

ORIGINAL ARTICLE

The relationship among metabolic rate of tree shrews (*Tupaia belangeri*) under cold acclimation

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Many small mammals inhabiting cold environments display enhanced capacity for seasonal changes in nonshivering thermogenesis (NST) and thermoregulatory maximum metabolic rate (MMR). However, it is not known how this plasticity remains in a mammal that rarely experiences extreme cold fluctuations. In order to answer this question, we determined body mass (M_b), basal metabolic rate (BMR), NST, and MMR on a tree shrews (*Tupaia belangeri*), acclimated to cold (5 °C) conditions. NST was measured as the maximum response of metabolic rate (NSTmax) after injection of norepinephrine (NE) in thermoneutrality minus BMR. Maximum metabolic rate was assessed in animals exposed to enhanced heat-loss atmosphere (He-O₂) connected with an open-flow respirometer. Body mass and metabolic variables increased significantly after cold acclimation with respect to control group but to a high extent (BMR, 87.97%; NST, 69.77%; and MMR, 32.35%). However, aerobic scope (MMR/BMR), and calculated shivering thermogenesis (ST) did not significantly change with control group. Our data suggest: 1). The body mass and the capacity of heat production in the cold acclimated group were higher; 2). The increase of BMR and MMR during cold acclimation was the main pattern of heat production in the tree shrews.

Key words: Cold acclimation; metabolic rate; *Tupaia belangeri*; thermogenesis plasticity

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The study of phenotypic plasticity has been a central topic in evolutionary ecology (Schlichting and Pigliucci, 1998). Phenotypic plasticity can be defined as reversible changes in the traits of organisms due to changes in internal or external environmental conditions (Naya et al. 2008). Eutherian nonhibernating small mammals shown several mechanisms to survive under cold environments. Thermogenic capacity include basal

metabolic rate (BMR), non-shivering thermogenesis (NST), and shivering thermogenesis (ST; Bozinovic and Rosenmann, 1989); the maximum metabolic rate (MMR) included the BMR, NST and ST. NST was summarized by Jansky (1973) is the most plastic of metabolic variables. However, cold acclimation may increase BMR or decrease as a strategy to minimize energy expenditure in an environment of diluted energy offer (Veloso and Bozinovic 1993). ST is

another way to produce heat; however, it is more energetically expensive than NST because energy is delivered into heat and muscular work. Indeed, it is generally believed that ST is used mainly during periods of low metabolic load (Jansky 1973; Lilly and Wunder 1979; Bockler and Heldmaier 1983).

Tree shrews, *Tupaia belangeri* (Mammalia: Scandentia: Tupaiidae), are the unique species 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 demonstrate that tree shrews shown a seasonal increased in body mass and thermogenic capacity to adapt to the increase of energy requirements for thermoregulation (Wang et al. 1994, Zhu et al. 2012), and seasonal variety in spermatogenesis of adult tree shrews (Cao, 1989). Tree shrews cold acclimated for 28 d decreased their serum leptin levels and increased thermogenesis (Zhang et al. 2011, 2012a), they acclimated to ward after cold acclimation for 28 d increased their serum leptin levels and decreased thermogenesis (Zhang et al. 2012b), short photoperiod can induce thermogenic capacity increased (Zhang et al. 2012c). However, we know nothing about the action of photoperiod with changes in serum leptin levels and its role in body mass regulation and thermogenesis in tree shrews.

Small mammals that regularly encounter seasonal variation conspicuous variables such as temperature, photoperiod and food availability in their habitats commonly alter their thermogenic capacity after cold acclimation (Hayes and Chappell 1986; Bozinovic et al. 1990; Kronfeld-Schor et al., 2000). We designed our study to examine how

regulate body temperature in relation to changes in cold conditions. That is, we measured the effect of changes in T_a on body mass (M_b), BMR, NST, and MMR. Considering the low variability of cold environment, we predict low plasticity on these variables.

MATERIALS AND METHODS

Animal

All animal procedures were licensed under the Animal Care and Use Committee of Institute of Zoology, the Chinese Academy of Sciences. Tree shrews, *T. belangeri* were captured (25°25'~26°22' N, 102°13'~102°57' E, 1679 m in altitude) around boscage at Luquan County in November 2009. The average yearly temperature was 15.6 °C, mean monthly temperature ranges from 7.8 °C in winter to 19.6 °C in summer. After being captured, then brought and bred at the School of Life Sciences, Yunnan Normal University, Kunming (1910 m in altitude). Each weight-matched tree shrew was housed individually in a wire cage (40 cm × 40 cm × 40 cm) with no bedding, all animals (23 males) were healthy adults. The photoperiod, ambient temperature and humidity were maintained at 12L: 12D (light on at 08.00 am), (25±1)°C, and 85%-92% relative humidity, respectively. They were kept for at least 2 weeks, and then were randomly assigned into two groups. The two groups were control group (0 d) and cold acclimation groups (28 d), and there are 8 animals in every group. The control group was acclimation to light (12L: 12D, lights on 0800 am) and temperature (25±1°C); the cold acclimation group was acclimation to light (12L: 12D, lights on 0800 am) and temperature (5±1°C). All pregnant, lactating or young individuals were excluded. Body mass in *T. belangeri* showed no significant variations before the experiment ($F_{(1, 14)}=1.57$, $P>0.05$, $n=16$). They were fed the mixed food; the food mixture

contained 25.0% crude protein, 6.3% crude fat, 4.6% crude fibre, 7.4% ash, and 0.96 KJ/g gross energy (Zou et al. 1991), with a ratio of every two-day interval feed appropriate apples, pears and other fruits, and water were provided *ad libitum*. The tree shrews were fed once daily at 10:00 am, additionally, with a ratio of 3:1 every two-day interval they received apples, pears and other fruits.

Measurement of Metabolic Rate (MR)

At the end of the acclimation period, metabolic rate was measured using open-system respirometry. A cylindrical, opaque respirometer chamber (760ml) 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 $30 \pm 0.5^\circ\text{C}$ (The thermal neutral zone (TNZ) of *T. belangeri* is $30 - 35^\circ\text{C}$, 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); 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 and all measurements were made daily between 1000 and 1400. The fractional concentrations of O_2 and CO_2 entering and exiting the chamber were measured using a gas analyzer (PowerLab ML206, ADInstruments Co. Sydney, Australia); air was dried (Drierite, W.A. Hammond Drierite Co., Xenia, OH, USA) prior to analysis. Ambient temperature (T_a) was measured via a T-type thermocouple situated within the respirometer chamber, just below the lid; unless otherwise noted, T_a was 30°C for all

measurements. Gas flow rate, and O_2 and CO_2 concentrations were recorded to computer during each experiment (PowerLab ML870, ADInstruments Co. Sydney, Australia). The animal's body mass was measured routinely before and after experiment 1. The method used for calculating the metabolic rate is detailed in Hill (1972):

$$\text{MR} = \frac{\text{FR} \times (\text{FiO}_2 - \text{FeO}_2) - \text{FR} \times \text{FeO}_2 \times (\text{FeCO}_2 - \text{FiCO}_2)}{1 - \text{FeO}_2}$$

Note: FR = flow rate (ml/min), FiO_2 = O_2 input fractional concentration, FiCO_2 = CO_2 input fractional concentration, FeO_2 = O_2 excurrent fractional concentration, FeCO_2 = CO_2 excurrent fractional concentration.

Maximum NST defined as the maximum metabolic response to norepinephrine (NE), and was measured as the highest oxygen consumption following by scapular subcutaneous injection norepinephrine (NE) bitartrate (Shanghai Harvest Pharmaceutical Co. LTD, Shanghai, China). The dosage of NE was calculated based on preliminary experiments and the equation described by Jansky (1973) and Heldmaier (1971): NE dosage (mg/kg) = $6.6\text{Mb}^{-0.458}$ (g). Maximum NST was considered the highest 3-min average after NE injection (Heldmaier et al. 1982; Van Sant and Hammond 2008). Oxygen consumption reached peak values within 15-30 min after NE injection (Zhang et al. 2011).

Maximum metabolic rate (MMR) was measured as described by Zhang et al. (2012d). We measured MMR in a He-O_2 atmosphere according to the procedure of Rosenmann and Morrison (1974) in an open-circuit respirometer, as described by Chappel and Bachman (1995). In brief, a mixture of He (79%) and O_2 (21%) was passed through a volumetric flowmeter before entering the chamber (i.e., a positive pressure system), which was maintained at 1000 ml/min. The MMR measured as in the case of

BMR measurements. Chamber temperature (-5.0 ± 0.5 °C) and T_b were measured. We defined instantaneous $\dot{V}O_2$ as the highest O_2 averaged over 2 min of the last 5 min of 15 min Heliox exposure.

Shivering Thermogenesis (ST)

We calculated ST in each acclimation using the equation $MMR = BMR + NST + ST$ for small eutherian mammals (Jansky 1973; Wunder and Gettinger 1996; Degen 1997).

Statistical analyses

Statistical analyses were performed using sigmaplot10.0, SPSS for Windows16.0 statistical package. Results are reported as mean \pm SE and $P < 0.05$ was considered to be statistically significant.

RESULTS

Body mass

Prior to acclimation, there was no significant difference in body mass between the control group and the cold acclimation group ($t = 0.087$, $P > 0.05$). During the 28 days, the body mass significantly increased 8.9% (Table 1). During the course of cold acclimation, there were significant differences in body mass within the treated group ($F = 4.321$, $P < 0.05$), but not within the control group ($F = 0.347$, $P > 0.05$).

Thermogenic capacity

BMR was influenced by ambient temperature.

Prior to acclimation, BMR in tree shrews showed no significant difference between groups ($t = 0.786$, $P > 0.05$). BMR in tree shrews gradually increased by 87.97% during the cold acclimation from 0 to 28 days (Table 1). NST was influenced by ambient temperature. Prior to acclimation, BMR in tree shrews showed no significant difference between groups ($t = 0.673$, $P > 0.05$). NST in tree shrews gradually increased by 69.77% during the cold acclimation (Table 1). During the course of cold acclimation, there were significant differences in body mass within the treated group ($F = 14.412$, $P < 0.05$), but not within the control group ($F = 0.532$, $P > 0.05$).

Basal metabolic rate, MMR and NST showed significant differences between the control group and the acclimation group (Table 1; Fig. 1). The mean values of $NST + BMR (= NST_{max})$ were significantly different between the control group and the acclimation group (Table 1; Fig. 1). Although Table 1 shows positive means of ST, most individuals had similar values of NST_{max} and MMR, which gave low, and even negative, magnitudes of ST in some cases. Mean calculated ST was no significant difference after both acclimations (Table 1). The MMR increased by 32.35% and was near twice as NST_{max} in tree shrews during cold acclimation (Table 1; Fig. 1).

Table 1: Body mass (M_b) and metabolic variable after cold acclimation

| | Control | Cold acclimation (28 days) | <i>P</i> values |
|---|-----------------|-----------------------------|-----------------|
| M_b (g) | 129.5 \pm 3.4 | 140.9 \pm 3.6 | $P < 0.05$ |
| BMR (ml O_2 g ⁻¹ h ⁻¹) | 1.58 \pm 0.13 | 2.97 \pm 0.34 | $P < 0.01$ |
| NST (ml O_2 g ⁻¹ h ⁻¹) | 3.54 \pm 0.11 | 6.01 \pm 0.23 | $P < 0.01$ |
| MMR (ml O_2 g ⁻¹ h ⁻¹) | 7.79 \pm 0.37 | 10.31 \pm 0.37 | $P < 0.01$ |
| ST (ml O_2 g ⁻¹ h ⁻¹) | 2.67 \pm 0.11 | 1.23 \pm 0.29 | $P > 0.05$ |
| MMR/ BMR | 4.93 | 3.47 | $P < 0.05$ |

Note. Shivering thermogenesis (ST) was computed for each individual as maximum metabolic rate (MMR) minus basal metabolic rate (BMR) minus nonshivering thermogenesis (NST). Nonshivering thermogenesis (NST) was computed for each individual as maximum nonshivering thermogenesis (NST_{max}) minus basal metabolic rate (BMR). Data are shown as mean \pm SE; $n = 8$ in all cases.

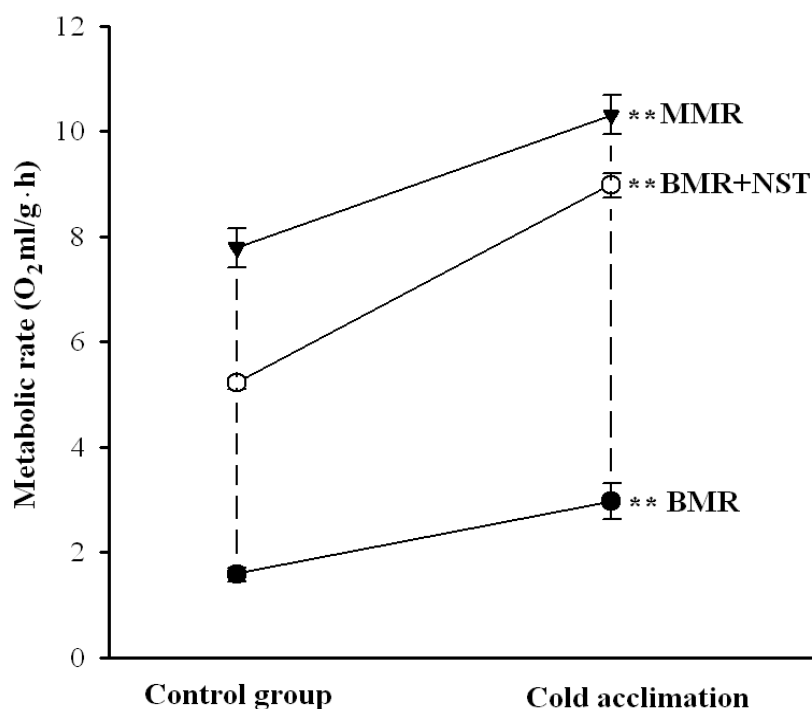


Figure 1. Maximum metabolic rate (MMR), nonshivering thermogenesis (NST), and basal metabolic rate (BMR) of tree shrews after cold acclimation. Asterisks denote significant differences between experimental group ($P < 0.01$).

DISCUSSION

Classic studies dealing with cold acclimation on the energetic features of small mammals of seasonal environments reported an increase in thermogenic capability, increasing survival probabilities. Classic studies dealing with cold acclimation on the energetic features of small mammals of seasonal environments reported an increase in thermogenic capability, increasing survival probabilities. For example, a reduced resting metabolic rate and a high NST capacity not only allows the maintenance of a low metabolic heat production during activity but also increases heat production quickly under cool to cold conditions (Degen, 1997).

Basal Metabolic Rate

Our values of BMR (1.58 ± 0.13 and 2.97 ± 0.34 ml O_2 $g^{-1} h^{-1}$, in control group and cold acclimation group individuals, respectively), and it increased by

87.98% after cold acclimation, are similar current to those previously reported for other rodents, but higher than those of other rodents, such as *Microtus agrestis* (McDevitt and Speakman, 1994).

Regarding the influence of acclimation regime, BMR has two aspects. First, it can be considered as an adaptive strategy to minimize energy expenditure when metabolic loads are high (i.e., BMR is regulated strategically). Indeed, several studies reported that BMR decreased after acclimation to low energy availability and/or high energy demands (Veloso and Bozinovic, 1993; Corp et al., 1997). Second, the high BMR detected after cold acclimation can be the by-product of enhanced metabolic expenditure triggered by food processing and oxygen delivery systems and by thermogenic tissue (i.e., BMR is regulated homeostatically). When animals are faced with low-quality food, it is reasonable to expect compensations in BMR through reducing the size of organs other than

those related to energy acquisition (Geluso and Hayes 1999). However, during cold acclimation, metabolically active organs cannot be reduced without limiting thermogenic capacity (Koteja 1996a, 1996b), and because of that, MMR is generally coupled with BMR (Bennet and Ruben 1979; Table 1). In those cases, BMR is predicted to increase after acclimation period to cold conditions (Wiesinger et al. 1990), as we found in cururos (Table 1). This result allows us to predict that cold-acclimated cururos increase organ masses to face with higher metabolic demands.

Maximum Metabolic Rate, Nonshivering Thermogenesis, and Shivering Thermogenesis

In extant eutherians, contribution of NST is higher in small eutherians, such as rodents, in comparison to larger eutherians (Cannon and Nedergaard, 2004). The body size of primitive eutherians is as summed to be small. Values of NST ($3.54 \pm 0.11 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$, in control group individuals) are close to other similarly sized rodents ($M_b = 60\text{--}250 \text{ g}$, NST = $0.67\text{--}3.30 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$, Jansky, 1973; Haim, 1996; Nespolo et al., 1999; Kronfeld-Schor et al., 2000; Haim et al. 1984; Hislop and Buffenestein, 1994; Goldman et al., 1999), but the values of NST ($6.01 \pm 0.23 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$, in cold acclimation group individuals) was fairly high compared with them. Unfortunately, comparisons are complicated because these authors used anesthetized animals, a procedure that underestimates NST (Ellison and Skinner, 1990; Wunder and Gettinger, 1996), which is clearly shown in the NST measurements done in anesthetized and awake naked mole rats (Goldman et al., 1999).

However, MMR (7.79 ± 0.37 and $10.31 \pm 0.37 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$, in control group and cold acclimation group individuals, respectively), the values of MMR was higher than NSTmax after cold acclimation

(Table 1; Figs. 1) give large ST. Observed differences between MMR and NSTmax are considered within the range of error of the measurements, so we conclude that ST is near the NSTmax. In the previous studies, shivering is the main way of thermogenesis after cold acclimation in birds (Saarela and Heldmaier 1987; Dietz et al. 1997) and in humans under cold environment (Giesbrecht et al. 1997). In contrast, the role of ST during cold acclimation is lower understood, suggesting that this way of heat production is used only when NST is insufficient (Jansky 1973; Lilly and Wunder 1979; Bockler and Heldmaier 1983; Nespolo et al. 1999).

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