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Geographic variation in basal thermogenesis in little buntings: Relationship to cellular thermogenesis and thyroid hormone concentrations

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ABSTRACT

Acclimatization to different ambient conditions is an essential prerequisite for survival of small passerine birds. Long-distance migration and winter acclimatization induce similar physiological and biochemical adjustments in passerines. To understand metabolic adaptations, the resting metabolic rate (RMR), the thermogenic properties of mitochondria in liver and muscle, and the activity of thyroid hormones were examined in field-captured little buntings (*Emberiza pusilla*) between Southeastern (Wenzhou) and Northeastern (Qiqihar) China from March to May in 2008 during their migration. Twelve birds were trapped from March to April in Wenzhou region, Zhejiang Province (27°29′N, 120°51′E) and eleven birds originated from April to May in Qiqihar region, Heilongjiang Province (47°29′N, 124°02′E). We found that RMRs of little buntings were significantly higher in Qiqihar than in Wenzhou. Consistently, mitochondrial state-4 respiration capacities and cytochrome c oxidase activities (COX) in liver and muscle, and circulating levels of plasma triiodothyronine (T_3) of little buntings were also significantly higher in Qiqihar than in Wenzhou. Variation in metabolic biochemical markers of liver and muscle, such as state-4 respiration and COX, and variation in thyroid hormone levels were correlated with variation in RMR. There was also a positive relationship between T_3 and metabolic biochemical markers. Little buntings mainly coped with a cold environment by enhancing thermogenic capacities through enhanced respiratory enzyme activities and plasma T_3. These results support the view that the primary means by which small birds meet energetic challenges of cold conditions is through metabolic adjustments.

1. Introduction

The necessity of maintaining a favorable energy balance is one of the major factors influencing the abundance and distribution of small birds, and a variety of morphological, physiological and behavioral adaptations are related to energy utilization (Weathers, 1979; Swanson and Garland, 2009). Metabolic rates (MR) are integrative measures of animal energetics and often vary among and within species in an apparently adaptive manner (McKechnie, 2008; Swanson, 2010). Elucidating the selection pressure(s) that drive the evolution of metabolic rate is necessary to understand the evolution of morphology, physiology, behavior, and life histories of birds (McNab, 2009; Lindsay et al., 2009a) and climate is one such selective factor implicated in the evolution of avian life-history traits (Guglielmo and Williams, 2003). Comparisons of standardized measures of metabolic rate among birds under conditions of variable environmental or ecological demands (e.g., different climates) can reveal how energy demands shape organismal metabolic rates. Basal metabolic rate (BMR) is the rate of energy transformation in a rested, fasted state in the absence of thermal stress and during the resting phase of the daily cycle, and is a standardized measure of minimum metabolic rate for animals maintaining normal physiological function. Consequently, BMR is one of the most important and often-used parameters for comparisons of energy metabolism among animals (McKechnie and Wolf, 2004; McKechnie et al., 2006; Barceló et al., 2009; McNab, 2009; Lindsay et al., 2009b; Swanson et al., 2012).

Phenotypic plasticity refers to variation in the phenotype generated by an environmental factor and defined by a reaction norm (Guglielmo and Williams, 2003). Phenotypic plasticity may involve phenotypic flexibility and/or developmental plasticity (irreversible changes that result from developmental processes) (McKechnie et al., 2006). Phenotypic flexibility refers to phenotypic changes that are reversible, temporary, and repeatable, such as acclimation and acclimatization (Piersma and Drent, 2003; Liknes and Swanson, 2011a,b). In birds, phenotypic flexibility in metabolic power output is an important component of thermoregulatory responses to seasonal environments and accommodating the elevated energy requirements associated with long-distance migration (Guglielmo and Williams, 2003; Bauchinger and Biebach,

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2005). In many species, adjustments of BMR comprise important components of seasonal acclimatization, and/or the physiological changes that precede migratory flights between geographically distant breeding and wintering grounds (McKechnie, 2008). Tropical birds typically have lower BMR than high-latitude birds, which has been explained as an adaptation to avoid heat stress and to conserve water (Weathers, 1997; Wiersma et al., 2007). In turn, the higher BMR of temperate and Arctic birds has been explained as a direct (low temperature) or indirect (food supply) result of the adaptation to a colder climate and a shorter breeding season, requiring higher metabolic capacity (Klaassen, 1995; Swanson, 2010). For a number of long-distance migrant birds, it has been suggested that their BMR in their tropical winter quarters is lower than that at their cold breeding grounds (Kersten et al., 1998; Lindström and Klaassen, 2003).

The liver is one of the largest and most metabolically active organs in endotherms, and it also has the potential to contribute to the thermogenic response (Villarin et al., 2003). Under basal metabolic conditions, the liver has been shown to contribute 25% of total heat production and seasonal changes in liver mass may be correlated with seasonal changes in BMR (Swanson, 1991; Li et al., 2001). However, little information on thermogenesis at the cellular level in the liver is available for birds. Potential mechanisms for elevating BMR in birds include increasing mitochondrial respiration or mass-specific aerobic enzyme capacity of liver (Huttemann et al., 2000). There is some evidence for increased mitochondrial respiration (state-4 respiration) or mass-specific aerobic enzyme capacity (cytochrome c oxidase) in cold or winter relative to control or summer individuals in some species (Li et al., 2001; Zheng et al., 2008a). Skeletal muscles have lower mass-specific metabolic rates at rest, but due to their total mass, contribute significantly to seasonal acclimatization through their role in thermogenesis (Zheng et al., 2008a; Swanson et al., 2009). Thyroid hormones (thyroxine T_4 and triiodothyronine T_3) play pivotal roles in development and energy metabolism of homoeothermic animals (Decuypere et al., 2005; Liu et al., 2006). Thyroid hormones can affect adaptive thermogenesis by influencing several aspects of energy metabolism, such as substrate cycling, ion cycling, and mitochondrial proton leakage (Yen, 2001; Decuypere et al., 2005; Zheng et al., 2008a).

The little bunting (*Emberiza pusilla*) is a small seed-eating passerine bird that has a very wide geographical range and inhabits vast areas of the continents of Europe and Asia. Little buntings breed in extreme north Eurasia, from central Scandinavia to Siberia, and north Asia, and migrate to winter in northeast India, central and southeast China and southeast Asia via Mongolia, southeast Russia and northeast China (MacKinnon and Phillipps, 2000). Little buntings are passage migrants only in Wenzhou and Qiqihar, and occur in these areas only during Spring and Autumn migratory periods. Little buntings have a high basal metabolic rate and thermal conductance, a wide thermal neutral zone and low lower critical temperature (Liu et al., 2001). Elevation of thyroid hormone levels in little buntings results in increases thermogenesis and physiological and biochemical markers of metabolism in liver and muscle along with decreases in body mass (Liu et al., 2006). Many studies have showed that migratory species change their body composition, organ masses and metabolic thermogenesis between winter grounds and breeding grounds (Hiebert, 1993; Burness et al., 1998; Swanson and Dean, 1999; Guglielmo and Williams, 2003; Petit et al., 2010). However, very few avian studies have looked at variation in thermogenesis at cellular or organismal levels for migratory species (Lundgren and Kiessling, 1986; Guglielmo et al., 2002; Swanson, 2010). These considerations led us to undertake a study of the little bunting that extends from the cellular to the organismal level and examines possible mechanisms mediating changes in thermogenesis. We measured RMR, mitochondrial respiration capacities (mitochondrial proton leakage) and COX activities (cellular aerobic capacity) in liver and muscle, and circulating level of plasma T_3 and T_4 of little buntings. We made these measurements in birds from two localities differing in climate during the same period of the annual cycle (spring migration). We predict that little buntings from the northern area (Qiqihar) will have higher levels of energy use (RMR and thermogenic capacity at the cellular level in liver and muscle) than those from the southern area (Wenzhou).

2. Materials and methods

2.1. Animals

Twenty-three little buntings (*E. pusilla*) used in our experiments were captured from the two regions in China during the spring migration period in 2008. Twelve birds were trapped from March to April in Wenzhou region, Zhejiang Province (27°29′N, 120°51′E) and eleven birds originated from April to May in Qiqihar region, Heilongjiang Province (47°29′N, 124°02′E) (Fig. 1).

In Wenzhou, the climate is warm-temperate with an average annual rainfall of 1700 mm spread across all months with slightly more precipitation during winter and spring. Mean daily maximum temperatures range from 39 °C in July to 8 °C in January, and mean temperature from March to April and from April to May is 14 °C and 18 °C, respectively (Zheng et al., 2008b). In Qiqihar, the annual mean temperature is 3.4 °C, average monthly air temperature in the coldest month (January) is −20.6 °C, and 22.9 °C in the warmest month (July). The mean temperature from April to May is 9.0 °C (Zheng et al., 2008a).

Body mass to the nearest 0.1 g was determined immediately upon capture with a Sartorius balance (model BT25S). Birds were transported to the laboratory on the day of capture, both in Wenzhou and Qiqihar, and caged for 1 or 2 days ($50\times30\times20$ cm^3) outdoors under natural photoperiod and temperature before measurements. Food and water were supplied ad libitum. The mean (±*SEM*) body mass of little buntings in Qiqihar and Wenzhou were 13.4 ± 0.4 g ($n=11$) and 14.7 ± 0.4 g ($n=12$), respectively.

2.2. Metabolic trials

Oxygen consumption was measured using the closed-circuit respirometer containing 3.6 L animal chambers (see Górecki, 1975), which were immersed in a water bath that maintained temperature at 25 ± 0.5 °C, which is within the thermal neutral zone for little buntings (Liu et al., 2001). The metabolic measurements at this temperature were obtained over a 60-min period that began after the birds had rested in their chambers for approximately an hour (Zheng et al., 2008a). At the time of measurement, they had fasted for 4 h to assure that they were in a postabsorptive state. H_2O and CO_2 were absorbed by silica gel and KOH, respectively. Recordings of oxygen consumption during bouts of activity were discarded before computing the metabolic rate of each individual. All measurements were made daily between 18:00 and 22:00 h. The reading interval for O_2 consumption was 5 min. Two consecutive stable and minimum readings were used to determine RMR. Metabolic rates were expressed as both whole-organism ($mL\ O_2\cdot h^{-1}$) and mass-specific ($mL\ O_2\ (g\cdot h)^{-1}$) rates and corrected to STP conditions (Schmidt-Nielsen, 1997). Body temperatures (T_b) of all individuals were recorded before and after each measurement. T_b was measured with a digital thermometer (Beijing Normal University Instruments Co.) in the cloaca at a depth of 1.5 cm (Zheng et al., 2008b).

2.3. Preparation of mitochondria

After metabolic measurements, the animals were sacrificed and blood was collected for serum preparation. The liver and pectoral muscle were quickly removed from the bunting carcass, placed in ice-cold sucrose-buffered medium, cleaned of any adhering tissue, blotted, and weighed. Liver samples were chopped coarsely with scissors, rinsed and resuspended in 5 vol of ice-cold medium. The samples were then homogenized using a Teflon/glass homogenizer. The samples used for muscle mitochondrial preparations were chopped,

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