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Thermoregulatory Development in Pups of Chevrier's Field Mouse (*Apodemus chevrieri*) in Hengduan Mountain Region

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Abstract.- The effect of huddling with nestlings and the resting metabolic rate (RMR) on body temperature (T_b) in pups of Chevrier's field mouse, *Apodemus chevrieri* from 4 to 48 days of age was evaluated at ambient temperatures (T_a) within and below the adult thermoneutrality range (30 and 20ºC, respectively). At 20 ºC, huddling behavior and higher RMR significantly increased pups' T_b until they were 20 days old. 16 days old pups showed an increase in their RMR, associated with on the set of independent thermoregulation. Pups older than 24 days showed a less variable T_b and their RMR decreased. 4 to 16 days old pups were spent more time huddled with their nest mates. The RMR differed between the two T_a evaluated. 20 days old pups reached adult T_b and spent significantly less time in contact with their mother and nest mates. Huddling did not have a significant effect on energy expenditure of young pups, being this related to the stable thermal conditions found in natural burrows and development mode of pups.

Keywords: Thermoregulatory development; huddling; resting metabolic rate (RMR); body temperature; pups; *Apodemus chevrieri*

INTRODUCTION

In mammals, the supply of a warm thermal environment to the nursing young is one of the most energy-demanding components during lactation (Hill, 1992). As regards the aspect, lactating females have several options for adjusting their energy use to compensate for changes in the availability of food resources or litter size. They can vary the energy invested per young such as the individual offspring body mass at natal emergence was inversely related to litter size (Millar, 1977). Females can also alter the thermoregulatory costs (heat loss) for nestlings by adjustments in the time they spend in the nest, the increase the rate of heat transfer to pups, indirect heating of the nest through an increase in females'heat loss rate and changes in the thermal isolation. They provide to the nest or its localization (Geiser and Kenagy, 1990; Hill, 1992). In addition, nursing young can optimise their energy allocation to growth and thermoregulation capacity. As regards strategies of pup development, the reproductive characteristics of mammalian was extremes of a continuum in the precocial or altricial young (Hill, 1992), which differ in the time pups become furred, possess capable eyesight or mature by their locomotor abilities, being precocial young more developmentally advanced when born and therefore achieving independence of their parents relatively early (Kunkele, 2000).

These two strategies differ in the pace of thermoregulatory development after birth. While precocial pups are capable of maintaining their T_b stable by shivering and non-shivering thermogenesis (Bruck and Hinckel, 1996), altricial pups are a low capacity to control their T_b, which falls rapidly when they face ambient temperatures (T_a) below thermo-neutrality (Bruck and Hinckel, 1996). In consequence, altricial pups can only overcome these limitations by means of behavioral strategies, nest insulation and heat transfer from their mothers (Cutrera *et al.*, 2003). One of the most frequent behaviors showed by young of small mammals at an early age is huddling, which is widely considered to be an important behavioral adjustment to cold temperatures (Alberts, 1978). Huddling was observed to reduce the energetic costs of maintaining a stable T_b in pups and adults of several small mammals' species exposed to low T_a, mainly

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by reducing the exposed surface area and heating the local microclimate (Bozinovic *et al.*, 1988). Although there are many studies concerning the several small mammals species' pups' thermoregulatory development, most of them have been conducted confronting pups to unlikely situations in the natural condition. As a result, the thermoregulatory capacity of pups, specially the altricial ones, has been underestimated leading to the early age nestlings are capable of keeping themselves warm.

Chevrier's field mouse, *Apodemus chevrieri* (Mammalia: Rodentia: *Apodemus*), which a typical species of Palaearctic, originated from the Europe or the Asian brink close to the Europe, they migrated westernward in recent years (Miclke, 1989), inhabiting Hengduan Mountain Region in southwest of China. This area locates at the boundary between the Palaearctic and Oriental region. It has been reported that *A. chevrieri* showed seasonal changes in body mass and digestive tract morphology (Wang *et al*., 2008, 2009), and, the evaporative water loss and energy metabolism has been studied (Zhu *et al*., 2008), furthermore, serum leptin involved in the energy intake regulation and thermoregulation in *A. chevrieri* under cold acclimation (Zhu *et al*., 2011). In this study was designed to investigate the changes of body temperature, metabolic rate and the capacity of thermoregulation in pups of *A. chevrieri*. We hypothesize there are two stages of thermoregulation in pups of *A. chevrieri*, at first, the capacity of thermoregulation increase; then, it keep constant.

MATERIALS AND METHODS

Animals stock and maintenance

Pregnant females were captured using plastic live traps in July and August, 2008 from Shilong village Jianchuan county of Yunnan Province, China (26.22°N, 99.48°E, and 2550-2615m). Captured individuals were carried to the laboratory and housed individually in plastic boxes (260mm× 160mm×150mm) with sawdust bedding under a constant light cycle (12:12 h light-dark cycle, lights 09:00 ~ 21:00) 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%. Animals were fed with standard feedstuff (produced by Kunming Medical College) and provide water with plastic bottles.

Measurement of metabolic rates

This experiment is operated in the laboratory of ecology in science of life department, Yunnan Normal University. Metabolic rates were measured using AD ML870 open respirometer at 30°C within the TNZ (thermal neutral zone) and 20°C as described previously, 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 500 ml, flow is 200 ml/min. *A. chevrieri* were stabilized in the metabolic chamber for at least 60 min prior to the BMR measurement, oxygen consumption was recorded for at least 60 min at 5 min intervals. Two stable consecutive lowest readings were taken to calculate BMR (Li and Wang, 2005). Calculate method of metabolic rate see detail in Hill (Hill, 1972). All metabolic measurements were performed more than 400 h. Animals were weighed before and after experiment, insert 3cm of a digital thermometer into rectum for about 45s to measure their body temperature, and regarded the measured temperature after experiment as T_b under that temperature.

Measurement of body temperaure

After exposured under 25°C for 40 minutes when huddling, the T_b of each pup were measured, and then the T_{bf} measured after exposured during 25°C for 40 minutes when mother and nest mates were absent (non-huddling). Horneothermy index (HI) and total thermal conductance were computed when data analyzed. HI= $(T_{bf}-T_a)/\ (T_{bi}-T_a)$ (Richlefs, 1987), where T_{bi} is the body temperature before exposured under 25°C measured. Total thermal conductance=RMR/ (T_b-T_a) (McNab, 1980).

Statistical analysis

The data were analyzed using SPSS for Windows 16.0, Sigmaplot 10.0, Independent samples *T*-test and One-way ANOVA were used to analyze the difference between *Tb*, RMR under two

Ta and changes of HI and total thermal conductance as the growth.The results is expressed by Mean±SE, *P*<0.05 means difference, *P*<0.01 means obvious difference.

RESULTS

Body temperature T_b

T_b of pups dropped lowly under 20°C before 24 days age reached adult level, showed significantly difference contrast to 30°C. After 24 days age, the T_b of pups could keep constantly (Fig.1).

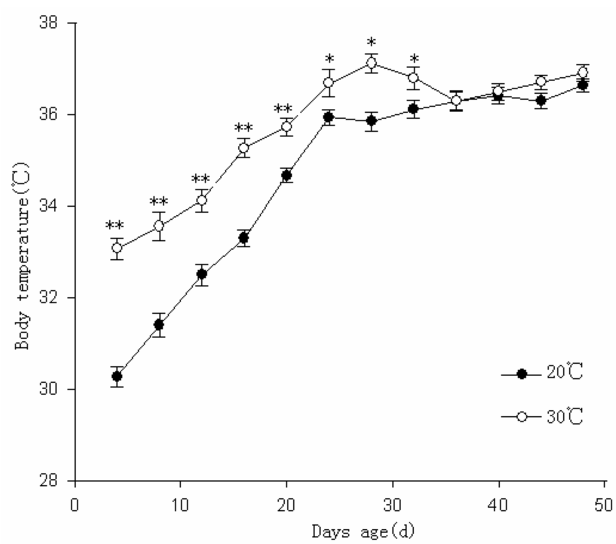

Fig. 1. The development of body temperature T_b under two different ambient temperature *Ta* in pups of *A. chevrieri*.
* Vs. control ($P < 0.05$), ** Vs. control ($P < 0.01$), Data are means ±SE.

RMR

RMR under two different T_a showed extreme significant from 4 to 48 days age, RMR under 20°C is lower than under 30°C (Fig.2). The results suggested that pups have weak thermogenesis ability to resist cold conditions after birth.

T_b under huddling and non-huddling

When huddling, T_b of pups could kept at a higher level contrast to non-huddling at the age of 8 and 16 days. However, at the 24 days age, T_b kept high when huddling as well as non-huddling (Fig.3).

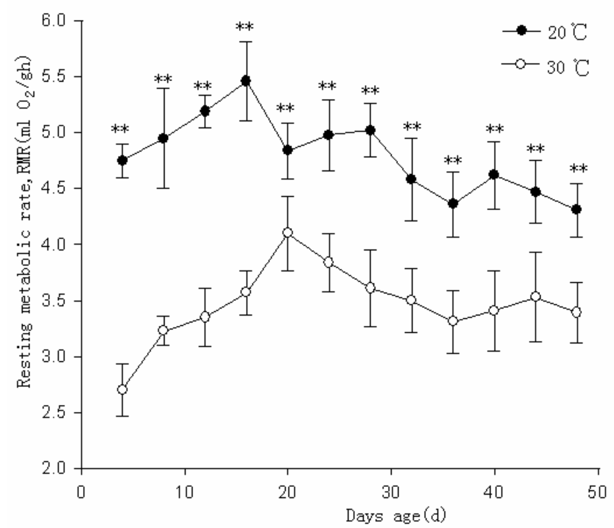

Fig. 2. The development of resting metabolic rate RMR under two different ambient temperature T_a in pups of *A. chevrieri*.
** Vs. control ($P < 0.01$), Data are means ±SE.

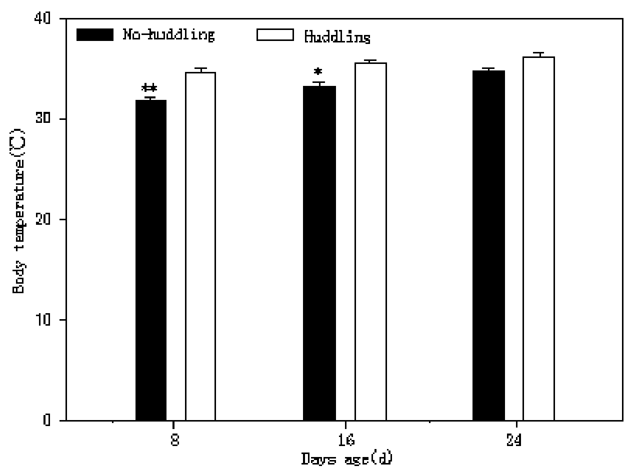

Fig. 3. The body temperature T_b under huddling or non-huddling in pups of *A. chevrieri*.
* Vs control ($P < 0.05$), ** Vs control ($P < 0.01$), Data are means ±SE.

HI and total thermal conductance

Both HI and total thermal conductance developed fast before 24 days age and then kept constant (Figs. 4, 5). It demonstrated that the thermoregulation capacity of pups developed perfect until 20 days age.

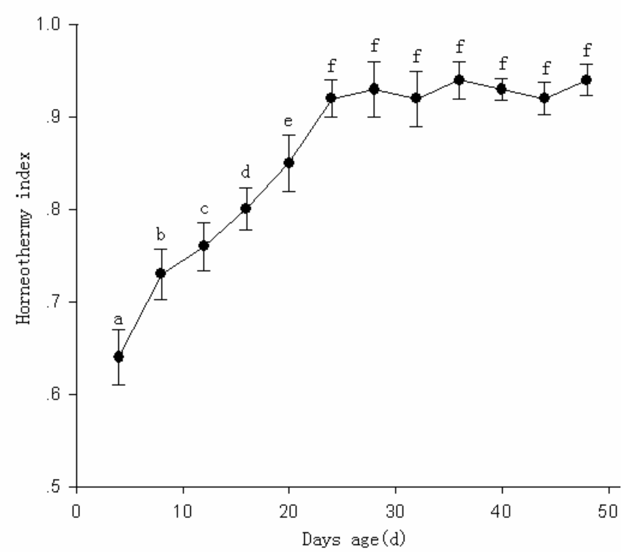

Fig. 4. The development of Horneothermy HI under 30°C ambient temperature T_a in pups of *A. chevrieri*. The same letter indicate no difference, the different letter indicate difference significant (p<0.05), Data are means ±SE.

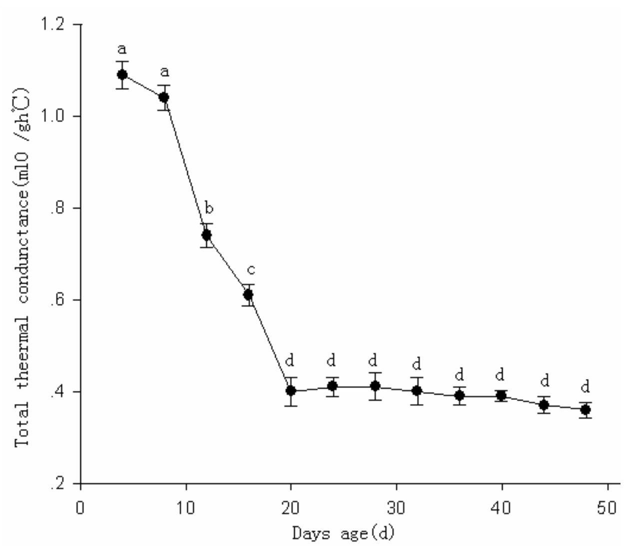

Fig.5 The development of total thermal conductance under 30°C ambient temperature *Ta* in pups of *A. chevrieri*. The same letter indicate no difference, the different letter indicate difference significant (p<0.05), Data are means ±SE.

DISCUSSION

One way to consider the implications of thermoregulation on energy expenditure of young endotherm during their development is to focus on how they use the chemical energy that is provided to them. There are certain energy-demanding processes that must be carried out using this energy but others, such as thermoregulation, can be carried out either with chemical resources given to the young or by other parental energy sources. In this way, avoiding the young's use of their own chemical energy resources for the latter processes could increase the energy available for the former (Cutrera *et al.*, 2003).

Given that heat generation by young endotherm is often limited and may reduce the availability of energy for growth, the brooding female becoming an important source of heat (Hill, 1992; Newkirk *et al.*, 1998). In this study, *A. chevrieri* pups showed a highly variable *Tb* and increase RMR under lower *Ta* at an early age 4–20 days. All of these data indicate that although they have a poor ability to thermoregulate during this period, but still can rely on their weak self-thermogenesis and untilize their energy to resist cold. As expected(what expected?), 16-day-old pups showed a marked increase in their RMR, indicating that pups to acquire physiological mechanisms of heat production at this age, resulting in an increase in their energy expenditure. In other species such as dwarf hamsters (scientific name?), independent thermoregulation via physiological mechanisms is energetically expensive and can reduce the energy budget that could be allocated to growth, which affects the pups' growth rate (Webb *et al*., 1990).

Nest-building is a common behavior in most species of small mammals. It represents a significant energy saving, and it contributes to the maintenance of the Tb of its inhabitants because of the nest's isolating properties (Vogt and Lynch, 1982; Bozinovic *et al.*, 1988). In this study, however, a nest was not provided to the animals during the experiments because it made it difficult to observe mother and pups' behavior. In most mammal species, nestling and weanling stages are potentially times of enormous natural selection (Hill, 1992). However, the altricial phase of development could also constitute an advantage in relation to the energetic budget of pups, considering that independent thermoregulation is energetically expensive and that maternal milk is pups' only energy source until they are approximately 15 days old (personal observe). Furthermore, pups of *A. chevrieri* go through the altricial phase of their development in the protected and thermally stable ambient of the burrow, where the lowest air temperatures, they are likely to encounter are only a few degrees below the adult thermoneutral zone (Zhu *et al*., 2008), and spend most of the time in contact with their mother and other nest mates in a nest.

As predicted, huddling among litter mates represent a significant reduction in pups' energy expenditure to independent thermoregulation.

This increase in fur density and body mass in older pups also contributes to reduce pups' thermal conductivity and. Consequently, the energy required for heat production would decrease in these more thermally protected pups. Weaning of *A. chevrieri* pups start at 20 days of age, when they reach adult *Tb*, reaching adult RMR 3-4 days after they were born. In accordance with these previous findings, older pups in the current study showed higher *Tb*, and they reached adult *Tb* 20 days of age. At this age, pups in this study had a RMR declined. Therefore, behavioral strategies seem to be essential for pups to maintain a stable *Tb* before they have acquired physiological thermoregulatory mechanisms. In this sense, there is strong evidence that supports the hypothesis that a high *Tb* is a key factor in facilitating pups' growth and maturation, affecting not only their growth rate but also other aspects of their development such as thermoregulatory maturity, the time they open their eyes and even the development of neural pathways (Horwitz *et al*., 1982; Hill, 1983, 1992; Zhu *et al*., 2008).

From this study, we get a conclusion that the pups of *A. chevrieri* inhabting Hengduan Mountaini Region employ these strategies to live through the cold condition: poor self-thermogenesis, huddling with other nestlings and the isolation of burrows. The capacity of thermoregulation in pups of *A. chevrieri* developed maturely at the age of 20 days when weaning.

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