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Thermogenesis, food intake and serum leptin in cold-exposed lactating Brandt's voles *Lasiopodomys brandtii*

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Summary

Lactation is the most energetically expensive period for mammals and is associated with increased metabolism and energy intake, but decreased thermogenic capacity. It is well known that small mammals increase both food intake and thermogenesis in the cold. The present study aimed to examine whether Brandt's voles *Lasiopodomys brandtii* could adjust energy intake and thermogenesis to accommodate simultaneous lactation and cold exposure. The voles were placed into two temperature treatments: warm (23±1°C) and cold (5±1°C). Animals at each temperature treatment were further divided into two groups: non-reproductive (NR) and lactating females. We found that lactating voles at peak lactation in the cold enhanced food intake by 2.6 g day^{-1} compared with those in the warm, and increased uncoupling protein 1 (UCP1) content in brown adipose tissue (BAT), to the same level as the cold-exposed NR females. Serum leptin levels decreased significantly during lactation and were positively correlated with body mass and fat mass. After correcting for the effects of body mass, residual serum leptin was negatively correlated with residual gross energy intake and residual RMR. In addition, residual serum leptin levels were positively correlated with UCP1 contents in the warm, but not in the cold. Together, these data suggest that lactating voles can increase thermogenic capacity and energy intake to meet the high energetic costs of simultaneous lactation and cold exposure. Further, serum leptin appears to be involved in the energy intake regulation and thermoregulation, but the thermoregulation in the cold may be mainly mediated by other factors.

Key words: Brandt's vole, *Lasiopodomys brandtii*, cold exposure, food intake, lactation, serum leptin, uncoupling protein 1 (UCP1).

Introduction

Lactation markedly increases energy requirements of the mother in most mammalian species and is associated with dramatic metabolic adjustments (Wade and Schneider, 1992; Barber et al., 1997). The increased energy requirements are usually met primarily by an increase in food intake (Wade and Schneider, 1992; Scantlebury et al., 2000). For example, food intake increased by fourfold in rats (Wade and Schneider, 1992) and threefold in Siberian hamster *Phodopus sungorus* (Bartness, 1997) at peak lactation. Although rodents increased food intake with the time of lactation, the sustained energy intake reached a plateau in late lactation (Johnson et al., 2001a). It has been suggested that the sustained energy intakes in lactation are limited either by the central machinery involved in acquisition, processing and allocation of energy, resources and waste products, or by the capacity of peripheral mammary glands (Hammond and Diamond, 1997; Bacigalupe and Bozinovic, 2001; Speakman and Król, 2005). Many studies have been performed to test between these two hypotheses; however, the evidence available at present does not unambiguously support either of them (Speakman and Król, 2005). The suppression of thermogenesis in brown adipose tissue (BAT), as shown by tissue hypotrophy, a decrease in mitochondrial biogenesis, and an impaired expression of genes encoding uncoupling protein 1 (UCP1) (Trayhurn et al., 1982; Wade et al., 1986; Schneider and Wade, 1987; Li and Wang, 2005a), is an important thermogenic characteristic during lactation. This adjustment has been considered as an energy-sparing mechanism to facilitate conversion of available energy for milk production (Smith and Grove, 2002). In addition, use of maternal reserves, which was thought to be a strategy during lactation only in large mammals (Oftedal, 2000), was also found in some rodents, such as laboratory rats (Barber et al., 1997), Siberian hamsters (Schneider and Wade, 1987), Syrian hamsters *Mesocricetus auratus* (Wade et al., 1986), mice (Johnson et al., 2001b), and Brandt's voles (*Lasiopodomys brandtii,* formerly *Microtus brandti*) (Liu et al., 2003).

A growing body of evidence indicates that leptin, primarily

secreted by adipocytes, acting on the leptin receptor OB-Rb in the hypothalamus, plays important roles in regulating both food intake and energy expenditure (Halaas et al., 1995; Friedman and Halaas, 1998; Schwartz et al., 2000). Circulating leptin concentrations are positively correlated with adiposity and positive energy balance (Halaas et al., 1995). During lactation, the daytime serum leptin concentration decreased by 20–75% and the nocturnal rise in serum leptin was attenuated in the rats (Woodside et al., 2000; Denis et al., 2003). The decrease in serum leptin acted as a starvation signal (Flier, 1998), and induced hyperphagia and suppression of thermogenesis during lactation (Xiao et al., 2004).

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 the winter lasts for more than 5 months and average annual temperature is 0–4°C. It has been demonstrated that Brandt's voles, in common with other small rodents, meet most of the energy demands for lactation by increasing food intake, using body reserves (Liu et al., 2003), and also by decreasing thermogenic capacity in BAT (Li and Wang, 2005a). In the wild, voles face combined stresses of lactation and cold exposure. It is well known that the development of large thermogenic capacities during cold exposure is an important mechanism to maintain body temperature in rodents (Cannon and Nedergaard, 2004), and the increase in food intake compensates for the added energy expenditure for thermogenesis (Bing et al., 1998). However, the rodents suppress thermogenic capacity in BAT (Trayhurn et al., 1982; Wade et al., 1986; Schneider and Wade, 1987; Li and Wang, 2005a) and increase food intake (Johnson et al., 2001a; Liu et al., 2003) during lactation. Thus the adjustment in thermogenesis and food intake is an interesting question during lactation and simultaneous cold stress. Some studies showed that in mice, food intake and resting metabolic rate (RMR) increased during lactation (Hammond et al., 1994; Johnson et al., 2001a; Johnson et al., 2001b), and food intake further significantly increased when the lactating mice were exposed to cold (Hammond and Kristan, 2000; Król and Speakman, 2003). No available data for integrated studies of the regulation of thermogenesis, food intake and serum leptin levels for wild rodents have been found, hence the present study aimed to examine whether Brandt's voles have the ability to adjust energy intake and thermogenesis to accommodate simultaneous lactation and cold exposure.

Materials and methods

Subjects and experimental design

Virgin female Brandt's voles *Lasiopodomys brandtii* (Radde 1861) (90–120 days old) that were the offspring of voles trapped in Inner Mongolian Grasslands in May 1999 and raised in Institute of Zoology, Chinese Academy of Sciences, were housed individually in plastic cages (30 cm×15 cm×20 cm) with sawdust as bedding. The voles were kept at 23±1°C, under a photoperiod of 12 h:12 h L:D (with lights on at 08:00 h). Commercial rabbit pellets (Beijing KeAo Feed Co., Beijing, China) and water were provided *ad libitum*. The voles were placed into two temperature treatments: warm (23±1°C) or cold (5±1°C). Each temperature-treatment group was further divided into two groups: non-reproductive (NR) and lactating females with natural litters of six pups.

The females were paired with males for 4 days to allow insemination, and then the males were removed. On the day of parturition, the females (lactating in the cold, LC: N=8) and their litters were transferred to a room at 5±1°C with the same photoperiod for 2 weeks, and provided with a small amount of cotton bedding (approximately 3 g) for nest material. Other lactating females (lactating in the warm, LW: N=8) remained at 23±1°C and 12 h:12 h L:D photoperiod. Non-reproductive females were kept either at 23±1°C (non-reproductive females in the warm, NW: N=8) or 5±1°C (non-reproductive females in the cold, NC: N=8) for the same period as the lactating ones. All the groups were given the same amount of cotton bedding. All animal procedures were licensed under the Institutional Animal Care and Use Committee of Institute of Zoology, Chinese Academy of Sciences.

Metabolic trials

Between 09:00 h and 17:00 h on day 11 of lactation or cold exposure, RMR was measured by using an established closed-circuit respirometer at 30±0.5°C (within the animals' thermal neutral zone) as described previously (Li and Wang, 2005b). 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, 2005b).

Energy intake

Dry food intake (DFI) was measured for the consecutive 3 days from days 12 to 14 of lactation, as described previously (Liu et al., 2003). During the test, voles were housed individually in stainless steel mesh metabolic cage (24 cm×24 cm×24 cm), 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 to constant mass, and separated manually. The caloric value of food and feces were determined by Parr1281 oxygen bomb calorimetry (Parr Instrument USA). Gross energy intake (GEI, kJ day^{-1}), digestible energy intake (DEI, kJ day^{-1}) and digestibility (%) were calculated (Grodzinski and Wunder, 1975; Liu et al., 2003) as follows:

$$\text{GEI} = \text{DFI} \times \text{food caloric value},$$

$$\text{DEI} = \text{GEI} - \text{dry feces mass} \times \text{feces caloric value},$$

$$\text{Digestibility} = \text{DEI} / \text{GEI} \times 100\%,$$

where dry food intake and dry feces mass are in g day^{-1} and calorific value in kJ g^{-1}.

Measurements of cytochrome c oxidase (COX) activity and UCP1 content

All voles were sacrificed between 09:00 h and 11:00 h by puncture of the posterior vena cava. Serum was collected and stored at –80°C. The interscapular BAT was carefully dissected, weighed and stored at –80°C. For measuring total mitochondrial protein content, COX activity and UCP1 content, BAT was homogenized as described previously (Zhao and Wang, 2005). The COX activity of BAT was measured by the polarographic method using oxygen electrode units (Hansatech Instruments Ltd, Norfolk, England) (Sundin et al., 1987). The mitochondrial protein content of BAT was measured with Folin phenol method using bovine serum albumin as standards (Lowry et al., 1951).

BAT mitochondrial protein (12 μg per lane) was separated in a discontinuous SDS-polyacylamide gel (12.5% running gel and 3% stacking gel) and blotted to a nitrocellulose membrane (Hybond-C, Amersham Biosciences, Buckinghamshire, UK). Efficiency of protein transfer was checked by staining gels and nitrocellulose membranes with Commassie Brilliant Blue and Ponceau Red, respectively. Unspecific binding sites were saturated with 5% non-fat dry milk in phosphate-buffered saline (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, Baltimore, PA, USA) as the secondary antibody. Enhanced chemoluminescence (ECL; Amersham Biosciences) was used for detection. UCP1 concentration was expressed as relative units (RU), determined by using Scion Image Software (Scion Corporation, Frederick, MA, USA) (Li and Wang, 2005a; Li and Wang, 2005b; Zhao and Wang, 2005; Zhang and Wang, 2006).

Serum leptin assays

Serum leptin levels were determined by radioimmunoassay (RIA) with a ^{125}I multi-species kit (Cat. No. XL-85K, Linco Research Inc., Missouri, USA) (Li and Wang, 2005b; Zhang and Wang, 2006). The lowest level of leptin that could be detected by this assay was 1.0 ng ml^{-1} when using a 100 μl sample (see manufacturer's instructions for multi-species leptin RIA Kit). Inter- and intra-assay variability for leptin RIA were <3.6% and 8.7%, respectively.

Morphology

After collecting trunk blood, the visceral organs, including heart, lung, liver, kidneys, spleen, uterus and gastrointestinal tract (stomach, small intestine, cecum, proximal colon and distal colon), were extracted and weighed (±1 mg). The stomach and intestines were rinsed with saline to eliminate all the gut contents, before being dried and weighed. The remaining carcass and all the organs were dried in an oven at 60°C to constant mass (at least 72 h), and then weighed again to obtain the dry mass. The difference between the wet carcass mass and dry carcass mass was the water mass of carcass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus.

Statistics

Data were analyzed using SPSS software (SPSS 1998). Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. The differences in body mass of the mother and pups during the experimental course were analyzed by repeated measures, followed by Least-Significant Difference (LSD) *post-hoc* tests. Group differences in RMR, energy intake (DFI, GEI, DEI), and morphological parameters were analyzed by a two-way analysis of covariance (ANCOVA) (temperature and lactation) with body mass as a covariate. Group differences in body mass of the mother, COX activity, UCP1 content and serum leptin levels were analyzed by a two-way analysis of variance (ANOVA). Data were further analyzed by one-way ANOVA or ANCOVA followed by LSD *post-hoc* tests. Group differences in the litter and pup masses were analyzed by Independent-samples *t*-test. Finally, linear regression analysis was performed to determine the correlation between residual serum leptin levels and residual GEI, and UCP1 content. Results are presented as means ± s.e.m.; $P<0.05$ was considered to be statistically significant.

Results

Body mass in the mothers and the litter mass

There were no differences in body mass among the four groups before mating ($F_{3,28}=0.05$, $P>0.05$; Fig. 1). However,

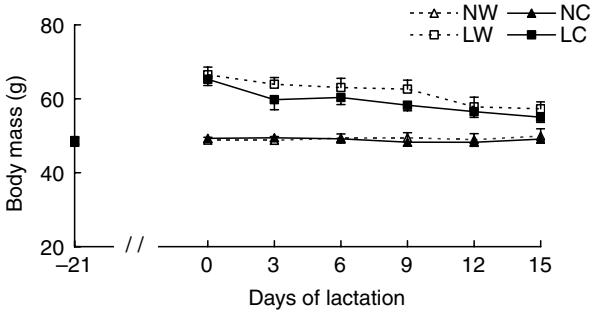

Fig. 1. Changes of body mass during the course of lactation and cold-exposure. Values are means ± s.e.m. (N=8). The lactating voles had higher body mass compared to non-reproductive (NR) voles, and body mass decreased significantly during the 15-day lactation compared to the NR voles, which had constant body mass throughout. Cold exposure did not influence body mass in either the NR or lactating voles. NW, non-reproductive voles in the warm; NC, non-reproductive voles in the cold; LW, lactating voles in the warm; LC, lactating voles in the cold.

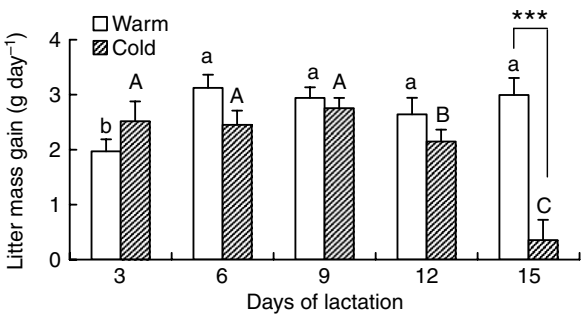

Fig. 2. Changes of litter mass gain during the course of 15-day lactation exposed to 23°C and 5°C. Values are means ± s.e.m. (N=8). The litter in the cold decreased mass gain from days 12 to 15, compared to that in the warm, which showed a relative constant mass gain. Different letters (a or b) above hatched bars indicate significant differences (P<0.05) in measurements within the warm group during the course of acclimation, and different letters (A, B, C) above solid bars indicate significant differences (P<0.05) within the cold-exposed group. ***P<0.001.

on the day of parturition, body mass of lactating voles averaged 65.9±1.3 g, and was higher than the NR (49.1±0.6 g) ($F_{1,28}$=127.3, P<0.001). Over the course of the 15 days lactation, the lactating females showed a steady decrease in body mass (LW: $F_{5,35}$=11.4, P<0.001; LC: $F_{5,35}$=10.8, P<0.001), compared to the NR females, which maintained a constant body mass (NW: $F_{5,35}$=0.54, P>0.05; NC: $F_{5,35}$=1.448, P>0.05). On day 15 of lactation, the lactating females were still heavier by 10–17% than the NR ($F_{1,28}$=127.3, P<0.001). Cold exposure did not affect body mass for both lactating and NR females at any time point (Day 0, $F_{1,28}$=0.06, P>0.05; Day 3, $F_{1,28}$=0.97, P>0.05; Day 6, $F_{1,28}$=0.68, P>0.05; Day 9, $F_{1,28}$=2.72, P>0.05; Day 12, $F_{1,28}$=0.30, P>0.05; Day 15, $F_{1,28}$=0.91, P>0.05).

Two pups from one litter in the cold died on day 14. This litter was still included except for the dead pups. The litters of pups in the cold had lower mass gain from days 12 to 15 ($F_{4,28}$=16.3, P<0.001), compared to the mass gain of those in the warm ($F_{4,28}$=6.17, P<0.001). On day 15 of lactation, the litter mass gain in the cold was decreased by 88% compared to that in the warm (t=5.46, d.f.=14, P<0.001; Fig. 2).

Morphology

Lactating females had higher water mass content of the carcass ($F_{1,27}$=30.2, P<0.001), liver and gastrointestinal tract (Tables 2 and 3), but lower dry carcass mass ($F_{1,27}$=34.4, P<0.001), fat mass ($F_{1,27}$=26.5, P<0.001) and body fat content ($F_{1,27}$=17.2, P<0.001; Table 1) than did NR females. Cold-exposed females had lower wet carcass mass ($F_{1,27}$=10.0, P<0.01), but higher values in kidney, small intestine and caudal colon (Table 2 and 3) compared to females in the warm. Fat free dry mass of the carcass was not affected by either lactation ($F_{1,27}$=0.14, P>0.05) or cold exposure ($F_{1,27}$=1.92, P>0.05; Table 1). The masses of kidney, stomach and small intestine were increased by the interactions of cold exposure and lactation (Table 2 and 3).

Energy intake

Cold-exposed females significantly increased their DFI (by 2.6 g day^{-1} or 16% in the lactating voles, and by 2.7 g day^{-1} or 36% in the NR voles; $F_{1,27}$=27.7, P<0.001), DEI (by 18.5 kJ day^{-1} or 10% in the lactating voles, and by 31.5 kJ day^{-1} or 42% in the NR voles; $F_{1,27}$=20.5, P<0.001; Table 4), compared to those in the warm. The lactating females increased DFI and GEI (by 8.6 g day^{-1} or 117% in the warm, and by 8.6 g day^{-1} or 86% in the cold; $F_{1,27}$=106.9, P<0.001), DEI (by 103.2 kJ day^{-1} or 136% in the warm, and by 90.2 kJ day^{-1} or 84% in the cold; $F_{1,27}$=96.1, P<0.001) compared to the NR females. Digestibility, however, was not changed during cold exposure ($F_{1,28}$=0.07, P>0.05) or lactation ($F_{1,28}$=3.42, P=0.075). The interaction of cold exposure and lactation was significant on DEI ($F_{1,27}$=4.69, P<0.05) and digestibility ($F_{1,28}$=6.15, P<0.05), but not on DFI or GEI ($F_{1,27}$=2.34, P>0.05; Table 4).

RMR and sustained energy intake

As expected, RMR was higher in voles exposed to cold than

Table 1. *Changes of body compositions in non-reproductive and lactating females exposed to 23°C and 5°C*

	Non-reproductive		Lactating		Statistical summary		
Parameters	23°C	5°C	23°C	5°C	T	L	T×L
Body mass (g)	49.9±2.0^{b}	49.1±1.0^{b}	57.3±1.8^{a}	55.0±1.4^{a}	ns	<0.001	<0.05
Wet carcass mass (g)	35.3±0.9^{a}	34.5±1.0^{a}	36.5±0.9^{b}	32.9±0.9^{c}	<0.01	<0.001	ns
Dry carcass mass (g)	18.0±0.8^{a}	17.8±1.1^{a}	15.6±0.6^{b}	13.3±0.5^{b}	ns	<0.001	ns
Water of carcass (g)	17.2±0.7^{a,b}	16.7±0.3^{a}	21.0±0.6^{c}	19.6±0.6^{b}	0.059	<0.001	ns
Fat free dry mass (g)	7.8±0.2	7.7±0.5	8.8±0.2	7.7±0.2	ns	ns	ns
Body fat mass (g)	10.2±0.9^{a}	10.0±1.0^{a}	6.8±0.5^{b}	5.6±0.4^{b}	ns	<0.001	ns
Body fat content (%)	55.7±3.0^{a}	55.8±3.4^{a}	43.2±1.8^{b}	41.8±2.0^{b}	ns	<0.001	ns

Values are means ± s.e.m. (N=8).

Values for a specific parameter that share different superscripts are significantly different at P<0.05, determined by a two-way ANCOVA and LSD *post-hoc* tests.

T, temperature; L, lactation; ns, not significant.

Table 2. *Mean wet organ masses in non-reproductive and lactating females exposed to 23°C and 5°C*

	Non-reproductive		Lactating		Statistical summary		
Parameters	23°C	5°C	23°C	5°C	T	L	T×L
Heart (g)	0.249±0.019	0.249±0.007	0.242±0.005	0.271±0.014	ns	ns	ns
Lungs (g)	0.401±0.028	0.382±0.029	0.390±0.030	0.421±0.035	ns	ns	ns
Liver (g)	1.950±0.138^{b}	1.805±0.064^{b}	2.776±0.101^{a}	2.577±0.094^{a}	ns	<0.001	ns
Kidney (g)	0.508±0.040	0.649±0.104	0.578±0.013	0.692±0.024	<0.05	ns	ns
Spleen (g)	0.048±0.009	0.045±0.009	0.048±0.005	0.051±0.005	ns	ns	ns
Uterus (g)	0.109±0.011	0.128±0.023	0.153±0.011	0.165±0.023	ns	ns	ns
Stomach (g)	0.354±0.028^{b}	0.330±0.017^{b}	0.436±0.014^{b}	0.465±0.017^{a}	ns	<0.05	<0.05
Small intestine (g)	0.656±0.072^{c}	0.658±0.035^{c}	1.087±0.040^{b}	1.368±0.105^{a}	<0.05	<0.001	<0.05
Cecum (g)	0.441±0.042^{b}	0.436±0.027^{b}	0.690±0.032^{a}	0.697±0.031^{a}	<0.05	<0.001	ns
Proximal colon (g)	0.156±0.012^{b}	0.153±0.008^{b}	0.248±0.025^{a}	0.250±0.013^{a}	ns	<0.01	ns
Distal colon (g)	0.207±0.024^{c}	0.227±0.014^{c}	0.381±0.021^{b}	0.422±0.019^{a}	<0.05	<0.001	ns

Values are means ± s.e.m. (N=8).

Values for a specific parameter that share different superscripts are significantly different at P<0.05, determined by a two-way ANCOVA and LSD *post-hoc* tests.

T, temperature; L, lactation; ns, not significant.

warm ($F_{1,27}$=21.1, P<0.001; Fig. 3). The lactating voles (Warm: 148.2±9.6 ml O_2 h^{-1}; Cold: 163.6±4.0 ml O_2 h^{-1}) had a higher RMR than NR voles (Warm: 96.6±4.5 ml O_2 h^{-1}; Cold: 126.2±5.4 ml O_2 h^{-1}) ($F_{1,27}$=20.0, P<0.001). There was no interaction of cold and lactation on RMR ($F_{1,27}$=2.55, P>0.05). Body mass was positively correlated with RMR (R^2=0.328, $F_{1,30}$=14.6, P<0.001). The sustained daily energy intake at peak lactation was 3.8 times of RMR in the warm, and 4.0 times in the cold.

Thermogenic capacity in BAT

Total BAT mass or relative to total body mass was lower in the lactating than that in the non-reproductive voles ($F_{1,28}$=6.31, P<0.05 and $F_{1,28}$=19.3, P<0.001, respectively; Table 5). But cold exposure or the interaction of cold exposure and lactation did not influence BAT mass in either the non-reproductive or lactating voles (P>0.05; Table 5).

Cold exposure increased mitochondrial protein content ($F_{1,28}$=42.5, P<0.001) and COX activities in BAT expressed per mg mitochondrial protein ($F_{1,28}$=13.5, P<0.001), per g BAT ($F_{1,28}$=13.0, P<0.001) and in whole BAT ($F_{1,28}$=19.1, P<0.001; Table 5) in both the non-reproductive and lactating voles. Lactation also significantly influenced BAT mitochondrial protein content ($F_{1,28}$=11.0, P<0.01) and COX activity per g BAT ($F_{1,28}$=13.9, P<0.001; Table 5). Moreover, the mitochondrial protein content in BAT was influenced by the interaction of cold exposure and lactation ($F_{1,28}$=37.3, P<0.001; Table 5).

Table 3. *Mean dry organ masses in non-reproductive and lactating females exposed to 23°C and 5°C*

	Non-reproductive		Lactating		Statistical summary		
Parameters	23°C	5°C	23°C	5°C	T	L	T×L
Heart (g)	0.059±0.003	0.054±0.007	0.055±0.002	0.065±0.003	ns	ns	ns
Lungs (g)	0.093±0.006	0.087±0.006	0.085±0.006	0.091±0.008	ns	ns	ns
Liver (g)	0.584±0.039^{b}	0.540±0.016^{b}	0.826±0.025^{a}	0.768±0.026^{a}	ns	<0.001	ns
Kidney (g)	0.124±0.008^{b}	0.132±0.007^{b}	0.137±0.004^{b}	0.173±0.005^{a}	<0.001	ns	<0.01
Spleen (g)	0.012±0.002	0.012±0.002	0.011±0.002	0.013±0.001	ns	ns	ns
Uterus (g)	0.032±0.005	0.035±0.004	0.031±0.003	0.038±0.004	ns	ns	ns
Stomach (g)	0.083±0.006^{b}	0.077±0.003^{b}	0.098±0.003^{b}	0.104±0.003^{a}	ns	<0.05	<0.01
Small intestine (g)	0.130±0.013^{c}	0.137±0.013^{c}	0.201±0.007^{b}	0.260±0.018^{a}	<0.05	<0.001	0.051
Cecum (g)	0.081±0.007^{b}	0.082±0.006^{b}	0.114±0.005^{a}	0.120±0.007^{a}	ns	<0.001	ns
Proximal colon (g)	0.032±0.002^{b}	0.030±0.002^{b}	0.044±0.004^{ab}	0.045±0.003^{a}	ns	<0.05	ns
Distal colon (g)	0.053±0.004^{c}	0.055±0.003^{bc}	0.077±0.003^{b}	0.089±0.004^{a}	<0.01	<0.001	ns

Values are means ± s.e.m. (N=8).

Values for a specific parameter that share different superscripts are significantly different at P<0.05, determined by a two-way ANCOVA and LSD *post-hoc* tests.

T, temperature; L, lactation; ns, not significant.

Table 4. *Changes of energy intake in non-reproductive and lactating females exposed to 23°C and 5°C*

	Non-reproductive		Lactating		Statistical summary		
Parameters	23°C	5°C	23°C	5°C	T	L	T×L
DFI (g day^{-1})	7.3±0.3^{d}	10.0±0.3^{c}	15.9±1.0^{b}	18.5±0.8^{a}	<0.001	<0.001	ns
GEI (kJ day^{-1})	124.1±5.5^{d}	168.9±5.6^{c}	269.6±16.4^{b}	313.7±13.0^{a}	<0.001	<0.001	ns
DEI (kJ day^{-1})	75.7±3.0^{c}	107.2±3.6^{b}	178.9±13.6^{a}	197.4±8.6^{a}	<0.001	<0.001	<0.05
Digestibility (%)	61.2±0.5^{b}	63.6±1.5^{b}	65.9±1.3^{a}	62.9±0.8^{b}	ns	ns	<0.05

Values are means ± s.e.m. (N=8).

Values for a specific parameter that share different superscripts are significantly different at P<0.05, determined by a two-way ANCOVA and LSD *post-hoc* tests.

DFI, dry food intake; GEI, gross energy intake; DEI, digestible energy intake; T, temperature; L, lactation; ns, not significant.

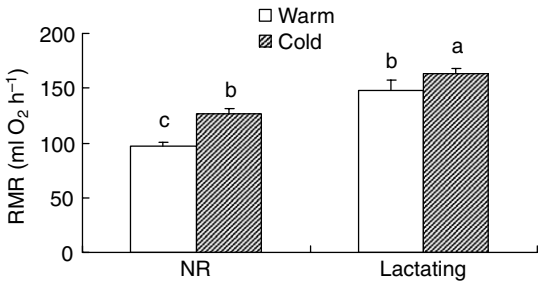

Fig. 3. Changes of resting metabolic rate (RMR) across non-reproductive (NR) and lactating females exposed to 23°C (warm) and 5°C (cold). Values are means ± s.e.m. (N=8). Values that share different superscripts are significantly different at P<0.05. RMR increased significantly in response to cold exposure and lactation.

UCP1 content in the cold was higher than that in warm ($F_{1,28}$=37.4, P<0.001; Fig. 4) both in the non-reproductive (by 16%) and lactating voles (by 75%). UCP1 content was also influenced by lactation ($F_{1,28}$=5.92, P<0.05; Fig. 4), such that the lactating voles decreased UCP1 content by 31% compared with NR in the warm (P<0.05), but there was no difference between these two groups in the cold (P>0.05). There was a significant interaction of cold and lactation on UCP1 content ($F_{1,28}$=10.5, P<0.01; Fig. 4). The lactating voles in the warm had the lowest UCP1 contents ($F_{3,28}$=17.9, P<0.001).

Serum leptin levels and the correlations with body fat mass, gross energy intake and UCP1 content

Serum leptin levels significantly decreased in the lactating voles compared with those in the NR voles ($F_{1,28}$=59.4, P<0.001), by 53% in the warm and by 43% in the cold. Although the difference was not significant between voles in the warm and cold ($F_{1,28}$=3.41, P=0.075), serum leptin levels in the cold decreased by 24% in NR voles, and by 6% in the lactating voles. There was no interaction of cold and lactation on serum leptin levels ($F_{1,28}$=2.08, P>0.05; Fig. 5).

Serum leptin was positively correlated with body mass (r^2=0.175, $F_{1,30}$=6.359, P<0.05) and fat mass (r^2=0.435, $F_{1,30}$=23.053, P<0.001), and negatively with gross energy intake (r^2=0.664, $F_{1,30}$=59.332, P<0.001) and RMR (r^2=0.473, $F_{1,30}$=26.974, P<0.001). After correcting for the effects of body mass, residual serum leptin was still negatively correlated with residual gross energy intake (r^2=0.598, $F_{1,30}$=44.7, P<0.001; Fig. 6A) and residual RMR (r^2=0.384, $F_{1,30}$=18.7, P<0.001; Fig. 6B). There was no relationship between residual serum

Table 5. *Changes in mitochondrial protein and cytochrome c oxidase in brown adipose tissue in non-reproductive and lactating females exposed to 23°C and 5°C*

	Non-reproductive		Lactating		Statistical summary		
Parameters	23°C	5°C	23°C	5°C	T	L	T×L
BAT mass (g)	0.193±0.012^{a,b}	0.223±0.016^{a}	0.171±0.014^{b}	0.170±0.008^{b}	ns	<0.01	ns
% body mass	0.393±0.025^{a}	0.457±0.039^{a}	0.302±0.030^{b}	0.309±0.013^{b}	ns	<0.001	ns
MP (mg g^{-1} BAT)	9.018±0.689^{b,c}	9.308±0.524^{b}	7.042±0.622^{c}	15.981±0.932^{a}	<0.001	<0.01	<0.001
COX							
nmolO_2 min^{-1} mg MP	285.85±14.45^{b,c}	302.75±15.38^{b}	401.47±25.26^{a}	239.73±21.61^{c}	<0.001	ns	<0.001
nmolO_2 min^{-1} g BAT	2515.8±97.9^{b}	2777.6±122.2^{b}	2797.8±261.2^{b}	3699.5±106.6^{c}	<0.001	<0.001	ns
nmolO_2 min^{-1} in whole BAT	485.7±36.0^{b}	610.9±34.9^{a}	460.1±34.9^{b}	626.2±26.8^{a}	<0.001	ns	ns

Values are means ± s.e.m. (N=8).

Values for a specific parameter that share different superscripts are significantly different at P<0.05, determined by a two-way ANOVA and LSD *post-hoc* tests.

MP, mitochondrial protein; COX, cytochrome *c* oxidase; BAT, brown adipose tissue; T, temperature; L, lactation; ns, not significant.

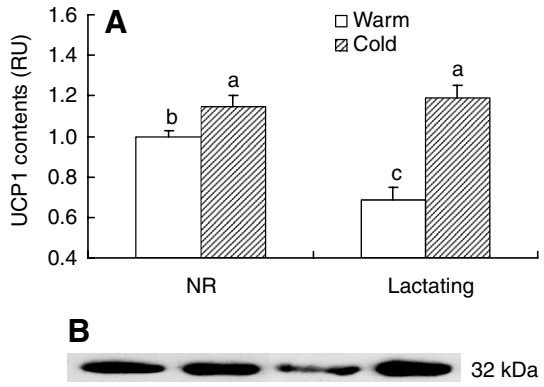

Fig. 4. Uncoupling protein 1 (UCP1) content (RU) across non-reproductive (NR) and lactating females exposed to 23°C (warm) and 5°C (cold). Means with the different letters within the four groups are significantly different at $P<0.05$. (A) Cold exposure increased UCP1 content both in the non-reproductive and lactating voles, although the lactating voles decreased UCP1 content by 31.3% in the warm. (B) Western blotting detection of UCP1 content for the non-reproductive or lactating Brandt's voles in the warm or in the cold. The blots from the left to right matched those in A.

leptin and UCP1 content (r^2=0.001, $F_{1,30}$=0.030, $P>0.05$). However, if the data in the warm and cold were analyzed separately, residual serum leptin was positively correlated with UCP1 content in the warm (r^2=0.543, $F_{1,14}$=16.6, P=0.001), but not in the cold (r^2=0.075, $F_{1,14}$=0.299, $P>0.05$; Fig. 6C).

Discussion

As expected (Cannon and Nedergaard, 2004), our data further support the notion that thermogenic capacity increased for the rodents to respond to the cold. Moreover, lactating Brandt's voles increased UCP1 content in the cold, to the same level as the cold-exposed NR females. Energy intake in the cold-exposed lactating females at peak lactation was increased, to compensate for the added energy demands of cold exposure.

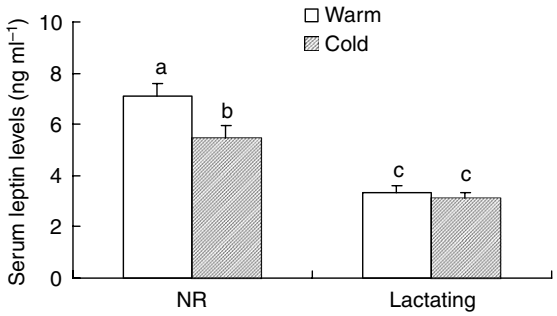

Fig. 5. Serum leptin levels across non-reproductive (NR) and lactating females exposed to 23°C (warm) and 5°C (cold). Values are means ± s.e.m. (N=8). Values that share different letters are significantly different at $P<0.05$. Serum leptin levels decreased significantly in the lactating voles, while cold exposure decreased serum leptin levels only in the non-reproductive voles, but did not in the lactating voles.

Serum leptin levels decreased in response to lactation, and were negatively correlated with energy intake, but not with UCP1 content. Our data suggest that the voles could respond to simultaneous cold and lactation by increasing their thermogenic capacity and energy intake. Serum leptin may be involved in the regulation of energy intake, but thermoregulation by leptin was complicated under different conditions.

Changes in body mass and body compositions

During pregnancy and lactation, nutritional requirements in small mammals are increased and the maintenance of body mass is critical to the survival and reproduction (Amico et al., 1998). The changes in body mass during pregnancy and lactation are species-specific. For example, laboratory rats (Leshner et al., 1972) and mice (Richard and Trayhurn, 1985; Johnson et al., 2001a) increased body mass and body fat during pregnancy and lactation. Siberian hamsters (Schneider and Wade, 1987; Bartness, 1997) and Syrian hamsters (Fleming, 1978; Wade et al., 1986) increased body mass, but decreased body fat mass during pregnancy and lactation. In the present study, Brandt's voles decreased body mass and body fat mass

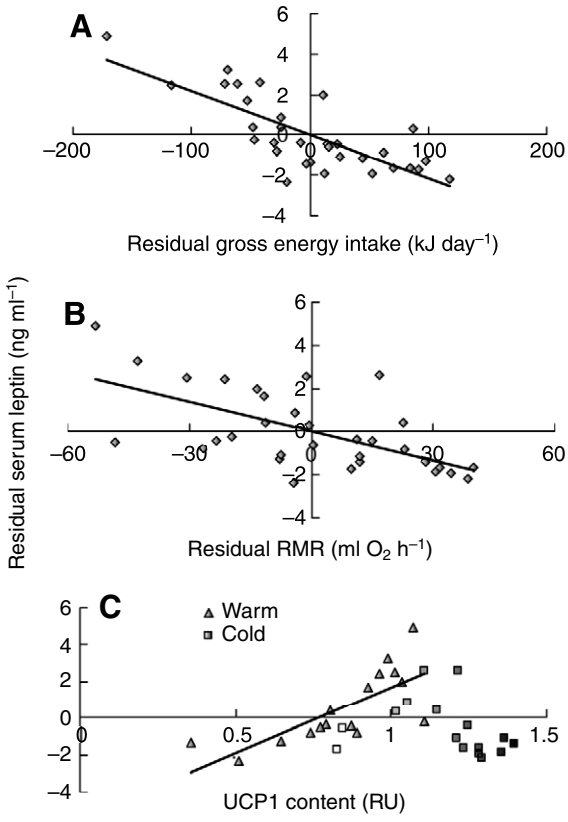

Fig. 6. Correlations of residual serum leptin with (A) residual gross energy intake (r^2=0.598, $F_{1,30}$=44.7, $P<0.001$), (B) residual resting metabolic rate (RMR) (r^2=0.384, $F_{1,30}$=18.7, P=0.001) and (C) uncoupling protein 1 (UCP1) content (positive only in the warm: r^2=0.543, $F_{1,14}$=16.6, P=0.001; but not in the cold: r^2=0.075, $F_{1,14}$=0.299, $P>0.05$) in non-reproductive (NR) and lactating females exposed to 23°C (warm) and 5°C (cold).

during lactation, consistent with the previous findings in the same species (Liu et al., 2003). These data suggest that mobilizing body fat may be one of metabolic compensations to meet the high nutrition (energy) requirements during lactation in some rodent species. In addition, modification of digestive organs, to meet the high energy intake and digestion, suggests that the sustained energy intake during lactation is not limited by the central machinery in Brandt's voles.

We also found that the litter mass decreased during cold exposure. This was supported by the findings in the cold-exposed lactating mice (Hammond and Kristan, 2000; Johnson and Speakman, 2001). The decrease in the litter mass may be the result of the decreased milk production or the greater daily energy expenditure of the pups in the cold. Johnson and Speakman found that cold-exposed lactating mice increased the volume of milk production and energy content, indicating that it was not the decrease in milk production that resulted in the decreased litter mass (Johnson and Speakman, 2001). Since we did not measure milk production and litter daily energy expenditure, however, we could not establish the factors that resulted in the decreased litter mass gains during cold exposure in Brandt's voles.

Changes in RMR and energy intake in response to cold exposure and lactation

The decrease in body mass during lactation was associated with changes in energy expenditure and intake. The lactating Brandt's voles increased RMR by 53%, as seen in other rodent species (Harder et al., 1996; Johnson and Speakman, 2001), and further increased it by 10% when exposed to cold. The increase in RMR was consistent with the increases in body mass, organ mass and the decomposition of fat reserves (Johnstone et al., 2005). The increased energy demand during lactation and/or cold exposure is met by the increase in food intake, as also shown for other small mammals (Wade and Schneider, 1992; Hammond et al., 1994; Bartness, 1997; Hammond and Kristan, 2000; Scantlebury et al., 2000; Johnson and Speakman, 2001).

The sustained gross energy intake at peak lactation was 4 times the measured RMR in cold-exposed female voles, which was similar to the previously suggested limitation on sustained metabolic rate (Drent and Dann, 1980), but was lower compared to the postulated limit of 7×RMR (Peterson et al., 1990; Hammond and Diamond, 1997). In the cold-exposed lactating MF1 mice, however, the sustained energy intake during late lactation was 9.4×RMR (Johnson and Speakman, 2001). This difference between Brandt's voles and MF1 mice may be related to their different litter sizes, as the former has a mean litter size of around 7 (Liu et al., 2003), while the latter is around 11 (Johnson and Speakman, 2001). Moreover, MF1 lactating mice in the cold increased food intake by 6.9 g compared with an average of 2.7 g increase in the non-reproductive females (Johnson and Speakman, 2001), while lactating Brandt's voles in the cold increased by the same amount of food as did the non-reproductive voles. Additionally, the cold-exposed lactating mice did not increase RMR, while the lactating Brandt's voles did, which may also result in the lower sustained metabolic rate in the voles.

Changes of thermogenesis in response to cold exposure and lactation

Thermogenic capacity in small mammals is enhanced in the cold (Cannon and Nedergaard, 2004), but suppressed during lactation (Trayhurn et al., 1982). In the present study, we found that the lactating voles decreased COX activity and UCP1 content, as shown in the previous study in Brandt's voles (Li and Wang, 2005a), and also decreased the absolute and relative BAT mass. These results have also been found in other wild rodents (Wade et al., 1986; Schneider and Wade, 1987; Nizielski et al., 1993). Inhibition of BAT thermogenesis has a significant energy-sparing effect on lactating (Trayhurn et al., 1982) and fasting animals (Sivitz et al., 1999). Alternatively, it can avoid overheating due to processing food and producing milk during lactation (Król and Speakman, 2003). We also found both non-reproductive and lactating voles increased BAT thermogenic capacity during cold exposure. These data suggest that cold-induced BAT thermogenesis is important for Brandt's voles to survive the cold early spring.

Roles of leptin in regulating energy intake and thermogenesis during cold exposure and lactation

It has been suggested that leptin, positively correlated with body fat content, plays roles in inducing hyperphagia and inhibiting thermogenesis during lactation (Schneider et al., 1998; Stocker et al., 2004; Xiao et al., 2004). Serum leptin levels in the lactating voles decreased significantly, coupled with higher energy expenditure (RMR), compared with the non-reproductive females. Similar results in the warm were found in rats (Amico et al., 1998; Woodside et al., 2000; Denis et al., 2003) and also in insectivorous bats *Eptesicus fuscus* (Kunz et al., 1999). These data support the hypothesis that the negative energy state results in the decrease in serum leptin during lactation (Vernon et al., 2002). Interestingly, in rats low leptin levels occur in the presence of large body fat stores (Brogan et al., 1999), which suggests that leptin synthesis and/or secretion was actively inhibited during lactation. Furthermore, our results show that after correcting for the effects of body mass, residual serum leptin was negatively related to residual energy intake. Similar results were also found in seasonally acclimatized Brandt's voles (Li and Wang, 2005b) and photoperiod acclimated root voles *Microtus oeconomus* (Wang et al., 2006). Other results have indicated that leptin administration to lactating mice or rats reduced their food intake (Woodside et al., 2000; Mistry and Romsos, 2002; Stocker et al., 2004). Together, these data may further support the hypothesis (Flier, 1998) that leptin acts as a 'starvation signal' to increase food intake during negative energy balance states such as fasting, cold exposure and/or lactation. It should be noted that, although serum leptin levels decrease little in lactating voles exposed to cold, food intake was further increased compared with that in the warm, which may suggest that the lactating voles were much sensitive to the levels of

leptin. Compared with cold-active mammals, hibernators ignore ‘satiety signal’ of leptin to allow extreme fat deposition during prehibernatory period (Kronfeld-Schor et al., 2000).

Although leptin is involved in regulating energy balance, the relationship between leptin and energy expenditure is still ambiguous. Leptin administration to mice or rats increased oxygen consumption, UCP1 mRNA and protein expression (Hwa et al., 1997; Scarpace and Metheny, 1998; Xiao et al., 2004). However, BAT thermogenesis was reduced in cold-acclimated rats when they were injected with exogenous leptin (Abelenda et al., 2003). Leptin administration to post-hibernatory Arctic ground squirrels did not alter RMR, BAT UCP1 mRNA and protein levels, but reduced food intake and weight gain (Boyer et al., 1997). The present study showed that serum leptin levels were negatively correlated with RMR (corrected for body mass) during lactation and cold exposure, in contrast to the proposed relationship between leptin and energy expenditure (Hwa et al., 1997). In previous studies on the cold-acclimated or seasonally acclimatized Brandt’s voles (Li and Wang, 2005b; Zhang and Wang, 2006), the decreased serum leptin levels were also associated with increased RMR and thermogenesis. Moreover, we found that serum leptin levels (corrected for body mass) were positively correlated with UCP1 content only in the warm-acclimated voles, but not in the cold. These data suggest that in warm-acclimated voles, leptin may be involved in decreasing energy expenditure by inducing the thermogenesis, while in the cold, the increase in thermogenesis activated by the sympathetic nerve may conceal the reduction of thermogenesis by decreased serum leptin. Although the exact relationship between leptin and energy expenditure could not be determined just by the correlation analysis, the recent finding that a leptin antagonist blocked leptin-mediated anorexic effects as well as the increase in BAT UCP1 protein (Zhang et al., 2006), confirms that leptin plays roles in regulating not only food intake but also thermogenesis.

Brandt’s voles can suppress thermogenesis during lactation. The conserved available energy might be used for milk production and/or to avoid overheating during lactation (Johnson et al., 2001a; Król and Speakman, 2003). During simultaneous cold exposure and lactation, however, the voles can increase thermogenesis. Serum leptin, secreted according to the body status and circumstances, was negatively related to the energy intake and RMR. These data suggest that Brandt’s voles can adjust energy intake and thermogenesis to accommodate simultaneous lactation and cold exposure, and serum leptin may potentially be involved in the regulation of energy intake and thermogenesis, but the thermoregulation in the cold may be mainly mediated by other factors.

List of abbreviations

BAT	brown adipose tissue
COX	cytochrome *c* oxidase
DEI	digestible energy intake
DFI	dry food intake
ECL	enhanced chemoluminescence
GEI	gross energy intake
LC	lactating in the cold
LW	lactating in the warm
NC	non-reproductive females in the cold
NR	non-reproductive
NW	non-reproductive females in the warm
RIA	radioimmunoassay
RMR	resting metabolic rate
RU	relative units
STP	standard temperature and pressure
UCP1	uncoupling protein 1

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