Photoperiodic regulation in energy intake, thermogenesis and body mass in root voles (*Microtus oeconomus*)

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Abstract

The present study was designed to examine whether photoperiod alone was effective to induce seasonal regulations in physiology in root voles (*Microtus oeconomus*) from the Qinghai–Tibetan plateau noted for its extreme cold environment. Root voles were randomly assigned into either long photoperiod (LD; 16L : 8D) or short photoperiod (SD; 8L : 16D) for 4 weeks at constant temperature (20 °C). At the end of acclimation, SD voles showed lower body mass and body fat coupled with higher energy intake than LD voles. SD greatly enhanced thermogenic capacities in root voles, as indicated by elevated basal metabolic rate (BMR), nonshivering thermogenesis (NST), mitochondrial protein content and uncoupling protein-1 (UCP1) content in brown adipose tissue (BAT). Although no variations in serum leptin levels were found between SD and LD voles, serum leptin levels were positively correlated with body mass and body fat mass, and negatively correlated with energy intake and UCP1 content in BAT, respectively. To summarize, SD alone is effective in inducing higher thermogenic capacities and energy intake coupled with lower body mass and body fat mass in root voles. Leptin is potentially involved in the photoperiod induced body mass regulation and thermogenesis in root voles.
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Keywords: Basal metabolic rate (BMR); Body mass; Energy intake; Leptin; Nonshivering thermogenesis (NST); Root vole (*Microtus oeconomus*); Short photoperiod (SD); Uncoupling protein-1 (UCP1)

1. Introduction

Seasonal change in photoperiod is an important environmental cue in regulating seasonal responses in several mammal species, which includes variations in body mass, adiposity, food intake and thermogenic capacities (Hagelstein and Folk, 1978; Król et al., 2005). Changes in thermogenic performance are primarily achieved by increases in the capacity for non-shivering thermogenesis (NST) originated from brown adipose tissue (BAT) (Jansky, 1973; Foster and Frydman, 1979). NST in small mammals is originated principally through activation of uncoupling protein-1 (UCP1) (Trayhurn et al., 1983). UCP1 is uniquely expressed in BAT where it uncouples electron transport through the respiratory chain from adenosine triphosphate production (Nicholls and Locke, 1984; Klingenberg and Huang, 1999) and

thus allows for the generation of heat upon sympathetic nervous system stimulation (Sell et al., 2004). It has been found that short photoperiod (SD) stimulated the increase in NST coupled with elevation in UCP1 content or UCP1 mRNA expression in many small mammals, such as Brandt's voles [*Lasiopodomys* (*Microtus*) *brandtii*] (Zhao and Wang, 2005), common spiny mice (*Acomys cahirinus*) (Kronfeld-Schor et al., 2000) and Siberian hamsters (*Phodopus sungorus*) (Demas et al., 2002).

Leptin, produced and secreted mainly by the adipocytes of mammals (Zhang et al., 1994; Dijk, 2001), is an important body mass regulator through effects on food intake and energy expenditure (Paracchini et al., 2005). It was found that lower serum leptin level was accompanied with higher food intake in rat (Flier, 1998) and Brandt's voles (Li and Wang, 2005), while Siberian hamsters showed lower leptin level and reduced food intake under winter-like conditions. Further, leptin administration can increase the energy expenditure (Collins et al., 1996; Hwa et al., 1997). However, leptin treatment in cold-acclimated rats

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decreased thermogenic capacities (Abelenda et al., 2003). The mechanisms by which leptin exerts its effects on metabolism are largely unknown and are likely quite complex.

Root voles (*Microtus oeconomus*) are an herbivorous and winter-active species, distributed widely from northwest Europe, eastward through north and central Asia to Alaska and northwest Canada (Brunhoff et al., 2003). In China it is mainly distributed in the Northwest (Wang and Wang, 1996). Root voles living on the Qinghai–Tibetan Plateau face more rigorous environment since they do not hibernate. It has been reported that root voles from Qinghai–Tibetan Plateau showed seasonal changes in body mass and metabolism (Jia and Sun, 1986; Wang and Wang, 1989; 1996). We have also found seasonal variations in serum leptin level and UCP1 content in this species (Wang et al., 2006). It was reported that cold exposure or cold exposure coupled with SD induced increases in thermogenesis with decreases in body mass (Wang et al., 1996, 1999). However, there is still a lack of studies integrating organismal and molecular regulations induced by photoperiod in this species. Further, energy intake and the role of leptin in photoperiod acclimated root voles were remained unexplored.

In the present study, we examined photoperiodic effects on several physiological, hormonal, and biochemical markers in root voles from the Qinghai–Tibetan plateau. We hypothesized that photoperiod alone acted as an environmental regulator of energy intake and thermogenesis. We predicted that root voles exposed to SD would decrease body mass and mobilize body fat while increase energy intake and thermogenesis, and leptin would be involved in the regulation of body mass, energy intake and thermogenesis.

2. Materials and methods

2.1. Animals

Root voles (*M. oeconomus*) were wild caught around the Haibei Alpine Meadow Ecosystem Research Station, the Chinese Academy of Sciences (37°29′–37°45′N, 101°12′–101°33′E, 3200–3500 m in altitude) at Menyuan County, Qinghai Province. After capture, animals were transported to the Northwest Plateau Institute of Biology, the Chinese Academy of Sciences in Xining, Qinghai, China (2275 m in altitude). Animals were housed singly in plastic cages ($290 \times 180 \times 160$ mm^3) with sawdust as bedding in a temperature controlled room (20 °C± 1) with a 12 L : 12 D cycle (lights on at 06:00 h) for 3 weeks to acclimate to laboratory conditions. Then animas were randomly divided into two groups ($n=10$ each group, 5 males and 5 females). One group was switched to a short photoperiod (SD, 8 L : 16 D with lights on 08:00 h) and the other group to a long photoperiod (LD, 16 L : 8 D with lights on 04:00 h). Food (rabbit pellet chow, KeAo Feed Co. Beijing) and water were provided *ad libitum*. Body mass of the voles was monitored twice a week.

2.2. Metabolic trials

All metabolic measurements were performed between 10:00 and 16:00 h (to minimize the effect of circadian rhythms) with an established closed-circuit respirometer (Wang and Wang, 1996).

Animals were fasted for 3 h and then kept in the metabolic chamber for 1 h stabilization. Oxygen consumption was recorded every 5 min for 60 min. The metabolic chamber size was 3.6 L. Temperature (± 0.5 °C) was controlled with water bath and KOH and silica gel were used to absorb carbon dioxide and water in the metabolic chamber. BMR was measured at 30 °C, which was within the thermoneutral zone for this species (28–32.5 °C; Wang and Wang, 2000). Two continuous stable lowest readings were used to calculate BMR. NST was induced by subcutaneous injections of norepinephrine (NE) bitartrate (Shanghai Harvest Pharmaceutical Co. LTD) and measured at 25 ± 1 °C. NE dosage was calculated according to Heldmaier (1971) as previously described: $(mg/kg)=6.6$ Mb$^{-0.458}$ (g) (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 Gettnger (1996) [NE $(mg/kg)=2.53$ W$^{-0.4}$], indicating that the NE dosage used in the present study was appropriate (Wang and Wang, 2006). Oxygen consumption was recorded every 5 min for 45–60 min and two stable consecutive highest recordings were taken for NST calculation (Wang and Wang, 1996, 2000; Li and Wang, 2005; Zhao and Wang, 2005). Generally the peak oxygen consumption will occur between 15 and 45 min later after NE injection. BMR and NST were measured respectively on the day before acclimation (marked as day 0) and once a week (7-day interval) thereafter. All data were corrected to standard temperature and pressure (STP) conditions.

2.3. Energy intake

Energy intake was measured in metabolic cages for 3 days as described previously (Liu et al., 2002; Song and Wang, 2003). During each test, food was provided quantitatively and water was provided *ad libitum*. Food residues and feces were collected during the 3-day test before the photoperiod acclimation (marked as week 0) and once a week (every fourth day) throughout the 4-week study period, and then oven-dried at 60 °C to get constant mass and separated manually. The caloric contents of food and feces were determined by Parr1281 oxygen bomb calorimetry (Parr Instrument, USA). Gross energy intake (GEI), digestible energy intake (DEI), and apparent digestibility of energy (hereafter referred to as digestibility) were calculated according to Grodzinski and Wunder (1975) and Liu et al. (2002):

$$\text{GEI (kJ day}^{-1}) = \text{dry matter intake (DMI) (g day}^{-1})$$
$$\times \text{ energy content of food (kJ g}^{-1});$$
$$\text{DEI (kJ day}^{-1}) = \text{GEI} - \text{dry mass of feces (g day}^{-1})$$
$$\times \text{ energy content of feces (kJ g}^{-1});$$
$$\text{Digestibility (\%)} = \text{DEI/GEI} \times 100\%.$$

2.4. Measurements of mitochondrial protein and UCP1 content

All subjects were sacrificed by decapitation between 09:00 and 11:00 h at the end of the acclimation. Interscapular BAT were removed and weighed immediately for mitochondrial protein preparation as described previously (Li et al., 2001; Li and Wang, 2005). Mitochondrial protein concentrations were determined by the Folin phenol method (Lowry et al., 1951) with bovine serum albumin as standard.
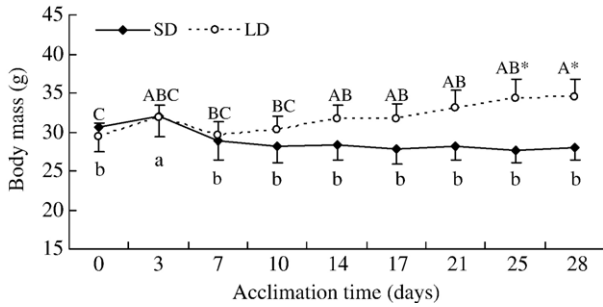
Fig. 1. Effects of photoperiod acclimation on body mass in root voles. Data were expressed as mean±S.E.M. $P<0.05$ was considered to be statistically significant.

UCP1 was measured as previously described (Li and Wang, 2005; Zhao and Wang, 2005) with 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). UCP1 content was quantified with Scion Image Software (Scion Corporation) and expressed in relative unit (RU).

2.5. Body fat analysis

The eviscerated carcass was oven-dried at 60 °C to get constant mass (BAT and the entire gastrointestinal tract was excluded before drying). Total body fat were extracted from the dried carcass by ether in a Soxhlet apparatus (Li and Wang, 2005).

2.6. Serum leptin levels

The blood sample was centrifuged at 2500×g 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 (Linco, St. Louis, MO, USA), which has been shown to be feasible in a previous study (Wang et al., 2006). Inter- and intra-assay variability for leptin RIA was

Table 1
Body mass, body fat and serum leptin levels in root voles acclimated to short photoperiod (SD) and long photoperiod (LD)

	SD	LD	P
Sample size	10	10	
Body mass			
Initial (g)	30.6±3.1	29.4±1.7	ns
Final (g)	28.0±1.6	34.4±2.4	<0.05
Dry carcass (g)	6.7±0.5	7.8±0.4	<0.05
Fat free dry carcass (g)	4.9±0.4	5.0±0.3	ns
Body fat			
g	1.870±0.168	2.628±0.252	<0.01
% body mass	6.7±0.2	7.6±0.3	<0.05
Serum leptin level (ng/mL)	3.792±1.451	4.323±1.448	ns
	(n=6)	(n=6)	

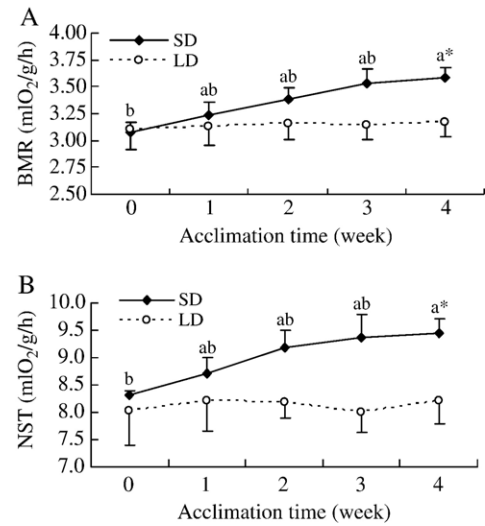Data were expressed as mean±S.E.M. $P<0.05$ was considered to be statistically significant.

Fig. 2. Photoperiodic responses of basal metabolic rate (BMR, A) and nonshivering thermogenesis (NST, B) in root voles. Data were expressed as mean±S.E.M. $P<0.05$ was considered to be statistically significant.

<3.6% and 8.7%, respectively. The lower and upper detection limits for leptin were 1.0 and 50 ng/mL.

2.7. Statistical analysis

Data were analyzed with the SPSS software package. Distributions of all variables were tested for normality using the Kolmogorov–Smirnov test. To remove the effects of body mass, data for BMR and NST were corrected by the 0.67 power of body mass in analysis as proposed for rodents previously (Heusner, 1984; Li and Wang, 2005). Throughout the acclimation, changes in body mass, BMR, NST and energy intake parameters (FMI, GEI, DEI; corrected by the 0.67 power of body mass) were analyzed by repeated measure one-way ANOVA followed by least-significant difference (LSD) post-hoc tests. Group differences in body mass and digestibility at each time point as well as UCP1 content were analyzed by two-way analysis of variance (ANOVA) (photoperiod by sex). Group differences in energy intake parameters, body fat mass and serum leptin levels were analyzed by a two-way analysis of covariance (ANCOVA) with body mass as a covariate. Pearson's correlation was performed to detect possible correlations among serum leptin and body mass, body fat mass, UCP1

Table 2
Effects of photoperiod on brown adipose tissue (BAT) mass and mitochondrial protein content in root voles

	SD	LD	P
Sample size	10	10	
BAT mass			
g	0.164±0.020	0.143±0.017	ns
Mitochondrial protein content			
mg/g BAT	2.544±0.313	1.514±0.098	<0.01
mg in total BAT	0.412±0.070	0.220±0.033	<0.05

Data were expressed as mean±S.E.M. $P<0.05$ was considered to be statistically significant.

content and GEI. All values were expressed as mean±S.E.M. and statistical significance was determined at $P<0.05$.

3. Results

Since no gender effects were found in any of the measured parameters, data from females and males were combined thereafter.

3.1. Body mass and body fat mass

No group differences were found between LD and SD voles ($F_{(1,16)}=0.112$; $P>0.05$; Fig. 1) before acclimation. From day 25 and thereafter, LD voles showed higher body mass than SD voles ($P<0.05$). At the end of the acclimation, LD voles were 22.8% heavier than SD voles. Except an increase on day 3 ($F_{(8,72)}=4.256$; $P<0.001$), SD voles showed relatively stable body mass throughout the acclimation period, whereas the LD voles showed a gradual increase over the 4-week acclimation ($F_{(8,72)}=3.657$; $P<0.01$). The body mass in LD voles on day 28 was significantly higher than that prior to day 10, and increased 17% as compared to the body mass prior to acclimation. Body fat mass in SD voles was lower than that in LD voles at the end of the acclimation ($F_{(1,15)}=12.987$, $P<0.01$ for body fat mass; $F_{(1,16)}=6.970$, $P<0.05$ for body fat percent; Table 1).
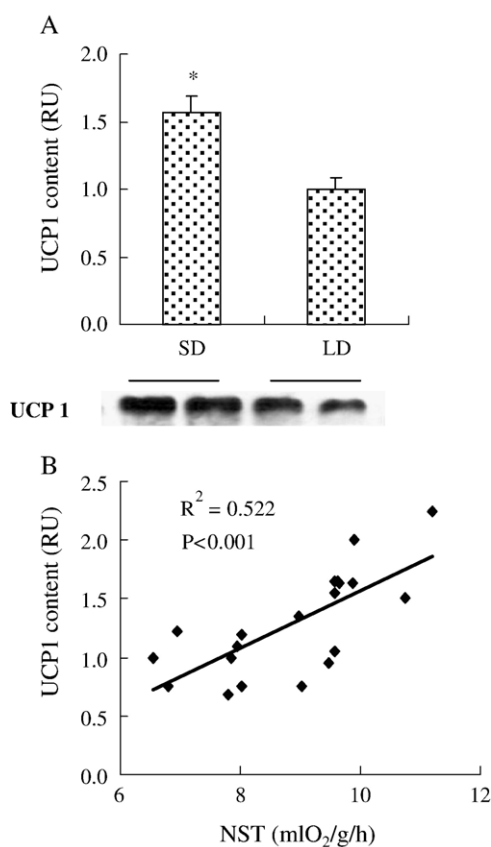

Fig. 3. Photoperiodic responses of uncoupling protein-1 (UCP1) content in brown adipose tissue (BAT) (A) and the correlation between UCP1 and nonshivering thermogenesis (NST) (B) in root voles. Data were expressed as mean±S.E.M. $P<0.05$ was considered to be statistically significant.
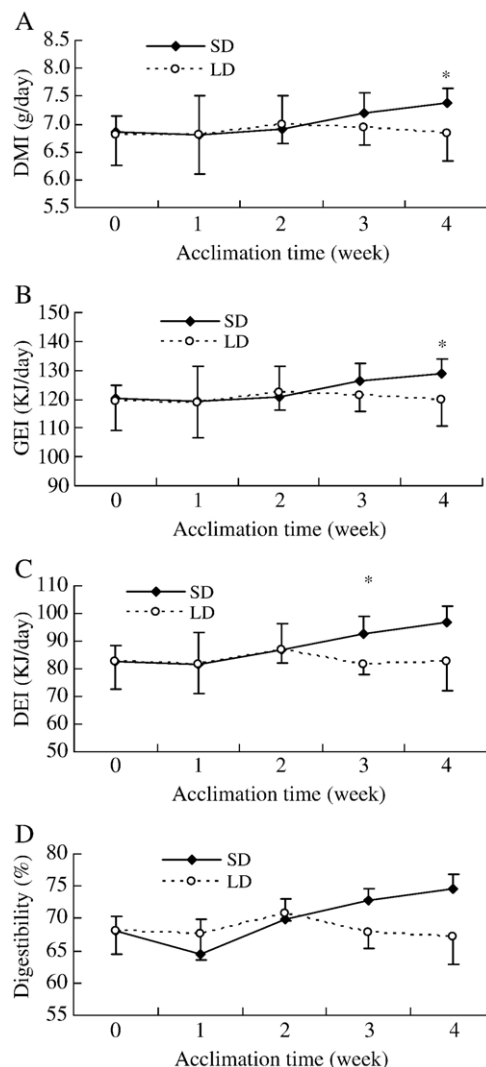

Fig. 4. Changes of dry matter intake (DMI; A), gross energy intake (GEI; B), digestible energy intake (DEI; C) and digestibility (D) in root voles acclimated to short photoperiod (SD, 8L:16D) and long photoperiod (LD, 16L:8D). Data were expressed as mean±S.E.M. $P<0.05$ was considered to be statistically significant.

At the end of the acclimation, the dry carcass mass in SD voles was higher than that in LD voles ($F_{(1,16)}=8.151$; $P<0.05$; Table 1). No differences were found in fat-free dry carcass mass between the two groups ($F_{(1,16)}=0.560$; $P>0.05$; Table 1).

3.2. Metabolic rate and BAT thermogenic capacities

Prior to acclimation, no group differences in BMR or NST were found between SD and LD voles ($F_{(1,16)}=0.146$, $P>0.05$ for BMR; $F_{(1,16)}=0.253$, $P>0.05$ for NST).

At the end of acclimation, BMR in SD voles was higher than in LD voles ($F_{(1,16)}=5.981$; $P<0.05$, Fig. 2A); in SD voles, BMR at week 4 was higher than that prior to acclimation ($F_{(4,36)}=5.179$; $P<0.05$); no variations in BMR in LD voles were found over the acclimation ($F_{(4,36)}=1.152$; $P>0.05$). RMR at the end of the acclimation in SD voles was higher than in LD voles ($F_{(1,15)}=10.714$; $P<0.01$).

At the end of the acclimation, the NST in SD voles was higher than in LD voles ($F_{(1,16)}=7.096$; $P<0.05$, Fig. 2B). Although no significant variations in NST were found in LD voles ($F_{(4,36)}=0.963$; $P>0.05$), the NST in SD voles at the end of the acclimation was higher than prior to acclimation ($F_{(4,36)}=3.937$; $P<0.01$). NST in SD voles at the end of the acclimation was higher than in LD voles ($F_{(1,15)}=19.298$; $P<0.001$).

BAT mass showed no difference between SD and LD voles ($F_{(1,15)}=0.804$; $P>0.05$; Table 2). Mitochondrial protein and UCP1 content in BAT in SD voles were higher than that in LD voles ($F_{(1,16)}=14.117$; $P<0.01$ for mitochondrial protein per g BAT, Table 2; $F_{(1,16)}=7.676$; $P<0.05$ for mitochondrial protein in whole BAT; $F_{(1,16)}=16.240$; $P<0.01$ for UCP1, Fig. 3A). UCP1 content was positively correlated with NST ($R^2=0.552$; $P<0.001$, Fig. 3B).

3.3. Energy intake

No differences were found in dry matter intake (DMI; Fig. 4A), gross energy intake (GEI; Fig. 4B), digestible energy intake (DEI; Fig. 4C) and digestibility (Fig. 4D) between SD and LD group prior to acclimation ($F_{(1,11)}=0.285$; $P>0.05$ for DMI; $F_{(1,11)}=0.285$; $P>0.05$ for GEI; $F_{(1,11)}=0.358$; $P>0.05$ for DEI; $F_{(1,11)}=0.201$; $P>0.05$ for digestibility). In SD voles, DMI ($F_{(1,11)}=6.036$; $P<0.05$) GEI ($F_{(1,11)}=6.036$; $P<0.05$) and DEI ($F_{(1,11)}=6.036$; $P<0.05$) were higher than in LD voles after the 4-week acclimation. No difference was found in digestibility between SD and LD voles at each time point throughout the 4-week acclimation ($P>0.05$). Across the
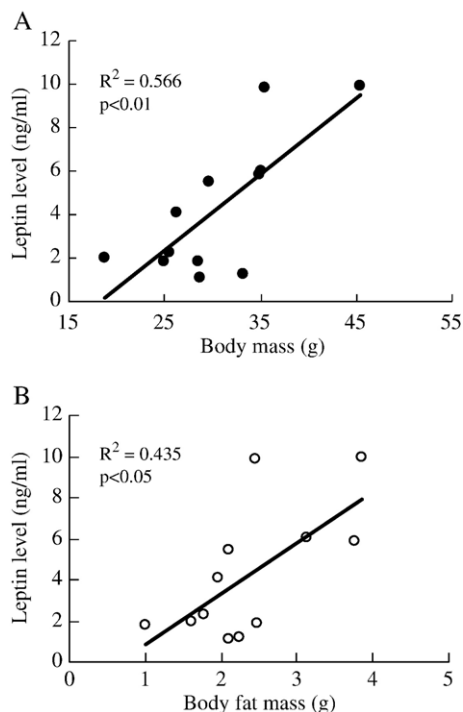

Fig. 5. Correlations between serum leptin level and body mass (A) and body fat mass (B) in root voles. $P<0.05$ was considered to be statistically significant.
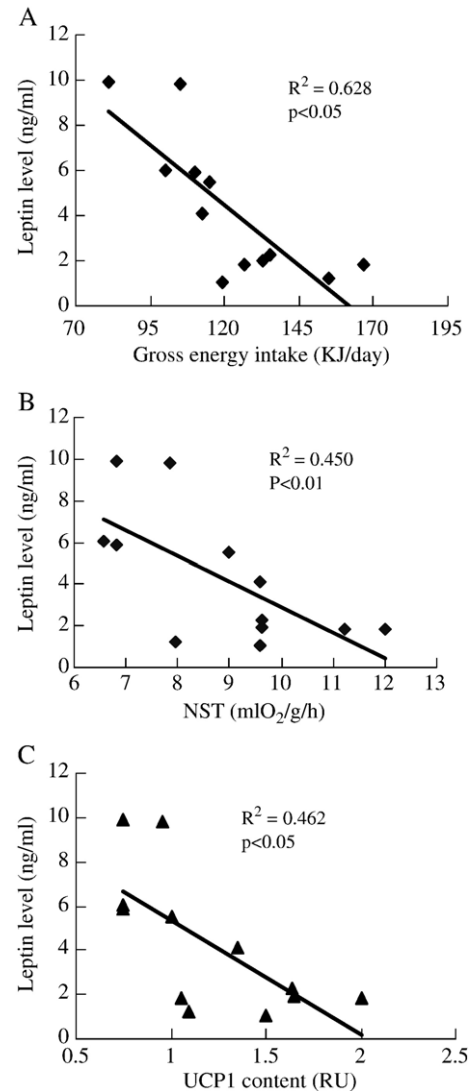

Fig. 6. Correlations between serum leptin level and gross energy intake (A), NST (B), and uncoupling protein 1 (UCP1) content (C) in root voles. $P<0.05$ was considered to be statistically significant.

4-week acclimation, no differences were found in any of the energy intake parameters either in SD or LD voles ($P>0.05$).

3.4. Serum leptin levels

No differences in serum leptin levels were found between LD and SD voles ($F_{(1,7)}=0.018$; $P>0.05$; $n=6$; Table 1). Serum leptin level was positively correlated with body mass ($R^2=0.566$; $P<0.01$; Fig. 5A) and body fat mass ($R^2=0.435$; $P<0.05$; Fig. 5B) whereas negatively correlated with energy intake ($R^2=0.628$; $P<0.05$; Fig. 6A), NST ($R^2=0.450$; $P<0.01$; Fig. 6B), and UCP1 content ($R^2=0.462$; $P<0.05$; Fig. 6C).

4. Discussion

Photoperiod is one of the environmental cues triggering seasonal responses in small mammals (Bartness and Wade, 1985; Genin and Perret, 2000). Some small mammals reduced body mass while

increased thermogenesis in SD conditions, such as meadow voles (*Microtus pennsylvanicus*) (Dark and Zucker, 1986), Siberian hamsters (Heldmaier et al., 1981; Demas et al., 2002), Brandt's voles (Zhao and Wang, 2005), white-footed mice (*Peromyscus leucopus*) (Lynch and Wichman, 1981), prairie voles (*Microtus ochrogaster*) (Wunder et al., 1977), and wood mice (*Apodemus sylvaticus*) (Klaus et al., 1988). However, collared lemming (*Dicrostonyx torquatus*) showed an increase in body mass coupled with a decrease in thermogenesis when exposed to SD (Nagy and Negus, 1993; Powel et al., 2002). Interestingly, both body mass and thermogenesis was stimulated in SD acclimated Syrian hamster (*Mesocricetus auratus*) (McElroy and Wade, 1986), while SD alone did not induce changes either in body mass or nonshivering thermogenesis (NST) in the white rat (*Rattus norvegicus*), indicating absence of adaptive capacities in body mass to photoperiod regulation (Hagelstein and Folk, 1978; Içten et al., 1998). Root voles in our study inhabited the extreme environmental conditions of Qinghai–Tibetan Plateau; since the temperature between day and night fluctuated greatly, it was not reliable for animals living here to depend solely on changes in environmental temperature to anticipate the forthcoming season (Wang et al., 1999), so root voles might have evolved to adjust physiological and behavioral traits responding to photoperiod alterations.

4.1. Changes in body mass

Our present study showed that SD voles had lower body mass and body fat mass compared with LD voles, which was also found in field voles (*Microtus agrestis*) (Król et al., 2005; 2006) and Brand't voles (Zhao and Wang, 2005). At the end of a 4-week acclimation, LD voles were 23% heavier than SD voles. Lower body mass in SD voles would contribute to reduce overall metabolic requirements when compared to LD voles (Wunder et al., 1977; McNab, 1983). In the present study, body fat mass in SD voles was 29% lower than LD voles. As dry carcass mass in SD voles was lower than in LD while no differences were found in fat free dry carcass mass between the two groups, so the lower body mass in SD voles was mainly due to the lower body fat content. It is important to note that the body mass in SD voles remained relatively stable through-out the whole acclimation, whereas the LD voles showed an increase of 17% in body mass at the end of the 4-week acclimation as compared to that prior to acclimation, revealing that SD imposed an inhibitory effect on body mass in root voles.

4.2. Changes in thermogenic capacities

BAT is the main site of NST production in small mammals (Foster and Frydman, 1979). In our present study, although no differences in BAT mass were found between SD and LD voles, NST was significantly elevated in SD voles. Further, UCP1 content in BAT was elevated by 57% in SD than LD voles. UCP1 content in BAT had a strongly regulated uncoupling activity, which was essential to the maintenance of body temperature in small mammals (Ricquier and Bouilloud, 2000). UCP 1 content or *ucp1* mRNA expression are strongly induced

by cold exposure or short photoperiod in some small mammals, such as Brandt's voles (Zhao and Wang, 2005), common spiny mice (*Acomys cahirinus*) (Kronfeld-Schor et al., 2000), Siberian hamsters (Praun et al., 2001; Demas et al., 2002), and Mongolian gerbils (*Meriones unguiculatus*) (Li et al., 2001; Li and Wang, 2005). Increases in UCP1 content and NST were also found in seasonally-acclimatized root voles (Wang et al., 2006). Our present study shows that the significantly elevated UCP1 content in BAT is positively correlated with NST, suggesting that UCP1 is the molecular basis for NST at organism level.

4.3. Roles of leptin during photoperiod acclimation

Leptin plays a critical role in body mass regulation (Zhang et al., 1994). Circulating leptin concentrations reflect levels of body adiposity as referred in the "adipostat hypothesis" (Schneider et al., 2000) found in photoperiod acclimated Brandt's voles (Zhao and Wang, 2006) and Siberian hamsters (Rousseau et al., 2002). However, plasma leptin concentrations did not accurately reflect the body fat content in fasted adult female Syrian hamsters (Schneider et al., 2000). In our result, although SD voles showed relatively lower serum leptin levels compared with LD voles, no significant differences were found between the two groups. We have also found that winter-caught root voles showed lower body mass and lower body fat as compared to summer-caught root voles (Wang et al., 2006). In the present study, further correlation analysis showed that serum leptin levels were positively correlated with body mass and body fat mass, indicating that leptin may act as "adiposity indicator" (Schneider et al., 2000), participate in the physiological transition from autumnal anabolism to wintertime catabolism and stimulate the mobilization of fat during the winter.

To regulate body mass, leptin influences energy balance via its effects on both food intake and energy expenditure (Friedman and Halaas, 1998; Concannon et al., 2001). Leptin administration is thought to increase energy expenditure as indicated by elevated expression of uncoupling protein (UCP1) or UCP1 mRNA level (Collins et al., 1996; Hwa et al., 1997). However, in cold-acclimated rats, chronic leptin administration induced decreases in thermogenesis (Abelenda et al., 2003). It has been reported that lower serum leptin levels were accompanied with higher energy intake in Brandt's voles, indicating that leptin acted as a starvation signal (Li and Wang, 2005; Zhao and Wang, 2005); whereas in Siberian hamsters and field voles, decline in leptin levels was coupled with reduction in food intake (Klingenspor et al., 1996; Król et al., 2006), which suggests the anorectic effect of leptin (Berthoud, 2005). The apparent complexity and contradiction in the roles of leptin in energy homeostasis indicate that the anorectic and thermogenic effects of leptin may be dissociated under different conditions (Król et al., 2006). Our present result showed that food intake and thermogenesis in SD voles were greatly elevated, which was also found in seasonally-acclimatized root voles (Wang and Wang, 1989, 1996; Wang et al., 1999, 2006), revealing that SD alone was effective in inducing seasonal acclimations in this species. Furthermore, correlation analysis showed that serum leptin levels were negatively correlated with

energy intake and NST as well as UCP1 content, indicating that leptin was potentially involved in SD-induced energy balance in this species. Further studies should elucidate the effects of exogenous leptin treatment on energy balance of root voles.

Taken together, SD voles showed lower body mass and body fat mass, higher thermogenic capacities and energy intake than LD voles. The present results support our hypothesis that photoperiod was an important environmental cue to induce adjustments in body mass, energy metabolism and thermogenesis in root voles at the absence of cold stress. Although no significant differences in serum leptin levels were found between the two groups, there were significant correlations between serum leptin levels with body (fat) mass, energy intake, and thermogenesis parameters, indicating that leptin was potentially involved in photoperiod-induced energy balance and body mass regulation. The physiological modification under different photoperiods would allow root voles to overcome the physiological challenges of the extreme environments in their habitat (Bozinovic et al., 2003). The metabolic and endocrine changes induced by SD may lead to energy preservation and enhancing the survival of root voles during the most challenging time of the year.

Acknowledgements

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