Effects of leptin infusion during peak lactation on food intake, body composition, litter growth, and maternal neuroendocrine status in female Brandt’s voles (Lasiopodomys brandtii)

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1State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beichen Xilu, Chaoyang District, Beijing, China; 2Graduate University of the Chinese Academy of Sciences, Beijing, China; and 3Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, Scotland, United Kingdom

Submitted 26 February 2010; accepted in final form 1 December 2010

Cui JG, Tang GB, Wang DH, Speakman JR. Effects of leptin infusion during peak lactation on food intake, body composition, litter growth, and maternal neuroendocrine status in female Brandt’s voles (Lasiopodomys brandtii). Am J Physiol Regul Integr Comp Physiol 300: R447–R459, 2011. First published December 1, 2010; doi:10.1152/ajpregu.00121.2010.—During lactation, female small mammals frequently reduce their fat reserves to very low levels. The function of this reduction is unclear, as calculations suggest that the contribution of the withdrawn energy from fat to the total energy balance of lactation is trivial. An alternative hypothesis is that reducing fat leads to a reduction in circulating adipokines, such as leptin, that play a role in stimulating the hyperphagia of lactation. We investigated the role of circulating leptin in lactation by repleting leptin levels using miniosmotic pumps during the last 7 days of lactation in Brandt’s voles (Lasiopodomys brandtii), a model small wild mammal we have extensively studied in the context of lactation. Repletion of leptin resulted in a dose-dependent reduction of body mass and food intake in lactating voles. Comparisons to nonreproducing individuals suggests that the reduced leptin in lactation, due to reduced fat stores, may account for ~16% of the lactational hyperphagia. Reduced leptin in lactation may, in part, cause lactational hyperphagia via stimulatory effects on hypothalamic orexigenic neuropeptides (neuropeptide Y and agouti-related peptide) and inhibition of the anorexigenic neuropeptide (proopiomelanocortin). These effects were reversed by the experimental repletion of leptin. There was no significant effect of leptin treatment on daily energy expenditure, milk production or pup growth, but leptin repletion did result in a reversal of the suppression of uncoupling protein-1 levels in brown adipose tissue, indicating an additional role for reducing body fat and leptin during peak lactation.

sustained energy intake; neuropeptide Y, agouti-related peptide, proopiomelanocortin, uncoupling protein-1

LACTATION IS WIDELY AGREED to be the most energetically demanding phase of the mammalian female life cycle (53, 65), particularly in small mammals. For example, over a period of ~18 days, the lactating female mouse (Mus musculus) increases her food intake by a factor of five (29, 33). At the same time, she remodels her morphology, growing the length of the alimentary tract by ~20% (~12 cm), doubling the size of the liver, and growing other internal organs like the pancreas. This is not just a generalized increase in size, because the lactating female also withdraws calcium from the major bones and almost all the lipids from her white adipose tissue (WAT) (25, 30, 36, 49, 68). Simultaneous to the behavioral and morphological changes, the female mouse also undergoes a radical alteration in her physiology. This includes increasing the capacity of the mammary glands to synthesize and secrete milk, combined with a profound reduction of the levels of uncoupling proteins-1 (UCP-1) and UCP-3 in brown adipose tissue (BAT) (55, 75, 87). The elevated food intake is closely coordinated with milk production and varies tremendously across individuals (37, 41, 86). Similar patterns of elevated intake and morphological/physiological changes in lactation are observed in many other species of small mammals (28, 45, 49, 58, 90, 92).

Food intake at peak lactation in small rodents appears to be limited. One explanation for this limit is that food intake is constrained by the nutrient uptake capacity of the alimentary tract. Milk production is then set by the capacity to take in food (16, 56, 82). An alternative view is that the system is limited by the capacity of the mammary glands to secrete milk (24, 26, 93). Neither of these hypotheses is well supported by the available data (25–28, 32, 33, 35, 46, 68, 77). Most recently, the suggestion has been made that limitations on the capacity of the female to dissipate heat generated as a byproduct of food intake (heat increment of feeding) and milk synthesis (36, 40, 41, 67) may limit food intake at peak lactation and hence lactation performance. Some studies have previously addressed the neuroendocrine changes that accompany the hyperphagia of lactation, showing, for example, that neuropeptide Y (NPY) and agouti-related peptide (AgRP) in the hypothalamus are both upregulated, while proopiomelanocortin (POMC) is downregulated (11, 14, 31, 43, 48, 57, 83) relative to nonreproductive animals. A potential primary role in integrating these responses may be played by the levels of circulating adipokines such as leptin (65, 67). As we have noted above, during lactation, females almost completely withdraw lipids from their WAT. Previous interpretations of this reduction in fat was that it provided energy to support the lactation energy demand. However, the contribution of the energy from WAT to the total demands of lactation is trivial (~2%) (65). Profoundly reducing body fat may primarily serve to decrease the levels of adipokines, thereby, in part, stimulating the hyperphagia characteristic of late lactation (65, 67). This interpretation of a role for peripheral leptin is supported by the fact that neuroendocrine changes in lactation occur in the system (NPY and AgRP) that is responsive to circulating leptin levels (see above). Moreover, food intake at peak lactation is negatively corre-
lated with circulating leptin levels (92). However, experimentally repleting leptin in lactating rats had no effect on food intake (89).

In the present paper, we experimentally investigated the role of leptin as a driver for lactational hyperphagia, by repleting circulating leptin levels during peak lactation in the Brandt’s vole (Lasiopodomys brandtii), a species we have previously studied extensively in the context of limitations on lactation performance (45, 86, 90, 91). Female Brandt’s voles are small rodents weighing 35–60 g when mature. In the wild, they are found throughout the Inner Mongolia grasslands of China, Mongolia, as well as the region of Beigaer Lake in Russia, where their diet is predominantly grass. They breed during the summer in burrows, and the females produce several litters of between 2 and 10 offspring from pregnancies lasting 21 days and lactations lasting 18 days. The offspring are altricial being naked and blind at birth. We monitored the effect of leptin repletion on food intake, body composition, milk production, and offspring growth. In addition we also measured expression of a range of hypothalamic neuropeptides selected because they have been previously linked with regulation of food intake (52, 61, 84) and also have been shown to be located in neurons that have the signaling form of the leptin receptor.

**MATERIALS AND METHODS**

All animal procedures were approved by the Animal Care and Use Committee of Institute of Zoology, the Chinese Academy of Sciences. Brandt’s voles (90–120 days old) were the offspring of the voles trapped in Inner Mongolian Grasslands in May 1999 and raised in Institute of Zoology, Chinese Academy of Sciences. Virgin voles were housed individually after they were 60 days old in plastic cages (30×15×20 cm) with sawdust and a little cotton as bedding. The voles were kept at 23 ± 1°C, under a photoperiod of 16:8-h light-dark cycles (lights on at 0400 h). Commercial rabbit pellets (Beijing KeAo Feed, Beijing, China) (crude protein: 20.8%, crude fat: 6.2%, neutral detergent fiber: 23.1%, acid detergent fiber: 12.5%, ash: 10%, caloric value: 17.5 KJ/g) and water were provided ad libitum. The females were paired with males for 1 wk to allow insemination, and then the males were removed. The day of parturition was designated as day 0. We performed two experiments. Experiment 1 included a range of leptin doses designed to span the level that would replete the levels of leptin in lactating females to the levels observed in nonreproductive animals. In experiment 2 we used a single dose informed by the results of experiment 1 but made more extensive measurements of the energy budgets in these animals, including quantification of energy expenditure and milk production. We did not normalize litter sizes to a standard size. While normalizing litters to a standard size may appear to remove variation due to this source, there are some complexities in this procedure that make it less than straightforward. First, even when manipulated to have equal numbers of pups, females may lose some pups leading to continued variability in litter size that needs to be accounted for. Second, there is some evidence that mammary growth in rodents occurs during pregnancy stimulated by the numbers of fetuses so the female is able to anticipate the lactational energy requirements. Thus normalizing litters to a constant size may remove the effect of the immediate number of pups being raised but introduces a new variable, which is the number of pups raised relative to the number that the female was expecting to raise. Overall then, standardizing litters to a standard size may not simplify the subsequent statistical treatment of the data to remove variations linked to litter sizes (actual or expected).

**Experiment 1**

Thirty females with a litter size of 6–8 were selected and randomly divided into five groups (1 infused with PBS and 4 leptin groups with different doses). Body mass, food intake, and litter weight were monitored daily up to day 17. During days 10–17 females were infused with recombinant murine leptin (Peprotech, London, UK) dissolved in PBS (pH 7.4) or PBS alone (control) using miniosmotic pumps (Alzet model 2001, capacity, 200 μl; release rate, 1 μl h; Durect, Cupertino, CA). The amount of leptin in the miniosmotic pumps was 50–300 μg, and the leptin concentration was 0.25–1.5 μg/μl. Due to the differences in individual body mass, the mean dose of leptin varied between 0.11 ± 0.01 and 0.64 ± 0.02 (means ± SE) μg g body mass−1·day−1 (Table 1). Female voles were anesthetized lightly with isoflurane, and the pump was implanted subcutaneously on the dorsal side on day 10 of lactation (after food intake was recorded). After surgery, voles continued suckling behavior within 30 min. On day 17 the dams were killed with CO2 asphyxiation. The blood samples were collected via posterior vena cava puncture and kept on ice for 30 min before being centrifuged at 4,000 g for 30 min at 4°C, and serum was sampled and stored at −75°C for leptin assay. As previously described (74), a slice of brain tissue was cut between the optic chiasm and the mammillary bodies, and the hypothalamus was dissected by a horizontal cut immediately below the anterior commissure and vertical cuts through the edge of the septum and periventricular sulci. The hypothalamus was immediately frozen in liquid nitrogen and stored at −80°C until subsequent analysis.

**Experiment 2**

In experiment 2 we aimed to use a single dose from experiment 1 to explore in more detail the energy budgets of the infused animals. Twenty-six females with litter sizes of 5–9 were selected and randomly divided into PBS (14 voles) and leptin-treated groups (12 voles). Maternal body mass, litter weight, and food intake were recorded daily throughout the experiment. During days 10–17 females were infused with recombinant murine leptin (dosage: 0.64 μg g body mass−1·day−1, implantation day 10) or PBS using Alzet

<table>
<thead>
<tr>
<th>Group</th>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target dose, μg·g body mass−1·day−1</td>
<td>PBS</td>
<td>0.1</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
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<tr>
<td>Sample size</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
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<tr>
<td>Maternal body mass day 10 of lactation</td>
<td>56.7 ± 2.5</td>
<td>54.2 ± 2.9</td>
<td>56.7 ± 2.5</td>
<td>55.1 ± 1.6</td>
<td>56.6 ± 1.8</td>
</tr>
<tr>
<td>Litter size</td>
<td>7.5 ± 0.3</td>
<td>7.3 ± 0.3</td>
<td>7.3 ± 0.4</td>
<td>7.7 ± 0.3</td>
<td>7.7 ± 0.3</td>
</tr>
<tr>
<td>Total leptin in pump, μg</td>
<td>0</td>
<td>50</td>
<td>100</td>
<td>200</td>
<td>300</td>
</tr>
<tr>
<td>Leptin concentration, μg/μl</td>
<td>0</td>
<td>0.25</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Dose, μg/day</td>
<td>0</td>
<td>6</td>
<td>12</td>
<td>24</td>
<td>36</td>
</tr>
<tr>
<td>Actual dose, μg·g body mass−1·day−1</td>
<td>0.00</td>
<td>0.11 ± 0.01</td>
<td>0.21 ± 0.01</td>
<td>0.44 ± 0.01</td>
<td>0.64 ± 0.02</td>
</tr>
<tr>
<td>Serum leptin concentration, ng/ml</td>
<td>2.1 ± 0.2</td>
<td>3.4 ± 0.7</td>
<td>8.4 ± 1.6</td>
<td>8.6 ± 0.6</td>
<td>9.0 ± 1.0</td>
</tr>
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Values are means ±SE. The highest doses of leptin were designed to bring circulating leptin levels during lactation back to the same levels observed in nonreproductive controls.
miniosmotic pumps as in experiment 1. In these animals, daily energy expenditure was measured between days 14 and 16 using the doubly labeled water (DLW) technique to estimate milk production.

**Energy Intake**

Food intake was measured in metabolic cages each day as described previously (47, 62). During each test, food and water were provided ad libitum. Food residues and feces were collected and then oven dried at 60°C to a constant mass and separated manually. Gross energy (GE) contents of food and feces were measured by a Parr 1281 oxygen bomb calorimeter (Parr Instruments). Gross energy intake (GEI), digestible energy intake (DEI), metabolizable energy intake (MEI), and digestibility (%) were then calculated according to the following equations (44): GEI (kJ/day) = dry matter intake (g/day) × GE content of food (kJ/g); DEI (kJ/day) = GEI (kJ/day) − (mass of feces (g/day) × GE content of feces (kJ/g)); MEI (kJ/day) = DEI × 98%; digestibility (%) = DEI/GEI × 100%.

**Daily Energy Expenditure and Milk Production**

We measured daily energy expenditure (DEE) using the DLW technique (8) on days 14 to 16 of lactation in animals from the second experiment. This method has been previously validated by comparison to indirect calorimetry in a range of small mammals including rodents (66) and provides an accurate measure of DEE over periods of several days. Daily variability in estimated energy metabolism suggests measurements spanning multiple 24-h periods may give a better estimate of average DEE (3, 72). Studies of lactating mammals suggest that recycling of isotopes between a mother and her offspring is negligible (60).

Lactating Brandt’s voles were weighed (±0.1 g) and injected subcutaneously with ~0.31 g of water containing enriched 18O (31.9/atom %) and 2H (19.0/atom %). Syringes were weighed before and after administration (± 0.0001 g) to calculate the mass of DLW injected. Blood samples (up to 70 µl by retroorbital sinus bleeding) were taken after 1 h of isotope equilibration to estimate initial isotope enrichments (39) and were also collected from unlabeled animals to estimate the background isotope enrichments (70). Blood samples were immediately heat sealed into 2×50 µl glass capillaries and stored at room temperature. A final blood sample was taken 48 h later (71) to estimate isotope elimination rates. Capillaries that contained the blood samples were then vacuum distilled, and water from the resulting distillate was used to produce CO2 (69) and H2 (38). The isotope ratios 18O:16O and 2H:1H were analyzed using gas source isotope ratio mass spectrometer (ISOCHROMMGas system and IsoPrime IRMS; Micromass, Manchester, UK). We ran three high-enrichment standards each day alongside the samples and corrected all the raw data to these standards.

Initial isotope dilution spaces (mole) were calculated by the intercept method and then converted to grams, assuming a molecular mass of body water of 18.020 and expressed as a percentage of body mass before injection. Final dilution spaces were inferred from the final body mass, assuming the same percentage of body water as measured for the initial dilution spaces. Isotope enrichments were converted to values of daily energy expenditure using a single pool model as recommended for animals under 10 kg (63). We assumed a fixed evaporation of 25% of the water flux (see equation 7.17 in Ref. 64), which minimizes error in a range of conditions (79–81). Energy equivalents of the rate of CO2 production were calculated using a conversion factor of 24.062 J·mol·1·CO2 derived from the Weir equation for a respiratory quotient of 0.85 (64). We used the DLW data to evaluate milk energy output, calculated from the difference between MEI and DEE (41). MEI and DEE were measured simultaneously on days 14 to 16 of lactation. This timing was based on the fact that previous studies have indicated that Brandt’s voles weaned their offspring completely at 18–21 days of lactation (90, 92). Subsequent to the present study, by partitioning total intake between the mother and her pups, we found that pups actually start to ingest food from day 12 of lactation, and maternal intake was already declining by days 14–16 (86). Animals appeared not to be affected by the DLW injections and bleeding, because their food intake and sucking behavior did not change.

**Maternal Body Composition**

After the 7 days of infusion in both experiments, the mothers were killed by CO2 overdose between 09:00 and 11:00 h. The interscapular BAT (IBAT) was immediately removed and dissected, weighed, and stored at −80°C until assayed. Blood samples were collected, clotted for 1 h, centrifuged at 4°C for 30 min at 4,000 g, and sera were then collected and stored at −80°C until assayed. After dissection of interscapular BAT, the visceral organs, including heart, liver, spleen, lung, kidneys, and gastrointestinal tract (containing contents) were dissected and weighed (±1 mg). The eviscerated carcass was weighed and then dried to constant weight at 60°C for determination of dry carcass mass. The difference between the wet carcass mass and dry carcass mass was the water mass of the carcass. Total body fat extraction was performed with a Soxtec Fat Extraction Systems (Soxtex Avanti 2050; Foss Analytical, Hoegaenae, Sweden), and then fat-free dry carcass mass was calculated from the difference between dry carcass mass and total body fat.

**BAT**

For measuring total mitochondrial protein and UCP-1 content, BAT was homogenized as described previously (94). Mitochondrial protein concentrations of BAT were determined by the Folin phenol method using bovine serum albumin as standard. BAT UCP-1 content was detected by Western blot analysis as described previously (45, 94). Briefly, interscapular BAT mitochondrial protein (20 µg per lane) was separated in a discontinuous SDS-polyacrylamide gel (12.5% running gel and 3% stacking gel) and blotted to a nitrocellulose membrane (Hybond-C, GE Healthcare, Piscataway, NJ). UCP-1 was detected by using a polyclonal rabbit anti-hamster UCP-1 (1:5000) (supplied by Dr. M. Klingenspor, Technische Universität München, Molecular Nutritional Medicine, Else Kröner-Fresenius Center, Freising-Weihenstephan, Germany) as a primary antibody, and goat anti-rabbit (1:5000) (ZSBG-BIO, Beijing, China) as the secondary antibody. Enhanced chemiluminescence (GE Healthcare) was used for detection. UCP-1 content was expressed as relative units, quantification of the blots was determined by using Quantity One Version 4.4.0 (Bio-Rad, Hercules, CA).

**Circulating Leptin Levels**

Serum leptin levels were measured by RIA with a 125I-labeled multispecies kit (cat. no. XL-85K; Linco Research, St. Charles, MO) (44, 94). The lower and upper limits of the assay kit were 1 and 50 ng/ml and the inter- and intra-assay variations were <8.7% and 3.6%, respectively. Serum leptin levels were determined in a single RIA in each experiment and expressed as nanograms per milliliter.

**Neuroendocrine Status**

Gene expression of several hypothalamic neuropeptides were measured as described previously (74). These hypothalamic neuropeptides were selected because they have been previously linked with regulation of food intake (18, 52, 61, 84) and have also been shown to be located in neurons that have the signaling function of the leptin receptor.

**Primer design.** To amplify partial cDNA fragments of Brandt’s voles, NPY, AgRP, POMC, cocaine- and amphetamine-regulated transcript (CART), and β-actin, the primers were designed based on the conserved regions of rat and mouse sequences available from GenBank. The fragments of Brandt’s voles, NPY, AgRP, POMC, CART, and β-actin, were attained by RT-PCR and sequenced. Homology analysis confirmed that these fragments from Brandt’s voles

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come from the target genes (74). Primers for real-time PCR were designed from the fragments of Brandt’s voles (78).

**Total RNA isolation and cDNA synthesis.** Total RNA was isolated from hypothalamus using Trizol reagent (Invitrogen), treated by RNase-free DNase I (Promega) for removing any possible DNA residue, and identified the RNA integrity by electrophoresis. Two micrograms of total RNA were transcribed using RT reagents (Fermentas, Glen Burnie, MD) in a 20-μl reaction volume. Total RNA 2 μg, and oligo(dt) 18 primer (0.5 μg/μl) 1 μl was added to diethylpyrocarbonate-treated water, and the final volume was 12 μl. The mixture was incubated at 70°C for 5 min, chilled on ice, and drops were collected by brief centrifugation. The tube was placed on ice, and the following components were added in order: 4 μl 5× reaction buffer, 1 μl Ribolock ribonuclease inhibitor (20 U/μl), 2 μl 10 mM dNTP mix. The mixture was incubated at 37°C for 5 min. Then 1 μl RevertAid M-MuLV reverse transcriptase (200 U/μl) was added. The mixture was incubated at 42°C for 60 min. The reaction was stopped by heating at 70°C for 10 min and then chilled on the ice.

**Real-time RT-PCR amplification.** Real-time PCR amplification was carried out at a final 12.5 μl volume, containing 6.25 μl SYBR Premix EX Tag (TaKaRa Biotechnology, Otsu, Shiga, Japan) 0.25 μl Rox, 0.25 μl 5 μM of each primer, 1 μl cDNA, and 4 μl deionized water. PCR amplification was performed in triplicate wells, using the following conditions: 10 s at 95°C, followed by 40 cycles consisting of 5 s at 95°C, 20 s at 60°C, and 20 s at 72°C. The fluorescence signals were read and collected at 72°C for each cycle. The real-time PCR was carried out on MX3000P Real-Time PCR Detection System (Stratagene). Melting curve analysis showed a single PCR product after amplification for four neuropeptides genes and β-actin, and ending products of PCR were further confirmed by DNA sequencing. We constructed standard curves for each gene via serial dilutions of cDNA (1- to 32-fold dilutions). Analysis of standard curves between target genes and β-actin showed that they had similar amplification efficiency, which ensures the validity of comparative relative quantity method. The quantities of gene mRNA was normalized with β-actin mRNA to compensate for possible variations from mRNA extraction and reverse transcription, and was then expressed as relative units.

**Statistics**

We compared the effects of the different leptin treatment groups in each experiment by using generalized linear modeling with day of lactation and litter size as covariates in the analysis. When an overall significant treatment effect was found, we probed the location of individual group differences using post hoc Tukey tests. We explored the relationships between continuous variables using linear regression analysis. In all cases, the data were tested for normality prior to analysis. Significance was set at \( P < 0.05 \).

**RESULTS**

**Circulating Leptin Levels**

The circulating levels of leptin at the end of the infusion period in the implanted animals in experiment 1 were not linearly connected to the administered dose (Table 1). The circulating leptin levels in the animals at the lowest dose were not significantly elevated above those observed in the PBS-treated animals. The circulating levels in the highest three dose groups were significantly higher than the PBS and lowest-dose group (\( P < 0.01 \)), but were not significantly different from each other (\( P > 0.05 \), and all averaged ~8 to 9 ng/ml). The levels of circulating leptin at the end of the infusion period in experiment 2 were significantly different (ANOVA; \( F = 15.7, P = 0.01 \)) between the PBS (mean = 1.5 ng/ml, SD = 1.3, \( n = 13 \)) and leptin-treated animals (mean = 15.8, SD = 13.0, \( n = 11 \)), but were extremely variable in the latter group. For comparison, the circulating leptin levels in nonreproductive animals averaged 7.1 ng/ml (mean ± SE = 0.5, \( n = 8 \) ) (91).

**Maternal Body Mass**

In experiment 1, female body masses were not significantly different between the control and leptin treatment groups between days 0 and 10 of lactation, prior to pump implantation, and also did not vary significantly with day of lactation or with litter size (day effect: \( F = 0.26, P = 0.97 \); group effect: \( F = 1.3, P = 0.27 \); litter size effect: \( F = 0.27, P = 0.60 \) ) (Fig. 1A). The mean female body weight over the first 10 days of lactation was 56.6 g (SD = 5.3, \( n = 30 \)). Following pump implantation, all of the treatment groups decreased in body mass. During this period, the body masses of the females were significantly related to the day of treatment (\( F = 5.98, P < 0.01 \)) and to the treatment group (\( F = 7.75, P < 0.01 \) ) (Fig. 1A) but there was no effect of litter size (\( F = 0.74, P = 0.39 \) ) and no day by treatment interaction (\( P > 0.05 \) ) (Fig. 1B). By day 17, at the end of infusions, the mass losses of the groups over the infusion period averaged 2.4 g (SD = 1.8) in the PBS group (4.6% of initial weight); 7.45 g (SD = 3.1), 13.4% of initial weight in the lowest dose leptin group; and 8.7 g (SD = 3.9), 10.2 g (SD = 2.3), and 9.7 g (SD = 2.6) in the three highest dose leptin treatment groups, respectively (ANOVA; \( F = 7.5, P < 0.001 \) ). These losses amounted to 15.6, 18.5, and 17.2% of their initial weights at the start of the infusion period. Pairwise comparisons using a post hoc Tukey test revealed that all the

![Graph A](http://ajpregu.physiology.org/)

**Fig. 1.** A: mean body mass (g) (means ± SE) of lactating female Brandt’s voles throughout lactation in experiment 1 separated into groups that were infused with leptin from day 10 onward. The broken line shows the day of surgery. PBS-treated group (●). Treatment groups 1–4 of increasing leptin concentration (○, ◊, □, △, respectively). **a** Values that do not share a common superscript letter differ significantly at \( P < 0.05 \), as determined by generalized linear modeling (GLM) and Tukey post hoc tests.

B: body mass loss between days 10 and 17 for individual lactating female Brandt’s voles infused with leptin between days 11 and 17 in relation to level of circulating leptin on day 17 of lactation.
mass losses of the groups treated with leptin were significantly different to the mass loss of the PBS-treated group, but that the differences between different leptin-dosed groups were not significant (P > 0.05). Nevertheless, individual mass loss was significantly related to the circulating leptin level at the end of the infusion period (regression: $r^2 = 0.45$, $F = 23.1$, $P < 0.001$). This relationship remained significant if the PBS group was excluded from the analysis (Fig. 1B: regression: $r^2 = 0.24$, $F = 7.17$, $P = 0.014$).

The pattern of maternal mass change in experiment 2 was similar to that observed in experiment 1 (Fig. 2A). Over the first 10 days of lactation there was no significant effect of day of lactation ($F = 0.2$, $P = 0.99$) or treatment group ($F = 0.09$, $P = 0.76$), but in this experiment, there was a significant relationship between maternal mass and litter size ($F = 7.28$, $P = 0.008$). None of the interactions between variables were significant (P > 0.05). Following pump implantation, both PBS and leptin treatment groups of animals lost mass (day effect: $F = 2.2$, $P = 0.04$), and the effect of litter size remained significant ($F = 41.6$, $P < 0.001$). The effect of treatment (PBS vs. leptin) was also highly significant ($F = 8.9$, $P < 0.01$). On average, the mass loss over the 7 days of the PBS-treated animals was 4.4 g (SD = 2.2, n = 14), while the leptin-treated animals lost on average 10.2 g (SD = 3.6, n = 12) (ANOVA; $F = 25.7$, $P < 0.001$). Post hoc Tukey tests revealed that the effects of treatment were significant from day 3 of treatment onward. Including all animals, there was a significant negative relationship between individual mass loss and the circulating leptin levels at the end of the experiment (Fig. 2B) (all individuals, regression: $r^2 = 0.61$, $F = 34.2$, $P < 0.001$), but this relationship marginally failed to reach significance if only the individuals that were treated with leptin were included ($F = 4.55$, $P = 0.062$).

Body composition analysis of the mothers on day 17 revealed that the greater losses in mass of the leptin-treated animals were contributed to by several effects (Table 2 and 3). In the experiment 1, we did not measure the full gut mass. All the other treatment effects on organ masses were minor and did not individually reach statistical significance. In experiment 2 (Table 3), the most significant difference between treatment and control groups was in the full gut mass. In leptin-treated animals, this averaged 7.4 g compared with 10.0 g in the controls ($F = 7.8$, $P = 0.01$). The difference (2.6 g) accounted

Table 2. Body composition of female voles at the end of lactation (day 17) in relation to dosage treatment with leptin over last 7 days of lactation (experiment 1)

<table>
<thead>
<tr>
<th>Group</th>
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<tr>
<td>Body mass, g</td>
<td>54.2 ± 3.2</td>
<td>46.7 ± 2.4</td>
<td>48.1 ± 3.3</td>
<td>45.0 ± 1.5</td>
<td>46.9 ± 2.0</td>
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<td>Wet carcass mass, g</td>
<td>32.19 ± 1.17</td>
<td>28.69 ± 1.34</td>
<td>30.43 ± 2.06</td>
<td>29.10 ± 0.98</td>
<td>29.70 ± 0.96</td>
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<td>Dry carcass mass, g</td>
<td>11.58 ± 0.91</td>
<td>10.41 ± 0.66</td>
<td>11.11 ± 1.07</td>
<td>10.78 ± 0.61</td>
<td>10.59 ± 0.41</td>
</tr>
<tr>
<td>Dry lean mass, g</td>
<td>7.57 ± 0.25</td>
<td>6.99 ± 0.18</td>
<td>7.37 ± 0.30</td>
<td>7.16 ± 0.18</td>
<td>7.07 ± 0.26</td>
</tr>
<tr>
<td>Dry fat, g</td>
<td>4.01 ± 0.80</td>
<td>3.42 ± 0.53</td>
<td>3.74 ± 0.82</td>
<td>3.62 ± 0.49</td>
<td>3.51 ± 0.35</td>
</tr>
<tr>
<td>Body water, %</td>
<td>64.3 ± 1.6</td>
<td>63.8 ± 1.3</td>
<td>63.8 ± 1.2</td>
<td>63.1 ± 0.9</td>
<td>64.3 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. None of the parameters was significantly related to treatment group. For treatment group details refer to Table 1.

*Fig. 2. A: mean body mass (g) (means ± SE) of lactating female Brandt’s voles throughout lactation in experiment 2 separated into groups that were infused with leptin from day 10 onward (●) and those infused with PBS (○). *P < 0.05. The broken line shows the day of surgery. B: body mass loss between days 10 and 17 for individual lactating female Brandt’s voles infused with leptin (●) or PBS (○) between days 11 and 17 in relation to level of circulating leptin on day 17 of lactation.*
Table 3. Body composition and wet organ masses of voles at the end of lactation (day 17) from experiment 2 for voles infused with PBS or leptin over the last 7 days of lactation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PBS, n = 14</th>
<th>Leptin, n = 12</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, g</td>
<td>55.8 ± 2.6</td>
<td>49.9 ± 2.3</td>
<td>0.098</td>
</tr>
<tr>
<td>Wet carcass mass, g</td>
<td>36.3 ± 1.8</td>
<td>33.8 ± 1.7</td>
<td>0.337</td>
</tr>
<tr>
<td>Dry carcass mass, g</td>
<td>15.1 ± 1.0</td>
<td>13.4 ± 1.1</td>
<td>0.264</td>
</tr>
<tr>
<td>Dry fat, g</td>
<td>7.0 ± 0.8</td>
<td>5.6 ± 0.8</td>
<td>0.241</td>
</tr>
<tr>
<td>Dry fat, %</td>
<td>18.7 ± 1.5</td>
<td>16.0 ± 1.6</td>
<td>0.238</td>
</tr>
<tr>
<td>Body water mass, g</td>
<td>21.2 ± 1.1</td>
<td>20.4 ± 0.7</td>
<td>0.577</td>
</tr>
<tr>
<td>Body water, %</td>
<td>58.7 ± 1.6</td>
<td>61.1 ± 1.5</td>
<td>0.293</td>
</tr>
<tr>
<td>Organ masses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAT, g</td>
<td>0.176 ± 0.025</td>
<td>0.126 ± 0.023</td>
<td>0.161</td>
</tr>
<tr>
<td>Heart, g</td>
<td>0.241 ± 0.009</td>
<td>0.236 ± 0.012</td>
<td>0.752</td>
</tr>
<tr>
<td>Liver, g</td>
<td>2.874 ± 0.217</td>
<td>2.286 ± 0.158</td>
<td>0.044</td>
</tr>
<tr>
<td>Spleen, g</td>
<td>0.055 ± 0.004</td>
<td>0.048 ± 0.005</td>
<td>0.348</td>
</tr>
<tr>
<td>Lung, g</td>
<td>0.321 ± 0.024</td>
<td>0.298 ± 0.017</td>
<td>0.441</td>
</tr>
<tr>
<td>Kidney, g</td>
<td>0.520 ± 0.015</td>
<td>0.490 ± 0.016</td>
<td>0.198</td>
</tr>
<tr>
<td>Gut, g</td>
<td>10.035 ± 0.686</td>
<td>7.420 ± 0.622</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Values are means ± SE. Note that gut includes all the gut contents.

for 30% of the total weight difference. The livers of the leptin-treated animals were on average 0.6 g lighter ($F = 4.54$, $P = 0.044$). The mean difference in wet carcass mass accounted for an additional 2.5 g of the overall group difference, while dry fat mass was 1.4 g lower in the leptin treatment group, although in these latter two cases the treatment effect was not significant. The remaining difference in overall mass (0.9 g) was contributed to by relatively minor nonsignificant effects in individual organ masses and overall water content (0.8 g) (Table 3).

Maternal Food and Energy Intake

In experiment 1 maternal food intake increased significantly from on average 12.7 g (SD = 2.9, $n = 30$) on day 2 of lactation to 16.9 g (SD = 3.0) on day 10 (Fig. 3A) (day effect: $F = 13.5$, $P < 0.01$) (Fig. 3A). The food intake was equivalent to a gross energy intake of 206.2 kJ/day (SD = 47.1) on day 2 and 274.4 kJ/day (SD = 48.7) on day 10. MEI values were 128.4 (SD = 29.3) and 170.9 kJ/day (SD = 30.3), respectively. Digestibility remained stable at ~62% throughout this period. Maternal food intake during the first 10 days of lactation was strongly affected by female body mass ($F = 71.8$, $P < 0.001$) and litter size ($F = 6.67$, $P = 0.01$). Unfortunately, once these effects had been taken into account there was also a significant effect of the groups to which the animals were randomized for leptin treatment ($F = 9.05$, $P < 0.01$). We could not anticipate this would be the case in advance and, consequently, had to retrospectively correct for it statistically. We adjusted the food intake of the different treatment groups following pump implantation (days 11 to 17) by subtracting the group average food intakes over the first 10 days of lactation. The (adjusted) food intake following pump implantation (Fig. 3A) was significantly related to the day of treatment ($F = 13.6$, $P < 0.001$), the litter size ($F = 20.8$, $P < 0.001$), and the treatment group ($F = 5.9$, $P < 0.001$: Fig. 3B), but there was no significant day by treatment interaction ($F = 0.62$, $P = 0.92$). (Note: the effect of treatment group on food intake without adjustment was even more significant: $F = 16.5$, $P < 0.001$). Adjusted food intake levels were negatively related to the leptin dose group (Fig. 3B) ($F = 5.74$, $P < 0.01$). However, pairwise comparisons revealed only the highest dose effect was significantly different to the PBS treatment (Fig. 3B).

In experiment 2 the daily food intake increased, on average, from 10.7 g (SD = 2.9) on day 2 of lactation to 15.3 g (SD = 3.2) on day 10 (day effect: $F = 4.03$, $P < 0.001$) (Fig. 3C). Food intake was also significantly related to the litter size ($F = 55.6$, $P < 0.01$; Fig. 3D) with those raising larger litters eating more food, but there was no significant effect of the treatment group ($F = 0.02$, $P = 0.976$). This intake was equivalent to a gross energy intake of 174.5 kJ/day (SD = 47.3) on day 1 to

Fig. 3. A: daily food intake (g/day) (means ± SE) of lactating female Brandt’s voles throughout lactation in experiment 1 separated into groups that were infused with leptin from day 10 onward. The broken line shows the day of surgery. PBS-treated group (○). Treatment groups 1–4 of increasing leptin concentration (△, ▽, ▼, □, respectively). For full details of treatment groups see Table 1 ($n = 6$ per group). B: mean adjusted daily food intake over last 7 days of lactation (g/day) (means ± SE) in relation to leptin treatment group in experiment 1. C: mean daily food intake (g/day) (means ± SE) of lactating female Brandt’s voles throughout lactation in experiment 2 separated into groups that were infused with leptin from day 10 onward (●) and those infused with PBS (○). The broken line shows the day of surgery. D: mean food intake (g/day) (means ± SE) over the last 7 days of lactation in relation to litter size in experiment 2. *$P < 0.05$. **Values do not share a common superscript letter differ significantly at $P < 0.05$, as determined by GLM and Tukey post hoc tests.
248.9 kJ/day (SD = 51.3) on day 10. Energy digestibility over the first 10 days of lactation averaged 59.8% (SD = 4.1) and was independent of the day (P > 0.05) and the treatment group (P > 0.05) but was significantly negatively related to the litter size (F = 15.48, P < 0.001). In general, the voles raising larger litters digested less of their gross intake; however, this effect was insufficient to offset their significantly greater gross intake. Over the first 10 days of lactation, the metabolizable energy intake therefore increased from, on average, 110.5 kJ/day (SD = 37.5) for voles raising litters of five to 160.8 kJ/day (SD = 31.8) for those raising litters of nine. Averaged across all litters, the MEI increased from 101.1 kJ/day (SD = 31.3) on day 2 to 146.8 (SD = 29.5) on day 10 of lactation. Following pump implantation, the food intake initially declined and then increased (Fig. 3C; day effect: F = 13.1, P < 0.001). There was a significant effect of litter size (F = 98.1, P < 0.001) and a significant effect of the treatment group (F = 6.6, P = 0.011) on the food intake. On average, across the 7 days of treatment the PBS-treated animals ate 17.6 g/day (285.5 kJ/day), compared with 16.2 g/day (263.1 kJ/day) in the leptin-infused animals, a reduction of 7.8%. Contrasting the period of early lactation, prior to pump implantation, the digestibility following leptin infusion was not related to the litter size (F = 0.82, P = 0.36) nor treatment group (F = 3.35, P = 0.069) but did vary significantly with the day of infusion (F = 3.58, P = 0.032), increasing from 62.7% (SD = 3.0) on day 11 to 65.4% (SD = 7.5) on day 16 before falling back to 61.5% (SD = 5.5) on day 17. Metabolizable energy intake following pump implantation was significantly related to the day of infusion (F = 10.8, P < 0.001), the litter size (F = 87.6, P < 0.001), and the treatment group (F = 4.3, P = 0.04). Post hoc Tukey tests revealed that food intake in the leptin-treated animals was significantly lower than that of the PBS-infused animals on days 3 to 6 of infusion. Over the whole 7 days of infusion, the intake of the PBS-treated group averaged 176.5 kJ/day (SD = 59.1), while the intake of the leptin-treated animals averaged 166.3 kJ/day (SD = 60.2), a reduction of 5.8%. The accumulated shortfall of metabolizable energy intake in the leptin-treated animals over the 7 days of infusion averaged 71.4 kJ.

**Pup Growth**

In experiment 1 during the first 10 days of lactation, the litter mass increased from an average of 21.9 g (SD = 2.9, n = 30) on day 1 to 49.74 g (SD = 5.0, n = 30) on day 10 (day effect: F = 467.17, P < 0.001) (Fig. 4A). Litter mass was also strongly dependent on litter size (F = 284.1, P < 0.001), but there was no litter size by day interaction (P > 0.05). As with food intake, once these effects had been accounted for there was also an effect of treatment group (F = 17.6, P < 0.01). So for analysis of the treatment effects, we adjusted the masses of the litters by subtracting the treatment group averaged pup masses after accounting for the effects of day and litter size.

Following pump implantation, the pups continued to grow from an average of 55.2 g (SD = 5.9, n = 30) on day 11 of lactation to 80.2 g (SD = 11.3, n = 30) on day 17 (day effect: F = 54.9, P < 0.001). There was again a significant effect of litter size (F = 81.4, P < 0.001) but no litter size by day interaction (P > 0.05). After adjustment for the treatment group effects prior to implantation (see above), there remained a small but significant treatment effect on litter mass (F = 3.7, P = 0.006). The effect however, was not related to the dose of leptin in any obvious fashion (Fig. 4B). Using the unadjusted data produced a very similar result.

In experiment 2 between days 0 and 10, the average litter mass increased from 23.2 g (SD = 5.8) to 50.9 g (SD = 14.4) (day effect: F = 16.0, P < 0.001) (Fig. 4C), but litter mass was independent of the treatment group (F = 1.45, P = 0.23). Following pump implantation, the litters continued to grow...
from on average 53.9 g on day 11 (SD = 15.2) to 83.6 g on day 17 (SD = 29) (day effect: \( F = 18.6, P < 0.001 \)) (Fig. 4C). There was a significant effect of litter size on litter mass gain between days 10 and 17 (\( F = 3.36, P = 0.029 \)) with the litters of five averaging an increase of 22.8 g over the 7 days (SD = 10.5), but litters of nine averaging an increase of 47.8 g (SD = 19.2) over the same period. There was no significant difference between the treatment groups (\( F = 0.21, P = 0.65 \)) (Fig. 4D) in mass gain over days 10 to 17, with leptin-treated mothers raising litters that increased by, on average, 34.7 g (SD = 16.6) compared with an increase of 31.8 g (SD = 16.0) for litters raised by PBS-treated mothers.

**Daily Energy Expenditure and Milk Production**

Daily energy expenditure data by DLW were available for only nine of the control and seven of the treatment animals. On average, the energy demands of the control PBS-treated animals averaged 122.3 kJ/day (SD = 14.3) and that of the treatment group averaged 115.6 kJ/day (SD = 11.5) (treatment effect: \( F = 0.3, P > 0.05 \)). Hence leptin treatment had no significant effect on energy expenditure. There was also no impact of litter size on DEE (regression: \( F = 0.38, P > 0.05 \)) but energy demands were positively related to maternal body mass on day 15 of lactation in the middle of the DLW measurement [Fig. 5A; regression: \( r^2 = 0.354, F = 7.66, P = 0.05 \); equation: DEE (kJ/day) = 1.78*body mass (g) + 21.1]. Combining the data on MEI and DEE we estimated the milk energy output of the females. There was no significant difference in milk energy output due to the leptin treatment (\( F = 0.18, P = 0.67 \)) or maternal body mass on day 15 (\( F = 1.52, P = 0.25 \)), but milk energy output was positively related to litter size [Fig. 5B; regression: \( r^2 = 0.34, F = 6.17, P = 0.029 \); equation: milk energy output (kJ/day) = 22.9*litter size (n) = 96.7].

**Maternal Neuroendocrine Status**

Leptin treatment had profound effects on the gene expression of key neuropeptides in the hypothalamus that have been previously linked to food intake. In experiment 1 hypothalamic gene expression of NPY decreased with increasing leptin dosage treatment group (\( F = 4.04, P = 0.002 \)) (Fig. 6A). In the highest dosage group, the suppression of NPY relative to the PBS-treated group was 70%. Post hoc Tukey tests revealed that intake in the highest dose group was significantly lower than in the PBS-treated or the lowest-dose leptin-treated group (\( P < 0.01 \)). At the individual level (either including or excluding the PBS group) there was a significant negative effect of the circulating leptin level at the end of the infusion period on the expression of NPY (with PBS excluded: \( F = 7.7, P = 0.011 \); Fig. 6B). Hypothalamic gene expression of AgRP was also reduced by leptin treatment (ANOVA: \( F = 5.45, P = 0.003 \)), but in this case, the pattern of gene expression was different (Fig. 6C). Post hoc Tukey tests revealed that expression was significantly reduced in all leptin treatment groups relative to PBS (all pairwise comparisons: \( P < 0.01 \)), but none of the treatment groups differed significantly from each other (\( P > 0.05 \)). Moreover, at the individual level, there was no significant relationship between circulating leptin levels and gene expression of AgRP (excluding the PBS-treated animals: \( P > 0.05 \)). For the anorexogenic neuropeptides there was a significant increase in expression of POMC in the hypothalamus in relation to leptin treatment (Fig. 6D) (ANOVA; \( F = 4.08, P = 0.011 \)). Post hoc pairwise comparisons (Tukey) revealed that the expression in the highest two leptin groups were significantly greater than the PBS-treated animals, and, on average, the expression in these two groups was, respectively, 2.6× and 2.5× the level in the PBS-treated animals. At the individual level (including or excluding the PBS-treated animals) there was a significant relationship between circulating leptin levels and expression of POMC (excluding the PBS group: \( r^2 = 0.37, F = 13.0, P < 0.01 \); Fig. 6E). There was no significant relationship between gene expression of CART in the hypothalamus and leptin treatment (Fig. 6F; ANOVA; \( F = 1.1, P > 0.05 \)).

**Maternal UCP-1 Protein Content in BAT**

In experiment 1 the level of UCP-1 protein relative to the mitochondrial protein levels in BAT was significantly positively related to leptin dose group (Fig. 7). There was also a positive association at the individual level between the circulating leptin levels and UCP-1 protein levels in BAT when the PBS-treated group was included into the analysis (regression: \( r^2 = 17.6, F = 5.97, P = 0.021 \)), but this was not significant if the PBS group was excluded (\( F = 0.71, P = 0.409 \)).
DISCUSSION

The patterns of food intake, maternal body mass, and litter mass during early lactation were consistent with previous studies of lactation in the Brandt’s vole (86, 90, 91). Consistent with the hypothesis that reduced body fatness in lactation serves to suppress leptin levels, which then drives the lactation hyperphagia, we found significant effects of the experimentally increased leptin levels during late lactation on the levels of several neuropetides in the hypothalamus (Fig. 6). These hypothalamic neuropetides have been previously linked with regulation of food intake (52, 61, 84) and are located in neurons that have the signaling form of the leptin receptor (50, 51). Both NPY and AgRP have both been shown previously to strongly stimulate food intake (6, 22), while the POMC and cocaine- and amphetamine-regulated transcript have a well-established strong suppressive effect on food intake (34, 78). Moreover, it has been shown that during lactation NPY and AgRP are both upregulated, while POMC is downregulated relative to nonreproducing controls (11, 14, 19, 43, 57). Consistent with some previous studies, we found that peripheral infusion of animals with recombinant murine leptin had significant effects on the gene expression levels of these key neuropetides: NPY, AgRP, and POMC. However, contrasting the previous suggestion that CART is also regulated by leptin levels (34), we found no impact of leptin repletion on hypothalamic CART gene expression in lactating voles. In addition, in one previous study, peripherally infused leptin in lactating rats had no effect on NPY or AgRP levels (89), but did recover POMC gene expression in the arcuate nucleus (89), similar to our data. Based on the established impacts of these neuropetides on food intake, the large changes observed in NPY, AgRP, and POMC neuropetides, in response to the leptin treatment, would have been anticipated to cause suppression of food intake and a reduction in body mass.

Consistent with this expectation, we recorded a significant suppression of MEI by ~10 kJ/day, compared with the average late lactation MEI of 150–170 kJ/day. On average, the effect of the leptin treatment, and its consequent effects on the neuropetide gene expression in the hypothalamus, resulted in a reduction of metabolizable energy intake by 7.8%. In comparison, the MEI of nonreproductive voles amounts to ~86 kJ/day (46, 90, 91). These data therefore suggest that ~16% of the increase in energy intake at peak lactation in the Brandt’s vole might be attributed to their suppression of leptin levels brought about primarily by the reduction in body fatness. In comparison, the energy in the fat that is withdrawn during lactation contributes ~1–2% of the lactational energy requirements.

Fig. 6. Hypothalamic gene expression of neuropeptide Y (NPY; A), agouti-related peptide (AgRP; C), proopiomelanocortin (POMC; D), and [cocaine- and amphetamine-regulated transcript (CART); F] of lactating female Brandt’s voles on day 17 of lactation in experiment 1 separated into groups that were infused with leptin from day 10 onward. In all plots, mean expression is expressed relative to the expression of PBS-infused animals (group 0). For full details of treatment groups see Table 1 (n = 6 per group). In B and E individual levels of hypothalamic gene expression for NPY and POMC (normalized with β-actin) are expressed in relation to the circulating leptin levels on day 17 of lactation. *Values that do not share a common superscript letter differ significantly at $P < 0.05$ as determined by 1-way ANOVA and Tukey post hoc tests.

Fig. 7. Relative protein content of uncoupling protein-1 (UCP-1) in brown adipose tissue (BAT) of lactating female Brandt’s voles on day 17 of lactation in experiment 1 separated into groups that were infused with leptin from day 10 onward. Mean protein levels are expressed relative to the expression of PBS-infused animals (group 0). For full details of treatment groups see Table 1 (n = 6 per group). *Values that do not share a common superscript letter differ significantly at $P < 0.05$, as determined by 1-way ANOVA and Tukey post hoc tests.
The finding here is therefore consistent with the suggestion that the primary role for reducing body fatness in lactation is not to directly provide fuel for the lactation process, but instead to reduce circulating leptin levels, which then modulate hypothalamic gene expression contributing to the hyperphagia of lactation (65).

While our data are consistent with this idea, it is clear from our data that reduced leptin and the consequent large changes in hypothalamic gene expression of NPY, AgRP, and POMC contributed in only a relatively minor way to the total increase in food intake observed during lactation. This small overall effect was consistent with that reported in previous studies that have infused lactating rats with leptin. Stocker et al. (73) fed rats on a low-protein-content diet (8% protein wt/wt) and infused them from day 14 of pregnancy until day 21 of lactation with leptin at a dose of 2 µg·g⁻¹·day⁻¹. Although food intake was significantly reduced in pregnancy by the leptin treatment (by ~20%), there was no effect on food intake in lactation relative to females fed the same diet and infused with saline. Similarly, Xu et al. (89) found that infused leptin also had no effect on food intake in lactating rats, despite effects on arcuate nucleus gene expression of POMC. However, in this latter study, the leptin was only infused for 2 days, and at an earlier stage of lactation. In our study, a significant effect of leptin on food intake was only apparent after 3 days of infusion at peak lactation. Consequently, the complete absence of an effect of leptin in that experiment may simply have been because the infusion was too short or the time it was administered was too early or both. This may also explain why more widespread effects on hypothalamic gene expression were not observed in that study either (89). However, differences in response between species may also reflect the large diversity that is apparent between small rodents. In general, studies of small rodents have tended to assume that their responses are homogenous when, in fact, there is wide variation among species with respect to the amount of fat that they store, the number of pups that they nurse, and the length of time during which the mother is the primary source of nutrients for the offspring. It is unlikely, therefore, that studies in one species can be considered indicative of responses across the group as a whole. These data therefore emphasize the importance of additional studies being performed in the future across a range of small mammals, in addition to the mouse and rat.

Together these data suggest that other neuronal pathways, apart from the classical leptin signaling pathway via the arcuate nucleus, must mediate the major part of the hyperphagic response in lactation, as also suggested elsewhere (89). One suggested potentially critical component driving lactational hyperphagia is the sustained levels of NPY in the dorsomedial nucleus, and the role of peripheral gonadotropin-releasing hormone (89). An additional potential candidate involved in lactation hyperphagia is prolactin, since prolactin is elevated in lactation and is known to stimulate food intake (20, 21, 85). However, it seems likely many other factors are also involved, such as orexins, growth hormone, and melanocortin receptors (7, 10–15, 59, 88), and peak lactation with its very high level of food intake may provide a useful model for dissecting the processes that regulate food intake more generally.

Although small, the magnitude of the effects of leptin treatment on intake and body mass during lactation were similar to the effects of leptin infusion on nonreproductive individuals. Short-tailed field voles (Microtus agrestis) kept in short photoperiods and infused with murine leptin for 7 days, using the same miniosmotic pumps that we used but at a slightly higher dosage rate (0.87 µg·g⁻¹·body mass⁻¹·day⁻¹) decreased their food intake by 0.35 g/day, which was an average of 5.2% lower than their preinfusion intake and 5.7% lower than the intake of voles simultaneously infused with PBS (35). There was no suppression of food intake at all in voles exposed to long photoperiods (35). For comparison, the breeding voles in our experiment were also in a long (16 h)-day photoperiod. In arctic ground squirrels (Spermophilus parryii) infusion of 0.45–0.85 µg·g⁻¹·body mass⁻¹·day⁻¹ resulted in a blunting of the hyperphagia that precedes hibernation by ~10% (5, 54). Atcha et al. (2) found a profound (~75% reduction) but very short-lived (1 day) effect of infused leptin on food intake in Siberian hamsters (Phodopus sugorovii). Overall, there was no significant effect of leptin infusion on the energy intake over the entire 14 days of infusion, although the effect in females was close to significance (P = 0.06). In contrast, Klingenspor et al. (32) reported a significant 14% reduction in food intake, independent of photoperiod for Siberian hamsters injected twice per day at a much higher dose of 6.5 µg·g⁻¹·body mass⁻¹·day⁻¹. In cold-exposed rats, leptin infusion at 0.1 µg·g⁻¹·body mass⁻¹·day⁻¹ for 7 days resulted in a 8% decrease in food intake (1). In lean mice, leptin infusion either peripherally or centrally depressed food intake and prevented the reduction in expenditure that normally accompanies reduced intake, thereby leading to reduced body weight (23). However, in mice fed a high-fat diet, peripheral injections or infusions and central leptin injections had no effect on food intake but did reduce body weight (4, 23, 27). In rats, a single intracerebroventricular injection of recombinant adeno-associated virus vector encoding the leptin gene in virgin female rats resulted in a lowered body weight and food intake throughout the subsequent pregestational, gestation, and lactation periods. The food intake was suppressed by ~25% during lactation, although the increase in leptin gene expression in the hypothalamus using this manipulation was considerably higher than in control animals (42).

During lactation, female rodents also suppress gene expression of UCP-1 and UCP-3 (55, 75–77, 87) in their BAT. This effect has also been previously confirmed for UCP-1 protein levels in the BAT of Brandt’s voles (45, 91). Our study suggests that the reduced leptin levels in lactation may be a primary factor causing this downregulation, because repletion of leptin significantly reversed the lactational suppression (Fig. 7) as also observed in other lactating rodents infused with leptin during lactation (87). Whether this was a direct effect of leptin on the BAT or an indirect effect via the brain, is presently uncertain. Some neurons in the arcuate nucleus of the hypothalamus project directly to the spinal cord, where they innervate preganglionic neurons that control sympathetic nervous system outflow (17), thereby potentially regulating UCP-1 activity (9), and since we have reported direct effects of the infusion on hypothalamic gene expression, an effect on UCP-1 via the brain is possible, in addition, or instead of, a direct peripheral effect mediated via leptin receptors on BAT. Reduced body fat and hence lowered circulating leptin may then not only play a role in stimulation of food intake in lactation, but also the inhibition of UCP-1 in BAT. This could have two benefits. First, it might spare energy usage that can be
devoted to lactation. A second effect, however, is that it may curb heat production in BAT that would constrain reproductive performance because of the heat dissipation limit on lactational food intake (37, 40, 67). Neither of these effects of UCP-1 suppression were supported in our data by an effect of leptin infusion on daily energy expenditure or a suppression of milk output. However, our sample size in this respect was small, and we probably had insufficient power to detect a subtle effect. Finally, although the mothers treated with leptin had significantly reduced food intake, the growth of their offspring was unaffected by the leptin treatment. This was because the animals supplemented the slightly reduced intake of energy following leptin infusion by withdrawing energy storage in their bodies. Hence, their milk energy output was unaffected by the treatment.

Perspectives and Significance

During lactation in small rodents, food intakes increase enormously above the levels observed in individuals that are not breeding. Our understanding of the hormonal and neuroendocrine factors that drive these very high levels of intake is improving, but it is still relatively basic. During lactation, female small rodents often withdraw almost all their body fat. Previous interpretations of this phenomenon have revolved around the suggestion that the energy from the withdrawn fat contributes to milk production. Quantification of this contribution, however, indicates it is trivial, hence an alternative interpretation is that by reducing fat levels, animals reduce the circulating leptin levels that are then permissive for the extreme hyperphagia. We investigated this hypothesis by repleting leptin levels in lactating Brandt’s voles and showed that ~16% of the increased intake could be attributed to decreased leptin levels. Future work needs to focus on the factors that drive the remaining 84% of the lactational hyperphagia. In particular, this is driven by altered circulating levels of additional adipokines that are changed when lactating animals withdraw their fat or is it due to additional currently unknown mechanisms? Discovering these mechanisms may be important in the context of the obesity epidemic because in nonbreeding individuals these mechanisms must be largely suppressed. Failure to completely suppress such mechanisms may then be part of the genetic contribution to the overconsumption that leads to obesity.

ACKNOWLEDGMENTS

We thank Dr. Paula Redman and Peter Thomson for assistance with isotope analyses, and Xiu-Ping Wang for help in taking care of the animals. We are grateful to all the members in the Animal Physiological Ecology Group for discussion and their help during the experiment.

GRANTS

This study was financially supported by the National Natural Science Foundation of China Grant 30025009, the National Basic Research Program of China Grant 2007BC109103, and the Chinese Academy of Sciences Grant KSCX2-YW-N-06 (to D.-H. Wang), by Natural Environment Research Council Grant NE/C004159/1 (to J. R. Speakman), and by the International Joint-Project of National Natural Science Foundation of China and Royal Society (to D.-H. Wang and J. R. Speakman).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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