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Energy metabolism, thermogenesis and body mass regulation in tree shrew (*Tupaia belangeri*) during subsequent cold and warm acclimation

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ABSTRACT

Environmental cues play important roles in the regulation of an animal's physiology and behavior. The purpose of the present study was to test the hypothesis that ambient temperature is a cue to induce adjustments in body mass, energy intake and thermogenic capacity, associated with changes in serum leptin levels in tree shrews (*Tupaia belangeri*). We found that tree shrews increased basal metabolic rate (BMR), energy intake and subsequently showed a significant decrease in body mass after being returned to warm ambient temperature. Uncoupling protein 1 (UCP1) content in brown adipose tissue (BAT) increased during cold acclimation and reversed after rewarming. The trend of energy intake increased during cold acclimation and decreased after rewarming; the trend of energy intake during cold acclimation was contrary to the trend of energy intake during rewarming. Further, serum leptin levels were negatively correlated with body mass. Together, these data supported our hypothesis that ambient temperature was a cue to induce changes in body mass and metabolic capacity. Serum leptin, as a starvation signal in the cold and satiety signal in rewarming, was involved in the processes of thermogenesis and body mass regulation in tree shrews.

1. Introduction

In endothermic small mammals, ability to survive in cold environment requires proper strategies and efficient thermoregulatory mechanisms (McNab, 2002). Energy metabolism is a critical component for distribution, abundance and reproductive success of rodents (Bozinovic, 1992). The animals' body mass, energy budget, thermogenesis and phenotypic plasticity are influenced by the environmental temperature (William and Christain, 2006). In particular, low ambient temperatures caused an increased energy consumption combined with a reduction of utilizable resources (Voltura and Wunder, 1998; Karasov and McWilliams, 2005). To cope with winter or cold conditions, different small mammals have different responses in their overall body mass change and enhance nonshivering thermogenesis (NST) (Nagy and Negus, 1993; Concannon et al., 2001; Bartness et al., 2002; Gündüz, 2002; Zhu et al., 2010; Zhang et al., 2011). An increase in NST could be attributed to the increased expression of uncoupling protein 1 (UCP1), which is the unique thermogenic protein found in the inner membrane of mitochondria in brown adipose tissue (BAT). In endothermic mammals, the function of brown adipose tissue is to transfer energy from food into heat; it is physiologically, both the heat produced and the resulting decrease in metabolic efficiency can be of significance. Both the acute activity of the tissue, i.e., the heat production, and the recruitment process in the tissue (that results in a higher thermogenic capacity) are under the control of norepinephrine released from sympathetic nerves (Cannon and Nedergaard, 2004). UCP1 uncouples the oxidation of fuel from adenosine triphosphate (ATP) production and transforms electrochemical energy into heat (Cannon and Nedergaard, 2004). Being the terminal enzyme in oxidative phosphorylation in mitochondria, cytochrome *c* oxidase (COX) is involved in mitochondrial energy metabolism (Kadenbach et al., 2000). The increased energy expenditure for thermogenesis in the cold may be compensated by hyperphagia in small mammals (Kenagy et al., 1989).

Leptin, a 16-kDa protein, is synthesized in the adipose tissue and secreted into the bloodstream. The discovery of leptin has improved our understanding of the relationship between adipose tissue and energy homeostasis (Campfield et al., 1995), it acts with the long-form OB-Rb receptors in the hypothalamic arcuate nucleus to regulate food intake and body mass (Friedman and Halaas, 1998). Leptin has been found to affect not only feed intake, but also the neuroendocrine-axis, metabolism and immunological processes (Hausman and Barb, 2010). It is also hypothesized that leptin contributes to maintaining body mass by regulating feed intake and energy expenditure (Friedman and Halaas, 1998). When animals were subjected to a chronic administration of leptin, some found that the animals' body mass decreased by the decrease in feed intake and the increase in energy utilization, as the female Wistar rats (Abelenda et al., 2003) and *Sus scrofa* showed (Ramsay et al., 2004). Furthermore, leptin administration was found to stimulate sympathetic nerve activity and increase body temperature, basal metabolic rate (BMR), nonshivering thermogenesis

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(NST), and uncoupling protein 1 (UCP1) mRNA expression in brown adipose tissue (BAT); indicating its potential involvement in the regulation of thermogenesis (Haynes et al., 1997; Scarpace and Metheny, 1998). Despite these findings, the role of leptin in energy expenditure and heat production is an area of current contention in animal or human physiology (Hukshorn and Saris, 2004), and contradictory results have been reported. In cold-exposed rats, low serum leptin levels were accompanied by an increase in UCP1 gene expression (Bing et al., 1998), and leptin administration reduced BAT thermogenesis (Abelenda et al., 2003). These studies reveal that the role of leptin in regulating energy intake and expenditure is complex. Some factors, such as abiotic environmental and physiological factors, altered energy metabolism in wild animals, and temperature influences animals' body mass, energy budget and thermogenesis (Bing et al., 1998; Voltura and Wunder, 1998; Abelenda et al., 2003).

Tree shrews, *Tupaia belangeri* (Mammalia: Scandentia: Tupaiidae), are unique species of small mammals; the remainder of the order's extant diversity is in mainland Southeast Asia, from China to Malaysia, and through the Malaysian and Indonesian islands of the Sunda region. *T. belangeri* live at the highest latitude, with the Yunnan–Kweichow Plateau being its northern limit (Wang et al., 1991). Previous studies described the physiological characteristics of *T. belangeri*, a pronounced seasonal cycle of resting metabolic rate (RMR), nonshivering thermogenesis (NST) and energy balance (Wang et al., 1994, 1995, 1999b; Zhang et al., 2001). A further study reported that thermogenesis was increased during cold acclimation in tree shrews (Wang et al., 1995; Zhang et al., 2011, 2012).

The present study was designed to investigate the role of cold acclimation and rewarming on the energy metabolism and body mass regulation in tree shrews. We hypothesized that ambient temperature was an important cue to influence body mass, and leptin was involved in the regulation of energy metabolism and body mass. We can predict that the tree shrew increases body mass during cold acclimation, but during rewarming, the body mass decreases. Leptin will be decreased as a signal to increase the energy intake in the cold and it is increased as a satiety signal to inhibit energy intake in the warm conditions.

2. Materials and methods

2.1. Animals and experimental designs

All animal procedures were licensed under the Animal Care and Use Committee of Institute of Zoology, the Chinese Academy of Sciences. The tree shrews, *T. belangeri*, were captured (25°25′–26°22′ N, 102°13′–102°57′ E, 1679 m altitude) around boscage at Luquan County in June 2010, then brought and bred at the School of Life Sciences, Yunnan Normal University, Kunming (1910 m altitude). All animals were healthy adults. Each tree shrew was housed individually in a wire cage (40 cm × 40 cm × 40 cm) with no bedding. The photoperiod, ambient temperature and humidity were maintained at 12L:12D (lights on at 08:00 h), 25 ± 1 °C, and 85%–92% relative humidity, respectively. They were fed with mixed food; the food mixture contained the following ingredients in proportion (by weight): 90 parts cornmeal, 5 parts milk, and 5 parts sugar, and together with water, were provided ad libitum.

2.2. Experiment 1

In order to test the effects of ambient temperature on body mass, sixteen weight-matched tree shrews were housed individually, kept for at least two weeks, and then randomly assigned into two groups. One group (the treated group: cold + warm) was transferred to cold (5 ± 1 °C) and maintained for 4 weeks and then returned to 25 ± 1 °C for further 4 weeks. The other group (control) remained at 25 ± 1 °C throughout the test. All animals were maintained under 12L:12D photoperiod with lights on at 08:00 h. In the experiment, the body mass, BMR, and energy intake were measured.

2.3. Experiment 2

We further tested the physiological actions of leptin in the metabolic responses to environment that remain to be completely elucidated and its role in regulating energy intake and body mass in tree shrews. This experiment was carried out with another 50 individuals (the 8 animals' death in the experiment and its causes, was unrelated to the experiment). They were acclimated to light (12L:12D, lights on 08:00 h) and temperature (5 ± 1 °C) for 28 days, then returned to light (12L:12D, lights on 08:00 h) and temperature (25 ± 1 °C) for further 28 days. On days 0, 1, 7, 28, 29, 35, and 56, the different individuals were sacrificed and these 7 groups were named C0, C1, C7, C28, RW1, RW7, and RW28, respectively (C, cold; RW, rewarming). The animals' body mass was measured routinely before they were killed. The experimental design is shown in Fig. 1. The shrews were killed between 09:00 and 11:00 h by decapitation. Blood was centrifuged at 600 g for 30 min, and produced the serum sample was stored at −20 °C for later measurement. The interscapular BAT was surgically removed, immediately frozen in liquid nitrogen, and stored at −80 °C for determining BAT cytochrome *c* oxidase (COX) activity and UCP 1 contents.

2.4. Gross energy intake

Food intake was measured in metabolic cages for 3 days a week as described previously (Liu and Wang, 2007). During each test, tree shrews were housed individually in a mesh metabolic cage (20 × 15 × 15 cm^3), in which food and water were provided *ad libitum*. Residual food and feces were collected, oven dried at 60 °C to a constant mass, and then separated manually. Energy contents of food were measured by YX-ZR/Q automatic calorimeter instrument (U-therm Industry Co. Ltd., Changsha, China). Gross energy intake (kJ/day) = dry food intake (g/day) × gross energy content of food (kJ/g).

2.5. Metabolic rate (MR)

At the end of the acclimation period, metabolic rate was measured using open-system respirometry. A cylindrical, opaque respirometer chamber (760 mL) was housed in an incubator (SPX-300, Shanghai Leaps Forward the Medical Instrument Co. Ltd., Shanghai, China) to maintain constant ambient temperature; ambient temperature was controlled with a temperature controlled cabinet at 30 ± 0.5 °C (the thermal neutral zone (TNZ) of *T. belangeri* is 30–35 °C, Wang et al., 1994). The air was forced through the chambers at a flow rate of 1000 mL/min using precision rotameters (7300 and 7400 series, Matheson, Montgomeryville, PA, USA). Flow rate was continuously monitored with a mass flowmeter (G265, Qubit Systems, Kingston, ON, Canada) and recorded to a computer; the subsample rate was approximately 200 mL/min. Each animal was generally in the metabolic chamber for at least 1.5 h, then we measured the BMR.

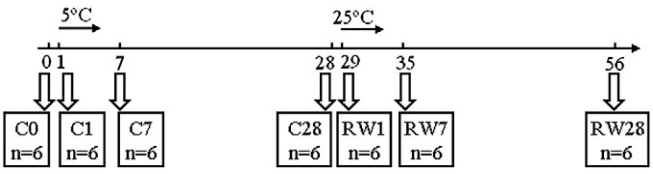

Fig. 1. The design of Experiment 2.Note: The experiment started with 50 tree shrews, bred at 25 ± 1 °C and under a photoperiod of 12L:12D. They were exposed to 5 ± 1 °C for 28 days and then returned to 25 ± 1 °C. On days 0, 1, 7, 28, 29, 35, and 56, different individuals were killed and the groups were named as C0, C1, C7, C28, RW1, RW7, and RW28 respectively (C0 = before cold acclimation; C1, C7, and C28 = days of cold acclimation; RW1, RW7, and RW28 = days after rewarming).