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Seasonal changes in body mass, energy intake and thermogenesis in Maximowiczi's voles (*Microtus maximowiczii*) from the Inner Mongolian grassland

Jing-Feng Chen · Wen-Qin Zhong · De-Hua Wang

Abstract Small mammals inhabiting temperate and arctic regions exhibit annual adaptive adjustments in physiology, anatomy, and behavior. No data on the physiology of Maximowicz's voles (*Microtus maximowiczii*) are available at present. Here we examined the seasonal changes in body mass, food intake, thermogenic capacity, serum leptin and thyroid hormone levels in wild-captured individuals from Inner Mongolian grassland, China. We further examined the effects of photoperiod on these parameters. Energy intake, resting metabolic rate, nonshivering thermogenesis (NST), and serum tri-iodothyronine (T3) levels increased while serum leptin and body mass decreased in the cold seasons. Serum T3 levels were positively correlated with NST and uncoupling protein 1 (UCP1) contents in brown adipose tissue, and leptin levels were negatively correlated with energy intake and resting metabolic rate. Furthermore, laboratory data showed these changes could be induced by short photoperiod alone. Taken together, our results indicate that Maximowicz's voles can increase thermogenic capacity and energy intake to cope with cold stress. Serum leptin seems to be involved in the regulation of energy intake and changes in T3 level may be important for the variations in NST and/or UCP1. Short photoperiod can serve as a seasonal cue for the winter acclimatization of energy balance in free-living Maximowicz's voles.

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Introduction

Survival of nonhibernating small mammals residing in temperate and arctic regions is strongly influenced by low temperature coupled with reduced food availability during winter (Merritt and Zegers 1991). With fluctuations in climate conditions, some winter-active rodent species showed reduction in body mass and body fat mass and increase in nonshivering thermogenesis (NST) (Mezhzherin 1964; Heldmaier 1989; Lovegrove 2005), such as Siberian hamsters (*Phodopus sungorus*) (Klingenspor et al. 2000), Brandt's voles (*Lasiopodomys brandtii*) (Li and Wang 2005a), and root voles (*Microtus oeconomus*) (Wang et al. 2006a). Brown adipose tissue (BAT) is the major site of NST (Jansky 1973; Foster and Frydman 1979; Lowell and Spiegelman 2000), which is innervated by sympathetic nervous system and modulated by hypothalamic-pituitary-thyroid axis (Silva 2006; Cannon and Nedergaard 2004). The capacity for NST in BAT entirely depends on uncoupling protein 1 (UCP1), a 32-kD carrier protein, which renders the inner membrane of the mitochondria "leaky" and hence releases energy in the form of heat rather than storing it as ATP (Nicholls and Locke 1984; Krauss et al. 2005).

Regulation of body mass and adiposity has been suggested to involve a lipostatic control system in which hormonal signals circulate in proportion to body fat reserves (Kennedy 1953; Schwartz et al. 2000). Leptin, a 167-amino acid product of the *ob* gene mainly in the adipocytes (Zhang et al. 1994), provides an attractive

candidate regulator of body mass by acting on the neuropeptide circuits in the hypothalamus (Friedman and Halaas 1998). Leptin was originally thought to act to prevent obesity but was later found to serve mainly as a mediator of adaptation to fasting in energy homeostasis (Ahima and Flier 2000). Most of these studies were carried out on laboratory rodents such as rats and mice. Therefore, more data for the role of leptin in regulating energy balance for wild seasonal mammals are needed. Available data showed that several seasonal mammals undergo annual cycles of food intake and adiposity coupled with seasonal changes in circulating leptin levels such as Siberian hamsters (Drazen et al. 2000), woodchucks (*Marmota monax*) (Concannon et al. 2001), collared lemmings (*Dicrostonyx torquatus*) (Johnson et al. 2004), Brandt's voles (Li and Wang 2005a), root voles (Wang et al. 2006a), plateau pikas (*Ochtona curzoniae*) (Wang et al. 2006b), and field voles (*Microtus agrestis*) (Król et al. 2006a). A positively functional relationship between leptin levels and thermogenic activity of BAT has been demonstrated in the post-cafeteria model of obesity and via administration of exogenous leptin (Scarpace et al. 1997; Commins et al. 2001).

Photoperiod is a more reliable environmental cue than temperature since its seasonality has astronomical precision. The effects of photoperiod on acclimation of thermoregulation, body mass and energy budgets have been demonstrated in many rodent species (Heldmaier et al. 1981; Bartness and Wade 1985; Rousseau et al. 2003). Generally, short photoperiod can markedly reduce body mass and enhance thermogenic capacity in some small mammals (Dark et al. 1983; Voltura and Wunder 1998); however, it can also significantly increase body mass in some small species such as collared lemmings (Powell et al. 2002) and golden hamsters (*Mesocricetus auratus*) (Jansky et al. 1986). Such species that show specific variations of photoperiod-mediated changes in body mass also involve interactions between peripheral and central hormonal signaling systems, as well as regulations of NST (Mercer and Tups 2003).

Maximowiczi's voles (*Microtus maximowiczii* Schrenk, 1859) mainly inhabit marsh, meadow and bank-forest areas of Northeastern China and the adjacent Russia and Mongolia. They are herbivorous and store food during the late autumn for overwintering. Because of their low population densities and limited distribution, a little is known about their ecophysiology. In the present study, we examined several behavioral, physiological, hormonal, and biochemical processes in captive seasonally acclimatized voles and photoperiod acclimated voles to examine the role of leptin in the regulation of energy balance and thermogenesis for seasonal and photoperiodic animals. We predicted that Maximowiczi's voles, like other sympatric species such as Brandt's voles (Li and Wang 2005a), will enhance their winter survival by making seasonal adjustments in body mass and thermogenesis associated with seasonal variations in serum leptin and thyroid hormones. We also predicted that similar changes can be induced under short photoperiod acclimation in Maximowiczi's voles.

Materials and methods

Animals and experimental protocol

Maximowiczi's voles used in experiment 1 were live trapped from the Inner Mongolian grassland Ecosystem Research Station of the Chinese Academy of Sciences (43°3′N, 116°41′E) in April 2005. The voles were housed under a natural photoperiod and ambient temperature. Only adult voles were used excluding pregnant or lactating females. The voles used in experiment 2 were first-generation born in captivity and raised under a 16L:8D photoperiod (lights on 04:30 a.m.) at an ambient temperature around 21°C (range 20–22°C). Each group included in this study was provided standard rabbit chow (Beijing Ke Ao Feed Co.) and water ad libitum. Voles from the field were supplemented with fresh China Aneurolepidium (*Aneurolepidium chinense*), Schmidt Sedge (*Carex schmidtti*) and slices of carrot during the 4-week habituation period after capture. Animals were maintained individually in plastic cages (30 × 15 × 20 cm). Sawdust was used as bedding material. All studies were conducted with the approval of the Animal Care and Use Committee of Institute of Zoology, the Chinese Academy of Sciences.

Experiment 1: seasonal acclimatization

To investigate the seasonal changes in body mass, energy intake, and thermogenesis, the voles were randomly assigned into one of the six experimental groups (each group included 3 females and 3–5 males). Experiments were performed in May, June, July, September, October and December under natural photoperiod and ambient temperature (Li and Wang 2005a). Energy intake, RMR, and NST were measured monthly. At the end of the experiment, animals were killed between 09:00 and 11:00 a.m. by puncture of the posterior vena cava. Blood was centrifuged at 4,000 rpm for 30 min, and serum was sampled and stored at −20°C for later measurement. The interscapular BAT was surgically removed and immediately frozen in liquid nitrogen and stored at −80°C for determining BAT cytochrome c oxidase (COX) activity and UCP1 content. An additional group of voles was also individually housed in natural environmental conditions in

May 2005, and their body mass were monitored weekly at 09:00 a.m. during the course of the experiment.

Experiment 2: short photoperiod acclimation

To investigate the effects of photoperiod on seasonal changes in the physiological parameters measured in experiment 1, the voles were randomly assigned into one of the two experimental groups that were acclimated either to short photoperiod (SD, 8L:16D with light on at 8:00 a.m., 3 males and 3 females) or to long photoperiod (LD, 16L:8D with lights on at 04:30 a.m., 3 males and 4 females). Body mass, metabolic parameters (RMR and NST), and energy intake were measured on the day before the start of photoperiod acclimation (Day 0) and the end of the test. After 4 weeks acclimation, animals were killed and blood samples and BAT were also collected between 09:00 and 11:00 a.m.

Metabolic trials

RMR and NST were measured in a closed-circuit respirometer (Grodzinski and Wunder 1975; Wang et al. 2000). The metabolic chamber size was 3.6 L. Chamber temperature was controlled within $\pm 0.5°C$ by a water bath. Carbon dioxide and water in the metabolic chambers were absorbed with KOH and silica gel, respectively. Animals were weighed before and after each test. To minimize the effect of circadian rhythms, all measurements were made between 9:00 a.m. and 5:00 p.m.

RMR was measured at $29 \pm 0.5°C$, which is within their thermoneutral zone (25–32.5°C) (Chen et al. 2006). After 60 min of habituation in the chamber, metabolic measurements were conducted for further 60 min at 5 min intervals. Two continuous stable minimum recordings were used to calculate RMR.

Maximum NST was defined as the total metabolic response to norepinephrine (NE) (Heldmaier et al. 1982) and was induced by a subcutaneous injection of NE at $25 \pm 0.5°C$, which is near the lower critical temperature (Chen et al. 2006). The mass-dependent dosage of NE (Shanghai Harvest Pharmaceutical Co. Ltd) was calculated according to the equation described by Heldmaier (1971). Oxygen consumption was recorded for further 60 min at 5 min intervals. The two consecutive stable maximal recordings of oxygen consumption were used to calculate the maximum NST (Wang and Wang 1996; Li et al. 2001). RMR and NST values were corrected to the standard temperature and air pressure (STP) conditions.

Energy budget

Food intake was measured in metabolic cages for 3 days as described previously (Liu et al. 2002). During each test,

food was provided quantitatively and water was provided ad libitum. Food residues and feces were collected from each animal after a 3-day test and separated after they were dried at 60°C to constant mass (Liu et al. 2002). The caloric values of food and feces were determined by an oxygen bomb calorimeter (Parr 1281, Parr Instrument, USA). Gross energy intake (GEI), digestible energy intake (DEI), and apparent digestibility of energy (hereafter referred to as digestibility) were calculated by the following equations (Grodzinski and Wunder 1975; Liu et al. 2002):

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

Measurement of cytochrome c oxidase activity, UCP1 and serum leptin

Mitochondrial protein was prepared as described in Wiesinger et al. (1989). Total mitochondrial protein content was determined by the Folin phenol method (Lowry et al. 1951) with bovine serum albumin as the standards. The COX activity was measured with the polarographical method using oxygen electrode units (Hansatech Instruments Ltd, England) (Zhao and Wang 2005).

BAT mitochondrial protein (5 μl:4 μg/μl) was diluted in 5 μl sample buffer and run on a SDS–polyacrylamide gel (3% stacking gel and 12.5% running gel) together with a prestained protein marker for about 2 h. Then the protein was 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 (1:5,000, UCP1 antibody was supplied by Dr. Klingenspor, Department of Biology, Philipps-University Marburg, Germany) for 2 h and then incubated with peroxidase-conjugated goat anti-rabbit IgG (1:5,000) (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. UCP1 content was expressed as relative units (RU) and quantified with Scion Image Software (Scion Corporation) and was expressed as relative units (RU) (Li and Wang 2005a, b).

Serum leptin levels were measured by radioimmunoassay (RIA) using the Linco[125] Muti-species Kit (St. Louis, MO, USA) and leptin values were determined in a single RIA. The lowest levels of leptin that can be detected by this
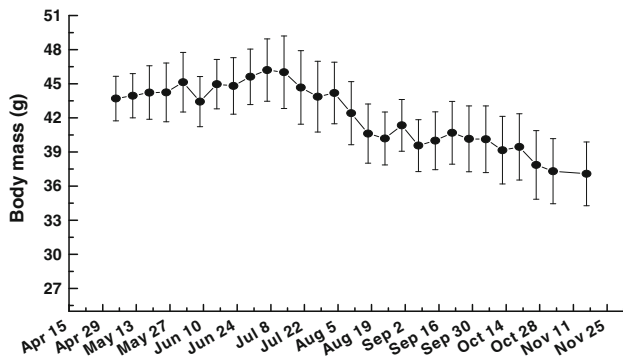
Fig. 1 Seasonal changes of body mass in Maximowiczi's voles (*Microtus maximowiczii*). Body mass of Maximowiczi's voles keep relative stable from 5 May to 28 July. From 4 August the voles' body mass declined slowly and reached the nadir in 17 November. Values are expressed as mean ± SEM (*n* = 10)

assay were 1.0 ng/ml when using a 100-μl sample size. Inter- and intra-assay variability for leptin RIA was <3.6 and 8.7%, respectively.

Serum tri-iodothyronine (T3) and thyroxine (T4) were quantified by radioimmunoassy using RIA kits (China Institute of Atomic Energy, Beijing, China). Intra- and inter-assay coefficients of variation were 2.4 and 8.8% for the T3, and 4.3 and 7.6% for T4, respectively.

Carcass composition analysis

The entire gastrointestinal tract was removed, and the eviscerated carcass (not including BAT) was dried to constant mass at 60°C for the determination of dry body mass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus (Li and Wang 2005a).

Statistical analysis

Data were analyzed using the SPSS software package (13.0). Distributions of all variables were tested for normality by the Kolmogorov–Smirnov test and data that were abnormally distributed were transformed to natural logarithms. The sample size in each gender was small; therefore, all data for both sexes were pooled together for the analyses. Seasonal data such as COX activity, mitochondrial protein content, thyroid hormones and UCP1 were analyzed by one-way analysis of variance (ANOVA) and significant group differences were further evaluated by LSD post hoc test. RMR, NST, body composition, serum leptin levels, and energy intake in both experiments were tested by analysis of covariance (ANCOVA) with body mass as the covariate. Seasonal changes of body mass were assessed by repeated measures analysis of variance (RM ANOVA). For experiment 2, group differences of thermogenic parameters and body composition were examined

by independent-samples *t* test, and differences over the course of the acclimation were analyzed by paired-samples *t* test. Pearson correlation analysis were used to detect the possible correlation of serum leptin levels with body mass, body fat mass, and UCP1 content, and also between UCP1 and NST, and serum T3 levels. Results are presented as mean ± SE (*n* sample size) in the test, and $P < 0.05$ was considered to be statistically significant.

Results

Experiment 1

Seasonal changes in body mass, RMR, and NST

Body mass changed with seasons ($F_{(28,252)} = 10.388$, $P < 0.01$, Fig. 1). Body mass kept relatively stable from 5 May (41.3 ± 2.0 g) to 28 July (39.6 ± 2.7 g) (LSD, $P > 0.05$), and then decreased gradually since 4 August, and reached the minimum on 17 November (31.1 ± 1.5 g). Compared with the initial body mass, the final body mass was reduced by 24.7% (Fig. 1).

Both RMR and NST showed marked seasonal changes (RMR, $F_{(5,34)} = 5.690$, $P < 0.01$; NST, $F_{(5,34)} = 2.877$, $P < 0.05$, Table 1). The lowest RMR was recorded in June as compared with that of other seasons. Similarly, the NST in June (176.1 ± 13.8 ml O_2/h) was lower than in other seasons (e.g., October: 219.8 ± 13.6 ml O_2/h, November: 219.1 ± 13.4 ml O_2/h).

Seasonal changes in energy intake and digestibility

Maximowiczi's voles showed seasonal variations in their DMI and GEI (DMI: $F_{(5,34)} = 10.507$, $P < 0.01$, Fig. 2a; GEI: $F_{(5,34)} = 10.602$, $P < 0.01$, Fig. 2b). GEI in November was 72% higher than that in July (LSD, $P < 0.01$). Same patterns were found in DEI ($F_{(5,34)} = 6.529$, $P < 0.01$, Fig. 2c). The digestibility also fluctuated markedly ($F_{(5,35)} = 6.011$, $P < 0.01$, Fig. 2d). From June to September the digestibility gradually decreased, then increased again from September to November. The digestibility in May was 12.6% higher than that in September.

Seasonal changes in mitochondrial protein content, COX activity and UCP1 content in BAT

There was significant difference in the absolute mass of BAT ($F_{(5,35)} = 2.543$, $P > 0.05$), but there was no significant difference in relative BAT mass among groups ($F_{(5,35)} = 4.308$, $P > 0.05$, Table 1). The mitochondrial protein content, BAT COX activity and UCP1 varied significantly among seasons (MP: $F_{(5,35)} = 5.746$, $P < 0.01$; COX: $F_{(5,35)} = 12.328$,

Table 1 Thermogenic parameters and body composition in seasonal acclimatized Maximowiczi's voles (*Microtus maximowiczii*)

Parameters	15 May	16 June	15 July	17 September	15 October	16 November	P
Sample size	6	6	7	8	7	7	
Body mass (g)	44.4 ± 3.3[a]	40.6 ± 2.9[ab]	45.7 ± 5.1[a]	35.9 ± 3.2[bc]	31.0 ± 1.5[c]	28.9 ± 1.5[c]	<0.01
RMR (ml O_2/h)	92.41 ± 5.36[bc]	79.69 ± 5.24[c]	86.86 ± 5.20[bc]	101.05 ± 4.49[b]	117.03 ± 5.19[a]	110.80 ± 5.21[ab]	<0.01
NST (ml O_2/h)	234.87 ± 14.29[a]	176.08 ± 13.77[c]	183.42 ± 13.80[bc]	218.30 ± 11.85[ab]	219.83 ± 13.57[ab]	219.05 ± 13.43[ab]	<0.05
BAT mass (g)	0.150 ± 0.016	0.111 ± 0.015	0.142 ± 0.015	0.104 ± 0.013	0.110 ± 0.015	0.137 ± 0.015	>0.05
BAT MP (mg/g BAT)	10.100 ± 0.950[b]	9.760 ± 1.860[b]	10.620 ± 1.330[b]	10.760 ± 0.800[b]	15.760 ± 1.230[a]	15.830 ± 0.880[a]	<0.01
COX activity (nmol O2/min/g BAT)	6,711.47 ± 214.2[b]	5,806.49 ± 244.33[c]	5,443.13 ± 166.52[bc]	6,378.4 ± 354.58[bc]	7,831.53 ± 172.24[a]	8,208.16 ± 315.03[a]	<0.01
UCP1 content (RU)	1.00 ± 0.13[ab]	0.72 ± 0.09[c]	0.67 ± 0.09[c]	0.84 ± 0.06[bc]	0.97 ± 0.04[ab]	1.19 ± 0.09[a]	<0.01
Serum leptin (ng/ml)	8.10 ± 1.46[ab]	9.30 ± 2.90[a]	7.22 ± 1.31[ab]	4.57 ± 0.67[bd]	2.73 ± 0.22[cd]	3.96 ± 1.32[cd]	<0.05
Serum T3 (ng/ml)	1.14 ± 0.10[bcde]	0.74 ± 0.13[e]	0.87 ± 0.22[de]	1.38 ± 0.17[acd]	1.55 ± 0.22[ac]	1.88 ± 0.20[a]	<0.01
Serum T4 (ng/ml)	34.710 ± 6.00[ab]	32.11 ± 3.79[ab]	25.47 ± 1.78[b]	37.31 ± 4.04[a]	23.21 ± 3.08[b]	22.37 ± 3.21[b]	<0.05
Wet carcass mass (g)	44.4 ± 3.3[a]	40.6 ± 2.9[a]	32.5 ± 2.9[ab]	25.5 ± 2.4[bc]	22.0 ± 1.1[abc]	19.5 ± 1.1[c]	<0.05
Dry carcass mass (g)	14.6 ± 1.0	12.9 ± 1.3	14.3 ± 1.5	10.3 ± 0.9	8.7 ± 0.4	8.6 ± 0.9	>0.05
Water of carcass (g)	17.0 ± 1.5	17.2 ± 2.8	18.2 ± 1.5	15.2 ± 1.6	13.2 ± 0.8	10.9 ± 0.5	>0.05
Body fat mass (g)	6.4 ± 0.7	5.5 ± 0.9	5.7 ± 0.9	3.7 ± 0.4	2.6 ± 0.1	2.8 ± 0.4	>0.05
Body fat content (%)	43.8 ± 2.7[a]	41.6 ± 3.6[ab]	38.5 ± 2.4[abc]	35.8 ± 2.0[bcd]	30.0 ± 1.2[cd]	32.8 ± 3.4[a]	<0.05

Different superscript letters represents statistically significant differences. Values are expressed as mean ± SEM

$P < 0.01$; UCP1: $F_{(5,35)} = 4.692$, $P < 0.01$). The mitochondrial protein content and COX activity in October and November were markedly higher than the other months. The COX activity in October and November were 35 and 41% higher than that in June, respectively. UCP1 in November was 40% higher than in June.

Seasonal changes in body composition and serum hormones

The wet carcass mass showed significant seasonal changes ($F_{(5,34)} = 2.919$, $P < 0.05$, Table 1). The highest values occurred in June and no significant differences were found among other groups. There were no marked variations among seasons in dry carcass mass ($F_{(5,34)} = 1.248$, $P > 0.05$), body fat mass ($F_{(5,34)} = 1.916$, $P > 0.05$), and water of carcass ($F_{(5,34)} = 1.409$, $P > 0.05$) but body fat content ($F_{(5,35)} = 3.170$, $P < 0.05$).

Seasonal alterations were also detected in serum leptin, T3, and T4 levels (leptin: $F_{(5,34)} = 3.122$, $P < 0.05$; T3: $F_{(5,35)} = 4.455$, $P < 0.01$; T4: $F_{(5,35)} = 2.580$, $P < 0.05$, Table 1). The serum leptin (9.3±2.9 ng/ml) in June was significantly higher than that in November (3.96 ± 1.32 ng/ml). However, T3 level in June (0.74 ± 0.13 ng/ml) was markedly lower than in November (1.88 ± 0.20 ng/ml). Serum T4 and leptin levels gradually decreased from warmer to colder months, and T4 showed a rise in September.

Correlation analysis showed that serum leptin levels were correlated positively with overall body mass ($r = 0.347$, $P < 0.05$; Fig. 3a) and body fat mass ($r = 0.54$, $P < 0.001$; Fig. 3b) but negatively with RMR ($r = -0.444$, $P < 0.01$; Fig. 3c) and energy intake ($r = -0.384$, $P < 0.05$; Fig. 3d), while no significant correlation of serum leptin levels with UCP1 content was found ($r = -0.224$, $P > 0.05$). Serum T3 level were positively correlated with NST ($r = 0.334$, $P < 0.05$; Fig. 3e) and UCP1 content ($r = 0.651$, $P < 0.05$; Fig. 3f). NST was positively correlated with UCP1 content ($r = 0.402$, $P < 0.05$).

Experiment 2

Effect of photoperiod on body mass, RMR, NST and energy intake

Prior to acclimation, no group differences (LD: 28.9 ± 2.4 g and SD: 29.7 ± 2.7 g, $df = 11$, $t = -0.244$, $P > 0.05$) were detected in body mass (Fig. 4). During the course of acclimation, no differences were observed between SD and LD groups ($df = 11$, $t = 1.593$, $P > 0.05$). SD voles showed relatively constant body mass throughout the acclimation period ($F_{(3,15)} = 1.606$, $P > 0.05$), whereas the LD voles showed 14% increase over the 4-week acclimation ($F_{(3,18)} = 7.617$, $P < 0.01$).
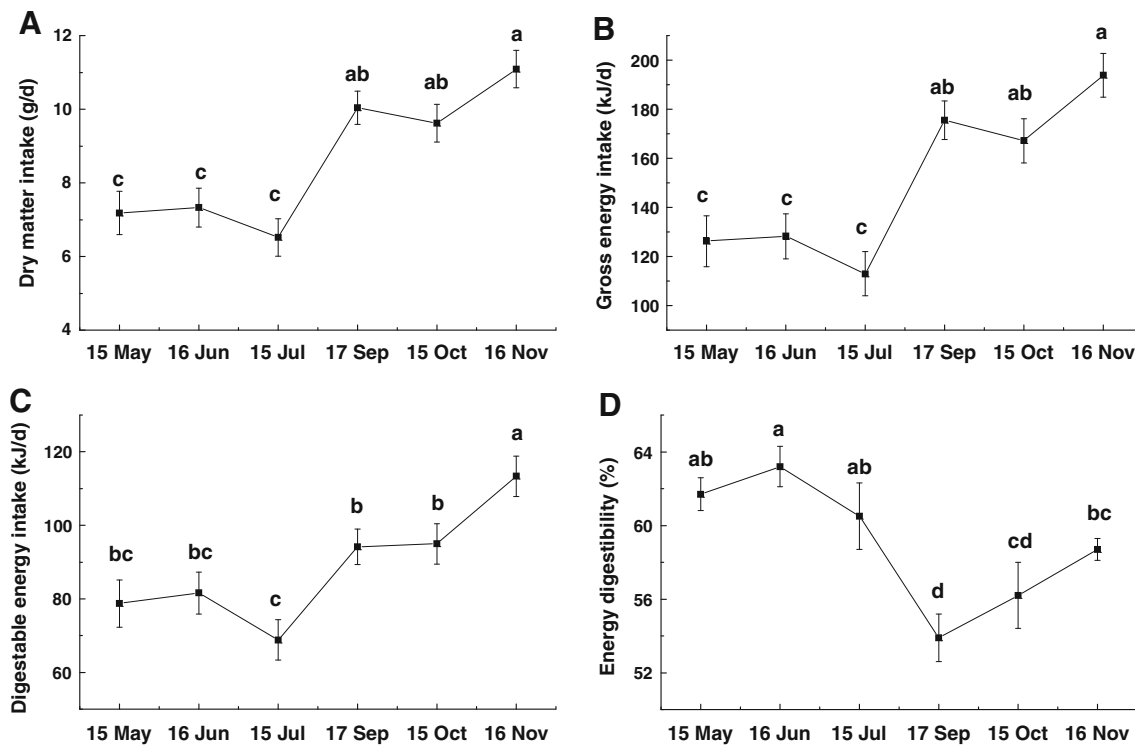
Fig. 2 Seasonal changes of dry matter intake (**a**), gross energy intake (**b**), digestible energy intake (**c**), and energy digestibility (**d**) in Maximowiczi's voles (*Microtus maximowiczii*). Different *superscript* *letters* denote statistically significant differences. Values are expressed as mean ± SEM

Initial RMR and NST showed no group differences prior to photoperiodic acclimation ($F_{(1,10)} = 0.042$, $P > 0.05$ for RMR; $F_{(1,10)} = 1.210$, $P > 0.05$ for NST, Table 2). At the end of acclimation, the SD voles showed a 11.1% increase (relative to the initial measurement) in NST, which was significantly higher than that of LD voles ($F_{(1,10)} = 8.513$, $P < 0.05$). However, no significant variations in RMR were noted ($F_{(1,10)} = 0.063$, $P > 0.05$).

No significant differences were found in DMI, GEI, DEI and digestibility between SD and LD group prior to acclimation ($F_{(1,10)} = 2.031$, $P > 0.05$ for DMI; $F_{(1,10)} = 2.031$, $P > 0.05$ for GEI; $F_{(1,10)} = 1.810$, $P > 0.05$ for DEI; $df = 11$, $t = -0.576$, $P > 0.05$ for digestibility; Table 2). At the end of the 4-week acclimation, the DMI, GEI and DEI in SD voles were significantly higher than those in LD voles ($F_{(1,10)} = 14.398$, $P < 0.01$ for DMI; $F_{(1,10)} = 2.031$, $P < 0.01$ for GEI; $F_{(1,10)} = 9.475$, $P < 0.01$ for DEI), but the digestibility in SD voles was lower than LD voles ($df = 11$, $t = 2.622$, $P < 0.05$).

Effect of photoperiod on mitochondrial protein content and COX activity

BAT mass and mitochondrial protein showed no differences between SD voles and LD voles ($df = 11$, $t = 0.652$, $P > 0.05$ for BAT mass; $df = 11$, $t = -0.102$, $P > 0.05$ for mitochondrial protein, Table 3). However, the COX activity showed significant differences ($df = 11$, $t = -3.185$, $P < 0.01$). The COX activity in SD voles was 32% higher than that of LD voles.

Effect of photoperiod on body composition and serum hormones levels

After the 4-week photoperiod acclimation, there were no significant differences in wet carcass mass ($F_{(1,12)} = 0.608$, $P > 0.05$), dry carcass mass ($F_{(1,12)} = 0.184$, $P > 0.05$), body fat mass ($F_{(1,12)} = 0.248$, $P > 0.05$, Table 3).

Serum T3 and T4 levels in LD voles were significantly lower than those in SD voles (serum T3: $df = 11$, $t = -2.413$, $P < 0.05$; serum T4: $df = 11$, $t = -2.561$, $P < 0.05$, Table 3). No difference was found in serum leptin levels between LD and SD voles ($F_{(1,12)} = 1.017$, $P > 0.05$, Table 3). A positive correlation between serum leptin levels and body fat mass was found ($r = 0.62$, $P < 0.05$).

Discussion

Seasonal changes in body mass and body fat mass

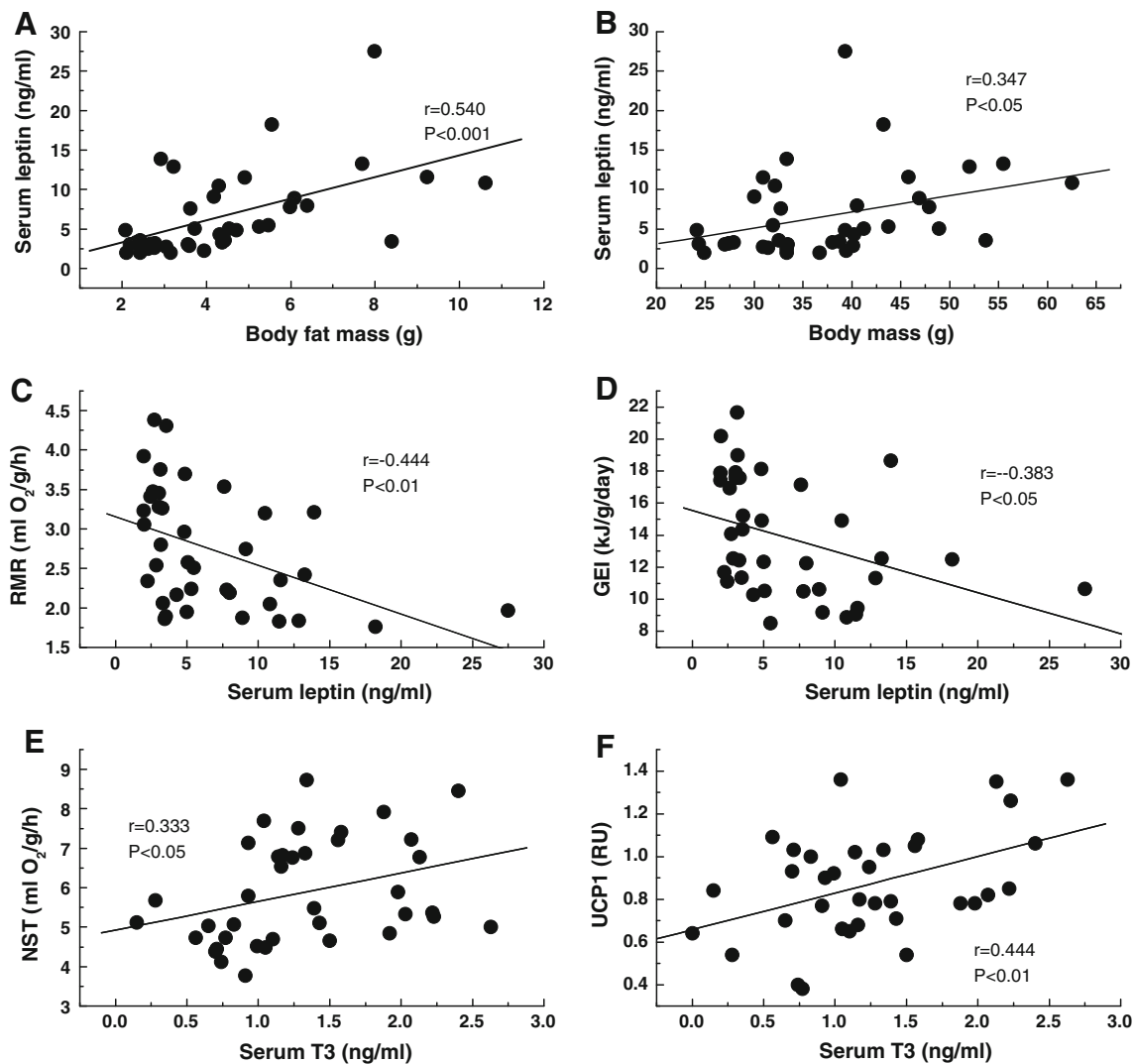Many winter-active small mammals residing in northern regions were reported to undergo a decline in body mass in

Fig. 3 The correlations between serum leptin and body fat mass (**a**), body mass (**b**), RMR (**c**), gross energy intake (**d**), and between serum T3 level and NST (**e**), and UCP1 (**f**). Values are expressed as mean ± SEM

winter (Dehnel 1949; Merritt 1986; Heldmaier 1989; Lovegrove 2005). However, some northern species showed an increase in body mass when exposed to winter-like conditions, such as collared lemmings (Nagy 1993) and short-tailed shrews (*Blarina brevicauda*) (Merritt 1986). In the present study, Maximowiczi's voles showed 25% reduction in body mass in winter, similar to Brandt's voles (Li and Wang 2005a), root voles (Wang et al. 2006a), Siberian hamsters (Heldmaier et al. 1981), and Masked shrews (*Sorex cinereus*) (Merritt 1995). In small mammals (<0.1 kg), body mass reduction in winter can decrease animals' total energy requirements (Heldmaier 1989), but it will also compromise their cold tolerance due to an increase in surface area-to-volume ratio (Speakman 1996; Jackson et al. 2001). It has been suggested that increased insulation coupled with reduced activity and a burrowing

habit may help them to counteract those disadvantages (Heldmaier 1989).

Accompanied with the reductions in body mass, Maximowiczi's voles also display a significant loss in body fat mass. Such changes were also documented in other rodent species including Siberian hamsters (Klingenspor et al. 2000), meadow voles (*Microtus pennsylvanicus*) (Dark and Zucker 1986), prairie voles (*Microtus ochrogaster*) (Kriegsfeld and Nelson 1996), Brandt's voles (Li and Wang 2005a), and root voles (Wang et al. 2006a).

Seasonal changes in thermogenic capacities

To cope with cold stress, small mammals mainly increase thermogenic capacity to maintain constant body temperature (Jansky 1973; Heldmaier et al. 1982). Our study found
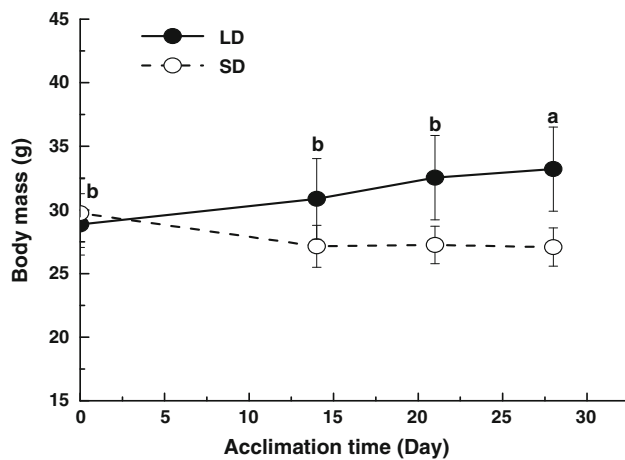
Fig. 4 The effect of photoperiod on body mass in Maximowiczi's voles (*Microtus maximowiczii*) during the acclimation. The body mass in LD voles showed a gradual and significant increase over the course of 4-week acclimation, but no differences were observed between SD and LD groups (*P* > 0.05). Different *superscript letters* identify statistically significant differences. Values are expressed as mean ± SEM

that NST and RMR of Maximowiczi's voles increased in colder months. The findings of elevated thermogenic capacity, indicated by the enhanced NST, is further supported by the other biochemical markers examined in the present study, including the MP content, the COX activity and UCP1 content, which is in agreement with the previous studies in Plateau pikas (Wang et al. 2006b), root voles (Wang et al. 2006a), Brandt's voles (Li and Wang 2005a), golden spiny mice (*Acomys russatus*) (Kronfeld-Schor et al. 2000), and tree shrews (*Tupaia belaneri*) (Li et al. 2001).

Generally, cold-induced NST is activated by the sympathetic nervous system but is modulated by THs (Lowell and Spiegelman 2000). 3,5,3′-triiodo-l-thyronine (T3) is the only active form of THs and its effects are mediated by nuclear T3 receptors, which chiefly affect the transcription of UCP1 (Cannon and Nedergaard 2004). In this study, our results demonstrated that the T3 concentration in winter was significantly higher than that in summer, which was consistent with the seasonal changes in NST and UCP1. Furthermore, correlation analysis showed that UCP1 protein contents and NST were positively correlated with T3

concentrations supporting that thyroid hormones are major modulators of cold-induced NST (Silva 2006).

Seasonal changes in serum leptin levels

Serum leptin levels displayed seasonal fluctuations. Furthermore, our results showed that changes in leptin expression in response to seasonal acclimatization were significant after the correction of the effect of body mass or body fat, which implies that leptin serves as an indicator of energy store, as well as a mediator of energy balance. Energy intake in Maximowiczi's voles increased significantly in fall/winter, similar as that in Brandt's voles (Li and Wang 2005a) and root voles (Wang et al. 2006c). Further experiments found that field voles acclimated under short photoperiod had a high sensitivity in response to exogenous leptin administration than those kept under long photoperiod, and this increased sensitivity to leptin may play a key role in their winter survival (Król and Speakman 2007). Maximowiczi's voles, as well as Brandt's voles, have food hoarding behavior for wintering. Interestingly, despite the doubled energy intake consumed from summer to winter, the voles' digestibility slightly decreased, which was consistent with that in the photoperiodic acclimation. This transition of digestive physiology in Maximowiczi's voles could be the result of a balance between the benefits of processing food through a digestive system with specific attributes and the cost of maintaining and carrying it (Liu and Wang 2007). The adjustment of digestive efficiency to photoperiod or seasonal changes was also found in field voles (Król et al. 2006b).

Leptin-modulated changes of energy expenditure have been suggested to link to thermogenic activity of brown adipose tissue, but the conclusion is controversial. It was reported that leptin administration caused increases in oxygen consumption and UCP1 gene expression in BAT of rat and mice (Scarpace et al. 1997; Commins et al. 2001); whereas Abelenda et al. (2003) showed that leptin administration to cold-acclimated rats decreased UCP1 protein concentrations in BAT and reduced thermogenesis. In addition, infusion of leptin has no effect on BAT UCP1 content in field voles and Brandt's voles under chronic cold

Table 2 The effects of photoperiod on the RMR, NST, energy intake in Maximowiczi's voles (*Microtus maximowiczii*) acclimated to either long (LD, light:dark, 16:8) or short photoperiod (SD, 8:16)

	0 day		*P*	28 days		*P*
	LD	SD		LD	SD	
RMR (ml O₂/h)	99.59 ± 5.34	101.20 ± 5.77	>0.05	102.10 ± 6.05	97.32 ± 6.58	>0.05
NST (ml O₂/h)	190.17 ± 4.87	198.05 ± 5.26	>0.05	190.14 ± 7.05	222.05 ± 7.67	<0.05
DMI (g/day)	7.57 ± 0.66	8.94 ± 0.71	>0.05	7.43 ± 0.60	10.87 ± 0.65	<0.01
GEI (kJ/day)	132.15 ± 11.45	156.20 ± 12.37	>0.05	129.71 ± 10.55	189.88 ± 1.43	<0.01
DEI (kJ/day)	75.78 ± 9.57	94.76 ± 10.34	>0.05	80.31 ± 6.95	112.49 ± 7.53	<0.05
Digestibility (%)	57.31 ± 1.12	59.23 ± 3.57	>0.05	61.90 ± 0.68	59.07 ± 0.83	<0.05

Values are expressed as mean ± SEM

Table 3 Thermogenic parameters and body composition in Maximowiczi's voles (*Microtus maximowiczii*) acclimated to either long (LD, light:dark, 16:8) or short photoperiod (SD, 8:16)

Parameters	LD	SD	P
Sample size	7	6	
Initial body mass (g)	28.9 ± 2.4	29.7 ± 2.7	>0.05
Final body mass (g)	33.91 ± 2.83	27.08 ± 1.34	0.064
Wet carcass mass (g)	24.95 ± 2.31	18.93 ± 1.19	>0.05
Dry carcass mass (g)	11.20 ± 1.23	7.95 ± 0.89	>0.05
Body fat mass (g)	4.55 ± 0.66	2.76 ± 0.69	>0.05
Body fat content (%)	39.97 ± 2.13	32.63 ± 4.00	>0.05
Mass out of carcass (g)	8.97 ± 0.68	8.15 ± 0.31	>0.05
BAT mass (g)	0.085 ± 0.011	0.072 ± 0.019	>0.05
BAT MP (mg/g BAT)	7.19 ± 0.86	7.36 ± 1.41	>0.05
COX activity (nmol O_2/min/g BAT)	7,900 ± 570.34	10,435.29 ± 543.21	<0.05
Serum T3 (ng/ml)	0.68 ± 0.1	1.04 ± 0.1	<0.05
Serum T4 (ng/ml)	24.48 ± 3.27	34.76 ± 2.03	<0.05
Serum leptin (RU)	7.55 ± 1.85	2.98 ± 0.96	<0.05

Values are expressed as mean ± SEM

exposure (Król et al. 2006a; Tang et al. 2009). In the present study, no correlations between serum leptin and UCP1 were found. Moreover, we have showed a negative correlation between leptin concentration and UCP1 protein contents in root voles and Brandt's voles (Wang et al. 2006a, b; Li and Wang 2005a). Under cold exposure, the increase in thermogenic capacity controlled by the sympathetic nervous system may offset the modulation effect of decreased serum leptin. That is to say, the anorectic and thermogenic effects of leptin may be dissociated (Król et al. 2006a). Another possibility is associated with the sensitivity of leptin as mentioned above (Tang et al. 2009). Our results also demonstrated that serum leptin levels of Maximowiczi's voles are negatively correlated with RMR, similar results found in the lactating voles (Zhang and Wang 2007) and insectivorous bats (*Eptesicus fuscus*) (Kunz et al. 1999) suggest that leptin might be involved in the regulation of RMR.

Roles of photoperiod in seasonal changes of body mass, thermogenesis, and serum hormones

Several small rodents have been reported to show reduction in body mass and increase in thermogenesis in SD conditions. This notion is supported by our data from the present study showing that LD voles displayed a steady increase in body mass whereas SD voles showed no changes in their body mass over the course of acclimation. BAT is the main site of NST production in small mammals (Foster and Frydman 1979). In our present study, although no differences in absolute BAT mass were found between SD and LD voles, NST was significantly elevated in SD voles. Changes of photoperiod affect seasonal adjustments of energy budgets in many small mammals (Bartness et al. 1989; Haim 1996; Powell et al. 2002). Short photoperiod induced the increase of energy intake in Macedonian mice (*Mus macedonicus*) (Haim et al. 1999), golden spiny mice (*Acomy russatus*) (Haim et al. 1994), and Levant voles (*Microtus guentheri*) (Banin et al. 1994). Our data also showed that energy intake in SD voles (kept at ∼21°C) was greatly elevated, similar to the findings under natural ambient temperature, revealing that SD alone was effective to induce seasonal acclimatization in this species. SD voles showed relatively higher serum T3 levels but lower serum leptin levels compared with LD voles, indicating that these two kinds of hormones were potentially involved in the regulation of SD-induced energy balance in this species.

We need to point out that in our experiment the short day exposure was only last about 4 weeks and this may devaluate our results. For photoperiod acclimation, usually short day acclimation requires 8–10 weeks for the animals to complete the changes in physiology (Bartness and Goldman 1989). We should also pay attention to the synchronization of seasonal changes, e.g., in Siberian hamsters, reproductive organs are reduced first, then body mass, and then the last event is the occurrence of daily torpor (Heldmaier and Lynch 1986).

Nonhibernating small mammals, which survive in the cold seasons and/or cold regions, have presumably developed several anatomical, behavioral and physiological strategies to enhance their survival (Wang and Wang 1996). In the present study, Maximowiczi's voles showed an increase in energy intake and thermogenesis in association with the decreases in body mass, body fat mass and serum leptin levels in winter and/or under winter (SD) conditions. Although Maximowiczi's voles display similar changes with the well-characterized seasonal rodent species such as field voles and Siberian hamsters in body mass and body fat mass during cold season, but their energy intake patterns are species-specific.

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