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Energy metabolism, thermogenesis and body mass regulation in Brandt's voles (*Lasiopodomys brandtii*) during cold acclimation and rewarming

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Abstract

Environmental cues play important roles in the regulation of an animal's physiology and behavior. The purpose of the present study was to test the hypothesis that ambient temperature was a cue to induce adjustments in body mass, energy intake and thermogenic capacity, associated with changes in serum leptin levels in Brandt's voles (*Lasiopodomys brandtii*). We found that Brandt's voles increased resting metabolic rate (RMR) and energy intake and kept body mass stable when exposed to the cold while showed a significant increase in body mass after rewarming. The increase in body mass after rewarming was associated with the higher energy intake compared with control. Uncoupling protein 1 (UCP1) content in brown adipose tissue (BAT) increased in the cold and reversed after rewarming. Serum leptin levels decreased in the cold while increased after rewarming, associated with the opposite changes in energy intake. Further, serum leptin levels were positively correlated with body mass and body fat mass. Together, these data supported our hypothesis that ambient temperature was a cue to induce changes in body mass and metabolism. Serum leptin, as a starvation signal in the cold and satiety signal in rewarming, was involved in the processes of thermogenesis and body mass regulation in Brandt's voles.

Keywords: Cold acclimation; Energy intake; Leptin; Rewarming; Resting metabolic rate (RMR); Uncoupling protein 1 (UCP1)

Introduction

In endothermic small mammals, ability to survive in cold environment requires proper strategies and efficient thermoregulatory mechanisms (McNab, 2002). Small mammals usually show seasonal changes in physiology and behavior (Bartness et al., 2002; Concannon et al., 2001; Klingenspor et al., 1996). To cope with winter or cold conditions, some small mammals will reduce their overall body mass and body fat and enhance nonshivering thermogenesis (NST) (Bartness et al., 2002; Concannon et al., 2001; Li and Wang, 2005a,b). The decline in body mass is an adaptive mechanism to reduce the energy requirements when food availability is limited and cold stress occurs (Wunder et al., 1977). An increase in NST could be attributed to the increased expression of uncoupling protein 1 (UCP1), which is the unique thermogenic protein found in the inner membrane of mitochondria in brown adipose tissue (BAT). UCP1 uncouples the oxidation of fuel from adenosine triphosphate (ATP) production and transforms electrochemical energy into heat (Cannon and Nedergaard, 2004; Himms-Hagen, 1985; Nicholls and Locke, 1984). The increased energy expenditure for thermogenesis in the cold may be compensated by hyperphagia in small mammals (Kenagy et al., 1989).

It has been indicated that circulating leptin, primarily synthesized and secreted from adipose tissue, acts with the long-form OB-Rb receptors in the hypothalamic arcuate nulceus to regulate food intake and body mass (Friedman and Halaas, 1998). Bing et al. (1998) found that decreased plasma leptin was accompanied by hyperphagia in cold-exposed rats. Exogenous leptin treatments could decrease food intake and increase energy utilization, thus resulting in the decline in body mass (Abelenda et al., 2003; Pelleymounter et al., 1995). Furthermore, leptin administration stimulated sympathetic nerve activity and

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increased animal's body temperature, basal metabolic rate (BMR), NST, and UCP1 mRNA expression in BAT, indicating a potential involvement in the regulation of thermogenesis (Haynes et al., 1997; Scarpace and Metheny, 1998). However, contradictory results have been reported. In the cold-exposed rats, low serum leptin levels were accompanied by an increase in UCP1 gene expression (Bing et al., 1998), and leptin administration reduced BAT thermogenesis (Abelenda et al., 2003). Thus, the role of leptin in regulating energy intake and expenditure is complex.

Brandt's voles (*Lasiopodomys brandtii*) are nonhibernating herbivores that mainly inhabit the grasslands of Inner Mongolia of China, Mongolia, and the region of Beigaer in Russia, where winter lasts for more than 5 months (Li and Wang, 2005a; Zhao and Wang, 2005). It has been reported that Brandt's voles showed seasonal changes in NST, energy intake, and body mass, indicating potential roles for photoperiod and temperature in the regulation of energy balance and body mass (Li and Wang, 2005a; Wang et al., 2003). Zhao and Wang (2005) have confirmed that short photoperiod, independent of ambient temperature and food availability, decreased body mass in Brandt's voles. It is well known that cold exposure increased BMR, NST, and energy intake in small mammals (Li et al., 2001), whereas available data of the rewarming effects on energy metabolism and body mass were limited. The present study was designed to investigate the role of cold acclimation and rewarming on the energy metabolism and body mass regulation in Brandt's voles. We hypothesized that ambient temperature was an important cue to influence body mass, and leptin was involved in the regulation of energy metabolism and body mass. We can predict that cold acclimation decreases body mass, while rewarming can increase body mass. Leptin will be decreased as a hungry signal to increase the energy intake in the cold while increased as a satiety signal to inhibit energy intake in the warm conditions.

Materials and methods

Animals and experimental designs

All animal procedures were licensed under the Animal Care and Use Committee of Institute of Zoology, the Chinese Academy of Sciences. Studies were carried out in adult male Brandt's voles. They were the offspring of Brandt's voles trapped in Inner Mongolian grasslands in May 1999 and raised in Institute of Zoology, the Chinese Academy of Sciences in Beijing (Li and Wang, 2005a; Zhao and Wang, 2005). Subjects were housed in groups (3–4) in plastic cages (30 × 15 × 20 cm^3) with sawdust bedding after being weaned, and were maintained at the room temperature of 23 ± 1°C, under a photoperiod of 12L:12D (with lights on at 0800). Subjects were fed with commercial rabbit pellets (Beijing KeAo Feed Co.) and water ad libitum.

Experiment 1

In order to test the effects of ambient temperature on body mass, sixteen weight-matched male voles were moved into individual cages and kept for at least 2 weeks and then were randomly assigned into two groups. One group (the treated group: Cold + warm) was transferred to cold (5 ± 1°C) and maintained for 4 weeks and then returned to 23 ± 1°C for further 4 weeks, the other group (Control) remained at 23 ± 1°C throughout the test. Photoperiod was kept at 12L:12D throughout the study. Body mass, RMR, and energy intake during the course of the experiment were measured.

Experiment 2

We further tested the response of leptin to the cold and its role in regulating energy intake, energy expenditure, and body mass in Brandt's voles. This experiment was carried out with another 50 male individuals. They were exposed to 5 ± 1°C for 28 days and then returned to 23 ± 1°C for further 28 days. On days 0, 1, 7, 28, 29, 35, and 56, different individuals were sacrificed and these 7 groups were named C0, C1, C7, C28, RW1, RW7, and RW28, respectively (C, cold; RW, rewarming). The experimental design was shown in Fig. 1. The voles were sacrificed between 0900 and 1100 by puncture of the posterior vena cava. Blood was centrifuged at 4000 rpm for 30 min, and serum was sampled and stored at −20°C for later measurement. The interscapular BAT was surgically removed and immediately frozen in liquid nitrogen and stored at −80°C for determining BAT cytochrome *c* oxidase (COX) activity and UCP1 contents.

Metabolic trials

Resting metabolic rate (RMR) was measured by using an established closed-circuit respirometer at 30 ± 0.5°C (within their thermal neutral zone) as described previously (Li and Wang, 2005a; Wang et al., 2003; Zhao and Wang, 2005). Briefly, the metabolic chamber volume was 3.6 l, and the temperature inside the chamber was maintained by a water bath. KOH and silica gel were used to absorb carbon dioxide and water respectively in the metabolic chamber. The voles were weighed before and after each test. After 60-min stabilization in the chamber, oxygen consumption was recorded for another 60 min at 5-min intervals. Two stable consecutive lowest readings were taken to calculate RMR and corrected to standard temperature and pressure (STP) (Li and Wang, 2005a; Zhao and Wang, 2005). All metabolic measurements were taken between 0900 and 1700 to minimize any effects of circadian rhythms.

Gross energy intake

Food intake was measured for 3 days once a week as described previously (Li and Wang, 2005a; Song and Wang, 2003). During each test, voles were housed individually in stainless steel mesh metabolic cage (24 × 24 × 24 cm^3), in which food and water were provided ad libitum. Uneaten food and feces were collected after the 3-day test, oven dried at 60°C and separated manually. The caloric values of food and feces were determined by Parr1281 oxygen bomb calorimeter (Parr Instrument, USA). Gross energy intake was calculated by the equation: Gross energy intake (kJ/day) = Dry food intake (g/day) × caloric value (kJ/g) of dry food (Li and Wang, 2005a,b; Song and Wang, 2003).

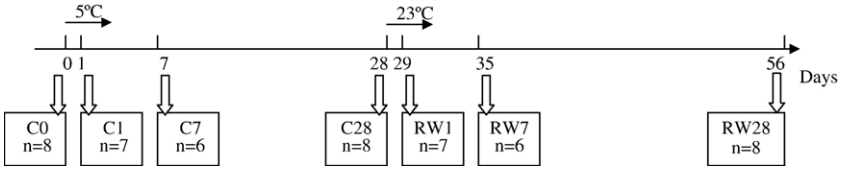

Fig. 1. The design of Experiment 2. The experiment started with 50 male Brandt's voles, raised at 23 ± 1°C and under a photoperiod of 12L:12D. They were exposed to 5 ± 1°C for 28 days and then returned to 23 ± 1°C. On days 0, 1, 7, 28, 29, 35, and 56, different individuals were killed and named as C0, C1, C7, C28, RW1, RW7, and RW28 respectively (C0 = before cold acclimation; C1, C7, and C28 = days of cold acclimation; RW1, RW7, and RW28 = days after rewarming).

Measurement of cytochrome c oxidase (COX) activity and UCP1

Mitochondrial protein concentrations were determined by the Folin phenol method (Lowry et al., 1951) with bovine serum albumin as the standards. The COX activity of BAT was measured with polarographic method using oxygen electrode (Hansatech Instruments LTD., England) (Sundin et al., 1987; Zhao and Wang, 2005).

UCP1 content was measured by Western blotting as described previously (Li and Wang, 2005a; Zhao and Wang, 2005). Total BAT protein (15 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, Amersham). To check for the efficiency of protein transfer, gels and nitrocellulose membranes were stained after transferring with Coomassie brilliant blue and Ponceau red, respectively. Unspecific binding sites were saturated with 5% nonfat dry milk in PBS. UCP1 was detected using a polyclonal rabbit anti-hamster UCP1 (1:5000) (supplied by Dr. M. Klingenspor, Department of Biology, Philipps-University Marburg, Germany) as a primary antibody and peroxidase-conjugated goat anti-rabbit IgG (1:5000) (Jackson Immuno. Inc., USA) as the second antibody. Enhanced chemoluminescence (ECL, Amersham Biosciences, England) was used for detection. UCP1 concentration was determined from area readings by using Scion Image Software (Scion Corporation) and was expressed as relative units (RU) (Li and Wang, 2005a; Zhao and Wang, 2005).

Serum leptin assays

Serum leptin levels were determined by radioimmunoassay (RIA) with the ^{125}I Multi-species Kit (Cat. No. XL-85K, Linco Research Inc.), which has been validated previously in Brandt's voles (Li and Wang, 2005a). The lowest level of leptin that can be detected by this assay was 1.0 ng/ml when using a 100-μl sample size. And the inter- and intra-assay variabilities for leptin RIA were <3.6% and 8.7%, respectively.

Carcass composition analysis

The entire gastrointestinal tract was removed, and the eviscerated carcass (not including BAT) was dried to constant weight at 60°C for determination of dry body mass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus (Li and Wang, 2005a).

Statistical analysis

Data were analyzed using SPSS software package. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. To remove the effects of body mass, data for RMR and gross energy intake were corrected by the 0.67 power of body mass as proposed previously for rodents (Heusner, 1984; Li and Wang, 2005a). All the data in the text, tables, and figures were presented as the measured data, and statistical description was presented for the standardized data with 0.67 scaling. Differences in body mass, RMR, and gross energy intake during the course of cold acclimation and rewarming were assessed by repeated measures analysis of variance (ANOVA), and significant differences were further evaluated with least-significant difference (LSD) post hoc tests. Group differences were assessed by independent-samples *t* test (Experiment 1) or one-way ANOVA with post hoc Duncan's multiple-range tests (Experiment 2). Pearson correlation analyses were used to detect possible associations of serum leptin levels with body mass, body fat mass, fresh and dry carcass mass, and UCP1 content. Results are presented as mean ± SEM, and $P < 0.05$ was considered to be statistically significant.

Results

Experiment 1: changes of body mass, RMR, and energy intake during the course of cold acclimation and rewarming

Prior to acclimation, there was no difference in body mass between the two groups (Control: 45.9 ± 0.53 g, Treated: 46.2 ± 0.42 g, $t = -4.405$, $df = 14$, $P > 0.05$; Fig. 2). During the first 4 days, the body mass in the treated voles kept stable. Interestingly, although from day 4 to 10 the treated voles showed a significant increase ($P < 0.05$) in body mass as compared to the control group, after day 10, the body mass in the treated voles was decreased and showed no difference with the control group until the end of cold exposure (day 28). After rewarming, the treated voles showed a steady increase in body mass from day 38 ($P < 0.05$) as compared to the control group. On day 56, the treated voles (65.4 ± 2.40 g) were 22% heavier compared with control (53.5 ± 1.22 g; $t = -4.406$, $df = 14$, $P < 0.001$). Over the course, significant differences were found within both the control ($F_{28,196} = 8.633$, $P < 0.001$; Fig. 2) and treated groups ($F_{28,196} = 27.425$, $P < 0.001$; Fig. 2).

RMR ($mlO_2/g{\cdot}h$) was influenced by ambient temperature (Fig. 3A). Prior to acclimation, there was no difference in RMR between the control and treated groups ($t = 0.179$, $df = 14$, $P > 0.05$). RMR in treated voles increased significantly and was 15% higher than that of control at the end of 4-week cold acclimation ($t = 5.404$, $df = 14$,

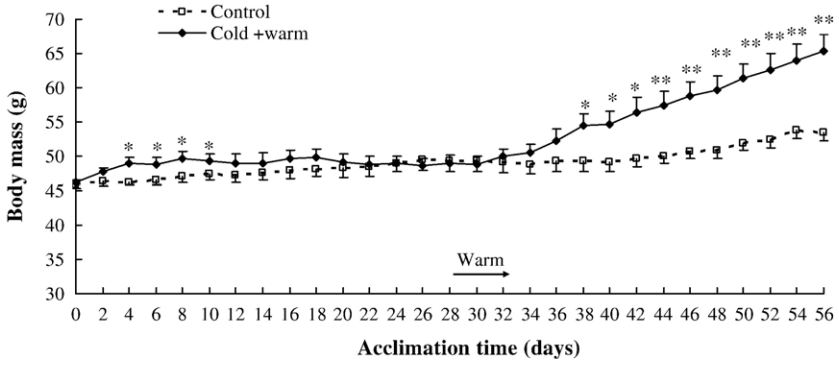

Fig. 2. Changes of body mass in cold-acclimated and rewarmed Brandt's voles. No significant differences in body mass were found during the 4-week cold acclimation, except for an increase from day 4 to day 10. However, body mass increased gradually after rewarming and was 22% higher than the control. The arrow represented the time when the voles were transferred back to the warm. Data are means ± SEM. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus control.

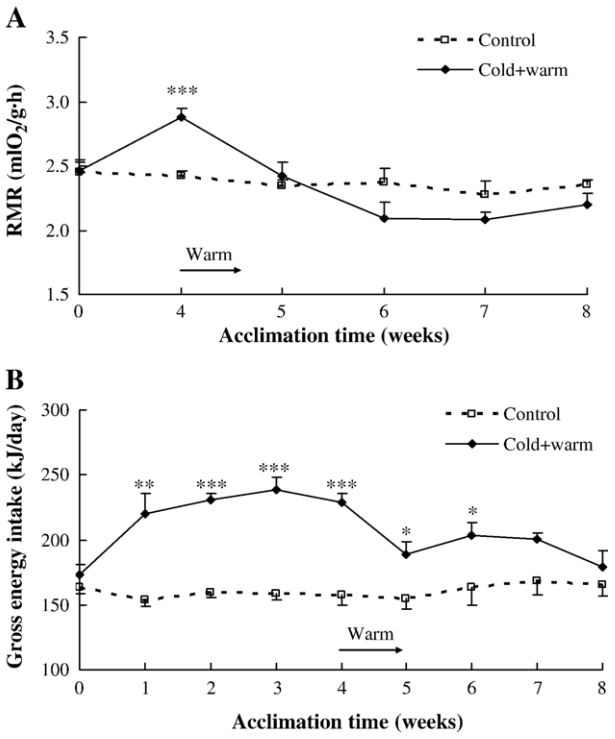

Fig. 3. Changes of resting metabolic rate (RMR) (A) and gross energy intake (B) in cold-acclimated and rewarmed Brandt's voles. (A) RMR increased markedly during cold acclimation and then returned to the control level after rewarming. (B) Gross energy intake increased significantly in the cold acclimation, and then decreased to the level of control until the third week after rewarming. The arrow represented the time when the voles were transferred back to the warm. Data are means ± SEM. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus control. The statistical analysis was performed for the data scaled with 0.67 power of body mass and repeated measure ANOVA and LSD were used to detect the changes during acclimation.

$P < 0.001$) and returned to the level of control in the first week after rewarming ($t = 1.254$, $df = 14$, $P > 0.05$). During the course of cold acclimation and rewarming, there were significant differences in RMR within the treated group ($F_{5,35} = 15.428$, $P < 0.001$), but not within the control group ($F_{5,35} = 0.403$, $P > 0.05$).

There was no difference in gross energy intake between treated and control voles prior to acclimation ($t = -1.597$, $df = 14$, $P > 0.05$; Fig. 3B) and gross energy intake in treated voles significantly increased in the first week compared to the control ($t = -4.111$, $df = 14$, $P < 0.001$; Fig. 3B). In the third week of cold acclimation, the gross energy intake was 51% higher than control ($t = -6.317$, $df = 14$, $P < 0.001$; Fig. 3B). When the voles returned to the warm conditions, energy intake was still higher than that in the control in the first week ($t = -2.447$, $df = 14$, $P < 0.05$), but there were no longer differences from the third week ($t = -1.284$, $df = 14$, $P > 0.05$). During cold acclimation, the treated voles increased energy intake significantly from 173.5 ± 7.1 kJ/day prior to acclimation to 238.9 ± 9.6 kJ/day in the cold ($P < 0.001$). After rewarming, the voles decreased energy intake and still maintained a relative high level by the third week ($P > 0.05$). There were no marked differences within the control group during the acclimation course ($F_{8,56} = 1.818$, $P > 0.05$; Fig. 3B).

Experiment 2

Changes in mitochondrial protein content, COX activity and UCP1 content during cold acclimation and rewarming

There were no differences in relative masses of BAT ($F_{6,49} = 1.272$, $P > 0.05$; Table 1) and liver ($F_{6,49} = 1.877$, $P > 0.05$; Table 1) during cold acclimation and rewarming, though the absolute masses of BAT and liver increased because of the increase in body mass after rewarming. BAT mitochondrial protein content and COX activity increased significantly during the cold acclimation and returned to the control levels after rewarming (Table 1). COX activity of liver in cold acclimation was 31% higher than the control and returned to the control level after rewarming, though no significant changes were found in liver mitochondrial protein content ($F_{6,49} = 0.938$, $P > 0.05$; Table 1) and COX activity ($F_{6,49} = 1.792$, $P > 0.05$; Table 1) among all the groups.

UCP1 content in BAT varied significantly during the course of cold acclimation and rewarming ($F_{6,49} = 3.180$, $P < 0.05$; Fig. 4). It demonstrated a 43% increase in the fourth week of cold acclimation and returned to the initial level in the first week of rewarming.

Changes in body compositions and serum leptin levels during cold acclimation and rewarming

Fresh and dry carcass masses, body fat mass, and fat content (body fat mass/dry carcass mass) decreased by 17%, 19%, 38%, and 23% respectively at the end of 4-week cold acclimation; however, all the compositions increased again in the fourth week after rewarming (Table 2).

Serum leptin levels decreased significantly by 52% on day 7 in the cold. After rewarming, leptin levels increased by 69% in the end compared with the primary levels ($F_{6,45} = 6.879$, $P < 0.001$; Fig. 5). Correlation analysis indicated that serum leptin levels were positively correlated with fresh carcass mass ($R^2 = 0.694$, $P < 0.001$; Fig. 6A), body fat mass ($R^2 = 0.670$, $P < 0.001$; Fig. 6B), dry carcass mass ($R^2 = 0.594$, $P < 0.001$; Fig. 6C), and body mass ($R^2 = 0.581$, $P < 0.001$; Fig. 6D).

Discussion

Ambient temperature plays an important role in mediating animals' physiology and behaviors. In the present study, Brandt's voles could keep stable body mass but decreased body fat content during cold acclimation. After rewarming, body mass increased again. These variations in body mass were associated with changes in RMR, energy intake, and other biochemical and hormonal markers. Brandt's voles increased energy intake and expenditure to adapt to the cold and made an attempt to compensate for the reduced body fat during cold acclimation by overeating after rewarming. UCP1 content in BAT (indicator of thermogenic capacity) increased with cold acclimation and decreased after rewarming. Serum leptin levels decreased during cold acclimation and increased during the

Table 1
Effects of cold acclimation and rewarming on mitochondrial protein (MP) and cytochrome *c* oxidase (COX) in the Brandt's voles (means ± SE)

Parameters	C0	C1	C7	C28	RW1	RW7	RW28	$F_{6,49}$	*P*
Brown adipose tissue (BAT)									
Mass (g)	0.153 ± 0.010^{c}	0.178 ± 0.026^{bc}	0.145 ± 0.017^{c}	0.143 ± 0.011^{c}	0.182 ± 0.013^{bc}	0.221 ± 0.021^{ab}	0.248 ± 0.016^{a}	5.895	<0.001
% body mass	0.323 ± 0.024	0.378 ± 0.040	0.306 ± 0.038	0.327 ± 0.035	0.369 ± 0.022	0.401 ± 0.031	0.378 ± 0.022	1.272	ns
MP (mg/g BAT)	8.556 ± 0.951^{b}	4.889 ± 0.416^{c}	4.861 ± 0.535^{c}	11.942 ± 0.522^{a}	5.957 ± 0.478^{c}	10.219 ± 1.364^{ab}	9.750 ± 0.383^{b}	15.478	<0.001
COX ($nmolO_2$/min·mg MP)	245.4 ± 35.1^{d}	448.9 ± 23.0^{a}	344.6 ± 24.7^{bc}	295.3 ± 13.1^{cde}	264.7 ± 15.2^{de}	378.3 ± 23.3^{b}	322.8 ± 17.1^{bcd}	9.320	<0.001
Liver									
Mass (g)	1.855 ± 0.097^{bc}	1.835 ± 0.106^{c}	1.997 ± 0.163^{bc}	2.012 ± 0.087^{bc}	1.942 ± 0.076^{bc}	2.207 ± 0.138^{ab}	2.463 ± 0.119^{a}	4.218	<0.01
% body mass	3.885 ± 0.193	3.974 ± 0.136	4.160 ± 0.210	4.578 ± 0.294	3.944 ± 0.096	4.020 ± 0.153	3.765 ± 0.164	1.877	ns
MP (mg/g liver)	16.351 ± 0.702	15.379 ± 0.605	15.469 ± 0.481	15.002 ± 0.722	15.130 ± 0.506	15.579 ± 1.283	16.923 ± 0.775	0.938	ns
COX ($nmolO_2$/min·mg MP)	55.542 ± 6.815^{ab}	64.527 ± 5.173^{ab}	69.397 ± 7.448^{ab}	72.690 ± 7.157^{a}	60.067 ± 5.194^{ab}	53.241 ± 9.346^{ab}	49.692 ± 3.983^{b}	1.792	ns

Values with the different superscripts within rows are significantly different ($P < 0.05$). C0 = before cold acclimation; C1, C7, and C28 = days of cold acclimation; RW1, RW7, and RW28 = days after rewarming.

rewarming course. Serum leptin levels were positively correlated with body mass and body fat mass. These data together indicate that ambient temperature was a cue to influence body mass, and serum leptin was involved in the regulating process of energy balance and body mass, suggesting that leptin can act as a starvation signal in cold and satiety signal in rewarming in Brandt's voles.

Body mass and energy metabolism

Seasonal variations in body mass are considered to be an important adaptive strategy for small mammals (Concannon et al., 2001). The seasonally acclimatized Brandt's voles showed the lowest body mass in winter (Li and Wang, 2005a), which was considered to be energetic advantages to reduce overall energy requirements. In the present study, the Brandt's voles acclimated to the cold for 4 weeks showed a decrease in body fat content, but no changes except for an initial increase in overall body mass. Consistent with this, many other herbivores, such as the prairie voles (*Microtus ochrogaster*), did not change body mass during 3-week cold acclimation, and the initial mass increase was attributed to the increased gastrointestinal content (Hammond and Wunder, 1995). In contrast, the Brandt's voles in short photoperiod showed a decrease in body mass compared to those in long photoperiod (Zhao and Wang, 2005). All these suggested that cold together with photoperiod can induce winter mass decline in Brandt's voles. Data from our study showed that body mass increased significantly after the voles were transferred back to the warm conditions, consistent with the previous study on seasonally acclimatized Brandt's voles (Li and Wang, 2005a), which showed the highest body mass in summer. Similar adjustments in body mass have also been found in other small rodents. Rats previously acclimated to 10°C showed a greater rate of increase in body mass during deacclimation (Hori et al., 1998). Further, the summer acclimatized short-tailed field voles (*Microtus agrestis*) increased body mass compared to winter acclimatized voles (McDevitt and Speakman, 1996). It should be noted that the voles compensate for the previous body fat loss by gaining overall body mass and increasing all carcass components above and beyond the

Table 2
Effects of cold acclimation and rewarming on body compositions in the Brandt's voles (means ± SE)

Parameters	C0	C1	C7	C28	RW1	RW7	RW28	$F_{6,49}$	*P*
Sample size	8	7	6	8	7	6	8		
Body mass (g)									
Initial	47.8 ± 0.70	47.6 ± 1.97	48.2 ± 1.82	46.5 ± 0.79	47.6 ± 0.65	47.5 ± 0.51	47.4 ± 0.40	0.628	ns
Final	47.8 ± 0.70^{c}	46.1 ± 1.98^{c}	47.7 ± 1.81^{c}	44.9 ± 1.82^{c}	49.4 ± 2.41^{bc}	54.8 ± 2.31^{b}	66.4 ± 2.35^{a}	14.429	<0.001
Carcass mass (g)									
Fresh	33.3 ± 0.72^{bc}	29.5 ± 1.22^{cd}	30.8 ± 1.10^{cd}	27.7 ± 1.24^{d}	32.7 ± 1.80^{bcd}	36.9 ± 1.99^{b}	46.9 ± 2.36^{a}	17.679	<0.001
Dry	14.5 ± 0.75^{bc}	12.2 ± 0.62^{c}	11.8 ± 0.34^{c}	11.7 ± 0.74^{c}	13.8 ± 1.13^{bc}	15.2 ± 1.00^{b}	18.6 ± 1.24^{a}	7.856	<0.001
Body water (g)	18.8 ± 0.50^{bc}	17.3 ± 0.65^{c}	19.0 ± 0.87^{bc}	16.0 ± 0.70^{c}	18.8 ± 0.71^{bc}	21.7 ± 1.29^{b}	28.3 ± 2.35^{a}	12.018	<0.001
Body fat									
% of dry body mass	47.9 ± 0.06^{a}	43.7 ± 2.12^{a}	35.3 ± 2.70^{b}	36.8 ± 2.17^{b}	45.9 ± 2.21^{a}	44.6 ± 3.56^{a}	46.4 ± 1.47^{a}	4.544	<0.001
g	6.99 ± 0.55^{ab}	5.37 ± 0.42^{bc}	4.18 ± 0.37^{c}	4.36 ± 0.45^{c}	6.47 ± 0.85^{b}	6.94 ± 0.92^{ab}	8.71 ± 0.71^{a}	6.683	<0.001
Fat free dry mass (g)	7.51 ± 0.40^{bc}	6.82 ± 0.33^{c}	7.61 ± 0.31^{bc}	7.37 ± 0.45^{bc}	7.36 ± 0.32^{bc}	8.26 ± 0.22^{b}	9.94 ± 0.61^{a}	6.347	<0.001
Mass out of carcass (g)	14.5 ± 0.47^{b}	16.6 ± 0.85^{ab}	16.9 ± 1.00^{a}	17.2 ± 0.85^{a}	16.8 ± 0.74^{ab}	17.9 ± 0.69^{a}	18.7 ± 0.75^{a}	3.201	<0.05

Values with the different superscripts within rows are significantly different ($P < 0.05$). C0 = before cold acclimation; C1, C7, and C28 = days of cold acclimation; RW1, RW7, and RW28 = days after rewarming.

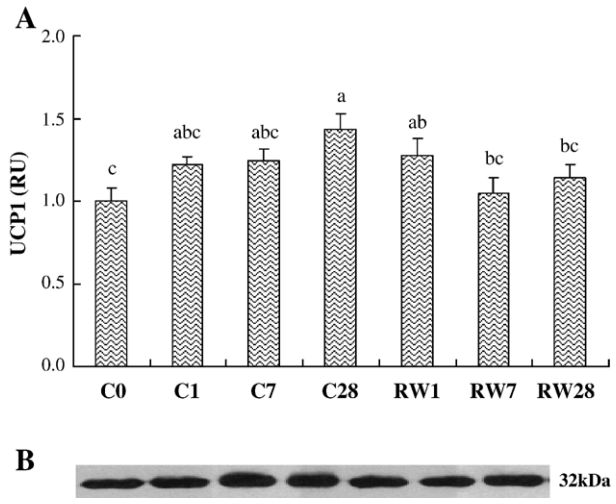

Fig. 4. Effects of cold acclimation and rewarming on uncoupling protein 1 (UCP1) content in Brandt's voles. (A) UCP1 content increased and was 43% higher at the end of cold acclimation as compared to that before cold acclimation and returned in the first week after rewarming. Data are means ± SEM. Means with the different superscripts within all the groups are significantly different ($P < 0.05$). C0 = before cold acclimation; C1, C7, and C28 = days of cold acclimation; RW1, RW7, and RW28 = days after rewarming. (B) Western blotting detection of UCP1 content in cold-acclimated and rewarmed Brandt's voles. The bands from left to right were consistent with those in panel A. Each band contains 15 μg mitochondrial protein.

masses prior to cold acclimation. The increase in body mass after rewarming possibly contributes to preparing for reproduction (Larkin et al., 2002).

The variations in body mass were associated with changes in energy intake and expenditure. It is evident that many winter-active small mammals enhance RMR and NST for survival in the cold (Klingenspor, 2003; Lovegrove, 2003). In the present study, RMR increased significantly during cold acclimation, which was consistent with the previous studies in Brandt's voles (Li et al., 1995, 2001) and other rodents, such as short-tailed voles (McDevitt and Speakman, 1994), root voles (*Microtus oeconomus*) (Wang and Wang, 1996; Wang et al., 1999), Mongolian gerbils (*Meriones unguiculatus*) (Li and Wang, 2005b; Wang et al., 2003), and Siberian hamsters (*Phodopus sungorus*) (Heldmaier et al., 1982; Wiesinger et al., 1990). Our data also indicated that RMR returned to the primary level after rewarming, which could be supported by the study on the deacclimated rats (Hori et al., 1998).

The changes in thermogenesis at the whole animal level were further supported by other biochemical markers examined in the present study, including the mitochondrial protein content, COX activity, and UCP1 content. Liver thermogenesis accounts for 20–25% of RMR (Couture and Hulbert, 1995). Our data indicated that the changes in liver COX activity were in parallel with the changes in RMR during cold acclimation and rewarming. UCP1 mRNA expression and production in BAT may be indicative of the thermogenic capacity (Cannon and Nedergaard, 2004; Nicholls and Locke, 1984), and the thermoregulatory role of UCP1 has been emphasized in UCP1-deficient mice, whose resistance to cold is impaired (Nedergaard et al., 2001). In the present study, UCP1 content increased markedly in the cold condition. The cold-induced increase in BAT UCP1 expression was also found in Siberian hamsters (Klingenspor et al., 1996; Sundin et al., 1987; von Praun et al., 2001), Mongolian gerbils, and ground squirrels (*Spermophilus dauricus*) (Li et al., 2001). UCP1 content decreased and returned to the primary level after rewarming, which could be supported by the deacclimated rats (Reichling et al., 1987). The decrease in UCP1 content during rewarming can avoid energy waste for thermogenesis.

The increased energy expenditure in the cold could be compensated by increasing energy intake and mobilizing reserves. Data from the present study showed that Brandt's voles could increase energy intake by 51% in response to the cold stress. Cold-induced hyperphagia was found in many other rodents, such as rats, mice (Bing et al., 1998; Hori et al., 1998), and Alaskan collared lemmings (*Dicrostonyx groenlandicus*) (Maier and Feist, 1991). However, Siberian hamsters decreased energy intake, which entered hibernation when winter came (Bartness and Wade, 1985). In our studies, energy intake in Brandt's voles was still higher in the first 2 weeks than the control during rewarming. These data suggest that Brandt's voles can compensate for the reduced body fat in the cold by overeating after rewarming.

Role of leptin in the regulation of energy metabolism and body mass

Leptin plays an important role in regulating food intake, energy expenditure, and body mass (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995). Our data showed that serum leptin were positively correlated with carcass mass, body fat mass, and body mass, supported by the notion that the secretion of leptin was in proportion to the body fat (Nagy et al., 1995). Serum leptin levels decreased on day 7 in the cold-acclimated Brandt's voles. Similar results were found

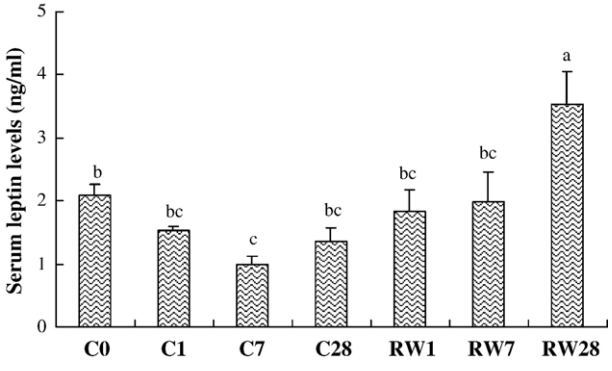

Fig. 5. Effects of cold acclimation and rewarming on serum leptin levels in Brandt's voles. Serum leptin levels decreased significantly by 52% on day 7 in the cold and increased by 69% at the end of rewarming compared with the primary level. Data are means ± SEM. Means with different superscripts within all the groups are significantly different ($P < 0.05$). C0 = before cold acclimation; C1, C7, and C28 = days of cold acclimation; RW1, RW7, and RW28 = days after rewarming.

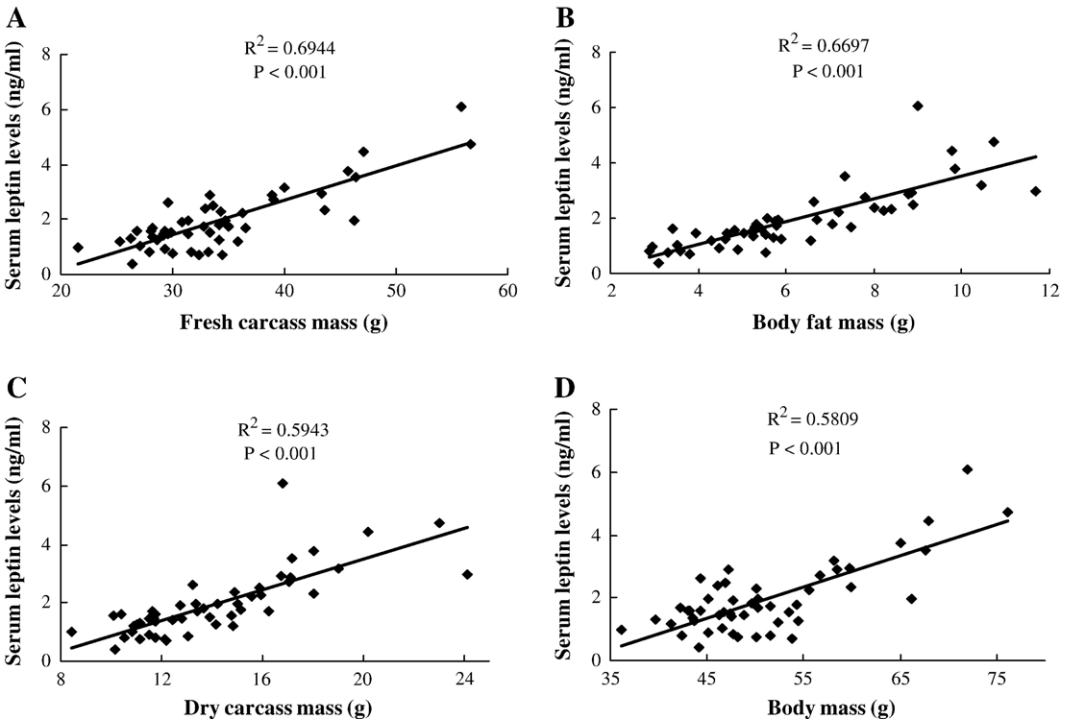

Fig. 6. Correlations of serum leptin levels with fresh carcass mass (A), body fat mass (B), dry carcass mass (C), body mass (D) in cold-acclimated and rewarmed Brandt's voles. Serum leptin levels were positively correlated with fresh carcass mass ($R^2 = 0.694$, $P < 0.001$), body fat mass ($R^2 = 0.670$, $P < 0.001$), dry carcass mass ($R^2 = 0.594$, $P < 0.001$), and body mass ($R^2 = 0.581$, $P < 0.001$).

in some rodent species, such as mice (Korhonen and Saarela, 2005), rats (Bing et al., 1998), and Siberian hamsters (Larkin et al., 2001; Ruf et al., 1993). The low leptin concentration can act as a starvation signal (Flier, 1998; Korhonen and Saarela, 2005; Li and Wang, 2005a) to enable the animal to increase energy intake in the cold in order to compensate for the increased energy expenditure for thermogenesis. After rewarming, serum leptin levels increased (as a satiety signal) and were even 69% higher in the fourth week than the primary levels, coupled with no significant decrease in energy intake. It is necessary to determine whether leptin sensitivity was changed after the voles were transferred back to the warm conditions by exogenous leptin administration. In Siberian hamster, it has been confirmed that leptin insensitivity induced the increased body mass in the long photoperiod (Rousseau et al., 2003).

It was confirmed that leptin administration stimulated UCP1 expression in BAT by increasing sympathetic outflow to BAT (Scarpace and Metheny, 1998). In the present study, however, the low serum leptin levels were accompanied by the high UCP1 contents during cold acclimation, while it was the opposite after rewarming. Leptin gene expression and production were downregulated by the sympathetic stimulation of white and brown fat which induced UCP1 expression in the cold exposure (Rayner and Trayhurn, 2001). Li and Wang (2005a) also found serum leptin was negatively correlated with UCP1 in seasonal acclimatized Brandt's voles. Furthermore, leptin administration to cold-acclimated rats reduced BAT thermogenesis (Abelenda et al., 2003). These divergent data at least suggest that leptin was involved in the regulatory process in thermogenesis and energy balance at different ambient temperatures. The exact relationship between leptin and UCP1 needs to be further validated.

In conclusion, Brandt's voles increased energy intake and thermogenesis and maintained stable body mass during cold acclimation. Voles can compensate for the reduced body fat caused by cold exposure by overeating when they returned to the warm conditions. Serum leptin levels, parallel with body fat, decreased in the cold and increased during rewarming, associated with the opposite changes in energy intake and expenditure. All these data suggest that ambient temperature was a cue to cause the changes in body mass and energy metabolism. Serum leptin can act as a starvation signal in cold and satiety signal in warm conditions to be involved in the process of monitoring body energy status and regulating energy balance in Brandt's voles.

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