Comparative Biochemistry and Physiology, Part A xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Leptin levels, seasonality and thermal acclimation in the Microbiotherid marsupial *Dromiciops gliroides*: Does photoperiod play a role?

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ARTICLE INFO

Article history:
Received 19 July 2016
Received in revised form 15 September 2016
Accepted 29 September 2016
Available online xxxx

Keywords:
Blood biochemistry
Dromiciops
Seasonal changes
Microbiotheria
Hibernation

ABSTRACT

Mammals of the Neotropics are characterized by a marked annual cycle of activity, which is accompanied by several physiological changes at the levels of the whole organism, organs and tissues. The physiological characterization of these cycles is important, as it gives insight on the mechanisms by which animals adjust adaptively to seasonality. Here we studied the seasonal changes in blood biochemical parameters in the relict South American marsupial *Dromiciops gliroides* ("monito del monte" or "little mountain monkey"), under semi-natural conditions. We manipulated thermal conditions in order to characterize the effects of temperature and season on a battery of biochemical parameters, body mass and adiposity. Our results indicate that monitos experience an annual cycle in body mass and adiposity (measured as leptin levels), reaching a maximum in winter and a minimum in summer. Blood biochemistry confirms that the nutritional condition of animals is reduced in summer instead of winter (as generally reported). This was coincident with a reduction of several biochemical parameters in summer, such as betahydroxybutyrate, cholesterol, total protein concentration and globulins. Monitos seem to initiate winter preparation during autumn and reach maximum body reserves in winter. Hibernation lasts until spring, at which time they use fat reserves and become reproductively active. Sexual maturation during summer would be the strongest energetic bottleneck, which explains the reductions in body mass and other parameters in this season. Overall, this study suggests that monitos anticipate the cold season by a complex interaction of photoperiodic and thermal cues.

1. Introduction

Mammals of the Neotropics are characterized by a marked annual cycle of activity, which is accompanied by several physiological changes at the levels of the whole organism, organs and tissues (Blank et al., 1990; Hofman, 2004). These changes are induced by peaks of activity and reproduction during spring and summer, and reductions in activity in fall and winter (Fournier et al., 1999; Heldmaier, 1993; Turbill et al., 2011); an extreme case being hibernation, where activity is drastically reduced during winter (Ruf and Geiser, 2015; Xu et al., 2013). In general, animals anticipate these changes by environmental cues, from which temperature and photoperiod appear as the most important (Hofman, 2004). Hence, characterizing the cascade of physiological changes that facilitate seasonal acclimatization at different levels of biological organization is central for understanding how organisms cope with environmental changes in temperate regions (Blank et al., 1990).

The measurement of blood metabolites permits a simple and repeatable characterization of the physiological status of free-ranging animals (see Albano et al., 2016; Artacho et al., 2007a; Franco et al., 2013; Hellgren et al., 1997; Swarnkar et al., 2000, and references therein). For instance, haematological analyses (e.g., quantification of blood cells, platelets and haemoglobin concentration) have permitted investigators to characterize the annual cycle in erythrocyte/leucocyte count that accompanies body mass changes during hibernation in bears (Delgiudice et al., 1991; Hissa et al., 1994), the effects of malnutrition in a population of swans during an environmental crisis (Artacho et al., 2007b) and to assess the health status of endangered species (Anderson et al., 2011; Christopher et al., 1999). Similarly, blood biochemistry (e.g., determination of the concentration of metabolites and specific enzymes in the serum) permits characterization of fuel utilization during the annual reproductive cycle (Pickering, 1986), migration (Albano et al., 2016; Jenni-Eiermann et al., 2002), hibernation (Otis et al., 2011) or fasting (Cherel et al., 1995). A great number of metabolites can be assessed from a single sample (see Coz-Rakvac et al., 2011; Franco et al., 2013; McKeon et al., 2011, and references therein), and the choice depends on the question being addressed. For instance,

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http://dx.doi.org/10.1016/j.cbpa.2016.09.025
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Please cite this article as: Franco, M., et al., Leptin levels, seasonality and thermal acclimation in the Microbiotherid marsupial *Dromiciops gliroides*: Does photoperiod play a role?..., Comp. Biochem. Physiol., A (2016), http://dx.doi.org/10.1016/j.cbpa.2016.09.025

some variables are good proxies of the nutritional status of an animal (e.g., betahydroxybutyrate, plasma proteins, see Artacho et al., 2007a; Jenni-Eiermann et al., 2002), others can give insight into organ damage (e.g., hepatic enzymes, see Artacho et al., 2007a; Smith et al., 1994), whereas others such as hormone concentration give information about metabolic signalization in the body (Wittert et al., 2005).

At the level of the whole organism, animals face the cold season by either increasing thermogenic capacity (Heldmaier et al., 1982; Sharbaugh, 2001), or by abandoning endothermy and entering into hibernation (Pulawa and Florant, 2000; Ruf and Geiser, 2015). Both are commonly known seasonal strategies of resistance and avoidance (Wunder and Merritt, 1984) by temperate mammals for adjusting to winter cold, and encompass different costs and benefits. Whereas enduring cold conditions in winter entails high energetic costs, especially during nutritional bottlenecks; hibernation can carry important survival costs (e.g., Kenagy et al., 1989; Sharbaugh, 2001). For both strategies, physiological adjustments are radically different. Whereas non-hibernating species can double or triple their thermogenic capacity when cold acclimated, with parallel increases in haematological parameters that improve blood oxygen transport during winter (Nespolo et al., 1999; Rosenmann and Ruiz, 1993), hibernating species experience a depression in most metabolites and haematological parameters (Andersen et al., 2000; Bouma et al., 2010; Franco et al., 2013). These variations are usually followed by cycles in body mass and adiposity, as animals lose fat reserves during winter and increase them during summer (Merritt et al., 2001; Sommer et al., 2016).

In 2004, Bozinovic and collaborators described the physiology of a highly seasonal mammal, the Microbiotherid marsupial “monito del monte” (*Dromiciops gliroides*) (Bozinovic et al., 2004; Palma and Spotorno, 1999). According to these findings, monitos are the first and only South American mammal known to hibernate (Hadj-Moussa et al., 2016). This species is restricted to the humid rainforests of southern South America and is the sole extant species of Microbiotheria, a relict mammalian order that is thought to represent the link between American and Australian marsupials (Mitchell et al., 2014; Palma and Spotorno, 1999). In contrast to other hibernators (e.g., Carey et al., 2003), monitos exhibit daily torpor in summer (Fonturbel et al., 2012; Nespolo et al., 2010). Profound variations in a number of physiological parameters, including immune function, haemoglobin, plasma proteins and fat metabolism have been described in torpid monitos (Franco et al., 2013). This metabolic depression is associated with 85 microRNAs located in the liver and muscle, which probably control the aforementioned physiological adjustments (Hadj-Moussa et al., 2016).

Considering the evolutionary status of monitos (i.e., the sole living representative of the Microbiotheria order, see Mitchell et al., 2014), several authors became interested in studying its ecology and physiology (see Fonturbel et al., 2012; Franco et al., 2011; Hadj-Moussa et al., 2016; Withers et al., 2012, and cited references). However, it is still unclear whether this marsupial exhibits a pattern of controlled physiological variations governed by anticipatory cues, as in other hibernators (Bradshaw and Holzapfel, 2007; Cudney and Place, 2012; Hope et al., 2000; Wittert et al., 2004), or if it simply responds just to the gradual reductions in temperature that precede the cold season (Cortes et al., 2009; Nespolo et al., 2010; Withers et al., 2012).

In order to explore the seasonal fluctuations in energy use and body condition in monitos, we measured a suite of biochemical variables from the blood at regular intervals throughout the year (Table 1). This suite of parameters represents nutritional status (BHB, COL, TRI, NEFA, PROT, ALB, GLU) and organ damage (CK and CREA) (see Table 1). As a proxy metric for the proportion of fat in the body (Cudney and Place, 2012; Li and Wang, 2007), we also measured leptin concentration. Leptin is a 16 kDa protein secreted by adipose tissue (an “adipokine”) in proportion to the animal's mass, i.e., larger proportions of adipose tissue in the body means increasing levels of leptin secretion. Leptin is primarily known for regulating fat deposits by inhibiting nutrient ingestion and inducing increases in energy expenditure (Cammisotto and Bukowiecki, 2002; Friedman and Halaas, 1998; Li and Wang, 2007).

Our experiment consisted of a two-way design aimed to test the effects of season and thermal acclimation on the biochemical parameters, wherein monitos were housed in an outdoor enclosure (a specially designed wood cabin, see Material and methods). The cabin was divided into two defined environmental chambers. One chamber was held constant at 20 °C, and the other chamber was open to the external environment, which resulted in varying ambient temperature (see Material and methods for details). Hence, we had two factors, season (four levels) and treatment (two levels). Through this design, we interpreted statistically significant effects as biological effects in the following fashion. First, main effects of season would be indicative of photoperiodic effects on the measured variable, because both groups were exposed to seasonal changes in day length. Second, main effects of treatment would be indicative of temperature effects (i.e., thermal acclimation), since differences are attributed to changes in ambient temperature. Third, statistically significant effects of both factors would mean that both, photoperiod and ambient temperature had an effect on the

Table 1
Biochemical, hormonal and metabolic parameters measured in this study, with a brief explanation of it meaning (see also Franco et al., 2014).

Parameter	Abbrev.	Significance
Beta-hydroxy-butyrate	*BHB*	A ketone body produced by lipid metabolism. It is increased in animals that are consuming their fat reserves.
Cholesterol concentration	*COL*	Energy source and also an important component of membranes during hibernation. Indicative of fat mobilization and energy use.
Triglyceride concentration	*TRI*	Main form of energy storage in vertebrates. Indicative of nutritional status and fat mobilization.
Non-esterified fatty acids	*NEFA*	Long-chain fatty acids that come from triglycerides. Indicative of nutritional status.
Total protein concentration	*PROT*	Concentration of proteins in the plasma. Indicative of nutritional status.
Albumin concentration	*ALB*	Main binding protein in the plasma. Indicative of nutritional status. It is increased in dehydrated individuals.
Globulin concentration	*GLO*	The second most abundant protein in plasma. A broad family of proteins associated with immune function.
Creatine kinase	*CK*	A by-product of muscle metabolism. It is increased in blood when myofibrils are damaged (i.e., it indicates muscle damage).
Creatinine concentration	*CREA*	A by-product of muscle metabolism, indicative of kidney function. High levels of creatinine indicate kidney damage.
Glucose concentration	*GLU*	Main form of usable energy in vertebrates. It is indicative of the nutritional status.
Leptin concentration	*LEP*	“Satiety” hormone that plays a key role in regulating energy intake and energy expenditure, by regulating appetite/hunger stimuli. It is produced in white fat (thus, it is a proxy of the fat amounts in the body).

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