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**ORIGINAL ARTICLE**

# Comparative study on the thermogenic mechanism in two rodents, *Eothenomys milletus* and *Apodemus chevrieri* in Hengduan Mountain region during cold exposure

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The thermogenic mechanisms were measured in the two small rodents, *Eothenomys milletus* and *Apodemus chevrieri* in Hengduan mountain region during cold exposure. The main results were as follows: 1. RMR and NST incredsed 68.34%, 36.34% during the cold exposure in *E.miletus*, RMR and NST incredsed 32.84%, 56.17% during the cold exposure in *A. chevrieri*, respectively. 2. During cold exposure, total protein content in liver, the total protein content(TP) and mitochodrnal protein content(MP) in brown adipose tissue (BAT), the content of uncoupling protein (UCP1) were increased in *E.miletus*, which increased 18.66%, 25.18%, 70.01%, 99.39%, respectively. Total protein content in liver, the total protein content(TP) and mitochodrnal protein content(MP) in brown adipose tissue (BAT), the content of uncoupling protein (UCP1) were increased in *A. chevrieri*, which increased 18.11%, 33.29%, 34.51%,235.95%, respectively. All of the results indicated that the mechanism of heat production for adaptative changes was similar in two rodents, but it showed different increased amplitude of heat production, it may considered that the difference of heat production was related to origin of species and inhabitats.

*Key words: Eothenomys miletus; Apodemus chevrieri; adaptive thermogenesis; cold exposure*

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Adaptive thermogenesis in small mammals can be divided into obligatory thermogenesis and facultative thermogenesis, the former mainly refers to the basic metabolic rate (BMR), the latter including nonshivering thermogenesis (NST) and shivering thermogenesis (ST) (Himms-Hagen, 1990). They had difference in the heat production of molecular mechanism, and regulation of BMR and NST in different species showed different functions. This difference is likely to relate to animal adaptation to the environment and evolution for small mammals (Klaus et al., 1988). Capacity of obligatory thermogenesis was lower in tropical species (Haim and Izbaki, 1993), the regulation of facultative thermogenesis was larger relatively at the same time (Oufara et al., 1987); It showed that not only obligatory thermogenesis is strong, but also NST is the main heat to resistance under low temperature for the northern species (Jansky, 1973), maintaining temperature stability in the environment temperature (Gordon, 1993), it is the most economical way to regulate the energy balance in heat production (Heldmaier et al., 1982; 1986), it had become a mode in adaptive thermogenesis regulation for north small mammals (Tomasi and Horton, 1992). However, regulation of adaptive thermogenesis in small mammals is also influenced by other factors, including body size (Kleiber, 1961), ecological or behavior characteristics (McNab, 1986), And it is likely to reflect on different levels, such as in tissues, cells and mitochondria and other biological molecules levels (Wunder, 1992). Capacity of the adaptive thermogenic and its regulation mechanisms in small mammals profoundly affect the survival of small mammals adapt to the characteristics and evolutionary pathways(Tomasi and Horton, 1992),different species of small mammals showed different the influencing factors for thermogenesis characteristics (Heldmaier et al., 1990).

Hengduan Mountains region is located the boundary between the Palaearctic region and the Oriental region, is the proper alp and gorge region; has abundant mammals, is been considered “the harbor in fourth ice age” (Zheng, 1993). Small mammals’ may show a different physiological and ecological character since the geographical diversity and climate diversity in Hengduan Mountains region. *Eothenomys miletus* is the inherent species in Hengduan mountains region, has special status in *Microtus*. It is also the host of rat epidemic disease of Yunnan province, who distributed in Jianchuan, Heqin, Baoshang *et al* of Yunnan province. *Apodemus chevrieri* is the inherent species in Hengduan mountains region (Zhu et al., 2008; 2011).

Therefore, we measured changes in thermogenesis including body mass, BMR, NST and biochemical indexes in *E. Miletus* and *A. chevrieri* under cold acclimation. We predict that *E. miletus* and *A. chevrieri* will show a decrease in BM and increase in thermogenesis during the cold exposure.

## MATERIALS AND METHODS

### ***Samples***

*E. miletus* and *A. chevrieri* were captured in farmland (26°15′-26°45′N; 99°40′-99°55′E; altitude 2,590m) in Jianchuan County, Yunnan province, 2011. Mean yearly temperature was 9.1°C; mean monthly temperature ranges form -4.0°C in January to 24.1°C in July.

*E. miletus* and *A. chevrieri* were bred at the School of Life Sciences, Yunnan Normal University Science Park, in individual plastic boxes (260 mm × 160 mm × 150 mm) without bedding material, and were maintained at room temperature (25 ± 1 °C) under a photoperiod of 12L:12D (lights on at 8:00 am). After 1 month stabilization, healthy adult animals were assigned to each of 2 groups, a control group (sampled at 0 days) and a cold-acclimated group (sampled at 7 days, 14 days, 21 days, 28 days). The acclimation conditions of the control group were as above; the cold acclimation groups were subjected to the same 12L: 12D light regime at 5 ± 1 °C. At the start and end of the experimental period, the body mass of each animal was measured. Body mass was not significantly different between the groups at the start of the experiment for both *E. miletus* and *A. chevrieri* ($P>0.05$). Food and water were provided ad libitum. All pregnant, lactating or young individuals were excluded.

### ***Measurement of metabolic rates***

Metabolic rates were measured by using AD ML870 open respirometer (AD Instruments, Australia) at 25°C within the TNZ (thermal neutral zone), gas analysis were using ML206 gas analysis instrument, the temperature was controlled by SPX-300 artificial climatic engine (±0.5°C), the metabolic chamber volume is 500ml, flow is 200 ml/min. Animals were stabilized in the metabolic chamber for at least 60 min prior to the BMR measurement, oxygen consumption was recorded for more than 60·min at 5·min intervals. Two stable consecutive lowest readings were taken to calculate BMR. Calculate method of metabolic rate is detailed by Hills (Hill 1972).

Nonshivering thermogenesis (NST) was induced by subcutaneous injection of norepinephrine (NE) (Shanghai Harvest Pharmaceutical Co. Ltd) and measured at 25 °C. Two consecutive highest recordings of oxygen consumption more than 60 min at each measurement were taken to calculate the NST. The doses of NE were approximately 0.8-1.0 mg/kg according to dose-dependent response curves that were carried out before the experiment (Zhu et al., 2011).

### ***Measurements of mitochondrial protein and UCP1 content***

Extraction method of mitochondria : guillotined animal, quickly removed the interscapular BAT and liver, and weight BAT and liver. Extraction method of mitochondria is detailed by Cannon and Lindberg (1979). Mitochondrial protein concentration were determined by the Folin phenol method (Lowry et al., 1951) with bovine serum album as standards. UCP1 content was measured by Western blotting. Total BAT protein (15 μg per lane) was separated in a discontinuous SDS-polyacrylamide 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 membranes were stained after transferring with Coomassie brilliant blue and Ponceau red, respectively. Unspecific binding sites were saturated with 5% nonfat dry milk in PBS. UCP1 was detected using a polyclonal rabbit anti-hamster UCP1 (1:5000) as a primary antibody and peroxidase-conjugated goat anti-rabbit IgG (1:5000) (Jackson Immuno. Inc., USA)as the second antibody. Enhanced chemoluminescence (ECL, Amersham Biosciences, England) was used for detection. UCP1 concentration was determined from area readings by using Scion Image Software (Scion Corporation) (Lin and Klingenberg, 1982).

### ***Statistical analysis***

Data were analyzed using SPSS 15.0 software package. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov-Smirnov and Levene tests, respectively, including independent t test. Results were presented as mean ± SEM, and $P < 0.05$ was considered to be statistically significant.

## RESULTS

It showed a significant difference in RMR and NST between cold acclimation group and control group in *E.miletus* ($P<0.01$). RMR and NST increased 68.34%, 36.34% during the cold exposure in *E.miletus*. During cold exposure, total protein content in liver, the total protein content (TP) and mitochodrnal protein content (MP) in brown adipose tissue (BAT), the content of uncoupling protein (UCP1) were increased in *E.miletus*, which increased 18.66%, 25.18%, 70.01%, 99.39%, respectively (Table 1).

It showed a significant difference in RMR and NST between cold acclimation group and control group in *A. chevrieri* ($P<0.01$). RMR and NST increased 32.84%, 56.17% during the cold exposure in *A. chevrieri*, respectively. Total protein content in liver, the total protein content(TP) and mitochodrnal protein content(MP) in brown adipose tissue (BAT), the content of uncoupling protein (UCP1) were increased in *A. chevrieri*, which increased 18.11%, 33.29%, 34.51%,235.95%, respectively (Table 2).

**Table1.** Effects of cold exposure on body mass and thermogenesis in *E. miletus*

| Parameters | Control (n=10) | Cold acclimation (28days) (n=10) | Significance |
|---|---|---|---|
| Body mass (g) | | | |
| Initial | 42.68±1.55 | 42.85±2.30 | >0.05 |
| Final | 42.80±1.63 | 34.56±2.05 | <0.01 |
| RMR ($mlO_2$/g.h) | 1.99±0.21 | 3.35±0.23 | <0.01 |
| NST ($mlO_2$/g.h) | 4.32±0.36 | 5.89±0.53 | <0.01 |
| Liver mass (g) | 1.81±0.24 | 2.03±0.24 | <0.05 |
| TP in liver (mg/g liver) | 328.44±13.37 | 389.72±11.46 | <0.01 |
| BAT mass (g) | 0.21±0.01 | 0.23±0.01 | <0.05 |
| TP in BAT (mg/gBAT) | 233.74±2.86 | 292.59±12.03 | <0.01 |
| Mp in BAT (mg/gBAT) | 17.69±0.731 | 31.49±0.77 | <0.01 |
| UCP1contents (mg/gBAT) | 70.56±8.96 | 140.69±10.22 | <0.01 |

**Table 2** Effects of cold exposure on body mass and thermogenesis in *A.chevrieri*

| Parameters | Control (n=10) | Cold acclimation (28days) (n=10) | Significance |
|---|---|---|---|
| Body mass (g) | | | |
| Initial | 38.35±1.45 | 38.41±1.33 | >0.05 |
| Final | 38.50±1.55 | 32.78±1.38 | <0.01 |
| RMR ($mlO_2$/g.h) | 3.41±0.63 | 4.53±0.69 | <0.01 |
| NST ($mlO_2$/g.h) | 5.75±0.56 | 8.98±1.01 | <0.01 |
| Liver mass (g) | 1.36±0.02 | 1.78±0.21 | <0.01 |
| TP in liver (mg/g liver) | 332.39±38.26 | 392.59±19.73 | <0.01 |
| BAT mass (g) | 0.17±0.02 | 0.21±0.04 | <0.05 |
| TP in BAT (mg/gBAT) | 226.58±32.39 | 302.02±6.12 | <0.01 |
| Mp in BAT (mg/gBAT) | 24.31±0.50 | 32.70±1.14 | <0.01 |
| UCP1contents (mg/gBAT) | 178.56±11.36 | 421.32±15.69 | <0.01 |

## DISCUSSION

Rest metabolic rate is an important indicator of physiological ecology, which can reflect the energy consumption level in different species or between individual in small mammals, had the important significance in the process of adaptation to the environment for animals (McNab, 1997), It is affected by many factors such as temperature, food, animal activity (Anderson et al., 2006; Terblanche et al., 2007). There has been a lot of reports suggests that the low temperature can lead to increase of RMR in many small rodents. In the present study, BMR increased significantly in both *E. miletus* and *A. chevrieri* during cold acclimation. It was consistent with other rodents, such as *Mesocricetus auratus* (Tomasi and Horwitz, 1987), *Gerbillus campestris* (Oufara et al., 1987), *Saccostomus campestris* (Haim et al., 1991), *Microtus agrestis* (McDevitt and Speakman, 1994).

Non shivering thermogenesis is a effectively and economically heat production mode for small mammals adapted to the cold environment (Jansky, 1973), It showed that many small mammals increases NST in cold acclimation (Himms-Hagen, 1990), such as *Phodopus sungorus* (Tomasi and Horton, 1992), *Mesoocricetus auratus* (Adolph and Lawrow, 1951). NST increased both in *E. miletus* and *A. chevrieri* during cold acclimation, it indicated that increase thermogenesis were a strategy to reply in a low temperature (Johnstone et al 2005; Zhu et al 2011). *A. chevrieri* increased NST significantly higher than in *E. miletus*, it showed that clod is a main factor to limit *E. miletus* diffused to the northern area (Zhu et al., 2010), and it may be related to its origins for *A. chevrieri*, *A. chevrieri* was the palaearctic animal, may retain some of the characteristics of small mammals in North.

The increase in thermogenesis at the animal individual level were further supported by other biochemical markers examined in the present study, including high mitochondrial protein content, and UCP1 content. Liver metabolism accounts for 20–25% of RMR (Brand et al., 1991), especially in small mammals, heat production capacity in liver occupies an important position (Porter and Brand, 1993). Our data indicated that the changes in liver were in parallel with the changes in RMR during cold acclimation both in *E. miletus* and *A. chevrieri*, which increased 18.11%, 18.11%, respectively. UCP1 mRNA expression and production in BAT may be an indicative of the thermogenic capacity (Didow and Hayward, 1969). In the present study, UCP1 content increased markedly in the cold condition. Data from the present study showed during cold exposure, total protein content in liver, the total protein content(TP) and mitochodrnal protein content(MP) in brown adipose tissue (BAT), the content of uncoupling protein (UCP1) were increased in *E.miletus*, which increased 18.66%, 25.18%, 70.01%, 99.39%, respectively. Total protein content in liver, the total protein content(TP) and mitochodrnal protein content(MP) in brown adipose tissue (BAT), the content of uncoupling protein (UCP1) were increased in *A. chevrieri*, which increased 18.11%, 33.29%, 34.51%,235.95%, respectively. It showed that *E. miletus* and *A. chevrieri* can improve the content of UCP1 to adapt to the low temperature environment, the two rodents appeared convergent adaptation.

However, several indicators of the BAT in *A. chevrieri* are higher than those in *E. miletus* in cold acclimation conditions. This may be related to their environment and origin of life. *E. miletus* is a burrowing type, there are food hoarding behavior, *A. chevrieri* is a burrowing type, it had significantly different environment to two rodents. In addition, *E. Miletus* and *A. chevrieri* in origin also had bigger difference, therefore, *E. Miletus* identified in the regulation of body temperature is very likely to keep with the climate characteristics of adaptation features, which mainly increased basal metabolic rate to survive in the cold condition. But for *A. chevrieri*, it was more sensitive to low temperature.

In conclusion, the results indicated that the mechanism of heat production for adaptative changes was similar in two rodents, but it showed different increased amplitude of heat production, it may considered that the difference of heat production was related to origin of species and inhabitants.

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