

Effects of photoperiod on energy budgets and thermogenesis in Mongolian gerbils (*Meriones unguiculatus*)

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Abstract

- (1) To investigate the role of photoperiod on the regulation of energy budgets and thermogenesis in Mongolian gerbils, body mass (BM), body fat mass (BFM), basal metabolic rate (BMR), nonshivering thermogenesis (NST), gross energy intake (GEI), mitochondrial cytochrome *c* oxidase (COX) activity and uncoupling protein1 (UCP1) content of brown adipose tissue (BAT), and serum triiodothyronine (T₃), thyroxine (T₄) and leptin levels were measured.
- (2) Short day (SD) gerbils showed higher levels of BMR, liver COX activity and T₄ concentration than Long day (LD) gerbils and, however, NST, BAT COX activity and UCP1 content, T₃ level did not differ between the two groups.
- (3) There was no group difference in BM, BFM and leptin levels. Leptin concentration was not correlated with BFM and GEI. GEI was significantly higher in SD than in LD gerbils.
- (4) Our data indicate that photoperiodic changes may provide cues altering energy budget, but not thermogenic capacity in Mongolian gerbils.

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Keywords: Basal metabolic rate (BMR); Body mass; Cytochrome *c* oxidase (COX); Leptin; Photoperiod; Nonshivering thermogenesis (NST); Thyroid hormones; Uncoupling protein1 (UCP1)

1. Introduction

Physiological adjustments to seasonal variations of environment are important adaptive strategies for small mammals (Wunder, 1984). Seasonal variation in photoperiod is most anticipated among many environmental factors including temperature, food quality and quantity. Many rodent species use the annual photoperiodic cycle as a cue for seasonal physiological changes, especially for energy balance and thermogenesis (Bartness and Wade, 1985; Bartness et al., 1989; Genin and Perret, 2000; Haim, 1996; Heldmaier et al., 1981; Wang et al., 1999).

In order to cope with changes in natural environment, many small mammals increase their thermogenic capacity in winter conditions (Haim and Izhaki, 1993; Heldmaier

et al., 1981; Wunder, 1984), and nonshivering thermogenesis (NST) is an important mechanism for heat production (Haim and Izhaki, 1993; Heldmaier, 1971; Heldmaier and Buchberger, 1985; Heldmaier et al., 1982; Li et al., 2001a; Lynch, 1970; Wang et al., 1999). Brown adipose tissue (BAT) is the main site of NST (Ricquier and Bouillaud, 2000). It has been shown that uncoupling protein 1 (UCP1), a 32 kDa protein uniquely expressed in the inner membrane of BAT mitochondria, plays an important role in heat production by uncoupling oxidative metabolism from ATP synthesis (Himms-Hagen and Ricquier, 1998; Rial and González-Barroso, 2001). As the terminal enzyme in oxidative phosphorylation in mitochondria, cytochrome *c* oxidase (COX, complex IV) is involved in mitochondrial energy metabolism (Kadenbach et al., 2000). It has also been demonstrated that several hormones are related to thermogenesis, e.g., thyroid hormones (triiodothyronine, T₃ and thyroxine, T₄) affect adaptive thermogenesis by

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influencing several aspects of energy metabolism, including substrate cycling, ion cycling and mitochondrial proton leaks (Krotkiewski, 2002; Wu et al., 1999). Photoperiodic changes, one of the important environment factors, affect thermogenic capacity in some small mammals such as plateau pikas (*Ochotona curzoniae*) and root voles (*Microtus oeconomus*) (Wang et al., 1999), Siberian hamsters (*Phodopus sungorus*) (Demas et al., 2002; Heldmaier et al., 1982) and bushy-tailed gerbils (*Sekeetamys calurus*) (Haim, 1996).

Seasonal fluctuations in body mass (BM) have been demonstrated to be dependent on photoperiod in some rodent species, such as Siberian hamsters (Bartness et al., 1989), meadow voles (*Microtus pennsylvanicus*) (Dark and Zucker, 1986), prairie voles (*Microtus ochrogaster*) (Kriegsfeld and Nelson, 1996), collared lemmings (*Dicrostonyx groenlandicus*) (Powell et al., 2002) and root voles (Wang et al., 1999). To maintain body temperature and energy balance in winter conditions, some species such as bushy-tailed gerbils (Haim, 1996) reduce energy expenditure and increase energy intake and thermogenesis under a short photoperiod. However, when acclimated to different photoperiods, bank voles (*Clethrionomys glareolus*) showed no significant change in any energetic parameters (Peacock et al., 2004). In collared lemmings, on the other hand, short photoperiod increases their body mass and decreases energy expenditure (Powell et al., 2002). Thus, there are species-specific responses in small mammals to photoperiod changes.

Leptin is the product of the obese gene (*ob*) and is expressed and secreted exclusively by adipocytes (Zhang et al., 1994). Leptin is considered to be an adipostatic signal regulating food intake (Halaas et al., 1995; Klingenspor et al., 1996; Pellemounter et al., 1995; Zhang et al., 1994), which makes it to be a likely candidate for involvement in endocrine regulation of seasonal changes in BM (Johnson et al., 2004). Seasonal fluctuations in serum leptin levels, energy intake, BM or body fat mass have been found in some seasonal acclimated rodents (Klingenspor et al., 1996, 2000; Li and Wang, 2005a; Li et al., 2004). The positive correlation between serum leptin levels and body fat mass has been found in many small mammals including Siberian hamsters (Klingenspor et al., 2000), collared lemmings (Johnson et al., 2004) and cold acclimated Mongolian gerbils (*Meriones unguiculatus*) (Li et al., 2004).

Mongolian gerbils inhabit mainly desert and semi-arid regions of northern China and Mongolia. In these regions the annual average temperature is -0.4°C , the average temperature in the coldest month is -22.3°C and in the warmest month is 18.8°C , with extreme minimum temperatures below -40°C (Chen, 1988; Wang et al., 2000). Mongolian gerbils survive in this harsh environment by a wide thermal neutral zone (Wang et al., 2000, 2003a), and show seasonal changes of thermogenesis and energy budgets in both captured Mongolian gerbils in Inner Mongolia (Wang et al., 2003a) and their breeding colony in outdoor enclosure, which implies an interaction of

temperature and photoperiod (Li and Wang, 2005b). Cold stimulates their NST capacity (Li et al., 2001b) and maximum metabolizable energy intake (Liu et al., 2002). However, changes of thermogenesis and energy budgets responding to photoperiod in the absence of cold ambient temperature are still unclear. If photoperiodic changes are important cues for seasonal acclimatization, then short photoperiod is expected to increase energy intake and thermogenesis in Mongolian gerbils. Further, energy intake might be mediated by leptin. We tested this hypothesis in the present study by measuring several physiological, hormonal and biochemical markers indicative of thermogenic capacity and energy budget responding to photoperiodic changes in captive gerbils maintained at constant ambient temperature.

2. Materials and methods

2.1. Subjects

The subjects used were the offspring of a captive colony that was trapped in Inner Mongolian grasslands ($41^{\circ}57'\text{N}$, $115^{\circ}16'\text{E}$, about 1500 m in altitude, and the natural photoperiod was around 8 h light in the midwinter and 16 h light in the midsummer), and brought to the animal facility in the Institute of Zoology, the Chinese Academy of Sciences in Beijing, China. Gerbils were raised in cages (30 cm \times 15 cm \times 20 cm; 3–4 per cage) in which food (standard rat pellets from Beijing Ke Ao Feed Co.) and water were provided ad libitum. All cages were maintained under a photoperiod of 12L:12D while the temperature was around $23 \pm 1^{\circ}\text{C}$. Prior to acclimation, 16 adult gerbils (120–150 days of age) were moved into individual cages with sawdust bedding for at least 2 weeks. They were then divided into two groups that were acclimated to two different photoperiod regimes—a long day group (LD, 16L:8D with lights on at 06:00 h, four males and four females) and a short day group (SD, 8L:16D with lights on at 08:00 h, four males and four females) for 4 weeks, in which all other housing conditions were unchanged. Body mass of the gerbils was monitored every other day during the photoperiod acclimation.

2.2. Metabolic trial

BMR and NST were measured in a closed-circuit respirometer (Gorecki, 1975; Wang et al., 2000). The metabolic chamber size was 3.6 L, and chamber temperature was controlled within $\pm 0.5^{\circ}\text{C}$ by a water bath. Carbon dioxide and water in the metabolic chamber were absorbed with KOH and silica gel, respectively. Gerbils were weighed before and after each test. All measurements were made between 09.00 and 17.00 h. In order to reduce the effect of circadian rhythms on the results, two SD gerbils and two LD gerbils were measured at the same time in four metabolic chambers.

BMR and NST were measured on the day before photoperiod acclimation began (day 0) and at 7 day intervals during the acclimation (total of five measurements). BMR was measured at 30 ± 0.5 °C, which is within their thermoneutral zone (26–38 °C) (Wang et al., 2000, 2003a). All subjects were fasted for 3 h before entering the metabolic chamber. After 60 min stabilization in the chamber, metabolic measurement was conducted for 60 min at 5 min intervals. Two continuous stable minimum recordings were used to calculate BMR. Maximum NST was defined as the maximum metabolic response to norepinephrine (NE), and was measured as the highest oxygen consumption following a mass-dependent subcutaneous injection of NE on the next day (Heldmaier et al., 1982). The NE (Shanghai Harvest Pharmaceutical Co. Ltd.) dose was calculated according to the equation (Heldmaier, 1971): $NE \text{ (mg/kg)} = 6.6 M_b^{-0.458} \text{ (g)}$. Maximum oxygen consumption occurred about 15–20 min after NE injection. Each measurement was conducted for 60 min at 5 min intervals at 25 °C (± 1 °C). Two continuous stable maximal recordings were used to calculate NST. BMR and NST were corrected to standard temperature and air pressure (STP) conditions.

2.3. Energy budget

Food intake was measured in the metabolism cages. Food was provided quantitatively and food residues and feces were collected from each animal over the last 3 days each week during photoperiod acclimation, and separated by hand after they were dried 60 °C to constant mass (Liu et al., 2002, 2003). The gross energy contents of the food and feces were determined by a Parr 1281 oxygen bomb calorimeter (Parr Instrument, USA). Gross energy intake (GEI), digestible energy intake (DEI) and apparent digestibility of energy (hereafter referred to simply as digestibility) were calculated as follows (Grodzinski and Wunder, 1975; Liu et al., 2002, 2003):

$$\begin{aligned} \text{GEI (kJ/day)} &= \text{dry matter intake (DMI) (g/day)} \times \text{GE content of food (kJ/g)}; \\ \text{DEI (kJ/day)} &= \text{GEI} - (\text{DMI (g/day)} \times \text{GE content of feces (kJ/g)}); \\ \text{Digestibility (\%)} &= \text{DEI/GEI} \times 100\%. \end{aligned}$$

2.4. Serum leptin levels and thyroid hormones

After 4 week acclimation, all gerbils were decapitated between 09:30–11:30 h. Trunk blood was collected for leptin and thyroid hormones measurement. Serum was separated from each blood sample. Serum leptin levels were determined by radio-immunoassay (RIA) using the Linco ^{125}I Multi-species Kit (Cat. No. XL-85 K, Linco Research Inc.). The lower and upper limits of the assay kit were 1 and 50 ng/ml, and the inter- and intra-assay variations were <3.6% and 8.7%, respectively.

Serum tri-iodothyronine (T_3) and thyroxine (T_4) were quantified by radioimmunoassay using RIA kits (China Institute of Atomic Energy, Beijing). Intra- and inter-assay coefficients of variation were 2.4% and 8.8% for the T_3 , and 4.3% and 7.6% for T_4 , respectively.

2.5. Isolation of BAT and liver mitochondria and measurements of COX activity

After trunk blood was collected, Liver and scapular BAT were removed and weighed, and then homogenized (1:15, w/v) with ice-cold medium A (250 mM sucrose, 10 mM TES, 1 mM EDTA, 64 μM BSA and pH 7.2). The homogenate was centrifuged (12096g for 10 min) at 4 °C, then the precipitate was suspended in ice-cold medium B (250 mM sucrose, 10 mM TES, 1 mM EGTA, 64 μM BSA and pH 7.2) and centrifuged (500g for 10 min) at 4 °C. The supernatant was centrifuged (8740g for 10 min) at 4 °C and the precipitate was resuspended (1:1, w/v) in ice-cold medium C (100 mM KCl, 20 mM TES, 1 mM EGTA and pH 7.2), and then used for measurements of mitochondrial protein content, COX activity and UCP1 contents.

Total mitochondrial protein content was determined by the Folin phenol method (Lowry et al., 1951) with bovine serum albumin as standard. COX activity was measured polarographically with oxygen electrode units (Hansatech Instruments Ltd., England) (Sundin et al., 1987).

2.6. Carcass and body fat content

After liver and scapular BAT were removed, we first extracted the gastrointestinal tract (stomach, small intestine, large intestine and cecum), and then the heart, lungs, pancreas, spleen, kidneys and urinary bladder. The remaining carcass was weighed (to 0.1 g) to determine wet mass, dried in an oven at 60 °C for 10 days to a constant mass, and then weighted (to 0.1 g) again to determine dry mass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus.

2.7. Western blotting

BAT mitochondrial protein (5 μl ; 4 $\mu\text{g}/\mu\text{l}$) was diluted in 5 μl sample buffer and run on an SDS-polyacrylamide gel (3% stacking gel and 12.5% running gel) together with a prestained protein marker for about 2 h. The protein was then transferred to a nitrocellulose membrane (Hybond-C, Amersham Biosciences, England). After blocking against non-specific binding by 5% skim milk at 4 °C overnight, the membrane was incubated with a rabbit polyclonal antibody to hamster UCP1 (1:5000, UCP1 antibody was supplied by Dr. M. Klingenspor, Department of Biology, Philipps-University Marburg, Germany) for 2 h, and then incubated with peroxidase-conjugated goat anti-rabbit IgG (1:5000) (Jackson Immuno-Research Laboratories, Inc., USA) for 2 h, washed in washing buffer and then incubated with an enhanced chemoluminescence kit (ECL,

Amersham Biosciences, England) for 5 min at room temperature. Signals were detected by exposing the membrane to autoradiography film. UCPI content was expressed as relative units (RU) and quantified with Scion Image Software (Scion Corporation)(Li and Wang, 2005a, b; Zhao and Wang, 2005).

2.8. Statistical analysis

Statistical analysis was carried out using the SPSS software package. Distributions of all variables were tested for normality using the Kolmogorov–Smirnov Test. Differences between groups in body mass, wet and dry carcass mass, body fat mass, mitochondrial protein content, COX activity, T_3 , T_4 and leptin levels, and UCPI content were analyzed by two-way analysis of variance (ANOVA) (photoperiod and sex), while BMR, NST, DMI and energy parameters (GEI, DEI, digestibility) were tested by analysis of covariance (ANCOVA) with body mass as the covariate. Further, differences in body mass, BMR, NST, DMI, GEI, DEI and digestibility over the course of the acclimation were analyzed by repeated measures, and significant differences were evaluated with the Turkey's honestly significant difference (HSD) post-hoc test. Statistic significance was assumed at $p < 0.05$.

3. Results

There were no significant differences in any measured parameters between two sexes and, therefore, data from male and female gerbils were combined.

3.1. BMR and NST

There were no significant differences in BMR ($\text{ml O}_2/\text{g h}$) ($F = 0.734$, $p > 0.05$; Fig. 1A) or NST ($\text{ml O}_2/\text{g h}$) ($F = 0.754$, $p > 0.05$; Fig. 1B) between the SD and LD treatments prior to acclimation. During the acclimation period BMR tended to increase in SD gerbils and to decrease in LD gerbils. After the 4 week acclimation, BMR in SD was significantly higher than that in LD gerbils ($F = 5.068$, $p < 0.05$), but NST was relatively stable ($F = 0.687$, $p > 0.05$).

3.2. Mitochondrial protein content and COX activity in BAT and liver, thyroid hormones and BAT UCPI

After the 4 weeks acclimation, there were no significant differences in mitochondrial protein content (mg/g tissue) of BAT and liver between SD and LD gerbils (BAT, $F = 2.329$, $p > 0.05$; liver, $F = 0.152$, $p > 0.05$; Table 1). SD gerbils had higher COX activities (nmol/min g tissue) in liver than LD gerbils ($F = 4.781$, $p < 0.05$), but there was no significant difference in BAT COX activity ($F = 1.518$, $p > 0.05$). Serum T_4 concentrations were higher in SD than that in LD gerbils ($F = 30.355$, $p < 0.01$) but not found in

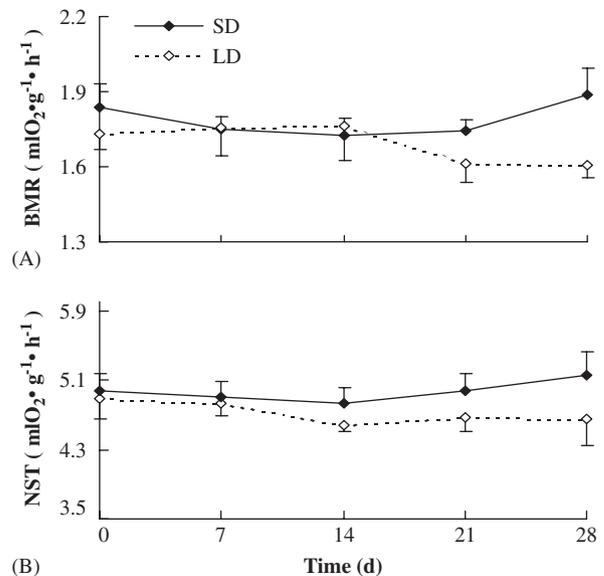


Fig. 1. Effects of photoperiod on basal metabolic rate (BMR; panel A) and nonshivering thermogenesis capacity (NST; panel B) in Mongolian gerbils (*Meriones unguiculatus*). On day 28, SD gerbils had significantly higher levels of BMR, but not NST, compared to LD gerbils. Data are means \pm SEM. *: $p < 0.05$.

T_3 concentration ($F = 1.597$, $p > 0.05$). Similarly, there was no significant difference in UCPI content between SD and LD gerbils ($F = 0.829$, $p > 0.05$; Table 1).

3.3. Energy budget

Prior to acclimation, there were no significant differences in DMI (g/day ; Fig. 2A), GEI (kJ/day ; Fig. 2B), DEI (kJ/day ; Fig. 2C) or digestibility ($\%$; Fig. 2D) between SD and LD gerbils (DMI, $F = 0.044$, $p > 0.05$; GEI, $F = 0.044$, $p > 0.05$; DEI, $F = 0.026$, $p > 0.05$; digestibility, $F = 0.032$, $p > 0.05$). After 4 weeks of acclimation, DMI, GEI and DEI in SD were significantly higher than that in LD gerbils (DMI, $F = 7.020$, $p < 0.05$; GEI, $F = 7.020$, $p < 0.05$; DEI, $F = 6.210$, $p < 0.05$). The increases were 35.0% in DMI, 34.0% in GEI and 36.1% in DEI in the SD gerbils, respectively, and 21.1%, 21.1% and 22.5% in LD gerbils, respectively. Digestibility remained relatively stable throughout photoperiod acclimation.

3.4. Body mass, body fat mass and serum leptin levels

There were no significant differences in body mass (g) between SD and LD gerbils prior to acclimation ($F = 0.194$, $p > 0.05$; Fig. 3). Both SD and LD gerbils tended to increase body mass during the course of acclimation, by 2.8% in SD and 5.6% in LD gerbils, respectively, but there was no significant difference at the end of the acclimation ($F = 1.137$, $p > 0.05$). After 4 weeks photoperiod acclimation, there were no significant differences in wet carcass mass ($F = 2.438$, $p > 0.05$), dry carcass mass ($F = 0.187$, $p > 0.05$), body fat mass ($F = 0.462$, $p > 0.05$) and serum leptin levels ($F = 0.368$, $p > 0.05$)

Table 1

Mitochondrial (Mt) protein content, cytochrome *c* oxidase (COX) activity of brown adipose tissue (BAT) and liver, uncoupling protein 1 (UCP1) content of BAT mitochondria and serum T₃, T₄ and leptin concentration in Mongolian gerbils acclimated to long photoperiod (LD; 16L:8D) and short photoperiod (SD; 8L:16D)

	SD	LD	<i>P</i>
Body mass (g)	53.45 ± 1.52	55.09 ± 1.58	ns
Wet carcass mass (g)	42.59 ± 1.52	45.40 ± 1.45	ns
Dry carcass mass (g)	15.87 ± 0.46	16.55 ± 0.65	ns
Body fat mass (g)	4.84 ± 0.39	5.48 ± 0.46	ns
Body fat content (%)	30.24 ± 1.62	32.72 ± 1.67	ns
BAT			
BAT mass (g)	0.176 ± 0.019	0.167 ± 0.016	ns
Mt protein content (mg/g tissue)	11.04 ± 1.36	8.72 ± 0.99	ns
COX activity			
nmol/min mg Mt protein	167.18 ± 14.08	186.58 ± 13.32	ns
nmol/min g tissue	1743.96 ± 116.77	1566.76 ± 126.69	ns
UCP ₁ content (relative unit, RU)	1.16 ± 0.09	1.00 ± 0.11	ns
Liver			
Liver mass (g)	1.973 ± 0.046	1.958 ± 0.102	ns
Mt protein content (mg/g tissue)	14.32 ± 0.56	14.05 ± 0.32	ns
COX activity			
nmolO ₂ /min mg Mt protein	84.29 ± 2.61	76.20 ± 2.47	0.05
nmolO ₂ /min g tissue	1205.61 ± 56.66	1066.24 ± 19.92	0.05
Serum			
T ₃ (ng/ml)	1.04 ± 0.05	0.88 ± 0.11	ns
T ₄ (ng/ml)	33.63 ± 3.26	12.92 ± 2.20	0.01
Leptin (ng/ml)	13.73 ± 3.19	18.74 ± 4.09	ns

Data are means ± SE.

between SD and LD gerbils (Table 1). Finally, serum leptin levels were not correlated with body fat mass ($r = 0.098$, $p > 0.05$) and GEI ($r = -0.229$, $p > 0.05$).

4. Discussion

4.1. Changes of BMR and liver COX activity responding to photoperiod

Changes of photoperiod are considered to be an important cue affecting BMR in many small mammals, including golden spiny mice (*Acomys russatus*) (Haim and Zisapel, 1995), Macedonian mice (*Mus macedonicus*) (Haim et al., 1999), Levant voles (*Microtus guentheri*) (Banin et al., 1994) and Brandt's voles (*Microtus brandti*) (Zhao and Wang, 2005). Mongolian gerbils showed seasonal changes of BMR, which was relatively higher in winter but lower in summer (Wang et al., 2003a). In the present study, SD gerbils, after 4 weeks photoperiodic acclimation, had higher BMR than LD gerbils. It is noted that at the 4 week time point, the BMR was increased by 2.7% in SD gerbils whereas decreased by 6.9% in LD gerbils compared to the baseline measurement. These data clearly indicated that gerbil's BMR was affected by changes of photoperiod. Further, liver, as an important energy-expending organ, is considered to make a large contribution to BMR (Selman et al., 2001). In our study,

liver COX activity in SD gerbils was significantly higher than that in LD gerbils, which was consistent with the changes in BMR. These data suggested that changes of BMR could be at least partially due to changes of mitochondrial respiration of the liver. Conversely, liver COX activity might potentially underlie the changes of BMR induced by photoperiodic changes.

4.2. Changes of NST, thyroid hormones, BAT COX activity and UCP1 content responding to photoperiod

Mongolian gerbils also showed increased NST in winter as adaptation to seasonal declines in environmental temperature (Wang et al., 2003a). Short photoperiod alone could induce enhancement in thermogenic capacity in several rodent species including Djungarian hamsters (Heldmaier et al., 1981), Brandt's voles (Zhao and Wang, 2005) and bushy-tailed gerbils (Haim, 1996). In the present study, however, we did not see any group differences in NST, indicating that photoperiodic cues alone could not alter NST capacity in the Mongolian gerbils, although we cannot exclude a possibility that a longer photoperiodic acclimation may induce changes in NST.

Enhancement of thermogenic capacity is generally indicated by increases in serum thyroid hormone levels (Li et al., 2001a; Tomasi and Mitchell, 1996), BAT COX activity (Heldmaier and Buchberger, 1985; Wang et al.,

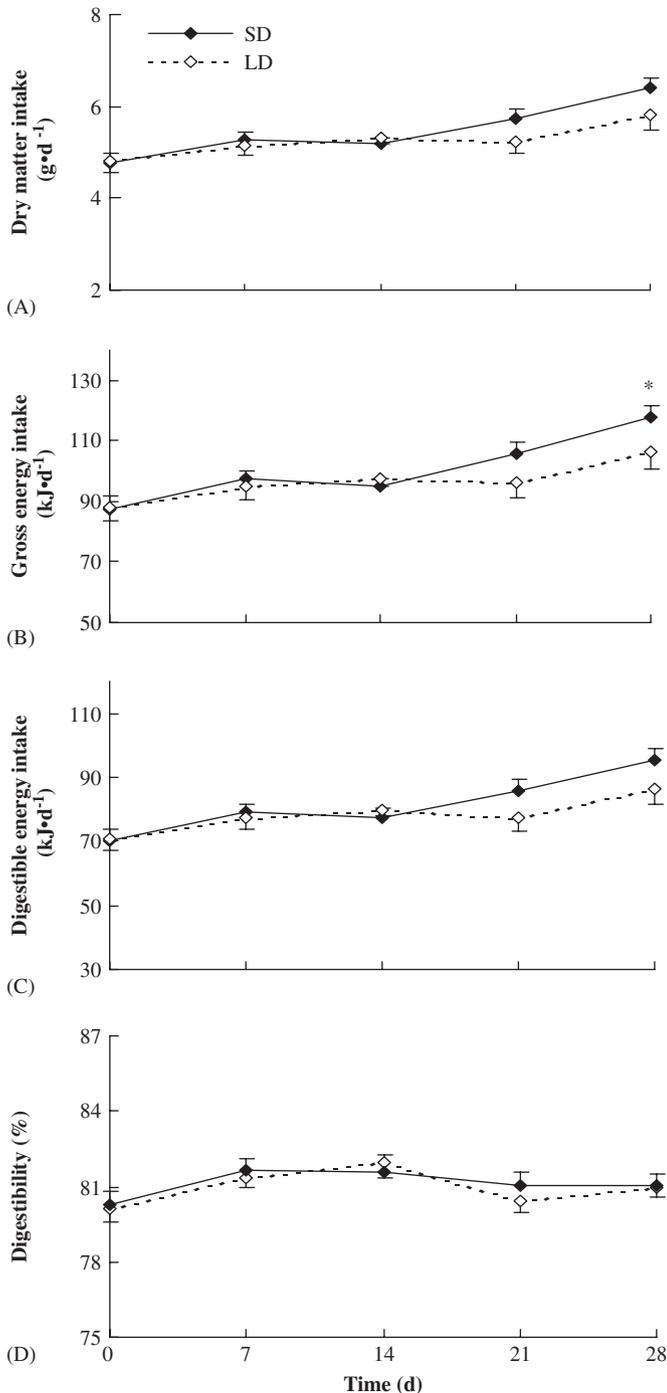


Fig. 2. Effects of photoperiod on dry matter intake (DMI; panel A), gross energy intake (GEI; panel B), digestible energy intake (DEI; panel C) and digestibility (panel D) in Mongolian gerbils (*Meriones unguiculatus*) acclimated to short photoperiod (SD; 8L:16D) or long photoperiod (LD; 16L:8D). On day 28, SD gerbils had significantly higher levels of DMI, GEI and DEI, but not digestibility, compared to LD gerbils. Data are means \pm SEM.

1999) and UCP1 expression (Jakus et al., 2002; Klingenspor, 2003; Li et al., 2001b; von Praun et al., 2001).

Thyroid hormones stimulate the increases of metabolism and thermogenesis in many rodent species (Freake and Oppenheimer, 1995; Klingenspor, 2003; Lanni et al., 2003;

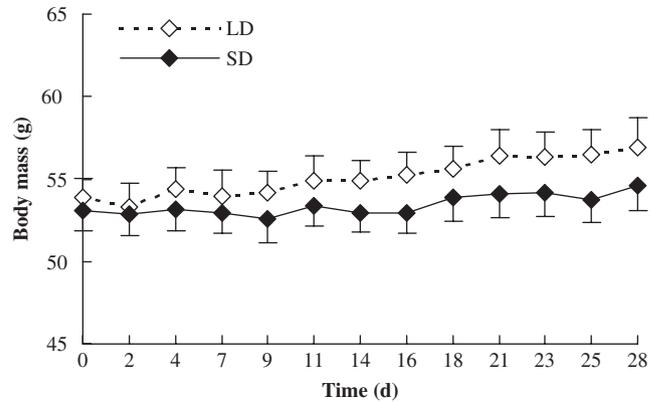


Fig. 3. Effects of photoperiod on body mass in Mongolian gerbils (*Meriones unguiculatus*). No significant differences were found between gerbils acclimated to long photoperiod (LD; 16L:8D) and short photoperiod (SD; 8L:16D) either prior to the experiment or during the course of photoperiod acclimation. Data are means \pm SEM.

Li et al., 2001b). In Mongolian gerbils, Petterborg et al. (1984) found no changes in either T_4 or T_3 in young males after 13 weeks of short photoperiodic acclimation. In our present study, serum T_4 concentration was significantly higher in SD than in LD gerbils, but group differences were not found in T_3 concentration. It seems that the T_4 's response to photoperiodic cues is age-dependent. T_4 can be converted to T_3 in peripheral tissues by deiodinases, such as Type II iodothyronine 5P-deiodinase (DII) in BAT (Lanni et al., 2003). It seemed that the DII activity was not affected by 4 weeks photoperiodic changes in the gerbils.

BAT is the main tissue involved in NST (Heldmaier and Buchberger, 1985). The increased composition and oxidative capacity of BAT were accompanied by enhancement of thermogenic capacity (Rafael and Vsiansky, 1985). Our data showed that BAT mitochondria protein content and COX activity were not affected by changes of photoperiod in the gerbils, consistent with the lack of changes in NST. Short photoperiod can induce increases in BAT *ucp1* mRNA (Demas et al., 2002) and UCP1 contents (Wang et al., 2003b) in some rodents. However, BAT *ucp1* mRNA levels were 50% lower in SD collared lemmings than in LD lemmings (Powell et al., 2002). The response of *ucp1* expression to photoperiodic changes seems to be species-specific. The lack of group differences in the UCP1 contents indicated that BAT COX activity and *ucp1* expression were not affected by photoperiodic changes in Mongolian gerbils.

4.3. Changes of energy intake, body mass and serum leptin levels responding to photoperiod

Changes of photoperiod affected seasonal adjustments of energy budgets in many small mammals (Bartness et al., 1989; Dark and Zucker, 1986; Haim, 1996; Klingenspor et al., 1996; Kriegsfeld and Nelson, 1996; Powell et al., 2002). Short photoperiod induced the increase of energy intake in Macedonian mice (Haim et al., 1999), golden spiny mice

(Haim et al., 1994), Levant voles (Banin et al., 1994) and bushy-tailed gerbils (Haim, 1996). Our data also showed energy intake were affected by changes of photoperiod, similar to the findings by Li et al. (2003), suggesting that photoperiod is an environmental cue for adaptive adjustments of energy budgets in the gerbils. For non-hibernating temperate small mammals, winter is often considered as the most energetically challenging period because low ambient temperatures require an increased energy intake (Speakman, 2000). For surviving the coming cold winter in field conditions, the increased energy intake induced by short photoperiod would contribute to maintaining a relatively high metabolic rate, which was an important strategy for maintaining the energy balance in Mongolian gerbils.

In the present study, we did not find any changes in body mass in Mongolian gerbils during the 4 weeks photoperiod acclimation, and this data is consistent with the finding in adult male gerbils by Karakas and Gunduz (2002). These data, together, suggested that body mass of adult gerbils was not affected by photoperiodic changes. However, it has been found that body mass decreased in young male gerbils under short photoperiod (Petterborg et al., 1984). It seemed that responses of body mass in Mongolian gerbils to photoperiodic changes were age-dependent. Interestingly, the gerbils did not change body mass under cold conditions independent of photoperiod (Li et al., 2004), and decreased body mass in a cold environment when exposed to short-day photoperiod (Gunduz, 2002), suggesting an interaction between short photoperiod and cold, not photoperiod alone, plays a role in body mass regulation in the gerbils.

Finally, leptin is involved in the endocrine regulation of energy balance and body mass (Johnson et al., 2004; Zhang et al., 1994). Our data showed no group differences in serum leptin levels and in body fat mass between LD and SD gerbils after 4 weeks of photoperiodic acclimation. Further, we did not see any correlation between serum leptin levels and body fat mass or gross energy intake in the gerbils. Changes of serum leptin levels in response to photoperiod seemed to be species-specific. Short photoperiod induced decreased serum leptin levels coupled with decreasing energy intake in Siberian hamsters (Klingenspor et al., 1996), and increased serum leptin levels coupled with increasing energy intake in collared lemmings (Johnson et al., 2004). It is interesting to note that cold-acclimated gerbils showed significant correlation between leptin and body fat mass or energy intake (Li et al., 2004), suggesting that leptin could be involved in the regulation of energy balance induced by the environmental factors other than photoperiod cues.

5. Summary

Seasonal changes of thermogenesis have been found in both captured Mongolian gerbils in Inner Mongolia (Wang et al., 2003a) and their breeding colony in outdoor enclosure (Li and Wang, 2005b). In our present study, we

found that several physiological, hormonal and biochemical variables indicative of thermogenic capacity, except for BMR and liver COX activity, were not affected by 4 weeks photoperiodic acclimation in the absence of cold stress, suggesting that photoperiodic changes may not provide cues altering adaptive thermogenesis. We did not find the differences in body mass and leptin levels in SD and LD gerbils, and leptin levels were not correlated with body fat mass and gross energy intake. Gross energy intake was significantly higher in SD than in LD gerbils. These data suggest that photoperiodic changes play an important role in the regulation of energy budgets in Mongolian gerbils.

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References

- Banin, D., Haim, A., Arad, Z., 1994. Metabolism and thermoregulation in the Levant vole *Microtus guentheri*: the role of photoperiodicity. *J. Therm. Biol.* 19, 55–62.
- Bartness, T.J., Wade, G.N., 1985. Photoperiodic control of seasonal body weight cycles in hamsters. *Neurosci. Biobehav. Rev.* 9, 599–612.
- Bartness, T.J., Elliott, J.A., Goldman, B.D., 1989. Control of torpor and body weight patterns by a seasonal timer in Siberian hamsters. *Am. J. Physiol.* 257R, 142–149.
- Chen, Z., 1988. Topography and climate of Xilin River basin. In: Academia, Sinica (Ed.), *Research on Grassland Ecosystem*. Inner Mongolia Grassland Ecosystem Research Station. Science Press, Beijing, pp. 13–22.
- Dark, J., Zucker, I., 1986. Photoperiodic regulation of body mass and fat reserves in the meadow vole. *Physiol. Behav.* 38, 851–854.
- Demas, G.E., Bowers, R.R., Bartness, T.J., Gettys, T.W., 2002. Photoperiodic regulation of gene expression in brown and white adipose tissue of Siberian hamsters (*Phodopus sungorus*). *Am. J. Physiol.* 282R, 114–121.
- Freake, H.C., Oppenheimer, J.H., 1995. Thermogenesis and thyroid function. *Annu. Rev. Nutr.* 15, 263–291.
- Genin, F., Perret, M., 2000. Photoperiod-induced changes in energy balance in gray mouse lemurs. *Physiol. Behav.* 71, 315–321.
- Gorecki, A., 1975. Kalabukhov-Skvortsov respirometer and resting metabolic rate measurement. In: Grodzinski, W. (Ed.), *Methods for Ecological Energetics*. Blackwell Scientific, Oxford, pp. 309–313.
- Grodzinski, W., Wunder, B.A., 1975. Ecological energetics of small mammals. In: Golley, F.B., Petruszewicz, K., Ryszkowski, L. (Eds.), *Small mammals: their productivity and population dynamics*. Cambridge University Press, Cambridge, pp. 173–204.
- Gunduz, B., 2002. Effects of photoperiod and temperature on growth and reproductive organ mass in adult male Mongolian gerbils, *Meriones unguiculatus*. *Turk. J. Biol.* 26, 77–82.
- Haim, A., 1996. Food and energy intake, non-shivering thermogenesis and daily rhythm of body temperature in the bushy-tailed gerbil *sekeetamys*

- calurus*: the role of photoperiod manipulations. *J. Therm. Biol.* 21, 37–42.
- Haim, A., Izhaki, I., 1993. The ecological significance of resting metabolic rate and non-shivering thermogenesis for rodents. *J. Therm. Biol.* 18, 71–81.
- Haim, A., Zisapel, N., 1995. Oxygen consumption and body temperature rhythms in the golden spiny mouse: responses to changes in day length. *Physiol. Behav.* 58, 775–778.
- Haim, A., Yedidia, I., Haim, D., Zisapel, N., 1994. Photoperiodicity in daily rhythms of body temperature, food and energy intake of the golden spiny mouse (*Acomys russatus*). *Isr. J. Zool.* 40, 145–150.
- Haim, A., Shabtay, A., Arad, Z., 1999. The thermoregulatory and metabolic responses to photoperiod manipulations of the Macedonian mouse (*Mus macedonicus*), a post-fire invader. *J. Therm. Biol.* 24, 279–286.
- Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., Friedman, J.M., 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269, 543–546.
- Heldmaier, G., 1971. Nonshivering thermogenesis and body size in mammals. *J. Comp. Physiol.* 73, 222–248.
- Heldmaier, G., Buchberger, A., 1985. Sources of heat during nonshivering thermogenesis in Djungarian hamsters: a dominant role of brown adipose tissue during cold adaptation. *J. Comp. Physiol. B* 156, 237–245.
- Heldmaier, G., Steinlechner, S., Rafael, J., Vsiansky, P., 1981. Photoperiodic control and effects of melatonin on nonshivering thermogenesis and brown adipose tissue. *Science* 212, 917–919.
- Heldmaier, G., Steinlechner, S., Rafael, J., Latteier, B., 1982. Photoperiod and ambient temperature as environmental cues for seasonal thermogenic adaptation in the Djungarian hamster, *Phodopus sungorus*. *Int. J. Biometeorol.* 26, 339–345.
- Himms-Hagen, J., Ricquier, D., 1998. Brown adipose tissue. In: Bray, G.A., Bouchard, C., James, W.P.T. (Eds.), *Handbook of Obesity*. Marcel Dekker, Inc., New York, NY, pp. 415–441.
- Jakus, P.B., Sipos, K., Kispal, G., Sandor, A., 2002. Opposite regulation of uncoupling protein 1 and uncoupling protein 3 in vivo in brown adipose tissue of cold-exposed rats. *FEBS Lett.* 519, 210–214.
- Johnson, M.S., Onorato, D.P., Gower, B.A., Nagy, T.R., 2004. Weight change affects serum leptin and corticosterone in the collared lemming. *Gen. Comp. Endocrinol.* 136, 30–36.
- Kadenbach, B., Huttemann, M., Arnold, S., Lee, I., Bender, E., 2000. Mitochondrial energy metabolism is regulated via nuclear-coded subunits of cytochrome *c* oxidase. *Free Radic. Biol. Med.* 29, 211–221.
- Karakas, A., Gunduz, B., 2002. Effect of different photoperiods on gonadal maintenance and development in Mongolian gerbils (*Meriones unguiculatus*). *Zool. Sci.* 19, 233–239.
- Klingenspor, M., 2003. Cold-induced recruitment of brown adipose tissue thermogenesis. *Exp. Physiol.* 88, 141–148.
- Klingenspor, M., Dickopp, A., Heldmaier, G., Klaus, S., 1996. Short photoperiod reduces leptin gene expression in white and brown adipose tissue of Djungarian hamsters. *FEBS Lett.* 399, 290–294.
- Klingenspor, M., Niggemann, H., Heldmaier, G., 2000. Modulation of leptin sensitivity by short photoperiod acclimation in the Djungarian hamster, *Phodopus sungorus*. *J. Comp. Physiol. B* 170, 37–43.
- Kriegsfeld, L.J., Nelson, R.J., 1996. Gonadal and photoperiodic influences on body mass regulation in adult male and female prairie voles. *Am. J. Physiol.* 270, 1013–1018.
- Krotkiewski, M., 2002. Thyroid hormones in the pathogenesis and treatment of obesity. *Eur. J. Pharmacol.* 440, 85–98.
- Lanni, A., Moreno, M., Lombardi, A., Goglia, F., 2003. Thyroid hormone and uncoupling proteins. *FEBS Lett.* 543, 5–10.
- Li, X.S., Wang, D.H., 2005a. Regulation of body weight and thermogenesis in seasonally acclimatized Brandt's voles (*Microtus brandti*). *Horm. Behav.* 48, 321–328.
- Li, X.S., Wang, D.H., 2005b. Seasonal adjustments in body mass and thermogenesis in Mongolian gerbils (*Meriones unguiculatus*): the roles of short photoperiod and cold. *J. Comp. Physiol. B* 175, 593–600.
- Li, Q.F., Sun, R.Y., Huang, C.X., Wang, Z.K., Liu, X.T., Hou, J.J., Liu, J.S., Cai, L.Q., Li, N., Zhang, S.Z., Wang, Y., 2001a. Cold adaptive thermogenesis in small mammals from different geographical zones of China. *Comp. Biochem. Physiol. A* 129, 949–961.
- Li, Q.F., Liu, X.T., Huang, C.X., Sun, R.Y., Lin, Q.S., 2001b. Thermogenic capacity and expression of uncoupling protein gene of brown adipose tissue from mongolian gerbils *Meriones unguiculatus* during cold acclimation. *Acta Zool. Sin.* 47, 388–393.
- Li, X.S., Wang, D.H., Yang, J.C., 2003. Effect of photoperiod on body weight and energy metabolism in Brandt's voles (*Microtus brandti*) and Mongolian gerbils (*Meriones unguiculatus*). *Acta Theriol. Sin.* 23, 304–311.
- Li, X.S., Wang, D.H., Yang, M., 2004. Effects of cold acclimation on body weight, serum leptin level, energy metabolism and thermogenesis in the Mongolian gerbil *Meriones unguiculatus*. *Acta Zool. Sin.* 50, 334–340 (In Chinese with English summary).
- Liu, H., Wang, D.H., Wang, Z.W., 2002. Maximum metabolizable energy intake in the Mongolian gerbil (*Meriones unguiculatus*). *J. Arid Environ.* 52, 405–411.
- Liu, H., Wang, D.H., Wang, Z.W., 2003. Energy requirements during reproduction in female Brandt's voles (*Microtus brandti*). *J. Mammal.* 84, 1410–1416.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Lynch, G.R., 1970. Effect of photoperiod and cold acclimation on non-shivering thermogenesis in *Peromyscus leucopus*. *Am. Zool.* 10, 308.
- Peacock, W.L., Krol, E., Moar, K.M., McLaren, J.S., Mercer, J.G., Speakman, J.R., 2004. Photoperiodic effects on body mass, energy balance and hypothalamic gene expression in the bank vole. *J. Exp. Biol.* 207, 165–177.
- Pelleymounter, M.A., Cullen, M.J., Baker, M.B., Hecht, R., Winters, D., Boone, T., Collins, F., 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 269, 540–543.
- Petterborg, L.J., Vaughan, M.K., Johnson, L.Y., Champney, T.H., Reiter, R.J., 1984. Modification of testicular and thyroid function by chronic exposure to short photoperiod: a comparison in four rodent species. *Comp. Biochem. Physiol.* 78A, 31–34.
- Powell, C.S., Blaylock, M.L., Wang, R., Hunter, H.L., Johanning, G.L., Nagy, T.R., 2002. Effects of energy expenditure and UCP₁ on photoperiod-induced weight gain in collared lemmings. *Obes. Res.* 10, 541–550.
- Rafael, J., Vsiansky, P., 1985. Photoperiodic control of the thermogenic capacity in brown adipose tissue of the Djungarian hamster. *J. Therm. Biol.* 10, 167–170.
- Rial, E., González-Barroso, M.M., 2001. Physiological regulation of the transport activity in the uncoupling proteins UCP₁ and UCP₂. *Biochim. Biophys. Acta* 1504, 70–81.
- Ricquier, D., Bouillaud, F., 2000. Mitochondrial uncoupling proteins: from mitochondria to the regulation of energy balance. *J. Physiol.* 529, 3–10.
- Selman, C., Lumsden, S., Bunker, L., Hill, W.G., Speakman, J.R., 2001. Resting metabolic rate and morphology in mice (*Mus musculus*) selected for high and low food intake. *J. Exp. Biol.* 204, 777–784.
- Speakman, J.R., 2000. The cost of living: field metabolic rates of small mammals. *Adv. Ecol. Res.* 30, 177–297.
- Sundin, U., Moore, G., Nedergaard, J., Cannon, B., 1987. Thermogenin amount and activity in hamster brown fat mitochondria: effect of cold acclimation. *Am. J. Physiol.* 252R, 822–832.
- Tomasi, T.E., Mitchell, D., 1996. Temperature and photoperiod effects on thyroid function and metabolism in cotton rats (*Sigmodon hispidus*). *Comp. Biochem. Physiol.* 113A, 267–274.
- von Praun, C., Burkert, M., Gessner, M., Klingenspor, M., 2001. Tissue-specific expression and cold-induced mRNA levels of uncoupling proteins in the Djungarian hamster. *Physiol. Biochem. Zool.* 74, 203–211.
- Wang, D.H., Sun, R.Y., Wang, Z.W., Liu, J.S., 1999. Effects of temperature and photoperiod on thermogenesis in plateau pikas

- (*Ochotona curzoniae*) and root voles (*Microtus oeconomus*). J. Comp. Physiol. B 169, 77–83.
- Wang, D.H., Wang, Y.S., Wang, Z.W., 2000. Metabolism and thermoregulation in the Mongolian gerbil (*Meriones unguiculatus*). Acta Theriol. 45, 183–192.
- Wang, D.H., Wang, Z.W., Wang, Y.S., Yang, J.C., 2003a. Seasonal changes of thermogenesis in Mongolian gerbils (*Meriones unguiculatus*) and Brandt's voles (*Microtus brandti*). Comp. Biochem. Physiol. 134A (Suppl. 1), S96 (abstract).
- Wang, D.H., Klingenspor, M., Heldmaier, G., 2003b. Short photoperiod acclimation augments uncoupling protein 1 expression and mitochondrial respiration in brown adipose tissue of Djungarian hamsters (*Phodopus sungorus*). Comp. Biochem. Physiol. 134A (Suppl. 1), S21 (abstract).
- Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., Troy, A., Cinti, S., Lowell, B., Scarpulla, R.C., Spiegelman, B.M., 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98, 115–124.
- Wunder, B.A., 1984. Strategies for, and environmental cueing mechanisms of, seasonal changes in thermoregulatory parameters of small mammals. In: Merritt, J.F. (Ed.), Winter Ecology of Small Mammals. Carnegie Museum of Natural History, Pittsburgh, pp. 165–172 (special publication).
- Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., Friedman, J.M., 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425–432.
- Zhao, Z.J., Wang, D.H., 2005. Short photoperiod enhances thermogenic capacity in Brandt's voles. Physiol. Behav. 85, 143–149.