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Thermogenic properties in three rodent species from Northeastern China in summer

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Abstract

1. The thermogenic properties of mitochondria in liver, brown adipose tissue (BAT) and muscle, and uncoupling protein 1 (UCP1) contents in BAT were studied in field-captured gray red-backed vole (*Clethrionomys rufocanus*), large Japanese field mouse (*Apodemus speciosus*) and striped field mouse (*Apodemus agrarius*) in summer from the Northeastern China.

2. The mitochondrial respiration capacities in liver, cytochrome *c* oxidase activities in BAT and muscle, and UCP1 contents in BAT were found to be significantly higher in gray red-backed vole than that in large Japanese field mouse and striped field mouse.

3. High thermogenic capacities in liver, BAT and muscle in gray red-backed vole can enable them to increase survivals. Different strategies were employed by gray red-backed vole, large Japanese field mouse and striped field mouse to adapt their environments.

Keywords: Uncoupling protein 1, UCP1; Brown adipose tissue, BAT; Gray red-backed vole, *Clethrionomys rufocanus*; Large Japanese field mouse, *Apodemus speciosus*; Striped field mouse, *Apodemus agrarius*

1. Introduction

Adaptive changes in thermogenic capacity are critical to the survival of small mammals in temperate zones (Nespolo et al., 2001; Woodley and Buffenstein, 2002). Associated with the adjustments in heat production, the respiratory capacities and metabolic enzyme activities in liver, muscle, brown adipose tissue (BAT) and other tissues (organs) would change correspondently. In particular, uncoupling protein 1 (UCP1), the thermogenic protein located in the inner membrane of BAT mitochondria (Cannon and Nedergaard, 2004), is the molecular basis of adaptive changes in nonshivering thermogenesis (NST), which is important for thermoregulation in small mammals.

The gray red-backed vole (*Clethrionomys rufocanus*) and the large Japanese field mouse (*Apodemus speciosus*) are mainly distributed in the northern China and cannot be found in the extremely arid areas, low latitudes and hot regions in China (Ma, 1986). The striped field mouse (*A. agrarius*) is distributed widely in China (Zhang and Wang, 1998). Within their distributional ranges the gray red-backed vole and the large Japanese field mice preferentially inhabit in forest. The dominant habitats of striped field mouse are grassland, farmland, old cultivated field and hillsides (Ma, 1986). General properties of basal metabolic rate (BMR) and NST in summer have been reported for these three rodent species (Liu et al., 2004). Gray red-backed vole and large Japanese field mouse had relatively high BMR and NST, relatively wide thermal neutral zone and low lower critical temperature. Striped field mouse showed the relatively low BMR, which was still higher than the predicted value based on their body mass, together with relatively narrow thermal neutral zone and low lower critical temperature (Liu et al., 2004). We predict that the species with high levels in BMR and NST will show higher mitochondrial respiration capacity and UCP1 contents. The present study aimed to compare the cellular

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and (or) molecular thermogenic properties in these three rodent species.

2. Materials and methods

2.1. Animals

Five gray red-backed voles (3 males, 2 females), six large Japanese field mice (3 males, 3 females) and seven striped field mice (4 males, 3 females) were live-trapped in the forest regions at Nehe County, Heilongjiang Province (48°29′N, 124°51′E) of China in June 2002. The mean annual temperature is 3.4 °C, and the mean temperature in the coldest month (January) is −20.6 °C, and 22.9 °C in the warmest month (July). The extreme minimum ambient temperature is below −38 °C for 7 months (from October to April). The annual amplitude of ambient temperature is 67.9 °C (from −32.2 to 35.7 °C) and the fluctuation between day and night is also great. Animals were kept in cages ($50 \times 30 \times 25\,cm^3$) under natural photoperiod (around 14L:10D) and temperature (around 20–24 °C). Laboratory mice chow pellets (Beijing Ke Ao Feed Co.) and water were supplied ad lib (Liu et al., 2004).

2.2. Preparation of mitochondria

After the measurements of metabolic trials (Liu et al., 2004), animals were killed by decapitation and blood was collected for hormone determination. Liver, BAT and muscle were carefully and quickly removed, with adhering tissues separated, then blotted, weighed, and placed in ice-cold sucrose-buffered medium and followed by homogenization for the isolation of mitochondria (Cannon and Lindberg, 1979). The protein content of mitochondria was determined by the Folin phenol method with bovine serum albumin as standard (Lowry et al., 1951).

2.3. Mitochondrial respiration, enzyme activity and hormone concentration

State 4 respiration in liver mitochondria was measured polarographically at 30 °C using an oxygen electrode (Estabrook, 1967). Succinate was used as the substrate in this study. The activity of cytochrome *c* oxidase was determined polarographically according to Sundin et al. (1987).

The concentrations of triiodothyronine (T_3) and thyroxine (T_4) in serum were determined by radioimmunoassay using RIA kits (China Institute of Atomic Energy). These kits were validated for all species tested by cross-activity. 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.

2.4. Uncoupling protein 1 (UCP1)

Total BAT protein (20 μg per lane) was separated in a discontinuous SDS-polyacylamide gel (12.5% running gel and 3% stacking gel), and blotted to a nitrocellulose membrane (Hybond C-Amersham). To check for the efficiency of protein transfer, gels and nitrocellulose membrane were stained after transfer with Coommassie brilliant blue and Ponceau red, respectively. UCP1 was detected using a polyclonal rabbit antibody against Siberian hamster UCP1 (1:5000) as primary antibodies and goat anti-rabbit (1:5000) as second antibodies (Klingenspor et al., 1996). We used enhanced chemoluminescence kit (ECL, Amersham) as detection system and unspecific binding sites were saturated with 5% degrease milk in PBS. UCP1 content was quantified by using the Scion Image and expressed in relative units (RU) (Li and Wang, 2005; Zhao and Wang, 2005).

2.6. Data analysis

Data were analyzed using the SPSS software package. Differences among groups were determined by one-way ANOVA followed with post hoc least significant difference (LSD). $P<0.05$ was considered to be statistically significant. All values in the text were presented as mean ± SE.

3. Results

3.1. Body mass and tissue mass

No significant differences were detected in body mass, wet mass of BAT and liver among the three rodent species ($P>0.05$, Table 1).

3.2. Protein content, mitochondrial respiration, and activity of cytochrome c oxidase in liver

There were no significant differences in protein contents and cytochrome *c* oxidase activities in liver among these three rodent species. Gray red-backed voles had higher mitochondrial state 4 respiration than large Japanese field mouse and striped field mouse ($F = 3.824$, $P<0.05$), while no significant differences were found between the two *Apodemus* species ($P>0.05$, Table 1).

3.3. Protein content, UCP1 relative content and activity of cytochrome c oxidase in BAT

The mitochondrial protein contents were significantly different among three species ($F = 4.595$, $P = 0.028$). Gray red-backed vole had higher mitochondrial protein contents than large Japanese field mice and striped field mice, whereas the two *Apodemus* species did not significantly differ from each other ($P>0.05$, Table 1). UCP1 contents showed significant differences among three species ($F = 4.595$, $P<0.05$). UCP1 contents in gray red-backed

Table 1
Thermogenic properties in liver, muscle and brown adipose tissue (BAT) for three rodent species

	C. rufocanus	*A. speciosus*	*A. agrarius*	*P* value
Sample size	5	6	7	
Body mass (g)	23.1±0.7	28.5±3.2	24.4±1.4	0.202
Liver (g)	1.18±0.07	1.18±0.12	1.45±0.17	0.311
% Body mass	5.14±0.27	4.18±0.19	5.95±0.68	0.050
Mito protein (mg/g)	27.19±0.89	25.14±1.81	21.63±2.13	0.129
State 4 respiratory				
(n mol O_2/min mg mito pro)	1.54±0.14	0.98±0.22	1.05±0.16	0.106
(n mol O_2/min g tissue)	41.58±3.40[a]	25.15±5.78[b]	23.5 6± 4.62[b]	0.045
Cytochrome *c* oxidase				
(n mol O_2/min mg mito pro)	76.00±8.57	84.17±9.26	76.43±7.05	0.740
(n mol O_2/min g tissue)	2060.00±235.91	2061.67±155.26	1595.71±149.33	0.115
BAT (g)	0.11±0.01	0.13±0.01	0.16±0.02	0.195
% Body mass	0.47±0.05	0.48±0.07	0.65±0.07	0.154
Mito protein (mg/g)	24.29±3.31[a]	14.83±2.13[b]	16.30±1.54[b]	0.028
Muscle				
Mito protein (mg/g)	8.39±0.85[a]	5.18±0.61[b]	4.62±0.54[b]	0.003
Cytochrome *c* oxidase				
(n mol O_2/min mg mito pro)	195.00±27.34	204.17±16.95	193.57±20.05	0.928
(n mol O_2/min g tissue)	1621.00±282.15[a]	1006.67±30.24[b]	836.43±48.83[b]	0.004

Data are presented as mean±SE, the different superscripts in the same row indicate significant differences.

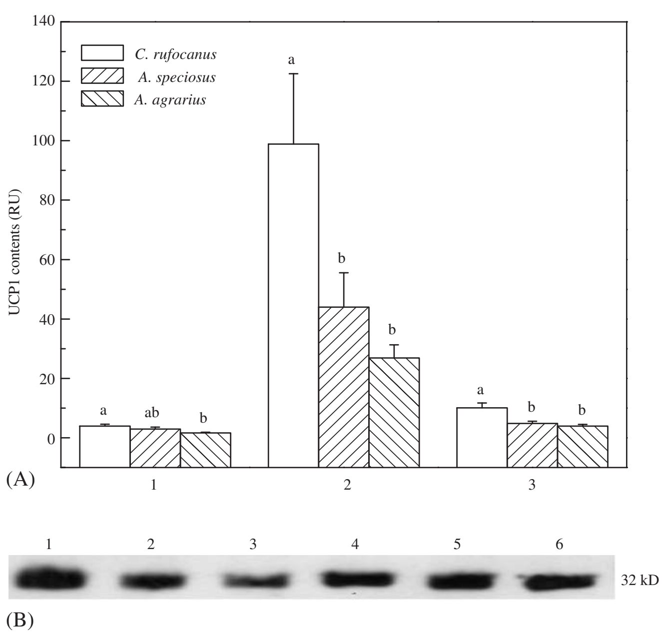

Fig. 1. Uncoupling protein 1 (UCP1) contents of brown adipose tissue (BAT) in three rodent species from Northeastern China. (A) UCP1 contents of three species. 1, RU/20 μg BAT mitochondrial protein; 2, RU/g BAT tissue; 3, RU/ total BAT tissue. RU, relative unit. (B) Western blot detection using 20 μg of BAT mitochondrial protein. 1 and 4 represent *C. rufocanus*, 2 and 5 represent *A. agrarius*, and 3 and 6 represent *A. peninsulae*.

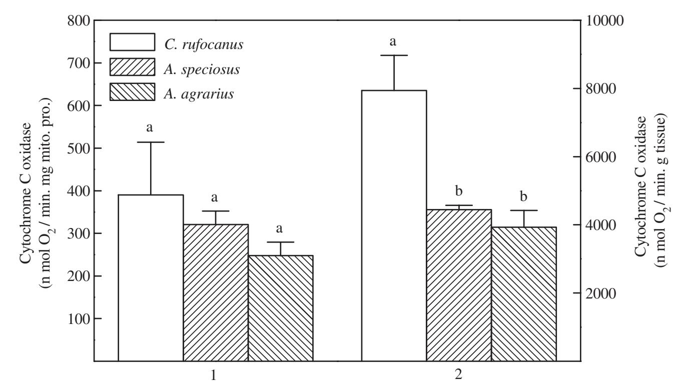

Fig. 2. Cytochrome *c* oxidase activity of brown adipose tissue (BAT) in three rodent species from Northeastern China. 1 represents specific activity and 2 represents per gram tissue activity.

vole were higher than that in large Japanese field mouse and striped field mouse ($F = 7.406$, $P < 0.01$ and $F = 10.461$, $P < 0.01$, respectively), and no significant differences were found between the latter two species ($P > 0.05$, Fig. 1). The specific activities of cytochrome *c* oxidase were not significantly different among three species (Fig. 2), but the activities per gram BAT showed significant differences among three species ($F = 10.803$, $P < 0.01$). Gray red-backed voles had higher activities than large Japanese field mouse and striped field mouse, but no significant differences were found between the latter two species ($P > 0.05$, Fig. 2).

3.4. Protein content and activity of cytochrome c oxidase in muscle

Among the three species, there were significant differences in protein contents and activities of cytochrome *c* oxidase (per gram tissue) in muscle ($F = 9.002$, $P < 0.01$ and $F = 8.273$, $P < 0.01$, respectively). Gray red-backed voles had higher protein contents and cytochrome *c* oxidase activities than large Japanese field mice and striped field mice, but no significant differences were found

Table 2
Comparison of serum T_3 and T_4 (ng/ml) levels for thee rodent species

	C. rufocanus	*A. speciosus*	*A. agrarius*	*P* value
Sample size	5	6	7	
Thyroxine (T_4)	26.08±4.78	23.07±5.97	30.77±3.26	0.553
Triiodothyronine (T_3)	2.13±0.19	2.37±0.13	2.26±0.12	0.491

Data are presented as mean±SE.

between the latter two species. The specific activities of cytochrome *c* oxidase in muscle of these species showed no significant differences ($P>0.05$, Table 1).

3.5. Serum T_3 and T_4

There were no significant differences in serum T_3 and T_4 concentrations among the three rodent species ($P>0.05$, Table 2).

4. Discussion

Enhancement in NST capacity is important for non-hibernating small mammals to maintain stable body temperature during cold acclimation or seasonal acclimatization (Corp et al., 1997; Li et al., 2001). Further, increase in BMR was regarded as an adaptive adjustment to cope with cold (Cooper and Withers, 2002; Lovegrove, 2003). From a geographic scale, we expect that small mammals living in boreal areas will show relatively high BMR, and there should be a cellular and molecular basis responsible for the adaptive changes at the whole animal level.

Liver was considered to contribute greatly to BMR (Coutre and Hulbert, 1995; Li et al., 2001; Villarin et al., 2003). Proton leak accounts for 20–30% of total hepatic oxygen consumption (Rolfe et al., 1999). In these three rodent species, there were no significant differences either in the mitochondrial protein contents or in cytochrome *c* oxidase activities in liver; however, the mitochondrial state 4 respiration in gray red-backed voles was the highest among the three species. Li et al. (2001) found that the mitochondrial protein contents, state 4 and state 3 respiration levels, and cytochrome *c* oxidase activities in liver was relatively lower in the small mammals of northern China, such as Brandt's voles (*Microtus brandti*), Mongolian gerbils (*Meriones unguiculatus*) and plateau pikas (*Ochotona curzoniae*), than the species of southern China, such as tree shrew (*Tupaia belangeri*) and greater vole (*Eothenomys miletus*). They explained that high cellular thermogenesis in liver has adaptive significance in seasonal acclimatization for the species of southern China (Li et al., 2001). The adaptive strategy in thermogenesis for northern species differed from southern species, as the species of northern China mainly depended on the enhancement in NST whereas the species of southern China depended on increase in RMR. In a previous study, we have found that these three rodent species showed higher metabolism than predicted values based on their body mass and the highest BMR in gray red-backed vole and the lowest in large Japanese field mouse (Liu et al., 2004). However, although no significant differences were found in thermogenic properties of liver tissue (either mitochondrial protein content or the cytochrome *c* oxidase activity) for three species in summer, we did find that gray red-backed vole had the largest gastrointestinal tract among the three species (Liu and Wang, unpublished data), indicating that other tissues such as digestive tract may contribute to the BMR in gray red-backed vole according to the statement of Koteja and Weiner (1993).

BAT is the main site of NST for small mammals. In the present study, we found that the mitochondrial protein in BAT was highest in gray red-backed voles and lowest in large Japanese field mice, which was consistent with the adjustment in whole animal NST (Liu et al., 2004). Although the specific activity of cytochrome *c* oxidase in gray red-backed vole showed no significant differences as compared to the other two species, the cytochrome *c* oxidase activity per gram BAT was higher than the two *Apodemus* species. UCP1 contents in grey red-backed voles were also higher than the other two species both per gram BAT and total tissue. High level of cytochrome *c* oxidase activity and UCP1 content in gray red-backed vole are the cellular and molecular basis of high level in NST.

Skeletal muscle mass makes up to nearly 40% of the body mass, therefore, it is a primary contributor to thermogenesis during cold exposure via BMR, shivering thermogeneis and even NST (Block, 1994). The muscle mitochondrial protein contents and cytochrome *c* oxidase activity in gray red-backed voles are higher than the two *Apodemus* species. Muscle is an important tissue (organ) of thermogenesis and the adaptive changes in thermogenic capacity play a role in locomotion and thermoregulation.

Thyroid hormones can stimulate the increase in BMR and NST (Lanni et al., 2003). It has been suggested that the adaptive action of thyroid hormone in maintaining homeostasis of body temperature is mainly through the stimulation of facultative thermogenesis in BAT, together with the activity of Na^+/K^+ ATPase and ADP/ATP exchange in the thyroid-hormone-sensitive tissues (Hoek, 1992; Liu et al., 1997). However, we did not find significant differences in serum T_3 and T_4 concentrations among the three rodent species. We do not know the reason at present.

Three rodent species in the present study mainly depend on the increase in NST and BMR to maintain homeothermy (Liu et al., 2004). Habitats, food habits and phylogeny can all affect NST and BMR (Koteja and Weiner, 1993). Food habits are one of the important factors that affect the metabolic rates. Animals feeding on grass and herbs will have high metabolic rates. High metabolic level was considered to be closely related to the consistency and abundance of food resources in animal's environment (McNab, 1986). In gray red-backed voles, which use the low-quality food, the high metabolic rate may be related to the extra metabolic cost of digestion or the contribution of symbiotic intestinal flora to the overall metabolic rate, or both. Metabolic costs in gray red-backed vole may be associated with a large digestive gut (Koteja and Weiner, 1993). Phylogeny is also an important factor for interpreting the differences between gray red-backed voles and two *Apodemus* species.

In summary, gray red-backed vole showed the highest mitochondrial respiration in liver, cytochrome *c* oxidase activity in BAT and muscle, and UCP1 content in BAT among the three rodent species. The cellular and molecular changes are the bases of thermogenesis adjustments at whole animal level. Our data implied that gray red-backed vole, large Japanese field mouse and striped field mouse adopt different strategies to adapt their environments.

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