

Adjusting energy expenditures to energy supply: food availability regulates torpor use and organ size in the Chilean mouse-opossum *Thylamys elegans*

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Abstract We studied how food abundance and consumption regulates torpor use and internal organ size in the Chilean mouse-opossum *Thylamys elegans* (Dielphidae), a small nocturnal marsupial, endemic in southern South America. We predicted that exposure to food rations at or above the minimum energy levels necessary for maintenance would not lead to any signs of torpor, while reducing food supply to energy levels below maintenance would lead to marked increases in frequency, duration and depth of torpor bouts. We also analyzed the relationship between food availability and internal organ mass. We predicted a positive relationship between food availability and internal organ size once the effect of body size is removed. Animals were randomly assigned to one of two groups and fed either 70, 100 or 130% of their daily energy requirement (DER). We found a positive and significant correlation between %DER and body temperature, and also between %DER and minimum body temperature. In contrast, for torpor frequency, duration and depth, we found a significant negative correlation with %DER. Finally, we found a significant positive correlation between the %DER and small

intestine and ceacum dry mass. We demonstrate that when food availability is limited, *T. elegans* has the capacity to reduce their maintenance cost by two different mechanisms, that is, increasing the use of torpor and reducing organ mass.

Keywords Torpor frequency · Body temperature · Food abundance · Energy requirements · Marsupials

Abbreviations

DER	Daily energy requirement
T_b	Mean body temperature
T_{bmin}	Minimum daily body temperature
Torpor _{freq}	Mean number of torpor bout per day
$T_{btorpor}$	Mean temperature during torpor events
T_s	Skin surface temperature

Introduction

The myriad of process and mechanisms by which animals regulate their energy budgets in face of spatio-temporal changes in environmental conditions gives a striking example of the importance of physiological flexibility (McNab 2002). An integrative approach is usually required to fully understand the physiological limitations behind patterns of energy use and regulation, and their consequences on organism performance. One diverse physiological mechanism widely employed by all major animal phyla to regulate their energy budgets is physiological dormancy (i.e. metabolic depression; Guppy and Withers 1999). In small endotherms, metabolic depression associated with deep

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(hibernation) and shallow (daily) torpor is a well-studied example (Geiser 2004; Bozinovic et al. 2004). Notwithstanding differences in the underlying mechanism responsible for, and the magnitude of, the metabolic depression, the energy savings accrued during torpor is hypothesized to increase survival under harsh environmental conditions (Geiser 1994, 2004). Furthermore, if animals activate the energy-saving mechanisms in advance (predictive response) or as a reactive response, the environmental cues triggering daily, shallow torpor seem to be a combination of three factors: reduced food availability, decreased ambient temperature and shortened photoperiod (Geiser 1986; 1988a, b, 1994; López-Calleja et al. 1997; Mzilikazi and Lovegrove 2004; Lovegrove et al. 1999).

Torpor physiology is generally similar among therian mammals (Hallam and Dawson 1993; Chappell and Dawson 1994; Gibson and Hume 2000; Holloway and Geiser 2001) although important differences are evident. For example, Cryan and Wolf (2003) demonstrated that during reproduction, female heterothermic bats defend euthermia, presumably to expedite embryonic growth, while males use torpor, presumably to minimize energy and water deficits. In general, two major patterns are recognized in regard to the relationship between torpor and food availability. Some species readily enter daily torpor when food is withheld or when it is restricted to levels below the minimum, necessary for maintenance (facultative torpor—McNab 1983). On the other hand, some species display torpor even when food is provided ad libitum (obligate torpor—McNab 1983). Facultative torpor, thus, is a flexible and opportunistic response to unpredictable environmental conditions. We study the physiological responses of the Chilean mouse-opossum (*Thylamys elegans* -Didelphidae) to food availability. Like some eutherian species, *T. elegans* has been suggested to make use of facultative torpor in order to adjust its energy expenditure when food availability or quality decreases (Sabat et al. 1995; Bozinovic and Méndez 1997; Bozinovic et al. 2005), but it is unknown how torpor is regulated by food availability. Given this information, and the suggestion that the amount of energy saved during torpor is dependent not only on the level of metabolic energy expenditure, but also on specific adjustments of torpor patterns (Holloway and Geiser 1995), we analyzed how the frequency, depth and duration of torpor bouts varied as a function of food availability. Overall, we predicted that exposure to food rations at or above the minimum, necessary for maintenance would not lead to any sign of torpor (increase in energy demand matched by food intake), while reducing food supply to levels below maintenance

would lead to marked increases in frequency, duration and/or depth of torpor bouts.

On the other hand, changes in internal organ mass comprise another way by which maintenance costs, and thus overall energy expenditures, can be adjusted to environmental conditions (Weiner 1992; Piersma and Lindstrom 1997; Starck 1999). In this sense, decreasing the mass of metabolically expensive organs when functional demand for them is low is considered an adaptive response that allows animals to maintain a positive energy budget during periods of low food availability (Karasov and Diamond 1983; Secor et al. 1994; Carey 2005; Naya and Bozinovic 2006). Consequently, the second objective of this work was to analyze the relationship between food availability and organ mass. Specifically, we predict a positive relationship between food availability and organ size, once the effect of body size is removed.

Material and methods

Animal collection

Mouse opossums were captured in central Chile (33°23'S, 70°31'W) with Sherman traps during the autumn-winters of 2005 ($n = 7$) and 2006 ($n = 6$). The vegetation at the study site is sclerophyllous, locally known as matorral. The climate is Mediterranean, with most precipitation and lowest temperatures concentrated from June to August, the austral winter. Precipitation is minimal and temperatures are highest from December to March, corresponding to the austral summer. The broadest daily range (from mean maximum to mean minimum) of air temperature is observed between December and February (16–17°C), and the narrowest between May and August (9–10°C).

Animal husbandry and experimental design

After capture, animals were brought to the laboratory, where they were individually maintained in wire cages. Each cage contained a food dispenser and shelter, provided by cardboard tubes filled with cotton. Animals were acclimated for one week after capture to prevailing natural conditions of temperature (12–18°C) and photoperiod, and feed ad libitum with commercial cat-food (Whiskas, Waltham®, UK). Before the onset of the experiments, a miniature (3 g) temperature data-logger (I-Buttons model DS1921L, Dallas Semiconductor, USA) was attached to the back of each animal (see Brown and Downs 2006). This was done by

removing a patch of fur and gently fixing the data-logger with super-bond glue. Data-loggers collected skin temperature once every 17 min, the shortest interval considering storage limitations for the three-week duration of the experiments. Skin temperatures were converted to internal (body) temperatures using empirically derived relationships between the two (see below).

The cages were then transferred to a temperature and photoperiod-controlled cabinet. Temperature was set at 12°C, which is the mean environmental temperature during winter months in the Mediterranean habitats of central Chile (www.meteochile.cl), and photoperiod to 12 h light:12 h dark cycle. Animals were randomly assigned to one of two groups. In the first group (hereafter referred to as Group 1; $n = 7$; four individuals collected during 2005 and three during 2006), animals received food, comprising 130% of their daily energy requirement (DER) for 1 week. Then, their rations were reduced to 100% of DER for 1 week, and, finally rations were reduced to 70% of DER during the last week. For the other group (hereafter referred to a Group 2; $n = 6$; three individuals collected during 2005 and three during 2006), we simply shifted the order of the initial and final food ration (first week 70% DER and third week 130% DER). The commercial cat food (Whiskas cat-food, Waltham®) used throughout the experiments consisted of 85% water, 8% crude protein, 2% lipids and < 1.5% crude fibers. To calculate the exact amount of food to be given during a certain phase we used the following equation: $\text{Food ration}(\text{g day}^{-1}) = \frac{\text{DEE}(\text{kJ day}^{-1})}{0.85 \times 3.4(\text{kJ g}^{-1})}$, where daily energy expenditure (DEE) was calculated according to the allometric equation for marsupials provided by Nagy (2001), $\text{DEE}(\text{kJ day}^{-1}) = 10.1 \times \text{g}^{0.59}$, 0.85 is the digestive assimilation efficiency for *T. elegans* feeding on meat (Sabat et al. 1995), and 3.45 is the energy content (in kJ g^{-1} wet basis) of the food.

Body mass was measured at the beginning of the experiment and at the end of each phase (1 week), and the new amount of the food to be given calculated accordingly. Food ration provided varied with the body mass of the animal. Consequently, we adjusted the food ration based on the treatment group (70, 100, and 130%). Animals were fed at the same time every day (ca. 17:00 h) and for the amount of food left over the following morning was also at the same time (9:00 h). Food intake was calculated as the daily difference between food given and left over, after correcting for evaporation in the latter. These daily values were transformed into energy equivalents (kJ day^{-1}) by

multiplying the amount eaten by the caloric content of the diet.

During these transitional periods between feedings, we also measured colonic body temperatures (T_b) of the mouse opossums by inserting the tip of type-T thermocouple ca 2 cm into the cloaca. By matching the reading of the thermocouple with the reading of the data-logger, we were able to construct a regression equation to estimate (T_b) from surface temperature or skin surface temperature (T_s), for all animals during the whole experiment ($T_b = -3.65 + 1.22 T_s$; $n = 15$; T_b min and max = 13.1 and 35.4°C, respectively; T_s min and max = 13.0 and 30.0°C, respectively; $r^2 = 0.96$; $P < 0.0001$).

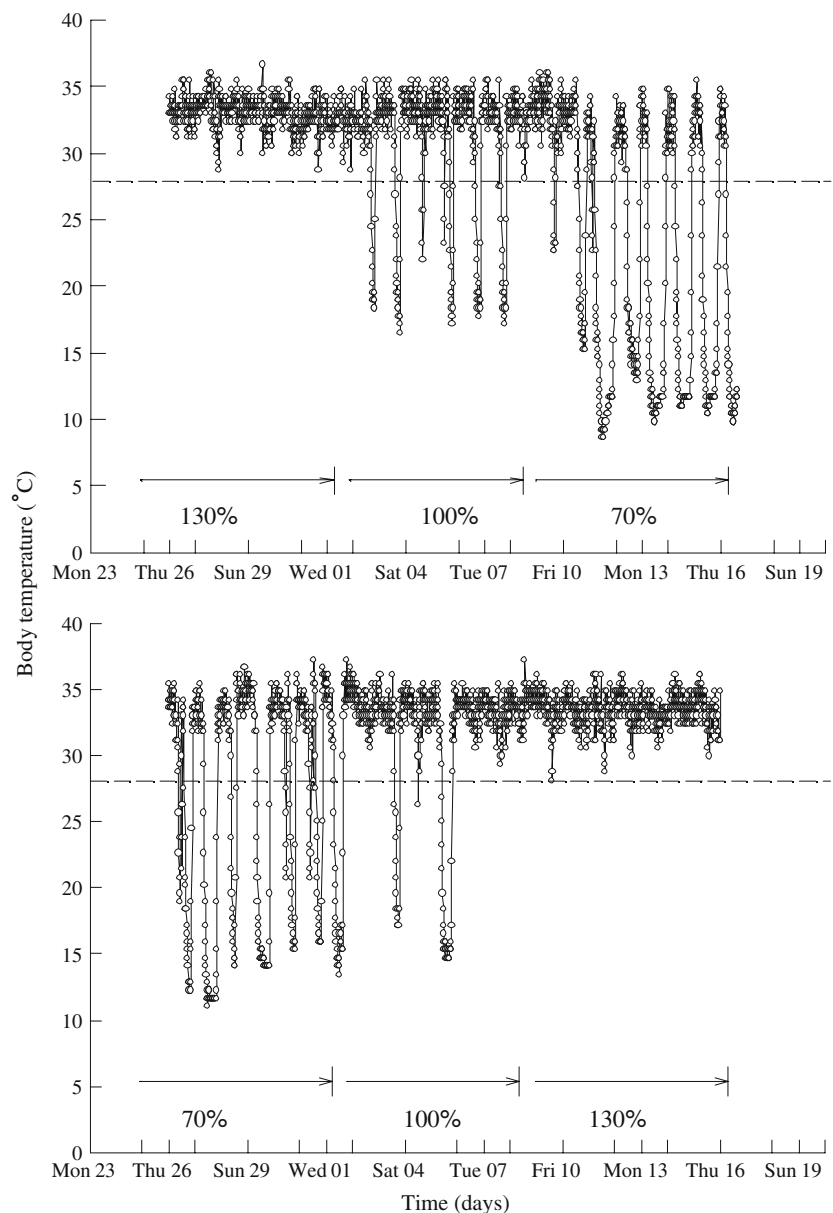
Organs measurements

All animals were killed at the end of the experiments. Consequently, we had seven animals (Group 1) after 1 week of being offered 130% of their daily ration, while the other six animals (Group 2) were killed after 1 week of being offered 70% of their daily ration. Wet and dry weights (after oven dried for 4 days at 60°C) of the following organs were measured: stomach, large and small intestine, ceacum, liver, spleen, lungs, kidneys, heart and the remaining carcass. We also measured the length and width of the small and large intestines, and length of stomach and ceacum. It should be noted that one-week is a long enough period to attain changes in the sizes of internal organs in response to changes in environmental conditions (see Naya et al. 2005).

Data analysis and statistics

We considered the Chilean-mouse opossum to be torpid when T_b decreased below 28°C. This value corresponds to the maximum T_b value observed by Geiser (1994) for torpid marsupials and was slightly lower (< 1°C) than the minimum T_b observed for animals kept with 130% of DER (see Fig. 1). The following variables were estimated for each animal during each of the three experimental phases: (a) Percentage of daily energy expenditure requirements consumed (%DER = $100 \times \text{food intake per DER}$), (b) mean body temperature (T_b , in °C), (c) minimum daily body temperature ($T_{b\text{min}}$, in °C), (d) Torpor frequency ($\text{Torpor}_{\text{freq}} = \text{mean number of torpor bout per day}$), (e) Torpor duration (mean time per day for which $T_b < 28^\circ\text{C}$, in min), and (f) Torpor depth (mean temperature during torpor events, $T_{b\text{torpor}}$, in °C). Correlations between %DER and torpor descriptors were evaluated for each group separately through a Pearson

Fig. 1 Time series of body temperature for a representative animal belonging from Group 1 (*upper panel*) and group 2 (*lower panel*). Note the relationship between the number and depth of torpor bouts and food offered as a percentage of predicted daily energy requirements (duration of food treatment shown by *arrows*)



product moment correlation. Differences in body mass between groups were evaluated by a RM-ANOVA, while differences between initial and final body mass, within groups, were evaluated separately by a *t*-test for dependent samples. The effects of the of food consumption on internal organs sizes were evaluated through Pearson product moment correlations between the %DER for the last week of the experiment and the organ dry mass. To remove the effect of body size, the residuals of a linear regression between each organ mass and the carcass dry mass were used in these correlations. Statistical significance was established at the 0.05 level. All the analyses were performed using the statistical package, STATISTICA® (2001) Version 6.0 for the Windows® operating system.

Results

The Chilean mouse-opossum did not show changes in body mass with respect to the different treatments. Indeed, no differences were observed in body mass between groups (Wilks $\lambda = 0.48$, $F_{4,8} = 2.13$, $P = 0.17$), or within groups ($t_6 = 0.14$, $P = 0.89$ for Group 1; $t_5 = 1.08$, $P = 0.33$ for Group 2; Fig. 2). Since the correlation between %DER and torpor descriptors did not differ between groups, we only report the results for pooled data. Positive and significant correlations were found between %DER and body temperature and between %DER and minimum body temperature (Fig. 3). In contrast, negative and significant correlations were found between %DER with torpor

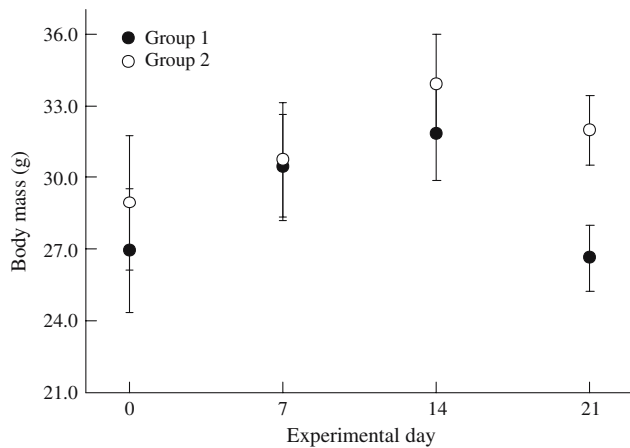


Fig. 2 Mean body mass of experimental groups over treatment period. Group 1: For 1 week animals received were fed 130% of their daily energy requirement (*DER*), then for 1 week at 100% *DER* and, finally, for 1 week receiving 70% *DER*. Group 2: Same as Group 1, except first week was 70% *DER* and third week was 130% *DER*

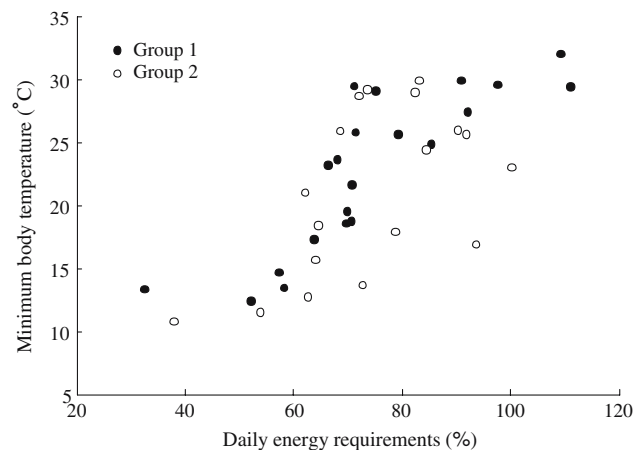


Fig. 3 a Relationship between body temperature and %*DER* [$T_b = 20.028(\pm 0.290) + 0.135(\pm 0.004) \%DER$, $t_{37} = 32.33$, $P = 0.0001$, $r^2 = 0.47$], and **b** between minimum body temperature and %*DER* [$T_{bmin} = 2.197(\pm 0.524) + 0.267(\pm 0.007) \%DER$, $t_{37} = 38.68$, $P = 0.0001$, $r^2 = 0.51$]. Errors are ± 1 SEM. T_b is the body temperature

frequency, duration and depth (Fig. 4). Finally, we found positive and significant correlations between the %*DER* and small intestine and ceacum dry mass, and a positive, but marginally significant correlation between the %*DER* and liver dry mass (Fig. 5).

Discussion

On an evolutionary and ecological timescale, it has been hypothesized that torpor use allows animals to regulate their energy budgets under different envi-

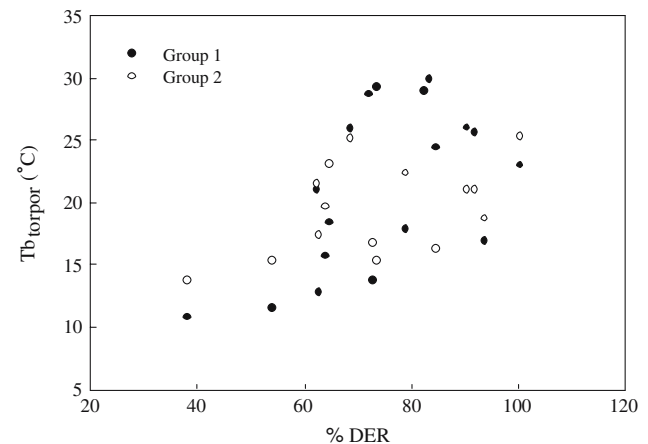
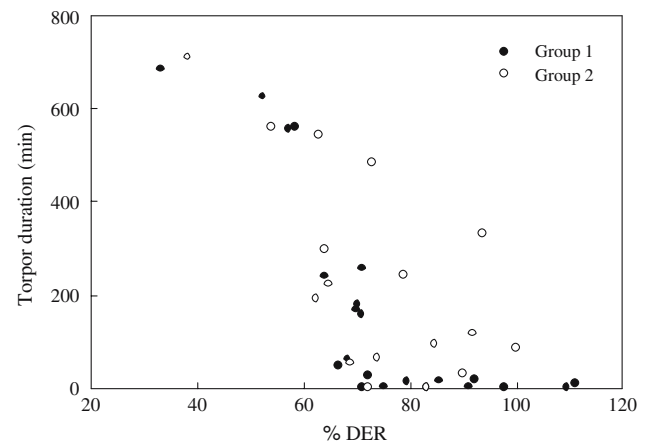
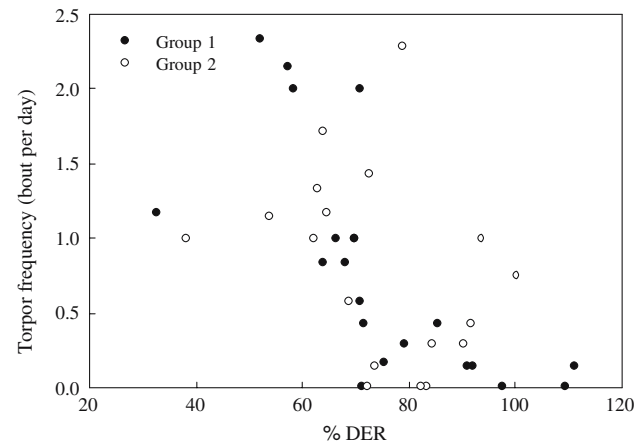


Fig. 4 a Relationship between torpor frequency and %*DER* [$Torpor_{freq} = 2.504(\pm 0.069) - 0.023(\pm 0.001) \%DER$, $t_{37} = 16.71$, $P = 0.0002$, $r^2 = 0.31$], **b** between torpor duration and %*DER* [$Torpor_{duration} = 909.50(\pm 18.267) - 9.58(\pm 0.239) \%DER$, $t_{37} = 41.09$, $P = 0.0001$, $r^2 = 0.53$], and **c** between torpor deep and %*DER* [$Torpor_{deep} = 13.645(\pm 0.352) - 0.096(\pm 0.005) \%DER$, $t_{37} = 10.59$, $P = 0.003$, $r^2 = 0.25$]. Errors are ± 1 SEM. Abbreviation as in Fig. 3

ronmental settings. In this vein, Geiser (2004) reviewed the patterns of metabolism and body temperature during torpor and hibernation in endotherms. After a

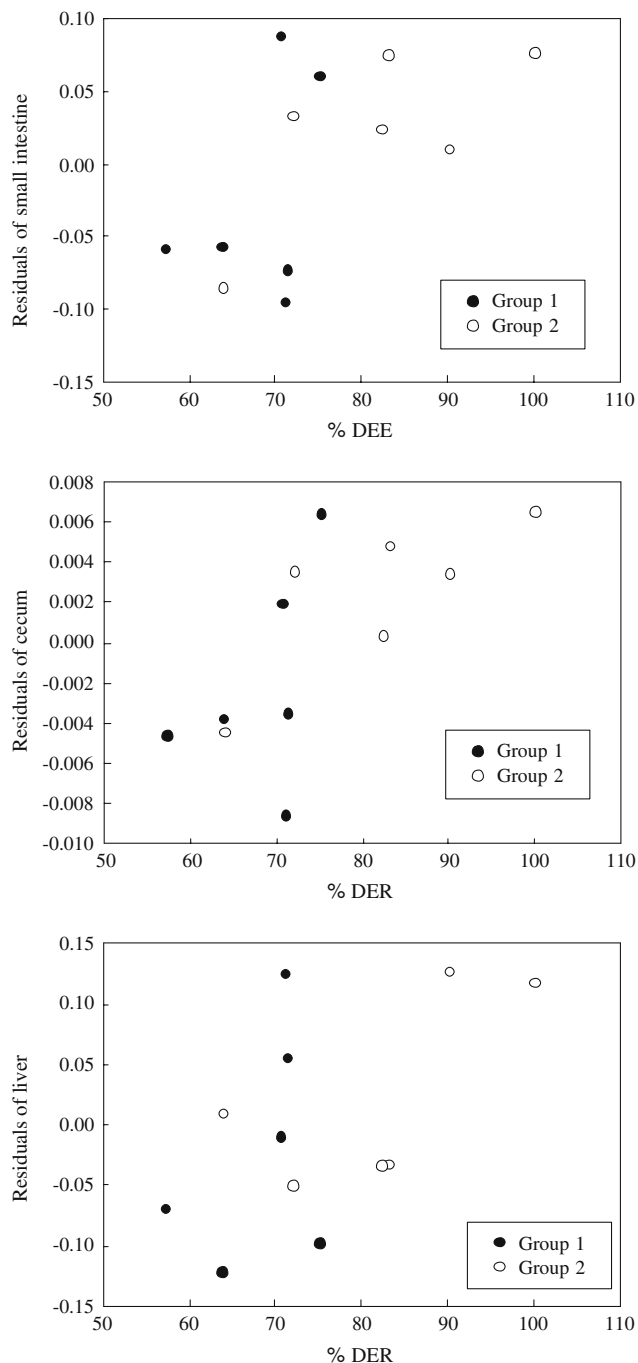


Fig. 5 **a** Relationship between the residual of small intestine dry mass and %DER (small intestine = $-0.267(\pm 0.032) + 0.004(\pm 0.0004) \%DER$, $t_{10} = 6.03$, $P = 0.034$, $r^2 = 0.38$), **b** between the residual of cecum dry mass and %DER (cecum = $-0.022(\pm 0.002) + 0.0003(\pm 3 \times 10^{-5}) \%DER$, $t_{10} = 9.12$, $P = 0.013$, $r^2 = 0.48$), and **c** between the residual of liver dry mass and %DER (liver = $-0.298(\pm 0.042) - 0.004(\pm 0.0005) \%DER$, $t_{10} = 4.37$, $P = 0.063$, $r^2 = 0.30$). Errors are ± 1 SEM. Abbreviation as in Fig. 3

comparative analysis, this author concluded that metabolic rate reductions depend on body mass, the patterns of torpor used and the state of torpor. Species

that enter daily torpor appear to rely mostly on the drop of Tb for metabolic rate reduction.

Our results show that the frequency of torpor in the Chilean mouse-opossum *T. elegans* strongly varied with food availability. That is, with amounts of food that exceed daily energy requirements, animals were rarely torpid as opposed to animals confronting lower food availability. Moreover, the relationship between the duration and depth of torpor bouts and %DER indicates that food availability influences these two parameters as opposed to just the frequency of torpor. This is in agreement with previous works that analyzed torpor patterns and food availability in other species (e.g., Silva-Duran and Bozinovic 1999; Mzilikazi and Lovegrove 2004; Lovegrove 2005; Lovegrove et al. 2001). On the other hand, Lovegrove et al. (1999) suggested that torpor facilitates avoiding risky foraging behavior in small mammals by overcoming long-term energy shortfalls generated by the variability of food availability in semi-arid habitats. Thereafter, torpor might diminish again, because animals must spend time searching for food (Humphries et al. 2001).

In addition, it has been shown that nutrient absorption in the digestive tract of torpid endotherms decreases when body temperature drops (Holloway and Geiser 1995). Therefore, in terms of energy savings, it would be risky to maintain torpor for long periods, given the low absorption of nutrients that it implicates. In this sense, it is known that, digestive enzymes in hibernating animals show an increase in their specific activity during periods of dormancy, which probably help to mitigate a food utilization problem (Carey 1990; Carey and Sills 1992; 1996; Ferraris and Carey 2000). In this vein, previous studies indicate that the Chilean-mouse opossum shows strong phenotypic flexibility in activity of intestinal enzymes, both as a seasonal (Sabat and Bozinovic 1994) and as a response to dietary acclimation (Sabat et al. 1995). Regarding internal organ mass, we found a positive correlation between the mass of the digestive tract and liver and the amount of food consumed. This result can be expected theoretically, because these organs are probably the most expensive to maintain in terms of energy and protein metabolism (McBride and Kelly 1990; Wang et al. 2001). Thus, a reduction in their sizes when food is limited can help animals to maintain positive energy budgets. Additionally, this reduction in organ size during food limited periods is in agreement with field studies on hibernation (e.g., Galluser et al. 1988; Hume et al. 2002) and with experimental works that evaluate the effect of starvation (e.g., Debnam and Levin 1975; Ferraris and Carey 2000; Goodman and Fleck 1980).

Weiner (1992) proposed a model, where energy flow is depicted as fluid flowing into a barrel through funnels acting in series and out of it through spouts working in parallel, where both might act as bottlenecks. The “barrel model” predicts that physiological limitation (e.g. digestion of food, the work capacity of different tissues) and environmental demands (e.g. food availability, environmental temperature) affect an organism’s capacity to acquire and expend energy. In particular, these limitations could be related to the central machinery used for acquiring, processing and allocating energy (represented by one of the funnels) or with the energy-consuming machinery (represented by the spouts; see also Bacigalupe and Bozinovic 2002). As indicated before, an alimentary/digestive bottleneck is supported by numerous observations and experiments demonstrating that changes in current energy demand and/or environmental food quality are associated with changes in the sizes of digestive organs—i.e. an increase in size of one of the funnels. However, another way to maintain a time-averaged energy budget over sufficiently longer periods while maintaining body mass constant is to reduce the flow through one or more of the spouts. Here, we demonstrate that when food availability is limiting, *T. elegans* specimen reduce their maintenance cost by two different mechanisms, that is, increasing the use of torpor and reducing organ mass.

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