Journal of Stress Physiology & Biochemistry, Vol. 9 No. 1 2013, pp. 148-156 ISSN 1997-0838

ORIGINAL ARTICLE

Effect of temperature on body temperature and resting metabolic rate in pups of *Eothenomys miletus*

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Received October 22, 2012

In order to investigate the ability of ambient temperature and thermoregulation in *Eothenomys miletus*, body temperature and resting metabolic rate (RMR) were measured during postnatal development (1-49 day) when *E. miletus* exposed different ambient temperature. The result showed that: body temperature and RMR of pups in *E. miletus* increased according to the increase of ambient temperature during 1 day to 7 day, showed character of poikilotherms; body temperature of pups were lower in low temperature(5°C, 10°C), relatively and RMR increased significantly when day age is 14 day, it indicated that the pups showed a certain degree of thermoregulation in this phase. Its thermoregulation ability developed quickly during 7 day to 14 day. RMR of pups was extreme significantly higher in low temperature than that in other temperature when day age was 21 day, it showed that the pups had some thermoregulation to low temperature stimulation. The RMR of pups was showed increasing trend in high temperature(35°C) when 28 day; on 35 day and 42 day, the thermal neutral zone were 22.5 to 30°C and approaching its adult level. All of these results indicated that pups of *E. miletus* in the different growing period had different thermogenesis and energy allocation to maintain stable to body temperature, thermogenesis was weaker in the early phase of postnatal development, most of energy is used to its growth. After pups were weaned, the ability of constant temperature and thermoregulation developed quickly to adjust variations of environment during postnatal development.

Key words: Eothenomys miletus; Body temperature; Resting metabolic rate

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Temperature plays an important role in animal's growth and development, pups will directly face the surrounding environment, energy acquisition and distribution, heat capacity, temperature regulation, heat loss are the main threats to their survival, therefore, rapid postnatal growth and physical function completion are very important (Leon, 1986; Krol and Speakman, 2003). Many studies of postnatal development in small mammals were reported, in accordance with birth thermostatic mechanism's development are divided into 3 categories: precocial, altricial and immature (Brück and Hinckel, 1996; Cannon and Nedergaard, 2004). Different molding to precocial pups, immature pups showed molding surface exposed at birth, incomplete development, many functions are not harmonious, weak physiological thermogenic capacity, cannot effectively prevent heat loss, temperature adjustment ability is poor (Hull,1973; Speakman, 2007; Speakman and Krol, 2005). Body temperature regulation in animal were divided into physiological thermoregulation and behavioral thermoregulation, behavioral thermoregulation has an important position for maintain high body temperature in immature pups, including acts of the huddling, nesting and swaddling (Hull,1973). Many researches about thermoregulation immature pups shown that: although their had weak physiological thermogenic capacity in the early period, but with the females care, parent cluster behavior as well as nest insulation effect, immature pups can still maintain high temperature (Hill, 1983; 1992), and high temperature is conducive to the rapid growth in immature pups (McManus, 1971).

Yunnan red-backed vole, *Eothenomys miletus* (Mammalia: Rodentia: Microtus), is a inherent specie of Hengduan Mountains region (Zheng, 1993). The Hengduan Mountains region is located the boundary between the Palaearctic region and the Oriental region. Environmental factors, such as short photoperiods and cold, are effective cues that influence body mass and thermogenesis, separately (Zhu et al., 2008, 2010a, 2010b, 2011, 2012). However, we know nothing about effect of temperature on body temperature and resting metabolic rate (RMR) in pups of *E. miletus*. We predicted that pups of *E. miletus* change their body mass and RMR under temperature acclimaton.

MATERIALS AND METHODS

Samples

Seven pregnant females were captured (26.22°N, 99.48°E, and 2, 550-2, 615m in altitude) around Shilong Village, Jianchuan County in July and August 2008, then brought and bred at the School of Life Sciences, Yunnan Normal University, Kunming (1, 910 m in altitude). Each mouse was housed individually in plastic boxes (260mm× 160mm×150mm) with sawdust bedding with no nest under a constant light cycle (12:12 h light-dark cycle, lights 0900-2100) and temperature (25±1°C), and maintained on a commercial standard rat pellet (produced by Kunming Medical College). Food and water were available *ad libitum*. Relative ambient humidity ranged from 60% to 75%. There were 33 pups were used in the experiment, all animals were healthy individual.

From the age of 1 day every three days to determine the body mass, continuous determination of up to 49 days of age, body mass was measured by using precision electronic balance (0.01g Scout SL SPN202F, USA).

Measurement of resting metabolic rates

Body temperature and RMR were measured every week from 1 day to 49 day in 5, 10, 15, 20, 25, 30 and 35°C (except for 35°C in 1 and 7 day). 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 rate 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 RMR. Calculate method of metabolic rate is detailed by Hills (Hill 1972). Body temperature were measured by digital thermometer (Sinan instrument of Beijing

Normal University, SN2202), 1.5cm was the probe inserted to the rectum, and read the body temperature after 45s.

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) and repeat metrical regression. Results were presented as mean ± SEM, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Body mass

Gestation period in *E. miletus* was about 15-18 days, litter sizes were from 2 to 6, but the majority litter size was 2, lactation period was 22 days. *E. miletus* shown relatively short gestation period, small litter size and longer lactation. Body mass showed significant differences from day 0 to 40 in pups of *E. miletus* ($P<0.01$), which shown S shape curve (Fig. 1).

Body temperature

Time and ambient temperature had significant effect of body temperature on pups of *E. miletus* (time: $F=6.352$, $P<0.01$, ambient temperature: $F=22.36$, $P<0.01$ interaction between time and ambient temperature: $F=11.25$, $P< 0.01$, Fig 2).

As the days of age increasing, body temperature in pups of *E. miletus* can gradually remain stable(Fig 2). At the age of 1 days and 7 days of age, body temperature increased in pups of *E. miletus* when ambient temperature increases. At the age of 14 days of *E. miletus* pups showed weaker ability to regulate body temperature in 15°C, body temperature was not high and relatively stable. At the age of 21 days, body temperature in pups of *E. miletus* were lowest at 20°C. At the age of 28 days and 35 days, body temperature in pups of *E. miletus* varies with ambient temperature increases. At the age of 42 days and 49n days, body temperature in pups of *E. miletus* were relatively stable, and showed relatively stable constant capability up 15°C.

RMR

Time and ambient temperature had significant effect on RMR in pups of *E. miletus* (time: $F=4.365$, $P<0.01$, ambient temperature: $F=15.34$, $P<0.01$ interaction between time and ambient temperature: $F=9.85$, $P< 0.01$, Fig 3).

RMR showed different adaptation characteristics in pups of *E. miletus* when the environment temperature changes. On 1 and 7 days of age, RMR increased as ambient temperature rises, showed similar cold-blooded animal characteristics, at the age of 14 days, RMR in pups of *E. miletus* at low temperatures (5, 10°C) showed increased heat production trend, and showed significantly higher RMR than other temperature under 15 and 20°C; at the age of 21 days, RMR in pups of *E. miletus* showed significantly higher than other temperature RMR in 5, 10°C ($P<0.01$), which indicated it have a certain heat regulation; at the age of 28 days, as the ambient temperature increases, RMR of *E. miletus* pups decreased gradually, at this time *E. miletus* pups showed some heat regulation ability; at the age of 35 days, RMR in pups of *E. miletus* were lower in 20 -30°C, and showed no significant difference ($P>0.05$); 42 and at the age of 49 days, RMR in pups has the minimum value in 25°C, RMR increased with the ambient temperature decreases below 25°C.

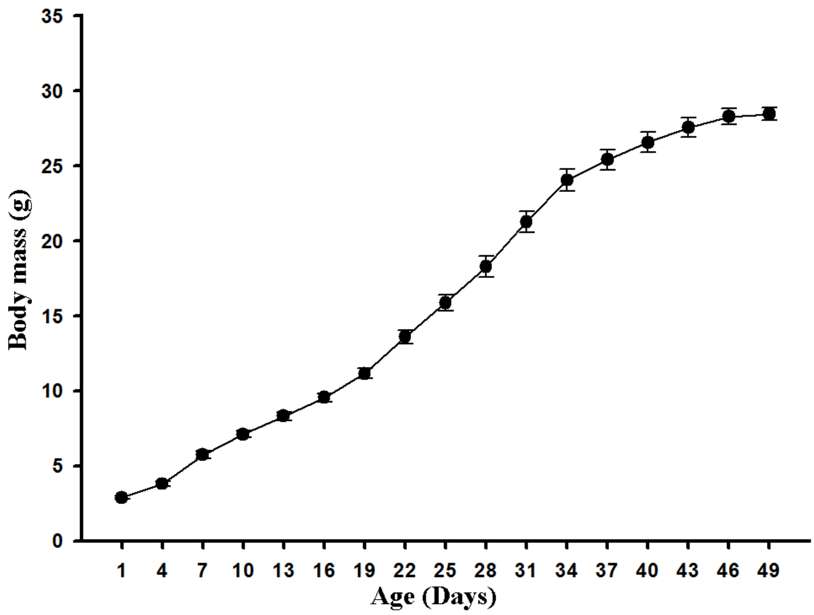

Figure 1: Changes of body mass during postnatal development in pups of *E. Miletus*

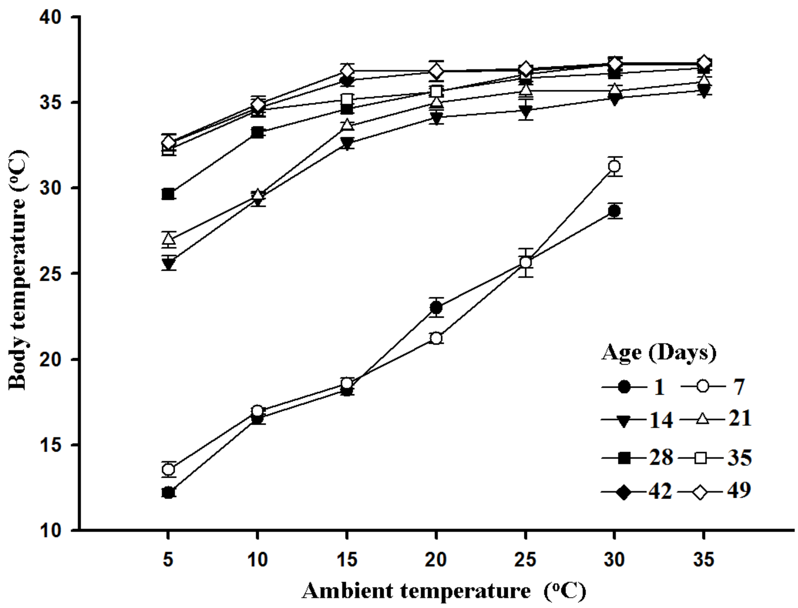

Figure 2: Changes of body temperature during postnatal development in pups under different ambient temperature of *E. Miletus*

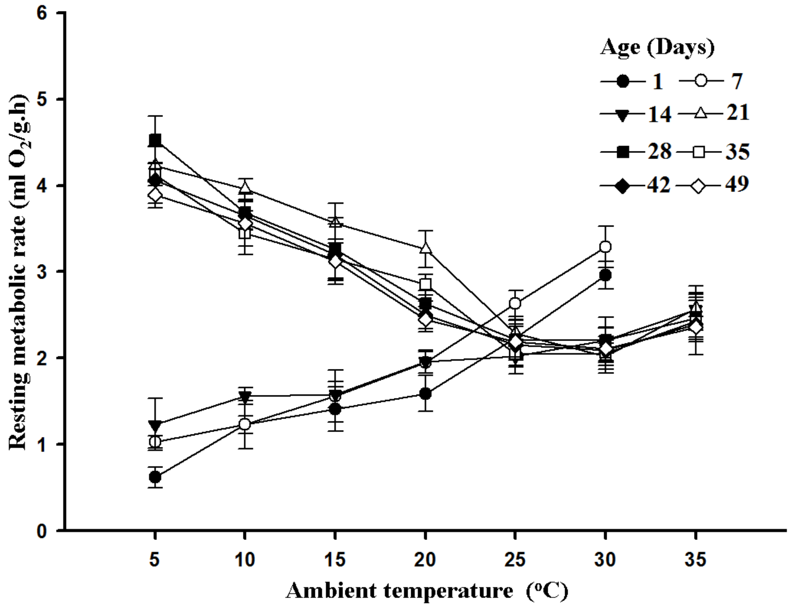

Figure 3: Changes of resting metabolic rate during postnatal development in pups under different ambient temperature of *E. Miletus*

DISCUSSION

Body mass

The growth of animal is one of the important research contents in the animal life history evolution, by comparing the different growth parameters between taxa or between species, it can reveal the evolutionary significance of animal's life history characteristics and animal's adaptability to environment (Millar, 1977; Case, 1978). *E. miletus* reached the maximum instantaneous growth rate time is 24 days, 2 days later than the *Apodemus chevrieri* (Liu et al., 2010), indicates that *E. miletus* had lower fetal development in terms of compared with *A. chevrieri*, cause body mass in pups were heavier when their born, but weaning weight were smaller than that of *A. chevrieri*, which may be associated with different species relationships. Lactation period of *E. miletus* was 22 days, weight growth slowing down after24 days, this may be associated with weaning food quality changes and their physiological heat production energy consumption increase.

Body temperature

E. miletus pups are born with poor body heat insulation ability, before 14 days, body temperature increased in *E. miletus* pups with ambient temperature rising, is greatly influenced by the environmental temperature, exhibited similar ectotherm features (Reading, 2003; Loman, 2002; Herczeg et al., 2006), body temperature in ectotherm varies with the ambient temperature change, which is not a good adaptation to changes in the external environment (Liu et al., 2010), *E. miletus* pups were different to ectotherm, changes of body temperature can be regarded as an important adaptive significance of characteristic, because of temperature regulation is a process of energy consumption, immature pups during early development can not carry out the physiology of thermoregulation effectively, mainly rely on the parent tending or other means to maintain body temperature, reduce the energy expenditure in the thermoregulatory will be increase energy for the growth (Hull, 1973; Hill, 1992).

Building process of thermostatic capacity in *E. miletus* pups were similar to *Peromyscus leucopus* (Hill, 1983), *Meriones unguiculatus* (McManus, 1971), from birth to building constant temperature mechanism is a gradual process, periods often has a rapidly mature stage of thermoregulatory capacity (Blumberg and Sokoloff, 1998; Sun and Zeng, 1987), *E. miletus* pups from 7 days to 14 days of age period, along with their physical development is gradually mature and thermogenesis mechanism complete, the thermoregulatory capacity growth is rapid (Liu et al., 2010), *E. miletus* pups maintain a higher body temperature during early development, which can slow growth speed.

Before the age of 10 days, *M. unguiculatus* pups can withstand body temperature reduced to close to 15°C (McManus, 1971), body temperature in *P. leucopus* drops to about 15°C, and was different to restore (Morhardt and Hudson, 1966), in extremely cold conditions, it only allows temperature moderately decreased to 28-30°C (Wickler, 1980). Low temperature tolerance pups is an adaptation to raise the survival rate of immature pups, body temperature regulation is very complex, its thermoregulatory capacity development can not be simply considered to adult thermoregulation ability level development process (Blumberg and Sokoloff, 1998). *E. miletus* pups belongs to the typical species in Hengduan mountain region, which located in the subtropical plateau, the annual temperature is small, but the temperature between days changes drastically, probably animal in a very short period of time, both to experience high temperature stress, but also to experience low temperature stress, development of thermoregulatory capacity in the pups is very important for its survival.

RMR

The 1 and 7 days of age, RMR increased in *E. miletus* pups as ambient temperature rises, also showed similar ectotherm features (Angilletta et al., 2002; Navas et al., 2008), although heat production ability is poorer under low temperature (5-20°C) of *E. miletus* pups, body temperature can be maintained at ambient temperature level or above, which can be inference of *E. miletus* pups that has certain response to low temperature in enhancing the production of heat capacity. *E. miletus* pups from at least 14 days age increased heat production response up to 5°C, at 14 days of age, *E. miletus* pups temperature began to show a constant period, at the age of 21 days, RMR was significantly higher than those of other temperature RMR *in E. miletus* pups at the low temperature of 5 and10°C, which shown that *E. miletus* pups at 21 day of age have been able to make a certain heat regulation on low temperature stimuli, consistent with immature pups characteristics under low temperature, different to hamsters (Blumberg, 1997). Our results also confirmed the previous research conclusion (Vinter et al., 1982).

In conclusion, All of these results indicated that pups of *E. miletus* in the different growing period had different thermogenesis and energy allocation to maintain stable to body temperature, thermogenesis was weaker in the early phase of postnatal development, most of energy is used to its growth. After pups were weaned, the ability of constant temperature and thermoregulation developed quickly to adjust variations of environment during postnatal development.

ACKNOWLEDGEMENT

This research was financially supported by National Science Foundation of China (No. 31071925; 31260097), Project of Basic research for application in Yunnan Province(2011FZ082).

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