

Short photoperiod enhances thermogenic capacity in Brandt's voles

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Abstract

Environmental cues play important roles in the regulation of an animal's physiology and behavior. In the present study, we examined the effects of short photoperiod (SD) on body weight as well as on several physiological, hormonal, and biochemical measures indicative of thermogenic capacity to test our hypothesis that short photoperiod stimulates increases in thermogenesis without cold stress in Brandt's voles. SD voles showed increases in basal metabolic rate (BMR) and nonshivering thermogenesis (NST) during the 4-week photoperiod acclimation. At the end, these voles (SD) had lower body weights, higher levels of cytochrome *C* oxidase (COX) activity and mitochondrial uncoupling protein-1 (UCP1) contents in brown adipose tissues (BAT), and higher concentrations of serum tri-iodothyronine (T_3) and thyroxine (T_4) compared to LD voles. No differences were found between male and female voles in any of the above-mentioned measurements. Together, these data indicate that SD experience enhances thermogenic capacity similarly in males and females of Brandt's voles.

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1. Introduction

Photoperiod acts as an environmental zeitgeber for seasonal acclimatization of thermoregulation in rodents [1–3]. It has been demonstrated that an animal's body weight [4], energy balance [3,5], and basal metabolic rate (BMR) [6] all are affected by photoperiod.

Nonshivering thermogenesis (NST) is an important mechanism for cold-exposed small mammals to generate heat [7], and this process is affected by photoperiod [3,8,9]. For example, acclimation to short photoperiod increases the NST capacity in a variety of rodent species including Djungarian hamsters (*Phodopus sungorus*) [8,10,11], bushy-tailed gerbils (*Sekeetamys calurus*) [3], wood mice (*Apodemus sylvaticus*) [12], kangaroo rats (*Dipodomys ordii*) [13], and root voles (*Microtus oeconomus*) [9]. It is

known that the brown adipose tissue (BAT) is a major site for NST [14], and that the thermogenic capacity of BAT can be enhanced by short photoperiod [2]. It is also known that mitochondrial respiration is accompanied by heat production as it is imperfectly coupled to ADP phosphorylation and almost completely uncoupled in activated brown adipocytes [14]. In addition, the uncoupling protein-1 (UCP1), a 32-kDa protein uniquely expressed in the inner membrane of BAT mitochondria, induces proton leakage which is considered to be an adaptation of mammalian tissues to nonshivering heat production [15]. It appears that enhancement of BAT thermogenic activity is primarily due to the functions of UCP1 [16]. Finally, thyroid hormones (tri-iodothyronine, T_3 and thyroxine, T_4) can affect adaptive thermogenesis by influencing several aspects of energy metabolism, such as substrate cycling, ion cycling, and mitochondrial proton leakage [17–19].

The Brandt's vole (*Microtus brandti*) is a typical herbivorous rodent species that mainly inhabits the Inner Mongolian steppe of China, the Republic of Mongolia, and

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the region of Beigaer Lake in Russia [20]. These animals show seasonal changes in body weight, BMR, and NST [21], indicating a potential role of ambient temperature and photoperiod in the regulation of thermogenesis. Indeed, Brandt's voles that acclimated to cold increased their thermogenic capacity and this process could be further enhanced by short photoperiod [22]. In the present study, by systematically measuring a variety of physiological, hormonal, and biochemical markers indicative of thermogenic capacity, we tested the hypothesis that short photoperiod enhances thermogenesis of the Brandt's voles in the absence of cold ambient temperature. We predicted that, as when exposed to cold, Brandt's voles acclimated to short photoperiods would show increases in BMR, NST, COX activity of BAT, and UCP1 and decreases in body weights in comparison to the conspecific individuals acclimated to long photoperiods. We also compared male and female voles to investigate potential sex differences in thermogenic adaptation to short photoperiod.

2. Materials and methods

2.1. Subjects

Subjects were male and female Brandt's voles (75–85 days old) that were the offspring of voles trapped in Inner Mongolian grasslands and raised in the Institute of Zoology, the Chinese Academy of Sciences. Subjects were housed in single sex groups (3–4) in plastic cages (30×15×20 cm) that contained sawdust bedding. Food (rabbit pellet chow; Beijing KeAo Feed Co.) and water were provided ad libitum. All cages were maintained under 12L:12D photoperiod and room temperature was kept at 23±1 °C. Subjects were moved into individual cages for at least two weeks, and then randomly assigned into one of two experimental groups that were acclimated either to short photoperiod (SD, 8L:16D with lights on at 0900, 4 males and 4 females) or to long photoperiod (LD, 16L:8D with lights on at 0500, 4 males and 4 females) for 4 weeks. Each subject's body weight was monitored every other day during the photoperiod acclimation.

2.2. Metabolic trial

Metabolic measurements were conducted on the day before the photoperiod acclimation began (Day 0) and again at 7-day intervals throughout the acclimation (total of 5 measurements). Metabolic rates were measured using a closed circuit respirometer as described previously [21,23,24]. Briefly, the metabolic chamber size was 3.6 L, and the chamber temperature was controlled within ± 0.5 °C by water bath. Carbon dioxide and water in the metabolic chamber were absorbed with KOH and silica gel. Subjects were weighted before and after each test. All measurements were made between 0900 and 1800. In order to minimize

the effect of circadian rhythms, two SD voles and two LD voles were measured at the same time with four metabolic chambers in each test.

BMR was measured at the temperature of 30±0.5 °C, which is within the thermoneutral zone for this species (27.5–32.5 °C) [21]. Subjects were fasted 3 h prior to being put into the metabolic chamber. After 60-min stabilization in the chamber, metabolic measurement was conducted for 60 min. Oxygen consumption was recorded at 5-min intervals. Two continuous stable minimum recordings were taken to calculate BMR. On the next day, NST was measured with the same order as BMR from the same subjects. Maximum NST was defined as the maximum metabolic response to norepinephrine (NE) [10] and was induced by a subcutaneous injection of NE at 25±1 °C. The mass-dependent dosage of NE (Shanghai Harvest Pharmaceutical Co. LTD) was calculated according to Heldmaier [25]. Two continuous stable maximal recordings were used to calculate maximum NST. Oxygen consumption reached peak values within 15–30 min after NE injection. BMR and NST were corrected to standard temperature and air pressure (STP) conditions and expressed as ml O₂ g^{-0.67} h⁻¹ [9,26,27].

2.3. Sample collection and isolation of mitochondria

After 4-week acclimation, subjects were sacrificed by decapitation between 0900 and 1100 h. Trunk blood was collected for thyroid hormone measurements. Scapular BAT was removed, weighed and homogenized (1:15, w/v) with medium A (containing 250 mM sucrose, 10 mM TES, 1 mM EDTA, 64 μM BSA, pH 7.2). The homogenate was centrifuged at 12096×g for 10 min at 4 °C, the supernatant was discarded, and the precipitate was resuspended with ice-cold medium B (containing 250 mM sucrose, 10 mM TES, 1 mM EGTA, 64 μM BSA, pH 7.2) and centrifuged at 500×g for 10 min at 4 °C. The supernatant was then centrifuged at 8740×g for 10 min at 4 °C, and the resulting pellet was resuspended (1:1, w/v) with ice-cold medium C (containing 100 mM KCl, 20 mM TES, 1 mM EGTA, pH 7.2) and subsequently used for Western blotting.

2.4. Measurements of cytochrome C oxidase (COX) activity and serum thyroid hormones

The COX activity of BAT was measured with the polarographic method using oxygen electrode units (Hansatech Instruments LTD., England) [28]. The mitochondrial protein content of BAT was measured with Folin phenol reagent with bovine serum albumin serving as standards [29]. Serum tri-iodothyronine (T₃) and thyroxine (T₄) concentrations were quantified by radioimmunoassay using RIA kits (China Institute of Atomic Energy, Beijing). This RIA kit was previously validated and used for Brandt's voles following the standard kit instructions [18]. 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.

2.5. Western blotting

Five microliters of BAT mitochondrial protein (4 $\mu\text{g}/\mu\text{l}$) was diluted in 5 μl sample buffer (0.125 M Tris-HCl, pH 6.8, 4% SDS, 0.2M DTT, 20% Glycerol, and 0.2% bromophenol blue) and run on a SDS-polyacrylamide gel (3% stacking gel and 12.5% running gel) together with a prestained protein marker for 2 h. Thereafter, the protein was transferred to a nitrocellulose membrane (Hybond-C, Amersham Biosciences, England). After blocking against non-specific binding using 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 with peroxidase-conjugated goat anti-rabbit IgG (1:5000) (Jackson Immuno. Inc., USA) for 2 h, washed in washing buffer (1 \times PBS, 0.05% Tween 20, 1% Triton X-100, 0.1% SDS), 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 an autoradiography film. UCP1 content was expressed as relative unit (RU) and quantified with Scion Image Software (Scion Corporation).

2.6. Data analysis

Statistical analysis was carried out using the SPSS software package. Group differences in body weight, BMR and NST, and BAT mass, COX activity, T_3 , T_4 , and UCP1 contents at the end of 4-week acclimation were analyzed by two-way analysis of variance (ANOVA) (photoperiod by sex). Further, differences in body weight, BMR, and NST over the course of acclimation were analyzed by two-way ANOVA with repeated measures, and significant differences were further evaluated with Least-Significant Difference (LSD) post-hoc tests. Statistical significance was determined at $p < 0.05$.

3. Results

3.1. Changes of body weight, BMR, and NST over the course of acclimation

Prior to acclimation, no group differences (LD voles: 47.66 ± 1.37 g and SD voles: 48.24 ± 1.65 g, $F_{(1,12)} = 0.07$, $p > 0.05$) or sex differences (female voles: 46.74 ± 1.91 g and male voles: 49.16 ± 0.75 g, $F_{(1,12)} = 1.24$, $p > 0.05$) were found in subjects' body weights (Fig. 1). However, LD voles showed a steady increase in body weight during acclimation compared to SD voles that kept relatively constant body weight. Over the course of acclimation, differences were found within both SD ($F_{(17,119)} = 1.95$, $p < 0.05$) and LD groups ($F_{(17,119)} = 28.76$, $p < 0.01$). LD voles had a higher body weight than SD voles on day 17 of the acclimation and

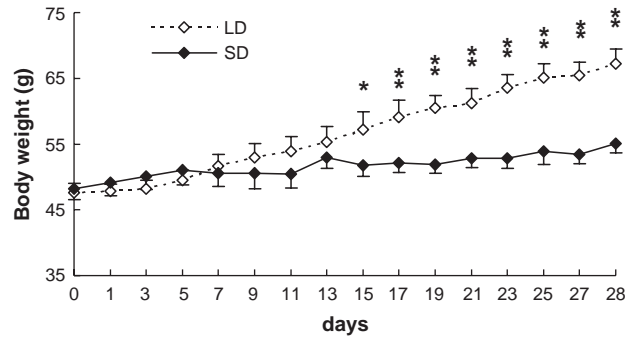


Fig. 1. The effects of photoperiod on body weight of Brandt's voles. On day 17 and thereafter, voles acclimated to long photoperiod (LD; 16L/8D) had significantly heavier body weight compared to voles acclimated to short photoperiod (SD; 8L/16D). Data are mean \pm S.E.M. * $p < 0.05$ and ** $p < 0.01$.

thereafter ($F_{(1,12)} = 6.07$, $p < 0.05$). At the end (Day 28), LD voles were 22% heavier compared to SD voles (LD voles: 67.24 ± 2.26 g and SD voles: 55.09 ± 1.44 g, $F_{(1,12)} = 20.73$, $p < 0.01$). No significant differences were found between sexes (female voles: 59.50 ± 2.36 g and male voles: 62.83 ± 3.37 g, $F_{(1,12)} = 1.55$, $p > 0.05$) or on sex-by-photoperiod interactions ($F_{(1,12)} = 0.53$, $p > 0.05$) at the end of the 4-week acclimation.

Short photoperiod also affected BMR ($\text{ml O}_2 \text{ g}^{-0.67} \cdot \text{h}^{-1}$) and NST ($\text{ml O}_2 \text{ g}^{-0.67} \cdot \text{h}^{-1}$) (Fig. 2). Although no group differences (BMR, SD voles: 7.95 ± 0.27 and LD voles: 8.06 ± 0.28 , $F_{(1,12)} = 0.25$, $p > 0.05$; NST, SD voles: 21.71 ± 0.84 and LD voles: 20.87 ± 0.94 , $F_{(1,12)} = 0.42$, $p > 0.05$) or sex differences (BMR, female voles: 8.31 ± 0.29 and male voles: 7.77 ± 0.23 , $F_{(1,12)} = 2.10$, $p > 0.05$; NST, female voles: 21.43 ± 0.92 and male voles: 21.16 ± 0.89 , $F_{(1,12)} = 0.04$, $p > 0.05$) were found in either measurement prior to acclimation, on day 28 SD voles showed a 20% increase (relative to the initial measurement) in BMR which was significantly higher than that of LD voles (SD voles: 9.53 ± 0.58 and LD voles: 7.97 ± 0.17 ; $F_{(1,12)} = 5.89$, $p < 0.05$). Similarly, SD voles showed a 13% increase over baseline in NST on day 28 of acclimation, which was significantly higher compared to LD voles (SD voles: 24.60 ± 0.88 and LD voles: 20.41 ± 1.32 ; $F_{(1,12)} = 7.57$, $p < 0.05$). In general, BMR and NST in LD voles (BMR, $F_{(4,28)} = 1.32$, $p > 0.05$; NST, $F_{(4,28)} = 0.27$, $p > 0.05$), and BMR in SD voles ($F_{(4,28)} = 2.08$, $p > 0.05$), fluctuated but did not change significantly during the course of acclimation. However, significant differences in NST were found in SD voles over the course of acclimation ($F_{(4,28)} = 3.30$, $p < 0.05$). Male and female voles did not show any significant differences in either measurement (BMR, female voles: 9.04 ± 0.52 and male voles: 8.52 ± 0.49 , $F_{(1,12)} = 0.26$, $p > 0.05$; NST, female voles: 21.96 ± 1.70 and male voles: 23.05 ± 0.88 , $F_{(1,12)} = 0.51$, $p > 0.05$) at the end of acclimation. No significant differences were found on sex-by-photoperiod interactions on BMR ($F_{(1,12)} = 0.00$, $p > 0.05$) or NST ($F_{(1,12)} = 2.63$, $p > 0.05$).

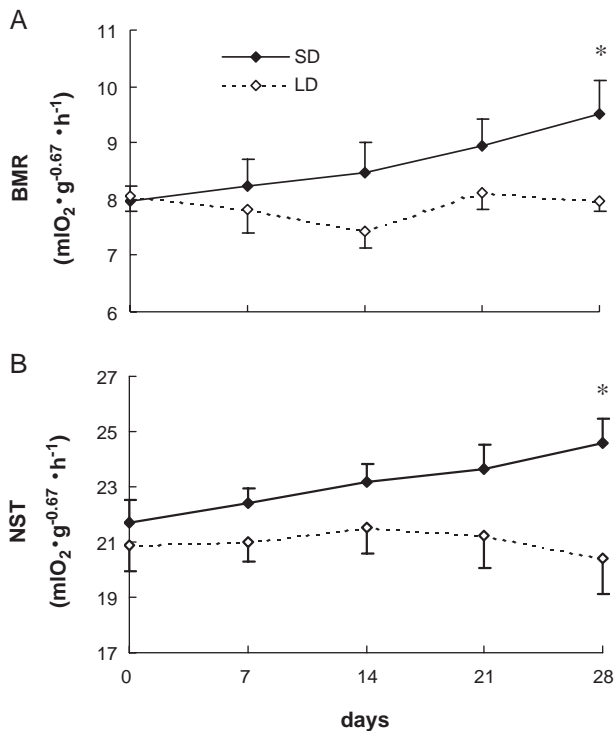


Fig. 2. The effects of photoperiod on the basal metabolic rate (BMR; panel A) and nonshivering thermogenesis capacity (NST; panel B) in Brandt's voles. Voles in short photoperiod (SD; 8L:16D), but not in long photoperiod (LD; 16L:8D), showed gradual increases in their BMR and NST over the course of acclimation. On day 28, SD voles had significantly higher levels of BMR and NST compared to LD voles. Data are mean \pm S.E.M. * $p < 0.05$.

3.2. Effects of photoperiod acclimation on COX activity, thyroid hormones, and UCP1 content

At the end of acclimation, SD and LD voles differed significantly on several measures (Table 1). LD voles had a higher body weight than SD voles, but the two did not differ in their BAT mass. SD voles also showed higher levels of BAT mitochondrial COX activity ($F_{(1,12)} = 5.90$, $p < 0.05$) and higher serum T_3 ($F_{(1,12)} = 13.35$, $p < 0.01$) and T_4 ($F_{(1,12)} = 12.16$, $p < 0.01$) concentrations compared to LD

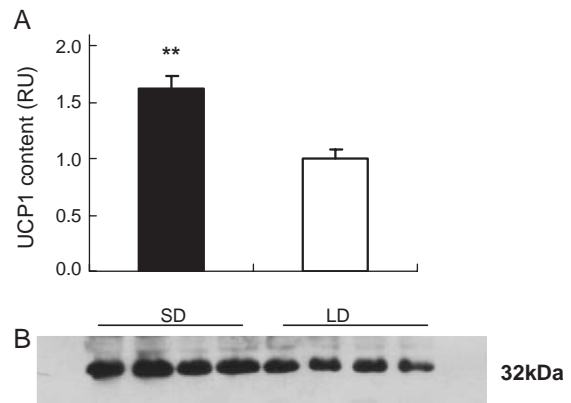


Fig. 3. The effects of photoperiod on the uncoupling protein 1 (UCP1) contents of the brown adipose tissue in Brandt's voles. (A) Voles acclimated to short photoperiod (SD; 8L:16D) had a higher level of UCP1 contents than did voles acclimated to long photoperiod (LD; 16L:8D). Data are mean \pm S.E.M. ** $p < 0.01$. (B) Autoradiograms displaying UCP1 labeling from LD and SD voles.

voles. Further, SD voles had a higher level of BAT UCP1 contents than did LD voles ($F_{(1,12)} = 20.42$, $p < 0.01$) (Fig. 3). Male and female voles did not differ in any of these measures.

4. Discussion

Photoperiod plays an important role in mediating an animal's physiology and behavior. In the present study, we found that alteration in photoperiod significantly influenced thermogenic capacity in Brandt's voles. Voles showed increased BMR and NST over the course of the 4-week SD acclimation. At the end, these voles (SD) had lower body weights, higher levels of BAT COX activity and UCP1 contents, and higher concentrations of serum T_3 and T_4 compared to LD voles. No gender differences were found in any of these measures. Together, these data suggest that SD experience enhances thermogenic capacity similarly in both male and female Brandt's voles.

Table 1

Mitochondrial protein content, cytochrome C oxidase (COX) activity of brown adipose tissue (BAT), and T_3 and T_4 concentrations in Brandt's voles acclimated to long photoperiod (LD; 16L:8D) and short photoperiod (SD; 8L:16D)

	SD		LD		P
	Female	Male	Female	Male	
Body weight (g)	54.40 \pm 1.72	55.78 \pm 2.52	64.60 \pm 2.39	69.88 \pm 3.67	0.01
BAT mass (g)	0.22 \pm 0.03	0.18 \pm 0.01	0.27 \pm 0.04	0.21 \pm 0.04	ns
BAT Mt protein (mg g ⁻¹ BAT)	6.59 \pm 0.65	6.07 \pm 0.66	6.76 \pm 0.84	6.09 \pm 0.65	ns
BAT activity of COX					
(nmol min ⁻¹ mg ⁻¹ Mt protein)	225.96 \pm 24.10	237.07 \pm 26.38	179.15 \pm 9.46	177.46 \pm 36.03	ns
(nmol min ⁻¹ g ⁻¹ tissue)	1357.33 \pm 84.63	1516.20 \pm 220.63	1101.93 \pm 98.93	1106.13 \pm 97.11	0.05
Serum T_3 (ng ml ⁻¹)	1.10 \pm 0.05	0.98 \pm 0.04	0.74 \pm 0.10	0.70 \pm 0.13	0.01
Serum T_4 (ng ml ⁻¹)	34.10 \pm 2.84	29.79 \pm 3.75	20.03 \pm 3.57	18.88 \pm 4.05	0.01
UCP1 (relative unit)	1.69 \pm 0.10	1.55 \pm 0.21	0.91 \pm 0.11	1.09 \pm 0.10	0.01

Data are mean \pm SE.

4.1. Changes of body weight, BMR, and NST during photoperiod acclimation

Seasonal changes in body weight are an important adaptive strategy for many small mammals [30,31]. Several environmental factors, such as temperature, photoperiod, and food quantity and quality, have been implicated in the regulation of seasonal variations in animals' body weights. For example, in Siberian hamsters [32] and meadow voles [33], LD animals were heavier than SD animals, indicating that exposure to short photoperiod decreased the animals' body weights. This notion is supported by our data from the present study showing that SD voles had significantly lower body weight compared to LD voles after 4 weeks of photoperiod acclimation. The time course data illustrated two interesting findings. First, no differences were found between the LD and SD voles in body weight until day 17 of acclimation, indicating a necessary time period by which SD photoperiod exerted significant effects on body weight of Brandt's voles. Second, over the course of acclimation, LD voles displayed a steady increase whereas SD voles showed no changes in their body weight, suggesting that such difference was due to the fact that SD voles failed to increase their body weight during acclimation. Interestingly, the opposite patterns were found in BMR and NST: SD voles displayed a gradual increase in the levels of BMR and NST whereas LD voles did not show any significant changes over the course of photoperiod acclimation, despite that both SD and LD voles were fed with the same type of food ad libitum. It should be noted that although two SD and two LD subjects were tested simultaneously each time to minimize potential effects of circadian rhythms on our measurements, we still cannot exclude the possibility that variations in the time of testing (conducted between 0900 and 1800) may have influences on our data. Nevertheless, we still found significant differences on metabolic parameters between SD and LD animals, suggesting that the photoperiod effects on thermogenesis were strong and significant. Finally, SD has been found to influence energy intake in rodents in a species-specific manner [34–37], and our recent data in Brandt's voles indicated that SD voles showed 31% higher energy intake (indicated by increases in food intake) than LD voles (Z.J. Zhao and D.H. Wang, unpublished data). Together, these data suggest that the lack of increase in body weight for SD voles was likely due to increased BMR and NST, rather than a decrease in animal's energy intake, during the acclimation.

Seasonal changes of BMR and NST are an important physiological adjustment for survival and reproductive success of many mammalian species. Enhanced thermogenic capacity, especially in NST, has been found to be associated with winter conditions in a variety of rodent species, including northern three-toed jerboas (*Dipus sagitta*), midgy gerbils (*Meriones meridianus*), desert hamsters (*Phodopus roborovskii*) and striped hamsters

(*Cricetulus barabensis*) [23], golden spiny (*Acomys russatus*) and common spiny mice (*Acomys cahirinus*) [38] and northern flying squirrels (*Glaucomys volans*) [39]. Short photoperiod could act independently and/or synergistically with cold temperature to enhance thermogenic capacity of small rodents [2,3,8–10,40]. Previous studies in Brandt's voles have shown that environmental temperature and photoperiod interact to regulate thermogenic capacity [9,22]. Our data from the present study indicated that photoperiod alone could affect BMR and NST of Brandt's voles and thus may play an important role in preparing animals for coming winter conditions in a natural setting.

4.2. Short photoperiod enhanced thermogenic capacity

In addition to BMR and NST, LD and SD voles in the present study also differed in several biochemical and hormonal markers indicative of their differences in thermogenic capacity. For example, BAT is a major organ for NST [14,41], and an increase in COX activity of BAT indicates enhanced NST capacity [11]. Early studies have shown that cold acclimation can induce an increase in COX activity of BAT accompanied by enhanced NST capacity in several rodent species including Brandt's voles [2,9,18]. Our data indicated that short photoperiod alone induced an increase in COX activity of BAT in Brandt's voles that also displayed enhanced NST capacity. This finding is in agreement with the finding from a study in cold-exposed root voles in which exposure to short photoperiod elevated COX activity and NST [9].

Changes in UCPI may indicate NST capacity in rodents. For example, cold induces an increase in BAT UCPI expression, together with enhanced NST, in Djungarian hamsters, Brandt's voles, Mongolian gerbils, and ground squirrels [18,42,43]. Exposure to short photoperiod increased BAT UCPI protein expression in common spiny mice [38] and UCPI mRNA expression in Djungarian hamsters [16], both of which were accompanied by increased NST capacity. In the present study, 4-week SD experience induced a significant increase in the levels of UCPI and NST in Brandt's voles. It has been suggested that photoperiod signals could be converted into biochemical signals through melatonin secretion via the pineal gland, and the sympathetic drive to BAT is involved in the mediation of SD-induced up-regulation of UCPI expression and NST capacity [44,45]. It is interesting to note that cold [18] and short photoperiod (present study) induced similar changes in UCPI expression, COX activity, and NST capacity in Brandt's voles, and such correlative changes in Brandt's voles are similar to that found in other species of rodents [9,16,38]. Therefore, it is possible that a common underlying mechanism mediated by UCPI and COX serves to regulate thermogenic responses to environmental stimulation in Brandt's voles as well as in other species of rodents. This speculation needs to be tested in further studies.

Finally, thyroid hormones can increase energy expenditure and stimulate basal thermogenesis by lowering metabolic efficiency. Metabolic adjustment of thyroid hormones may correlate with thermogenic capacity in some cold-exposed rodents [18]. Our data showed that short photoperiod alone could increase serum T₃ and T₄ concentrations in Brandt's voles. These data are consistent with the previous finding in cotton rats (*Sigmodon hispidus*), further supporting the notion that thyroid hormones are involved in the thermogenic enhancement induced by photoperiod [17]. Interestingly, UCP1 has been suggested to be a potential candidate for mediating thyroid thermogenesis [46].

4.3. Photoperiod effects on thermogenic capacity are not sexually dimorphic

In the present study, although SD had significant effects on thermogenic capacity in Brandt's voles, no sex differences were found in any of the above-mentioned measures indicative of thermogenic capacity. Several previous studies examined potential sex differences in the effects of photoperiod on thermogenic capacity. For example, no sex differences were found in body weight and resting metabolic rates in bank voles [36] and in SD-induced elevation in BMR of gray mouse lemurs (*Microcebus murinus*) [37]. Our data seem to support this finding; male and female Brandt's voles responded to SD similarly in their thermogenic capacity. It should be pointed out that a drawback of the present study was the small sample size, and therefore, more studies should be performed to carefully address this issue.

5. Conclusion

Brandt's voles mainly live in habitats in which environmental conditions show remarkable seasonal variations [21,47]. In winter, these animals show enhanced thermogenic capacity that is considered to be an important adaptation for their survival [21]. In the present study, we found that short photoperiod, in the absence of changes in ambient temperature, significantly elevated several physiological, hormonal, and biochemical markers all indicative of enhanced thermogenic capacity in both male and female Brandt's voles. These data suggest that photoperiod could serve as an environmental cue to alter animal's thermogenic adaptation, and thus may play an important role in the survival and reproductive success of this species.

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