Role of hypoleptinemia during cold adaptation in Brandt’s voles
(Lasiopodomys brandtii)

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Submitted 30 March 2009; accepted in final form 31 August 2009

Tang G-B, Cui J-G, Wang D-H. Role of hypoleptinemia during cold adaptation in Brandt’s voles (Lasiopodomys brandtii). Am J Physiol Regul Integr Comp Physiol 297: R1293–R1301, 2009. First published September 2, 2009; doi:10.1152/ajpregu.00185.2009.—Brandt’s voles Lasiopodomys brandtii exhibit large increases in non-shivering thermogenesis to cope with chronic cold exposure, resulting in compensatory hyperphagia and fat mobilization. These physiological events are accompanied by a remarkable reduction in serum leptin levels. However, the role of hypoleptinemia in cold adaptation in this species is still unknown. In the present study, we tested the hypothesis that hypoleptinemia contributes to increases in food intake and brown adipose tissue (BAT) thermogenesis by modifying hypothalamic neuropeptides in cold-exposed Brandt’s voles. Adult male voles were transferred to 5°C for 28 days. Accompanied by a decrease in serum leptin levels, hypothalamic agouti-related protein (AgRP) mRNA levels were significantly increased, but there were no changes in the long form of leptin receptor (Ob-Rb), suppressor of cytokine signaling 3 (SOCS3), neuropeptide Y (NPY) mRNA, proopiomelanocortin (POMC), and cocaine- and amphetamine-regulated peptide (CART) mRNA levels in the hypothalamus. When cold-exposed voles were returned to warm (23°C) for 28 days, body mass, food intake, serum leptin, and AgRP mRNA were restored to control levels. Leptin administration in cold-exposed voles decreased food intake as well as hypothalamic AgRP mRNA levels. There were no significant effects of leptin administration on hypothalamic Ob-Rb, SOCS3, NPY, POMC, CART mRNA, and uncoupling protein 1 levels under cold conditions. These results suggest that hypoleptinemia partially contributes to cold-induced hyperphagia, which might involve the elevation of hypothalamic AgRP gene expression.

Peptidergic neurons within the hypothalamus produce a number of neuropeptides, which can be divided into two categories: 1) orexigenic neuropeptides, such as neuropeptide Y (NPY) and agouti-related protein (AgRP), which can stimulate food intake and inhibit energy expenditure, and 2) anorectic neuropeptides, such as proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated peptide (CART), which can inhibit food intake and stimulate energy expenditure (14, 36). Two types of arcuate nucleus (ARC) neurons express the long form of leptin receptor Ob-Rb (16). Leptin regulates these neurons in a reciprocal manner by inhibiting NPY/AgRP neurons while stimulating POMC/CART neurons (41). Leptin levels parallel changes in nutritional status and are well-positioned to signal energy insufficiency (14). In a state of negative energy balance, such as fasting or lactation, a decrease in leptin levels elicits an increase in food intake and a decrease in energy expenditure by stimulating NPY/AgRP expression and suppressing POMC/CART expression (2, 10, 15, 39, 46).

In contrast to fasting and lactating mice or rats, cold-exposed mice or rats increase both food intake and thermogenesis (5, 7), which poses a seeming paradox for leptin-responsive neuropeptides. As for these neuropeptides, they could not allow the increase in food intake and thermogenesis simultaneously. Limited studies showed that leptin-responsive neuropeptides exhibited different alternations during cold exposure from those seen during fasting and lactation (7, 17, 20, 22, 33). However, the underlying mechanisms remain poorly understood. Therefore, the main objective of this study was to investigate the role of hypoleptinemia and these neuropeptides in cold adaptation in Brandt’s voles.

We hypothesize that hypoleptinemia can contribute to increases in food intake and brown adipose tissue (BAT) thermogenesis by modifying hypothalamic neuropeptides in cold-exposed Brandt’s voles. We examined serum leptin levels and the gene expression of NPY, AgRP, POMC, and CART in the hypothalamus of Brandt’s voles on day 2 and day 28 of cold exposure. In addition, we examined the effect of rewarming on neuropeptide gene expression on day 2 and day 28 after rewarming. Furthermore, to confirm the role of hypoleptinemia during cold adaptation, we treated cold-exposed Brandt’s voles with exogenous leptin for 7 days. Suppressor of cytokine signaling 3 (SOCS3) is a target gene increased by activation of Ob-Rb, and it plays a key role in the regulation of leptin signaling by feedback inhibition of the leptin receptor (4, 8). Both Ob-Rb and SOCS3 have been commonly regarded as key factors in leptin signal transduction (8, 29, 30). Thus, we also investigated the effect of cold exposure and/or leptin treatment on Ob-Rb and SOCS3 gene expression.

SMALL ENDOTHERMIC MAMMALS inhabiting temperate and arctic regions exhibit extraordinary physiological adaptations to harsh winter to ensure their survival (31, 43, 45). Brandt’s vole (Lasiopodomys brandtii) is a small endothermic mammal, which mainly inhabits the Inner Mongolia grasslands of China, Mongolia, and the Baikal region of Russia, where winter lasts for >5 mo. In the Xilin River Valley, a major habitat of Brandt’s vole in Inner Mongolia, the lowest temperature is −47.5°C (11). In winter, Brandt’s vole decreases body mass (25) and ceases reproduction (26, 27, 44). During cold exposure (4–5°C), captive Brandt’s voles met most of their energy demand not only by increasing food intake, but also by mobilizing body fat (24, 48). The increase in food intake and the decrease in body fat are closely related with circulating leptin levels, which decreased by 52% under cold acclimation (48). However, the role of hypoleptinemia during cold adaptation in this species is still not known.

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http://www.ajpregu.org 0363-6119/09 $8.00 Copyright © 2009 the American Physiological Society R1293
Materials and Methods

Animals

All animal procedures were licensed under the Animal Care and Use Committee of Institute of Zoology, the Chinese Academy of Sciences. Brandt’s voles were from our laboratory colony and were maintained at 23 ± 1°C with a 16:8-h light-dark photoperiod (lights on at 0400). Voles were weaned at 18–20 days of age and then housed with same-sex siblings in plastic cages (30×15×20 cm) with sawdust bedding. Commercial rabbit pellets (51.1% crude fat, 24.3% crude protein, 25.0% neutral-detergent fiber, and 13.6% acid-detergent fiber [Beijing KeAo Feed]) and water were provided ad libitum. Adult voles (90–120 days old) were housed singly for 1 mo (16:8-h light-dark cycle and 23 ± 1°C) before cold (16:8-h light-dark cycle and 5 ± 1°C) exposure.

Experimental Protocols

Experiment 1: effects of cold and rewarming acclimation on body mass and food intake. Fourteen adult male voles were divided randomly into a Warm group (7 voles, maintained at 23 ± 1°C for 8 wk) and a Cold-Warm group (7 voles) that were first exposed to cold (5 ± 1°C) for 4 wk and then returned to warm (23 ± 1°C) for another 4 wk. Body mass was recorded every 3 days during the course of the acclimation. Food intake was measured for 3 consecutive days once a week as described previously (48).

Experiment 2: effects of cold and rewarming acclimation on serum leptin levels, uncoupling protein 1 (UCP1) content in interscapular BAT and hypothalamic gene expression. This experiment was performed to test our prediction that Brandt’s vole would display plastic changes in the levels of leptin, UCP1, and hypothalamic gene expression in response to cold and rewarming acclimation. Thirty-five adult male voles were divided randomly into five groups: C0 (7 voles, the control, no treatment); C2 (7 voles, cold exposure for 2 days); C28 (7 voles, cold exposure for 28 days); RW2 (7 voles, warm exposure for 28 days, and then returned to warm for 2 days); and RW28 (7 voles, cold exposure for 28 days, and then returned to warm for 28 days). Voles were killed between 0900 and 1100. Trunk blood was collected and centrifuged at 4,000 rpm for 30 min, and the serum was stored at −80°C for leptin assay. Interscapular BAT (IBAT) was dissected and weighed.

As previously described (7), 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 perihypothalamic sulcus. The hypothalamus was immediately frozen in liquid nitrogen and stored at −80°C until subsequent analysis.

Experiment 3: effects of leptin administration under warm and cold conditions on energy intake, hypothalamic gene expression, and UCP1 content in IBAT. To test the predication that exogenous leptin would suppress hyperphagia and the enhancement of UCP1 in cold-acclimated voles, we divided randomly 28 adult male voles into four groups: WP (8 voles, maintained at 23 ± 1°C for 21 days and then treated with PBS for 7 days); WL (6 voles, maintained at 23 ± 1°C for 21 days and then treated with leptin for 7 days); CP (7 voles, maintained at 5 ± 1°C for 21 days and then treated with PBS for 7 days); and CL (7 voles maintained at 5 ± 1°C for 21 days and then treated with leptin for 7 days). Voles were anesthetized with isoflurane and implanted with a miniosmotic pump (Alzet model 2001; capacity, 200 µl; release rate, 1 µl/h; Durect, Cupertino, CA) containing either recombinant murine leptin (Peprotech, London, UK) dissolved in PBS (pH 7.4) or PBS alone (sham procedure) subcutaneously on the dorsal side. The amount of leptin in the miniosmotic pumps was ~140 µg, and the mean dose of leptin was 0 ± 0.11 (±SE) µg/g body weight. Body mass was recorded throughout the experiment. Food intake was measured daily during leptin infusion (days 22–28). Gross energy (GE) contents of food and feces were measured by a Parr 1281 oxygen bomb calorimeter (Parr Instrument). Gross energy intake (GEI) and digestible energy intake (DEI) were then calculated according to the following equations (25, 49):

\[
\text{GEI (kJ/day)} = \text{dry matter intake (g/day)} \times \text{GE content of food (kJ/g)}
\]

\[
\text{DEI (kJ/day)} = \text{GEI (kJ/day)} - [\text{mass of feces (g/day)} \times \text{GE content of feces (kJ/g)}]
\]

On day 28, voles were killed and serum, BAT, and hypothalamus samples were taken and stored at −80°C. The heart, liver, spleen, lung, kidneys, and gastrointestinal tract were dissected for body composition analysis.

Body Composition Analysis

After dissection of the hypothalamus and IBAT, the visceral organs, including heart, lungs, liver, kidneys, spleen, and gastrointestinal tract (containing contents) were extracted and weighed (±1 mg).

Body fat extraction was performed with a Soxtect Fat Extraction System (Avanti 2050; FOSS, Höganäs, Sweden) with petroleum ether following the manufacturer’s directions.

Serum Leptin Level Assay

Serum leptin levels were measured by RIA with a 125I multispecies kit (cat. no. XL-85K; Linco Research), which had been validated previously in Brandt’s voles (25, 49). The lower and upper limits of the assay kit were 1 and 50 ng/ml and the inter- and intra-assay variations were <3.6% and 8.7%, respectively. Serum leptin levels were determined in a single RIA and expressed as nanograms per milliliter.

Measurement of UCP1 Content in IBAT

Mitochondrial protein concentrations of BAT were determined by Folin phenol method (28) with bovine serum albumin as standard. IBAT UCP1 content was measured by Western blot analysis as described previously (25, 49). Specifically, total IBAT mitochondrial protein (20 µg/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; Amersham, Buckinghamshire, UK). IBAT was detected using a polyclonal rabbit anti-hamster UCP1 (1:5,000) (supplied by Dr. M. Klingenspor, Department of Biology, Philipps University, Marburg, Germany) as a primary antibody, which had been validated previously for use in Brandt’s voles (25), and goat anti-rabbit (1:5,000) as the second antibody (19). Enhanced chemiluminescence (Amersham) was used for detection. Quantification of the blots was determined with the use of a Quantity One version 4.4.0 (BioRad, Hercules, CA). UCP1 content was expressed as relative units.

Real-time RT-PCR Assay of Hypothalamic Gene Expressions of Ob-Rb, SOCS3, NPY, AgRP, POMC, and CART

Total RNA isolation and cDNA synthesis. Total RNA was isolated from the hypothalamus using Trizol Reagent (cat. no. 159596-026; Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. To denature any contaminating DNA, RNA samples were treated with RNase-free DNase I (cat. no. M6101; Promega, Madison, WI) for 30 min at 37°C. Equal volumes (4 µg) of total RNA was transcribed into first-strand cDNA for each sample by using reverse transcription kit (cat. no. 1622; Fermentas International, Burlington, ON, Canada) according to the manufacturer’s instruction.

Real-time RT-PCR. Primers set for β-actin and six hypothalamic genes were designed for real-time PCR as previously described (40) (Table 1). Real-time PCR was completed using the SYBR Green master qPCR kit (cat. no. DRR041D; Takara Bio) in the MX3000P quantitative PCR system (Stratagene, Cedar Creek, TX). Real-time RT-PCR was carried out in 12.5 µl reaction agent composed of 6.25 µl 2× SYBR Premix EX Taq master mix, 1 µl cDNA templates and 0.2 µmol/l primers. Each sample was analyzed in triplicate. The conditions for the reaction were as follows. Initial
**Table 1. Gene-specific primers used for real-time RT-PCR**

<table>
<thead>
<tr>
<th>Primers</th>
<th>Oligonucleotide Sequence, 5’→3’</th>
<th>Product Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ob-Rb, forward</td>
<td>CTG AGA GGG GTT CTC TCT GT</td>
<td>147</td>
</tr>
<tr>
<td>Ob-Rb, reverse</td>
<td>AGA TCG TTC CCG TAG GTC TAG</td>
<td>114</td>
</tr>
<tr>
<td>SOCS3, forward</td>
<td>GCC GCT CCA TCT CTT CAT AG G</td>
<td>116</td>
</tr>
<tr>
<td>SOCS3, reverse</td>
<td>GCC GCT CCA TCT CTT CAT AG G</td>
<td>116</td>
</tr>
<tr>
<td>NPY, forward</td>
<td>TGC CTC TGT CCC TGG TGT G</td>
<td>116</td>
</tr>
<tr>
<td>NPY, reverse</td>
<td>TGC CTC TGT CCC TGG TGT G</td>
<td>116</td>
</tr>
<tr>
<td>AgRP, forward</td>
<td>CCG TGG TCC CCA GAG TGG CC</td>
<td>114</td>
</tr>
<tr>
<td>AgRP, reverse</td>
<td>CCG TGG TCC CCA GAG TGG CC</td>
<td>114</td>
</tr>
<tr>
<td>POMC, forward</td>
<td>ATG TAG GAC TTC GGC CAA AGC</td>
<td>134</td>
</tr>
<tr>
<td>POMC, reverse</td>
<td>ATG TAG GAC TTC GGC CAA AGC</td>
<td>134</td>
</tr>
<tr>
<td>CART, forward</td>
<td>TGG AAG CTG GCT TTA GCA AC</td>
<td>145</td>
</tr>
<tr>
<td>CART, reverse</td>
<td>TGG AAG CTG GCT TTA GCA AC</td>
<td>145</td>
</tr>
<tr>
<td>β-actin, forward</td>
<td>TGT TGC GTC AGA ACA AGG</td>
<td>200</td>
</tr>
<tr>
<td>β-actin, reverse</td>
<td>TGT TGC GTC AGA ACA AGG</td>
<td>200</td>
</tr>
</tbody>
</table>

Ob-Rb, long form of leptin receptor; SOCS3, suppressor of cytokine signaling 3; NPY, neuropeptide Y; AgRP, agouti-related protein; POMC, proopiomelanocortin; CART, cocaine- and amphetamine-regulated peptide.

denaturation at 95°C for 10 s, followed by 40 cycles of 95°C for 5 s, 60°C for 20 s, and 72°C for 20 s. At the end of the experiments, melting curve analysis showed that there were no nonspecific amplifications. PCR products were further confirmed by DNA sequencing. Standard curves were constructed for each gene via serial dilutions of cDNA (2-fold dilutions). Analysis of standard curves between target genes and β-actin showed that they had similar amplification efficiency, which confirmed the validity of the comparative quantity method. Data derived from Mx3000P quantitative software were expressed as relative amounts, calculated by normalizing the amount of target gene mRNA levels to the amount of β-actin mRNA levels. No amplification was detected in the absence of template or in the no RT control.

**Statistical Analyses**

Data were analyzed using SPSS 13.0 software (SPSS, Chicago, IL). Prior to all statistical analyses, data were examined for normality of variance using the Kolmogorov-Smirnov test. For experiment 1, during the course of acclimatation, differences in body mass were analyzed by repeated-measures ANOVA, while differences in food intake were analyzed by repeated-measures ANCOVA with body mass as covariate. For experiment 2, group differences in serum leptin levels, UCP1 content in IBAT, and hypothalamic gene expressions were assessed by one-way ANOVA, followed by the Tukey’s honestly significant difference test for comparisons. For experiment 3, the effects of leptin treatment and temperature on body mass were assessed by repeated-measures ANOVA. Differences in food intake were analyzed by repeated-measures ANCOVA with body mass as covariate. The effects of leptin treatment and temperature on serum leptin levels, UCP1 content, hypothalamic gene expression, and body composition were assessed by two-way ANOVA followed by least-significant difference (LSD) post hoc tests. Group differences in body composition were assessed by two-way ANCOVA with body mass as covariate. Finally, Pearson correlation analysis was performed to determine the correlation between serum leptin levels and hypothalamic neuropeptides and the correlation between hypothalamic neuropeptides and food intake. Data are expressed as means ± SE, and \( P < 0.05 \) was considered to be statistically significant.

**RESULTS**

**Experiment 1**

**Body mass.** During the first 4 wk of cold exposure (days 0 to 28), body mass changes of Cold-Warm voles were not significantly different from those of Warm voles (interaction group × day, \( P = 0.062 \); group effect, \( P = 0.104 \); day effect, \( P = 0.653 \); Fig. 1). After the animals were rewarmed (days R3 to R28), Cold-Warm-acclimated voles exhibited greater mass increase than Warm voles (interaction group × day, \( P < 0.05 \); group effect, \( P = 0.147 \); day effect, \( P < 0.001 \); Fig. 1).

**Food intake.** During cold exposure (days 0 to 28), the cold-exposed voles exhibited substantially greater food intake increase than Warm voles (interaction group × day, \( P < 0.001 \); group effect, \( P < 0.01 \); day effect, \( P = 0.628 \); Fig. 2). After the animals were rewarmed (days R3 to R28), food intake of Cold-Warm-acclimated voles decreased to those of the control voles (interaction group × day, \( P = 0.706 \); group effect, \( P = 0.751 \); day effect, \( P = 0.978 \); Fig. 2).

**Experiment 2**

**Serum leptin levels.** Cold exposure induced a significant decrease in serum leptin levels, such that at the end of cold exposure, serum leptin level in voles of the C28 group was decreased by 69% compared with that in the C0 group (\( P < 0.01 \); Fig. 3). After the animals were rewarmed, leptin levels were increased and returned to the level of C0 group at the end of the acclimation (Fig. 3).

**IBAT UCP1 expression.** Cold exposure also induced a significant increase in IBAT UCP1 levels by 53% in the C28 group compared with the C0 group (\( P < 0.05 \); Fig. 3). After the animals were rewarmed, UCP1 levels remained elevated in the RW2 and RW28 groups (Fig. 3).

**Hypothalamic gene expression.** Hypothalamic AgRP mRNA expression showed significant variations among groups; AgRP mRNA level was higher in C28 group, but then it decreased and was restored to the control level in RW28 group (\( P < 0.01 \); Fig. 4). There were no significant changes in mRNA expression of hypothalamic NPY, POMC, CART, Ob-Rb, and SOCS3 (Fig. 4).

**Experiment 3**

**Body mass.** Prior to leptin treatment (days 0–21), voles in the Warm group exhibited a greater mass increase than cold-exposed voles (interaction group × day, \( P < 0.05 \); group effect, \( P < 0.05 \); day effect, \( P = 0.379 \); Fig. 5).

During leptin treatment (days 22 to 28), leptin-treated voles decreased body mass compared with PBS controls (temperature effect, \( P = 0.01 \); leptin treatment effect, \( P < 0.05 \); day effect, \( P < 0.001 \); interaction temperature × day, \( P = 0.981 \); interaction leptin treatment × day, \( P < 0.001 \); interaction temperature × leptin treatment × day, \( P = 0.413 \)). After 7 days of treatment, the mean absolute body mass decrease was 6.3 g in WL voles and 6.0 g in CL voles compared with controls. This decrease represents 14.6% and 15.1% of control body mass under warm and cold conditions, respectively.

**Energy intake.** There were no significant differences in gross or digestible energy intakes between control and leptin-treated voles in either warm or cold groups on day 21. In response to leptin treatment, leptin-treated voles in the warm and cold groups decreased their gross energy intake (temperature effect, \( P < 0.001 \); leptin treatment effect, \( P < 0.01 \); day effect, \( P = 0.007 \); Fig. 6A) and digestible energy intake (temperature effect, \( P < 0.001 \); leptin treatment effect, \( P < 0.01 \); day effect, \( P = 0.01 \); Fig. 6B) compared with PBS controls. There were no
interaction effects of temperature, leptin treatment, or time of the treatment on gross and digestible energy intakes.

**Body composition.** There were significant effects of cold and leptin treatment on carcass and body fat mass, which were smaller in cold-exposed voles and leptin-treated voles (Table 2). The masses of heart and spleen were significantly decreased in cold-exposed voles compared with warm voles, but there were no significant effects of leptin treatment (Table 2). The masses of lungs and kidneys were not affected by either cold or leptin treatment (Table 2). There were no interaction effects of cold and leptin treatment on masses of carcass, body fat, BAT, liver, heart, lung, spleen, kidneys, or alimentary organs (Table 2).

**IBAT UCP1 expression.** No significant effects of either temperature or leptin treatment were found on IBAT UCP1 expression (interaction leptin infusion × temperature, \( P = 0.12 \); leptin treatment effect, \( P = 0.221 \); temperature effect, \( P = 0.073 \); Fig. 7).

**Hypothalamic gene expression.** Gene expression of hypothalamic NPY, Ob-Rb, and SOCS3 were not affected by either cold or leptin treatment (Fig. 8). Hypothalamic POMC (\( F_{1,22} = 4.93, P = 0.037 \)) and CART mRNA (\( F_{1,22} = 11.88, P = 0.002 \)) were upregulated in cold-exposed voles. There were no interaction effects between cold and leptin treatment on NPY, Ob-Rb, SOCS3, POMC, or CART. There was a significant effect of temperature and infusion on hypothalamic AgRP mRNA expression, with higher levels of expression in cold-exposed voles (interaction leptin treatment × temperature, \( P = 0.004 \); leptin infusion effect, \( P = 0.014 \); temperature effect, \( P < 0.001 \); Fig. 8).

Serum leptin levels were negatively correlated with AgRP mRNA levels (\( r = 0.172, P < 0.05 \)) (Fig. 9). No significant relationships were found between leptin levels and other hypothalamic genes. There was a significant positive relationship between AgRP mRNA and gross energy intake (\( r = 0.575, P < 0.01 \)) (Fig. 10).

**DISCUSSION**

Chronically cold-exposed Brandt’s voles showed similar physiological adjustments to cold-exposed rats. Sustained thermogenesis led to compensatory hyperphagia and body fat loss. Hypoleptinemia partially contributes to cold-in-
duced hyperphagia. Elevation of AgRP gene expression may be involved in the process.

Ob-Rb and SOCS3 are key factors in modulation of leptin sensitivity (22, 29, 30, 42). In the present study, there were no significant changes in hypothalamic Ob-Rb and SOCS3 gene expression, suggesting that leptin sensitivity remained unchanged during cold exposure. Warm-acclimated voles and cold-exposed voles exhibited similar response in body mass (WL voles decreased the body mass by 12.7%; CL voles decreased the body mass by 11.2%), and food intake (WL voles decreased the food intake by 14.7%; and CL voles decreased the food intake by 20.2%) in response to exogenous leptin, which showed that cold did not affect leptin sensitivity in Brandt’s voles.

Leptin-responsive ARC neurons, such as NPY/AgRP and POMC/CART neurons, are known to be important mediators of metabolic and endocrine responses to leptin.
of leptin action on the regulation of energy intake (36). In this study, hypothalamic NPY, POMC, and CART mRNA levels remained stable despite the reduction in leptin levels during cold acclimation. This was in contrast to the state of starvation and lactation, in which decreased leptin levels typically stimulate NPY/AgRP expression and depress POMC/CART expression. It seems that the regulation of NPY, POMC, and CART might be mediated through a leptin-independent pathway under cold conditions. Consistent with this assumption, a previous study showed that short-term (1.5 h) cold exposure reduced NPY gene expression in the paraventricular nucleus, dorsomedial nucleus, and ARC (32), while long-term cold exposure resulted in unchanged hypothalamic NPY (7). Another study showed that ARC CART mRNA levels increased during cold exposure despite a reduction in leptin levels (20).

Interestingly, cold exposure induced large and progressive increases in AgRP mRNA, suggesting that AgRP may be involved in the induction of cold-induced hyperphagia in Brandt’s voles. To test the role of hypoleptinemia and AgRP in cold-induced hyperphagia, we treated the cold-exposed voles with exogenous leptin. Leptin treatment induced a significant reduction in food intake in cold-exposed voles. However, food intake in cold leptin-treated voles was still higher than that in WP voles. Similar results were also reported in leptin-treated rats under cold conditions (1). These results are consistent with the hypoleptinemia being partially driven by the hyperphagia. Other undetermined factors must be involved in the regulation of food intake under cold conditions. The most likely candidate is norepinephrine. Norepinephrine has a stimulatory effect on food intake (3, 23). It has been shown that norepinephrine increased in cold-exposed Brandt’s voles (47). Under cold conditions, we did not detect significant effects of leptin treatment on the gene expression of NPY, POMC, CART, Ob-Rb, and SOCS3. Similar to the change in food intake, hypothalamic AgRP mRNA in cold leptin-treated voles decreased after 7-day leptin administration. There was a positive correlation between AgRP mRNA levels and gross energy intake. In addition, there was also a negative correlation between serum leptin and AgRP mRNA levels.

For small mammals, UCP1-mediated adaptive thermogenesis plays a critical role in coping with cold (9, 19, 37).

### Table 2. Effects of cold exposure (5°C) and leptin treatment (7 days) on body compositions (wet mass) in male Brandt’s voles

<table>
<thead>
<tr>
<th>Parameters</th>
<th>23°C</th>
<th>5°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>Leptin</td>
<td>PBS</td>
</tr>
<tr>
<td>Body mass</td>
<td>47.7 ± 1.1</td>
<td>47.0 ± 0.6</td>
</tr>
<tr>
<td>Carcass</td>
<td>32.638 ± 1.068*</td>
<td>29.503 ± 0.944b</td>
</tr>
<tr>
<td>Body fat</td>
<td>7.655 ± 0.873a, 3.618 ± 0.859c,e</td>
<td>5.226 ± 0.735b</td>
</tr>
<tr>
<td>BAT</td>
<td>0.066 ± 0.0077a</td>
<td>0.033 ± 0.0066a</td>
</tr>
<tr>
<td>Liver</td>
<td>1.914 ± 0.134a</td>
<td>1.445 ± 0.087</td>
</tr>
<tr>
<td>Heart</td>
<td>0.189 ± 0.009b</td>
<td>0.179 ± 0.009b</td>
</tr>
<tr>
<td>Lung</td>
<td>0.406 ± 0.002</td>
<td>0.373 ± 0.001</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.043 ± 0.007</td>
<td>0.032 ± 0.001</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.44 ± 0.022a,b</td>
<td>0.395 ± 0.023b</td>
</tr>
<tr>
<td>Alimentary organs</td>
<td>7.856 ± 1.346a,b</td>
<td>5.537 ± 0.005b</td>
</tr>
</tbody>
</table>

Values are means ± SE in grams. T, temperature; L, leptin treatment; ns, not significant; BAT, brown adipose tissue. Alimentary organs include stomach, small intestine, large intestine, and caecum. Values for a specific parameter that share different superscripts are significantly different at P < 0.05, determined by a two-way ANCOVA and post hoc LSD test.
In this study, there were large increases in UCP1 levels accompanied by progressive reduction in leptin levels during cold exposure. To determine the effect of hypoleptinemia on UCP1 in cold-exposed voles, we treated these voles with exogenous leptin. Interestingly, there was no significant effect of 7-day leptin administration on IBAT UCP1 protein expression. Consistent with this result, leptin treatment in cold-acclimated field voles (Microtus agrestis) had no effect on UCP1 mRNA expression (21).

Hypothalamic neuropeptides play important roles in regulating BAT thermogenesis. Intracerebroventricularly administered NPY and AgRP inhibit BAT thermogenesis, while intracerebroventricularly administered α-MSH and CART stimulate BAT thermogenesis in rats (6, 13, 18, 20, 38). Unchanged gene expression of hypothalamic NPY, POMC, and CART, together with increased gene expression of AgRP suggest that these neuropeptides might not be involved in the modification of UCP1 in cold-exposed voles. However, the suggestion needs further support since we did not measure protein expression of these neuropeptides.

Together, these results showed a critical role of hypoleptinemia and a complex and versatile regulation of energy balance in cold-exposed voles. Facing artificially increased circulating leptin levels, cold-exposed voles decreased energy intake. It seems that cold-exposed leptin-treated voles do not have enough energy to further increase thermogenesis when faced with high leptin levels, which typically induce BAT thermogenesis at thermoneutrality or room temperature (12, 34). This is logical since exogenous leptin resulted in reduction in energy intake and body fat stores were very low due to the chronic...
cold exposure. Similarly, Abelenda et al. (1) reported that there was no further increase in lipolysis in rats under similar experimental conditions. Cold-exposed voles and rats exhibit similar changes in food intake, UCP1, body fat, and body mass after treatment with exogenous leptin, suggesting they probably share the same conservative mechanisms in cold adaptation.

Trade-offs between energy intake and energy expenditure regulation in cold-exposed voles when treated with exogenous leptin also suggest the anorexic and thermogenic role of leptin and neuropeptides were independently regulated. In keeping with this, the anorexic and thermogenic responses to exogenous leptin were not regulated synchronously during the development of leptin resistance in mildly obese rats (35). Chronic central nervous system administration of AgRP in rats also suggested that the AgRP regulation of food intake and BAT UCP1 were mediated by independent pathways (38).

**Perspectives and Significance**

Endothermic animals living in cold environments maintain normothermia and energy balance by activating mechanisms that increase heat production and food ingestion. These physiological adaptations generate a unique situation characterized by increased food intake but body mass loss. Characterization of hypothalamic mechanisms that integrate thermogenic capacity and feeding behavior may help in understanding some features of cold adaptation in endotherms. Leptin is an adipocyte-derived hormone that communicates the status of body energy stores to the brain to regulate feeding behavior and energy balance. Hypoleptinemia seems to be a common character of cold-acclimated endotherms. The present study investigated the role of hypoleptinemia in the regulation of food intake and BAT thermogenesis and examined the changes in gene expression of several hypothalamic neuropeptides downstream of leptin signaling. Results from this and other studies indicated that the effects of leptin on energy balance were different between animals acclimated to warm and cold environments. Future studies should investigate the differences in hypothalamic mechanisms underlying leptin action between warm- and cold-acclimated animals. In addition, investigation should also be focused on the interplay between leptin and stress hormones under cold conditions. These physiological data can help us understand the mechanisms that determine the limits of physiological flexibility and homeostasis and perhaps help predict the impact of climate change on endotherms.

**ACKNOWLEDGMENTS**

We thank all members of Animal Physiological Ecology Group for their discussion and help with the experiments. We are grateful to Professor Ian D. Hume, the University of Sydney, Australia, for improving the English expression of the text. We also thank Dr. Martin Klingenspor at the Department of Biology, Philipps-University, Marburg, Germany, for supplying the hamster UCP1 antibody. We also thank the three anonymous reviewers for their critical comments and suggestions.

**GRANTS**

This study was supported by National Natural Science Foundation of China Grant 30625009 and National Basic Research Program of China Grant 2007BC109103 (to D.-H. Wang).

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