

Seasonal thermogenesis and body mass regulation in plateau pikas (*Ochotona curzoniae*)

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Abstract Changes in photoperiod, ambient temperature and food availability trigger seasonal acclimatization in physiology and behavior of many animals. In the present study, seasonal adjustments in body mass and in several physiological, hormonal, and biochemical markers were examined in wild-captured plateau pikas (*Ochotona curzoniae*) from the Qinghai-Tibetan plateau. Our results showed that plateau pikas maintained a relatively constant body mass throughout the year and showed no seasonal changes in body fat mass and circulating levels of serum leptin. However, nonshivering thermogenesis, cytochrome c oxidase activity, and mitochondrial uncoupling protein 1 (UCP1) contents in brown adipose tissues were significantly enhanced in winter. Further, serum leptin levels were positively correlated with body mass and body fat mass while negatively correlated with UCP1 contents. Together, these data suggest that plateau pikas mainly depend on increasing thermogenic capacities, rather than decreasing body mass, to cope with cold, and leptin may play a potential role in their thermogenesis and body mass regulation.

Keywords Basal metabolic rate · Nonshivering thermogenesis · Cytochrome c oxidase · Leptin · Plateau pika (*Ochotona curzoniae*)

Introduction

Proper adjustments in the morphology, physiology, and behavior of small mammals ensure survival in seasonal environments (Merritt 1986). It has been demonstrated that many small mammals respond to winter-associated environmental cues by reducing body mass and body fat mass together with enhancing thermogenesis (Heldmaier 1989; Bartness et al. 2002; Lovegrove 2005). Winter decline in body mass is considered to be an adaptive mechanism for the reduction of energy requirements when food availability is limited and cold stress occurs (Wunder et al. 1977; Merritt 1986; Lovegrove 2005). In some species, however, body mass is increased in winter. Animals seem to use several energy-conserving tactics such as torpidity (hibernation) and communal nesting to alleviate a need for body mass reduction (e.g., Nagy 1993; Merritt et al. 2001; Larkin et al. 2002). Further, there are some winter-active small mammals showing no significant seasonal changes in body mass. For example, wood mice (*Apodemus sylvaticus*) (Klaue et al. 1988), spiny mice (*Acomys cahirinus*) (Khokhlova et al. 2000), cold-resistant populations of golden spiny mice (*Acomys russatus*) (Brout et al. 1978), and white-footed mice (*Peromyscus leucopus*) (Lynch 1973) usually maintain constant body mass throughout the year.

Small mammals largely depend on nonshivering thermogenesis (NST) to cope with decreased ambient temperature (Jansky 1973). The brown adipose tissue

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(BAT) is known as the major site for NST (Foster and Frydman 1979). The capacity for NST in BAT depends on the concentration of uncoupling protein 1 (UCP1), a 32-kDa carrier protein uniquely located on the mitochondrial inner membrane of brown adipocytes. Through UCP1-mediated proton leakage from the mitochondrial respiratory chain, energy that is derived from metabolic fuels is dissipated as heat (Nicholls and Locke 1984; Krauss et al. 2005), which, in turn, is essential for effective thermoregulation in small mammals (Argyropoulos and Harper 2002). It has been found that many small mammals showed enhanced NST (Rafael et al. 1985a; Merritt and Zegers 1991; Jefimow et al. 2004) associated with increased BAT mass, mitochondrial protein (MP) concentrations, cytochrome c oxidase (COX) activity, and UCP1 mRNA level and protein expression in winter conditions (Rafael et al. 1985b; Wang et al. 1999; Li et al. 2001; Li and Wang 2005).

Leptin, a 167-amino acid product of the OB gene mainly in the adipocytes (Zhang et al. 1994), is an important regulator of body mass via its control on food intake and energy expenditure by acting on neuropeptide circuits in the hypothalamus (Friedman and Halaas 1998; Concannon et al. 2001). In many seasonal mammals, annual cycles of food intake and adiposity are coupled with seasonal changes in circulating leptin levels (Klingenspor et al. 1996). Leptin is thought to increase energy expenditure by increasing thermogenesis in BAT, including increased expression of UCP1 or UCP1 mRNA expression (Hwa et al. 1997; Commins et al. 2001). However, controversial reports exist. It has been suggested that leptin administered to cold-acclimated rats reduces both food intake and BAT thermogenesis (indicated by decreased UCP1 concentration in BAT) (Abelenda et al. 2003).

Plateau pikas (*Ochotona cuizoniae*, also called the black-lipped pika; Lai and Smith 2003), are the dominant species in alpine meadows on the Qinghai-Tibetan plateau, China. They are small herbivorous lagomorphs, which are winter active and do not store food during the cold seasons. Given the fact that it lives at high altitude under extremely harsh environmental conditions, including extreme cold stress and low biological productivity, this lagomorpha species may serve as an ideal model to study winter-adaptive strategy. It has been reported that plateau pikas showed changes in BMR and relatively stable NST across seasons (Wang and Wang 1990, 1996), and there were seasonal variations in mass, structure and components of BAT (Wang and Wang 1989; Wang et al. 1993a). However, the variations at the molecular level of BAT (UCP1) and the role of leptin in seasonal changes of body mass

and thermogenesis of this species is still unknown. Further, a more comprehensive and in-depth study (integrative study from molecular to organismal level) is needed to understand the plateau pika's adaptive strategies to the cold alpine environment. Therefore, in the present study we examined several physiological, hormonal, and biochemical markers of plateau pikas that were wild-caught from the Qinghai-Tibetan plateau throughout the year. It is hoped that this information will present an insight into the crucial physiological modifications that allow plateau pikas to adapt to the extremely harsh alpine environment.

Materials and methods

Animals

Subjects were live-trapped male and female plateau pikas from alpine meadows around the Haibei Alpine Meadow Ecosystem Research Station of the Chinese Academy of Sciences (37°29'–37°45'N, 101°12'–101°33'E, 3,200–3,500 m altitude) in Menyuan County, Qinghai Province. The research station is located on the Qinghai-Tibetan plateau, which is noted for its high altitude and extremely harsh environmental conditions, such as low ambient temperature, strong solar radiation and a short period for plant growth. Climatic characters for the alpine meadow from 1980 to 1996 are shown in Table 1. The annual mean temperature is around -0.9 to -2.5°C (Li 1998). It has been reported that the highest ambient temperature is around 24.2°C (July), and the lowest falls to -37.1°C (December). There is no frostless season, and some thickness of permafrost can be found in extensive areas including mountains and grasslands in August. Plateau pikas were captured during April, July and October 2004 and January 2005 (five male and five female adult pikas at each time point excluding pregnant or lactating individuals as well as young pikas less than 100 g in body mass). Animals were transported to the Northwest Plateau Institute of

Table 1 Ambient temperature and precipitation at Haibei Alpine Meadow Ecosystem Research Station of the Chinese Academy of Sciences. Data were adapted from Li (1998)

	April	July	October	January
Ambient temperature ($^{\circ}\text{C}$)				
Mean	-0.5	9.9	-1.0	-15.2
Mean maximum	6.6	16.5	8.1	-3.5
Mean minimum	-6.5	3.5	-7.2	-24.0
Extreme maximum	17.1	24.2	18.0	6.1
Extreme minimum	-20.0	-3.5	-21.5	-35.2
Precipitation (mm)	37.1	117.9	32.6	4.1

Biology (Chinese Academy of Sciences in Xining, Qinghai, China) for experimental analysis. Between capture and metabolic analysis, the animals were kept individually in plastic cages ($320 \times 215 \times 170 \text{ mm}^3$) with sawdust as bedding in a room with natural temperature and photoperiod. The subjects were fed ad libitum with natural forage and carrots as food and water resources. After the basal metabolic rate (BMR) and NST measurement, subjects were sacrificed via decapitation within 4 days after capture for all the four seasons, and blood and tissue samples were taken for measurement of physiological parameters.

Metabolic trials

Metabolic measurements were conducted 1 day after the subjects had been transported to the laboratory in Xining to allow the animals to recover from transportation. The BMR was measured with an established closed-circuit respirometer (Wang and Wang 1996; Li and Wang 2005; Zhao and Wang 2005) at 30°C , which is within the thermoneutral zone for this species ($25\text{--}30^\circ\text{C}$; Wang et al. 1979, 1993b, 1999). Briefly, the metabolic chamber size was 3.6 l, and the chamber temperature was controlled by a water bath ($\pm 0.5^\circ\text{C}$). Potassium hydroxide and silica gel were used to absorb carbon dioxide and water in the metabolic chamber. Animals were fasted for 3 h prior to being put into the metabolic chamber. After 60 min stabilization in the chamber, oxygen consumption was recorded for 60 min at 5-min intervals. The two stable consecutive lowest readings were used to calculate BMR. The animals' body temperature and body mass were measured before and after each test. Body temperature was measured with a digital thermometer (Beijing Normal University Instruments) inserted into the rectum at a depth of 3 cm ($\pm 0.1^\circ\text{C}$). All metabolic measurements were performed between 1000 and 1700 hours to minimize the effect of circadian rhythms. NST was induced by subcutaneous injections of norepinephrine bitartrate (NE; 0.2 mg ml^{-1} diluted with physiological saline solution; Shanghai Harvest Pharmaceutical) at $25 \pm 1^\circ\text{C}$. The dosage of NE was calculated based on the equation described by Heldmaier (1971): $\text{NE dosage (mg kg}^{-1}) = 6.6 \text{ Mb}^{-0.458} \text{ (g)}$, which was suggested to be able to induce the highest NST in plateau pikas (Wang and Wang 1996; Wang et al. 1999). Further, the NST value obtained was close to what might be expected from the dosages suggested by Wunder and Gettinger [$\text{NE (mg kg}^{-1}) = 2.53 \text{ W}^{-0.4}$; Wunder and Gettinger (1996)], indicating that the NE dosage used in the present study was appropriate (Wang and Wang 2006). The two consecutive highest recordings of oxygen consumption were taken to calculate the maxi-

mum NST (Song and Wang 2003; Li and Wang 2005). All metabolic parameters were corrected to standard temperature and pressure.

Measurements of COX activity and MP content

The interscapular BAT was removed and weighed immediately after animals were sacrificed by decapitation between 0900 and 1200 hours. MP was prepared as described previously (Wang et al. 1999; Li et al. 2001). COX activity was measured with the polarographic method using oxygen electrode units (Hansatech Instruments, England) (Li and Wang 2005; Zhao and Wang 2005). MP concentrations were determined by the Folin phenol method (Lowry et al. 1951) with bovine serum albumin as the standard.

Measurement of UCP1 and serum leptin

Total BAT protein ($20 \mu\text{g}$ 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. UCP1 was detected using a polyclonal rabbit anti-hamster UCP1 (1:5,000, supplied by M. Klingenspor, Department of Biology, Philipps-University, Marburg, Germany) as a primary antibody and peroxidase-conjugated goat anti-rabbit (1:5,000, Jackson Immuno, USA) as the second antibody (Klingenspor et al. 1996). We used enhanced chemoluminescence (Amersham) for detection and unspecific binding sites were saturated with 5% non-fat dry milk in PBS. UCP1 content was expressed in relative unit and quantified with Scion Image Software (Scion) (Li and Wang 2005; Zhao and Wang 2005).

The blood sample was centrifuged at 4,000 r.p.m. for 30 min, and then serum was sampled and stored at -75°C . Serum leptin levels were measured by radioimmunoassay (RIA) using the Linco 125-I multispecies kit (St. Louis, Mo.) and leptin values were determined in a single RIA. The detection limit for leptin was 1.0 ng ml^{-1} when using a $100\text{-}\mu\text{l}$ sample size (according to the instructions for the multi-species leptin RIA kit). Inter- and intra-assay variability for leptin RIA were <3.6 and 8.7% , respectively.

Carcass composition analysis

The eviscerated carcass (interscapular BAT excluded and the entire gastrointestinal tract removed) was

dried at 60°C to get constant mass for body fat mass analysis. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus.

Data analysis

Statistical analyses were performed using the SPSS software package (13.0 version). Distributions of all variables were tested for normality by the Kolmogorov–Smirnov test and data that were not normally distributed were transformed to natural logarithms. Data were analyzed by two-way ANOVA (season by sex) and significant group differences were further evaluated using the least-significant difference post hoc test. BMR, NST, BAT mass, fat mass and serum leptin level were tested by analysis of covariance with body mass as the covariate. Pearson correlation was used to detect possible associations between serum leptin and body mass, body fat mass, and UCP1. All values were expressed as mean \pm SEM and $P < 0.05$ was considered to be statistically significant.

Results

No significant sex differences were found on any of the measurements. Therefore, data from males and females were combined.

Body mass and body temperature

The plateau pikas showed no seasonal variations either in body mass (Table 2; $F_{3,32} = 1.032$; $P > 0.05$) or in body temperature (Table 2; $F_{3,32} = 0.141$; $P > 0.05$).

Thermogenesis variations

BMR and NST data were shown as oxygen consumption per individual ($\text{ml O}_2 \text{ h}^{-1}$). No significant seasonal changes were found in BMR per individual (Fig. 1a; $F_{3,32} = 0.903$; $P > 0.05$) or in adjusted BMR with body mass as covariate (Fig. 1b; $F_{3,31} = 0.965$; $P > 0.05$).

However, NST showed marked difference among seasons. The NST per individual was significantly lower in July (Fig. 1a; $F_{3,32} = 3.954$; $P < 0.05$). After adjustment by body mass, NST in October and in January was significantly higher than that in July (Fig. 1b; $F_{3,31} = 3.969$; $P < 0.05$).

BAT wet mass (Fig. 1c; $F_{3,31} = 8.046$; $P < 0.001$), dry mass (Fig. 1c; $F_{3,31} = 9.640$; $P < 0.001$) and BAT mass/body mass (%) (Fig. 1c; $F_{3,32} = 6.104$; $P < 0.01$) showed significant seasonal variations in plateau pikas. In all measurements, the lowest value was found in July as compared to that of other periods.

There was no marked seasonal difference in MP content in a unit of BAT (Table 3; $F_{3,32} = 0.659$; $P > 0.05$); however, significant seasonal variations were found in MP content in the whole BAT which showed the lowest value in July (Table 3; $F_{3,32} = 4.458$; $P < 0.05$).

COX activity in BAT also showed significant seasonal variations. The COX activity per gram MP (Table 3; $F_{3,32} = 6.371$; $P < 0.01$) and per gram BAT (Table 3; $F_{3,32} = 4.451$; $P < 0.05$) in July and January was significantly higher than that in April and October. However, when expressed as total COX activity in whole BAT, COX activity in April was lower than that in January but higher than that in July and October (Table 3; $F_{3,32} = 4.232$; $P < 0.05$), while data for the latter two months did not differ from each other. Finally, the UCP1 content in BAT was significantly lower in July than that in October and January (Fig. 1d; $F_{3,32} = 4.382$; $P < 0.05$).

In the liver, the MP content remained constant across seasons (Table 3; $F_{3,32} = 0.764$; $P > 0.05$) but COX activity showed significant seasonal changes either per gram MP (Table 3; $F_{3,32} = 9.089$; $P < 0.001$) or per gram liver (Table 3; $F_{3,32} = 5.854$; $P < 0.01$). The COX activity level in July was significantly lower than that at the other three time points.

Body fat mass and serum leptin level

Body fat mass (Table 2; $F_{3,31} = 0.869$; $P > 0.05$), body fat content (defined as lipid % body mass) (Table 2;

Table 2 Seasonal changes of body mass, body fat mass, body fat content and serum leptin level in plateau pika. Values are means \pm SEM

	April	July	October	January
Sample size	10	10	10	10
Body mass (g)	135.9 \pm 4.5	128.2 \pm 4.9	132.2 \pm 2.7	129.6 \pm 1.4
Body temperature (°C)	39.8 \pm 0.1	40.3 \pm 0.2	40.2 \pm 0.2	39.8 \pm 0.2
Body fat mass (g)	5.33 \pm 0.62	4.39 \pm 0.28	5.20 \pm 0.83	3.75 \pm 0.20
Body fat content (%)	4.0 \pm 0.4	3.4 \pm 0.2	3.9 \pm 0.7	2.9 \pm 0.2
Serum leptin level (ng ml ⁻¹)	6.44 \pm 0.69	5.11 \pm 0.41	6.12 \pm 0.74	4.39 \pm 0.46

Fig. 1 Seasonal changes of basal metabolic rate (BMR; **a** ml O₂ h⁻¹ for original data and **b** ml O₂ h⁻¹ for adjusted means with body mass as covariate), nonshivering thermogenesis (NST; **a** for ml O₂ h⁻¹ with original data and **b** ml O₂ h⁻¹ for adjusted means with body mass as covariate), brown adipose tissue (BAT) mass (**c**) and uncoupling protein 1 (UCP1) contents (**d**) in plateau pikas (*Ochotona curzoniae*). Values are means ± SEM. *P* < 0.05 was considered to be statistically significant

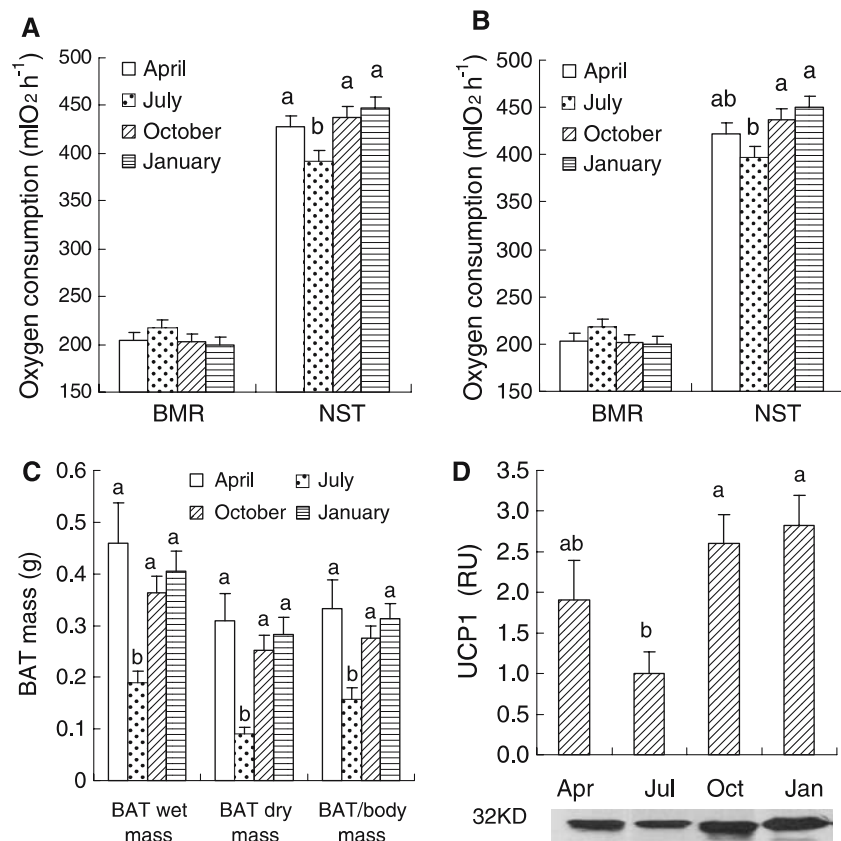


Table 3 Seasonal changes of cytochrome c oxidase (COX) activity and mitochondrial protein (MP) in brown adipose tissue (BAT) and liver of plateau pika. Values are means ± SEM. Means within rows followed by different letters are significantly different (*P* < 0.05)

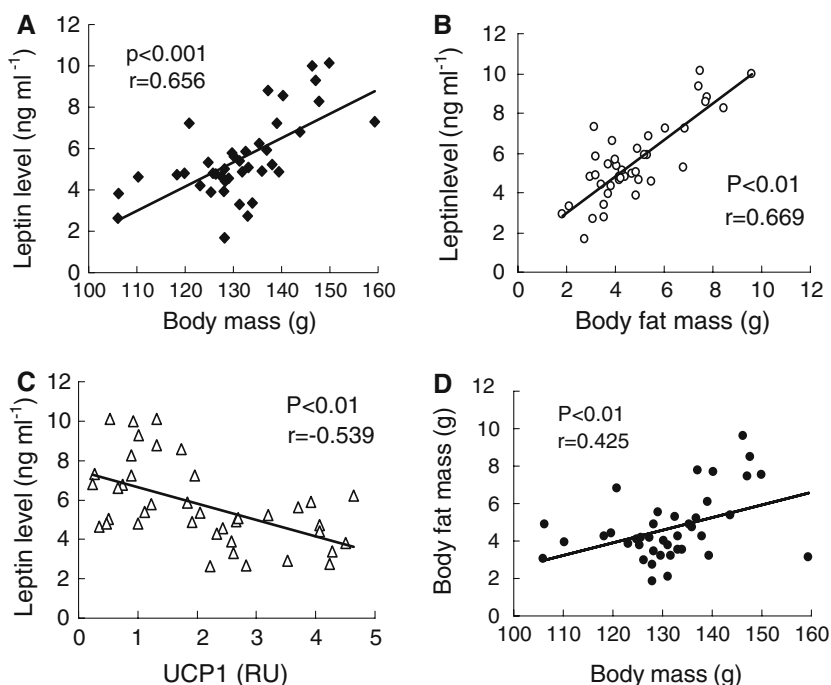
		April	July	October	January
Sample size		10	10	10	10
BAT MP content	mg g ⁻¹ BAT	6.42 ± 0.65	8.75 ± 0.65	6.92 ± 0.64	7.78 ± 0.64
	mg in whole BAT	2.96 ± 0.11 a	1.66 ± 0.11 b	2.52 ± 0.10 a	3.15 ± 0.17 a
BAT COX activity	nmol O ₂ min ⁻¹ mg ⁻¹ MP	249.16 ± 23.63 b	291.22 ± 23.40 a	214.61 ± 23.14 b	294.74 ± 23.22 a
	nmol O ₂ min ⁻¹ g ⁻¹ BAT	1,518.43 ± 243.53 b	2,543.67 ± 241.22 a	1,481.93 ± 238.53 b	2,358.24 ± 239.31 a
	nmol O ₂ min ⁻¹ in BAT	698.48 ± 43.23 b	483.30 ± 23.34 c	539.42 ± 54.32 c	955.09 ± 23.46 a
Liver MP content	mg g ⁻¹ liver	10.97 ± 0.91	9.96 ± 0.90	11.85 ± 0.89	11.15 ± 0.89
Liver COX activity	nmol O ₂ min ⁻¹ mg ⁻¹ MP	93.62 ± 5.01 a	64.37 ± 4.96 b	80.07 ± 4.90 a	91.14 ± 4.92 a
	nmol O ₂ min ⁻¹ g ⁻¹ liver	992.46 ± 85.21 a	637.81 ± 84.40 b	926.26 ± 83.46 a	1,060.26 ± 83.73 a

$F_{3,32} = 2.345$; $P > 0.05$), and serum leptin level (Table 2; $F_{3,31} = 2.063$; $P > 0.05$) showed no significant seasonal variations. Correlation analysis indicated that serum leptin levels were positively correlated with changes in overall body mass (Fig. 2a; $r = 0.656$; $P < 0.001$), body fat mass (Fig. 2b; $r = 0.846$; $P < 0.001$), and negatively correlated with UCP1 contents (Fig. 2c; $r = 0.539$; $P < 0.01$). Finally, body fat mass was positively correlated with changes in overall body mass (Fig. 2d; $r = 0.425$; $P < 0.01$).

Discussion

Small winter-active mammals employ a wide array of strategies to cope with seasonally changing environments. These include, but are not limited to, adjustments in body mass, metabolic rates, behavior, and associated hormonal and biochemical activities (Wunder et al. 1977; Wunder 1984; Merritt 1986; Jackson et al. 2001). Plateau pikas are small winter-active lagomorphs native to the Qinghai-Tibetan Plateau. Given

Fig. 2 Correlation of serum leptin levels with body mass (a), body fat mass (b) and UCP1 contents (c), and correlation between body fat mass and body mass (d) in plateau pikas (*O. curzoniae*). Values are means \pm SEM. $P < 0.05$ was considered to be statistically significant



the fact that these animals live at a high altitude in an extremely harsh environment, they must have evolved an adaptive strategy for winter survival.

Seasonal variations in body mass and body fat mass

Many small mammals inhabiting seasonal environments undergo winter body mass declines or slowed growth, which are considered to be energetically advantageous reducing overall energy requirements during periods of food scarcity and cold stress (Wunder et al. 1977; McNab 1983; Merritt 1986; Heldmaier 1989; Lovegrove 2005). However, some mammals maintained a stable body mass or even showed an increase in body mass, when exposed to winter-like conditions (Klaus et al. 1988; Nagy 1993). Data from our present study indicate that plateau pikas showed no winter reduction in their body mass, which is consistent with the previous findings (Wang and Wang 1990, 1996). It is interesting that the American pikas (*Ochotona princeps*), which remained active in winter, also showed no seasonal changes in body mass (MacArthur et al. 1973), though the ecology of the two pika species is different. American pikas inhabit areas which are covered by snow in winter and they can feed on stored food under the snow cover (Wunder 1992). The relatively warm microenvironment and stored food may alleviate the need to reduce body mass in winter. However, plateau pikas live in alpine meadows characterized by strong winds and are rarely covered by snow. Further, plateau pikas do not catch food in winter so

they must forage (Wang and Wang 1996). It has been suggested that increased insulation coupled with reduced activity and a burrowing habit of plateau pikas may help conserve energy, and thus reduce the need to decrease energy requirements through body mass reduction (Wang et al. 1993b; Wang and Wang 1996).

Seasonal changes in body fat mass are found to be species-specific. For example, collared lemmings (*Dicrostonyx groenlandicus*) increase their body fat mass in cold conditions (Nagy et al. 1995) whereas the Siberian hamster (*Phodopus sungorus*) shows winter reduction in body fat mass (Klingenspor et al. 2000), suggesting that specific strategies may be employed by each species to cope with winter-associated cold stress and limited food availability. Our data show no seasonal variations in body fat mass as well as a positive correlation between body mass and body fat mass, again supporting the notion that maintaining stable body mass and body fat mass may serve as a specific strategy employed by plateau pikas (Wang and Wang 1996).

Seasonal variations in thermogenic capacities and body temperature

To cope with winter cold stress, many small winter-active mammals increase thermogenic capacity to maintain constant body temperature (Heldmaier et al. 1982; Wunder 1984; Merritt 1995). In the present study, plateau pikas showed no seasonal changes in BMR (decreased by 8%) but had an increased level of NST

(by 24.6%) in January as compared to July. These data suggest that an enhanced capacity for NST, rather than an elevated BMR, is an important adaptive mechanism for this species to ensure winter survival. Lack of seasonal changes in BMR (Merritt and Zegers 1991; Bozinovic et al. 2004) and enhanced NST capacities in winter conditions (Heldmaier et al. 1982; Merritt 1995) have also been found in other small rodents. Our present study showed that plateau pikas maintained high level of NST with BAT deposited throughout the year, while this was not the case for American pikas. It was reported that American pikas might use BAT in autumn, whereas in winter when their habitat is covered by insulating snow levels, they lose the ability to develop NST with the loss of BAT. The well-insulated warm microenvironment coupled with the elevated BMR in winter (Wunder 1992) may alleviate the need to develop NST for American pikas.

It should be noted, however, that in a previous study, although no significant seasonal changes in NST were observed, NST was enhanced by 8.5% from summer to winter (Wang and Wang 1996). One possibility for this discrepancy could be the differences in sampling time and the statistical method used for data analysis. In the present study, all animals were wild-caught in the corresponding months, while in previous study, pikas were caught monthly and data from June to August and from December to February were pooled to give summer and winter data, respectively. Combining data may weaken the seasonal variations in NST. The differences in food availability in these two studies might also contribute to the differences in NST.

The notion of elevated thermogenic capacity, indicated by the enhanced NST, is further supported by the other biochemical markers examined in the present study, which include BAT mass, the MP content, the COX activity and UCP1 content. Our data show that the BAT mass, MP content, and the COX activity increased significantly during the winter (January) in comparison to that of the summer (July), suggesting that plateau pikas increased the total respiratory capacity of BAT and the liver in winter. This finding is in agreement with previous studies in plateau pikas (Li et al. 2001) and root voles (Wang et al. 2006) as well as in other rodent species including white-footed mice (*P. leucopus*; Lynch 1973), yellow-necked field mice (*Apodemus flavicollis*; Klause et al. 1988), wood mice (*Apodemus sylvaticus*; Klause et al. 1988), tree shrew (*Tupaia belangeri*; Li et al. 2001), and golden spiny mice (*Acomys russatus*; Kronfeld-Schor et al. 2000). There has been an increase in the documentation of data integrated from molecular to organismal level. Elevated UCP 1 content or mRNA expression in a cold or short-

ened photoperiod has been documented for common spiny mice (*Acomys cahirinus*; Kronfeld-Schor et al. 2000), Brandt's vole (*Lasiopodomys brandtii*; Li and Wang 2005, Zhao and Wang 2005, Zhang and Wang 2006) and Mongolian gerbil (*Meriones unguiculatus*; Li et al. 2001). In our study, the UCP1 content in BAT showed significant seasonal variations, with the lowest found in summer as compared to other seasons. It is noted that there was no further increase in thermogenic variables with a decrease in environment temperature from autumn to winter, and this may indicate a limited response of these characters in wild plateau pikas.

Our data show no significant seasonal variation in body temperature. Body temperature was quite high, 39.6–40.5°C, for plateau pikas. This was consistent with the body temperature of 40.1°C (range 37.9–42.7°C) for the American pikas, which also showed no seasonal variations in body temperature (MacArthur et al. 1973), suggesting that the relatively high and stable body temperature may serve as a specific character in pikas. However, it is disadvantageous for the animals to maintain a high body temperature in such a cold environment since heat loss is increased, so the pikas have evolved high levels of BMR, NST and good insulation (MacArthur et al. 1973; Wang et al. 1979; Wang and Wang, 1996, 1990) coupled with behavioral thermoregulation (MacArthur et al. 1974) to maintain body temperature. A similar mechanism was found in Gansu pikas (*Ochotona cansus*, Wang et al. 1991), and root voles (*Microtus oeconomus*, Wang and Wang 1990; Wang et al. 2006) from the same study site, indicating that a high metabolic rate might be a common adaptive strategy for small mammals living on the Qinghai-Tibetan plateau.

Seasonal changes of serum leptin levels

In seasonal mammals, annual cycles of adiposity are correlated with changes in circulating leptin levels (Klingenspor et al. 2000; Rousseau et al. 2003). It has been postulated that leptin may convey information concerning fat mass to the central nervous system via interactions with receptors in the hypothalamus, and thus regulates adiposity (Johnson et al. 2004), which may serve as part of an adaptive mechanism for increasing the odds of winter survival when food availability is decreased and adipose tissue stores are at their nadir (Bartness et al. 2002). Our study showed that serum leptin levels did not change seasonally, which was consistent with the changes in body mass and body fat mass, although this differs from results for other small mammals such as Brandt's voles (Li and Wang 2005) and Siberian hamsters (Klingenspor et al.

2000), as well as the sympatric species root voles (Wang et al. 2006). Further analysis showed that the serum leptin level was positively correlated with changes in body mass and body fat mass; this was also demonstrated in several other rodent species, such as Brandt's voles (Li and Wang 2005; Zhang and Wang 2006, Zhao and Wang 2005), collared lemmings (Nagy et al. 1995), and Siberian hamsters (Klingenspor et al. 2000), and suggests that leptin acted as an adiposity signal in body mass regulation.

To regulate body mass, leptin influences both sides of the energy balance via its effects on both food intake and energy expenditure. It was reported that *ob/ob* mice could not maintain body temperature when exposed to cold (Trayhurn et al. 1977) because of the deficient leptin system, suggesting the involvement of leptin in body heat production (Friedman and Halaas 1998). Although it was demonstrated that leptin is able to affect energy expenditure, the available reports are controversial. Some studies showed that leptin would stimulate thermogenesis by increasing UCP1 mRNA levels or UCP1 protein expression in BAT (Scarpace et al. 1997; Tajima et al. 2005). However, other studies found that leptin administration to cold-acclimated rats induced reduction in thermogenesis coupled with a decrease in UCP1 concentration in BAT (Abelenda et al. 2003). Furthermore, it was reported that chronic cold exposure would stimulate sympathetically driven thermogenesis in BAT while decreasing the plasma leptin concentration (Bing et al. 1998), suggesting a complex role of leptin in the regulation of energy balance under cold stress. Our data show a negative correlation between leptin level and UCP1 content, and a similar phenomenon was found in seasonally acclimated Brandt's voles (Li and Wang 2005a) and root voles (Wang et al. 2006), indicating that leptin might be potentially involved in energy expenditure across seasons in the plateau pikas. However, experiments on exogenous leptin administration are needed to clarify the exact role of leptin in body mass and thermogenesis regulation in plateau pikas.

The plateau pikas on the Qinghai-Tibetan plateau maintain constant body mass, body fat mass and serum leptin levels while displaying a significantly enhanced thermogenic capacity to survive in winter conditions. Leptin is potentially involved in the regulation of body mass and energy expenditure in pikas. The present results suggest that the observed physiological regulation from the organismal, hormonal levels to the cellular level of this alpine small mammal are critically important and allow plateau pikas to successfully overcome the physiological challenges of an extremely harsh environment in a cold alpine meadow.

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