Effects of Temperature, Photoperiod and Food Quantity on Body Mass and Thermogenesis in *Apodemus chevrieri*

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**ABSTRACT**

To investigate the influence of different ecological factors in *Apodemus chevrieri* during the process of seasonal changing, we designed a three-factor experiment of temperature, photoperiod and food quantity in *A. chevrieri* in the present study. Animals were divided into 8 groups randomly: moderate temperature, longer photoperiod with no food-restricted group; moderate temperature, longer photoperiod with food-restricted group; moderate temperature, shorter photoperiod with no food-restricted group; lower temperature, longer photoperiod with food-restricted group; lower temperature, longer photoperiod with food-restricted group; lower temperature, shorter photoperiod with no food-restricted group; lower temperature, shorter photoperiod with food-restricted group, which were acclimated for 4 weeks. Body mass, resting metabolic rate (RMR) and non shivering thermogenesis (NST), leptin levels and hypothalamic neuropeptide gene expression were measured in each group. The results showed that body mass in *A. chevrieri* was significantly affected by temperature, food and photoperiod, which was decreased by low temperature, food restriction and short photoperiod. RMR and NST were significantly affected by temperature and food quantity. The content of leptin was significantly affected by temperature, photoperiod and food quantity. Low temperature, short photoperiod and food restriction all decreased the content of leptin. Expression levels of NPY and AgRP were significantly affected by temperature and food quantity. Low temperature and food restriction up-regulated the expression levels of NPY and AgRP. While POMC expression was only affect by food quantity, CART expression was significantly different under the influence of temperature and food quantity. These results suggested that temperature, photoperiod and food quantity had different effects on physiological indexes and energy balance in *A. chevrieri*, and leptin was involved in the regulation of body mass in *A. chevrieri*.

**INTRODUCTION**

The animals living in wild often face challenges from ecological factor, such as temperature, photoperiod and food quantity. Therefore, survival strategies need to be adjusted in time to obtain the best survival opportunity (Karakas et al., 2005; Hu et al., 2017). When seasonal changes occur, the most significant change is environmental temperature. The temperature change cause adjustments in body mass, thermogenesis and this series of changes are species-specific (Szafranska et al., 2013). Low temperature is the main factor inducing the enhancement of resting metabolic rate (RMR) and non shivering thermogenesis (NST). Most animals showed the enhancement of thermogenesis when facing seasonal changes or under cold temperature (Mineo et al., 2012). Temperature affects the body mass of mammals usually in three cases: Low temperature has no significant effect on body mass, or low temperature decline body mass, or increase body mass. For example, temperature has no significant effect on the body mass in striped hamster (*Cricetulus barabensis*), but low temperature significantly increases its food intake and thermogenesis (Zhao et al., 2010). Low temperature significantly reduced the body mass in female *Peromyscus maniculatus* and *Eothenomys melanogaster* and promoted the increase of metabolic rate.
(Rezende et al., 2004; Xu et al., 2010). However, *Tupaia belangeri* increased its body mass under low temperature (Zhang et al., 2014). Photoperiod is also an important signal of seasonal change, and HPV axis recognized the changes of photoperiod, and then stimulates hormone secretion, adjusting food intake and energy metabolism in mammals (Zhang et al., 2015). There are three main types of mammalian body mass changes response to photoperiod: body mass is not affected by photoperiod changes, short photoperiod inhibits body mass, and body mass gain under short photoperiod (Gao et al., 2013). Generally, metabolisms of mammals under short photoperiod were higher than those under long photoperiod. *Microtus oeconomus* showed a decreasing trend in body mass and enhanced thermogenesis under short photoperiod (Wang et al., 2006). Similarly, NST in *Lastiopodomys brandtii* was enhanced under short photoperiod, while the serum leptin level was significantly affected by photoperiod (Lu et al., 2007). Change of food quantity is also an important factor affecting their survival when mammals face seasonal changes (Yom-Tov and Yom-Tov, 2005; Zhao et al., 2011). Studies have shown that *Eothenomys milletus* can reduce body mass, metabolic rate to cope with food shortage (Zhu et al., 2014). *Mus musculus* become more active and consume more energy after food restriction, resulting in lower body mass and fat mass (Kirkwood and Shanley, 2005).

Leptin is a protein mainly expressed in adipose tissue, which regulates animals' weight and energy metabolism by binding to specific receptors in hypothalamus (Friedman and Halaas, 1998). The changes of environmental factors affect the leptin content. For example, low temperature and short photoperiod reduce the leptin content in *T. belangeri*, *L. brandtii* and *Meriones unguiculatus* (Zhang et al., 2014; Li and Wang, 2007). It is reported that serum leptin levels in mammals decrease during food restriction (Rousseau et al., 2003). The hypothalamus regulates the feeding and energy of animals through neuropeptide Y (NPY), agouti related protein (AgRP), pro-opiomelanocortin (POMC) and cocaine and amphetamine regulated transcription peptide (CART) (Morton et al., 2006).

*Apodemus chevrieri*, is a typical Paleoboreal animal (Corbet, 1978). Previous studies reported that *A. chevrieri* lost weight and increased thermogenesis under cold acclimation (Zhu et al., 2011). Short photoperiod inhibited the body mass in *A. chevrieri*, stimulated the increase of thermogenesis and reduced the concentration of serum leptin (Zhu et al., 2013a). Food restriction decreased body mass and thermogenic capacity in *A. chevrieri* significantly (Zhu et al., 2013b). Based on the above research, the three factor experiment of temperature, photoperiod and food quantity in *A. chevrieri* was designed to determine the relationship between temperature, photoperiod, food quantity and energy balance of *A. chevrieri*, we hypothesized that temperature, photoperiod and food quantity will affect the energy metabolism in *A. chevrieri*, and the effects of those three ecological factors were different, further clarify the relationship between leptin and body mass regulation, and better understand the impact of seasonal changes in energy metabolism in *A. chevrieri*.

**MATERIALS AND METHODS**

**Animals**

The experimental animals were collected in 2020 in Jianchuan County, Dali City, Yunnan Province (99°75’E, 26°43’N, altitude 2590 m). After disinfecting and killing fleas, the captured animals were brought back to the single cage in the animal feeding room of Yunnan Normal University, and housed individually in a wire cage (26 cm×16 cm×15 cm). Water and foods were provided ad libitum.

The three factor experiment of temperature, photoperiod and food quantity in *A. chevrieri* was designed. The animals were randomly divided into 8 groups (n=48, ♀:17, ♂: 31): moderate temperature, longer photoperiod with no food-restricted group (normal temperature LC), 25 °C, 16L: 8D, free feeding and drinking; moderate temperature, shorter photoperiod with no food-restricted group, 25°C, 8L: 16D, free feeding and drinking (normal temperature SC); moderate temperature, longer photoperiod with food-restricted group, 25°C, 16L: 8D, the amount of food was 80% of the daily intake, and free drinking (normal temperature LF); moderate temperature, shorter photoperiod with food-restricted group, 25 °C, 8L: 16D, the amount of food was 80% of the daily intake, and free drinking (normal temperature SF); low temperature, longer photoperiod with no food-restricted group, 5 °C, 16L: 8D, free feeding and drinking (low temperature LC); low temperature, shorter photoperiod with no food-restricted group, 5 °C, 8L: 16D, free to feeding and drinking (low temperature SC); low temperature, longer photoperiod with food-restricted group, 5 °C, 16L: 8D, the amount of food was 80% of the daily intake, and free drinking water (low temperature LF); low temperature, shorter photoperiod with food-restricted group, 5 °C, 8L: 16D, the amount of food was 80% of the daily intake, and free drinking (low temperature SF); 8 groups of animals were domesticated under their respective conditions for 4 weeks, the number of animals in each group was 6. Body mass was measured on day 0 and 28, and the animals were killed after 28 days. Thermogenic capacity, serum leptin content and hypothalamic neuropeptide gene expression were measured on day 28.
Measurement of RMR and NST

Body mass, RMR, and NST were measured using the metabolic system (BXY-R, Sable Systems). *A. chevrieri* were acclimated to calorimetry cages prior to 30 min the study and data collection, see details for specific measurement methods in Zhang et al. (2011).

Measurement of serum leptin levels and hypothalamic neuropeptide gene expression

After the experiment, the animals were killed, and the serum leptin content was measured by leptin radioimmunoassay kit (Linco company, USA) (Zhu et al., 2012a). The gene expression of hypothalamic neuropeptide NPY, AgRP, POMC and CART was determined, the primers for amplification sequence and measurement methods are detailed in the literature (Zhang et al., 2015).

Statistical analysis

Data were analyzed using the software package SPSS 22.0. Prior to all statistical analyses, data were examined for assumptions of normality and homogeneity of variance using Kolmogorov–Smirnov and Levene tests, respectively. Since no gender effects were found on almost all measured parameters, data from females and males were combined. The change of body mass was tested by Three-Way ANOVA. The differences of RMR and NST, leptin content and hypothalamic neuropeptide expression between groups were tested by Three-Way ACNOVA, in which body mass was used as covariate. Results are presented as means ± SE, and \( p < 0.05 \) was considered to be statistically significant.

RESULTS

Body mass

There was no significant difference of body mass in *A. chevrieri* in 8 groups before the experiment \( (p > 0.05) \). After 28 days, body mass in *A. chevrieri* was significantly affected by temperature, food quantity and photoperiod. Low temperature, food restriction and short photoperiod reduced body mass in *A. chevrieri* (temperature: \( F_{1,40} = 6.65, p < 0.01 \); photoperiod: \( F_{1,40} = 2.15, p < 0.05 \); food quantity: \( F_{1,40} = 9.87, p < 0.01 \), Fig. 1).

RMR and NST

RMR was significantly affected by temperature and food quantity after 28 days in *A. chevrieri* (temperature: \( F_{1,40} = 12.32, p < 0.01 \); food quantity: \( F_{1,40} = 15.32, p < 0.01 \)). RMR in low temperature group was higher than that of normal temperature group, and food restriction decreased RMR (Fig. 2). On day 28, NST was significantly affected by temperature, food quantity and photoperiod (temperature: \( F_{1,40} = 9.36, p < 0.01 \); photoperiod: \( F_{1,40} = 3.21, p < 0.05 \); food quantity: \( F_{1,40} = 6.57, p < 0.01 \)). Low temperature and short photoperiod increased NST, while food restriction decreased NST in *A. chevrieri* (Fig. 2).

Fig. 1. Effect of temperature, photoperiod and food quantity on body mass in *Apodemus chevrieri*.

Serum leptin levels and hypothalamic neuropeptide gene expression

Serum leptin levels was significantly affected by temperature, photoperiod and food quantity after 28 days of domestication in *A. chevrieri* (temperature: \( F_{1,40} = 12.32, p < 0.01 \); photoperiod: \( F_{1,40} = 5.63, p < 0.01 \); food quantity: \( F_{1,40} = 32.32, p < 0.01 \)). Low temperature, short photoperiod and food restriction decreased the leptin content (Fig. 3). NPY expression was significantly affected by temperature and food quantity (temperature: \( F_{1,40} = 17.63, p < 0.01 \); food quantity: \( F_{1,40} = 13.32, p < 0.01 \); AgRP expression was also significantly affected by temperature and food quantity (temperature: \( F_{1,40} = 8.93, p < 0.01 \); food quantity: \( F_{1,40} = 6.28, p < 0.01 \)). Low temperature and food restriction increased the expression of NPY and AgRP (Fig. 4). POMC expression was significantly affected by food quantity (\( F_{1,40} = 15.32, p < 0.01 \)). CART expression was significantly affected by temperature and food quantity (temperature: \( F_{1,40} = 21.32, p < 0.01 \); food quantity: \( F_{1,40} = 35.62, p < 0.01 \)).
Low temperature and food restriction reduced CART expression (Fig. 4).

Fig. 3. Effect of temperature, photoperiod and food quantity on serum leptin levels in Apodemus chevrieri.

Fig. 4. Effect of temperature, photoperiod and food quantity on hypothalamic neuropeptide genes expression in Apodemus chevrieri.

**DISCUSSION**

Body mass of mammals will adjust to varying degrees when environmental changing (Akhtar et al., 2017). It is reported that some small rodents lose body mass and increase thermogenic capacity under low temperature or short photoperiod (Chen et al., 2012). For example, Microtus maximowiczii and Lasiopodomys brandtii decreased body mass significantly when exposed to cold temperature and short photoperiod (Li and Wang, 2007). Body mass of some animals showed the opposite trend. For example, body mass in T. belangeri increased when they were affected by low temperature and short photoperiod (Zhu et al., 2012a). Quantity and quality of food often become an important factor limiting their survival in mammals. Studies have shown that food restriction can lead to weight loss of most small mammals, including M. unguiculatus, C. barabensis and E. miletus (Zhang and Wang, 2008; Zhao et al., 2015; Hou et al., 2020). This is mainly because hunger increases during the period of food shortage, resulting in increased food consumption, leading to body fat mass reduction and weight loss (Gutman et al., 2008). In the present study, low temperature, short photoperiod and food restriction reduced the body mass in A. chevrieri, which is consistent with the results of weight loss in winter during seasonal changes (Zhu et al., 2012b), indicating that A. chevrieri needs to reduce body energy consumption by reducing body mass in winter, so as to resist the stress of extreme conditions and obtain the best survival opportunity.

Temperature affects the metabolism of mammals, which is mainly reflected under low temperature, promotes the increase of metabolism (Wanner et al., 2017). Ochotona curzoniae and Microtus oeconomus in the Qinghai Tibet Plateau of China mainly rely on increasing their metabolic rate to cope with the extreme cold environment and improve their cold tolerance (Wang et al., 2006; Karol et al., 2014). During cold acclimation, animals will significantly increase their thermogenic capacity, such as Mus musculus and Phodopus roborovskii (Mineo et al., 2012; Chi and Wang, 2011). The change of photoperiod also affects energy consumption in many small mammals (Powell et al., 2002). Affected by the short photoperiod in winter, RMR and NST in C. barabensis were higher than those of in summer (Zhao et al., 2010). Short photoperiod not only reduced the body mass in Phodopus roborovski, but also increased their adaptive thermogenesis (Zhang et al., 2015). However, M. unguiculatus have different effects on photoperiod (Li and Wang, 2005). In our results, RMR in A. chevrieri under low temperature group was higher than that of normal temperature group; Low temperature and short photoperiod increased NST in A. chevrieri, while food restriction decreased RMR and NST. The results were similar to that of smallest mammals. These results showed that A. chevrieri needed to increase thermogenesis under conditions similar to winter; when food resource is limited, it is necessary to reduce the energy consumption for RMR and NST to maintain the relative energy balance.

Leptin further affects the synthesis and release of appetite neuropeptides by binding receptors in hypothalamus through blood circulation, inhibiting food intake and promoting energy consumption (Fink et al., 2007). Temperature, photoperiod and food quantity affected leptin content and hypothalamic neuropeptide expression. For example, leptin decreased in winter in E. miletus as a hunger signal, allowing increased energy...
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intake (Ren et al., 2020). Under starvation, the decrease of leptin level usually stimulates the expression of NPY/AgRP and inhibits the expression of POMC/CART (Tang et al., 2009). The results of the present study showed that the leptin content in *A. chevrieri* is significantly affected by temperature, photoperiod and food quantity after 28 days of domestication. Low temperature, short photoperiod and food restriction all reduce the leptin content in *A. chevrieri*, which is consistent with the previous research results of seasonal changes and food restriction in *A. chevrieri* (Zhu et al., 2012b, 2013b), indicating that leptin may be involved in body mass changes caused by temperature, photoperiod and food restriction. NPY and AgRP expressions were significantly affected by temperature and food quantity, indicating that temperature and food quantity were important factors affecting their survival. POMC expression was significantly affected by food quantity; CART expression was significantly affected by temperature and food quantity. These results show that *A. chevrieri* can actively increase the expression of appetite promoting neuropeptides, promote food intake and meet the survival needs of the body under the condition of low temperature and food shortage.

**CONCLUSION**

In conclusion, different physiological indexes in *A. chevrieri* were affected by temperature, photoperiod and food quantity to varying degrees, suggesting that *A. chevrieri* can adapt to different environmental conditions by adjusting the changes of body mass and thermogenic capacity. Moreover, leptin and hypothalamic neuropeptide expression may be involved in the regulation of body mass in *A. chevrieri*.

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**Ethics statement**

The protocol and study were approved by the Animal Care and Use Committee of the School of Life Sciences, Yunnan Normal University (No. 13-0901-011).

**Statement of conflict of interest**

The authors have declared no conflict of interest.

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