Effects of photoperiod history on body mass and energy metabolism in Brandt's voles (*Lasiopodomys brandtii*)

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Summary

Many small mammals respond to seasonal changes in photoperiod via alterations in morphology, physiology and behaviour. In the present study, we tested the hypothesis that the preweaning (from embryo to weaning) photoperiod experience can affect subsequent development in terms of body mass and thermogenesis. Brandt's voles (*Lasiopodomys brandtii*) were gestated and reared to weaning under either a short (SD, 8 h:16 h L:D) or a long photoperiod (LD, 16 h:8 h L:D) at a constant ambient temperature (23°C). At weaning, male juveniles were either maintained in their initial photoperiod or transferred to the alternative photoperiod for 8 weeks. Postweaning SD voles had a lower body mass but higher thermogenic capacity compared with LD voles. At the same time, preweaning photoperiod conditions had long-lasting effects on thermogenic capacity later in life. Serum leptin concentration was positively correlated with body mass and body fat mass, whereas it was negatively correlated with energy intake and uncoupling protein 1 content in brown adipose tissue. Our results suggest that postweaning development in terms of body mass and thermogenesis is predominantly influenced by the postweaning photoperiod, while the preweaning photoperiod experience could chronically modify thermogenesis but not body mass. Furthermore, serum leptin, acting as a potential adipostatic signal, may be involved in the regulation of both energy intake and energy expenditure.

Introduction

Many small mammals living in temperate and arctic regions have evolved a suite of strategies to adapt to the environment, involving anatomy, physiology and behaviour (Bartness et al., 2002; Concannon et al., 2001; Heldmaier and Steinlechner, 1981; Merritt et al., 2001). Photoperiod is often regarded as the primary proximate cue used to mediate these adjustments (Kauffman et al., 2003; Kriegsfeld and Nelson, 1996; Nagy et al., 1993a; Nagy et al., 1993b). Specifically, the integration of absolute day-length and changing day-length allows animals to determine precisely the time of year (Nagy et al., 1993a; Nagy et al., 1993b).

In several rodent species, postweaning body mass has been reported to be programmed by earlier photoperiod history (Gower et al., 1997; Horton, 1984; Lee and Zucker, 1988; Nagy et al., 1993a; Nagy et al., 1993b; Stetson et al., 1986). Studies concerning the effects on energy expenditure of photoperiod history have been conducted predominantly in adult animals. Powell et al. (Powell et al., 2002) found that when long photoperiod (LD) collared lemmings (*Dicrostonyx groenlandicus*) were exposed to a short photoperiod (SD), resting energy expenditure and uncoupling protein 1 (UCP1) mRNA levels in brown adipose tissue (BAT) decreased. In some species such as Siberian hamsters (*Phodopus sungorus*),

SD acclimation increased non-shivering thermogenesis (NST) and UCP1 content (Demas et al., 2002; Heldmaier et al., 1981). NST is an important mechanism for thermoregulation in small mammals (Jansky, 1973) and BAT is a major site for NST (Ricquier and Bouillaud, 2000). The enhancement of BAT function is fulfilled by increasing BAT mass, mitochondrial protein (MP) content (Martin et al., 1989; Trayhurn et al., 1982) and, most importantly, increased expression of UCP1. UCP1 is a 32 kDa protein, uniquely located in the inner membrane of BAT mitochondria, that can induce proton leakage, which results in heat generation (Rial and Gonzalez-Barroso, 2001). In addition, cytochrome *c* oxidase (COX) as the terminal enzyme in oxidative phosphorylation in mitochondria is also involved in mitochondrial energy metabolism (Kadenbach et al., 2000). To our knowledge, there has been no integrative study assessing the effects of preweaning photoperiod history on body mass and thermogenesis regulation from molecular to organismal levels.

Body mass growth and energy balance during development are fundamental for physiological functions in adulthood. Leptin, a hormone secreted primarily by adipocytes, plays an important role in energy balance and reproductive status (Ahima and Flier, 2000; Fox et al., 2000; Oates et al., 2000; Schneider et al., 2000; Zhang et al., 1994). In several species of wild rodent, seasonal fluctuations of serum leptin and its gene

expression are tightly coupled with the dynamics of food intake and adiposity, indicating leptin's potential role in underlying the seasonal changes in body mass and energy balance (Concannon et al., 2001; Klingenspor et al., 1996; Li and Wang, 2005). Further, under laboratory conditions, exogenous leptin administration induced a decrease in food intake and an increase in energy expenditure (Abelenda et al., 2003; Pelleymounter et al., 1995; Scarpace et al., 1997). However, the effects of leptin on thermogenesis are somewhat paradoxical. Leptin can increase BMR (basal metabolic rate), NST and UCP1 mRNA expression in *ob/ob* mice (Pelleymounter et al., 1995; Scarpace et al., 1997), but low serum leptin level was accompanied by an increase in UCP1 gene expression in cold-acclimated rats (Bing et al., 1998).

Brandt's voles (*Lasiopodomys brandtii*) are herbivores that mainly inhabit the typical steppe in Inner Mongolian grasslands of China, Mongolia, and the region of Beigaer in Russia. They breed seasonally, with the breeding season lasting from March to August (Liu et al., 2003; Wan et al., 2002). Seasonal variations in body mass, BMR and NST have been observed (Wang et al., 2003). In seasonally acclimatized voles, energy intake, serum leptin level and body fat mass change on a seasonal basis, which indicates the potential role of ambient temperature and photoperiod in the regulation of energy balance and body mass (Li and Wang, 2005). Further, it has been shown that photoperiod and temperature are important cues to induce these changes (Li and Wang, 2005; Li and Wang, 2007; Zhang and Wang, 2006). SD alone can decrease body mass, body fat mass and serum leptin level, while increasing BMR, NST, energy intake, COX activity and UCP1 content in BAT (Zhao and Wang, 2005; Zhao and Wang, 2006). SD can also delay the growth in body mass of weaning voles (Liu and Fang, 2001). We still have no knowledge about the energy expenditure and energy intake in response to photoperiod in juveniles. The effects of photoperiod history on the physiological, biochemical and hormonal markers during development in Brandt's voles remain unknown.

We hypothesized that the preweaning (from embryo to weaning) photoperiod experience can affect the subsequent development in terms of body mass and thermogenesis in Brandt's voles. We predicted that (1) postweaning responses to photoperiod in terms of body mass and thermogenesis development were primarily determined by the postweaning photoperiod; (2) the preweaning photoperiod experience has long-lasting effects on body mass and thermogenesis regulation even in adulthood; and (3) leptin is involved in the regulation of both energy intake and energy expenditure.

Materials and methods
Subjects
All animal procedures were licensed under the Animal Care and Use Committee of the Institute of Zoology, Chinese Academy of Sciences. Thirty-two male Brandt's voles (*Lasiopodomys brandtii*, Radde) were obtained from a breeding colony established in May 1999 from a wild population that inhabits the Inner Mongolian grasslands of China. They were gestated and weaned under a photoperiod of either 16 h:8 h L:D (with lights on at 05:00 h; long photoperiod, LD) or 8 h:16 h L:D (with lights on at 08:00 h; short photoperiod, SD), and room

temperature remained constant throughout the photoperiod manipulation (23±1°C). To eliminate maternal effects on the body mass of the offspring, we chose dams with similar body mass (50±4 g) under different photoperiods to become pregnant. At weaning (22 days of age), 16 male pups (from at least 10 litters) in each photoperiod were randomly assigned into two groups. When two pups were from the same litter, they were split between the two postweaning treatments. After determination of energy intake for 3 days and oxygen consumption for 2 days to determine baseline values, voles were exposed to the different photoperiod regimes. In other words, at 27 days of age, one-half of the 16 male pups in each photoperiod were switched to the alternative photoperiod whilst the other half remained in their preweaning photoperiod. Thus, four groups were formed: (1) chronically long photoperiod (LL), (2) preweaning long but postweaning short photoperiod (LS), (3) chronically short photoperiod (SS), and (4) preweaning short but postweaning long photoperiod (SL). Eight weeks after photoperiod transfer, animals were killed to determine the variation of physiological, biochemical and hormonal markers.

After weaning, all voles were raised individually in plastic cages (30 cm × 15 cm × 20 cm) with sawdust bedding. Commercial rabbit pellets (Beijing KeAo Feed Co., Beijing, China) and water were provided *ad libitum*. Before photoperiod transfer, we determined body mass (±0.01 g) using a digital balance (Sartorius, Goettingen, Germany) at birth, at day 15 (the time when animals began to eat solid food) and at weaning to examine the effect of preweaning photoperiod on preweaning growth. After photoperiod transfer, body mass was monitored once a week. Energy intake, resting metabolic rate (RMR) and NST were each measured from 22 days of age, at the 1st week, 3rd week and 8th week after photoperiod transfer, and each measurement lasted for 5 days. Serum leptin level, body composition/organ morphology, MP content, COX activity and UCP1 content in BAT were measured *post mortem*.

Metabolic trials
Oxygen consumption was measured using an established closed-circuit respirometer (Wang and Wang, 1996; Wang et al., 2000). RMR was measured at 30±0.5°C [thermal neutral zone: 28–38°C (Wang et al., 2003)]. The volume of the metabolic chamber was 3.6 l and the temperature was maintained with a waterbath (Zhao and Wang, 2005). Carbon dioxide and water were absorbed by KOH and silica gel, respectively. Voles were put into the metabolic chamber for about 60 min to stabilize, then the oxygen consumption was recorded for another 60 min with 5 min intervals. The two stable consecutive lowest readings were used to calculate RMR. The next day, NST stimulated by subcutaneous injection of norepinephrine (NE; Shanghai Harvest Pharmaceutical Co. Ltd, Shanghai, China) was measured at 25±1°C. The dosage of NE (in mg kg^{-1}) was calculated according to the equation described for body mass (M_b; in g) by Wunder and Gettinger (Wunder and Gettinger, 1996): NE dosage=$2.53M_b^{-0.4}$. This dosage can induce the maximum NST in Brandt's voles (Wang and Wang, 2006). The two stable consecutive highest values were taken to calculate maximum NST (Li and Wang, 2005; Wang and Wang, 1996). NST capacity (NST$_{cap}$) was calculated as the maximum NST minus RMR at thermal neutral zone (Jansky, 1973; Klaus et al., 1988).

All metabolic measurements were performed between 09:00 and 17:00 h and oxygen consumption was corrected to the standard temperature and pressure (STP) condition.

Energy intake

Animals were housed in metabolic cages made of stainless steel mesh (24 cm × 24 cm × 24 cm). Food was provided in quantitative excess and water was provided *ad libitum*. The faeces and food residues were collected 3 days later, oven-dried at 70°C to constant mass and separated manually. The caloric value of food and faeces was determined by Parr 1281 oxygen bomb calorimetry (Parr Instrument, Moline, IL, USA). Parameters were calculated as follows (Grodzinski and Wunder, 1975; Liu et al., 2003):

Dry food provided =
$$\text{fresh food provided} \times [1 - (30\,g - \text{dry mass})/30\,g] ,$$

$$\text{DMI} = (\text{dry food provided} - \text{dry food residue})/3\,\text{days} ,$$

$$\text{GEI} = \text{DMI} \times \text{caloric value of dry food} ,$$

$$\text{DEI} = \text{GEI} - \text{gross energy of faeces} ,$$

$$\text{Apparent digestibility} = 100 \times \text{DEI/GEI} ,$$

where DMI is dry matter intake (g day^{-1}); GEI is gross energy intake (kJ day^{-1}); DEI is digestible energy intake (kJ day^{-1}); dry food provided (g); fresh food provided (g); dry mass (g); dry food residue (g); caloric value of dry food (kJ g^{-1}); gross energy of faeces (kJ day^{-1}); and apparent digestibility (%).

Serum leptin assays

Serum was separated by centrifugation (1500 *g*, 30 min, 4°C). Leptin concentration was determined by radioimmunoassay (RIA) with a ^{125}I multi-species leptin RIA kit (Linco, St Louis, MO, USA), which has been shown to be feasible for use with Brandt's voles (Li and Wang, 2005; Zhao and Wang, 2006). The lowest level of leptin that can be detected is 1.0 ng ml^{-1} when using a 100 µl sample (manufacturer's instructions). Inter- and intra-assay variability for leptin RIA was <3.6% and 8.7%, respectively.

Body composition and organ mass analysis

At the end of the experiment (89 days of age), voles were killed between 14:00 and 16:00 h by puncture of the posterior vena cava without anaesthetization. After trunk blood and interscapular BAT were collected, the organs such as the liver, heart, lung, spleen, kidneys and testes were dissected out to determine wet mass (±0.0001 g; using a digital balance, Sartorius). Then the entire gastrointestinal tract was removed, and the remaining carcass, together with organs, was dried in an oven at 70°C for 10 days to a constant mass, and weighed again to determine dry mass. Total body fat was extracted from the dried carcass by ether extraction in a Soxhlet apparatus (Li and Wang, 2005). Total water mass was calculated as the difference between wet carcass mass and dry carcass mass.

Measurement of mitochondrial protein content, COX activity and UCP1 content in BAT

Mitochondria were isolated according to the description of Wiesinger et al. (Wiesinger et al., 1989) and mitochondrial protein concentration was determined by the Folin phenol method (Lowry et al., 1951) with bovine serum albumin as the standard. COX activity was measured with the polarographic method using an oxygen electrode (Hansatech Instruments Ltd, King's Lynn, Norfolk, UK) (Li and Wang, 2005; Sundin et al., 1987; Zhao and Wang, 2005). Briefly, it was measured at 25°C in 1.98 ml of respiration medium (100 mmol l^{-1} KCl, 20 mmol l^{-1} Tes, 1 mmol l^{-1} EGTA, 2 mmol l^{-1} MgCl$_2$, 4 mmol l^{-1} KH$_2$PO$_4$ and 60 µmol l^{-1} bovine serum albumin, pH 7.2). A 10 µl aliquot taken from the supernatant and 10 µl cytochrome *c* (60 mg ml^{-1}) were added to the electrode and COX activity was measured in a final volume of 2 ml. COX activity was expressed in terms of MP (nmol O$_2$ min^{-1} mg^{-1} MP) and BAT mass (nmol O$_2$ min^{-1} g^{-1} tissue).

UCP1 content was measured by Western blotting as described previously (Klingenspor et al., 1996; Li and Wang, 2005; Zhao and Wang, 2005). In short, after discontinuous SDS-polyacrylamide gel electrophoresis, BAT protein was blotted onto a nitrocellulose membrane (Hybond-C, Amersham Biosciences, Bucks, UK). Non-specific binding sites were saturated with 5% fat-free dry milk in PBS, then UCP1 was detected using a polyclonal rabbit anti-hamster UCP1 antibody (1:5000 dilution; supplied by Dr M. Klingenspor, Philipps-University Marburg, Germany) as a primary antibody and peroxidase-conjugated goat anti-rabbit IgG (1:5000 dilution; Jackson ImmunoResearch Labs Inc., West Grove, PA, USA) as the secondary antibody. The primary antibody has been shown to be highly specific in Brandt's voles (Li and Wang, 2005; Zhang and Wang, 2006; Zhao and Wang, 2005). UCP1 concentration was determined with Scion Image Software (Scion Corporation, Frederick, MD, USA) and are expressed as relative units (r.u.) (Li and Wang, 2005; Zhao and Wang, 2005).

Data analysis

Data were analysed using SPSS software (SPSS 1988, Chicago, IL, USA). Body mass during lactation was analysed using Student's *t*-tests for independent samples, while two-way ANOVA was employed with preweaning photoperiod, postweaning photoperiod and associated interaction as factors thereafter. These tests were also employed to analyse RMR and NST$_{cap}$, and ANCOVA was employed to analyse energy intake, as described in the corresponding tables (Tables 1 and 2). Differences in serum leptin concentration, carcass mass and visceral organ mass were analysed by two-way ANCOVA with the final body mass as covariate. In addition, serum leptin concentration was adjusted for body mass and/or body fat mass to detect the group difference. Two-way ANOVA was used to examine the group differences for body fat mass and BAT mass (no correlations with body mass were detected), MP content, COX activity of BAT, and UCP1 content. All the differences in each parameter were further evaluated by LSD *post hoc* tests, with the significance level adjusted to account for the number of comparisons (Bonferroni correction). Pearson's correlations were performed to determine the correlations between serum leptin concentration and body mass, body fat mass, digestible energy intake and UCP1 content, and between NST$_{cap}$ and UCP1 as well. To remove the effect of body mass, the correlation between serum leptin concentration and energy intake was achieved by using the residuals.
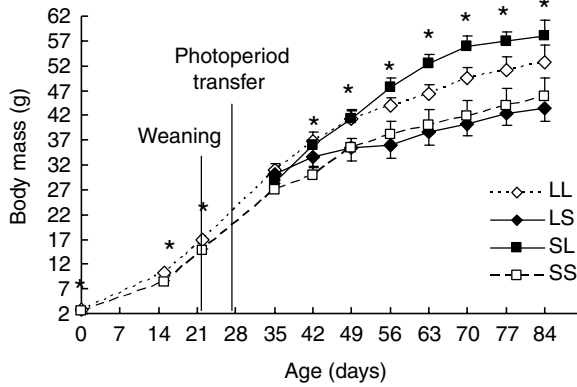
Fig. 1. Effects of photoperiod transfer on body mass in Brandt's voles. During lactation, short photoperiod (SD) voles showed significantly lower body mass than long photoperiod (LD) voles. After weaning, the differences among the four groups occurred from 42 days of age to the termination of the experiment. Data are shown as means ± s.e.m. (N=8). *Significant difference between two groups (during lactation) or among four groups (during the postweaning photoperiod acclimation). LL, chronically long photoperiod; LS, preweaning long but postweaning short photoperiod; SL, preweaning short but postweaning long photoperiod; SS, chronically short photoperiod.

Results

Body mass

During lactation, SD pups had a significantly lower body mass than LD pups (Fig. 1). After weaning, preweaning photoperiod only affected body mass in the 1st week after photoperiod transfer (35 days of age: $F_{1,28}$=6.5, P=0.017), while postweaning photoperiod had effects on body mass from the 2nd week post-switch and through to the end of the experiment (42 days of age: $F_{1,28}$=9.4, P=0.005; 84 days of age: $F_{1,28}$=10.5,

P=0.003). The differences among the four groups occurred from 42 days of age (LL *vs* SS: P=0.004) and achieved a maximum on week 6 (70 days of age; LS *vs* SL: P<0.001; SL *vs* SS: P=0.001). SL voles were 26% heavier than SS voles at that time. No interaction between pre- and postweaning photoperiod existed at any time point.

RMR and NST$_{cap}$

No differences between LD and SD voles were found for either RMR or NST$_{cap}$ at weaning (Table 1). There were significant effects of postweaning photoperiod on RMR at the 1st week ($F_{1,28}$=9.6, P=0.004) and the 3rd week post-switch ($F_{1,28}$=19.2, P<0.001). At both time points, SL voles had significantly lower RMR than SS voles (P=0.001). However, preweaning photoperiod effects on NST$_{cap}$ were detected in the 3rd week ($F_{1,28}$=9.2, P=0.005), and postweaning effects on NST$_{cap}$ were detected in the 8th week ($F_{1,28}$=8.9, P=0.006), and in both cases it was SD exposure that tended to increase NST$_{cap}$ (Table 1). Consistent with this, at the end of the experiment (week 8) the highest NST$_{cap}$ was in SS voles, although the difference between them and the other groups was not statistically significant in every case (Table 1). No interaction between pre- and postweaning photoperiod existed for either RMR or NST$_{cap}$ at any time point.

Energy intake

There were no differences between the LD and SD voles in energy intake parameters at weaning (Table 2). Neither pre- nor postweaning photoperiod effects were detected for any of these parameters; however, there was a significant interaction for the DEI and digestibility at week 8 (DEI: $F_{1,25}$=11.7, P=0.002; digestibility: $F_{1,28}$=28.3, P<0.001). When preweaning LD voles were transferred to SD, DEI and digestibility increased in week 8, but no changes occurred in voles switched from SD to LD.

Table 1. *Effects of photoperiod manipulation on resting metabolic rate and non-shivering thermogenesis capacity in Brandt's voles*

| | | | | | *F* value or *t* value | | | *P* value | | |
	LL	LS	SS	SL	Pre	Post	Pre×post	Pre	Post	Pre×post
RMR (ml O$_2$ h^{-1})										
Weaning	62.2±2.7		64.2±1.8		−0.6[A]	–	–	0.562	–	–
1st week	85.3±2.0[b]	89.0±4.3[a,b]	100.4±2.1[a]	82.5±4.6[b]	1.5	9.6	4.2	0.228	**0.004**	0.051
3rd week	85.1±6.3[b,c]	102.8±5.5[a,b]	105.7±4.5[a]	79.0±3.5[c]	0.1	19.2	0.8	0.750	**<0.001**	0.385
8th week	104.4±3.5	100.1±4.0	113.2±7.6	114.5±6.7	4.1	0.2	0.1	0.052	0.627	0.802
NST$_{cap}$ (ml O$_2$ h^{-1})										
Weaning	105.1±7.4		127.8±12.3		−1.6[A]	–	–	0.123	–	–
1st week	132.6±17.3	133.3±14.4	145.8±15.7	144.5±8.9	0.7	0.1	0.1	0.406	0.943	0.982
3rd week	89.7±17.9[c]	137.9±14.1[b]	163.0±17.7[a]	162.9±14.8[a]	9.2	2.2	2.2	**0.005**	0.142	0.148
8th week	81.2±14.7[b]	117.9±10.2[a,b]	127.7±16.4[a]	85.8±10.3[b]	0.3	8.9	0.1	0.588	**0.006**	0.848

Analysis of resting metabolic rate (RMR) and non-shivering thermogenesis capacity (NST$_{cap}$) was conducted without body mass as a covariate because of the lack of a correlation between RMR or NST$_{cap}$ and body mass in the present experiment, so independent-samples *t*-tests were performed to compare RMR and NST$_{cap}$ at weaning while two-way ANOVA was employed for data for the 1st, 3rd and the 8th week.

LL, voles under preweaning long photoperiod (LD) and postweaning LD; LS, voles under preweaning LD and postweaning short photoperiod (SD); SS, voles under preweaning SD and postweaning SD; SL, voles under preweaning SD and postweaning LD. Pre, preweaning photoperiod effect; post, postweaning photoperiod effect; pre×post, the interaction between pre- and postweaning photoperiod. Weaning voles experienced only the preweaning photoperiod of either LD or SD, so the statistical analysis was conducted on two groups.

[A]The *t* value derived from independent-samples *t*-test. Data are the raw data shown as means ± s.e.m. (N=8 for each group). The Bonferroni correction was applied to the significance level. Significant differences at the 95% confidence level are in bold type. Values with different superscript letters within rows are significantly different.

Table 2. *Effects of photoperiod manipulation on dry matter intake, gross energy intake, digestible energy intake and digestibility in Brandt's voles*

	LL	LS	SS	SL	Pre	Post	Pre×post	Pre	Post	Pre×post
					F value or *t* value			*P* value		
DMI (g day^{-1})										
Weaning	5.55±0.18		5.82±0.15		0.0	–	–	0.900	–	–
1st week	7.34±0.38	7.27±0.42	7.56±0.26	6.42±0.31	3.0	1.9	1.5	0.095	0.186	0.231
3rd week	6.89±0.42	6.82±0.32	8.30±0.36	6.89±0.24	1.6	4.1	2.9	0.220	0.055	0.104
8th week	7.10±0.49	8.72±0.46	8.94±0.69	8.14±0.45	5.7	0.4	3.8	0.025	0.523	0.062
GEI (kJ day^{-1})										
Weaning	95.97±3.10		100.30±2.71		0.0	–	–	0.866	–	–
1st week	125.59±6.04	125.82±7.16	131.48±4.49	109.12±5.13	3.0	2.1	1.5	0.097	0.160	0.234
3rd week	117.82±7.80	117.71±5.45	138.40±5.99	117.13±3.80	2.0	3.1	1.6	0.168	0.091	0.222
8th week	118.87±8.39	146.48±7.74	153.68±11.88	137.65±7.50	5.6	0.3	3.2	0.026	0.561	0.086
DEI (kJ day^{-1})										
Weaning	52.91±2.85		68.78±2.70		0.2	–	–	0.639	–	–
1st week	75.93±4.54	73.34±4.71	78.54±3.35	58.37±5.54	1.6	1.6	1.0	0.222	0.211	0.398
3rd week	67.11±4.04	80.79±4.42	83.74±4.27	56.91±2.87	0.3	1.0	0.3	0.592	0.318	0.613
8th week	57.94±3.16[b]	89.51±5.15[a]	78.72±4.15[a]	79.11±7.35[a]	1.9	1.0	11.7	0.183	0.326	**0.002**
Digestibility (%)										
Weaning	54.5±1.7		68.4±1.5		−1.8[A]	–	–	0.216	–	–
1st week	60.2±1.3	58.2±1.0	59.6±0.7	52.8±3.1	0.1	0.3	0.2	0.799	0.567	0.908
3rd week	57.3±1.3	68.7±2.4	60.4±1.4	48.6±1.8	3.5	1.1	1.3	0.086	0.315	0.256
8th week	49.4±2.5[c]	61.2±1.9[a]	57.3±0.5[b]	51.0±1.0[b,c]	0.5	2.7	28.3	0.494	0.112	**<0.001**

Differences in dry matter intake (DMI), gross energy intake (GEI) and digestible energy intake (DEI) at weaning were examined by one-way ANCOVA with weaning body mass as covariate, or by two-way ANCOVA (with body mass at the corresponding time point as covariate) after weaning. Analysis of digestibility was conducted with no body mass correction because of the lack of correlation between them, so independent-samples *t*-test was performed to compare digestibility at weaning while two-way ANOVA was employed on data for the 1st, 3rd and 8th week.

[A]The *t* value derived from independent-samples *t*-test. Data are the raw data listed as means ± s.e.m. (*N*=8 for each group). The Bonferroni correction was applied to the significance level. Significant differences at the 95% confidence level are in bold type. Values with different superscript letters within rows are significantly different.
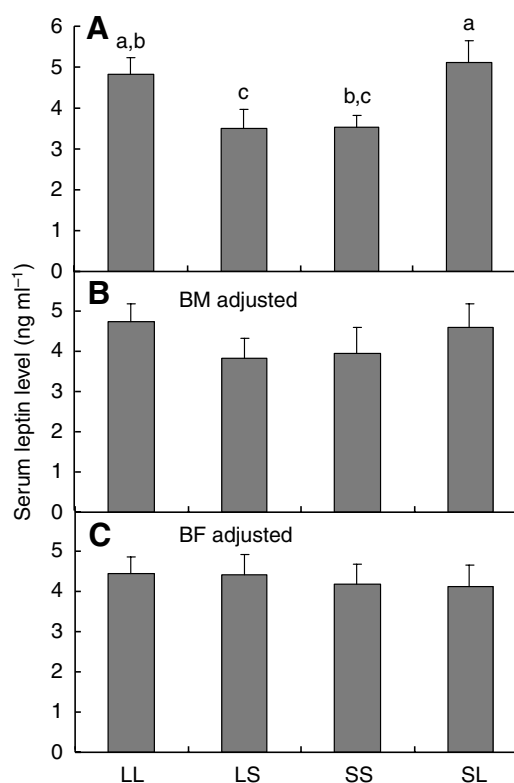
Serum leptin concentration

Neither pre- nor postweaning photoperiod affected absolute serum leptin level and body mass- and/or body fat mass-adjusted residuals (Fig. 2A,B,C), but there was an interaction between the two factors in absolute values ($F_{1,25}$=8.9, *P*=0.006). Postweaning SD voles tended to show lower serum leptin levels, but preweaning photoperiod may modify the concentrations. SL voles had the highest serum leptin levels whereas LS voles had the lowest (*P*=0.007; Fig. 2A). All these differences disappeared after including mass as a covariate (Fig. 2B,C). Correlation analysis demonstrated that residual serum leptin concentration was correlated negatively with the residual DEI (*r*=–0.542, *P*=0.002; Fig. 3).

Body composition and visceral organ mass

No pre- or postweaning photoperiod effects were found on

Fig. 2. Effect of photoperiod transfer on serum leptin concentration in Brandt's voles in original data (A), and after removing the effects of body mass (BM, B) and body fat mass (BF, C). Photoperiod transfer affected the serum leptin level but no differences were detected after removing the effects of body mass and body fat mass. Different letters above the bars represent a significant difference. Data are shown as means ± s.e.m. (*N*=8 except for SS where *N*=5).
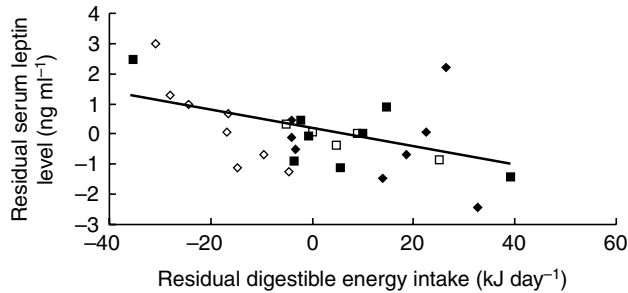
Fig. 3. Correlation between serum leptin concentration and digestible energy intake in Brandt's voles. Serum leptin concentration was negatively correlated with digestible energy intake after adjusting for differences in body mass. Open diamond, LL group; filled diamond, LS group; open square, SS group; filled square, SL group. The line represents the relationship between parameters for all the voles ($r=-0.542$, $P=0.002$; $N=8$ except for SS where $N=5$).

body composition or any visceral organ mass (Table 3). Interaction between the two factors was significant for body fat mass (absolute mass, g: $F_{1,\ 28}=34.3$, $P<0.001$; fat content, %: $F_{1,28}=15.2$, $P=0.001$) and dry paired testes mass ($F_{1,25}=21.5$, $P<0.001$). SL voles had the highest body fat mass while LS voles had the lowest ($P<0.001$). LL voles had the highest dry paired testes mass whereas SS voles had the lowest ($P=0.002$). Serum leptin concentration was correlated positively with body mass ($r=0.529$, $P=0.003$; Fig. 4A) and body fat mass ($r=0.681$, $P<0.001$; Fig. 4B).

Mitochondrial protein content, COX activity and UCP1 content in BAT

Neither pre- nor postweaning photoperiod had an effect on BAT mass, BAT MP content or COX activity in BAT; however, there was an interaction between the two factors on BAT mass (absolute mass: $F_{1,28}=8.2$, $P=0.008$), although no group differences were detected (Table 4).

SD during both the pre- and the postweaning period increased

Table 3. *Wet and dry carcass mass, body fat mass, body water mass, and wet and dry visceral organ mass in Brandt's voles under different photoperiodic manipulation*

	LL	LS	SS	SL	*F* value or *t* value			*P* value		
					Pre	Post	Pre×post	Pre	Post	Pre×post
Carcass mass (g)										
Wet	38.87±2.03	29.80±2.14	30.51±1.65	47.00±3.24	0.4	0.3	0.0	0.521	0.567	0.829
Dry	15.91±0.75	12.59±0.76	13.66±1.18	18.44±1.87	2.8	0.3	0.5	0.108	0.566	0.486
Body fat										
Mass (g)	8.06±1.67[a]	4.77±1.31[b]	5.59±1.80[b]	9.37±1.97[a]	3.1	0.2	34.3	0.585	0.668	**<0.001**
Content (%)	50.31±6.65	38.05±9.55	40.62±8.05	52.48±10.26	0.6	0.1	15.2	0.449	0.948	**0.001**
Body water										
Mass (g)	22.96±1.67	17.21±1.59	16.85±1.07	28.56±1.93	2.6	0.0	1.5	0.121	0.870	0.230
Content (%)	58.70±1.88	57.25±2.07	55.42±2.52	61.11±2.32	4.0	0.1	1.0	0.059	0.717	0.318
Liver mass (g)										
Wet	2.053±0.185	1.520±0.151	1.281±0.066	2.522±0.186	2.7	0.2	2.3	0.114	0.695	0.145
Dry	0.638±0.087	0.473±0.044	0.406±0.027	0.791±0.063	1.4	2.6	4.4	0.250	0.133	0.048
Heart mass (g)										
Wet	0.250±0.012	0.216±0.013	0.195±0.012	0.279±0.014	0.4	0.2	0.1	0.518	0.646	0.763
Dry	0.063±0.004	0.056±0.003	0.051±0.004	0.070±0.003	0.7	3.1	0.0	0.413	0.091	0.876
Lung mass (g)										
Wet	0.253±0.029	0.246±0.033	0.201±0.011	0.258±0.009	8.1	0.2	0.7	0.009	0.641	0.408
Dry	0.058±0.007	0.057±0.007	0.049±0.003	0.062±0.003	5.6	0.1	1.7	0.027	0.824	0.208
Spleen mass (g)										
Wet	0.043±0.005	0.034±0.005	0.032±0.004	0.055±0.005	0.2	0.0	0.3	0.694	0.974	0.562
Dry	0.011±0.001	0.009±0.001	0.011±0.003	0.012±0.001	0.1	1.0	0.9	0.789	0.336	0.350
Kidney mass (g)										
Wet	0.472±0.031	0.401±0.033	0.336±0.016	0.561±0.034	2.1	0.1	1.4	0.164	0.794	0.258
Dry	0.130±0.010	0.118±0.009	0.093±0.005	0.147±0.009	1.9	0.2	0.1	0.187	0.679	0.761
Paired testes mass (mg)										
Wet	900.4±120.0	472.3±110.0	396.2±90.9	1020.0±30.0	1.2	0.1	7.3	0.289	0.779	0.013
Dry	165.2±27.3[a]	73.5±49.1[b]	63.6±38.5[b]	161.6±13.0[a]	3.6	0.0	21.5	0.073	0.911	**<0.001**

Body fat content was calculated as g g^{-1} dry carcass mass and is expressed as a percentage. Body water content was calculated as g g^{-1} wet carcass mass and is expressed as a percentage.

Data are the raw data listed as means ± s.e.m. ($N=8$ for each group). The Bonferroni correction was applied to the significance level. Significant differences at the 95% confidence level are in bold type. Values with different superscript letters within rows are significantly different.
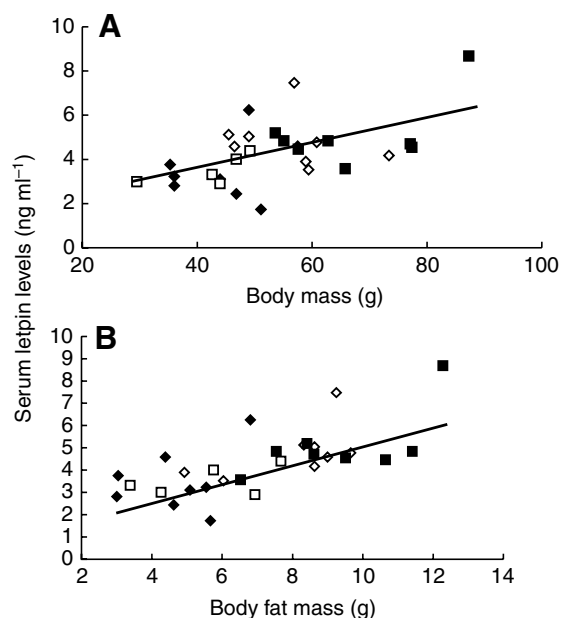
Fig. 4. Correlation between serum leptin concentration and body mass (A) and body fat mass (B) in Brandt's voles. Serum leptin concentration was correlated positively with body mass (r=0.529, P=0.003) and body fat mass (r=0.681, P<0.001; N=8 except for SS where N=5).
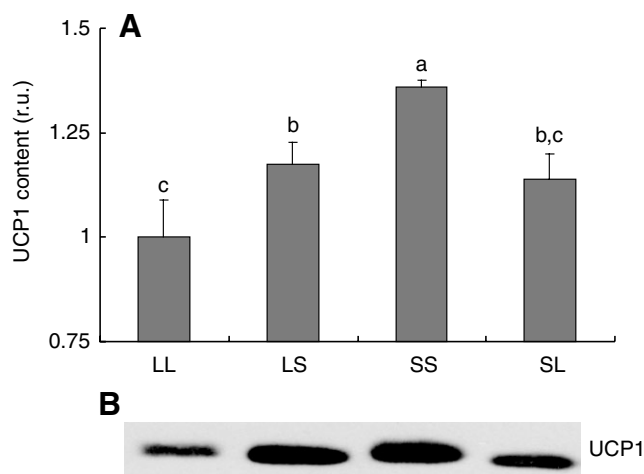

Fig. 5. Effect of photoperiod transfer on uncoupling protein 1 (UCP1) content in Brandt's voles. (A) SD during both the pre- and the postweaning period increased UCP1 content, with no significant interaction. Data are means ± s.e.m. (N=8). r.u., relative units. Different letters above columns signify means that differed significantly. (B) Autoradiograms displaying UCP1 labelling from individuals from each of the four groups.

UCP1 content, with no significant interaction (preweaning: $F_{1,28}$=7.3, P=0.012; postweaning: $F_{1,28}$=10.8, P=0.003; pre × post: $F_{1,28}$=0.1, P=0.703; Fig. 5). UCP1 content was positively correlated with NST$_{cap}$ (r=0.614, P<0.001; Fig. 6), and serum leptin concentration correlated negatively with UCP1 content (r=–0.419, P=0.024; Fig. 7).

Discussion

Changes in body mass throughout photoperiod manipulation

It has been shown that LD or summer conditions can stimulate a gain of body mass in adult Brandt's voles, whereas

SD or winter conditions can inhibit mass gain (Li and Wang, 2005; Zhang and Wang, 2006; Zhao and Wang, 2005; Zhao and Wang, 2006). The present study further supports the previous findings (Li and Wang, 2005; Zhang and Wang, 2006; Zhao and Wang, 2005; Zhao and Wang, 2006). At the termination of the experiment, postweaning SD voles showed significantly lower body mass compared with LD voles. These findings are consistent with field observations. The breeding season of Brandt's voles extends from March until August, and the offspring of the spring cohort (simulated by SL and LL groups in this experiment) grew rapidly while the late summer cohort (simulated by LS and SS groups) showed growth inhibition

Table 4. *Effect of photoperiod manipulation on the mass, mitochondrial protein content and cytochrome c oxidase activity of brown adipose tissue in Brandt's voles*

	LL	LS	SS	SL	F value or t value			P value		
					Pre	Post	Pre×post	Pre	Post	Pre×post
Wet BAT mass										
Absolute mass (g)	0.18±0.01	0.12±0.01	0.11±0.01	0.25±0.07	0.7	1.2	8.2	0.415	0.274	**0.008**
Relative mass (%)	0.33±0.03	0.27±0.02	0.25±0.02	0.38±0.11	0.1	0.4	2.7	0.808	0.543	0.114
MP content (mg g^{-1} BAT)	3.78±0.33	3.65±0.60	3.76±0.27	4.38±0.30	0.0	3.4	3.1	0.871	0.078	0.092
COX activity										
MP	380.0±26.5	694.6±58.4	562.1±47.2	443.6±31.1	0.2	1.3	6.5	0.689	0.259	0.017
BAT	1393.2±109.6	1921.6±98.8	2053.5±140.3	1945.6±195.0	5.9	2.2	5.1	0.022	0.147	0.032

Cytochrome *c* oxidase (COX) activity is given in terms of mitochondrial protein (MP; nmol O$_2$ min^{-1} mg^{-1} MP) and brown adipose tissue (BAT) mass (nmol O$_2$ min^{-1} g^{-1} BAT).

Data are the raw data listed as means ± s.e.m. (N=8 for each group). The Bonferroni correction was applied to the significance level. Significant differences at the 95% confidence level are in bold type.
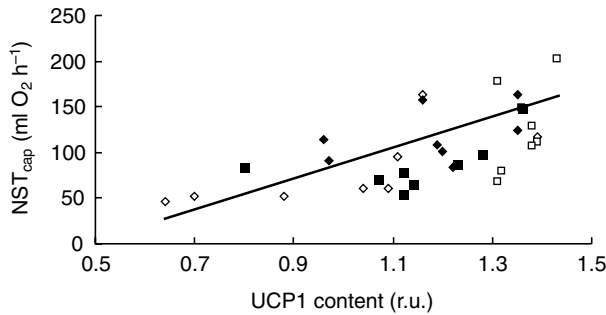
Fig. 6. Correlation between non-shivering thermogenesis capacity (NST_{cap}) and UCP1 content in Brandt's voles. NST_{cap} was positively correlated with UCP1 content ($r=0.614$, $P<0.001$; $N=8$).

(Wan et al., 2002). A similar pattern was found in montane voles (*Microtus montanus*) (Negus et al., 1986), Siberian hamsters (Stetson et al., 1986) and meadow voles (*Microtus pennsylvanicus*) (Lee and Zucker, 1988). Preweaning photoperiod had no significant effect on the voles' gain in body mass later in life. Our results suggest that later development in body mass is predominantly programmed by postweaning photoperiod, while preweaning photoperiod plays no role in body mass development. These results are different from those reported for collared lemmings (Nagy et al., 1993a).

Effects of photoperiod history on thermogenic capacity

Little information on the thermogenic responses to photoperiod is available for photoperiod-transferred juvenile animals. In adults of most rodent species, SD enhances UCP1 content in BAT as reported for common spiny mice (*Acomys cahirinus*) (Kronfeld-Schor et al., 2000), Siberian hamsters (Demas et al., 2002) and Brandt's voles (Zhao and Wang, 2005). In the present study, the response to postweaning photoperiod was similar to that described in the above-mentioned results but opposite to the SD-induced declines in UCP1 mRNA level and resting energy expenditure in collared lemmings (Powell et al., 2002). Of particular interest in our study was that we found evidence that SD both before and after weaning significantly increased UCP1 content; SS voles had
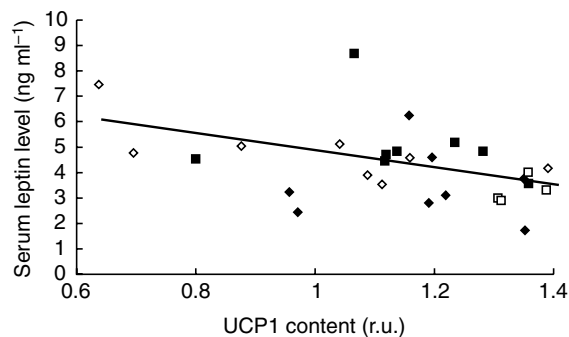

Fig. 7. Correlation between serum leptin concentration and UCP1 content in Brandt's voles. Serum leptin concentration was negatively correlated with UCP1 content ($r=-0.419$, $P=0.024$; $N=8$ except for SS where $N=5$).

the highest content of all groups (Fig. 5). Consistent with this, we also found evidence that SD both before and after weaning significantly increased NST_{cap}, and SS voles also had the highest mean value for this parameter (Table 1). Overall, there was a postive correlation between UCP1 content and NST_{cap} (Fig. 6), which further confirms the role of UCP1 as the molecular basis for NST_{cap}. In Brandt's voles, postweaning photoperiod might play a major role, and preweaning photoperiod exerted long-term effects on thermogenesis. Juvenile Brandt's voles showed responses to photoperiod similar to those of adults in both resting metabolism and NST_{cap} (Zhao and Wang, 2005). These data suggest that photoperiod can serve as an environmental cue to induce winter-like thermogenic characteristics at both the organismal and molecular level, in both juvenile and adult voles.

The underlying mechanism of body mass regulation

Leptin plays an important role in the regulation of body mass and energy balance (Ahima and Flier, 2000; Zhang et al., 1994). Few data are available for wild small mammals at present. Adult collared lemmings display decreases in serum leptin concentration relative to body fat mass when transferred from SD to LD (Johnson et al., 2004). In Brandt's voles, although the serum leptin level was highest in SL groups, no group differences were detected after removing the effects of body mass and body fat mass. This indicates that serum leptin level can be regarded as a signal for changes in body mass, especially body fat mass, in Brandt's voles.

The effect of leptin in regulating body mass is different between Brandt's voles and collared lemmings. In lemmings, body mass loss as a consequence of photoperiod transfer from SD to LD was tightly coupled with hypoleptinaemia. Johnson et al. (Johnson et al., 2004) consequently inferred hypersensitivity to leptin under LD and/or hyposensitivity under SD. In Brandt's voles, however, LS voles showed 17% lower body mass than LL voles, and SL voles showed 26% higher mass than SS voles despite the stable leptin levels relative to body fat mass. The body mass gain and the larger degree of change under postweaning LD may suggest a lower leptin sensitivity under LD but higher leptin sensitivity under SD. Klingenspor et al. (Klingenspor et al., 2000) reported that Siberian hamsters that were exposed to a short photoperiod had a higher sensitivity to exogenous leptin administration than animals that were exposed to a long photoperiod, and the increased sensitivity to leptin may play an important role in the animal's winter survival. Because exogenous leptin treatment has not been performed, such a conclusion for Brandt's voles is somewhat tentative so far.

SD induced an elevation in energy intake in adult bushy-tailed gerbils (*Sekeetamys calurus*) (Haim, 1996), but resulted in a decrease in energy intake in Siberian hamsters (Heldmaier, 1989; Klingenspor et al., 2000). However, SD had no effect on energy intake in collared lemmings (Nagy et al., 1994), bank voles (*Clethrionomys glareolus*) (Peacock et al., 2004) or field voles (*Microtus agrestis*) after correcting for differences in body mass (Król et al., 2005). In the present study, changing the direction of the photoperiod caused differences in digestible energy intake when juveniles had grown up. Preweaning photoperiod and postweaning photoperiod interacted, resulting

in the higher digestible energy intake of LS voles than LL voles. These enhancements in energy intake induced by winter-like day-lengths can be fulfilled by their food-hoarding habit in autumn so as to meet the high thermogenic demand in winter. It has been suggested that species-specific changes in energy intake, as mentioned above, may involve interactions between a network of central and peripheral hormonal signalling systems (Kalra et al., 1999; Mercer and Tups, 2003).

In the present study, serum leptin concentration was negatively correlated with energy intake after adjusting for body mass effects, which suggests that leptin may be involved in energy intake regulation. These findings are consistent with the previous studies in acclimatized Brandt's voles (Li and Wang, 2005) and photoperiod-acclimated voles (Zhao and Wang, 2006) as well as other studies performed on *ob/ob* mice (Pelleymounter et al., 1995; Scarpace et al., 1997).

Beside the unambiguous function of leptin in energy intake, the effects of serum leptin concentration on energy expenditure appear complex. A negative correlation between serum leptin concentration and UCP1 content in Brandt's voles was observed, which implies a potential role for leptin in energy expenditure regulation. A similar relationship was also found in seasonally acclimatized Brandt's voles (Li and Wang, 2005) and root voles (*Microtus oeconomus*) (Wang et al., 2006). When performing correlation analysis between residuals of serum leptin concentration and NST_{cap}, however, no correlation was observed ($r=-0.113$, $P=0.558$). The relationship between leptin and thermogenesis requires further analysis.

Lastly, our photoperiod manipulation caused a difference in body mass at birth between LD and SD voles, which indicated the effects of prenatal photoperiod. The role of gestational photoperiod in body mass regulation has been demonstrated in some species such as collared lemmings (Gower et al., 1994) and Siberian hamsters (Stetson et al., 1986; Stetson et al., 1989). It should be noted that it was difficult to remove the effects of photoperiod during the prenatal period in our present manipulations.

Taken together, postweaning SD voles showed lower body mass in association with higher thermogenic capacity than postweaning LD voles. At the same time, preweaning photoperiod conditions had long-term effects on thermogenic capacity. In addition, serum leptin, acting as a potential adipostatic signal, may be involved in the regulation of both energy intake and energy expenditure. These findings partly support our hypothesis that preweaning photoperiod experience has long-term effects on development in terms of thermogenesis, but not in terms of body mass.

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