaneous or internally located visceral WAT. Consequently, the goal of the present experiments was to determine whether male and female mice respond differentially to two distinct fat-reducing manipulations: CR and fat pad-specific surgical lipectomy. Specifically, we investigated energy intake and energy expenditure, the two sides of the energy balance equation. We further measured leptin and uncoupling protein-1 (UCP-1) mRNA of brown fat because both are important regulators of energy balance in mice. CR is a stressor besides metabolic challenger, evidenced by its potent ability to increase circulating levels of glucocorticoids. To determine whether such a stress itself could affect the regulation of energy homeostasis and body fat distribution, we also measured the circulating corticosterone levels to examine whether there are important differences in this response in male and female mice.

MATERIALS AND METHODS

Animals

Adult 12-wk-old male and female FVBN mice obtained from the Jackson Laboratory (Bar Harbor, ME) were individually housed 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. Mice were provided with ad libitum access to

Address for reprint requests and other correspondence: R. J. Seeley, 2170 E. Galbraith Road, Dept. of Psychiatry, Univ. of Cincinnati, Cincinnati, OH 45237 (e-mail: randy.seeley@uc.edu).

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pelleted rodent chow (Harlan Teklad, rodent diet 8604; 3.4 kcal/g) and water unless otherwise noted. All animal procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Experimental Design

Experiment 1: CR. Male and female mice received ad libitum food (ad libitum mice; \(n = 10\) males and \(n = 10\) females), received 60% of the amount of food that was consumed daily by ad libitum mice for 6 days (CR mice; \(n = 10\) males and \(n = 10\) females), or were comparably calorie restricted and then returned to ad libitum food for 3.5 days (CR-ad libitum mice; \(n = 10\) males and \(n = 10\) females). Vaginal smears were performed between 1000 and 1100 each day for the determination of the phase of the estrous cycle in the females (4). Food intake and body weight were recorded daily at 1500, and 60% of the average food intake of the ad libitum mice that day was given to CR mice or CR-ad libitum mice during their CR phase within 1 h before dark onset. All CR mice and their ad libitum counterparts had energy expenditure assessed on day 6 of CR, and all CR-ad libitum mice had energy expenditure assessed on day 3 after return to ad libitum food, the dynamic recovery period during which the sexual dimorphism may be most prominent. CR and CR-ad libitum mice were killed on the following day, whereas ad libitum mice were kept alive for assessing daily food intake and were killed on the same day as the last subgroup of ad libitum and CR mice. Body fat and lean tissue distribution were assessed after death for all mice. Specifically, assessments occurred 9 days after the first subgroup of CR mice started CR for ad libitum mice, after 6-day CR for CR mice, and the 4th day after returning to ad libitum access to food for CR-ad libitum mice (Fig. 1A).

Experiment 2: lipectomy. Male and female mice were either underwent sham operation (\(n = 9\) males and \(n = 8\) females) or underwent bilateral removal of retroperitoneal WAT (RWAT; \(n = 8\) males and \(n = 10\) females) or subcutaneous inguinal WAT (IWAT; \(n = 9\) males and \(n = 8\) females). Food intake and body weight were monitored weekly during recovery. Whole body composition was measured before, 2 wk after surgery (when animals had recovered from the surgery and resumed normal food intake and other behaviors), and 12 wk after surgery (when compensation for lipectomy-induced adipose tissue loss was complete; Ref. 29). Energy expenditure was assessed 2 wk after surgery because this is the dynamic phase of the compensatory response to fat loss. The most striking finding from our previous studies was a marked decrease in norepinephrine turnover of interscapular brown adipose tissue (IBAT), thus suggesting a decrease in thermogenesis, an energy sparing response during this phase (70). Fat distribution was assessed after death (Fig. 1B).

Lipectomy and Sham Surgeries

Bilateral subcutaneous IWAT or intra-abdominal RWAT fat pads were exposed and carefully dissected (IWATx or RWATx, respectively) as much as possible without damaging adjacent muscle tissue or organs. Half of the sham-operated animals received similar procedures as IWATx and the other half received similar procedures as RWATx; in both cases the WAT was identified but not removed. Because there were no differences between these two control groups, their data were combined for the analyses.

Body Mass and Food Intake Measurements

Body weight and food intake were measured to the nearest 0.01 g. Food intake was estimated by the difference of the weight of the food hopper over 24 h (CR experiment) or 1 wk (lipectomy experiment). The difference, corrected for spillage, represented the daily or weekly food intake. Food intake data were converted to calories and were adjusted for body mass.

Indirect Calorimetry

Energy expenditure was measured by indirect calorimetry. Mice were placed into individual metabolic chambers at the end of the light cycle and remained in the chambers for 22 h with ad libitum access to water and food for ad libitum mice or with the appropriate amount of food for CR mice. The estrous cycles of female mice were monitored during acclimation so that the coming diestrous phase could be reliably predicted. We started CR after estrous, and all female mice were at the diestrous phase on day 6. All female mice were assessed at the diestrous phase of the cycle for the following reasons. Our female mice had 5-day estrous cycles with proestrous lasting 6–8 h, estrous lasting ~12 h, and diestrous lasting ~2–3 days. Thus we opted to assess mice during diestrous to reduce variance resulting from different phases of the cycles. A second advantage is that CR may reduce cyclicity in mice by increasing the length of their diestrous cycle. Consequently, comparing all female mice in their diestrous phase results in the best “apples to apples” comparison.

Mice in experiment 1 were assessed in a Physioscan System (Accuscan Instruments, Columbus, OH), and mice in experiment 2 were assessed in an Oxymax System (Accuscan Instruments) because of the relative availabilities of the systems when the experiments were in progress. Physioscan and Oxymax systems are designed along the
same principles to estimate the same key parameters. Whole animal heat production was determined as calories per hour. Volume of consumed oxygen ($V_{O2}$) was determined as milliliters per kilogram body weight per minute (ml·kg$^{-1}$·min$^{-1}$) and was subsequently adjusted to kilograms of lean mass. All mice from the same cohort were measured by the same system, and heat production and $V_{O2}$ data were compared within each cohort but not across instruments or cohorts.

**Death, Blood Withdrawal, and Tissue Harvest**

All mice were fasted for 3 h before being killed by FetalPlus administration (Vortech Pharmaceuticals, Dearborn, MI; 100 mg/kg i.p.) followed by decapitation. Blood samples were centrifuged, and serum was stored at $-20°C$ until assay. IBAT were quickly removed and snap frozen on dry ice for later measurement of UCP-1 gene expression. The fat distribution of carcasses was assessed by the pelting technique (see below).

**Body Composition and Body Fat Distribution**

A mouse-specific NMR Echo MRI whole body composition analyzer (EchoMedical Systems, Houston, TX) was used to assess body fat, body water, and lean mass (73) in conscious mice, providing longitudinal data. At the end of experiments, the relative amounts of subcutaneous and visceral fat were assessed by the pelting technique, whereby the skin, attached fat, and fat on the surface of any skeletal muscle was removed from each carcass, functionally separating each mouse into two distinct portions: 1) the pelt portion containing skin and subcutaneous fat and 2) the carcass portion including all muscle, skeleton, organs, and intramyocellular and visceral fat (9). NMR was then used to determine the amounts of fat and lean tissue of the pelt and carcass portions.

**Quantitative Real-Time PCR Analysis of UCP-1 Gene Expression From IBAT**

IBAT UCP-1 gene expression was measured in experiment 1. This assessment was not made in experiment 2 because UCP-1 gene expression was not expected to differ from controls following full compensation by 12 wk postsurgery. IBAT were dissected and homogenized, and total RNA was isolated with Tri reagent (MRC, Cincinnati, OH). After DNase treatment (Ambion, Austin, TX), cDNA was synthesized by an iScript kit (BioRad, Hercules, CA) and verified by the constitutively expressed ribosomal protein L32 RT-PCR amplification products in agarose gel (L32 RT-PCR primer forward primer is 5′-GCC AGG AGA CGA CAA AAA and reverse primer is 5′-GTC GGT CCT TCC TTG GTG TA. The mouse L32 quantitative real-time PCR (Q-PCR) forward primer is 5′-GCC AGG AGA CGA CAA AAA and reverse primer is 5′-AAT CCT CTT GCC CTG ATC CT. The mouse UCP-1 Q-PCR forward primer is 5′-GCG CCC TTG TAU ACA ACA AA and reverse primer is 5′-GTC GTG CCT TCC GTG TAG TA. Q-PCR was performed in triplicate with a BioRad iCycler and iQ SYBR green Supermix (BioRad), with two-step amplification at 95°C for 10 s and annealing temperature of 61.2°C for 30 s for 40 cycles. The average threshold cycle ($C_T$) of each set of triplicates was calculated, and the $ΔC_T$ was calculated for each sample by subtracting the average $C_T$ of L32 from the average $C_T$ of UCP-1. $ΔC_T$ was used for data analysis. For relative quantification, the $ΔC_T$ was averaged for the ad libitum group and was then subtracted from the $ΔC_T$ of each CR sample to generate the $ΔΔC_T$. The $ΔΔC_T$ was used to calculate the approximate difference (in fold; Applied Biosystems’ instruction).

**Assays**

Circulating leptin and corticosterone concentrations were measured with $^{125}$I RIA kits (leptin: Linco Research, St. Charles, MO; corticosterone: MP Biomedicals, Orangeburg, NY). The coefficients of variation of intra- and interleptin assays were 3% and 4%, and intra- and intercorticosterone assays were 4.4% and 6.5%. The sensitivity was 0.5 ng/ml for leptin assay and 25 ng/ml for corticosterone assay.

**Statistical Analysis**

Data are expressed as means ± SE. Comparisons among multiple groups were made with appropriate one-way or two-way ANOVA. Post hoc tests of individual groups were made with 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**

**Estrous Cycle of Female Mice**

Our CR paradigm was not severe or long enough to affect estrous cycle length. Estrous cycle length was 4.9 ± 0.3 days for ad libitum mice throughout the experiment, 4.9 ± 0.2 days before CR and 4.9 ± 0.4 days during CR for CR mice, and 5.3 ± 0.4 days before CR and 5.3 ± 0.3 days during CR for CR-ad libitum mice.

**Body Mass**

In experiment 1, body mass decreased in CR mice relative to ad libitum mice, becoming significant by day 3 in males and by day 4 in females. By day 6, male CR and CR-ad libitum mice had lost an average of 15.80 ± 0.79% of body mass, whereas female CR and CR-ad libitum mice had lost an average of 10.74 ± 0.89% of body mass. Mice of both sexes regained body mass to pre-CR levels on the first day of refeeding (Fig. 2, A and B). In experiment 2, equivalent amounts of RWAT or IWAT were dissected from males and females (Table 1). Specifically, male RWATx mice lost 4.43 ± 0.73% of total fat mass and 0.38 ± 0.07% of body weight, male IWATx mice lost 7.47 ± 0.54% of total fat mass and 0.67 ± 0.03% of body weight, female RWATx mice lost 4.15 ± 0.33% of total fat mass and 0.44 ± 0.05% of body weight, and female IWATx mice lost 8.96 ± 0.77% of total fat mass and 0.84 ± 0.05% of body weight. There was no significant change of total body weight after surgery (Fig. 2, C and D).

**Body Composition**

Six days of CR significantly decreased the percentages of both fat and lean mass in male and female mice. Male mice regained both fat and lean mass, and female mice regained only their lean mass after 3.5 days of return to ad libitum feeding (Fig. 3). Although mice of both sexes lost comparable percentages of fat mass during CR (Fig. 3A), male CR mice lost a significantly greater percentage of lean tissue than females over the 6 days (Fig. 3B). After return to ad libitum feeding, males overcompensated and attained a significantly higher amount of body fat than ad libitum controls, and they also restored a significantly greater percentage of fat mass than did females (Fig. 3A). This overrestoration of body fat was not seen in females (Fig. 3B), who tended to underrestore total body fat after being returned to ad libitum feeding, although total body fat in CR-ad libitum females was not significantly different from that of ad libitum females by the end of the experiment (Fig. 3A).
In experiment 2, male and female mice did not change their body fat (Fig. 3, C and E). There was a tendency toward mild fat reduction in female sham mice at 2 wk postsurgery vs. that shown in male sham mice (P = 0.242; Fig. 3C), possibly due to nonspecific effects of the surgical procedure, and a tendency showing that lipectomized male and female mice nonsignificantly reduced a comparable percentage of fat at 12 wk postsurgery (RWATx males, P = 0.167; RWATx females, P = 0.204; IWATx males, P = 0.343; IWATx females, P = 0.423; Fig. 3E). Over the 2 wk postsurgery, there was a tendency for the lipectomized males to increase and for the lipectomized females to decrease their lean mass, such that the change of percent lean body mass was significantly different between sexes (Fig. 3D). This suggests a faster growth and recovery for male than for female mice at 3 mo of age. Lean mass of both lipectomized and sham-operated mice increased over the experiment (Fig. 3F).

Fat and Lean Tissue Distribution

During CR, male mice decreased both subcutaneous and visceral fat (Fig. 4A) and lean tissue (Fig. 4C), whereas female mice conserved their subcutaneous fat by exclusively losing visceral fat (Fig. 4B) and visceral lean tissue (Fig. 4D). After they were returned to ad libitum feeding, male mice recovered lost fat by accumulating both subcutaneous and visceral fat (Fig. 4A), whereas female mice moderately restored visceral lean tissue (Fig. 4D) without accruing increased visceral adipose mass (Fig. 4B), suggesting that, unlike males, females lack the capability to restore visceral adiposity after fat loss. We are interested in the differences between males and females in terms of time courses and

Table 1. Amount of fat removed in lipectomy study

<table>
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<tr>
<th>Fat Removed, g</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>Sham</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RWATx</td>
<td>0.0857±0.0160</td>
<td>0.0844±0.0104</td>
</tr>
<tr>
<td>IWATx</td>
<td>0.1543±0.0107</td>
<td>0.1680±0.0136</td>
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Values are means ± SE. IWATx, dissected subcutaneous inguinal white adipose tissue; RWATx, dissected intra-abdominal, retroperitoneal white adipose tissue.
degrees of fat restoration. Thus we examined body composition on the 4th day after CR mice were returned to ad libitum feeding when the differences between males and females were clearest. However, it is likely that, given sufficient time, female mice would eventually restore the lost fat as male counterparts do. No difference in fat distribution was found among any of the lipectomy groups 12 wk after surgery (data not shown).

Fig. 3. Fat and lean mass percent change in CR and lipectomy studies. A and B: fat (A) and lean (B) mass change in CR study. C–F: fat (C) and lean (D) mass change 2 wk after surgeries and fat (E) and lean (F) mass change 12 wk after surgeries in lipectomy study. Values are means ± SE. *Statistically significant difference between treatments within sex (P < 0.05). †Statistically significant difference between sexes (P < 0.05).
Energy Intake and Energy Expenditure

Torpor (characterized by being cold to the touch, curled posture, low frequency of breathing, and the absence of food and water intake) was not observed in any animal. On the first day of return to ad libitum feeding, male CR-ad libitum mice significantly increased their caloric intake, whereas female CR-ad libitum mice did not change caloric intake. On subsequent days, male CR-ad libitum mice had no further change of food intake, whereas female CR-ad libitum mice had decreased caloric intake (Fig. 5A). The overall mean caloric intake across the 3-day recovery was significantly increased in males but decreased in females (data not shown).

Between weeks 2 and 5 after lipectomy, male RWATx mice significantly increased their caloric intake, and male IWATx mice had a comparable increase that did not reach significance. In contrast, female RWATx and IWATx mice had no change of intake (Fig. 5B). Intake of all lipectomized mice was comparable to that of controls 6 wk after surgery (data not shown).

Energy expenditure was measured on day 6 of CR and on day 3 of return to ad libitum feeding in experiment 1 and between postsurgical weeks 2 and 3 in experiment 2. CR significantly decreased whole animal heat production by 10.10\% in males and by 21.93\% in females (Fig. 5C). Female RWATx mice also had significantly reduced whole animal heat production (Fig. 5C). Female CR and RWATx mice had decreased VO_2 (Fig. 5D and F), whereas none of the male CR or lipectomy groups had significantly altered VO_2. Male and female CR-ad libitum mice had heat production and VO_2 results similar to the ad libitum groups during refeeding (Fig. 5D).

Q-PCR Analysis of IBAT UCP-1 Gene Expression

UCP-1 gene expression was significantly decreased in IBAT of female CR mice but was not changed in CR males. UCP-1 gene expression of CR females was significantly lower than that of CR males. There was a tendency toward a slight decrease of UCP-1 mRNA levels in IBAT of male and female CR-ad libitum mice compared with the same sex ad libitum groups (Table 2).

Plasma Leptin

Ad libitum and CR female mice had higher plasma leptin levels than ad libitum and CR male mice. CR significantly reduced and refeeding significantly increased plasma leptin in male mice. CR and refeeding had no effect on plasma leptin levels in females relative to ad libitum-fed controls, although they had less total fat (Table 3).

Circulating Corticosterone

Ad libitum females had significantly higher baseline corticosterone than males, and CR significantly elevated corticosterone in both sexes. After 3 days of refeeding, plasma corticosterone returned to normal in both sexes (Table 4). No sex differences were found between male and female CR groups or CR-ad libitum groups.

DISCUSSION

To provide energy for compensatory increases in deficient lipid stores, animals must either increase energy intake or decrease energy expenditure. In the present series of experi-
ments, we used two distinct approaches to reduce fat mass, and we found that males and females use different behavioral strategies to restore fat after loss via either CR or lipectomy. Similar to what has been reported in rats (74), calorically restricted female mice had a greater decrease of energy expenditure and suppression of IBAT UCP-1 gene expression than males. It is noteworthy that male CR mice also significantly decreased nonnormalized whole animal heat production. When all parameters were considered together, the energy conservation response was less but not completely absent in males than that shown in females. In contrast, CR males but not

Table 2. IBAT UCP-1 gene expression

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<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>∆Cr</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum</td>
<td>−6.16±0.36</td>
</tr>
<tr>
<td>CR</td>
<td>−6.28±0.26</td>
</tr>
<tr>
<td>CR-ad libitum</td>
<td>−6.03±0.12</td>
</tr>
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</table>

Values are means ± SE. CR, caloric restriction; ∆Ct, change in threshold cycle; UCP-1, uncoupling protein-1. *Statistically significant difference between groups within sex (P < 0.05). †Statistically significant difference between sexes within group (P < 0.05).
females had increased caloric intake after return to ad libitum feeding. Furthermore, RWATx male but not female mice had increased food intake, and RWATx female but not male mice had decreased heat production and VO₂. These data collectively imply that the primary strategy of males is to gain fat by increasing energy intake, whereas that of females is to gain fat by decreasing energy expenditure.

We found distinct patterns of body fat distribution between male and female mice during CR and recovery, with females conserving subcutaneous fat during CR by decreasing visceral fat, whereas males lost both subcutaneous and visceral fat simultaneously. In addition, although male mice restored fat in both sites after they were returned to ad libitum intake following CR, female mice were less capable of regaining visceral fat than males during refeeding. Analogous to these findings in mice, diet-induced reduction of body fat is more significant in men than in women (42, 54, 77).

To begin to understand why females have less hyperphagia after CR or lipectomy than males, we assessed plasma leptin in experiment 1. Consistent with previous reports (8), ad libitum male mice had lower circulating leptin than ad libitum female mice. Also consistent with previous reports (16, 23, 38, 55, 64, 66), leptin levels correlated more highly with subcutaneous fat than with total adiposity. Plasma leptin decreased with CR and increased again after refeeding in males. This dynamic change of leptin likely contributes to the robust increase of food intake of male mice upon refeeding, particularly during the initial period after they are returned to ad libitum food. In contrast, females had relatively stable subcutaneous WAT, and they had no change of leptin in response to CR or refeeding. The relatively stable levels of leptin may be an important factor for the lack of hyperphagia and reduced ability to restore visceral fat after fat loss relative to what occurs in males. In contrast to the CR paradigm, leptin does not appear to serve as a signal for the lipectomy-induced fat loss because both ob/ob and db/db mice, which lack leptin signaling, are able to compensate for lipectomy-induced fat loss (29). The signal for the fat loss in the lipectomized mice could therefore be neural. Previous work has found that specific sensory denervation of WAT mimics the fat mass increase seen after lipectomy (69), suggesting that sensory innervation of WAT could be the signal for fat loss.

In addition to being a metabolic challenge, CR is also a significant stressor. This is evidenced by increased glucocorticoid levels that occur in various CR paradigms, including our own (5, 25, 36, 68). Thus a CR-related stress response could affect the regulation of energy homeostasis and body fat distribution (12). Unlike what occurred with leptin, both male and female CR mice increased their corticosterone levels in parallel. Thus differences in corticosterone are not likely to be a major contributor to different recovery strategies between male and female mice.

On a relative basis, females conserved their energy expenditure more than males, whereas males increased their food intake after CR and refeeding in experiment 1 and during the early postsurgery period in experiment 2. Previous studies reported increased (43), similar (13, 30, 70, 71), or decreased (21) food intake by lipectomized animals and unchanged or increased body weight-adjusted heat production postlipectomy (30). Species that undergo annual cycles of fat gain and loss, including ground squirrels (Spermophilus lateralis; Ref. 13), Syrian hamsters (Mesocricetus auratus; Ref. 28), and Siberian hamsters (Phodopus sungorus; Refs. 70, 71), have precise lipectomy-induced compensation (48), but they lack food intake responses to energy challenges such as food deprivation (1, 2, 15) or glucoprivation (1, 14, 53). Thus a likely mechanism underlying lipectomy-induced compensation in these species is decreased energy expenditure, as suggested by decreased sympathetic activity of BAT (70), and thus decreased thermogenesis. These studies collectively support the hypothesis that differences in sympathetic activity may contribute to the observed differences between males and females.

The CR-ad libitum female mice regained their body weights but did so without an apparent increase of food intake or decrease of VO₂ or heat production. IBAT UCP-1 mRNA from CR-ad libitum mice was measured on day 4 of refeeding, and no significant difference was found between CR-ad libitum and ad libitum groups. Any dynamic gene regulation may occur earlier on the day 1 or 2 after refeeding. The tendency toward decreased UCP-1 expression in female CR-ad libitum mice, although not reaching statistical significance, suggests a possible reduction in thermogenesis on refeeding in the CR-ad libitum females, an effect that was not great enough to be detected by indirect calorimetry. The present study is limited in that only heat production and VO₂ were assessed as indicators of energy expenditure. Total energy expenditure, however, also includes resting metabolic rate, thermogenesis, locomotor activity, and diet-induced thermogenesis. Future studies will need to assess all aspects of energy expenditure in addition to addressing the central nervous system mechanisms that are differentially engaged in males and females to account for these important differences.

The decreased UCP-1 expression in IBAT of CR females suggests an important decrease in sympathetic drive. Many hormones and neuropeptides involved in regulation of energy balance alter sympathetic activity and thermogenesis. Hormones and neuropeptides such as corticotrophin-releasing hormone (18), cholecystokinin (83), enterostatin (51), leptin (11), bombesin (3), cocaine- and amphetamine-regulated transcript

### Table 3. Circulating leptin levels

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<thead>
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<th>Female</th>
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<tr>
<td>Ad libitum</td>
<td>1.39±0.21</td>
<td>2.42±0.30†</td>
</tr>
<tr>
<td>CR</td>
<td>0.62±0.07*</td>
<td>2.39±0.45†</td>
</tr>
<tr>
<td>CR-ad libitum</td>
<td>2.73±0.22*</td>
<td>2.52±0.25</td>
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Values are means ± SE. *Statistically significant difference between treatments within sex (P < 0.05). †Statistically significant difference between sexes within group (P < 0.05).

### Table 4. Circulating corticosterone levels

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<th>Female</th>
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<tbody>
<tr>
<td>Ad libitum</td>
<td>311.78±50.75</td>
<td>489.10±70.58†</td>
</tr>
<tr>
<td>CR</td>
<td>639.22±77.85*</td>
<td>750.20±64.22*</td>
</tr>
<tr>
<td>CR-ad libitum</td>
<td>268.50±28.43</td>
<td>383.80±52.26</td>
</tr>
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</table>

Values are means ± SE. *Statistically significant difference between treatments within sex (P < 0.05). †Statistically significant difference between sexes within group (P < 0.05).
(47), proopiomelanocortin products such as α-melanocyte-stimulating hormone and adrenal corticotrophic hormone (17), and orexin B (82) increase sympathetic activity. Hormones and neuropeptides such as neuropeptide Y (20), proopiomelanocortin product β-endorphin (17, 19), orexin A (82), galanin (51), and melanin-concentrating hormone (57) reduce sympathetic activity. Whether differences between males and females in any of these systems account for the different responses to CR is a key question for future research.

The present results are consistent with other studies that have identified differences in the behavioral and metabolic strategies used by males and females to regulate energy balance. When overfed with a palatable high-fat diet either chronically (62) or acutely (63), female rats gain more body weight than males because of a greater conservation of energy expenditure with lower activation of thermogenesis in BAT. Similarly, when rats are calorie restricted, females conserve energy by decreasing V\textsuperscript{O}_2 and thermogenesis, whereas males do not (74). This sexual dimorphism is also seen in several genetic mouse models. For example, we previously observed that, although syndecan-3-deficient mice resist diet-induced obesity on a high-fat diet, males accomplish it by decreasing caloric intake; however, females have a disproportionate increase in energy expenditure (72). In a separate model, both male and female mice with targeted disruption of the gene encoding granulocyte macrophage-colony stimulating factor have greater fat mass than wild-type controls, with the males achieving this via a pronounced increase in intake, whereas females have decreased energy expenditure (61). Collectively, these examples point to unique strategies for body weight regulation in males and females in which males predominantly adjust energy intake and females predominantly alter expenditure to regulate energy homeostasis.

These different strategies to regulate energy balance are presumably due to differential evolutionary and sexual selection pressures (34). Fat stores are typically greater in female mammals, whereas lean tissue is typically greater in males. This is consistent with a greater role of females in pregnancy, lactation, and caretaking, whereas male reproductive roles often involve competition for territory and mates. Because of these evolutionary pressures, females are relatively inclined to store fat when food is abundant and are adapted to survival by resisting fat loss when food is scarce. Consistent with this point of view, the ad libitum female mice in the present experiments had twice as much body fat but only two-thirds of the lean tissue as the males; in addition, the females had greater ability to withstand fat loss via conserving energy. Given these different regulatory strategies, it may be necessary to design quite different therapeutic approaches to produce safe and efficacious weight loss in males vs. females.

**Perspectives**

Understanding the response to CR- or lipectomy-induced fat loss in rodents would contribute to our knowledge and strategies for therapeutic programs targeting body fat in humans. Both CR and suction-assisted lipectomy have been used to decrease body fat in men and women. Successful long-term weight management is difficult to achieve, however, because the majority of men and women who lose weight with diets regain their lost weight over time (24, 75). In addition, men usually lose more body weight and fat than women from diet-induced weight-reducing programs (42, 77). Suction-assisted lipectomy that removes large amounts of body fat has been used as an alternative approach to decrease body fat (46). Weight gain and fat return also have been reported however (6). To understand the mechanisms of fat recovery from CR or lipectomy in men and women and the possible sex difference during the recovery, we used CR and lipectomy to reduce adiposity and studied the sexual dimorphic responses to the fat loss. The lipectomy procedure in the present study was not designed to mimic human liposuction procedures. In most instances, only subcutaneous but not visceral fat is removed in the liposuction procedure in obese humans, whereas both subcutaneous and visceral fat were manipulated in the present study. The magnitude of our surgical lipectomy manipulation was small (4.15–8.96% of total fat) and consistent with that of a previous study (29). Although the amount of fat that was removed was small, it was not trivial, since we found that either energy intake or energy expenditure was changed after lipectomy, depending on the sex of the mouse, and this did not happen after the sham procedure.

Obese mouse models can be used to study sexually dimorphic regulation of energy balance. Two noninvasive and nondrug-treated obese mouse models that have been generally used are diet-induced obese and genetically obese mice. Consumption of a high-fat diet makes mice obese but also changes the homeostatic regulation of energy balance independent of body weight change (79) by causing insensitivity to some satiety signals such as cholecystokinin (67), insulin (35), ghrelin (44), and leptin (44, 50). Diet-induced obesity also causes diet-induced hyperphagia (80) and diet induced thermogenesis (45). Such changes would mask the responses of animals such that the use of high-fat diet-induced obesity is not appropriate. The present study is, to our knowledge, the first to be performed to ascertain sexual dimorphism in response to fat loss in mice. Future experiments can take advantage of a number of genetic mouse models of obesity to test specific hypotheses about the underlying differences between male and female mice that lead to these important differences.

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