Journal of Thermal Biology 34 (2009) 60–69

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Adaptive thermogenesis in Brandt's vole (*Lasiopodomys brandti*) during cold and warm acclimation

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ARTICLE INFO

Article history:
Received 4 June 2008
Accepted 4 November 2008

Keywords:
Brandt's vole
Lasiopodomys brandti
Cold acclimation
Warm acclimation

ABSTRACT

Brandt's voles (*Lasiopodomys brandti*) exposed to cold (5±1 °C) or warm (23±1 °C) showed some physiological and biochemical variations which might be important in adaptation to their environments. Cold acclimation induced increases in resting metabolic rate (RMR) and the serum triiodothyronine (T_3) level, the state-4 respiration of liver and muscle mitochondria were activated after 7 days when animals exposed to cold, and the activity of cytochrome c oxidase (COX) of liver and muscle mitochondria tended to rise with cold exposure. RMR and T_3 level decreased during warm acclimation. The state-4 respiration of liver mitochondria declined after 3 days and muscle after 7 days when animals exposed to warm, and the activities of COX of liver and muscle mitochondria tended to decrease with warm acclimation. The cold activation of liver and muscle mitochondrial respiration (regulated by T_3) was one of the cytological mechanisms of elevating RMR. Both state-4 respiration and COX activity of brown adipose tissue (BAT) mitochondria increased significantly during cold acclimation and decreased markedly after acclimated to warm. The uncoupling protein 1 (UCP1) contents in BAT increased after exposure to cold and decreased after warm acclimation. Nonshivering thermogenesis (NST) plays an important role in the process of thermoregulation under cold acclimation for Brandt's voles. Changes in thermogenesis is a important way to cold adaptation for Brandt's voles in natural environments.

1. Introduction

Endothermic animals living in a cold environment maintain normothermia and energy balance through an activation of mechanisms that increase heat production and heat conservation. It has been demonstrated that an animal's body weight, energy balance and metabolic rate all are affected by temperature (Cooper and Withers, 2002; Lovegrove, 2003; Oufara et al., 1988). Temperature also acts as an environmental zeitgeber for seasonal acclimatization of thermoregulation in rodents (Merritt and Zegers, 1991). Heat production is commonly classified as either obligatory or facultative thermogenesis (Himms-Hagen, 1990; Lowell and Spiegelman, 2000). The obligatory thermogenesis results from the widespread metabolic activity in many tissues and serves to maintain normal body temperature, which in homeotherms is usually higher than ambient temperature, and corresponds to the basal metabolic rate (BMR) or resting metabolic rate (RMR) (Himms-Hagen, 1990). The facultative thermogenesis is primarily produced in the brown adipose tissue (BAT) and skeletal muscle (Himms-Hagen, 1990). BAT is an important organ of nonshivering thermogenesis (NST) induced by either cold or food (Cannon and Nedergaard, 2004).

Although animals in nature may function only rarely at basal levels of energy expenditure, this variable has been useful as a physiological standard for assessing the energy costs of thermoregulation and increments in energy expenditure due to activity in the wild (Corp et al., 1997; McNab, 1994; Nagy et al., 1999; Wunder, 1984). For example, acclimation to lower temperature increases the RMR in a variety of small mammal species including tree shrew (*Tupaia belangeri*), greater vole (*Eothenomys miletus*), Mongolian gerbil (*Meriones unguiculatus*), Daurian ground squirrel (*Spermophilus dauricus*), and plateau pika (*Ochotona curzoniae*) (Li et al., 2001). It is known that skeletal muscle and liver are major oxygen consumers, and their combined activities at thermoneutrality take up near 40% of cardiac oxygen delivery (Zaninovich et al., 2003). It is also known that mitochondrial state-4 respiration is accompanied by heat production as it is imperfectly coupled to ADP phosphorylation in activated liver and muscle (Porter, 2001). In addition, thyroid hormones (thyroxine, T_4 and triiodothyronine, T_3) can affect obligatory thermogenesis by influencing several aspects of energy metabolism (Tomasi, 1991;

Abbreviations: RMR, resting metabolic rate; NST, nonshivering thermogenesis; BAT, brown adipose tissue; UCP1, uncoupling protein 1

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doi:10.1016/j.jtherbio.2008.11.001

Decuypere et al., 2005). Potential mechanisms for elevating obligatory thermogenesis include decreased carrier protein affinities for T_4, increasing mass-specific aerobic enzyme capacity, ion cycling, and mitochondrial proton leak (Lanni et al., 2003; Tomasi and Mitchell, 1996). BAT is the main site of facultative thermogenesis in small rodents and probably most eutherian mammals during the early postnatal period. Furthermore, this tissue is also traditionally regarded as being of particular significance in maintenance of the euthermic state in the cold (Himms-Hagen, 1990; Nedergaard et al., 2001). The key element for the energy dissipation capacity of BAT is uncoupling protein 1 (UCP1), a 32 kDa protein uniquely expressed in the inner membrane of BAT mitochondria. UCP1 dissipates the proton gradient created by the respiratory chain, thereby accelerating respiration (Lanni et al., 2003; Li et al., 2001; Lowell and Spiegelman, 2000; Wang et al, 2006a, b).

Brandt's voles (*Lasiopodomys brandti*) live mainly in grasslands of Inner Mongolia of China, the Republic of Mongolia, and the region of Beigaer Lake in Russia (Zhang and Wang, 1998). In these habitats, climate shows remarkable seasonal variation with winter lasting for 5 months. It has been reported that Brandt's voles showed relatively stable BMR and seasonal changes in NST (Wang et al., 2003), increased NST was associated with an increase in UCP1 mRNA expression in BAT (Li et al., 2001), elevated cytochrome c oxidase (COX) activity and UCP1 content of BAT in short photoperiod (Zhao and Wang, 2005), and increased energy intake and thermogenesis in association with decreases in body weight, body fat mass, and serum leptin levels in winter conditions, indicating a potential role of ambient temperature in the regulation of thermogenesis (Li and Wang, 2005a). Indeed, Brandt's voles that acclimated to cold increased their thermogenic capacity and this process could be further enhanced by short photoperiod (Li et al., 1995). In the present study, by systematically measuring a variety of physiological, hormonal, and biochemical markers indicative of thermogenic capacity, we tested the hypothesis that thermogenesis enhances at cold ambient temperature of the Brandt's voles and decreases in warm conditions. We predicted that, as when exposed to cold, Brandt's voles would show increases in RMR, state-4 respiration of liver and muscle mitochondria, and the activity of COX of liver and muscle mitochondria in comparison to the conspecific individuals acclimated to warm ambient temperature, and reversed results in warm acclimation.

2. Materials and methods

2.1. Animals

Subjects were 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 plastic cages ($30 \times 15 \times 20$ cm) under natural photoperiod (14L:10D) and room temperature was kept at 23 °C. 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 2 weeks, and then randomly assigned into one of two experimental groups that were acclimated either to cold acclimation or to warm acclimation. Cold-acclimated groups were maintained at 5 °C for 1, 7, 14 and 21 days and control group was maintained at 23 °C. Warm-acclimated groups were maintained at 5 °C for 2 months and transferred to 23 °C for 1, 7, 14 and 21 days. Control group was maintained at 5 °C.

2.2. Metabolic trials

At the end of the acclimation period, RMR in the animals were measured. RMR was measured using the closed-circuit respirometer at 25 °C (±0.5 °C) according to Górecki (1975). 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. Animals were fasted 4 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 RMR. Maximum NST was defined as the maximum metabolic response to norepinephrine (NE) and was induced by a subcutaneous injection of NE at 25 °C. The mass-dependent dosage of NE (Shanghai Harvest Pharmaceutical Co. Ltd.) was calculated according to Heldmaier (1971): NE dosage $(\mathrm{mg/kg}) = 6.6 M_b^{-0.458}(\mathrm{g})$. Two continuous stable maximal recordings were used to calculate maximum NST. Oxygen consumption reached peak values within 15–30 min after NE injection. RMR and NST were expressed as $\mathrm{ml\,O_2\,h^{-1}}$ and corrected to standard temperature and air pressure (STP) conditions.

2.3. Sample collection and isolation of mitochondria

On day after metabolic measurement, the animals were killed by decapitation, and blood was collected for serum preparation. The liver and muscle were quickly removed, placed in ice-cold sucrose-buffered medium, cleaned of any adhering tissue, blotted, and weighed, followed by homogenization for the isolation of mitochondria (1:15, w/v) with medium A (containing 250 mM sucrose, 10 mM TES, 1 mM EDTA, 64 AM BSA, pH 7.2) (Cannon and Lindberg, 1979). The BAT was weighed and followed by homogenization for the isolation of mitochondria (1:15, w/v) with medium A (containing 250 mM sucrose, 10 mM TES, 1 mM EDTA, 64 μM BSA, pH 7.2) (Cannon and Lindberg, 1979). 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 B (containing 100 mM KCl, 20 mM TES, 1 mM EGTA, pH 7.2) and subsequently used for Western blotting. The protein content of mitochondria was determined by the Folin phenol method with bovine serum albumin as standard (Lowry et al., 1951).

2.4. Mitochondrial respiration

State-4 respiration in liver and muscle mitochondria was measured at 30 °C in 1.96 ml of respiration medium (225 mM sucrose, 50 mM Tris/HCl, 5 mM $MgCl_2$, 1 mM EDTA and 5 mM KH_2PO_4, pH 7.2) with a Clark electrode (Hansatech Instruments Ltd., England, DW-1), essentially as described by Estabrook (1967). These are the major parameters of isolated mitochondrial respiration rates, reflecting oxidative phosphorylation capacity. State-4 respiration was run in 1 h and substrate dependent, and succinate as the substrate in our experiments.

2.5. Measurements of COX activity and serum thyroid hormones

The COX activity of liver and muscle mitochondria was measured with the polarographic method using oxygen electrode units (Hansatech Instruments Ltd., England) according to Sundin et al. (1987). Serum triiodothyronine (T_3) and thyroxine (T_4) concentrations were quantified by radioimmunoassay using RIA kits (China Institute of Atomic Energy, Beijing). These kits were

validated for all species tested by cross activity (Zhao and Wang, 2005). 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.

2.6. Western blotting

Five microliters of BAT mitochondrial protein (4 μg/μl) was diluted in 5 μl sample buffer (0.125 M Tris–HCl, pH 6.8, 4% SDS, 0.2 M DTT, 20% glycerol, and 0.2% bromophenol blue) and run on a SDS-polyacryladmide 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 againstnon-specific binding using 5% skim milk at 4 °C overnight, the membrane was incubated with a rabbit polyclonal antibody to hamster UCP1 (1:5000) 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 × 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 (Klingenspor et al., 1996). 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) (Zhao and Wang, 2005).

2.7. Analysis of data

Data were analyzed using the SPSS software package. Differences among groups were determined by one-way ANOVA and $P<0.05$ was taken to be statistically significant. All values in the text were presented as mean ± SE. Least significant difference (LSD) was used for statistical analysis of different groups.

3. Results

3.1. Body mass, RMR and NST

3.1.1. Cold acclimation

Results presented in Table 1 showed that the body weights in all voles were not markedly affected after cold acclimation in 3 weeks. The RMR ($ml\,O_2\,h^{-1}$) and corrected RMR ($ml\,O_2\,g^{-0.67}\,h^{-1}$) were significantly affected by lower temperature ($F_{(4,25)}=12.438$, $P<0.001$, for RMR, and $F_{(4,25)}=13.027$, $P<0.001$, for corrected RMR with 0.67 power of body mass). LSD tests showed that the RMR increased markedly and enhanced 64% after 3 weeks; and the corrected RMR increased 22% after 1 day acclimated to cold and 71% after 3 weeks, respectively (Table 1). The NST ($ml\,O_2\,h^{-1}$) and corrected NST were significantly affected by lower temperature ($F_{(4,25)}=3.677$, $P<0.05$, for NST, and $F_{(4,25)}=3.075$, $P<0.05$, for corrected NST). LSD tests showed that the NST increased markedly after 3 weeks; and the corrected NST increased significantly after 14 days and enhanced 46% after 3 weeks compared with controls (Table 1).

3.1.2. Warm acclimation

Significant differences were not found in body weight among the five groups after acclimation, but the body weight was significant increased over the warm acclimation in 3 weeks for voles (paired samples t-test, $t=4.086$, $P<0.001$). The RMR and corrected RMR were significantly decreased in warm acclimation (one-way ANOVA, $F_{(4,21)}=10.663$, $P<0.001$, for RMR, and $F_{(4,21)}=13.773$, $P<0.001$, for corrected RMR). LSD tests showed that after 3 days acclimated to warm, the RMR decreased significantly and reduced to 63% after 3 weeks; and the corrected RMR decreased to 59% (Table 2). The NST and corrected NST were significantly decreased in warm acclimation ($F_{(4,21)}=3.330$, $P<0.05$, for NST, and $F_{(4,21)}=3.516$, $P<0.05$, for corrected NST). LSD tests showed that after 7 days acclimated to warm, the NST decreased significantly compared with control, and reduced to 57% after 3 weeks; and the corrected RMR decreased to 74% after 3 weeks compared with control voles (Table 2).

3.2. Liver: wet weight, protein content, mitochondrial respiration and activities of COX

3.2.1. Cold acclimation

At the end of acclimation, cold-acclimated voles and control differed significantly on several measures (Table 3, Fig. 1). The relative weight of liver among different vole groups did not differ, and liver mitochondrial protein content increased after 1 day exposed to cold, and decreased after 3 weeks. The voles after acclimated to cold for 7 days and 3 weeks had a higher level of liver mitochondrial state-4 respiration than control group (one-way ANOVA, $F_{(4,25)}=20.595$, $P<0.001$ as specific (n mol O/min mg protein), $F_{(4,25)}=13.797$, $P<0.001$ as per gram tissue (n mol O/min g tissue)) (Table 3). Voles acclimated to cold also showed

Table 1
Metabolic thermogenesis in Brandt's voles during cold acclimation.

Parameters	23 °C	5 °C			
		1 day	3 days	7 days	21 days
Sample size (n)	6	6	6	7	5
Body weight (g)					
Initial	63.8 ± 3.5	51.7 ± 3.3	52.3 ± 4.6	53.8 ± 2.5	60.0 ± 1.2
Final	65.2 ± 3.0	51.2 ± 3.0	52.0 ± 4.6	52.9 ± 2.7	61.0 ± 1.4
RMR					
$ml\,O_2\,h^{-1}$	128.6 ± 5.9^{a}	133.8 ± 13.3^{a}	145.0 ± 10.7^{ab}	160.2 ± 6.0^{b}	210.7 ± 4.2^{c}
$ml\,O_2\,g^{-0.67}\,h^{-1}$	7.84 ± 0.28^{a}	9.54 ± 0.75^{b}	10.39 ± 1.92^{bc}	11.27 ± 0.40^{c}	13.42 ± 0.27^{d}
NST					
$ml\,O_2\,h^{-1}$	272.4 ± 13.7^{a}	253.6 ± 33.9^{a}	299.4 ± 20.3^{a}	317.6 ± 29.6^{ab}	382.9 ± 9.7^{b}
$ml\,O_2\,g^{-0.67}\,h^{-1}$	16.67 ± 0.99^{a}	17.99 ± 1.97^{ab}	21.43 ± 1.41^{abc}	22.55 ± 2.50^{bc}	24.42 ± 0.83^{c}

Data are presented as mean ± SE. The same superscripts in the same row indicate no significant differences.

Table 2
Metabolic thermogenesis in Brandt's voles during warm acclimation.

Parameters	5 °C	23 °C			
		1 day	3 days	7 days	21 days
Sample size (*n*)	6	5	5	5	5
Body weight (g)					
Initial	58.8±1.3	58.4±3.0	58.1±2.6	59.4±4.6	64.4±4.2
Final	59.8±1.7	60.1±3.0	60.0±2.8	60.9±4.8	66.0±3.6
RMR					
ml O_2 h^{-1}	201.4±3.9^{a}	181.9±19.2^{a}	149.2±2.1^{b}	138.4±8.4^{b}	127.3±7.1^{b}
ml O_2 $g^{-0.67}$ h^{-1}	13.02±0.32^{a}	11.74±1.22^{a}	9.66±0.41^{b}	8.83±0.24^{bc}	7.68±0.28^{c}
NST					
ml O_2 h^{-1}	367.3±15.3^{a}	328.2±41.0^{ab}	301.0±27.2^{ab}	290.6±18.5^{b}	272.7±16.9^{b}
ml O_2 $g^{-0.67}$ h^{-1}	23.71±0.92^{a}	20.98±2.07^{ab}	19.37±1.51^{bc}	18.76±1.53^{bc}	16.58±1.2^{c}

Data are presented as mean±SE. The same superscripts in the same row indicate no significant differences.

Table 3
Metabolic thermogenesis of liver, BAT and muscle in Brandt's voles during cold acclimation.

Parameters	23 °C	5 °C			
		1 day	3 days	7 days	21 days
Sample size (*n*)	6	6	6	7	5
Liver					
Wet mass (g)	2.44±0.19^{bc}	1.87±0.20^{ab}	1.66±0.20^{a}	1.62±0.14^{a}	2.74±0.31^{c}
%BW	3.73±0.17	3.82±0.56	3.17±0.15	3.04±0.16	4.55±0.63
Mit. protein (mg/g tissue)	22.46±0.65^{ab}	26.32±1.43^{c}	24.86±1.29^{bc}	25.90±0.61^{c}	20.55±0.78^{a}
State-4 respiration (n mol O/min mg protein)	14.33±3.60^{a}	15.68±1.78^{a}	21.04±2.27^{a}	33.31±4.27^{b}	46.63±2.89^{c}
(n mol O/min g tissue)	319.7±29.8^{a}	403.1±35.0^{a}	523.6±66.0^{a}	860.2±108.4^{b}	965.5±90.2^{b}
BAT					
Wet mass (g)	0.18±0.03	0.15±0.02	0.11±0.02	0.16±0.03	0.23±0.03
%BW	0.27±0.08	0.29±0.07	0.21±0.02	0.29±0.05	0.38±0.05
Mit. protein (mg/g tissue)	14.65±1.87^{a}	15.50±0.98^{a}	16.04±1.03^{a}	18.00±0.88^{ab}	22.20±3.17^{b}
State-4 respiration (n mol O/min mg protein)	1.89±0.52^{a}	5.63±0.73^{ab}	7.21±1.53^{bc}	8.89±0.88^{bc}	11.34±3.63^{c}
(n mol O/min g tissue)	28.49±10.27^{a}	85.14±8.55^{ab}	110.93±18.39^{bc}	157.86±12.79^{cd}	216.09±50.34^{d}
Muscle					
Mit. protein (mg/g tissue)	4.77±0.28^{a}	5.12±1.00^{a}	6.18±0.57^{ab}	6.26±0.83^{ab}	7.30±0.69^{b}
State-4 respiration (n mol O/min mg protein)	2.03±0.92^{a}	4.32±1.67^{b}	3.17±0.60^{ab}	6.44±1.39^{c}	7.86±1.24^{c}
(n mol O/min g tissue)	9.46±1.55^{a}	35.02±6.34^{b}	19.15±1.30^{a}	39.71±6.50^{b}	56.38±3.87^{c}

Data are presented as mean±SE. The same superscripts in the same row indicate no significant differences.

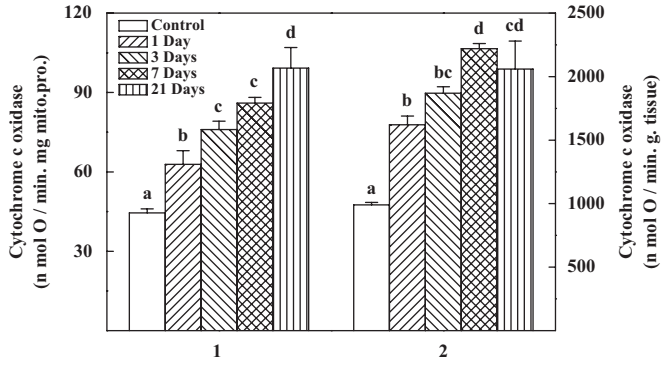

Fig. 1. Effects of cold acclimation on mitochondrial COX activity of liver in Brandt's vole. 1 represents specific activity and 2 represents per gram tissue activity.

higher levels of activities of COX in liver than did control voles (one-way ANOVA, $F_{(4,25)}=23.810$, $P<0.001$ as specific (n mol O/min mg protein), $F_{(4,25)}=28.108$, $P<0.001$ as per gram tissue (n mol O/min g tissue)) (Fig. 1). The specific activities of COX were increased 41% after 1 day exposed to cold (62.83±5.20), and reached 123% after 3 weeks (99.20±7.77) compared with their controls (44.50±1.54), respectively. Compared with controls (994.9±16.4), the activities as per gram tissue had similar variations, increased 63% after 1 day (1622.7±74.9) and 107% after 3 weeks (2057.3±217.7), respectively (Fig. 1).

3.2.2. Warm acclimation

Compared with 5 °C group, the wet weight of liver in warm groups did not differ, and the mitochondrial protein contents decreased after 1 day acclimated to warm and kept relatively

constant during acclimation. The warm-acclimated voles showed a significant decrease in liver mitochondrial state-4 respiration compared with the control voles (one-way ANOVA, $F_{(4,21)} = 17.863$, $P<0.001$ as specific, $F_{(4,21)} = 12.008$, $P<0.001$ as per gram tissue) (Table 4). Warm temperature also affected markedly the COX activities of liver in different groups as specific (one-way ANOVA, $F_{(4,21)} = 12.651$, $P<0.001$) or as per gram tissue activities (one-way ANOVA, $F_{(4,21)} = 11.679$, $P<0.001$). The specific activities of COX were decreased to 41% after 7 days acclimated to warm (42.68 ± 0.63) compared with their controls (103.64 ± 7.76), and the activities as per gram tissue decreased to 50% (1035.6 ± 279.4) compared with control group (2087.7 ± 181.3), respectively (Fig. 2).

3.3. Brown adipose tissue: wet mass, protein content, mitochondrial respiration, activities of COX and UCP1

3.3.1. Cold acclimation

BAT wet weight and relative weight (expressed as g/100 g body) among different vole groups did not differ (wet: $F_{(4,25)} = 1.951$, $P>0.05$, and relative: $F_{(4,25)} = 1.653$, $P>0.05$), but BAT mitochondrial protein content increased at the end of acclimation ($F_{(4,25)} = 3.039$, $P<0.05$) (Table 3). The mitochondrial state-4 respiration of BAT enhanced significantly after 3 days exposed to cold and showed a steady increase during acclimation ($F_{(4,25)} = 4.604$, $P<0.01$), and enhanced 6 times after 3 weeks acclimated to cold (Table 3). Cold acclimation voles also showed higher levels of BAT mitochondrial COX activity ($F_{(4,25)} = 12.903$, $P<0.001$ as specific activity (n mol O/min mg protein), and $F_{(4,25)} = 72.171$, $P<0.001$ as per gram tissue activity (n mol O/min g tissue)). LSD tests showed that the specific activities of COX were increased 69% after 1 day exposed to cold (121.87 ± 13.00), and reached 133% after 3 weeks (167.89 ± 57.91) compared with their controls (72.20 ± 17.86), respectively. The activities as per gram tissue had similar variations, increased 89% after 1 day (1870 ± 80) and 246% after 3 weeks (3420 ± 160) compared with controls (990 ± 50), respectively (Fig. 3). Cold acclimation significantly affected the UCP1 contents (RU: $F_{(4,25)} = 4.922$, $P<0.01$, RU/g BAT: $F_{(4,25)} = 8.216$, $P<0.001$, RU/total tissue: $F_{(4,25)} = 9.317$, $P<0.001$). UCP1 (RU) levels were not different between 7 days (6.64 ± 1.76) and 3 weeks (7.92 ± 1.89) cold groups, although they were markedly elevated compared with those in the control

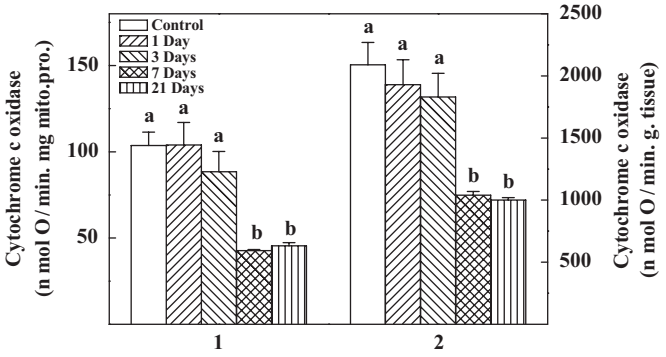

Fig. 2. Effects of warm acclimation on mitochondrial COX activity of liver in Brandt's vole. 1 represents specific activity and 2 represents per gram tissue activity.

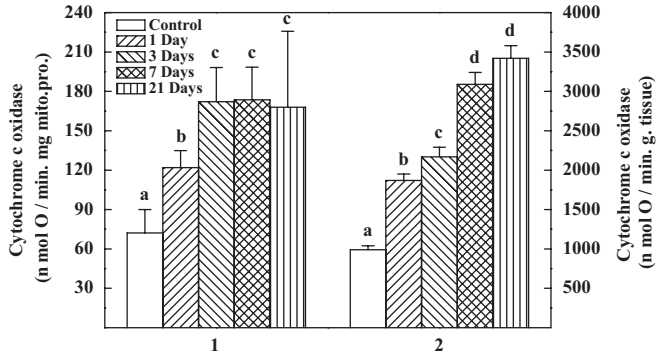

Fig. 3. Effects of cold acclimation on mitochondrial COX activity of BAT in Brandt's vole. 1 represents specific activity and 2 represents per gram tissue activity.

Table 4
Metabolic thermogenesis of liver, BAT and muscle in Brandt's voles during warm acclimation.

Parameters	5 °C	23 °C			
		1 day	3 days	7 days	21 days
Sample size (n)	6	5	5	5	5
Liver					
Wet mass (g)	2.74 ± 0.27	1.99 ± 0.04	2.10 ± 0.19	2.25 ± 0.20	2.46 ± 0.23
%BW	4.61 ± 0.49^{a}	3.35 ± 0.19^{b}	3.48 ± 0.16^{b}	3.74 ± 0.33^{ab}	3.72 ± 0.21^{ab}
Mit. Protein (mg/g tissue)	20.09 ± 0.79^{ab}	18.89 ± 1.07^{a}	21.18 ± 1.03^{ab}	24.26 ± 0.53^{c}	22.01 ± 0.58^{bc}
State-4 respiration (n mol O/min mg protein)	44.47 ± 3.21^{a}	43.55 ± 6.86^{a}	32.21 ± 1.18^{b}	14.92 ± 0.76^{c}	14.25 ± 1.65^{c}
(n mol O/min g tissue)	904.42 ± 95.93^{a}	820.90 ± 125.74^{a}	682.54 ± 42.94^{a}	361.59 ± 18.51^{b}	312.38 ± 35.49^{b}
BAT					
Wet mass (g)	0.22 ± 0.03	0.22 ± 0.01	0.20 ± 0.03	0.26 ± 0.07	0.17 ± 0.04
%BW	0.38 ± 0.03	0.35 ± 0.02	0.34 ± 0.05	0.43 ± 0.13	0.27 ± 0.04
Mit. protein (mg/g tissue)	21.58 ± 2.66^{a}	23.35 ± 2.45^{a}	15.60 ± 0.90^{b}	13.13 ± 0.83^{b}	15.06 ± 4.99^{b}
State-4 respiration (n mol O/min mg protein)	11.69 ± 2.28^{a}	8.05 ± 1.08^{ab}	8.11 ± 2.49^{ab}	2.69 ± 1.20^{c}	4.54 ± 0.99^{bc}
(n mol O/min g tissue)	233.33 ± 34.47^{a}	183.80 ± 22.50^{ab}	131.22 ± 46.07^{b}	33.65 ± 13.07^{d}	69.13 ± 22.88^{cd}
Muscle					
Mit. protein (mg/g tissue)	7.49 ± 0.59^{a}	7.41 ± 0.92^{a}	4.73 ± 0.52^{b}	5.46 ± 1.32^{ab}	4.94 ± 0.28^{b}
State-4 respiration (n mol O/min mg protein)	7.52 ± 1.17^{ab}	5.99 ± 1.66^{ab}	9.33 ± 3.98^{a}	3.01 ± 1.86^{c}	4.38 ± 1.10^{bc}
(n mol O/min g tissue)	53.37 ± 4.36^{a}	44.64 ± 11.74^{a}	42.08 ± 5.92^{a}	13.91 ± 0.35^{b}	21.35 ± 2.33^{b}

Data are presented as mean ± SE. The same superscripts in the same row indicate no significant differences.

(1.02±0.04) and 1 day (3.58±0.46) cold group. UCP1 (RU/g BAT) showed a similar response to cold exposure as did UCP1 (RU), with a significantly higher value in 7 days (119.56±29.84) and 3 weeks (149.87±25.53) cold groups than 1 day (54.91±2.32) and control (15.06±2.32) groups, but with no difference between the latter two groups. In addition, UCP1 (RU/total tissue) was also calculated and significantly affected by cold acclimation at 5 °C. They were increased by 514% and 994%, respectively, in voles after 7 days (17.99±6.03) and 3 weeks (32.04±5.15) in the cold compared with that value of control (2.93±0.93) at 23 °C (Fig. 5).

3.3.2. Warm acclimation

Both BAT wet mass ($F_{(4,21)}=0.743$, $P>0.05$) and relative mass ($F_{(4,21)}=0.775$, $P>0.05$) were not significantly changed by acute or chronic cold exposure. However, the BAT mitochondrial protein contents were markedly affected by warm acclimation ($F_{(4,21)}=4.659$, $P<0.01$) (Table 4). In the 7 days and 3 weeks groups, the contents of BAT mitochondrial protein were decreased to 61% and 70%, respectively, compared with the corresponding values in control group, but no significant difference existed between control and 1 day group. The mitochondrial state-4 respiration of BAT was significantly lower in 7 days and 3 weeks warm groups than in the control ($F_{(4,21)}=3.983$, $P<0.05$), no difference existed among one, 3 days and control groups (Table 4). BAT mitochondrial COX activity, a regulatory factor during BAT thermogenesis, was significantly affected by warm acclimation at 23 °C ($F_{(4,21)}=13.899$, $P<0.001$ as specific activity, and $F_{(4,21)}=155.851$, $P<0.001$ as per gram tissue activity). The specific activity was decreased to 68% in 1 day group (112.77±11.90) compared with control group (166.25±16.55), and further significantly decreased in 3 weeks group (71.36±8.88) by 57% compared with control group. The total activities showed a similar response to warm acclimation as did specific activity, with a significantly decrease in 1 day group than control group, and further significantly decreased in 7 days and 3 weeks group, but with no difference between the latter two groups (Fig. 4). The UCP1 contents showed significant variations in different warm-acclimated groups compared with control group (RU: $F_{(4,21)}=2.724$, $P<0.05$, RU/g BAT: $F_{(4,21)}=4.429$, $P<0.01$, RU/total tissue: $F_{(4,21)}=3.963$, $P<0.05$). LDS tests showed that in the 3 weeks warm (0.9±0.11) group, UCP1 (RU) was decreased by 89% compared with the corresponding value in control (7.96±2.34) group, but no significant difference existed among control, 1 day (7.52±2.27), 3 days (4.28±2.38) and 7 days (5.33±2.98) groups. In addition, the UCP1 contents (RU) as g BAT and total tissue were also calculated. One-way ANOVA indicated that a significant difference did occur in UCP1 (RU/g BAT). UCP1 content after warm acclimation in 3 weeks was the lowest. Control and 1 day groups

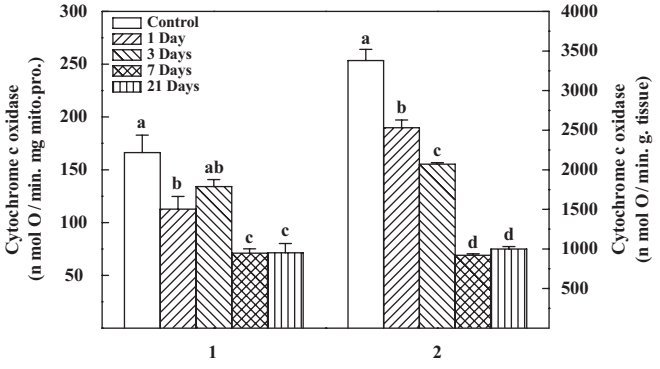

Fig. 4. Effects of warm acclimation on mitochondrial COX activity of BAT in Brandt's vole. 1 represents specific activity and 2 represents per gram tissue activity.

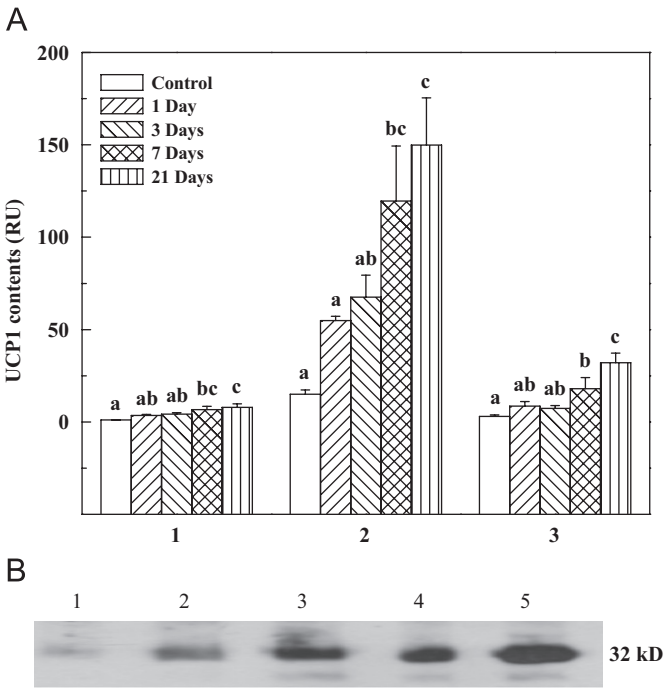

Fig. 5. Effects of cold acclimation on UCP1 contents of BAT in Brandt's vole. (A) Comparison of UCP1 contents during cold acclimation. (1) RU/20 μg BAT mitochondrial protein; (2) RU/g BAT tissue; (3) RU/total tissue. (B) Western blot detection using 20 μg of BAT mitochondrial protein. Band 1, 2, 3, 4 and 5 represent control, 1, 7, 14 and 21 days.

showed no significant differences in UCP1 contents, and 3 days and 7 days also showed no significant differences. Compared with control (167.07±46.86), UCP1 contents in 3 days (66.15±16.95), 7 days (66.09±14.53) and 3 weeks (12.84±1.53) decreased by 60%, 60% and 92%, respectively (Fig. 6). The UCP1 (RU/total tissue) showed a similar response to warm acclimation, with a significantly lower value in the 3 weeks warm group than control group. Total tissue content was decreased by 65% in 3 days warm group (13.52±3.51) compared with control group (38.51±11.64), and further significantly decreased in 3 weeks group (2.15±0.37) by 94% compared with control (Fig. 6).

3.4. Muscle: protein content, mitochondrial respiration and activities of COX

3.4.1. Cold acclimation

The protein contents of muscle increased significantly after 1 day exposed to cold and kept relatively constant contents during cold acclimation (one-way ANOVA, $F_{(4,25)}=2.941$, $P<0.05$) (Table 3). The mitochondrial state-4 respiration of muscle enhanced significantly after 1 day exposed to cold and showed a steady increase during acclimation (one-way ANOVA, $F_{(4,25)}=20.595$, $P<0.001$ as specific, $F_{(4,25)}=13.732$, $P<0.001$ as per gram tissue) (Table 3). One and 3 weeks voles acclimated to cold also had a higher COX activity than control voles as specific (one-way ANOVA, $F_{(4,25)}=6.672$, $P<0.01$) or as per gram tissue activities (one-way ANOVA, $F_{(4,25)}=52.334$, $P<0.001$). The specific activities of COX were increased 38% after 7 days exposed to cold (146.47±15.76), and reached 64% after 3 weeks (174.34±18.58) compared with their controls (106.17±6.09), respectively. The activities as per gram tissue had similar variations, increased 43% after 1 day (711.4±31.8) and 145% after 3 weeks (1222.7±16.0) compared with controls (498.3±10.0), respectively (Fig. 7).

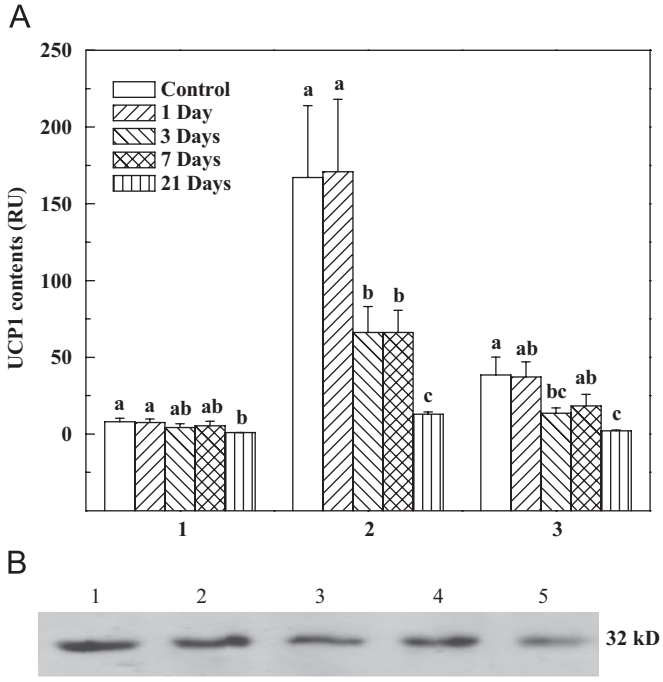

Fig. 6. Effects of warm acclimation on UCP1 contents of BAT in Brandt's vole. (A) Comparison of UCP1 contents during warm acclimation. (1), RU/20 μg BAT mitochondrial protein; (2) RU/g BAT tissue; (3) RU/total tissue. (B) Western blot detection using 20 μg of BAT mitochondrial protein. Band 1, 2, 3, 4 and 5 represent control, 1, 7, 14 and 21days.

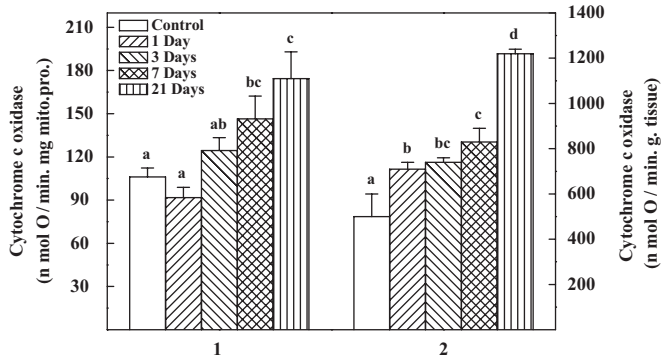

Fig. 7. Effects of cold acclimation on mitochondrial COX activity of muscle in Brandt's vole. 1 represents specific activity and 2 represents per gram tissue activity.

3.4.2. Warm acclimation

The muscle protein contents decreased significantly after 3 days acclimated to warm and kept relatively constant contents during warm acclimation (one-way ANOVA, $F_{(4,21)}=2.899$, $P<0.05$) (Table 4). The voles after acclimated to warm for 7 days and 3 weeks had a lower level of muscle mitochondrial state-4 respiration than control group (one-way ANOVA, $F_{(4,21)}=17.863$, $P<0.001$ as specific, $F_{(4,21)}=7.466$, $P<0.01$ as per gram tissue) (Table 4). Voles acclimated to warm also showed lower levels of activities of COX in muscle than did control voles as specific (one-way ANOVA, $F_{(4,21)}=12.651$, $P<0.001$) and as per gram tissue (one-way ANOVA, $F_{(4,21)}=11.679$, $P<0.001$) (Fig. 4). The specific activities of COX were decreased 55% after 7 days acclimated to warm (92.09 ± 13.16) compared with their controls (166.69 ± 14.62), and the activities as per gram tissue decreased 36% (434.8 ± 22.4) compared with controls (1205.8 ± 21.4) (Fig. 8).

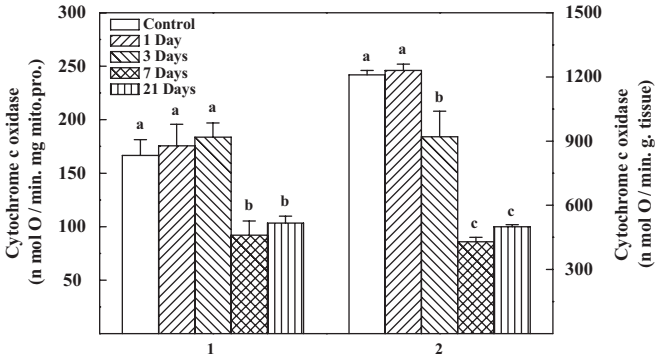

Fig. 8. Effects of warm acclimation on mitochondrial COX activity of muscle in Brandt's vole. 1 represents specific activity and 2 represents per gram tissue activity.

3.5. Serum triiodothyronine (T_3) and thyroxine (T_4)

3.5.1. Cold acclimation

Control voles averaged 11.38 ± 1.27 ng/ml serum T_4 and 1.18 ± 0.13 ng/ml serum T_3. Serum T_4 levels in control and acclimated groups were not significantly different, but an LDS test revealed that T_3 levels in 7 and 21 days were significantly higher than the values obtained for control voles (through one-way ANOVA test showed that control group was not significantly different from the others at the 0.05 level) (Table 5).

3.5.2. Warm acclimation

Serum T_4 levels were not significantly affected after 3 weeks warm acclimation, but T_3 levels were markedly decreased (one-way ANOVA, $F_{(4,25)}=2.972$, $P<0.05$). LSD tests showed that T_3 levels were reduced after 7 days acclimated to warm and decreased to 66% of the level in control (Table 6).

4. Discussion

Temperature plays an important role in mediating an animal's physiological and behavior. In the present study, we found that the alteration in temperature significantly influenced thermogenic capacity in Brandt's voles. Voles showed increased RMR, physiological and biochemical markers in cold acclimation and the reversed results were found in warm acclimation.

4.1. Changes of body mass, RMR and NST during cold and warm acclimation

Seasonal changes in body weight are an important adaptive strategy for many small mammals (Gottreich et al., 2000). Body mass showed an increase (Wang et al., 1995), no change (Reynolds and Lavigne, 1988), or even decrease (Merritt, 1995) during winter or cold acclimation. We found that the body mass of Brandt's voles showed no changes during cold and warm acclimation. The no change or decrease in body mass may reduce the energy requirements of the individuals, or increase the RMR/unit body mass, which was advantageous in maintaining body temperature and was, therefore, an adaptive strategy to cold (Li et al., 2001; Reynolds and Lavigne, 1988).

Cold produced a surface heat loss in the animals. In order to maintain a stable body temperature, endothermic mammals need to expend considerable energy for their thermoregulation, and the enhancement of BMR was thought to be an adaptive mechanism to cold (McDevitt and Speakman, 1994; Tomasi and Horwitz, 1987). The increase of RMR has been found to be associated with

Table 5
Serum T_3 and T_4 in Brandt's voles during cold acclimation.

Parameters	23 °C	5 °C			
		1 day	3 days	7 days	1 day
Sample size (n)	6	6	6	7	5
Serum T_3 (ng/ml)	1.18 ± 0.13^{a}	1.55 ± 0.12^{ab}	1.52 ± 0.03^{ab}	1.74 ± 0.20^{b}	1.83 ± 0.21^{b}
Serum T_4 (ng/ml)	11.38 ± 1.27	15.99 ± 1.04	16.57 ± 1.10	18.54 ± 2.89	15.64 ± 2.10

Data are presented as mean ± SE. The same superscripts in the same row indicate no significant differences.

Table 6
Serum T_3 and T_4 in Brandt's voles during warm acclimation.

Parameters	5 °C	23 °C			
		1 day	3 days	7 days	1 day
Sample size (n)	6	5	5	5	5
Serum T_3 (ng/ml)	1.78 ± 0.18^{a}	1.51 ± 0.10^{ab}	1.45 ± 0.13^{ab}	1.23 ± 0.10^{b}	1.17 ± 0.16^{b}
Serum T_4 (ng/ml)	15.52 ± 1.72	13.85 ± 2.48	15.48 ± 2.20	14.31 ± 1.71	11.49 ± 1.55

Data are presented as mean ± SE. The same superscripts in the same row indicate no significant differences.

cold acclimation in a variety of small mammal species, including tree shrews, greater voles, Mongolian gerbils and plateau pikas (Li et al., 2001), short-tailed voles (*M. agrestis*) (McDevitt and Speakman, 1994) and hamsters (*Mesocricetus auratus*) (Tomasi and Horwitz, 1987). The decrease of RMR also has been found to be associated with warm acclimation in small mammal species, including plateau pikas (Liu and Li, 1996) and gerbils (*Gerbillus campestris*) (Oufara et al., 1988). Cold acclimation caused a 68% increase of RMR in tree shrews, a 25% increase in greater voles, and a 21% increase in plateau pikas (Li et al., 2001). Previous studies in Brandt's voles have shown that environmental temperature and photoperiod interact to regulate thermogenic capacity (Li et al., 1995). Our data from the present study indicated that temperature alone could affect RMR of Brandt's voles. The RMR and corrected RMR increased markedly in the cold-acclimated voles and enhanced 84% and 71% after 3 weeks, respectively, and decreased significantly in the warm-acclimated voles and reduced to 57% and 59% after 3 weeks, respectively, which indicated the temperature may play an important role in preparing voles for coming winter conditions in a natural setting (Li et al., 2001). Brandt's voles showed improved cold tolerance as well as elevated RMR in cold acclimation compared with warm acclimation. Improved cold tolerance in cold-acclimated small mammals is widespread in species wintering in cold temperate climates and is generally correlated with increased thermogenic capacity (Li et al., 2001; Wang et al, 2006a, b). Increased cold tolerance in this species is most likely attributable to increased nonshivering endurance, and endurance is closely linked to increases in NST (Li and Wang, 2005a). Small mammals largely depend on NST to cope with cold (Heldmaier et al., 1982). Winter- or cold-induced enhancement in NST has been reported in some small mammals species under natural, semi-natural and laboratory conditions (Li et al., 2001; Bozinovic et al., 2004; Li and Wang, 2005b; Wang et al, 2006a, b; Zhang and Wang, 2006). Our present study found that Brandt's voles showed significantly higher NST in cold-acclimated group than that in warm-acclimated group, which was consistent with the previous findings (Li et al., 2001). The decrease in cold tolerance and NST of Brandt's voles in warm acclimation might be explained by a reduction in thermogenic tissue mass, as an adaptation to conserve energy during the reproductive season, or as a response to physiological stress associated with reproduction (Li and Wang, 2005c).

4.2. Thermogenesis at cellular level during cold and warm acclimation

The RMR of an animal is the sum of the metabolic rates of its organs and other metabolically active structures. Organs such as gastrointestinal tract, liver and kidneys have high mass-specific energy metabolism and may contribute significantly to RMR (Scott and Evans, 1992; Klaassen et al., 2004). Because, liver is one of the largest and most metabolically active organs in mammals, its thermogenesis was considered to contribute greatly to RMR (Coutre and Hulbert, 1995; Li et al., 2001; Villarin et al., 2003). Under basal metabolic conditions, proton leak accounts for 20–30% of total hepatic oxygen consumption (Rolfe et al., 1999). Early studies have shown that cold acclimation can induce an increase in state-4 respiration and COX activity of liver accompanied by enhanced RMR in several small mammal species including tree shrews, greater voles, Mongolian gerbils and Brandt's voles (Li et al., 2001), Our data indicated that low temperature induced an increase in state-4 respiration and COX activity of liver in Brandt's voles that displayed enhanced RMR, and warm temperature brought a decrease in state-4 respiration and COX activity of vole's liver that also showed decreased RMR. These results indicate that temperature reflects changes in liver oxidative metabolism. These findings are in agreement with the findings from a study in cold- and warm-acclimated plateau pikas changed state-4 respiration and COX activity of liver and RMR (Liu and Li, 1996). It is showed that the cold activation of liver mitochondrial respiration is one of the cytological mechanisms of elevating RMR.

BAT is the main site for NST in small mammals. The adaptive changes in BAT were achieved through modifications in the mass of BAT, mitochondrial contents and the concentrations of UCP1 (Nedergaard et al., 2001; Cannon and Nedergaard, 2004). UCP1 had a strongly regulated uncoupling activity (Ricquier and Bouilloud, 2000). It has been found that some small mammals showed enhanced NST associated with increased UCP1 mRNA level or UCP1 protein contents in cold or winter conditions, such as common spiny mice (*Acomys cahirinus*) (Kronfeld-Schor et al., 2000), Daurian ground squirrel, Mongolian gerbil, Brandt's vole (Li et al., 2001; Li and Wang, 2005b), plateau pika (Wang et al., 2006a) and root vole (*Microtus oeconomus*) (Wang et al., 2006b). In this study, UCP1 protein contents in BAT were lower in warm

acclimation and kept higher in cold acclimation, which was consistent with the changes in NST. Our present study showed that UCP1 protein content in BAT was increased by 677% in 3 weeks cold-acclimated group as compared with that in warm-acclimated group as RU, 891% as RU/g BAT and 994% as RU/ total tissue, respectively, whereas in plateau pikas UCP1 protein content was elevated by 183% in winter than that in summer (Wang et al., 2006a), and UCP1 contents of Mongolian gerbils under semi-natural conditions increased 77% in winter than that in summer (Li and Wang, 2005b) and 194% higher in winter than in summer for root voles in the field (Wang et al., 2006b), which might indicate that the extreme harsh environment where the Brandt's voles live, required more cost for thermoregulation. The notion of elevated thermogenic capacity, indicated by the enhanced NST, is further supported by the other biochemical markers examined in the present study, which include the mitochondrial protein content, mitochondrial state-4 respiration and the COX activity. Our data show that the BAT mitochondrial protein content, mitochondrial state-4 respiration, and the COX activity increased significantly during the cold acclimation in comparison to that of the warm acclimation, suggesting that Brandt's voles increased the total respiratory capacity of BAT in the cold. This finding is in agreement with previous studies in tree shrews, plateau pikas, Brandt's voles, Daurian ground squirrels, Mongolian gerbils (Li et al., 2001) and root voles (Wang et al., 1999) as well as in other rodent species including yellow-necked field mice (*Apodemus xavicollis*) and wood mice (*Apodemus sylvaticus*) (Klause et al., 1988), and golden spiny mice (*Acomys russatus*) (Kronfeld-Schor et al., 2000). There has been an increase in the documentation of data integrated from molecular to organismal level.

Skeletal muscles have lower mass-specific metabolic rates (Scott and Evans, 1992), but contribute significantly to RMR due to their overall mass (Weber and Piersma, 1996). In accordance with the concept of symmorphosis proposed by Weber and Piersma (1996), the present results support the notion that the respiratory capacities of muscle essentially are determined by the amount of mitochondria and oxidase activity (Rasmussen et al., 2004). There were also differential changes in muscle state-4 respiration and COX activity during cold and warm acclimations. An increase in the state-4 respiration and COX activity indicates an increase in mitochondrial protein and indicates in RMR (Weber and Piersma, 1996). The state-4 respiration of Brandt's voles with cold-acclimated tests exhibited a 287% increase compared with their controls. The COX activities of Brandt's voles with cold-acclimated tests exhibited a 64% increase as specific activity and a 145% increase as per gram tissue after 3 weeks compared with controls, respectively. In contrast, all other warm-acclimated voles with either 1 week or with 3 weeks showed a decrease in state-4 respiration and COX activity.

4.3. Changes of serum triiodothyronine (T_3) and thyroxine (T_4) during cold and warm acclimations

Thyroid hormones can increase energy expenditure and stimulate basal thermogenesis by lowering metabolic efficiency (Tomasi, 1991). On metabolism, they are critical in the central regulation of body temperature, stimulating thermogenesis, and regulating cellular metabolism (Wrutniak-Cabello et al., 2001; Zaninovich et al., 2003). Metabolic adjustment of thyroid hormones may correlate with thermogenic capacity in some cold-exposed rodents (Li et al., 2001; Liu et al., 1997). Li et al. (2001) reported that when tree shrews, greater voles and Mongolian gerbils exposed to cold, the serum T_3 concentrations increased 188%, 48% and 325%, respectively. Our data showed that cold acclimation could increase serum T_3 concentrations and warm acclimation could decrease serum T_3 concentrations in Brandt's voles. These data are consistent with the previous finding tree shrews, greater voles and Mongolian gerbils, further supporting the notion that thyroid hormones are involved in the thermogenic enhancement induced by temperature (Li et al., 2001).

The Brandt's vole is a dry grassland rodent and the adaptive principle to cold is typical of boreal small mammals. Cold acclimation induced an increase in liver and muscle mitochondrial state-4 respiration and COX activity. The lower state-4 respiration and COX activity of liver and muscle mitochondria from warm-acclimated voles may explain their low RMR, whereas the higher state-4 respiration and COX activity of liver and muscle mitochondria from cold-acclimated voles may explain their higher RMR. The increase of mitochondrial protein, state-4 respiration, COX activity and UCP1 content in BAT during cold acclimation may also explain their higher NST. As a result of this great adaptability of liver, BAT and muscle mitochondria, the voles seemed to be able to live in a wide range of ambient temperatures in its natural habitat.

Acknowledgments

We wish to thank Dr. Xing-Sheng Li for his assistance on this study. This study was financially supported by the National Natural Foundation of China (nos. 30430140 and 30625009) to DHW and the Chinese Academy of Sciences (KSCX2-YW-N-06).

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