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Effects of photoperiod and temperature on the body mass, thermogenesis, and serum leptin levels of *Apodemus draco* (Rodentia: Muridae) in the Hengduan Mountain region, China

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Abstract

Background: Environmental cues play important roles in the regulation of physiology and behavior in small mammals. In the present study, we performed a factorial experiment (temperature × photoperiod) in which the South China field mouse *Apodemus draco* (Rodentia: Muridae) was acclimated to different photoperiods (a long photoperiod of 16 h light/8 h dark and a short photoperiod of 8 h light/16 h dark) and temperatures (warm at 30°C and cold at 5°C) to test the hypothesis that photoperiod, temperature, or both together can trigger changes in serum leptin levels, body mass, thermogenesis, and energy intake.

Results: Body mass, the resting metabolic rate (RMR), nonshivering thermogenesis (NST), and energy intake significantly decreased in the cold condition. Cold exposure induced increases in mitochondrial protein contents, cytochrome C oxidase (COX) activity, and α-glycerophosphate oxidase (α-GPO) in the liver and brown adipose tissue (BAT). There were no significant differences in mitochondrial protein contents, COX, or α-GPO under different photoperiods. Cold also induced an increase in uncoupling protein 1 in the BAT but showed no significant differences with photoperiod.

Conclusions: All of the results indicated that *A. draco* was more sensitive to temperature. Further, serum leptin levels were involved in the processes of thermogenesis and body mass regulation in *A. draco*.

Keywords: *Apodemus draco*; Photoperiod; Temperature; Serum leptin levels; Uncoupling protein 1 (UCP1)

Background

Energy metabolism is a critical component in the distribution, abundance, and reproductive success of rodents (Bozinovic 1992), which can perhaps be driven by changes in environmental cues, such as photoperiod and temperature (Heldmaier et al. 1989; Li and Wang 2005a; Lovegrove 2005; Atiénzar et al. 2012; Yoshida et al. 2012). However, most previously published research indicated that different rodent species showed different physiological sensitivities to photoperiod and temperature (Klingenspor et al. 2000; Peacock et al. 2004; Li and Wang 2007). Leptin, a 16-kDa protein, is synthesized in adipose tissues of mammals (Silva 2006). Leptin was found to affect food intake, the neuroendocrine axis, metabolism, and immunological processes (Barb and Kraeling 2004), and it was hypothesized to contribute to maintaining body mass by regulating food intake and energy expenditure (Friedman and Hallas 1998). Exogenous leptin caused a significant decrease in body mass by restraining energy intake and increasing energy expenditure (Abelenda et al. 2003). Previous studies showed that leptin as a starvation signal contributes to energy intake during winter-like conditions (Flier 1998; Li and Wang 2005b). Environmental cues, such as photoperiod and temperature, affect serum leptin levels associated with body mass, and short photoperiods or cold induced reductions in leptin levels in both serum and tissues (Hardie et al. 1996; Klingenspor et al. 1996).

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Uncoupling protein 1 (UCP1), a membranous 33-kDa protein, is uniquely expressed in brown adipose tissue (BAT) (Zhang and Wang 2006). In brown adipocytes, activated UCP1 provides an alternative way for protons to reenter the mitochondrial matrix, thereby separating, or 'uncoupling', the oxidation of fuel from ATP synthesis and transforming electrochemical energy into heat (Cannon and Nedergaard 2004). It was reported that leptin administration can enhance the expression of BAT-UCP1 messenger RNA (mRNA), indicating the potential involvement of leptin in thermogenesis (Scarpace et al. 1997). Nevertheless, contradictory results were reported (Bing et al. 1998; Abelenda et al. 2003).

The Hengduan Mountain region is located at the boundary between the Palaearctic region and the Oriental region in China. It has an alpine climate with high mountains and gorges. The average temperatures are 5.4°C in spring, 23.9°C in summer, 16.6°C in fall, and −3.8°C in winter; the solar durations are 6.49 h/day in spring, 5.61 h/day in summer, 5.93 h/day in fall, and 7.07 h/day in winter (Zhu et al. 2012b). The diversity and abundance of mammals is high, and it is considered to be 'a harbor in the fourth ice age'. Therefore, small mammals from the region may differ from those from other regions. The South China field mouse *Apodemus draco* (Mammalia: Rodentia: Muridae) is an indigenous species in the Hengduan Mountain region. Evaporative water loss and energy metabolism in *A. draco* were reported (Li et al. 2009). Effects of cold acclimation on energy metabolism and body mass regulation in *A. draco* were also studied (Zhu et al. 2012a, 2013). In the present study, we hypothesized that photoperiod, temperature, or both together can trigger changes in serum leptin levels, body mass, thermogenesis, and energy intake in *A. draco*. We predicted that short photoperiods and cold would cause increases in thermogenesis and energy intake with a decrease in body mass. Leptin was investigated due to its possible involvement in regulating energy intake and expenditure.

Methods

Samples

A. draco individuals were captured in a farmland (26°15′ to 26°45′N, 99°40′ to 99°55′E at an elevation of 2,590 m) in Jianchuan County, Yunnan Province, in July 2011. The annual average temperature is 9.1°C, with a minimum average temperature of −4.0°C in January and a maximum average temperature of 24.1°C in July.

A. draco was bred for two generations at the School of Life Science, Yunnan Normal University (Kunming, China) and was individually housed in plastic boxes (260 × 160 × 150 mm). *A. draco* was maintained at a room temperature of 25°C ± 1°C, under a photoperiod of 12 h light (L)/12 h dark (D; with lights on at 8:00 a.m.). After 1 month of acclimatization, the animals were randomly divided into the following four experimental regimes: a long photoperiod (LD; 16 h L/8 h D) and warm (30°C), an LD and cold (5°C), a short photoperiod (SD; 8 h L/16 h D) and warm, and an SD and cold (n = 10 mice/group) for 4 weeks. At the end of the experiment, all animals were sacrificed at 9:00 a.m. to 11:00 a.m. by decapitation. Blood was centrifuged at 4,000 rpm for 30 min after a 30-min interval. Blood serum was collected and stored at −75°C for hormone determination. All animals were dissected to evaluate organ morphology, and pregnant, lactating, or young individuals were excluded from the present study. All animal procedures were licensed under the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences (Kunming, China).

Measurement of metabolic rates

Metabolic rates were measured using an AD ML870 open respirometer (AD Instruments, Castle Hill, Australia) at 25°C within the thermal neutral zone (TNZ), and a gas analysis was performed using an ML206 gas analysis instrument (AD Instruments, Castle Hill, Australia). The temperature was controlled by an SPX-300 artificial climatic incubator (to ±0.5°C) (Shanghai Boxun Company, Shanghai, China); the metabolic chamber volume was 500 ml, and the airflow rate was 200 ml/min. *A. draco* was allowed to acclimatize to the metabolic chamber for at least 60 min prior to the resting metabolic rate (RMR) measurement, and oxygen consumption was recorded for at least 120 min at 1-min intervals. Ten stable consecutive lowest readings were taken to calculate the RMR (Li and Wang 2005a). Details of the method used for calculating the metabolic rate are given in Hill (1972).

Nonshivering thermogenesis (NST) was induced by a subcutaneous injection of norepinephrine (NE; Shanghai Harvest Pharmaceutical, Shanghai, China) and measured at 25°C. Two consecutive highest recordings of oxygen consumption in each 60-min measurement were taken to calculate NST (Li and Wang 2005a). Doses of NE were approximately 0.8 to 1.0 mg/kg according to dose-dependent response curves that were created before the experiment and the equation described by Heldmaier (1971): norepinephrine dosage (mg/kg) = 6.6 $M^{-0.458}$ (g).

Energy intake

Energy intake for each experimental group was measured by food trials (Song and Wang 2002). Each animal was housed in a metabolic cage (20 × 15 × 15 cm). The animals were allowed to feed on a fixed quantity of food for a fixed time (10:00 a.m. to 11:00 a.m.); on the next day, the animals were weighed, and leftover food was measured. Residual food was dried at 65°C for at least 72 h until the mass had stabilized, and it was then weighed to the nearest 0.1 g and then reweighed to determine the dry mass. The energy content of the sample

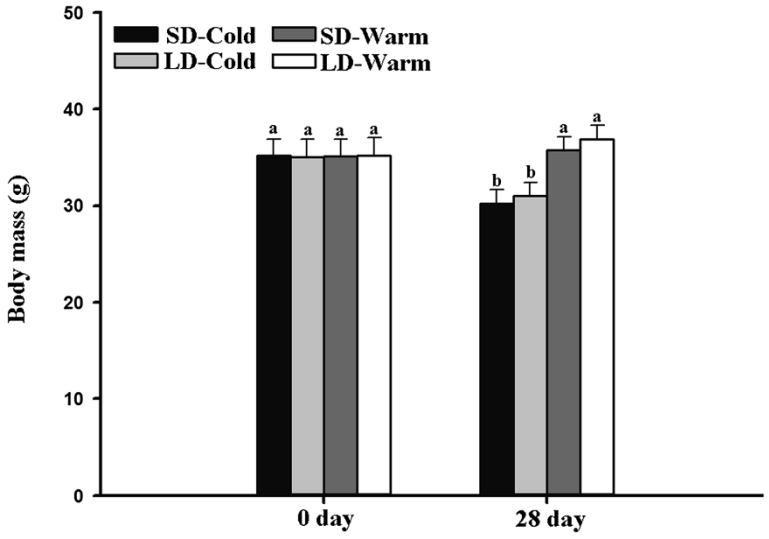

Figure 1 Effects of photoperiod and temperature on the body mass of *A. draco*. Different lowercase letters in a given row indicate a significant difference ($p < 0.05$).

was measured with a YX-ZR/Q automatic calorimeter (U-therm Industry, Changsha, China). The caloric value of the diet fed to these animals was 18.0 ± 0.8 kJ/g. The calculation of energy intake was according to Drozdz (1975): Energy intake (kJ/day) = Food (g/day) × Energy content (kJ/g).

Morphology

On day 28, after collecting trunk blood, the visceral organs, including the liver, BAT, heart, lungs, kidneys, spleen, and gastrointestinal tract (stomach, small intestine, cecum, and large intestine), were extracted and weighed (to ±1 mg). The stomach and intestines were rinsed with saline to eliminate all gut contents and then weighed. The remaining carcass and all organs were dried to a constant mass in an oven at 60°C (for at least 72 h) and then weighed again to obtain the dry mass. The difference between the wet and dry carcass masses was the water content. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus (Zhang and Wang 2007).

Measurement of serum leptin levels

Serum leptin levels were determined by a radioimmunoassay (RIA) with the ^{125}I Multi-species Kit (cat. no. XL-85K, Linco Research, St. Charles, MO, USA). The lowest level of leptin that could be detected by this assay was 1.0 ng/ml when using a 100-μl sample size. The inter- and intra-assay variabilities for the leptin RIA were <3.6% and 8.7%, respectively.

Measurement of the COX activity, α-GPO activity, and UCP1 content

The mitochondrial protein concentration was determined by the Folin phenol method (Lowry et al. 1951) with bovine serum albumin as a standard. Cytochrome C oxidase (COX; EC 1.9.3.1) and α-glycerophosphate oxidase (α-GPO; EC 1.1.3.21) activities were measured by a polarographic method using oxygen electrode units (Hansatech Instruments, Norfolk, UK) (Sundin et al. 1987).

All animals were sacrificed and dissected to evaluate the BAT in an ice bath. The UCP1 content was measured by Western blotting. Total BAT proteins (15 μg per lane) were separated in a discontinuous sodium dodecyl sulfate

Table 1 Effects of photoperiod and temperature on body mass and serum leptin levels in *A. draco*

Parameter	SD-cold (n = 10)	SD-warm (n = 10)	LD-cold (n = 10)	LD-warm (n = 10)
Body mass (g)	30.22 ± 1.45 b	35.75 ± 1.38 a	31.05 ± 1.36 b	36.88 ± 1.46 a
Body fat mass (g)	3.39 ± 0.16 b	3.96 ± 0.13 a	3.48 ± 0.16 b	4.03 ± 0.14 a
Wet carcass mass (g)	20.13 ± 0.89 a	22.98 ± 1.21 a	20.36 ± 1.02 a	23.02 ± 1.23 a
Dry carcass mass (g)	8.19 ± 0.75 a	10.65 ± 0.72 a	9.62 ± 0.59 a	10.84 ± 0.95 a
Water of carcass (g)	11.93 ± 0.56 a	12.33 ± 0.46 a	10.75 ± 0.56 a	12.18 ± 0.56 a
Serum leptin levels (ng/ml)	2.26 ± 0.08 b	2.49 ± 0.12 a	2.29 ± 0.09 b	2.55 ± 0.12 a

Different lowercase letters in a given row indicate a significant difference ($p < 0.05$). SD, short photoperiod; LD, long photoperiod.

Table 2 Effects of photoperiod and temperature on wet organ masses of *A. draco*

Parameter	SD-cold (n = 10)	SD-warm (n = 10)	LD-cold (n = 10)	LD-warm (n = 10)
Heart (g)	0.270 ± 0.026 a	0.237 ± 0.012 a	0.238 ± 0.010 a	0.232 ± 0.009 a
Lungs (g)	0.327 ± 0.017 a	0.307 ± 0.034 a	0.320 ± 0.011 a	0.310 ± 0.016 a
Liver (g)	1.977 ± 0.136 a	1.597 ± 0.173 b	1.752 ± 0.071 a	1.575 ± 0.046 b
BAT (g)	0.227 ± 0.020 a	0. 195 ± 0.038 b	0.220 ± 0.012 a	0.193 ± 0.010 b
Kidneys (g)	0.203 ± 0.017 a	0.190 ± 0.020 a	0.193 ± 0.006 a	0.183 ± 0.010 a
Spleen (g)	0.020 ± 0.001 a	0.019 ± 0.003 a	0.020 ± 0.002 a	0.020 ± 0.001 a
Stomach (g)	0.411 ± 0.016 a	0.402 ± 0.015 a	0.395 ± 0.016 a	0.397 ± 0.010 a
Small intestine (g)	0.763 ± 0.024 a	0.701 ± 0.045 b	0.743 ± 0.023 a	0.655 ± 0.030 b
Cecum (g)	0.440 ± 0.015 a	0.423 ± 0.026 a	0.438 ± 0.025 a	0.421 ± 0.022 a
Large intestine (g)	0.353 ± 0.020 a	0.340 ± 0.032 a	0.342 ± 0.016 a	0.336 ± 0.011 a

Different lowercase letters in a given row indicate a significant difference ($p < 0.05$). BAT, brown adipose tissue; SD, short photoperiod; LD, long photoperiod.

(SDS)-polyacrylamide gel (12.5% running gel and 3% stacking gel) and blotted onto a nitrocellulose membrane (Hybond-C, Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). To check for the efficiency of protein transfer, the gels and nitrocellulose membranes were respectively stained with Coomassie brilliant blue and Ponceau red after being transferred. Nonspecific binding sites were saturated with 5% nonfat dry milk in phosphate-buffered saline (PBS). UCP1 was detected using polyclonal rabbit UCP1 (1:5,000) as the primary antibody (Abcam, Shanghai, China) and peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG; 1:5000) (Jackson ImmunoResearch, West Grove, PA, USA) as the second antibody. Enhanced chemiluminescence (ECL, Amersham) was used for detection of the UCP signal. The UCP1 content was determined from area readings using Scion Image Software (Scion Corporation, Frederick, MD, USA) and was expressed as relative units (RU) (Li and Wang 2005a).

Statistical analysis

Data were analyzed using the software package SPSS 15.0 (SPSS, Chicago, IL, USA). Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance using the Kolmogorov-Smirnov and Levene tests, respectively. The body mass, RMR, NST, and energy intake before the experiment were analyzed by a one-way analysis of variance (ANOVA). The metabolic rate, serum leptin levels, body fat mass, UCP1 content, and other parameters were analyzed by a two-way analysis of covariance (ANCOVA) with body mass as the covariate. To detect possible associations of serum leptin with body fat mass, RMR, NST, and energy intake, we used a Pearson correlation analysis. Differences in RMR, NST, organ mass, and digestive tract functionality in the four groups were analyzed by a one-way ANCOVA with body mass as the covariate. Since no gender effects were found for almost any of the measured parameters, data from females and males were combined except where specified. Results are presented as the mean ± the standard error of the mean (SEM), and $p < 0.05$ was considered statistically significant.

Results

Body mass, body composition, and serum leptin levels

For *A. draco*, there was no significant effect of photoperiod or temperature on body mass among the four

Table 3 Effects of photoperiod and temperature on dry organ masses of *A. draco*

Parameter	SD-cold (n = 10)	SD-warm (n = 10)	LD-cold (n = 10)	LD-warm (n = 10)
Heart (g)	0.046 ± 0.001 a	0.043 ± 0.005 a	0.043 ± 0.002 a	0.044 ± 0.002 a
Lungs (g)	0.070 ± 0.001 a	0.068 ± 0.001 a	0.069 ± 0.001 a	0.070 ± 0.001 a
Liver (g)	0.502 ± 0.045 a	0.410 ± 0.026 b	0.483 ± 0.020 a	0.395 ± 0.016 b
Kidneys (g)	0.043 ± 0.001 a	0.042 ± 0.001 a	0.042 ± 0.001 a	0.040 ± 0.001 a
Spleen (g)	0.005 ± 0.001 a	0.004 ± 0.001 a	0.004 ± 0.001 a	0.004 ± 0.001 a
Stomach (g)	0.096 ± 0.011 a	0.096 ± 0.018 a	0.094 ± 0.014 a	0.093 ± 0.014 a
Small intestine (g)	0.033 ± 0.002 a	0.028 ± 0.007 b	0.030 ± 0.002 a	0.029 ± 0.003 b
Cecum (g)	0.046 ± 0.002 a	0.045 ± 0.003 a	0.045 ± 0.004 a	0.044 ± 0.005 a
Large intestine (g)	0.044 ± 0.003 a	0.043 ± 0.003 a	0.045 ± 0.007 a	0.045 ± 0.007 a

Different lowercase letters in a given row indicate a significant difference ($p < 0.05$). SD, short photoperiod; LD, long photoperiod.

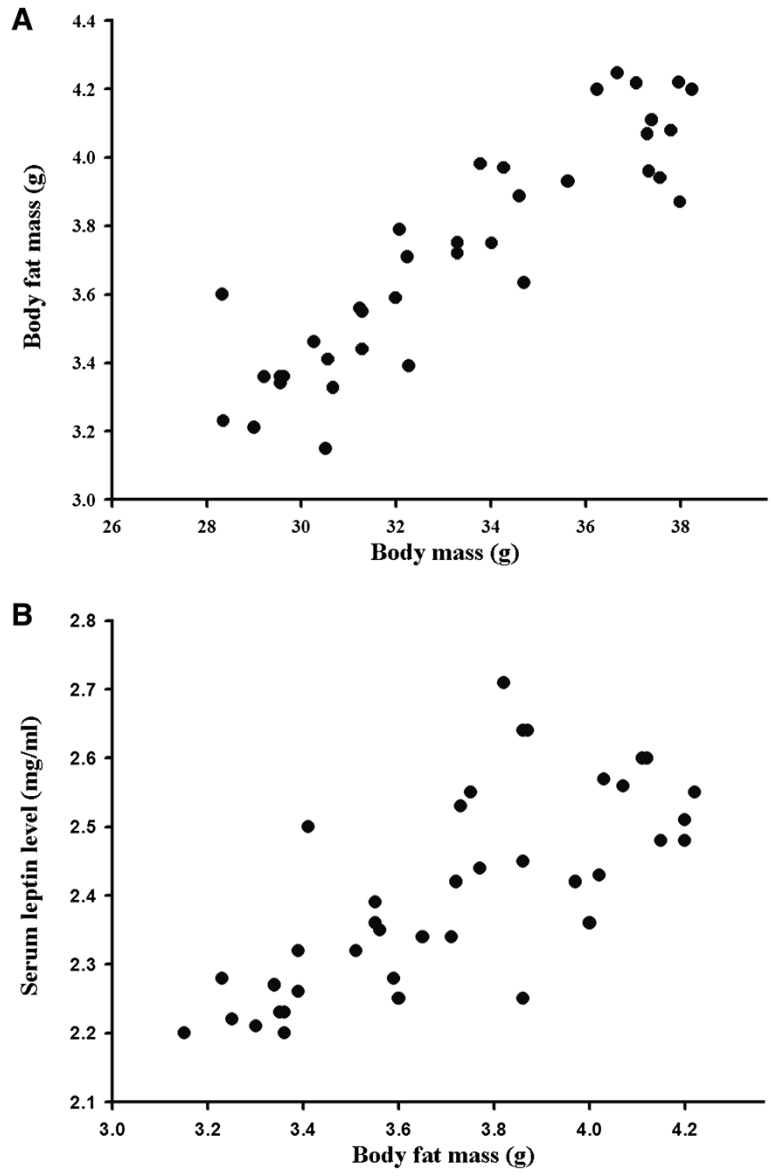

Figure 2 Correlations of body mass (A) and serum leptin levels (B) with body fat mass in *A. draco*.

groups on day 0 of the experiment ($p > 0.05$). SD-cold induced a decrease in body mass compared with LD-warm. At the end of 4 weeks, body mass showed significant differences (photoperiod: $F_{1,34} = 1.13$, $p > 0.05$; temperature: $F_{1,34} = 32.35$, $p < 0.01$; interaction: $F_{1,34} = 0.18$, $p > 0.05$; Figure 1). Temperature had significant effects on the body fat mass (photoperiod: $F_{1,34} = 1.36$, $p > 0.05$; temperature: $F_{1,34} = 9.63$, $p < 0.01$; interaction: $F_{1,34} = 0.10$, $p > 0.05$; Table 1). Temperature had significant effects on the wet liver mass (photoperiod: $F_{1,34} = 0.29$, $p > 0.05$; temperature: $F_{1,34} = 9.32$, $p < 0.01$; interaction: $F_{1,34} = 0.22$, $p > 0.05$), BAT (photoperiod: $F_{1,34} = 0.27$, $p > 0.05$; temperature: $F_{1,34} = 7.69$, $p < 0.01$; interaction: $F_{1,34} = 0.09$, $p > 0.05$), and small intestine (photoperiod: $F_{1,34} = 0.46$, $p > 0.05$; temperature: $F_{1,34} = 10.03$, $p < 0.01$; interaction: $F_{1,34} = 0.31$, $p > 0.05$; Table 2). Temperature also had significant effects on the dry liver mass (photoperiod: $F_{1,34} = 0.39$, $p > 0.05$; temperature: $F_{1,34} = 5.98$, $p < 0.01$; interaction: $F_{1,34} = 0.25$, $p > 0.05$) and dry mass of the small intestine (photoperiod: $F_{1,34} = 0.38$, $p > 0.05$; temperature: $F_{1,34} = 6.21$, $p < 0.01$; interaction: $F_{1,34} = 0.34$, $p > 0.05$; Table 3). There was a positive correlation between the body fat mass and body mass in *A. draco* ($r = 0.756$, $p < 0.01$; Figure 2A).

Temperature had a significant effect on serum leptin levels ($F_{1,34} = 12.25$, $p < 0.01$; Table 1) after 28 days of acclimation in *A. draco*. Photoperiod and the interaction

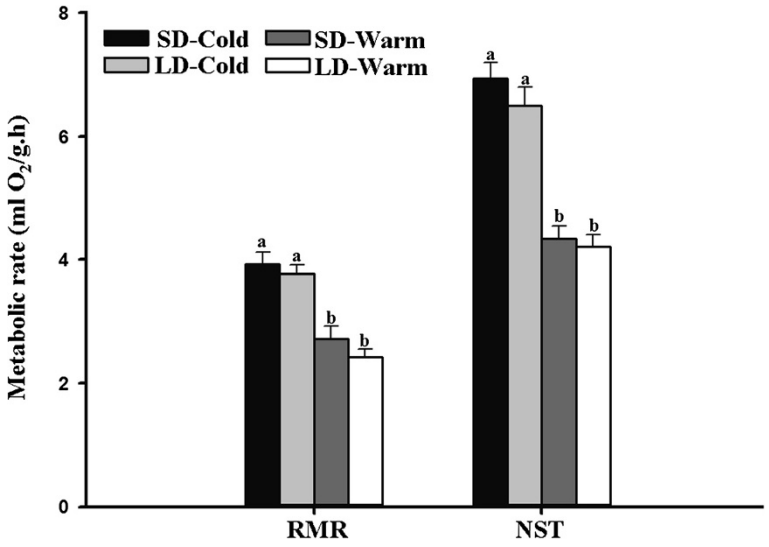

Figure 3 Effects of photoperiod and temperature on the resting metabolic rate and nonshivering thermogenesis in *A. draco*. Different lowercase letters in a given row indicate a significant difference ($p < 0.05$).

of photoperiod and temperature had no effect on serum leptin levels in *A. draco* (photoperiod: $F_{1,34} = 1.23$, $p > 0.05$; interaction: $F_{1,34} = 0.71$, $p > 0.05$). There was a positive correlation between serum leptin levels and the body fat mass ($r = 0.747$, $p < 0.01$; Figure 2B).

RMR, NST, and energy intake

Before acclimation, no differences were found among the different groups in the RMR of *A. draco* ($p > 0.05$). Over the course of acclimation, temperature had a significant effect on the RMR (photoperiod: $F_{1,34} = 1.26$, $p > 0.05$; temperature: $F_{1,34} = 14.85$, $p < 0.01$; interaction: $F_{1,34} = 0.57$, $p > 0.05$; Figure 3). Before acclimation, no differences were found among the groups for the NST of *A. draco* ($p > 0.05$). Over the course of acclimation, temperature had a significant effect on the NST (photoperiod: $F_{1,34} = 0.62$, $p > 0.05$; temperature: $F_{1,34} = 7.48$, $p < 0.01$; interaction: $F_{1,34} = 0.78$, $p > 0.05$; Figure 3). Before acclimation, no differences were found among groups in energy intake by *A. draco* ($p > 0.05$). Over the course of acclimation, energy intake in the cold groups was higher, and the effect of cold persisted to the end (photoperiod: $F_{1,34} = 0.69$, $p > 0.05$; temperature: $F_{1,34} = 6.53$, $p < 0.01$; interaction: $F_{1,34} = 0.16$, $p > 0.05$; Figure 4). There were

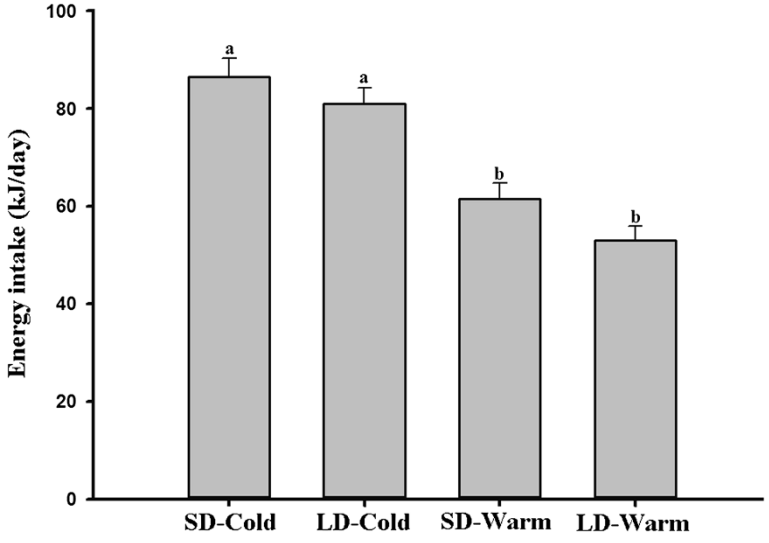

Figure 4 Effects of photoperiod and temperature on energy intake in *A. draco*. Different lowercase letters in a given row indicate a significant difference ($p < 0.05$).

negative correlations between serum leptin levels and the RMR (r = −0.659, p < 0.01; Figure 5A) and NST (r = −0.874, p < 0.01; Figure 5B).

Thermogenic capacity of the liver and BAT

For *A. draco*, temperature, but not photoperiod, had significant effects on the mitochondrial protein, and COX and α-GPO activities in the liver and BAT (Table 4). Temperature, but not photoperiod, also had a significant effect on the UCP1 content in BAT (photoperiod: $F_{1,34}$ = 0.63, p > 0.05; temperature: $F_{1,34}$ = 4.95, p < 0.01; interaction: $F_{1,34}$ = 0.19, p > 0.05; Table 4).

Discussion

Body mass, body composition, and serum leptin levels

Phenotypic plasticity is known as the capacity to change in response to different environmental conditions, which is important for it determines how much variation an individual can tolerate (Bush et al. 2008; Goldberg et al. 2012). It was demonstrated that many small mammals respond to winter-associated environmental cues by reducing their body mass and body fat mass together with enhancing thermogenesis (Merritt et al. 2001; Lovegrove 2005; Wang et al. 2006), such as *Meriones unguiculatus* (Li and Wang 2005a), *Microtus brandti* (Li and Wang 2005b), Djungarian hamster (Steinlechner et al. 1983),

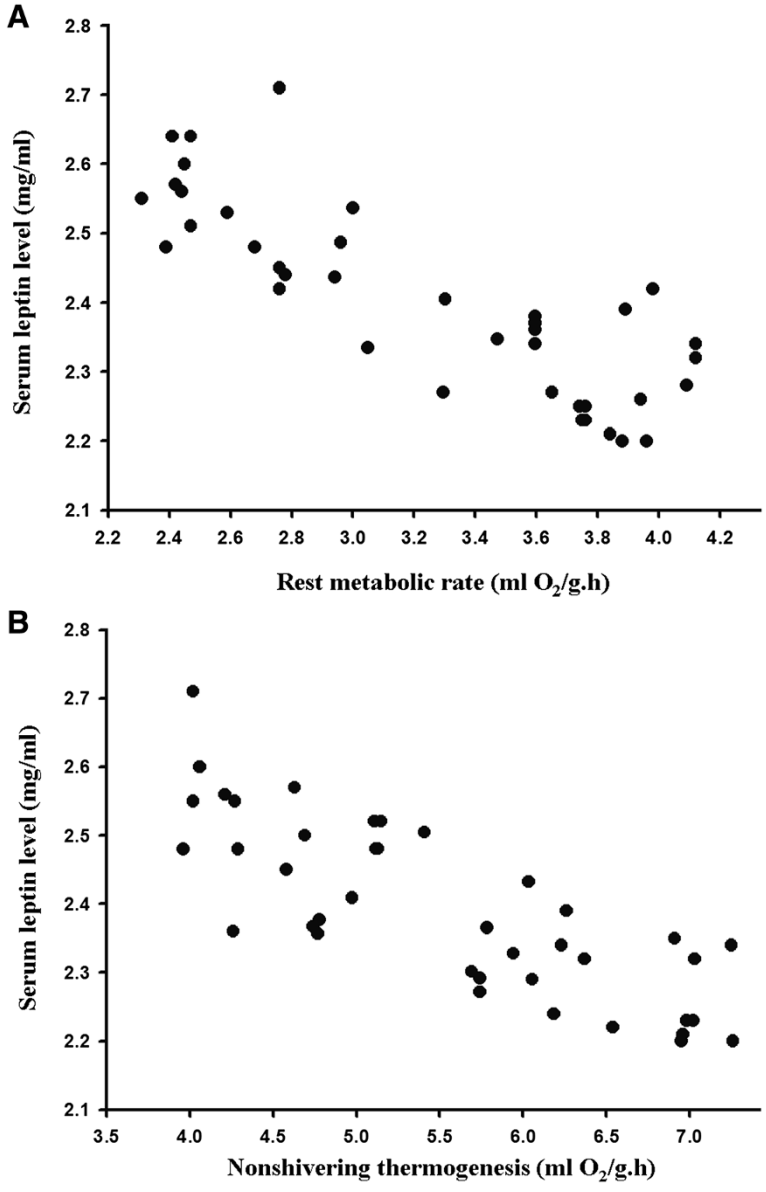

Figure 5 Correlations of serum leptin levels with the RMR (A) and NST (B) in *A. draco*.

Table 4 Effects of photoperiod-temperature on mitochondrial protein and enzyme activity in the liver and BAT

Parameter	SD-cold (n = 10)	SD-warm (n = 10)	LD-cold (n = 10)	LD-warm (n = 10)
Liver				
Mitochondrial protein (mg/g)	25.81 ± 2.31 a	18.16 ± 2.06 b	24.53 ± 2.36 a	17.88 ± 2.59 b
COX (nmol O_2/min·mg)	62.05 ± 5.62 a	49.28 ± 5.14 b	59.34 ± 6.02 a	47.16 ± 2.68 b
α-GPO (nmol O_2/min·mg)	65.59 ± 4.56 a	38.96 ± 3.65 b	59.49 ± 4.21 a	36.91 ± 3.02 b
BAT				
Mitochondrial protein (mg/g)	23.55 ± 2.35 a	15.36 ± 1.95 b	20.23 ± 2.14 a	15.67 ± 2.08 b
COX (nmol O_2/min·mg)	135.26 ± 9.25 a	87.23 ± 6.32 b	130.26 ± 6.95 a	86.53 ± 2.65 b
α-GPO (nmol O_2/min·mg)	426 ± 15.21 a	156.25 ± 10.12 b	401.59 ± 16.02 a	151.29 ± 11.69 b
UCP1 (relative units)	1.55 ± 0.12 a	1.00 ± 0.09 b	1.48 ± 0.13 a	0.91 ± 0.08 b

Different lowercase letters in a given row indicate a significant difference ($p < 0.05$). UCP1, uncoupling protein 1; SD, short photoperiod; LD, long photoperiod.

and *Eothenomys miletus* (Zhu et al. 2010a, b). In contrast, other mammals maintain a stable body mass or even show an increase in body mass when exposed to winter-like conditions (Steinlechner et al. 1983), such as *Dicrostonyx groenlandicus*, *Mesocricetus auratus*, and *Akodon azarae* (Nagy 1993). In the present study, we showed that temperature is an important environmental cue that can cause *A. draco* to significantly reduce its body mass in cold conditions as a decreased body mass is important for decreasing absolute energy requirements. Body mass declines in winter-like conditions are considered to be an adaptive mechanism for reducing energy requirements when stress occurs (Li and Wang 2007).

As potential components of the adipostatic mechanism in body mass regulation, serum leptin levels and body fat mass were also influenced by cold in *A. draco* (Table 1). This suggests that mobilizing body fat may be a metabolic compensation to meet the high energy requirements during cold acclimation (Zhang and Wang 2007). *A. draco* decreased its serum leptin levels and body fat mass in the cold and seemed to be very sensitive to cold temperatures. The decrease in serum leptin levels after cold exposure was also verified in cold-exposed rats (Bing et al. 1998). Comparable changes in serum leptin levels and body fat mass indicated that leptin plays a signaling role in changes of body energy reserves during seasonal adaptations (Klingenspor et al. 2000; Rousseau et al. 2003). Leptin levels may reflect the fattiness content and could serve as a signal to the brain to regulate food intake, energy expenditure, and resistance to obesity (Schwartz et al. 2000). There was a positive correlation between serum leptin levels and body fat mass (Friedman and Hallas 1998). Subsequent experiments in rodents supported the tenets of the lipostasis theory that the serum 'satiety signal' is at a higher concentration in obese animals than in lean animals (Coleman 1978). The present study also showed similar results. In addition, modification of the masses of the liver and digestive organs (Tables 2 and 3), to meet high energy intake and digestion, suggests that sustained energy intake during cold acclimation is not limited by the central machinery in *A. draco*.

RMR, NST, and energy intake

It is evident that many small mammals which are active in winter enhance their RMR and NST for survival in the cold (Heldmaier et al. 1989). A short photoperiod and/or cold can cause a decrease in the seasonal RMR of some small mammals, such as *Clethrionomys glareolus* (Heldmaier et al. 1989). Our present results showed that cold is an important environmental cue that can influence *A. draco* to significantly increase its RMR and NST in such conditions. It was shown that the NST capacity of *Phodopus sungorus* in winter increased by 70% compared to that in summer (Heldmaier et al. 1982). The plateau pika (*Ochotona curzoniae*) and root vole (*Microtus oeconomus*) which live on the Qinghai-Tibet Plateau (Wang and Wang 1996) and Brandt's voles (*M. brandti*) that live in grasses of Inner Mongolia (Li and Wang 2005b) also showed similar patterns of adaptation. The increased energy expenditure in the cold can be compensated for by increasing energy intake and mobilizing body fat.

It was pointed out that leptin might be a starvation signal to induce increased energy intake in rats under winter-like conditions (Flier 1998), and similar results were observed in other small mammals (Bing et al. 1998; Abelenda et al. 2003; Peacock et al. 2004; Li and Wang 2005b). Our present results showed that cold is an important environmental cue that can influence *A. draco* to significantly increase its energy intake in these conditions. Lower serum leptin levels were associated with increased energy intake. When serum leptin levels decreased, the RMR and NST increased in a cold condition, and they both showed significant negative correlations. This suggests that leptin is involved in cold-induced energy balance in *A. draco*. The present study supports our hypothesis that changes in the

thermogenic capacity driven by temperature might be mediated by leptin.

Thermogenesis in the liver and BAT

Increased thermogenesis in *A. draco* was further supported by other biochemical markers examined in the present study, including high mitochondrial protein content, COX activity, and UCP1 content. Liver metabolism accounts for 20% to 25% of the RMR (Couture and Hulbert 1995). Our data indicated that changes in COX activity in the liver were parallel to those in the RMR during cold acclimation. UCP1 mRNA expression and production in BAT may be indicative of the thermogenic capacity (Cannon and Nedergaard 2004), and the thermoregulatory role of UCP1 was emphasized in UCP1-deficient mice (Nedergaard et al. 2001). In the present study, the UCP1 content markedly increased in a cold condition, and the increased UCP1 content was used to increase the thermogenic capacity of the NST, which may be regulated by the nervous system. A cold-induced increase in BAT UCP1 expression was also found in Siberian hamsters (Von et al. 2001) and Mongolian gerbils and ground squirrels (*Spermophilus dauricus*) (Li et al. 2001). Furthermore, temperature, but not photoperiod, had a significant effect on total protein, mitochondrial protein, and COX and α-GPO activities in the liver and BAT of *A. draco* (Table 4). Taken together, *A. draco* seems to be more sensitive to cold than to short photoperiod. Our results were similar to those of previous studies, in which we found that a short photoperiod was an effective cue that influenced body mass and thermogenesis in *E. miletus* in the Hengduan Mountain region, but *E. miletus* was sensitive to temperature when acclimating to different photoperiods and temperatures (Zhu et al. 2011).

Conclusions

In conclusion, all results indicated that *A. draco* mobilizes its body fat mass, alters its body composition, and increases the RMR and energy intake to regulate its body mass and energy balance in cold conditions. Results showed that cold is an effective cue that induced a decrease in serum leptin levels and increases in protein contents, COX activity, and α-GPO activity in the liver/BAT to cope with winter-like conditions. However, there were no significant differences in thermogenic responses in *A. draco* between the long and short photoperiods, indicating that the low latitude and high elevation of the Hengduan Mountain region may lead *A. draco* to be more sensitive to temperature than to photoperiod in seasonal adaptation. Furthermore, leptin may potentially be involved in regulating the body mass, energy intake, and thermogenesis in *A. draco*.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

WZ carried out the studies of body mass, thermogenesis, and energy intake and drafted the manuscript. LZ carried out the serum leptin levels and enzyme activity. GY participated in the design of the study and performed the statistical analysis. ZW conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

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