Thermoregulatory capacities and torpor in the South American marsupial, *Dromiciops gliroides*

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A B S T R A C T

During periods of adverse conditions small endotherms depend on a continuous supply of food and energy to maintain body temperature. Thus, rapid and reversible phenotypic modifications at different organizational levels are key for an efficient use of resources and survival. In this study, we provide a quantitative description of thermoregulatory capacities and energy-saving strategies in the Chilean marsupial *Dromiciops gliroides*. In particular, we evaluated the effect of thermal acclimation on basal metabolic rate (BMR), thermal conductance (C) and torpor patterns, as well as the presence of non-shivering thermogenesis (NST) as a rewarming mechanism in this marsupial. Non-significant effects of thermal acclimation were observed in BMR, C and body mass, but cold-acclimated individuals exhibited significantly longer torpor bouts. Also, minimum body temperature during torpor, inter-bout body temperature and arousal rewarming rate were lower in cold-acclimated animals. Furthermore, we found that *D. gliroides* did not display NST in response to Norepinephrine. Hence, despite the high regulation of torpor of other species, *D. gliroides* shows low flexibility in the ability to adjust energy expenditure and insulation properties, and (as in other marsupials) NST do not seems to be important as thermo-regulatory mechanism.

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1. Introduction

Phenotypic flexibility represents reversible within-individual phenotype adjustments to environmental change (Via et al., 1995; Pigliucci, 2001; Piersma and Drent, 2003). Among the immense repertoire of flexible responses, energy acquisition and allocation are described as the most environmentally-sensible functions at the level of the organism (Maddocks and Geiser, 2000; Humphries et al., 2005; Larivee et al., 2010; Chi and Wang, 2011; Gutowski et al., 2011; Fletcher et al., 2012). One example of important allocation challenges occurs in temperate rain-forests, where small endotherms are faced with strong changes in cold exposure and energy availability. Endotherms depend on continuous supply of food and energy to maintain body temperature (T_b) by which rapid and reversible phenotypic modifications are key for efficient use of resources (Weiner, 1992; Angilletta, 2009). Hence, phenotypic

flexibility in behavioural, physiological and morphological traits and energy-saving strategies are critical for survival.

In general, as a response to cold exposure, small mammals can modulate phenotype at multiple organization levels, including total heat production (Tomlinson et al., 2007; McKechnie and Swanson, 2010), absorption/processing capacities (Hammond and Janes, 1998; Hammond et al., 2001; del Valle et al., 2004), fur properties oriented to reduce heat loss (see Gordon (2012), for review) and/or the set-point at which T_b is regulated (Geiser and Kenagy, 1988; Frey, 1991). In particular, high levels of basal metabolic rate (BMR) and increases in body mass (M_b), as well low values of thermal conductance (C) are associated with cold-acclimation and winter-acclimatization (Nespolo et al., 2003a, 2003b; Sheriff et al., 2009; Vèzina et al., 2011; Zhang et al., 2012; Zhu et al., 2012).

Basal metabolic rate (BMR) represents the endothermic cost of living in absence of thermoregulatory constraints (Hulbert and Else, 2000, 2004). There is considerable evidence documenting significant increase in BMR during cold acclimation (Nespolo et al., 2002; Klaassen et al., 2004; Novoa et al., 2005; Vèzina et al., 2006; Zhang et al., 2012). The variation in BMR after thermal acclimation is explained by changes in size and activity of metabolically active

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tissues such as brown fat, small intestine, heart and liver (Konarzewski and Diamond, 1995; Campbell and MacArthur, 1998; Bacigalupe et al., 2004). Minimal thermal conductance (C), on the other hand, represents the ease of hate transference between body and environment and is the inverse of insulation. Typically, cold-induced variation in C is interpreted as changes in fur properties to reduce heat loss (Aschoff, 1981; Piersma et al., 1995; Schleucher and Withers, 2001).

Variation in thermogenic capacities represents an important mammalian response when facing energetic constraints. Shivering (ST) and non-shivering thermogenesis (NST) are the main mechanisms associated with thermoregulation in small mammals (Bicego et al., 2007; Morrison et al., 2008; Nowack et al., 2013). Heat production by ST is the consequence of rapid and isometric muscle contractions (Banet et al., 1978). On the other hand, NST is a source of heat associated with brown adipose tissue (BAT), which is induced by cold exposure (Haim and Izhaki, 1993; Klingenspor, 2003; Cannon and Nedergaard, 2004; Jansky et al., 2008). BAT is composed by small adipocytes with by high density of mitochondria that are activated by norepinephrine (NE) (Takeuchi et al., 1995; Thomas and Palmiter, 1997; Palou et al., 1998). Heat production in BAT involves fatty acid oxidation and uncoupling of oxidative phosphorylation. A BAT-specific protein, the uncoupling protein 1 (UCP1) is the responsible to dissipate the proton gradient across the mitochondrial inner-membrane to transform directly energy in heat than adenosine triphosphate (Ricquier et al., 1991; Matthias et al., 2000; Nedergaard et al., 2001). In eutherian mammals, the expression of UCP1-mediated NST is highly flexible, principally because of cold exposure (Nespolo et al., 2001a, 2001b; Van Sant and Hammond, 2008; Mineo et al., 2012; Nowack et al., 2013; Oelkrug et al., 2012; Zhu et al., 2012). In marsupials, however, elicitation of NST after cold exposure and NE injection is not conclusive. While physiological evidence for NST was documented by some authors (Kabat et al., 2003; May, 2003b; Rose and Ikonomopoulou, 2005), most of marsupials present no response to cold induced NST (Nicol et al., 1997; Opazo et al., 1999). Furthermore, the role of uncoupling proteins and BAT is not clear. In the few species were BAT and UCP-1 genes have been described, these not show thermogenic properties (Kabat et al., 2003; May, 2003a; Jastroch et al., 2008; Polymeropoulos et al., 2012). For marsupials and birds, heat production by NST could be the consequence of metabolic activity in the liver and white adipose tissue (Rose et al., 1999; Villarin et al., 2003; Talbot et al., 2004; Teulier et al., 2010).

Torpor or transitory heterothermy is the physiologically controlled reduction of metabolic rate and body temperature experienced by small endotherms when facing periods of low temperature and/or food resources (Geiser and Ruf, 1995; Geiser, 2004; Heldmaier et al., 2004; Melvin and Andrews, 2009). This phenotype is characterized by drastic reductions in several physiological processes, provoking an important saving in the energy cost of maintenance (Carey et al., 2003; Geiser, 2004). Heterothermic therian mammals (i.e. placental and marsupials) express different patterns of torpor, where daily torpor and hibernation represent the extreme of a continuum (Wilz and Heldmaier, 2000; Lovegrove et al., 2001a, 2001b; Melvin and Andrews, 2009).

Arousal from torpor represents an important constraint on these energy-saving strategies (Fons et al., 1997; Karpovich et al., 2009). In fact, periodical arousals may compromise as much as 75% of total winter energy budget (Thomas et al., 1990; Holloway and Geiser, 1995; Lovegrove et al., 1999). The functional significance of arousal includes elimination of metabolic waste (Geiser and Kenagy, 1988), immune system reactivation (Prendergast et al., 2002) and water balance (Thomas and Geiser, 1997; Withers et al., 2012). In therian mammals, physiological mechanisms associated with arousal from torpor are different. For placental mammals,

NST play a key role during arousal from torpor, while for marsupials ST and liver activity could represents the main thermogenic mechanisms (Stone and Purvis, 1992; Fons et al., 1997; Lovegrove et al., 1999; Opazo et al., 1999; Toien et al., 2001; Villarin et al., 2003; Talbot et al., 2004; Bicego et al., 2007; Teulier et al., 2010). Despite these differences in thermogenic mechanisms, there appears not to be major differences between marsupials and placental mammals in heat production rates during rapid rewarming (Heldmaier, 1978; Geiser and Baudinette, 1990; Stone and Purvis, 1992).

An intra-specific variation in torpor pattern and thermoregulatory capacities has been documented by field and laboratory studies (Wassmer and Wollnik, 1997; Cruz-Neto and Bozinovic, 2004; Lehmer and Biggins, 2005; Solick and Barclay, 2007; Dunbar and Brigham, 2010). According to these authors, ambient temperature and energy availability are the most immediate factors explaining this variation (Geiser and Heldmaier, 1995; Buck and Barnes, 2000; Wilz and Heldmaier, 2000; Lovegrove et al., 2001a; Landry-Cuerrier et al., 2008).

Dromiciops gliroides, the only living species of the order Microbiotheria is an endemic marsupial that inhabits the South American temperate forest (Lobos et al., 2005; Martin, 2010; Nilsson et al., 2010; Franco et al., 2011). In this environment, *Dromiciops* faces strong changes in cold exposure and energy availability, which represents energetic bottlenecks for this omnivorous species (Amico and Aizen, 2000; Rodriguez-Cabal et al., 2008). To face this challenge, *D. gliroides* exhibits physiological adaptations to a generalist diet, with a facultative insectivory/frugivory (Cortes et al., 2011; Fonturbel et al., 2012). *Dromiciops* has been described as the only known hibernator in South America, which also exhibits short bouts of daily torpor, whose depth and duration depends principally on ambient temperature and nutrient availability (Bozinovic et al., 2004; Nespolo et al., 2010). Besides the available information about the role of the energetic constraints imposed by the environment, detailed information about the relationship between thermal acclimation, thermoregulatory capacities and torpor is absent in this species. Consequently, the aim of this study was to explore the effects of thermal acclimation on basal metabolic rate, non-shivering thermogenesis, thermal conductance, rewarming rate (RR) and torpor patterns in *D. gliroides*.

2. Material and methods

2.1. Animals

Eleven adult individuals of *D. gliroides* were captured near Valdivia, Chile (39°48′S, 73°14′W; 9 m.a.s.l) during the austral summer (January–February 2009). Modified tomahawk traps were located in trees and shrubs, 1 m above ground, and baited with bananas. Individuals were transported to the laboratory immediately after capture. Animals were individualized and housed in plastic cages ($45 \times 30 \times 20$ cm^3) with 2 cm of bedding. Before thermal acclimation, all individuals were maintained in a climatic controlled chamber (Pitec Instrument, Chile) at 20 ± 1 °C with a 12:12 photoperiod. Food (mix of mealworms and fruits) and water were provided *ad libitum*. The same conditions, excepting the bedding, were maintained during thermal acclimation.

2.2. Thermal acclimation and torpor

To elicit non-shivering thermogenesis and to characterize the effects of ambient temperature on torpor, we located two groups of animals during 1 month in two climatic chambers. The first group was maintained at 18 ± 1 °C (operationally defined as "warm"-acclimated). The other group was maintained at 8 ± 1 °C

("cold"-acclimated). These temperatures were chosen because they represent average environmental temperature experienced by *D. gliroides* during summer and winter, respectively (Franco et al., 2011).

During thermal exposures, a temperature data logger (temperature iButton, model DS1921G, 3 g, 16 mm diameter, Dallas semiconductors, USA) was attached to the back of each animal. Although these devices do not record core temperature, previous experiences with *Dromiciops* and also with *Thylamys elegans* have shown that attached data loggers on bare skin are good proxies of internal temperature, a less disturbing procedure than surgically inserted loggers or thermocouple insertion (Bozinovic et al., 2007; Nespolo et al., 2010). Unfortunately, Unfortunately, we cannot ignore the fact that T_{skin} could fluctuate whereas core temperatures remains constant (Schmidt-Nielsen, 1995).

The data logger measured skin temperature (T_{skin}) every 2 min, and then the information was downloaded to the computer every two days, at the same time when we renewed food and water. Rewarming rates were estimated as the rate of change of T_{skin} (dT_{skin}/dt) considering two points in the rewarming curve. The first point was the last measure of the minimum skin temperature during torpor ($T_{skin\ min}$). The second point was the last reading at the end of arousal, when the animal was normothermic (Fig. 1). At the end of the acclimation, animals were submitted to metabolic rate measurements to obtain basal metabolic rate, non-shivering thermogenesis and thermal conductance after both thermal exposures. Torpor incidence were computed as the amount of time spent of torpor in comparison to the total time registered by temperature data logger along thermal exposure.

2.3. Basal metabolic rate and non-shivering thermogenesis

According to Cooper and Withers (2009) the gold-standard method for measuring BMR is during a minimum of four hours. These are the ideal conditions to measure BMR, especially when the mean BMR is needed for comparative studies (when the mean for the species is used) (Cooper and Withers, 2009). When treatments are tested and BMR being used as a response variable within an experiment, we believe using shorter measurements

(we used 180 min) are good approximation for BMR (see Nespolo et al., 2001a; Clarke et al., 2010 and references therein). Hence, we define operationally BMR for the purposes of this experiment, but we are careful to indicate that the values could be somewhat overestimating the real BMR.

After thermal acclimation, animals were starved during 6 h before metabolic rate measurements. Metabolic rate was measured following the procedures previously explained by Bockler and Heldmaier (1983), Opazo et al. (1999) and Nespolo et al. (2001a), consisting in (1) the measurement of carbon dioxide production (V_{CO_2}) during 180 min at thermoneutral and resting conditions ($T_a=30\ °C$) (BMR); (2) intramuscular injection of saline solution (0.9%) (SS) or intramuscular injection of norepinephrine bitartrate (Sigma®) solution (NE); and (3) a new V_{CO_2} record during 30 min (NST) (Opazo et al., 1999; Nespolo et al., 2001a). Hence, each animal was measured twice, on time with saline solution (control) and other the norepinephrine treatment, with an interval of two days. The order of measurements (i.e., NE or SS) was randomized. The norepinephrine solution doses were calculated according to Wunder and Gettinger (1996). We used the same volume of saline solution for control measurements.

2.4. Respirometry

To estimate carbon dioxide production (V_{CO_2}) we used a flow-through respirometry system composed by a Li–Cor 6262·H₂O/CO₂. Ambient air was pumped to the system with a flow-rate of $1000\pm1\ ml\ min^{-1}$ controlled by a Sierra mass-flow controller, located upstream of the metabolic chamber, but after two columns with H_2O and CO_2 scrubbers (Drierite and Baralyme, respectively). The metabolic chamber (1 L) was located in a PELT-5 temperature controller (Sable Systems International, Inc.), and ambient temperature (T_A) was set to 30 °C and registered continuously during the record. Income air passed through columns with CO_2 and H_2O scrubbers (Drierite and Baralyme, Barium Hydroxide), and then through the mass flowmeter, then into the metabolic chamber, then injected into the Li–Cor 6262 analyser using a flow rate of $250\ ml\ min^{-1}$.
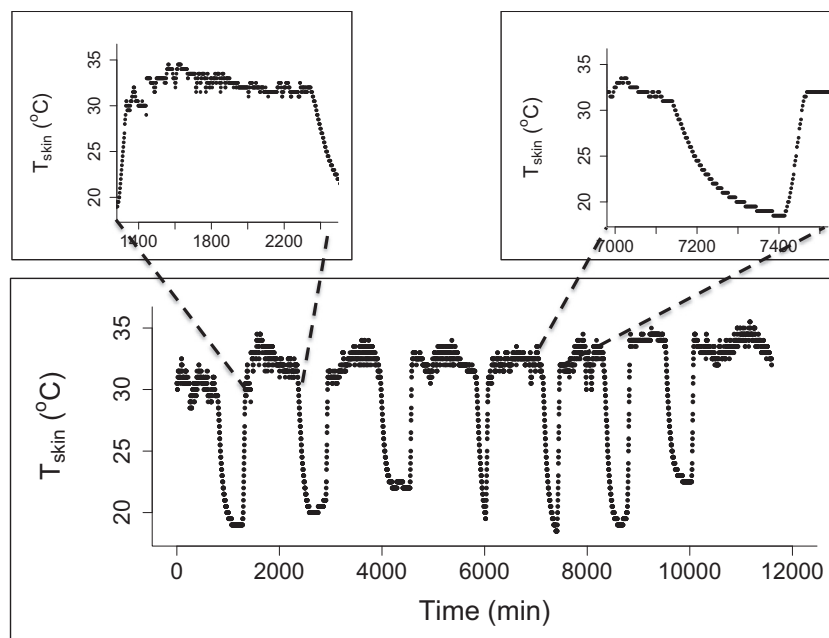

Fig. 1. Representative graphic of skin temperature registered with a data-logger during 8 days in a warm-acclimated individual. A detailed record of body temperature during torpor (right top panel) and inter-bout periods (left top panel) is displayed in the zoom. The black arrows on the bottom panel shows the point considered to the rewarming rate estimation (dT_b/dt).

Each record was corrected for drift deviations by base line. To obtain the final V_{CO_2} values, we computed the average of the minimum five-minute steady-state recording. In the case of NST, we computed the maximum five-minutes recording. From V_{CO_2} data, we then computed minimal thermal conductance as: $C = V_{CO_2}/(T_{skin} - T_a)$, where T_a is the environmental temperature.

2.5. Statistics

Data analysis was performed using R platform 2.15.0 (R Development Core Team, 2009) and results are shown as mean ± s.e. For the analysis, values of torpor patterns and re-warming rates represent an average of the multiple torpor events experimented for each individual along the month of acclimation. Since the basal metabolic rate ($r = 0.01$, $p = 0.71$) and torpor patterns (RR, $r = 0.15$, $p = 0.29$; T_{skin}, $R = 0.14$, $p = 0.32$; torpor bout length, $r = 0.19$, $p = 0.24$) were not correlated with body mass (M_b), different variables were analysed by one-way ANOVA.

3. Results

The comparison of M_b did not show significant differences between temperatures (Table 1). However, the percentage of torpor use during the acclimation was significantly different between temperatures. Cold-exposed individuals experienced torpor 3.3 times more than warm-exposed individuals (ANOVA, $F_{1,9} = 38.26$; $p < 0.01$; Table 1). Also, significant differences in torpor bout length were appreciated (ANOVA, $F_{1,9} = 16.92$; $p < 0.05$; Table 1, Fig. 2A). During torpor, $T_{skin\ min}$ was significantly lower in cold than warm-exposed animals (ANOVA, $F_{1,9} = 178.78$; $p < 0.01$; Table 1, Fig. 2B). Similarly, we found differences between treatments in T_{skin} during inter-bout periods, being higher in warm-exposed individuals (ANOVA, $F_{1,9} = 19.33$; $p < 0.01$; Table 1). The rewarming rate (RR) was significantly higher in warm-exposed individuals (ANOVA, $F_{1,9} = 15.22$; $p < 0.01$; Fig. 3, Table 1), although no differences were observed in basal metabolic rate between cold and warm-exposed individuals, as well as in V_{CO_2} between animals injected with norepinephrine or saline solution (Fig. 4).

4. Discussion

In mammals, classical adaptive response to cold includes flexibility in thermoregulatory capacities and the utilization of energy saving strategies. For the case of placental mammals, evidence suggests that non-shivering thermogenesis represents

Table 1
Results for one-way ANOVAs testing the effects of thermal acclimation on body mass (M_b), basal metabolic rate (BMR), thermal conductance (C), torpor bout length, torpor frequency, mean normothermic skin temperature (T_{skin}) and minimum skin temperature during torpor ($T_{skin\ min}$) and rewarming rate (RR) in *Dromiciops gliroides*. Sample sizes are provided in parentheses.

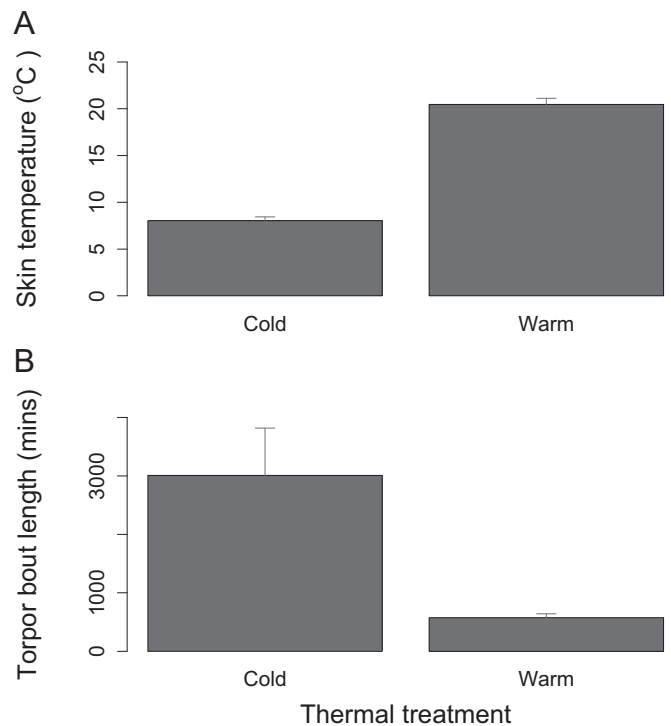| Trait	Cold (4)		Warm (7)		$F_{(1,9)}$	p
	Mean	s.e	Mean	s.e		
M_b (g)	25.5	1.61	30.1	2.48	1.27	0.28
BMR (ml CO_2 min^{-1})	0.38	0.02	0.32	0.50	0.76	0.41
C (ml CO_2 min^{-1} °C^{-1})	0.09	0.02	0.06	0.01	3.18	0.11
Torpor bout length (min)	3009.4	808.5	575.26	63.32	16.92	< 0.05
Torpor frequency (%)	69.98	6.17	20.95	4.84	38.26	< 0.01
T_{skin}	27.69	1.15	31.67	0.26	19.33	< 0.01
$T_{skin\ min}$	8.03	0.41	20.46	0.65	178.78	< 0.01
RR (°C min^{-1})	0.15	0.01	0.24	0.02	15.22	< 0.01

Fig. 2. (A) Torpor bout length and (B) minimum skin temperature during torpor in individuals acclimated during 30 days at 8 ± 1 °C (cold acclimation) and 18 ± 1 °C (warm acclimation). Sample size for cold and warm-acclimation are $n = 4$ and $n = 7$, respectively. Values are expressed in means ± s.e.
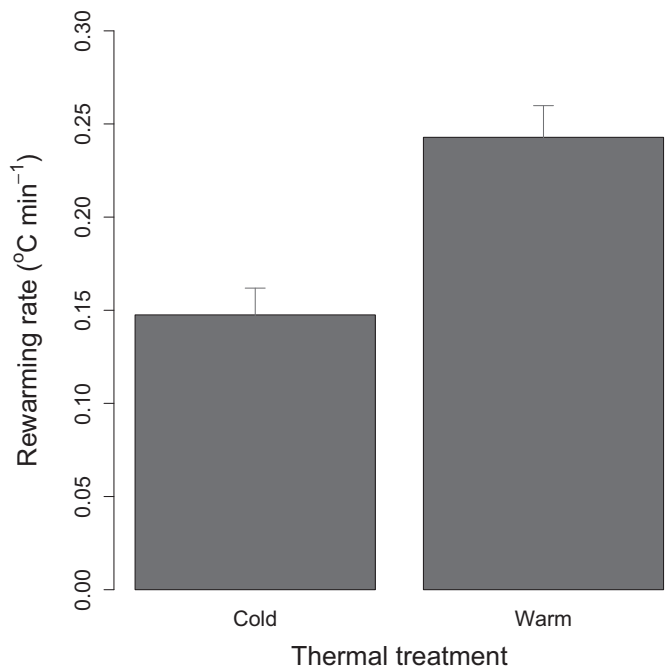

Fig. 3. Rewarming rate in torpid individuals acclimated during 30 days at 8 ± 1 °C (cold acclimation) and 18 ± 1 °C (warm acclimation). Sample size for cold and warm-acclimation are $n = 4$ and $n = 7$, respectively. Values are expressed in means ± s.e.

an important heat production mechanism after acute cold exposure (Kronfeld-Schor et al., 2000; Merritt et al., 2001; Nowack et al., 2013). Although thermogenetic responses to NA injection have been reported for some Australian marsupials, the presence of UCP-1 and BAT-induced NST in marsupials is controversial
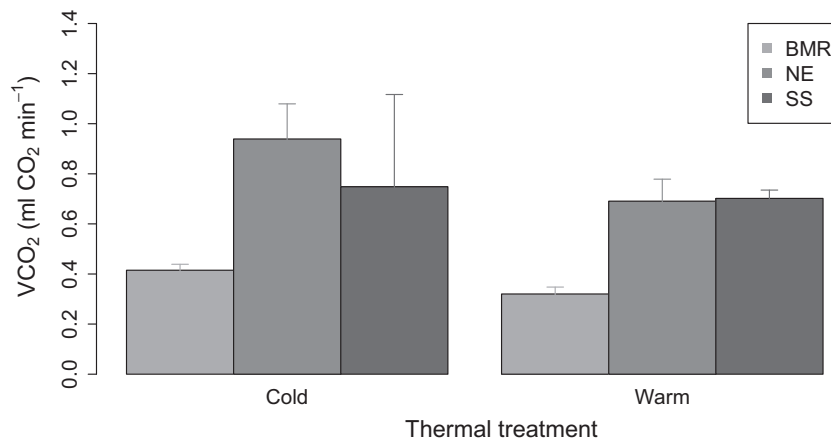
Fig. 4. Basal metabolic rate (BMR) and norepinephrine (NE) and saline solution (SS) effect on metabolic rate in cold and warm-acclimated individuals. Metabolic measurements were made at thermoneutral zone ($T_A = 30$ °C). Sample size for cold and warm-acclimation are $n = 4$ and $n = 7$, respectively. Values are expressed in means $\pm$ s.e.

(Kabat et al., 2003; May, 2003b; Rose and Ikonomopoulou, 2005; Polymeropoulos et al., 2012). However, NST expression is not restricted only to BAT. Several other tissues can contribute to NST (Granneman et al., 2003; Ukropec et al., 2006), which suggests the presence of BAT is not essential for NST. Our results indicate that norepinephrine injected individuals showed 2.29 times more metabolic activity than non-injected individuals. However, NE injection did not have significantly larger effects than saline solution on metabolic rate (Fig. 4; see also Opazo et al., 1999). Hence, the absence of effects of norepinephrine injection on metabolic rate could discard BAT-induced NST as a heat production mechanism for *D. gliroides.* Unfortunately, we cannot ignore the fact that our procedure (saline injection) involves considerable handling effect, which could mask non-acute metabolic effects of NE. If this is the case for *D. gliroides,* further research is needed to show whether NE has effects on metabolism.

Our results showed absence of changes in BMR after cold acclimation in *D. gliroides.* This finding is contrary with previous studies documenting an increase in BMR as a typical response to cold acclamation in endotherms (Nespolo et al., 2001b; Sharbaugh, 2001; Cruz-Neto and Bozinovic, 2004). This increase in BMR typically reflect reversible changes in size and activity of metabolically active internal tissues (e.g. small intestine, heart and liver) and/or thermogenic capacities (e.g. skeletal muscle and BAT activity) (Bozinovic et al., 1990; McDevitt and Speakman, 1994; Liknes and Swanson, 1996; Nespolo et al., 2002; Li and Wang, 2005; Zhao and Wang, 2005; Song and Wang, 2006). Hence, the absence of flexibility in BMR could be interpreted as an absence of changes at the organ and tissue level, such as increase in intestine mass and other metabolically active organs (Konarzewski and Diamond, 1995). However, this result is does not agree with a previous study, reporting adaptive phenotypic plasticity in the activity of intestinal enzymes and digestive organs as a response to diet acclimation (Cortes et al., 2011). It could be the case, however, that changes in tissue mass do not parallel detectable metabolic changes (Geluso and Hayes, 1999).

Energy saving-strategies represent another important response to overcome the energetic demands for thermoregulation in cold environments. Episodes of seasonally (i.e. hibernation) or daily torpor, in small mammals, constitute an important adaptation to endure periods of low temperature and/or food availability (Geiser, 2004; Heldmaier et al., 2004; Franco et al., 2012). Former studies showed that *D. gliroides* experiences both seasonal and daily torpor (Bozinovic et al., 2004; Nespolo et al., 2010; Franco et al., 2012). This strategy could represent up to 92% of energy savings. Also, our results show that cold exposition can significantly alter

the profundity and duration of torpor. In fact, in cold acclimated individuals, time spent in torpor increased by 330% in the cold (Fig. 2A). After one month of cold exposition, T_{skin} min during torpor was 13 °C lower in cold than warm-acclimated individuals (Fig. 2B). Our findings are in agreement with previous studies in marsupials, reporting an inverse relationship between T_A and bout length and torpor deepness (Fig. 2) (Rambaldini and Brigham, 2008; Stawski and Geiser, 2010).

Interestingly, we found that thermal acclimation had an effect on body temperature during inter-bout events, with lower values in cold-acclimated individuals. In contrast to placental mammals, marsupials presents larger short-term variation in T_b (Gemmell et al., 1997). Information available for *D. gliroides* shows that this species present daily range up to 10 °C and a low time-consistency (i.e. repeatability) of this trait (Cortes et al., 2009; Nespolo et al., 2010; Franco et al., 2012). Our values for daily range of T_b are in accordance with this result. The thermal amplitude experienced by *Dromiciops* was interpreted as the consequence of an imperfect control of T_b (Nespolo et al., 2010). However, this variability in regulation of T_b could represent an energetic advantage during cold acclimation, being important to reduce the energy requirements to stay active during inter-bout periods (Mortola and Lanthier, 2004; Vaanholt et al., 2007; Chi and Wang, 2011; Mortola, 2013). These results, however, must be taken with caution as we used skin temperature as proxy of body temperature, with non-constant ambient temperature. Although previous experiences with *D. gliroides* have shown that attached data loggers on bare skin are good proxies of internal temperature (Nespolo et al., 2010), it is well known that T_{skin} could fluctuate whereas core temperatures remains constant (Schmidt-Nielsen, 1995).

Arousal from torpor is usually accompanied by a rapid rewarming, which represents an important constraint on the net energy saved during torpor (Fons et al., 1997). The magnitudes of RR computed in this study are lower (0.10–0.25 °C min^{-1}, cold and warm acclimated-individuals, respectively; Fig. 3) than those expected for marsupials of the same size (Geiser and Baudinette, 1990). Our results showed that warm-acclimated individual rewarmed 39% faster than individuals acclimated to cold conditions. It has been established that lower RR will represent higher energetic requirements to arousal, which could be critical for animals unable to perform BAT-mediated NST (Geiser and Kenagy, 1988; Cooper and Withers, 2004). In fact, Oelkrug et al. (2012) reported differences in the efficiency of rewarming between wild type and UCP1-ablated mice. These authors found that compared to wild type, UCP1-ablated individuals spent more time

to arousal from torpor requiring an additional 60% of energy for the same increase in body temperature. A plausible explanation for these higher energetic requirements is an increase in heat loss to the environment (Utz et al., 2007). We did not consider the role of passive rewarming, then our results need to be taken with care as we could be overestimating the energetic cost of arousal (Lovegrove et al., 1999).

Summarizing, in this study we investigated the physiological flexibility in thermoregulatory capacities and the use of energy-saving strategies associated to thermal acclimation in the South American marsupial, *D. gliroides*. Our work shows: (1) low flexibility in their ability to adjust energy expenditure and insulation properties, (2) that NST is not present a thermogenic mechanism and (3) high regulation of torpor in *D. gliroides*. Taken together, these results suggest that torpor represents an efficient strategy to save energy, while modulation of thermoregulatory capacities is not a useful mechanism to avoid energetic restrictions of cold conditions in this marsupial species.

Acknowledgments

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