

Seasonal changes in thermogenesis and body mass in wild Mongolian gerbils (*Meriones unguiculatus*)

Zhi-Qiang Zhang^{a,b,c}, De-Hua Wang^{a,*}

^a State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, 100101 Beijing, China

^b College of Animal Science and Technology, Anhui Agriculture University, 230036 Hefei, China

^c Graduate School of the Chinese Academy of Sciences, 100049 Beijing, China

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Abstract

Seasonal adjustments in body mass (BM), nonshivering thermogenesis (NST) and several physiological, hormonal, and biochemical markers were measured in wild-trapped Mongolian gerbils (*Meriones unguiculatus*) from Inner Mongolia, China. Sexual differences were detected in BM, NST, brown adipose tissue (BAT) mass, and mitochondrial protein content. BM and NST in males were higher in winter (January) and spring (May) than in summer (August), and BM of females was also the highest in winter, but NST remained relatively constant throughout the year. Cytochrome c oxidase activity and mitochondrial uncoupling protein 1 (UCP1) content in BAT were enhanced in winter in males or females, respectively. Serum leptin concentration was the lowest in winter and positively correlated with BM and body fat mass but was negatively correlated with BAT UCP1 content. These data suggest that wild Mongolian gerbils do not depend on a decrease in BM, but instead increase their thermogenic capacity to cope with cold stress. Leptin may be involved in the seasonal regulation in energy balance and thermogenesis in field Mongolian gerbils.

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1. Introduction

In temperate zones, most small mammals increase their capacity of nonshivering thermogenesis (NST) in winter or winter-like conditions (Feist and Rosemann, 1976; Heldmaier et al., 1990; Merritt and Zegerts, 1991; Li and Wang, 2005a,b; Wang et al., 2006a,b) through uncoupling respiration in brown adipose tissue (BAT) (Foster and Frydman, 1978; Cannon et al., 1982). The uncoupling is mainly regulated by uncoupling protein 1 (UCP1), which is located in the inner mitochondrial membrane of BAT (Cannon and Nedergaard, 2004). Short photoperiod is considered to be an important cue for the induction of an increase in BAT UCP1 in wood mice (*Apodemus sylvaticus*) (Haim et al., 1995a,b), short-tailed vole (*Microtus agrestis*) (McDevitt and Speakman, 1996),

common spiny mice (*Acomys cahirinus*) (Kronfeld-Schor et al., 2000) and Brandt's voles (*Lasiopodomys brandtii*) (Zhao and Wang, 2005, 2006b), although not all species show the same responses (McDevitt and Speakman, 1994b; Kronfeld-Schor et al., 2000; Zhao and Wang, 2006a). Likewise, cold acclimation can significantly enhance BAT UCP1 content or UCP1 mRNA expression (Wang et al., 2000b; Praun et al., 2001; Li et al., 2001; Zhang and Wang, 2006a). Some information is available on seasonal patterns of thermogenesis and body mass (BM) using integrative approaches from organismal to molecular levels in wild small mammals (Klaus et al., 1988; Kronfeld-Schor et al., 2000; Wang et al., 2006a,b).

Leptin is a hormone secreted mainly by the adipose tissue in mammals (Zhang et al., 1994). It functions as an important regulator of BM via the control for energy intake and expenditure by acting on neuropeptide circuits in the hypothalamus (Friedman and Halaas, 1998; Concannon et al., 2001). Serum leptin levels are lower under winter-like conditions in Siberian hamsters (*Phodopus sungorus*) (Klingenspor et al.,

* Corresponding author. Tel.: +86 10 6480 7073; fax: +86 10 6480 7099.

E-mail address: wangdh@ioz.ac.cn (D.-H. Wang).

2000) and woodchucks (*Marmota monax*) (Concannon et al., 2001) during seasonal acclimatization, coupled with an increase in food intake. However, the relationship between leptin and thermogenesis is still not clear. Leptin administration to cold-acclimated rats reduces both food intake and BAT UCP1 mRNA levels (Abelenda et al., 2003), and serum leptin level is negatively correlated with UCP1 content in some seasonal small mammals (Li and Wang, 2005a; Wang et al., 2006a,b). However, no significant correlations have been found between leptin and UCP1 level in cold-acclimated Mongolian gerbils (*Meriones unguiculatus*) (Li et al., 2004) or field voles (*M. agrestis*) (Król et al., 2006).

The Mongolian gerbil is a small rodent distributed in the desert and semiarid regions of Mongolia and Northern China (Walker, 1968). Only a few studies have been performed on wild Mongolian gerbils (Weiner and Górecki, 1981; Wang et al., 2000a; Zhang and Wang, 2006b; Zhang et al., 2006), and seasonal patterns of thermogenesis and BM in the field are presently not well understood. Here we measure seasonal changes in thermogenic properties integratively from organismal to molecular levels including NST, BAT mass, mitochondrial protein (MP) content and cytochrome c oxidase (COX) activity from wild-caught Mongolian gerbils, and explore the potential role of leptin in the regulation of BM and thermogenesis. We hypothesize that, similar to other small mammals, Mongolian gerbils will change their thermogenesis seasonally and serum leptin will change with BM or body fat mass. We predict that Mongolian gerbils will show a decrease in BM and increase in thermogenesis in the cold season and that serum leptin level will be lower in winter than summer.

2. Materials and methods

2.1. Animals

This study was carried out in Taipusiqi County (41°58'N, 115°17'E, 1300–1800 m in altitude), Inner Mongolia, China. This area is a mixture of grassland and farmland. The annual mean temperature is -0.4 °C, average monthly T_a in the coldest month (January) is -22.3 °C and 18.8 °C in the warmest month (July). The extreme low temperature is -47.5 °C in January and the extreme high is 35.3 °C in July. Average annual precipitation is 350 mm and the plant growth period is 150–180 days (Chen, 1988).

All experimental procedures were licensed by the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. A total of 38 males and 37 females, after excluding pregnant, lactating or young individuals (less than 35 g in BM), included 7–11 adult males and 7–12 females in each of summer (August 2004), fall (October 2004), winter (January 2005), and spring (May 2005). The animals were live-trapped in an area 30 km from the field laboratory of Taipusiqi County. Captured gerbils were transported to the field laboratory and kept individually in plastic cages (30×15×20 cm) with cotton as bedding under natural temperature and photoperiod, and offered natural forage with some carrots in each season. Food and water were provided *ad libitum*.

2.2. Metabolic trials

Resting metabolic rate (RMR) was measured with an established closed-circuit respirometer at 30 °C within the TNZ (26–38 °C, Wang et al., 2000a) on the day after capture. Gerbils were stabilized in the metabolic chamber for at least 60 min prior to the RMR measurement. The metabolic chamber volume was 3.6 L. A water bath was used to control the temperature (± 0.5 °C) of the metabolic chamber and KOH and silica gel were used to absorb carbon dioxide and water in the chamber. Oxygen consumption was recorded for 60 min at 5 min intervals. The two stable consecutive lowest readings were used to calculate the RMR. BM was measured before and after each measurement.

Nonshivering thermogenesis (NST) was induced by subcutaneous injection of norepinephrine (NE) (Shanghai Harvest Pharmaceutical Co. Ltd.) and was measured at 2 °C below the TNZ. The dosage of NE was calculated according to the equation of Heldmaier (1971): NE dosage (mg/kg) = $6.6 \text{ Mb}^{-0.458}$ (Mb is body mass in gram). In previous experiments, Li and Wang (2005b) and Zhao and Wang (2006b) demonstrated that this dosage of NE maximized metabolic responses in Mongolian gerbils. Further, Wang and Wang (2006) showed that NST induced by this dosage was close to what might be expected from the dosage suggested by Wunder and Gettinger (1996) [NE (mg/kg) = $2.53 \text{ Mb}^{-0.4}$]. Two consecutive highest recordings of oxygen consumption at each measurement were taken to calculate the maximum NST (Li and Wang, 2005b). All metabolic measurements were performed between 0800 and 1800 h to minimize the effect of circadian rhythms. RMR and NST were corrected to standard temperature and pressure (STP) conditions.

2.3. Measurements of COX activity and MP content

After RMR and NST measurement, gerbils were sacrificed and blood and tissue samples were taken for later measurements. Interscapular BAT was removed, and each pad was trimmed of connective tissue and weighed immediately (Zhang et al., 2006), then stored at -55 °C.

COX activity was measured with the polarographic method using oxygen electrode units (Hansatech Instruments Ltd., England) according to Li and Wang (2005a) and Zhao and Wang (2005). MP was prepared as described by Wang et al. (1999) and Li et al. (2001). MP contents were determined by the Folin phenol method (Lowry et al., 1951) with bovine serum albumin as standard.

2.4. Measurement of UCP1

Total BAT MP (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) as described by Li and Wang (2005a) and Zhao and Wang (2005). Briefly, to check for the efficiency of protein transfer, gels and nitrocellulose membranes were stained with Coomassie brilliant blue and Ponceau red,

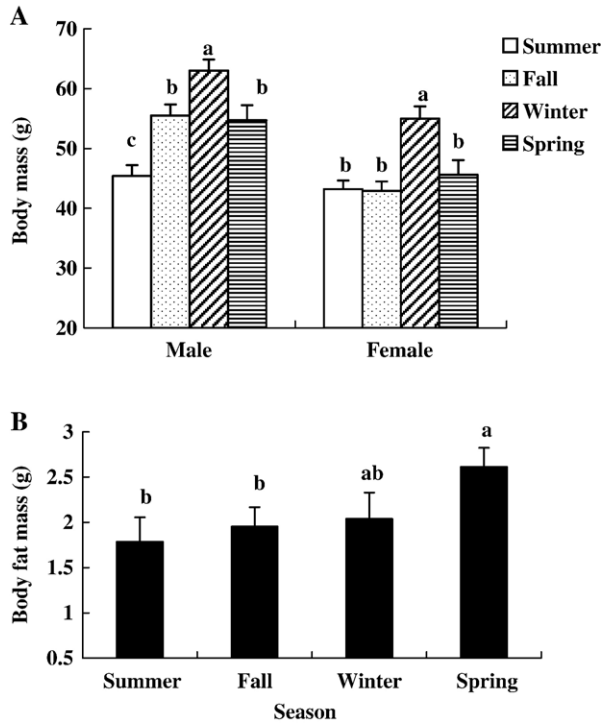


Fig. 1. Seasonal changes of body mass (A) and body fat mass (B) in *Meriones unguiculatus*. Different letters above the bars are significantly different ($n=7-12$, mean \pm SE).

respectively. 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 (1:5000, Jackson Immuno. Inc., USA) as the second antibody (Klingenspor et al., 1996). We used enhanced chemoluminescence (ECL, Amersham) for detection, and unspecific binding sites were saturated with 5% non-fat dry milk in phosphate-buffered saline. UCP1 protein content was quantified with Scion Image Software (Scion Corporation) and expressed in relative units (RU) (Li and Wang, 2005a; Zhao and Wang, 2005).

2.5. Measurement of serum leptin

Blood samples were centrifuged at 1500 \times g for 30 min and the serum was sampled and stored at -20 °C. Serum leptin concentrations were measured by radioimmunoassay (RIA) using the Linco 125 I Multispecies Kit (Linco, St. Louis, MO, USA). The detection limit for leptin was 1.0 ng/mL when using a 100 μ L sample size (Instructions for Multi-species leptin RIA Kit). Inter- and intra-assay variabilities for leptin RIA were <3.6% and 8.7%, respectively. It has been demonstrated that this kit is valid for the measurement of leptin levels in Mongolian gerbils (Li et al., 2004; Zhao and Wang, 2006b).

2.6. Body fat analysis

The eviscerated carcass (excluding interscapular BAT and the gastrointestinal tract) was oven-dried at 58 °C to constant

mass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus (Li and Wang, 2005a).

2.7. Statistics

Statistical analysis was performed using the SPSS software package (SPSS, 1988). Distributions of all variables were tested for normality by the Kolmogorov–Smirnov test. Data that were not normally distributed were transformed to natural logarithms. Sexual differences were analyzed by Two-way (season by gender) analysis of variance (Two-way ANOVA for BM, BAT MP content, COX activity and UCP1 content or Two-way ANCOVA for RMR, NST, BAT mass and body fat mass as well as serum leptin level). If no gender difference was detected, differences among seasons were further analyzed by One-way ANOVA, except RMR, NST, BAT mass, body fat mass and serum leptin concentration, which were tested by ANCOVA with BM as the covariate. Group differences were evaluated using the least-significant difference (LSD) *post hoc* test. Possible correlations between serum leptin and BM and body fat mass, as well as between NST and MP content and COX activity in whole BAT and UCP1 content were examined by Pearson correlation. Possible correlations between RMR and NST and leptin level, as well as between leptin level and UCP1 content were carried out by partial correlation after controlling for BM. Data are presented as mean \pm SE in the text and the significance level was $p<0.05$.

3. Results

3.1. BM and body fat mass

BM of male gerbils was higher than that of females across the seasons except for summer, and increased by 29% and 23% from summer to winter in males and females, respectively (Two-way ANOVA: $F(1,67)=30.646$, $p<0.001$; $F(3,67)=16.596$, $p<0.001$, Fig. 1A). Body fat mass did not show any gender difference (Two-way ANCOVA: $F(1,62)=1.661$, $p=0.202$, Fig. 1B). It was higher in winter and spring and lower in summer (One-way ANOVA: $F(3,62)=2.637$, $p=0.057$, Fig. 1B), and was positively correlated with BM (Pearson correlation: $r^2=0.173$, $n=71$, $p<0.001$).

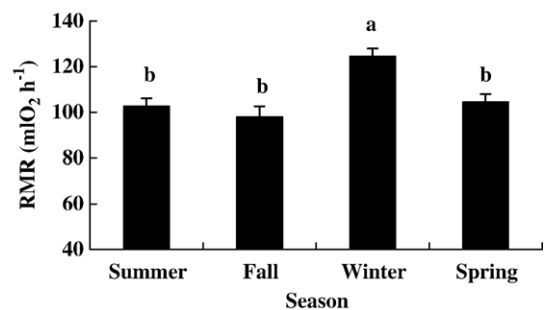


Fig. 2. Seasonal changes of resting metabolic rate (RMR, mL O₂ h⁻¹ for adjusted means with body mass as covariate) in *Meriones unguiculatus*. Different letters above the bars are significantly different ($n=7-12$, mean \pm SE).

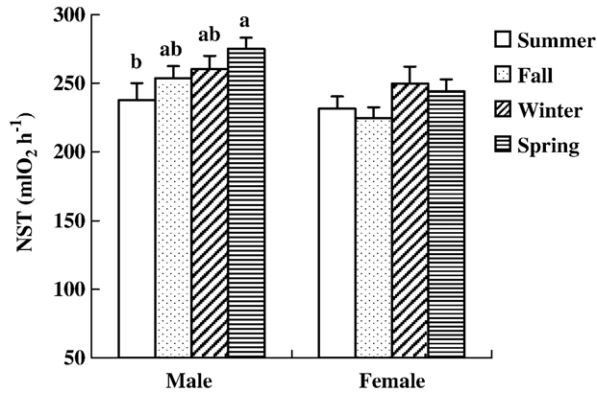


Fig. 3. Seasonal changes of nonshivering thermogenesis (NST, $\text{mLO}_2 \text{ h}^{-1}$ for adjusted means with body mass as covariate) in *Meriones unguiculatus*. Different letters above the bars are significantly different ($n=7-12$, mean \pm SE).

3.2. RMR and NST

No gender differences were found in RMR or NST in any season (Two-way ANOVA: $F(1,66)=2.939$, $p=0.091$; $F(1,67)=3.464$, $p=0.067$) except that NST in males was higher than females in spring (Two-way ANCOVA: $F(1,66)=4.455$, $p=0.039$; t -test: $t=3.219$, $df=18$, $p<0.05$). RMR with BM as the covariate showed significant seasonal variations, and was higher in winter than in any other season (Two-way ANCOVA:

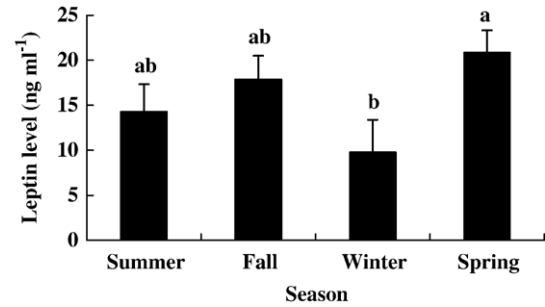


Fig. 4. Seasonal changes of serum leptin levels in *Meriones unguiculatus*. Different letters above the bars are significantly different (male: $n=6-8$, female: $n=5-6$, mean \pm SE).

$F(3,66)=5.278$, $p=0.003$, Fig. 2). NST maintained relatively stable throughout the year (Two-way ANOVA: $F(3,67)=2.135$, $p=0.104$). After adjustment for BM, NST in males was higher in winter and spring than in summer, but not in females (Two-way ANCOVA: $F(3,66)=3.142$, $p=0.031$, Fig. 3). No significant correlations were found between RMR and NST (Partial correlation: $r^2=0.054$, $n=71$, $p=0.054$) or leptin concentration (Partial correlation: $r^2=0.017$, $n=43$, $p=0.410$) after controlling for BM, or between NST and MP content (Pearson correlation: $r^2=0.005$, $n=71$, $p=0.542$), COX activity in whole BAT (Pearson correlation: $r^2=0.002$, $n=71$, $p=0.916$), or UCP1 content (Pearson correlation: $r^2=0.018$, $n=36$, $p=0.429$).

Table 1

Seasonal changes of brown adipose tissue (BAT) mass, mitochondrial protein (MP) and uncoupling protein 1 (UCP1) content, and cytochrome c oxidase (COX) activity in wild Mongolian gerbils (*Meriones unguiculatus*)

	Summer	Fall	Winter	Spring	p value*		
					S	G	S×G
BAT mass (mg)							
Male	10.5 \pm 1.8 ^b	14.1 \pm 1.6 ^a	12.0 \pm 1.8 ^a	12.9 \pm 1.5 ^a	<0.05	<0.01	ns
Female	12.8 \pm 1.7 ^b	15.6 \pm 1.5 ^{bc}	17.1 \pm 1.8 ^a	20.2 \pm 1.6 ^{ab}			
BAT MP content							
mg g ⁻¹ BAT							
Male	6.9 \pm 0.2 ^b	12.3 \pm 0.7 ^a	10.5 \pm 0.6 ^a	10.6 \pm 0.8 ^a	<0.01	<0.01	ns
Female	10.4 \pm 0.7 ^b	12.6 \pm 0.9 ^{ab}	15.1 \pm 0.8 ^a	10.9 \pm 1.2 ^b			
mg in whole							
BAT							
Male	0.6 \pm 0.1 ^c	2.0 \pm 0.1 ^a	1.6 \pm 0.1 ^b	1.4 \pm 0.1 ^b	<0.01	<0.01	<0.01
Female	1.1 \pm 0.1 ^c	1.6 \pm 0.1 ^b	2.8 \pm 0.1 ^a	2.1 \pm 0.2 ^a			
BAT UCP1 content (RU)							
Male	0.5 \pm 0.2 ^c	1.2 \pm 0.2 ^b	2.1 \pm 0.2 ^a	1.7 \pm 0.1 ^a	<0.01	ns	<0.05
Female	1.3 \pm 0.2	1.3 \pm 0.2	1.7 \pm 0.3	1.5 \pm 0.2			
BAT COX activity							
nmol O ₂ min ⁻¹ mg ⁻¹ MP							
Male	265 \pm 23 ^b	388 \pm 38 ^a	372 \pm 23 ^a	347 \pm 16 ^a	<0.01	ns	ns
Female	302 \pm 31 ^b	389 \pm 17 ^a	308 \pm 14 ^{ab}	349 \pm 7 ^a			
nmol O ₂ min ⁻¹ g ⁻¹ BAT							
Male	19.4 \pm 1.7 ^b	22.2 \pm 1.7 ^b	31.4 \pm 1.7 ^a	23.5 \pm 1.8 ^b	<0.01	ns	ns
Female	16.5 \pm 1.5 ^b	23.0 \pm 0.9 ^{ab}	27.7 \pm 1.6 ^a	20.5 \pm 2.1 ^{ab}			
nmol O ₂ min ⁻¹ in whole BAT							
Male	184 \pm 26 ^b	694 \pm 69 ^a	653 \pm 80 ^a	552 \pm 52 ^a	<0.01	ns	<0.01
Female	387 \pm 61 ^c	494 \pm 50 ^{bc}	732 \pm 90 ^{ab}	812 \pm 94 ^a			

ANOVA for the effects of season (S), gender (G), and season \times gender interaction (S \times G) on BAT mass, MP and UCP1 content, and COX activity. * The significant level was set at $p<0.05$; ns = not significant.

Data are presented as mean \pm SEM. Different superscripts within the same row represent significant differences among seasons.

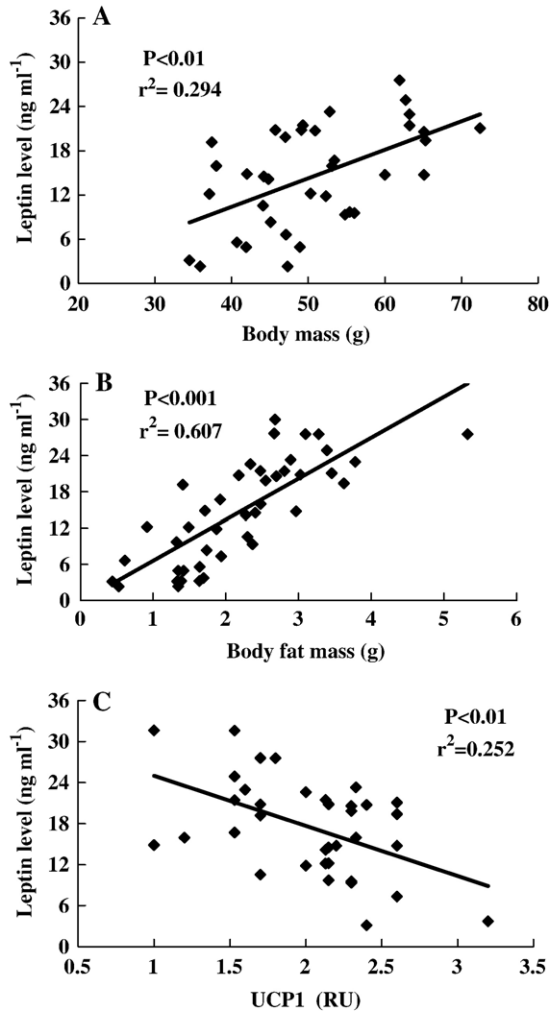


Fig. 5. Correlation of serum leptin levels with body mass (A) and body fat mass (B), as well as partial correlation of leptin levels with uncoupling protein 1 content (C) controlling for body mass in *Meriones unguiculatus*. (Leptin, male: $n=6-8$, female: $n=5-6$; UCP1, male: $n=5-6$, female: $n=3-4$, mean \pm SE).

3.3. BAT Mass, MP and UCP1 content, and COX activity

BAT mass in females was higher than that in males in spring (Two-way ANCOVA: $F(1,66)=9.547$, $p=0.003$); it was higher in winter than summer ($F(3,66)=3.041$, $p=0.035$; Table 1).

MP content in BAT in females was higher than in males in summer and winter (Two-way ANOVA: $F(1,67)=13.722$, $p<0.001$; t -test: $t=-4.546$, $df=14$, $p<0.05$; $t=-4.716$, $df=16$, $p<0.05$). MP content in whole BAT in females was also higher than that in males (Two-way ANOVA: $F(1,67)=22.604$, $p<0.001$) in summer (t -test: $t=-3.125$, $df=14$, $p<0.05$), winter (t -test: $t=-7.056$, $df=16$, $p<0.05$) and spring (t -test: $t=-2.731$, $df=18$, $p<0.05$) but the opposite was observed in fall (t -test: $t=2.305$, $df=19$, $p<0.05$). However, male and female gerbils showed similar seasonal changes in MP content in whole BAT, which was the lowest in summer and highest in winter (Two-way ANOVA: $F(3,67)=11.752$, $p<0.001$) (Table 1).

No significant gender differences were detected in UCP1 content (Two-way ANOVA: $F(1,29)=0.373$; $p=0.546$) or

COX activity (Two-way ANOVA: $F(1,67)=3.305$, $p=0.086$). Both UCP1 content (Two-way ANOVA: $F(3,29)=6.101$, $p=0.002$; Table 1) and COX activity (Two-way ANOVA: $F(3,67)=4.731$, $p=0.005$, Table 1) were higher in winter and spring than that in summer.

3.4. Serum leptin

No significant gender difference was found in serum leptin concentration (Two-way ANCOVA: $F(1,43)=1.984$, $p=0.166$). Serum leptin concentration was higher in spring than that in winter, but there were no differences among summer, fall and spring (Two-way ANCOVA: $F(3,43)=2.931$, $p=0.044$, Fig. 4). Serum leptin concentration was positively correlated with BM (Pearson correlation: $r^2=0.303$, $n=37$, $p<0.01$, Fig. 5A) and body fat mass (Pearson correlation: $r^2=0.607$, $n=43$, $p<0.001$, Fig. 5B), but was negatively correlated with UCP1 content after controlling for BM (Partial correlation: $r^2=0.252$, $n=36$, $p<0.01$, Fig. 5C).

4. Discussion

Mongolian gerbils showed elevated thermogenic capacities at different levels, in agreement with our hypothesis and predictions, except that there were sexual differences in NST and BAT MP content. Contradictory to our expectations, the gerbils showed an increase in BM, which was inconsistent with a reduction in serum leptin concentration in winter.

4.1. Seasonal changes in RMR and NST

Male Mongolian gerbils caught in the wild increased RMR and NST in cold seasons, consistent with previous results for the same species during cold acclimation and under seasonally acclimated conditions in captivity (Li et al., 2001; Li and Wang, 2005b). It is also similar to other small mammals living in cold regions (Wunder et al., 1977; Feist and Feist, 1986; Tomasi and Horwitz, 1987; McDevitt and Speakman, 1994a; Wang et al., 2006b), but differs from captive Brandt's voles and plateau pikas (*Ochotona curzoniae*), which maintained relatively stable RMR under seasonally acclimated conditions (Li and Wang, 2005a; Wang et al., 2006a). Although Mongolian gerbils and Brandt's voles are sympatric species in Inner Mongolian grasslands, and both are non-hibernators with similar MB that live solitarily in burrows, they show different patterns in RMR. McNab (1986) contended that most of the variation in basal rate of metabolism in eutherians could be accounted for by the combined influences of BM, food habits, behavior and climate. On this basis, different food habits may be responsible for differences in RMR between these two small rodents. Mongolian gerbils mainly feed on plant leaves and stems in spring and summer, and seeds in fall and winter. In contrast, Brandt's voles feed on plant leaves and stems throughout the year.

Seasonal changes in NST in the male Mongolian gerbils in the present study were similar to those seen in acclimatized captive Brandt's voles (Li and Wang, 2005a), suggesting that they employ common mechanisms to enhance heat production

in winter and spring in response to dramatically altered environmental conditions. In contrast, female gerbils maintained relatively stable NST throughout the year. This gender difference may be related to the restricted activities of females in winter and spring compared with males (Wang et al., 1998); activity times in male adult gerbils were twice those of females (Wang et al., 1998). Greater NST in male Mongolian gerbils in winter and spring would enable them to withstand the extreme circadian differences in ambient temperature on the surface of their native habitats in semi-desert and desert regions.

NST capacity depends mainly on UCP1 in BAT mitochondria (Cannon and Nedergaard, 2004). In the present study, the seasonality in COX activity and UCP1 content is similar to the seasonal changes in NST in Mongolian gerbils. Similar results were found in several other species of small mammal (Klaus et al., 1988; Kronfeld-Schor et al., 2000; Wang et al., 2006a,b). However, in contrast to our findings with the gerbils, Brandt's voles (Li and Wang, 2005a) and *Acomys russatus* (Kronfeld-Schor et al., 2000) showed no marked seasonal changes in BAT UCP1 content. These results suggest that there are species-specific molecular mechanisms of thermoregulatory heat production. Previous studies demonstrated that NST and the gene expression of BAT UCP1 increased by 41% and 189% under short photoperiod and cold conditions (Li and Wang, 2005b) and during cold acclimation (Li et al., 2001), respectively, but photoperiodic acclimation had no effect on NST or BAT UCP1 content (Zhao and Wang, 2006a), or on BAT MP content or COX activity (Wang et al., 2000b) in Mongolian gerbils.

In the present study, no significant correlations were found between NST and BAT COX activity and UCP1 content in Mongolian gerbils. However, with the increase in NST, BAT COX activity and UCP1 increased by 143% and 65% respectively between summer and winter. Wild Mongolian gerbils might mainly rely on the winter-associated enhancement of thermogenic capacity, to adapt to the large ambient temperature changes in their natural environment.

4.2. BM and leptin

Many small mammals, such as shrews and some Avicolid species, show a decline in BM in winter (Dehnel, 1949; Klaus et al., 1988; Heldmaier et al., 1990; Merritt and Zegerts, 1991; Concannon et al., 2001). In contrast, our Mongolian gerbils showed an increase in BM in winter *versus* summer. There are two possible explanations for this difference: One is that Mongolian gerbils cache food in the fall for wintering. The other is that Mongolian gerbils are still active and can conserve energy by huddling in burrows in winter. Although ambient temperatures are very low during winter and spring, burrow temperatures are relatively stable (Wang et al., 1998). In winter, Mongolian gerbils remain in their burrows most of the time and only venture outside for periods of a few minutes when the weather is fine (Wang et al., 1998, personal observations). Thus, they could be expected to show a winter increase in BM by feeding on cached seeds in the favorable environment of the burrow. A larger BM can be expected to decrease the rate of heat loss of individuals and thus increase their capacity to tolerate cold conditions (Haim and

Izhaki, 1993). In contrast, in captive Mongolian gerbils acclimatized to seasonal conditions outdoors, BM was lower in winter than spring and summer (Li and Wang, 2005b). This difference may be related to differences in cold stress and diet. The captive gerbils suffered from lack of shelter in the outdoor conditions, and thus were probably exposed to colder conditions than wild Mongolian gerbils in their burrows in winter. Also, the captive gerbils were restricted to standard rat chow throughout the year, in contrast to the seasonally changing diet of wild Mongolian gerbils.

We found that Mongolian gerbils showed a positive correlation between serum leptin concentration and body fat mass, similar to findings in several other species of small mammals from highly seasonal environments, such as Brandt's voles (Li and Wang, 2005a), Siberian hamsters (Klingenspor et al., 2000), plateau pikas and root voles (Wang et al., 2006a,b). These findings suggest that leptin may be responsible for seasonal adjustments in BM as a potential adiposity signal. That our Mongolian gerbils showed an increase in BM in winter and spring suggests that Mongolian gerbils use leptin as a regulatory signal to maintain energy balance. It has been demonstrated that Mongolian gerbils show a reduction in serum leptin concentration during cold acclimation. This reduction was associated with unchanged BM but increased energy intake (Li et al., 2004). Digestible energy intake increased in winter over summer in captive animals seasonally acclimatized outdoors (Li and Wang, 2005b). We suggest that serum leptin in Mongolian gerbils may act as a starvation signal in winter to increase food intake in response to greater energy requirements, and as a satiety signal in summer to decrease food intake to avoid obesity.

There was a negative correlation between serum leptin concentration and BAT UCP1 content in our wild Mongolian gerbils. Similar relationships have also been found in seasonally acclimatized captive Brandt's voles (Li and Wang, 2005a), and in wild plateau pikas and root voles (Wang et al., 2006a,b), indicating that leptin is involved in thermoregulation in many seasonal small mammals. Likewise, low serum leptin concentrations were found to be associated with increased BAT UCP1 gene expression in rats during cold acclimation (Bing et al., 1998), and leptin administration to cold-acclimated mice reduced both food intake and BAT thermogenesis (Abelenda et al., 2003). However, although leptin is supposed to increase energy expenditure during the rest phase, this is not necessarily linked to BAT. For example, when leptin concentrations were high, UCP1 expression increased in rats by increasing sympathetic outflow to BAT (Scarpace et al., 1997; Scarpace and Metheny, 1998). In the common shrew (*Sorex araneus*), leptin concentrations in the interscapular BAT were highest in mid-winter (Nieminen and Hyvärinen, 2000). The role of leptin in thermogenesis in Mongolian gerbils needs further study using administration of exogenous leptin.

The results presented in this study show that Mongolian gerbils mainly employ enhanced NST, as well as increased BAT COX activity and UCP1 content to survive the long cold winters in their natural habitat. They tend to increase BM in winter while reducing serum leptin concentrations, suggesting that leptin may act as a factor in regulating seasonal fluctuations in BM. Enhanced thermogenic capacities coupled with increased BM are important for winter survival in this species.

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