Aerobic power, huddling and the efficiency of torpor in the South American marsupial, *Dromiciops gliroides*

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Summary

During periods of cold, small endotherms depend on a continuous supply of food and energy to maintain euthermic body temperature (Tb), which can be challenging if food is limited. In these conditions, energy-saving strategies are critical to reduce the energetic requirements for survival. Mammals from temperate regions show a wide range of such strategies, including torpor and huddling. Here we provide a quantitative description of thermoregulatory capacities and energy-saving strategies in *Dromiciops gliroides*, a Microbiotherid marsupial inhabiting temperate rain forests. Unlike many mammals from temperate regions, preliminary studies have suggested that this species has low capacity for control and regulation of body temperature, but there is still an incomplete picture of its bioenergetics. In order to more fully understand the physiological capacities of this “living fossil”, we measured its scope of aerobic power and the interaction between huddling and torpor. Specifically, we evaluated: (1) the relation between basal (BMR) and maximum metabolic rate (MMR), and (2) the role of huddling on the characteristics of torpor at different temperatures. We found that BMR and MMR were above the expected values for marsupials and the factorial aerobic scope (from VCO₂) was 6.0±0.45 (using VCO₂) and 6.2±0.23 (using VO₂), an unusually low value for mammals. Also, repeatability of physiological variables was non-significant, as in previous studies, suggesting poor time-consistency of energy metabolism. Comparisons of energy expenditure and body temperature (using attached data-loggers) between grouped and isolated individuals showed that at 20°C both average resting metabolic rate and body temperature were higher in groups, essentially because animals remained non-torpid. At 10°C, however, all individuals became torpid and no differences were observed between grouped and isolated individuals. In summary, our study suggests that the main response of *Dromiciops gliroides* to low ambient temperature is reduced body temperature and torpor, irrespective of huddling. Low aerobic power and low time-consistency of most thermoregulatory traits of *Dromiciops gliroides* support the idea of poor thermoregulatory abilities in this species.

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Key words: Huddling, Maximum metabolic rate, Marsupials, Thermoregulation, Torpor

Introduction

Thermoregulation and energy expenditure are phenomena of paramount relevance for survival and reproduction. Whereas the way animals thermoregulate define their ecto- or endotherm condition (hence determining a great deal of their mode of life), energy expenditure is determinant for fitness because surplus energy in excess of maintenance costs can be allocated to offspring (Burton et al., 2011). Both processes affect ecological patterns such as abundance and distribution in space and time. Thus, thermoregulation and energy-saving strategies are critical in determining the energetic requirements for survival (Willmer et al., 2005). This is especially important for small endotherms in temperate regions, as they depend on a continuous supply of food to maintain high body temperature (t_b) (McNab, 1978; Boyer and Barnes, 1999; Geiser, 2011; Humphries et al., 2005). In many birds and mammals the capacity to tolerate cold conditions is strongly determined by structural attributes such as body mass and insulation, and on behavioral processes such as searching for refuges or thermoregulatory ‘shuttling’ (Sharbaugh, 2001; Bustamante et al., 2002; Boix-Hinzen and Lovegrove, 1998).

In addition to these individual responses, animals that live in groups can use another strategy to reduce rates of heat loss: huddling. Also known as social thermoregulation (Arnold, 1993; Jefimow et al., 2011), huddling represents up to 53% of energy saving during cold, both in birds and mammals (Gilbert et al., 2010). However, how huddling interacts with other energy saving strategies such as torpor (Frey, 1991; Namekata and Geiser, 2009; Jefimow et al., 2011) has been little studied. In placental mammals, such as Siberian hamsters (*Phodopus sungorus*), Alpine marmots (*Marmota marmota*) and four-striped grass mice (*Rhombomys pumilio*), huddling affects the length and depth of daily torpor (Arnold, 1988; Jefimow et al., 2011; Scantlebury et al., 2006). This is also true in marsupials (Namekata and Geiser, 2009; Frey, 1991) and birds (Wojciechowski et al., 2011), where huddling appears to increase energy savings by permitting reduced thermoregulatory heat production while maintaining a higher body temperature, which reduces risks of death.
A high capacity for aerobic power production is a key evolutionary innovation of endotherms, which is related with the capacity of sustained metabolic work. High aerobic power output is essential for intense, sustained activities such as endurance locomotion, prey search, predator escape and foraging. Maximal aerobic power is mostly determined by the maximum rate of oxygen consumption or CO$_2$ production (MMR), a variable that was defined as “the best single measure of aerobic capacity” (Garland and Bennett, 1990). Hence, MMR is very informative when limits to energy expenditure are characterized. A standard index of flexibility in aerobic metabolism is Factorial Aerobic Scope (FAS), the ratio between MMR and BMR (basal metabolic rate). Factorial aerobic scope in marsupials typically ranges from 5 to 10 (Hinds et al., 1993). Mammalian body temperature and BMR are the lowest in monotremes, intermediate in marsupials and maximum in eutherians (Polymeropoulos et al., 2012), and FAS is maximum in marsupials (Hinds et al., 1993).

Heterothermic physiological strategies, such as daily and seasonal torpor, are important phenomena aiding in energy savings in endotherms. These are dramatic reductions in body temperature, which affects essentially all biological functions, producing hypometabolism, over periods from a few hours to months (Turbill et al., 2011; for a review see Geiser, 2004; Heldmaier et al., 2004). Since energy expenditure is severely reduced during torpor, it has been estimated that animals save up to 80% (Geiser, 2011; Geiser and Turbill, 2009) of energy costs compared to remaining euthermic, which obviously has an considerable impact on energy budgets (Kenagy, 1989; Kenagy et al., 1989), and presumably on fitness (Turbill et al., 2011). Numerous studies have characterized in detail the mechanistic basis of torpor and hibernation, and its ecological and evolutionary consequences in a wide variety of species (Melvin and Andrews, 2009; Geiser, 2008; Heldmaier et al., 2004; Carey et al., 2003; and references therein). However, there are few data on whether other energy saving strategies such as huddling, interact with torpor in reducing energy expenditure.

Within the mammalian lineage, marsupials diverged from eutherians about 150 million years ago (Bininda-Emonds et al., 2007; Nilsson et al., 2010). They apparently originated in Asia, dispersed to the Americas and colonized Australia, passing through Antarctica during the Cretaceous, 140 million years ago (Luo et al., 2003). After the last formation of the Panama isthmus, the great American interchange apparently caused the extinction of several marsupial orders in South America (Apesteguia and Ares, 2010). Dromiciops gliroides, the “Monito del Monte”, is the sole living representative of one of these orders (Microbiotheria). D. gliroides is a small, omnivorous marsupial strongly associated with temperate rain forests of southern Chile and Argentina. It lives in trees, hibernates in holes either solitarily or in groups, and consumes fruits, insects and vegetable material (Franco et al., 2011). In addition to being a “living fossil”, D. gliroides represents the missing link between Australian and American marsupial fauna (Palma and Spotorno, 1999). Moreover D. gliroides is the sole South American mammal known to exhibit seasonal torpor or hibernation (Bozinovic et al., 2004). Because of these reasons, D. gliroides has attracted the interest of many researchers. Its frugivorous habits make it an important disperser of endemic species of vines and trees (Amico and Aizen, 2000). Its diet shifts seasonally to insects in relation to food availability, and it exhibits exceedingly high physiological plasticity in nutrient processing capacities (Cortés et al., 2011).

Although these studies described several aspects of the thermoregulatory physiology of D. gliroides, there is still an incomplete picture of its basic bioenergetics, such as time-consistency of basal metabolic rate and maximum capacities for aerobic power production, and the interaction of these traits with energy saving strategies such as torpor. A preliminary survey of inter-individual variation of several physiological capacities of this species found very low repeatability for energy metabolism and body temperature (Cortés et al., 2009). With some exceptions (Russell and Chappell, 2007; Dohm et al., 2001), repeatability studies have shown that physiological traits derived from respirometric records have high inter-individual consistency (Labocha et al., 2004; Chappell et al., 1995; Nespolo and Franco, 2007), thus making the low repeatability in D. gliroides an unexpected result. Several methodological problems could generate low repeatability, such as bias and error in the respirometry technique. Moreover, previous studies (e.g., Cortés et al., 2009) did not measure “true” BMR, which strengthens the possibility that residual error due to feeding, or growth affected repeatability estimations. Alternatively, this species could be exhibiting natural within-individual variation in physiological parameters. Here we address two additional aspects of the bioenergetics of D. gliroides, aerobic capacity (i.e., BMR, MMR and FAS), the role of huddling in energy savings during torpor, and confirm its low inter-individual variation. Intuitively, and according to studies in other species, huddling should increase the energy savings of torpor. However, physiological characteristics of D. gliroides have suggested that body temperature (and torpor) in this species do not appear to be modulated by environmental cues other than ambient temperature, making this expectation uncertain.

Materials and Methods

Animals

Twenty adult individuals of D. gliroides were captured near Valdivia, Chile (39°48’S, 73°14’W; 9 m) during the austral summer in December–January 2009, using Tomahawk-style live traps constructed with wire-mesh, with a single door (30×10×10 cm, local manufacture). Traps were placed in trees and shrubs 1–2 m above ground and baited with bananas. Traps were checked daily at dawn, and captured individuals were transported to the laboratory on the day of capture. The animals were housed in plastic cages (45×30×20 cm) with 2 cm of bedding and nests of moss and cardboard tubes. Cages were maintained in a climate controlled chamber at 20±1°C (standard error), with a 12:12 hour photoperiod for two weeks. Water and food (a mixture of peach compote, strawberry baby food and mealworms) were available ad libitum. Procedures associated with capture and animal handling were performed according guidelines recommended by the American Society of Mammalogists (Gannon and Sikes, 2007) and were approved and authorized by the Chilean Agriculture and Livestock Bureau (Servicio Agrícola y Ganadero).

Respirometry measurements

Each individual was fasted for 12 hours before respirometry trials and each trial lasted 3 hours. All measurements were performed with a respirometry system consisting of a Li-Cor 6262 CO$_2$ analyzer (LiCor, USA). The CO$_2$ analyzer was calibrated periodically against a known gas sample of 107 ppm for CO$_2$. For some tests we simultaneously measured oxygen consumption (VO$_2$) using an Oxzilla analyzer (Sable Systems, Henderson, Nevada, USA). We used cylindrical metabolic chambers of 1000 ml and a flow rate of 1000±10 ml min$^{-1}$ regulated by a Sierra mass-flow controller (Sierra Instruments, USA), located upstream of the metabolic chamber. Incurrent air was passed through two columns of H$_2$O and CO$_2$ scrubbers (Drierite and Soda lime, respectively). The metabolic chamber was located in an incubator, and ambient temperature ($T_a$) was continuously recorded by a Cole Parmer (USA) thermocouple located inside the incubator. Dry, CO$_2$-free air from the mass flowmeter passed through the metabolic chamber and then through a Gast (Gast Manufacturing, USA) pump (i.e. negative pressure). After that, a subsampler (Intelligent Subsampler, Sable Systems, Las Vegas, NV, USA) injected 200 ml min$^{-1}$ of excruent air into the LiCor 6262. If VO$_2$ was being measured, the sample air then passed through a small column of desiccant and then to the Oxzilla analyzer. No animal urinated or defecated within the chamber during the trials. Data-acquisition was carried out with the
Expe-Data (Sable Systems), set for an averaging sampling rate of one sample per second. From the respirometric records and according to the configuration of the system (i.e. flowmeter was upstream from the chamber, both CO2 and water were scrubbed, and use of flow-mass controllers), we computed the following variables (Withers, 1977):

- Rate of CO2 production ($\dot{V}V_{\text{CO2}}$), as:

$$\dot{V}V_{\text{CO2}} = (\text{FeCO2} - \text{FiCO2}) / \text{FR}$$

Where $\dot{V}V_{\text{CO2}}$ is expressed in terms of ml CO2 min$^{-1}$; FiCO2 is the input fractional concentration of CO2; FeCO2 is the excurrent fractional concentration of CO2; FR is the flow rate (ml min$^{-1}$).

The fractional concentration of CO2 was corrected before calculation for water dilution as:

$$\text{UCO2} = \text{BP} - \text{WVP}$$

Where UCO2 is the uncorrected CO2 signal; BP is the barometric pressure (kPa); and WVP is the water vapor partial pressure (kPa; obtained from the Oxzilla).

Oxygen consumption was calculated as:

$$\dot{V}O_2 = \text{FR} \times (\text{FiO2} - \text{FeO2}) / (1 - \text{FeO2} \times (1 - \text{RQ}))$$

Where FiO2 and FeO2 are initial and final oxygen concentrations and RQ is the respiratory quotient (assumed to $\approx 0.85$). Use of this constant RQ introduces a maximum error of 3% in $\dot{V}O_2$ across the expected physiological range of RQ.

Basal Metabolic Rate (BMR) and Maximum Metabolic Rate (MMR)

BMR and MMR were determined according to protocol described above. The complete BMR trials lasted 3 hours. We used a test temperature (30°C) within thermoneutrality (Bozinovic et al., 2004) to elicit BMR. To calculate BMR, we computed the average of the lowest steady-state value that was maintained for a minimum of 30 minutes of recording. To estimate MMR, forced exercise $\dot{V}V_{\text{CO2max}}$ (and in some cases, $\dot{V}V_{\text{O2max}}$) was obtained by running D. gliroides in a cylindrical metabolic chamber (running wheel) of 1000 ml, which was rotated for 15 minutes to provide sufficient time to elicit MMR. This trial was performed at 20°C, the wheel speed was low at the start of the test and then increased every 30 seconds until the animal exhausted. All the individuals measured showed behavioral signs of exhaustion at the end of exercise (loss of coordination, failure to maintain speed, stable or declining metabolic rate despite speed increases) but none were injured. To be sure those individuals attained MMR; we finished each record when the decline in metabolic rate was evident (usually after 10–15 minutes of measurement). For $\dot{V}O_2$ measurements, we applied the “instantaneous” correction (Bartholomew et al., 1981) to account for mixing characteristics of the chamber.

Huddling experiments

Fifteen adult individuals of D. gliroides were used for the huddling experiment. Animals were maintained as described above. We identified animals as active when they were resting or awake and responded to handling. In contrast, animals in torpor were lethargic and did not respond to handling. The system configuration is described above. To estimate the effect of huddling on energy saving in D. gliroides,
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Body mass 67.5501 39.0803 0.63, F 13,27

Body temperature 0.0618 1.65865 0.11, F 10,23

higher values in grouped individuals in both variables (Figs 3 and 5).

we performed two treatments: grouped individuals (3 individuals per group) and single individuals and the response variables were metabolic rate (at a per gram basis) and body temperature. We repeated each trial five times (n=5), randomizing individuals in each subsequent measurement. We tried to maximize the number of combinations of individuals. Body temperature was obtained from data loggers (iButtons, DS1922L-F5, Dallas Maxim Integrated Products, UK, 3 g, 17 mm diameter, 6 mm thick), these devices were synchronized, programmed (resolution ± 0.5˚C; temperature measured every 10 minutes; total recording 16 hours) and attached with masking tape around the body, on the abdomen of the animals. No animal resulted injured with such procedure, which was easier and less invasive than abdominal implants (Nespolo et al., 2010) without significant reductions in precision (Bozinovic et al., 2007). This variable has been widely studied in placental mammals, mainly rodents (Hayes, 1989; Buck and Barnes, 2000; Rezende et al., 2004; Weibel et al., 2004; Chappell et al., 2004; Ochocinski and Taylor, 2005; Rezende et al., 2005; Gebczyński and Konarzewski, 2009). Our study provides the first detailed data on MMR in D. gliroides. Marsupials are known as having comparatively high MMR and low basal metabolic rate (BMR), which makes their factorial aerobic scope (FAS) unusually large.

Discussion
Thermoregulatory heat production is a large part of daily energy expenditures in many small endotherms (Tubill et al., 2011). They may also be confronted with seasonal changes in temperature and food availability (Körntner and Geiser, 1998), which create energy demands for thermoregulation that become prohibitively high.

Maximal aerobic power, measured as the maximal rate of oxygen consumption or CO₂ production (MMR), represents one of the most important factors that influence endurance capacity. This variable has been widely studied in placental mammals, mainly rodents (Hayes, 1989; Buck and Barnes, 2000; Rezende et al., 2004; Weibel et al., 2004; Chappell et al., 2004; Ochocinski and Taylor, 2005; Rezende et al., 2005; Gebczyński and Konarzewski, 2009). Our study provides the first detailed data on MMR in D. gliroides. Marsupials are known as having comparatively high MMR and low basal metabolic rate (BMR), which makes their factorial aerobic scope (FAS) unusually large (Hinds and MacMillen, 1984; Hinds et al., 1993). Although comparison of our results with previous reports of BMR in D. gliroides is complicated by the fact that different techniques were used, volumes of respiratory gases can be converted into power units using respiratory quotient and the appropriate conversion factor for different nutrients, as reported in Walsberg and Wolf (Walsberg and Wolf, 1995). Using that value, the reported resting

Table 1. Descriptive statistics for metabolic traits of D. gliroides (data from the first measurement). Basal Metabolic Rate (BMR; as the rate of CO₂ production and O₂ consumption), Body Mass at the moment of BMR measurement (Mb₁) Maximum Metabolic Rate (MMR; as the rate of CO₂ production and O₂ consumption), Body Mass at the moment of MMR measurement (Mb₂), Factorial Aerobic Scope (FAS) from the rate of CO₂ production and O₂ consumption, respiratory quotient from BMR measurements (RQBMR) and also from MMR measurements (RQMMR).

| Trait              | N  | Mean   | Min.  | Max.  | s.e.  | c.v.   |
|--------------------|----|--------|-------|-------|-------|--------|
| BMR (ml CO₂ min⁻¹) | 10 | 0.721  | 0.55  | 1.08  | 0.05  | 21%    |
| BMR (ml O₂ min⁻¹)  | 10 | 0.809  | 0.40  | 1.35  | 0.08  | 31%    |
| Mb₁ (g)            | 14 | 22.3   | 17.6  | 31.9  | 1.70  | 23%    |
| Mb₂ (g)            | 14 | 4.49   | 2.60  | 7.38  | 0.35  | 29%    |
| MMR (ml CO₂ min⁻¹) | 14 | 4.90   | 3.31  | 7.29  | 0.35  | 27%    |
| MMR (ml O₂ min⁻¹)  | 14 | 24.6   | 17.0  | 33.8  | 1.72  | 26%    |
| FAS (CO₂)          | 14 | 6.02   | 4.55  | 7.1   | 0.23  | 12%    |
| FAS (O₂)           | 14 | 6.18   | 4.93  | 9.54  | 0.45  | 23%    |
| RQMMR              | 10 | 0.93   | 0.79  | 1.4   | 0.05  | 19%    |
| RQBMR              | 14 | 0.91   | 0.73  | 1.1   | 0.03  | 11%    |

Table 2. Repeatability of metabolic variables (intraclass correlation coefficient, τ), computed as the ratio between inter-individual variance and total variance from one-way ANOVA.

| Variable         | Between-individual variance | Within-individual variance | τ   |
|------------------|-----------------------------|---------------------------|-----|
| MMR              | 0.01506                     | 0.07863                   | 0.16, F₁₅,₂₉=1.53, P<0.05 |
| Body temperature | 0.0618                      | 1.65865                   | 0.11, F₁₀,₂₃=1.38, P=0.24 |
| Body mass        | 67.5501                     | 39.0803                   | 0.63, F₁₃,₂₇=6.04, P>0.01 |
metabolism of *D. gliroides* varies from 4.3 miliwatts/g in a closed, manometric system at Ta = 30˚C (Bozinovic et al., 2004), to 9.5 miliwatts/g using open-flow, CO2 records at 20˚C (Nespolo et al., 2010). On the other hand, Withers et al. reported 7.8 miliwatts/g at 30˚C (Withers et al., 2012). Our estimated BMR was 12.1 miliwatts/g (95% confidence interval: 11.98–12.97; from our sample of CO2 production) and 10.9 miliwatts/g (95% confidence interval: 10.7–11.0; from our sample of O2 consumption), falling well above the predicted values by mass (7.96 miliwatts/g) (Hinds et al., 1993; Cooper and Withers, 2006). Applying similar considerations, our calculated MMR is 75.2 miliwatts/g (95% confidence interval: 74.6–75.8 miliwatts/g; from our sample of CO2 consumption), which is 112% of the expected value for marsupials, and 82.1 miliwatts/g (95% confidence interval: 81.5–82.8 miliwatts/g; from our sample of O2 consumption), which is 122% of the expected value for marsupials (Hinds et al., 1993).

Hence, a combination of comparatively high BMR and MMR, statistically not different to the expectation, produced in *D. gliroides* an unusually low FAS (~6.2; close to reptiles) (Hinds et al., 1993) – to the best of our knowledge, the lowest known FAS in a mammal (Hinds et al., 1993; Willmer et al., 2005). This result, however, need further confirmation as our BMR measurements could be overestimated because of record duration. Since the values that we obtained either from CO2 and O2 measurements gave similar FAS values, the only way this could have happened is because of too short metabolic trials (three hours). However, we imitated previous studies where typical duration of BMR records was 2–3 hours of duration (e.g., McNab, 2000; Westman et al., 2002; Polymeropoulos et al., 2012).

Contrary to the general trend for whole-animal aerobic metabolism (Nespolo and Franco, 2007), Cortés et al. suggested that *D. gliroides* presents low repeatability and time-consistency in several thermoregulatory traits (Cortés et al., 2009). Low repeatability may result from measurement problems (low sample size, high sampling error), or low-consistency of physiological capacities. Further studies also showed that this species shows remarkable variation in ‘normothermic’ body temperature (ca. 10˚C) (Nespolo et al., 2010; Withers et al., 2012). In the present study we used different techniques and measured different physiological variables (i.e., BMR and MMR), and again found that the repeatability of energy metabolism in *D. gliroides* was not significant. We also found that body temperature presented low repeatability and was highly variable even in a single measurement. It should be noticed that the definition of BMR assumes that body temperature is homogeneous across measurements. Therefore, variation in Tb could be affecting the consistency in BMR, making this measure unrepeatable. Whereas this fact could question this repeatability estimation of BMR, it strengthens the conclusion that this species show low inter-individual variation in thermoregulatory traits. Further studies are certainly needed to confirm this conclusion and to explore the time-consistency of BMR with longer measurement times, larger sample sizes and perhaps controlling BMR by changes in Q10 due to Tb variations.
Our results also suggest that *D. gliroides* maintains a small differential between rates of heat production and loss, entering in torpor easily in the predominating temperatures of its temperate forest habitat. In addition, Withers et al. studied the water economy, reporting that this species does not differ from other marsupials (Withers et al., 2012). During torpor, *D. gliroides* exhibits negative water balance, which would explain periodical arousals during seasonal torpor or hibernation (Withers et al., 2012; Nespolo et al., 2010; Jefimow et al., 2011; Wojciechowski et al., 2011).

To overcome energetic constraints, many small mammals have physiological and behavioral energy-saving strategies (Bozinovic and Merritt, 1991). In *D. gliroides* huddling appears not to interact with the initiation or characteristics of torpor, as it does not affect its deepness or duration. Hence, our results suggest that ambient temperature is the main criterion for torpor induction in *D. gliroides*, with a secondary effect of food availability (Nespolo et al., 2010; Bozinovic et al., 2007).

Huddling has been reported as a mechanism to save energy in small eutherian mammals such as Darwin’s leaf-eared mouse (*Phyllotis darwini*) (Bustamante et al., 2002), the Alpine marmot (*Marmota marmota*) (Arnold, 1988), Abert’s squirrel (*Sciurus aberti*) (Edelman and Koprowski, 2007), the striped mouse (*Rhabdomys pumilio*) (Schradin et al., 2006), the Indiana bat (*Myotis sodalis*) (Boyles et al., 2008), the Cape ground squirrel (*Xerus inauris*) (Wilson et al., 2010), and the neotropical bat (*Noctilio albiventris*) (Roverud and Chappell, 1991). Such behavior is also observed among some Australian marsupials, such as brush-tailed phascogales (*Petaurus breviceps*), whose origin is *Dromiciops gliroides* (Boyer, B. B. and Barnes, B. M. (1999). Molecular and metabolic aspects of mammalian hibernation. *BioScience* 49, 713-724).

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