Effects of different temperature and reproductive experiences on energy metabolism in *Eothenomys miletus*

Gong Xue-Nai, Zhang Han, Zhu Wan-Long

1. Key Laboratory of Ecological Adaptive Evolution and Conservation on Animals-Plants in Southwest Mountain Ecosystem of Yunnan Province Higher Institutes College, School of Life Science of Yunnan Normal University, Kunming; 650500, China.
2. Engineering research center of sustainable development and utilization of biomass energy Ministry of Education, Kunming, 650092, China.
3. The Key Laboratory of Biomass Energy and Environmental Biotechnology in Yunnan Province, Kunming; 650092, China.

**ABSTRACT**

In order to investigate the effects of environmental temperature and reproductive experience on the energy metabolism during lactation of *Eothenomys miletus*, three consecutive reproductions of *E. miletus* were exposed to different temperatures. The first, second and third reproductions of *E. miletus* were exposed to 30 °C, 20 °C and 10 °C, respectively. Body mass, energy intake, resting metabolic rate (RMR), nonshivering thermogenesis (NST), litter size and mass, cytochrome c oxidase (COX) activity in brown adipose tissue (BAT) and serum thyroid hormone were measured. The results showed that compared with the first reproduction group, energy intake, RMR, NST, COX activity, triiodothyronine (T3) levels of *E. miletus* in the third reproduction group increased significantly, while litter mass decreased significantly during weaning. All of the results showed that *E. miletus* reproduces at lower temperatures were in a negative energy balance, and there was a trade-off between self-sustainment and energy allocation of feeding offspring; thermogenesis increased and reproductive output decreased at lower temperatures. Moreover, *E. miletus* may perceive changes in environmental temperature and reduce reproductive investment under the condition of different temperatures, which accorded with the prediction of the "seasonal investment hypothesis".

**INTRODUCTION**

The survival and reproduction of small mammals were affected by environmental temperature (Hammond and Kristan 2000). The decrease of temperature will increase the energy demand of animals, and the energy demand of animals breeding at lower temperature will further increase, while the maternal body meets the energy demand of offspring growth and development. In many studies on the energetics of reproduction in lactating animals and experimental designs, it also needs additional energy expenditure to produce thermogenesis in order to maintain body temperature regulation (Zhang and Wang 2007; Gong et al. 2018). Wild small mammals may be able to sense seasonal changes in reproductive value, thus showing selective reproductive investment, namely the "seasonal investment hypothesis" (Speakman and Król 2005). How animals perceive seasonal changes in environmental conditions and then determine their "seasonal investment" remains unclear. The environmental temperature has the characteristics of seasonal variation, which has been reported that there is a negative correlation between temperature and animal reproductive investment, such as the reproductive output of *Lasiopteryx brandti* (Zhang and Wang 2007) and *Mus musculus* (Król and Speakman 2003) in warm conditions (30 °C) were lower than that of animals in cold conditions (5 °C, 8 °C). It is not clear that temperature changes will determine the selective reproductive investment in animals.

*Eothenomys* (Mammalia: Rodentia: Cricetidae) is a genus of rodent in China, and were the typical animals in Hengduan mountain regions. *E. miletus* is an inherent species in southwest China, specifically in Hengduan mountain regions (Miller 1896; Zhu et al. 2016a). Although there had been many studies on the energetics of reproduction in lactating *E. miletus* (Zhu et al. 2014; 2016b). But the relationship between energy balance and reproductive output of different reproductive experiences and temperatures of *E. miletus* was not clear. In the present study, energy metabolism in different reproductive experiences and temperatures of *E. miletus* were measured, testing the "seasonal investment hypothesis".

**MATERIALS AND METHODS**

**Animals and experimental designs**

*E. miletus* were obtained from a laboratory colony, which were captured in farmland (26°15′~26°45′N; 99°40′~99°55′E; altitude 2,590 m) in Jiangchuan County, Yunnan province, 2010. *E. miletus* were maintained at a room temperature of 25±1 °C, under a photoperiod of 12L:12D (with lights on at 08:00, food (standard rabbit pellet chow; produced by Kunming Medical University, Kunming) and water were provided ad libitum. A total of 88 females (all virgin voles) were selected adaptation at 30 °C for 4 weeks, then adult males were put in, pregnant females were separated from males before delivery. A total of 52 females were pregnant, delivered and lactating, random selection of 10 to measure the energy metabolism as the 1st lactation periods (Lac 1) under a room temperature of 30±1 °C; remaining 42 in weaning after 2 weeks to male rats and paired, 29 female pregnancy, childbirth and lactation, select 10 determination energy budget, as 2nd lactation periods (LAC 2, n = 10) under a room temperature of 20±1 °C; remaining 19 in weaning after 2 weeks to male rats and paired, 11 female pregnancy, select 10 determination energy budget, as 3rd lactation periods (LAC 3, n = 10) under a room temperature of 10±1 °C. All animals procedures were compliant with the Animal Care and Use Committee of the School of Life Science, Yunnan Normal University. This study was approved by the Committee (13-0901-011).
Statistical analysis
Data were analyzed using the software package SPSS 15.0. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance using Kolmogorov-Smirnov and Levene tests, respectively. Body mass, food intake, litter size, litter mass, RMR, NST, COX activity and serum thyroid hormone were analyzed with one-way ANOVA with each experimental group followed by LSD post hoc tests. Results are presented as means ± SEM and P < 0.05 was considered to be statistically significant.

RESULTS

Body mass and food intake
There was a significant change in body mass on day 0 in different lactation groups (F=5.58, P<0.01, Fig. 1). On day 22, body mass in three groups showed significant differences (F=3.21, P<0.05). Food intake on day 0 and 22 had significant differences among three groups (Day 0: F=8.23, P<0.01; Day 22: F=4.87, P<0.01, Fig. 2).

Litter size and litter mass
Litter size on day 0 had no significant differences among three groups (P>0.05), and reproductive experiences had no significant differences on litter size on day 22 (P>0.05, Fig. 3). Litter mass also showed no significant differences among three groups on day 0 (Fig. 4), but it had significant differences on day 22 (F=3.15, P<0.05).

RMR, body fat mass, COX activity and serum thyroid hormone
Reproductive experiences and temperature had significant differences on RMR and NST on day 22 (RMR: F=2.89, P<0.05; NST: F=3.33, P<0.05, Table 1). Wet or dry masses of carcass, body fat mass showed no significant differences on day 22 (Table 1). COX activity in BAT showed significant differences on day 22 among three groups (F=2.78, P<0.05). Significant differences was also found in T3 levels (F=3.58, P<0.01), but T4 and prolactin levels in serum had no significant differences on day 22 (P>0.05, Table 1).

DISCUSSION
Reproduction of small mammals was a complex and energy-consuming process. Lactation period is the highest energy requirement stage in the whole reproductive period. Animals generally use their energy storage and increase their food intake to meet the increased energy requirement during the reproductive period (Garton and Harder 1994). It was found that the food intake increased significantly of E. miletus, while body mass decreased significantly, which was similar to previous studies (Zhu et al. 2016b). Food intake increased significantly in Lac 3 compared with that of other two groups, suggesting that cold temperature could increase food intake. Lower temperature exposure also increased food intake of lactating Lasiopodomys brandti (Zhang and Wang 2007) and Mus musculus (Kröl and Speakman 2003). These results indicate that energy intake of small mammals during lactation were significantly affected by environmental temperature. Mammals under lower temperature need to increase their food intake to cope with the dual energy needs of lower temperature and lactating offspring (Zhang and Wang 2007).

In the present study, the reproductive output of E. miletus decreased significantly with the decrease of ambient temperature. Previously study showed that there was no significant changes in energy intake and reproductive output of E. miletus bred four times at room temperature during lactation (Zhu et al. 2016c), indicating that the reproductive capacity of E. miletus with different reproductive experiences was similar. Therefore, the reason for the significant decrease of reproductive output in the third reproductive group under lower temperature may not be related to the reproductive capacity of the animals themselves, but mainly affected by the environmental temperature, thus, different environmental temperature may be the main reason for the significant decrease of reproductive output, which was consistent with the prediction of the “seasonal investment hypothesis” (Koivula et al. 2003). Lower temperature exposure increases the energy demand of animals. It was found that the RMR and NST of E. miletus increased significantly in the third reproductive group compared with that of other two reproductive group. BAT COX activity and serum T3 level also increased significantly, suggesting that thermogenesis increased at lower temperature. Small mammals need additional energy expenditure to produce heat and maintain body temperature regulation when facing lower temperature environment during reproductive period, but this may lead to reduced energy expenditure and reproductive output for lactation (Zhao 2011). In the current study, litter mass on day 22 in Lac 3 group were significant lower that of other two reproductive group.

CONCLUSION
In conclusion, food intake, litter mass increased significantly, and body mass decreased significantly during lactation. Food intake, litter mass and thermogenesis were significantly affected by ambient temperature. There is a trade-off between self-sustainment and energy allocation of feeding offspring. Thermogenesis increases and reproductive output decrease at lower temperatures. E. miletus may reduce changes in environmental temperature and reduce reproductive investment under the condition of different temperatures, which accords with the prediction of the “seasonal investment hypothesis”.

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Fig. 2: Effects of reproductive experience and temperature on food intake in *Eothenomys miletus*.

Fig. 3: Effects of reproductive experience and temperature on litter size in *Eothenomys miletus*.

Fig. 4: Effects of reproductive experience and temperature on litter mass in *Eothenomys miletus*.
### Table 1: Effects of reproductive experience and temperature on RMR, NST, body fat mass, COX activity and serum thyroid hormone in *Eothenomys miletus*.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lac 1</th>
<th>Lac 2</th>
<th>Lac 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RMR (mlO₂/h)</strong></td>
<td>110.52 ± 2.23</td>
<td>111.25 ± 1.69</td>
<td>120.36 ± 2.36</td>
</tr>
<tr>
<td><strong>NST (mlO₂/h)</strong></td>
<td>165.36 ± 3.36</td>
<td>166.89 ± 2.25</td>
<td>189.65 ± 2.48</td>
</tr>
<tr>
<td><strong>Wet carcass mass (g)</strong></td>
<td>18.65 ± 0.51</td>
<td>17.96 ± 0.54</td>
<td>16.58 ± 0.32</td>
</tr>
<tr>
<td><strong>Dry carcass mass (g)</strong></td>
<td>5.42 ± 0.12</td>
<td>5.43 ± 0.21</td>
<td>4.98 ± 0.24</td>
</tr>
<tr>
<td><strong>COX activity (nmol/mg.min)</strong></td>
<td>40.22 ± 2.56</td>
<td>41.56 ± 3.36</td>
<td>48.96 ± 4.25</td>
</tr>
<tr>
<td><strong>T₃ (ng/ml)</strong></td>
<td>1.26 ± 0.03</td>
<td>1.33 ± 0.05</td>
<td>1.81 ± 0.05</td>
</tr>
<tr>
<td><strong>T₄ (ng/ml)</strong></td>
<td>55.63 ± 2.58</td>
<td>54.36 ± 3.25</td>
<td>50.14 ± 2.12</td>
</tr>
<tr>
<td><strong>Prolactin (U/ml)</strong></td>
<td>88.89 ± 6.65</td>
<td>92.35 ± 5.12</td>
<td>95.34 ± 4.89</td>
</tr>
</tbody>
</table>

**REFERENCES**