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Seasonal variation of metabolism in lizard *Phrynocephalus vlangalii* at high altitude

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ABSTRACT

Seasonal acclimatization is important for animals to live optimally in the varying environment. *Phrynocephalus vlangalii*, a species of lizard endemic in China, distributes on Qinghai-Tibet Plateau ranging from 2000 to 4600 m above sea level. To dissect how this lizard mediate metabolism to adapt various season, the preferred body temperature (Tb), standard metabolic rate (SMR), mitochondrial respiration rates and activities of four metabolic enzymes in this species were tested in different seasons (spring, summer, and autumn). The results showed that the preferred Tb was the lowest in spring and the highest in summer. SMR, maximal mitochondrial respiration rates in liver and skeletal muscle were the highest in spring. Similarly, higher activities of lactate dehydrogenase (LDH), citrate synthase (CS) and cytochrome *c* oxidase (CCO) activities of liver and skeletal muscle were observed in spring. However, β-hydroxyacyl coenzyme A dehydrogenase (HOAD) activities of liver and skeletal muscle were higher in autumn. On the whole, seasonal variation of metabolism is the highest in spring and the lowest in summer. Seasonal variation of metabolism is the opposite of preferred body temperature, this may be one of the mechanisms to adapt to the environment in *P. vlangalii*. Our results suggested that *P. vlangalii* at high altitude has certain adaptive characteristics on metabolism in different seasons.

1. Introduction

For most parts of the Earth, environmental changes with seasonality mainly include temperature, photoperiod, food availability and precipitation. The season change is an important factor to affect animals to live and reproduction. Many organisms experience considerable seasonal changes in environmental conditions and physiological demands (Zheng et al., 2008), especially in ectotherms. When faced with seasonal fluctuations, ectotherms show a variety of strategies, from behavior and physiology to biochemistry for coping with environmental changes (Guderley and St-Pierre, 2002). Over the recent decades, many studies (Berner and Bessay, 2006; Chamane and Downs, 2009; De Souza et al., 2004; Seebacher et al., 2003; Tsuji, 1988; Williard and Harden, 2011) have been reported that the behavior and physiology of ectotherms change significantly in different seasons. Most of these studies are mainly focused on body temperature regulation, standard metabolic rate, mitochondrial metabolism and metabolic enzyme activities.

Ectothermic animals often experience seasonal fluctuations in body temperature. In the face of seasonal changes of environment, ectotherms especially terrestrial reptiles rely on behavioral mechanisms to maintain a proper preferred body temperature (Tb), which favors optimal cellular function and whole-animal performance under natural conditions (Angilletta, 2009; Huey and Stevenson, 1979). Meanwhile, seasonal differences in Tb can be accompanied by changes in metabolism (Glanville and Seebacher, 2006; Seebacher et al., 2009). The standard metabolic rate (SMR) which is the minimum rate of energy expenditure needed to sustain life at a specified temperature, during resting and in a post-absorptive state (Niewiarowski and Waldschmidt, 1992). Many ectotherms can adjust their SMR in different seasons. In reptiles, the extent and direction of these seasonal fluctuations in SMR are thought to be linked to the seasonality of activity and energy requirements (Zari, 2013). For example, some lizards showed the higher SMR during high-activity seasons (Naya et al., 2008; Toledo et al., 2008). Tsuji (1988) reported that the winter-dormant population of *Sceloporus occidenta* in cold region showed depressed SMR in winter and an annual peak during reproduction period in spring, but the winter-active population showed intermediate SMR in winter, annual peak in spring and the lowest SMR in summer.

The seasonal metabolic adjustments were closely related to the mitochondrial oxidative capacities and metabolic enzymes, such as lactate dehydrogenase (LDH), cytochrome *c* oxidase (CCO) and citrate synthase (CS). Adjustment of metabolic enzyme activities may be of particular importance in seasonal changes. It has been reported that seasonal acclimation to cold (or decreasing) temperatures enhanced the oxidative capacity of mitochondria in rainbow trout (*Oncorhynchus mykiss*)

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(Doucet-Beaupré et al., 2010; Guderley and St-Pierre, 2002) and catfishes (*Silurus meridionalis*) (Yan and Xie, 2011). In addition, the activities of metabolic enzymes showed significant seasonal changes in fish (Thibault et al., 1997), amphibians (Berner and Bessay, 2006; Berner and Puckett, 2010) and reptiles (Seebacher et al., 2004; Williard and Harden, 2011). *Alligator mississippiensis* compensated for lower winter temperatures by increasing enzyme activities, and the activities of cytochrome *c* oxidase and lactate dehydrogenase were significantly greater in winter compared with summer (Seebacher et al., 2003).

Although considerable studies on seasonal changes of ectotherms have been reported in many species under laboratory and wild conditions, only a few data on seasonal variation of metabolism and adjustments in ectotherm animals are reported (Southwood et al., 2006; Yan and Xie, 2011). Limited works focus on the physiological and biochemical levels to explore seasonal metabolism adaptaion in wild ectotherms living on extreme environments. *Phrynocephalus vlangalii* is a small viviparous lizard endemic to China and wildly distributed on Qinghai-Tibet Plateau at elevations ranging from 2000 to 4600 m above sea level (Zhao and Adler, 1993), which is a perfect model to dissect the adaptive mechanism in extreme environments. Seasonal acclimatization is a complex phenomenon driven by a multitude of metabolic processes (Clarke, 1993), whether or not it has the same regulatory mechanisms with thermal acclimation in nature conditions is unknown. In this study, we measured body temperature, SMR, mitochondrial respiration rates and metabolic enzyme activities in three different seasons (spring, summer and autumn). Our aim was to assess the behavior and physiology response to various seasons in *P. vlangalii* and test the hypothesis how the metabolism change with thermoregulation and physiological or biochemical variables.

2. Materials and methods

2.1. Animals

Phrynocephalus vlangalii, belong to Phrynocephalus Agamidae (Agaminae), Sauria, Iguania, Squamata (lizards). In the present study, adult male lizards (*P. vlangalii)* were collected in spring (2014, later April), summer (2014, early July) and autumn (2014, early September) from Maqu (33°57′N, 102°05′E), Gansu province, China. This area belongs to fell-field with the average altitude about 3450 m. Local climate data, which include air temperature, precipitation, and sunshine duration, are shown in Table 1, which were provided by the Chinese Climatic Data Centre (CDC) for the years from 1984 to 2014. In each season, about 50 lizards were captured by hand or noose and randomly divided into two groups, one group was used for the experiments of preferred Tb and SMR in field research station near the collecting site; the other group was brought to the laboratory at Lanzhou University (36°05′N, 103°86′E) within 24 h after capture for further measurement of enzyme activity and mitochondrial respiration rate experiments. The mean body mass was 6.65 ± 0.09 g (spring, N = 20), 6.99 ± 0.17 g (summer, N = 20) and 7.78 ± 0.14 g (autumn, N = 20). All experimental procedures in this study were approved by the Institutional Animal Care and Use Committee at Lanzhou University.

Table 1
Climatic data of Maqu from 1984 to 2014.

	Mean temperature (°C)	Amount of precipitation (mm)	Sunshine duration (h)
April	2.78	26.79	227.18
May	6.25	71.45	221.20
June	9.53	103.03	194.08
July	11.56	129.82	214.68
August	10.87	102.42	224.01
September	7.59	84.59	178.77
Annual values	2.02	591.9	2584

2.2. Preferred body temperature (Tb)

The preferred Tb was measured according to a method modified from He et al. (2013). Briefly, about 25 lizards were maintained in a chamber (1.50 m long, 0.60 m high, and 0.50 m wide), and the bottom of the chamber was covered with 5 cm depth of sand and there were no special shelters for lizards. A 150 W incandescent bulb was fixed at one end of the chamber, which can heat the surface of sand and establish a temperature gradient approximately from 20 °C to 55 °C. The heat was available from 0830 h to 1830 h every day. Within the temperature gradient, all lizards could freely select the preferred Tb by behavioral thermoregulation. 15 lizards were randomly chosen from the chamber to measure preferred Tb at 1000 h, 1200 h, 1400 h, 1600 h and 1800 h respectively. The probe (1.5 mm diameter), which connected to a digital electronic thermometer, was inserted about 3–5 mm into the cloaca and measurement was finished <15 s, in order to maximally limit possible influence of heat transfer between the human hand and the lizard. During the experimental period, lizards were fed with mealworms and water ad lib.

2.3. Standard metabolic rate (SMR)

SMR was assessed by a small animal respiratory measurement open-flow system (RP1LP, Qubit, Canada). The method was performed according to Yue et al. (2012) with some modifications. In the local field research station, 10 lizards were fasted for 2 days to ensure a postabsorptive state before assessment. In order to minimize the activities of lizard, SMR measurements were conducted in a dark room from 2000 h to 0600 h. SMR of lizards were determined at 20 °C and 30 °C in each season. At each temperature, SMR under resting conditions was measured more than 30 min after the lizard was equilibrated to the experimental temperature for 30–40 min. Through the production of carbon dioxide (CO_2), SMR was expressed by mg CO_2 min^{-1} g^{-1}.

2.4. Tissue collection

In laboratory lizards were sacrificed via cranial and spinal pithing. Liver and skeletal muscle were removed, washed with saline, then immediately frozen in liquid nitrogen and transferred to a −80 °C freezer for determination of enzyme activity. Additional samples of liver, skeletal muscle were collected to isolate mitochondria.

2.5. Mitochondrial respiration rate and mitochondrial cytochrome c oxidase

Mitochondria were isolated and assayed according to Kayes et al. (2009) with the following modifications. After weighing, tissues (liver and skeletal muscle about 0.15 g) were homogenized by glass homogenizer in 4 volumes of isolation buffer (140 mM KCl, 20 mM Hepes, 10 mM EDTA, 5 mM $MgCl_2$, 0.5% BSA, pH 7.3) and the homogenate was centrifuged at 3000 rpm for 5 min at 4 °C. Then the supernatant was collected and centrifuged at 12,000 rpm for 10 min at 4 °C. At last, the supernatant was discarded and the mitochondrial pellet was resuspended in 800 μl assay medium (140 mM KCl, 20 mM Hepes, 5 mM KH_2PO_4, 0.5% BSA, pH 7.3).

Respiration rates of the mitochondria were measured using a Clarke-type electrode (Hansatech Instruments Ltd., Norfolk, UK) at constant temperatures (20 °C and 30 °C) by a circulator bath. 250 μl of mitochondrial suspension was added to the respiration chamber. State 2 oxygen consumption rate was measured by adding pyruvate (2.5 mM final concentration), and malate (5 mM final concentration) to spark the TCA cycle. ADP (3.8 mM final concentration) was then added to the chamber and state 3 respiration (ATP synthesis) was recorded until the ADP was exhausted the respiration state changed to state 4. The respiratory control ratio (RCR) was calculated by dividing state 3 by state 4 respiration.