Seasonal variations of thermoregulatory and thermogenic properties in *Eothenomys miletus* and *Apodemus chevrieri*

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*Eothenomys miletus* and *Apodemus chevrieri* are typical species of small mammals inhabiting in Hengduan mountains region. The characteristics of thermoregulation and thermogenesis of two mammals were measured to search their physiological and ecological characteristics of adaptations to this region in different seasons. All results indicated: the body weight of *E. miletus* and *A. chevrieri* in summer was separately 47.29±0.73g, 32.74±0.54g, and their body weight in winter was separately 39.28±0.61g, 31.70±0.76g; the thermal neutral zones (TNZ) of *E. miletus* and *A. chevrieri* in summer were separately 25~32.5°C and 25~30°C, and their TNZ in winter were all of 22.5~27.5°C; their basal metabolic rates (BMR) in summer were respectively 3.76±0.07ml O$_2$/g.h, 4.58±0.09mlO$_2$/g.h, and their BMR in winter were respectively 4.46±0.04mIO$_2$/g.h, 5.23±0.01mIO$_2$/g.h; their maximum nonshivering thermogenesis (NST) in summer was respectively 5.70±0.18mlO$_2$/g.h, 7.12±0.31mlO$_2$/g.h, and their NST in winter was respectively 6.67±0.05mlO$_2$/g.h, 7.42±0.04mlO$_2$/g.h; their NST scope (NST/BMR) in summer was separately 1.52±0.05, 1.46±0.04, and their NST scope in winter was separately 1.49±0.01, 1.42±0.01. Their thermogenic characteristics and thermoregulatory styles possibly reflected features of small rodents in Hengduan mountains region which have lower body temperatures and NST scope, higher BMR, Cm and NST and could keep their body temperatures stable in narrower ambient temperatures comparing with other rodents. Body temperature, Cm BMR and NST of *A. chevrieri* were higher than these of *E. miletus*. *A. chevrieri* could keep body temperature stable in a wider range of ambient temperatures than *E. miletus*. NST scope of *E. miletus* was higher than it of *A. chevrieri*. Their TNZ and the ambient temperature range in which they could keep C stable in winter were narrower than these indexes in summer. The body temperature and body weight in winter were lower comparing with the summer. The BMR, F-value and NST$_{max}$ in winter were significantly higher than the summer. The TNZ in winter was shifted to the lower ambient temperature comparing with the summer.

*Key words: Eothenomys miletus; Apodemus chevrieri; Thermoregulation; Thermogenesis*
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Their thermogenic characteristics and thermoregulatory styles possibly reflected features of small rodents in Hengduan mountains region which have lower body temperatures and NST scope, higher BMR, Cm and NST and could keep their body temperatures stable in narrower ambient temperatures comparing with other rodents. Body temperature, Cm BMR and NST of *A. chevrieri* were higher than these of *E. miletus*. *A. chevrieri* could keep body temperature stable in a wider range of ambient temperatures than *E. miletus*. NST scope of *E. miletus* was higher than it of *A. chevrieri*. Their TNZ and the ambient temperature range in which they could keep C stable in winter were narrower than these indexes in summer. The body temperature and body weight in winter were lower comparing with the summer. The BMR, F-value and NST max in winter were significantly higher than the summer. The TNZ in winter was shifted to the lower ambient temperature comparing with the summer.

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regulation is the animal to maintain the body temperature in certain range process by physical (behavior) or physiological way (Ge, 2002). Heat regulation characteristics of small mammals were significant influenced by ecological or behavioral characteristics, and profoundly affects the animal energy distribution and its evolutionary path (Tomasi and Horton, 1992).

Thermoregulation and thermogenesis characteristics of small mammals in different seasons were different, these differences were mainly in body mass, body temperature, BMR, the appearance of these differences is the main cause of the animal stress with low temperature, stress caused by the different food in different seasons. It showed that body mass of small mammals in the winter can be reduced up to 50% (Lovegrov, 2003). The northern three-toed jerboa studies had shown the seasonal temperature from spring to fall gradually reduced, the spring animal resting metabolic rate minimum (Bao et al., 2000). Small mammals, especially rodents, the BMR affected by seasons, some mammals showed high BMR in winter than that in summer, such as Sylvilagus audubonii, Lepus californicus, Lepus townsendi (Degen, 1997). Adaptation of Animal to cold environmental were always used by changes in basal metabolic rate, reduce the heat conduction and increased fur thickness, another way is nonshivering thermogenesis (NST). Nonshivering thermogenesis is constant in animal muscle activity and increased heat production under cold exposure, small mammals using the mechanism is NST, shivering thermogenesis is only in extreme cold as an auxiliary heat source. When body mass was more than 3kg, the importance of NST is very small, but in small mammals, especially less than 200g of the body mass, NST is much more important than shivering thermogenesis (Heldmaier et al., 1990).

Hengduan Mountains region is located the boundary between the Palaeartic region and the Oriental region, is the proper alp and gorge region; has abundant mammals, is been considered “the harbor in fourth ice age” (Wu and Wang, 1985). Small mammals’ may show a different physiological and ecological character since the geographical diversity and climate diversity in Hengduan Mountains region. E. 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 (Zheng, 1993). Here we measure seasonal changes in thermogenesis including body mass, BMR, NST from E. miletus and A. chevrieri. We hypothesize that, similar to other small mammals, E. miletus and A. chevrieri will change their thermogenesis seasonally. We predict that E. miletus and A. chevrieri will show a decrease in BM and increase in thermogenesis in the cold season.

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.

A total of 152 included summer (June 2011) (n=80) and winter (December 2011) (n=72) of E. miletus, a total of 151 included summer (June 2011) (n=79) and winter (December 2011) (n=72) of A.
chevrieri. Between capture and metabolic analysis, the animals were kept individually in plastic cages (350×300×250mm³) in a room with natural temperature and photoperiod, summer (23.9 °C), winter (-3.8 °C). 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 (Heldmaier, 1971). 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.

**Thermal conductance(C) and F value**

Thermal conductance was evaluated as C=RMR/(Tb-Ta) (McNab, 1980). In this equation, RMR is the basal metabolic rate (ml O₂/g.h), Tb is the body temperature (°C), Ta is the environment temperature(°C). F value can be calculated as F=(BMR/Kleiber predicted BMR)/ (C/Bradly predicted C) (McNab,1970). In this equation, predicted BMR was Kleiber’s (1961) body mass predicted value; predicted C was Bradly’s(1980)body mass predicted value.

**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 analysis of covariance(ANCOVA), repeat metrical regression and independent t test. Results are reported as mean ± standard deviation for each species. Predicted BMR was Kleiber’s (1961) body mass predicted value; predicted C were Herried (1967) and Bradly’s (1980) body mass predicted value. Results were presented as mean ± SEM, and P < 0.05 was considered to be statistically significant.

**RESULTS**

**Body mass**

Body mass in *E. miletus* was 47.29±0.73g (n=80) in summer, was 39.28±0.61g (n=72) in winter, showed significant difference between different seasons (t=8.734, P<0.01), body mass in summer was 22.47% higher than that in Winter. Body mass in *A. chevrieri* was 32.74±0.54g (n=79) in summer, was 31.70±0.76g (n=72) in winter, showed significant difference between different seasons (t=1.012, P>0.05), body mass in summer was 8.58% higher than that in Winter (Table 1).

**BMR**

The relation between BMR and Ta (5 °C ~35 °C) of *E. miletus* in summer is represented by the following equation: BMR =7.643-0.118Tₐ (r=-0.821,
P<0.01). Using \( W^{0.73} \) rectification, the relation is: BMR =21.548 - 0.334 \( T_o \), \( (r=-0.825, P<0.01) \). The relation between BMR and Ta (5 °C ~35 °C) of A. chevrieri in summer is represented by the following equation: BMR =8.167-0.106\( T_o \), \( (r=-0.824, P<0.01) \). Using \( W^{0.73} \) rectification, the relation is: BMR =20.965-0.276\( T_o \), \( (r=-0.869, P<0.01) \) (Fig 1).

Using analysis of repeat metrical regression, when Ta between 25 °C and 32.5 °C, BMR in E. miletus were not significant correlation (P>0.05) in summer, so the thermal neutral zone (TNZ) was 25 °C and 32.5 °C. In the TNZ, BMR of E. miletus was 3.76±0.07 ml/o.h. In the TNZ, by the ANCOVA, BMR of E. miletus and body mass showed significant correlation: BMR=33.0554W^{0.5703} \( (r=-0.767, P<0.01) \). Using analysis of repeat metrical regression, when Ta between 25 °C and 30 °C, BMR in A. chevrieri were not significant correlation (P>0.05) in summer, so the thermal neutral zone (TNZ) was 25 °C and 30 °C. In the TNZ, BMR of A. chevrieri was 4.58±0.09 ml/o,g.h. In the TNZ, by the ANCOVA, BMR of A. chevrieri and body mass showed significant correlation: BMR=33.0554W^{0.5703} \( (r=-0.767, P<0.01) \).

The relation between BMR and Ta(5 °C ~35 °C) of E. miletus in winter is represented by the following equation: BMR=8.497-0.106\( T_o \), \( (r=-0.884, P<0.01) \). Using \( W^{0.73} \) rectification, the relation is: BMR=21.016 - 0.267\( T_o \), \( (r=-0.736, P<0.01) \). The relation between BMR and Ta (5 °C ~35 °C) of A. chevrieri in winter is represented by the following equation: BMR=8.497-0.106\( T_o \), \( (r=-0.884, P<0.01) \). Using \( W^{0.73} \) rectification, the relation is: BMR=22.075-0.293\( T_o \), \( (r=-0.828, P<0.01) \).

Using analysis of repeat metrical regression, when Ta between 22.5 °C and 27.5 °C, BMR in E. miletus were not significant correlation (P>0.05) in winter, so the thermal neutral zone (TNZ) was 22.5 °C and 27.5 °C. In the TNZ, BMR of E. miletus was 4.46±0.04 ml/o,g.h. In the TNZ, by the ANCOVA, BMR of E. miletus and body mass showed significant correlation: BMR=1.046W^{0.389} \( (r=0.935, P<0.01) \). Using analysis of repeat metrical regression, when Ta between 22.5 °C and 27.5 °C, BMR in A. chevrieri were not significant correlation (P>0.05) in winter, so the thermal neutral zone (TNZ) was 22.5 °C and 27.5 °C. In the TNZ, BMR of A. chevrieri was 5.23±0.01 ml/o,g.h. In the TNZ, by the ANCOVA, BMR of A. chevrieri and body mass showed significant correlation: BMR=4.677W^{0.034} \( (r=0.932, P<0.01) \).

BMR showed significant difference between different seasons in E. miletus \( (t=6.701, P<0.01) \), it also showed significant difference between different seasons in A. chevrieri \( (t=6.337, P<0.01) \).

**NST**

NST of E. miletus was 5.70±0.18ml o,g .h in 25 °C in summer, was Heldmaier’s body mass predicted value’s 112.31±2.54%. NST of A. chevrieri was 7.12±0.31 ml o,g .h in 25 °C in summer, was Heldmaier’s body mass predicted value’s 118.29±2.75%. NST of E. miletus was 6.67±0.05 ml o,g .h in 25 °C in winter, was Heldmaier’s body mass predicted value’s 127.44±1.35%. NST of A. chevrieri was 7.42±0.04 ml o,g .h in 25 °C in summer, was Heldmaier’s body mass predicted value’s 107.29±2.75% (Table 1).

NST showed significant difference between different seasons in E. miletus \( (t=-4.878, P<0.01) \), it also showed significant difference between different seasons in A. chevrieri \( (t=-4.441, P<0.01) \). The value of NSTmax/BMR were 1.52±0.05 and 1.46±0.04 for E. miletus and A. chevrieri in summer, and were 1.49±0.01 and 1.42±0.01 in winter, respectively.
Thermal conductance (C) and F value

Down the TNZ, thermal conductance of *E. miletus* and *Ta* showed no significant correlation in summer, average value was 0.28±0.005 ml O$_2$·g·h.$^{-1}$·°C$^{-1}$, was Herried's body mass predicted value's 191.13±2.60%, was Bradly's body mass predicted value's 196.44±2.59%. Up the 30 °C, thermal conductance increased when the environment temperature ascended, the relation between the thermal conductance of *E. miletus* and *Ta* was: 

\[ C = 0.2226 + 0.0053T_a \] 

\( r = 0.822, P<0.01 \) (Fig 2). Down the TNZ, thermal conductance of *A. chevrieri* and *Ta* showed no significant correlation in summer, average value was 0.32±0.009 O$_2$·g·h.$^{-1}$·°C$^{-1}$, was Herried's body mass predicted value's 186.85±5.06%, was Bradly's body mass predicted value's 186.58±5.09%. Up the 30 °C, thermal conductance increased when the environment temperature ascended, the relation between the thermal conductance of *A. chevrieri* and *Ta* was: 

\[ C = 0.2125 + 0.0086T_a \] 

\( r = 0.881, P<0.01 \) (Fig 2). In the TNZ, F value of *E. miletus* in summer was 0.88±0.05. In 5 °C and 35 °C, F value of *E. miletus* declined when the environment temperature ascended, they showed significant correlation: 

\[ F = 3.745 - 0.099T_a \] 

\( r = -0.997, P<0.01 \); In the TNZ, F value of *A. chevrieri* in summer was 1.10±0.05. In 5 °C and 35 °C, F value of *A. chevrieri* declined when the environment temperature ascended, they showed significant correlation: 

\[ F = 3.922 - 0.1T_a \] 

\( r = 0.974, P<0.01 \).
Table 1 Effects of the body mass, thermogenic abilities in *E. miletus* and *A. chevrieri* in different seasons

<table>
<thead>
<tr>
<th></th>
<th>Summer</th>
<th>Winter</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. miletus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>47.29±0.73</td>
<td>39.28±0.61</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMR (ml O₂/g.h)</td>
<td>3.76±0.07</td>
<td>4.46±0.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NST (ml O₂/g.h)</td>
<td>5.70±0.18</td>
<td>6.67±0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Thermal conductance (C)</td>
<td>0.28±0.005</td>
<td>0.31±0.005</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>F value</td>
<td>0.88±0.05</td>
<td>1.03±0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>Apodemus chevrieri</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>32.74±0.54</td>
<td>31.70±0.76</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMR (ml O₂/g.h)</td>
<td>4.58±0.09</td>
<td>5.23±0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NST (ml O₂/g.h)</td>
<td>7.12±0.31</td>
<td>7.42±0.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Thermal conductance (C)</td>
<td>0.32±0.009</td>
<td>0.32±0.005</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>F value</td>
<td>1.10±0.05</td>
<td>1.26±0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Figure 1: The relationship between basal metabolic rate and ambient temperatures in *E. miletus* and *A. chevrieri* in different seasons.
DISCUSSION

Body mass

Ambient temperature plays an important role in animals’ physiology and behaviors. It has been demonstrated that many small mammals, such as Phodopus sungorus, Sorex, respond to winter-associated environmental cues by reducing body mass (Genoud, 1988; Zhan and Wang, 2004; Liu et al., 2003). Our present results showed that cold temperature is an important environmental cue that can influence E. miletus and A. chevrieri to reduce their body mass significantly. Body mass decline in winter conditions is considered to be an adaptive mechanism for the reduction of absolute energy requirements when stress occurs (Heldmaier et al., 1986; Downs, 1985; Wunder, 1985).

BMR and NST

BMR of mammals associate with the selective factors, such as climate, habitat (Rezende et al., 2004). Generally speaking, BMR of mammals in torrid zone are lower than them in temperature zone, and BMR of mammals in temperature zone are lower than them in frigid zone. Animals live in frigid zone, especially in polar region, most animals have higher BMR (Koteja and Weiner, 1993). BMR in E. miletus and A. chevrieri were higher compare with some small mammals, BMR of two species in summer were Kleiber’s body mass predicted value’s 287.36±4.32%, 320.49±4.96%, respectively. They are higher than some rodents lived in low altitude in north region, Microtus pennsylvanicus (141%), M.ochrogaster (129%), M.richardson (131%), M.breweri (110.4%) (Kurta and Ferkin, 1991), also higher than M. oeconomus (214%) which live in alpine region(Wang and Wang, 2000). All of these may cope with their adaptation characteristics of two species: although two species habitat’s latitude are lower than some species live in north region, but their habitat’s altitude are close, along with the increase of altitude, manace of low temperature.
gradually increase, it is equal to increased the latitude (Wang et al., 1994), so two species have higher BMR levels. Furthermore, because of the daily difference in temperature are greater in Hengduan mountains region, two species suffer high temperature and low temperature every day, and the ability of their non-shivering thermogenesis (NST) are relatively lower, it lead to adopt high metabolic rate to fit the menace of low temperature. In addition, the habitat of two species has no snow cover in winter and abundant food resource, so these also provide some conditions for them to adopt higher energy strategy (McNab, 1986).

The variations in body mass were associated with changes in energy intake and expenditure. It is evident that many winter-active small mammals enhance NST for survival in the cold or winter (Heldmaier et al. 1986; Jansky, 1973). In present study, NST of E. miletus and A. chevrieri showed seasonal plasticity, E. miletus and A. chevrieri in the wild increased NST in cold seasons; it was also similar to other small mammals living in cold regions (Bao et al., 2001; Li et al., 1994; Haim and Izbaki, 1993). These results supported the hot disappearing limit hypothesis; animals increased heat production in winter, heat loss was increased, energy intake has also increased, animals decreased heat production in summer, heat loss was restricted, energy intake has also limited.

**Thermal conductance(C) and F value**

Thermal conductance of two species maintain invariability between 5 °C and 25 °C, higher than typical species of north region obviously, are close to the level of species live in torrid zone, such as *Tupaia belangeri* (199.9%) (Wang et al., 1994). One hand, it is relate to theirs behaviors; another hand, it is connect with the difference in temperature of theirs habitat. Because of particular geography and climate in Hengduan mountains region, two species endure more menace of low temperature, tolerance of hot temperature were feeble, so it appeared excessive hot temperature and thermal conductance increase when the environment temperature was low (Wang et al., 2006). F value is show the ability of thermoregulation (Gordon, 1993), can be weight the relatively ability of energy metabolism of mammals in different temperatures. F value in *E. miletus* and *A. chevrieri* were lower than rats live in north region, such as *Micrurus pennsylvanicus*, *M. ochrogaster*, *M. richardson*, but higher than species live in torrid zone (Kurta and Ferkin, 1991; Wang et al., 1999). All of these illuminate that ability of maintain body temperature invariableness of two species were finite.

In conclusion, thermogenic characteristics and thermoregulatory styles in *E. miletus* and *A. chevrieri* possibly reflected features of small rodents in Hengduan mountains region which have lower body temperatures and NST scope, higher BMR, Cm and NST$_{max}$ and could keep their body temperatures stable in narrower ambient temperatures comparing with other rodents. Body temperature, Cm BMR and NST$_{max}$ of *A. chevrieri* were higher than these of *E. miletus*. *A. chevrieri* could keep body temperature stable in a wider range of ambient temperatures than *E. miletus*. NST scope of *E. miletus* was higher than it of *A. chevrieri*. Their TNZ and the ambient temperature range in which they could keep C stable in winter were narrower than these indexes in summer. The body temperature and body weight in winter were lower comparing with the summer. The BMR, F-value and NST$_{max}$ in winter were significantly higher than the summer. The TNZ in winter was shifted to
the lower ambient temperature comparing with the summer.

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REFERENCES


Kurta, A., Ferkin, M. (1991) The correlation of...
demography and metabolic rate: a test using the vole (Microtus breweri) and the meadow vole (Microtus pennsylvanicus). Oecologia, 87: 102-105.


