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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.

Keywords: Basal metabolic rate (BMR); Nonshivering thermogenesis (NST); Cytochrome *C* oxidase (COX); Thyroid hormone; Uncoupling protein 1 (UCP1)

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 O_2 $g^{-0.67}$ 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 12 096×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 (T_3) and thyroxine (T_4) 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 T_3, and 4.3% and 7.6% for T_4, respectively.