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Interplay among energy metabolism, organ mass and digestive enzyme activity in the mouse-opossum *Thylamys elegans*: the role of thermal acclimation

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Summary

The potential for thermal acclimation in marsupials is controversial. Initial studies suggest that the thermoregulatory maximum metabolic rate (MMR) in metatherians cannot be changed by thermal acclimation. Nevertheless, recent studies reported conspicuous seasonality in both MMR and in basal metabolic rate (BMR). We studied the role of thermal acclimation in the Chilean mouse-opossum, *Thylamys elegans*, by measuring MMR and BMR before and after acclimation to cold or warm conditions. Following acclimation we also measured the mass of metabolically active organs, and the activity of a key digestive enzyme, aminopeptidase-N. No significant effect of thermal acclimation (i.e. between cold- and warm-acclimated animals) was observed for body mass, MMR, body temperature or factorial aerobic scope. However, the BMR of cold-acclimated animals was 30 % higher than for warm-acclimated individuals. For organ mass, acclimation had a significant effect on the dry mass of caecum, liver and kidneys only. Stepwise multiple regression using pooled data showed that 71 % of the variation in BMR is explained by the digestive organs. Overall, these results suggest that MMR is a rather rigid variable, while BMR shows plasticity. It seems that *T. elegans* cannot respond to thermal acclimation by adjusting its processes of energy expenditure (i.e. thermogenic capacity and mass of metabolically active organs). The lack of any significant difference in aminopeptidase-N specific activity between warm- and cold-acclimated animals suggests that this response is mainly quantitative (i.e. cell proliferation) rather than qualitative (i.e. differential enzyme expression). Finally, as far as we know, this study is the first to report the effects of thermal acclimation on energy metabolism, organ mass and digestive enzyme activity in a marsupial.

Key words: basal metabolic rate, maximum metabolic rate, marsupial, thermal acclimation, organ mass, *Thylamys elegans*, aminopeptidase-N.

Introduction

The capacity of small endotherm-homeotherms to tolerate cold conditions depends on such structural attributes as body mass and insulation, and on active processes such as behavioral thermoregulation and their capacity to increase oxygen consumption (V_{O_2}) above the basal metabolic rate. Small eutherians can increase their thermogenic capacity after short periods of cold exposure, a process known as phenotypic flexibility (Hammond et al., 2001), which has been the subject of a large amount of research in the last few decades (Hayes and Chappell, 1986; Campbell and MacArthur, 1996; Hammond et al., 2001 and references therein). Short-term (i.e. days) as well as long-term (i.e. months) exposure to cold and changes in photoperiod (Rezende et al., 2000) induce a range of physiological changes from the cellular level to the organismal level (Puchalski et al., 1987; Wiesinger et al., 1990; Deveci et al., 2001). These modifications occur in three ways: (1) organs such as skeletal muscles and brown adipose tissue increase their total heat production capacity (Jansky, 1973; Puchalski et al., 1987; Hammond et al., 2001), (2) intestines and food-processing organs enhance their absorption/processing capacities (Hammond and Janes, 1998), and (3) systems responsible for the supply of oxygen and nutrients to skeletal muscles and brown adipose tissue (i.e. cardio-respiratory system) increase their efficiency. As a result of this last process, the heart and lungs enlarge in cold-acclimated individuals (Hammond et al., 2001). At the organismal level, the increase in thermoregulatory maximum metabolic rate (MMR) is a consequence of the first process (Rosenmann and Morrison, 1975; Rafael et al., 1985; Hayes, 1989; Hammond et al., 2001), and probably increases survival of natural populations during the cold season, or during short-term exposure to cold conditions (Hayes and O'Connor, 1999). Nevertheless, this increase in MMR imposes costs associated with the maintenance of energetically expensive metabolic machinery, which are reflected in the high basal metabolic rate (BMR) of cold-acclimated animals compared

with warm-acclimated animals (Konarzewski and Diamond, 1995).

Small eutherian mammals and birds are classic examples of animals that use the processes listed above (for a review, see McNab, 2002). However, it is not well known what happens in other endotherms such as marsupials. The physiology of marsupials is similar to eutherians in several respects (Hallam and Dawson, 1993; Chappell and Dawson, 1994; Gibson and Hume, 2000; Holloway and Geiser, 2001), but with respect to BMR and thermal acclimation, there are some differences. The BMR in marsupials is comparatively low, which makes their factorial aerobic scope (FAS=MMR/BMR) unusually high (for marsupials, FAS is near 8; for birds and mammals, FAS=5) (see Hinds and MacMillen, 1984; Smith and Dawson, 1985; Hinds et al., 1995). In addition, the body temperature of marsupials is considerably lower than in eutherians (Hume, 1999). Interestingly, in some marsupial species, thermal acclimation does not induce MMR changes (Smith and Dawson, 1985; Dawson and Olson, 1988), and this was attributed to the absence of brown adipose tissue (BAT) (Dawson and Olson, 1988; Rose et al., 1999). However, cold acclimation can increase the speed and reduce energy expenditure of re-warming after torpor (Opazo et al., 1999), which suggests that acclimation could induce some changes in the thermogenic capacity of marsupials, even in the absence of BAT. Birds do not have BAT either, but they do exhibit changes in MMR in response to thermal acclimation (Swanson, 2001), which demonstrates that BAT is not the only way of changing thermogenic capacity during thermal acclimation.

To evaluate the underlying causes of differences in organismal performance, the phenotype needs to be investigated simultaneously at several levels. This integrative approach correlates organismal performance at one level with changes at the lower level in the same individual subjected to a variety of experimental treatments (e.g. Garland, 1984; Garland and Else, 1987; Hammond and Janes, 1998; Chappell et al., 1999; Konarzewski et al., 2000; Hammond et al., 2001). We used this approach to assess the effects of thermal acclimation in a small didelphid marsupial, the mouse-opossum (*Thylamys elegans*), which inhabits Mediterranean environments of central Chile. This species shows conspicuous phenotypic flexibility in the activity of its intestinal disacharidases, both on a seasonal basis (Sabat and Bozinovic, 1994) and in response to diet acclimation (Sabat et al., 1995).

Our studies are useful not only for determining the underlying causes of the observed differences in organismal performance in a wild endothermic species, but also because little is known about the flexibility of MMR in marsupials. For example, in an exhaustive review on marsupial nutrition and energetics there is no mention of interspecific variability or intraspecific plasticity in MMR (Hume, 1999), whereas ecophysiological variables such as field metabolic rate, water turnover and BMR received a thorough analysis (Hume, 1999).

The aim of this work was to determine the effect of laboratory thermal acclimation of *T. elegans* on (1) aerobic metabolism (BMR and MMR), (2) mass of metabolically active organs and (3) digestive enzymatic activity. Since *T. elegans* is mainly an insectivorous species (Sabat et al., 1993), and the intestinal aminopeptidase-N shows clear phenotypic flexibility in birds and mammals (Sabat et al., 1998, 1999), we chose this enzyme to determine if physiological flexibility exists at this level.

Materials and methods

Experimental design

Study animals and acclimation conditions

Twelve male and eight female mouse-opossum *Thylamys elegans* (L.) with a mean body mass (M_b) of 31.1±3.5 g (±S.D.) were live-caught using Sherman traps at Quebrada de la Plata (33 °31′S, 70 °50′W) during September–October 2001, and transferred to the laboratory on the same day of capture. Animals were individually housed in plastic cages (60 cm×60 cm×20 cm) filled with approximately 30 cm of litter, and fed with Puppy Chow (Purina®) and water *ad libitum*. Temperature and photoperiod were held constant at 22±2 °C and 12 h:12 h L:D photoperiod, for 2 weeks. After the initial laboratory habituation, ten randomly chosen individuals were acclimated to 30.0±2.0 °C (i.e. warm) in a climatic chamber for one month. The remaining ten animals were acclimated to 10.0±2.0 °C (i.e. cold) for the same amount of time, which was long enough to reach an asymptotic physiological state (Rezende et al., 2000). The 12 h:12 h L:D photoperiod was maintained for both conditions and food was provided *ad libitum*. We chose these acclimation temperatures to reflect the extremes of the natural temperature range in the habitat where the sample organisms were captured (Di Castri and Hajek, 1976).

Physiological measurements (i.e. BMR and MMR, see below) were made before and after acclimation. At the end of each measurement we recorded body mass, using an electronic balance (sensitivity ±0.1 g), and rectal body temperature (T_b) using a Cole-Parmer copper-constant thermocouple.

Basal metabolic rate (BMR)

Prior to BMR measurements animals were fasted for 6 h. BMR was determined according to the following protocol. Oxygen consumption (V_{O_2}) was measured in a computerized (Datacan V) open-flow respirometry system (Sable Systems, Henderson, Nevada, USA). Measurements of animals were made in steel metabolic chambers of 1000 ml, at ambient temperature (T_a) 30.0±0.5 °C, which is within the thermoneutral zone for this species (M. Rosenmann, personal communication). The metabolic chamber received dried air at a rate of 505 ml min^{-1} from mass flow controllers (Sierra Instruments, Monterey, California, USA), which was enough to ensure adequate mixing in the chamber. Air passed through CO_2-absorbent granules of Baralyme and Drierite before and after passing through the chamber and was monitored every 5 s by an Applied Electrochemistry O_2-analyzer, model S-3A/I

(Ametek, Pittsburgh, Pennsylvania, USA). Oxygen consumption values were calculated using equation 4a of Withers (1977). Since *T. elegans* is a nocturnal species, all metabolic trials were completed during the rest phase of activity (between 08.00 and 16.00 h).

The complete V_{O_2} trial lasted 2.5 h and BMR was taken as the lowest sample registered during the last hour of V_{O_2} recording, which was comparable with previous results obtained using the same species (e.g. Sabat et al., 1995; Opazo et al., 1999), and yielded the same values as the lowest obtained during a 5 min period over the complete V_{O_2} record. Previous BMR measurements to determine the optimal time to reach minimum metabolism indicated that this species reaches a steady state after 15–20 min, with no changes of V_{O_2} >15 % in the following 3 h.

Maximum metabolic rate (MMR)

We measured MMR in a He-O_2 atmosphere following the procedure of Rosenmann and Morrison (1974), using an open circuit respirometer, as described by Chappel and Bachman (1995). In brief, a mixture of He (80 %) and O_2 (20 %) was passed through a volumetric flowmeter before entering the chamber (i.e. a positive pressure system), and was maintained at a rate of 1000±3 ml min^{-1}. Such a flow rate prevented the partial oxygen pressure from falling below 150 Torr, a value far above hypoxia (Rosenmann and Morrison, 1975). The mixture passed through CO_2-absorbent granules of Baralyme and Drierite before and after passing through the chamber, which was tightly sealed with teflon and vaseline. The chamber temperature (0.0±0.5 °C) was continuously recorded. To be sure that individuals attained MMR, we (1) finished each record when the decline in V_{O_2} was evident (usually after 8–10 min of measurement), and (2) measured T_b after each trial to ensure animals reached hypothermia (T_b<30 °C in all cases; normothermic T_b=34 °C, see Results).

Organ masses and enzyme assays

After the second set of metabolic measurements all animals were killed by decapitation and dissected abdominally. We extracted first the colon and small intestine, and then heart, lungs, liver and kidneys, and the remaining carcass was weighed. Organs were washed with 0.9 % NaCl solution and immediately weighed (fresh mass). Stomach, caecum and total intestine were weighed with and without content. Except for the small intestine, all organs were dried at 60 °C to constant mass (48 h), and weighed again (dry mass). No significant trends (i.e. acclimation effect, dependence with M_b, metabolism or enzyme activity) on total content and water content of fresh organs were observed (data not shown).

We weighed the entire small intestine before cutting it into four sections of equal length (defined operationally as duodenum, jejunum A, jejunum B and ileum) for statistical comparisons. The sections were washed with 0.9 % NaCl and stored in criovials with liquid nitrogen. To measure intestinal aminopeptidase-N activity, tissues were thawed and homogenized (30 s in an Ultra Turrax T25 homogenizer at maximum setting) in 20 volumes of 0.9 % NaCl solution. We measured enzyme activity in the whole-tissue homogenate to avoid any underestimation of activity.

Aminopeptidase-N assays were done using L-alanine-*p*-nitroanilide as a substrate. Briefly, 100 µl of homogenate diluted with 0.9 % NaCl solution were mixed with 1 ml of assay mix (2.04 mmol l^{-1} L-alanine-*p*-nitroanilide in 0.2 mol $µl^{-1}$ NaH_2PO_4/Na_2HPO_4, pH 7). The reaction was incubated at 37 °C and arrested after 10 min using 3 ml of ice-cold acetic acid (2 mol l^{-1}), and absorbance was measured at 384 nm. Standardized intestinal enzymatic activities were calculated on the basis of absorbance. The protein content of intestinal homogenates was determined using Coomassie-Plus Protein Assay (Pierce, Rockford, Il, USA). Enzyme activity was standardized to rate per g wet tissue of intestine and per mg protein (Sabat et al., 1998), and the activities of all enzymes are presented as standardized hydrolytic activity (UI g^{-1} wet tissue, and UI mg^{-1} protein, where UI=µmol hydrolyzed min^{-1}).

Statistical analyses

We performed all analyses both with and without sex as a factor. Since the results were similar we report only the latter values here. For analyses of V_{O_2} (i.e. BMR and MMR) and factorial aerobic scope (FAS=MMR/BMR), there were two factors: thermal acclimation and laboratory effect (i.e. each individual was measured twice, before and after thermal acclimation). Since V_{O_2} is correlated with M_b, we included it as a changing covariate in repeated-measures analysis of covariance (ANCOVA) (StatSoft, 1996). The effect of acclimation on organ masses was tested by ANCOVA with carcass mass as the covariate, to avoid confounding effects due to the part-whole correlation (see Christians, 1999). The correlation among organ masses was assessed using residuals from the linear regression between organ mass and M_b of pooled data (i.e. cold- and warm-acclimated individuals). No transformations were necessary to meet analysis of variance (ANOVA) assumptions (see below).

Aminopeptidase-N activity (i.e. UI mg^{-1} protein; no dependence on M_b) was measured in the four sections of the small intestine, which were considered as repeated-measures (Meynard et al., 1999). The effect of thermal acclimation and position on this variable was evaluated using a repeated-measures ANOVA. This analysis was repeated for total enzyme activity (UI per total intestine) using ANCOVA and M_b as covariates.

To assess the association between organ masses and V_{O_2}, we performed stepwise multiple regressions for BMR and MMR separately, using residuals of organ masses as independent variables, and residuals of V_{O_2} as the dependent variable.

All data were tested for homogeneity of variance using the Levene test and for normality using the Kolmogorov–Smirnov test. For ANCOVA analyses we tested for parallelism between both levels of the factor (i.e. acclimation temperature) using a test of interaction with covariate (StatSoft, 1996).

Results

Body mass and metabolic rates

There were no significant differences in M_b between acclimation groups (Table 1). However, we observed a significant laboratory effect on animals, with increased M_b after time spent in the laboratory in comparison with M_b at capture (ANOVA, $F_{1,13}$=32.9, P=0.001). Body temperature was not significantly different between acclimation groups (34.2±0.7 and 34.0±0.6 °C for warm- and cold-acclimated individuals, respectively; ANOVA, $F_{1,10}$=0.04, P=0.8). There were no significant differences in BMR between the two groups measured before acclimation. After acclimation, BMR was significantly higher in cold-acclimated individuals than warm-acclimated animals (ANCOVA, $F_{1,12}$=5.21, P=0.041). There were marginally significant differences in BMR measured before and after thermal acclimation (ANCOVA, $F_{1,12}$=4.19, P=0.063, Table 1). However, the interaction between BMR and the laboratory effect was not significant (i.e. BMR in the laboratory was higher than at capture, in both groups). The same analysis for MMR and FAS did not yield significant effects, i.e. neither variable changed with thermal acclimation or showed any laboratory effect (Table 1).

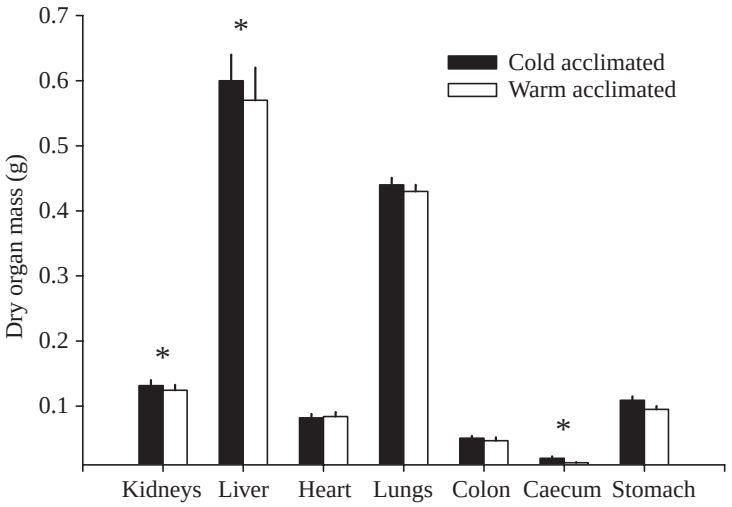

Fig. 1. Dry mass of organs from warm- and cold-acclimated *T. elegans* (mean ± S.E.M.). An asterisk indicates significant differences after correction for carcass dry mass (ANCOVA, P<0.05). Sample size as in Table 1.

Organ masses

Analyses using fresh organ mass gave similar results as for dry mass, thus, for organs other than small intestine (for which we have only fresh mass, since it was used for the enzyme assay), we report only the results of the dry mass analyses. Carcass dry mass showed no significant difference between cold- and warm-acclimated individuals (cold=14.1±1.02 g; warm=16.1±1.5 g, ANOVA, $F_{1,17}$=1.16, P=0.296). We observed significant differences in the dry mass of some organs between acclimation groups, however (Fig. 1). Masses were higher in cold acclimated individuals for kidneys (ANCOVA, $F_{1,15}$=10.73, P=0.005), caecum (ANCOVA, $F_{1,11}$=7.80, P=0.017), and liver (ANCOVA, $F_{1,16}$=6.38, P=0.022).

We did not observe significant effects of thermal acclimation on the wet mass of small intestine (cold=26.8±2.8 g; warm=19.8±2.2 g; ANCOVA, $F_{1,16}$=0.984, P=0.098, Table 2) or colon (ANCOVA, $F_{1,13}$=3.29, P=0.093), although masses tended to be greater for cold-acclimated individuals than for warm-acclimated individuals. We did not observe significant differences in the dry mass of heart, lungs or stomach between acclimation groups (Fig. 1).

Correlations of residuals (from linear regressions with M_b) between the dry mass of various organs (Table 3) demonstrated the following significant positive associations: kidneys with liver, colon, caecum and stomach; liver with colon and stomach; heart with lungs; and colon with caecum and stomach (see Table 3 for statistics). However, after a Bonferroni correction, just colon and liver remained significantly correlated. Stepwise multiple regressions between residuals of BMR (pooled data from both acclimation groups) and residuals of organ mass (kidneys, liver, heart, lungs, colon, caecum, stomach, carcass, and fresh small intestine) revealed that 77 % of the variance in BMR is explained by the mass of colon, small intestine and heart (multiple r^2=0.77, P=0.011). A similar analysis for MMR did not give significant results (P=0.15).

We repeated the stepwise regression analysis for BMR using only residuals of digestive organ mass as independent variables (stomach, liver, kidneys, small intestine, colon and caecum)

Table 1. *Body mass, basal metabolic rate, maximum metabolic rate and factorial aerobic scope of field-caught and laboratory acclimated Chilean mouse-opossum* Thylamys elegans

	Field				After acclimation			
Treatment	M_b (g)	BMR (ml O_2 h^{-1})	MMR (ml O_2 h^{-1})	FAS	M_b (g)	BMR (ml O_2 h^{-1})	MMR (ml O_2 h^{-1})	FAS
Cold	31.1±3.5 (9)	19.9±2.3 (9)	294.2±18.2 (8)	13.5±0.8 (8)	42.5±5.0 (9)	34.5±3.9 (9)	371.9±26.0 (8)	11.5±2.1 (8)
Warm	31.1±1.9 (6)	20.4±2.1 (6)	263.5±25.8 (8)	13.5±1.0 (6)	38.2±3.8 (6)	26.9±3.4 (6)	333.3±30.4 (8)	11.8±2.0 (6)

Values are means ± S.E.M. (N).
Sample sizes are shown in parentheses in each case.
Warm, acclimated to 30 °C; cold, acclimated to 10 °C.

Table 2. *Wet mass and standardized aminopeptidase-N activity in warm- (30 °C) and cold- (10 °C) acclimated mouse-opossum* Thylamys elegans

	Enzyme activity (UI mg^{-1} protein)		Wet mass (g)	
Section	Cold	Warm	Cold	Warm
Duodenum	0.250±0.016	0.254±0.020	0.89±0.145	0.61±0.092
Jejunum A	0.292±0.017	0.287±0.019	0.70±0.097	0.54±0.061
Jejunum B	0.335±0.047	0.314±0.043	0.56±0.085	0.41±0.064
Ileum	0.287±0.028	0.270±0.033	0.38±0.068	0.34±0.070

Values are means ± S.E.M. (N=10 for cold- and 9 for warm-acclimated individuals).

UI, μmol hydrolysed min^{-1}. See text for details.

Table 3. *Correlation matrix (r^2) of residuals of organ dry mass*

	Residual element					
Organ	Li	H	Lu	Co	Ca	St
Kidneys[a]	**0.324***	0.070	0.029	**0.327***	**0.397***	**0.427*****
Liver[a]	–	0.172	0.121	**0.504*****	0.217	**0.406*****
Heart[a]		–	**0.491*****	0.001	0.088	0.074
Lungs[a]			–	0.043	0.073	0.001
Colon				–	**0.544*****	**0.307***
Caecum					–	0.136
Stomach						–

[a]Significantly correlated with M_b.

Data from both acclimation treatments were pooled (N=19).

Significant correlations are shown in bold (*P<0.05, **P<0.01, ***P<0.001). After a Bonferroni correction, only the correlation between colon and liver was significant.

and residuals of BMR as the dependent variable. We found that 71 % of the variance in BMR was explained by colon, small intestine and kidneys (r^2=0.71, P=0.028). The stepwise multiple regression between residuals of MMR and residuals of thermogenic organ mass (heart, lungs and carcass) were marginally significant (r^2=0.42, P=0.06).

Digestive aminopeptidase-N

There were no significant differences in aminopeptidase-N activity between acclimation groups when expressed as UI mg^{-1} protein (ANOVA, $F_{1,17}$=0.9, P=0.78, Table 2), but there was a significant effect of position along the small intestine on enzyme activity (ANOVA, $F_{3,51}$=3.78, P=0.016, Table 2). The *a posteriori* Scheffe test revealed that jejunum B had the largest enzyme activity compared with the other three small intestine sections (P=0.017, Table 2). Cold-acclimated individuals presented significantly higher total aminopeptidase-N activity (using M_b as a covariate) than warm-acclimated animals (ANCOVA, $F_{1,16}$=16.88, P<0.001).

Discussion

Among mammals, marsupials are known to have comparatively low BMR values (for references, see Hume, 1999). Our results indicate that *T. elegans* is not the exception since its mass-specific BMR (range: 0.640–0.812 ml O_2 g^{-1} h^{-1}; Table 1) is close to that of other similarly sized marsupials (Didelphids, Dasyiurids, Petaurids and Burriamid) (see Hume, 1999). One consequence of having a low BMR is an increased FAS and comparatively low T_b. Actually, marsupials present greater (thermoregulatory) FAS than eutherians and birds (FAS of marsupials=8–9 compared with FAS of eutherians=5–6) (Dawson and Dawson, 1982; Hinds et al., 1995). In this study we found that (1) FAS of *T. elegans* is even greater than in marsupials in general (11.5–13.5, Table 1), and (2) FAS is not affected by thermal acclimation. This last finding is not common among eutherian mammals, and FAS is significantly modified by thermal acclimation in several rodent species (Rosenmann et al., 1975; Nespolo et al., 1999) and some marsupials (Gibson and Hume, 2000, and references therein). The observed absence of flexibility in FAS in *T. elegans* is probably due to the lack of flexibility in MMR (see below).

It is a recognized fact that most small eutherian mammals change their thermogenic capacity after cold acclimation (e.g. Rosenmann et al., 1975; Hammond et al., 2001; Merritt et al., 2001, and references therein). This phenotypic flexibility (*sensu* Hammond et al., 2001) is observed at different levels of the physiological phenotype. For example, during intense thermogenesis and/or exercise, total thermogenic capacity (in eutherians) is driven by non-shivering thermogenesis (NST) and shivering thermogenesis (ST) (Jansky, 1973), and each of these components present changes after thermal acclimation (e.g. Jansky, 1973; Rosenmann et al., 1975; Bockler and Heldmaier, 1983). Nevertheless, NST is considered the most plastic component since it depends on the metabolism of BAT, which is specialized to hypertrophy during cold acclimation (Kronfeld-Schor et al., 2000). Marsupials do not demonstrate NST, at least not in the same way as small eutherians (i.e. associated with BAT) (Rose et al., 1999), which may explain why thermal acclimation did not have an effect on MMR in *T. elegans*. It could also be argued that the thermal gradient we selected for this study (10–30 °C) was not steep enough to induce a change in MMR. However, previous studies have shown that this same thermal gradient is enough to elicit large and significant differences in MMR in several species of rodents (Nespolo et al., 2001). Moreover, our results are in agreement with previous reports on marsupials (Smith and Dawson, 1985; Dawson and Olson, 1988).

Recently, Holloway and Geiser (2001) documented significant seasonal differences in MMR for sugar gliders *Petraurus breviceps*. However, MMR and BMR values in their study are reported (and statistically compared) in terms of mass-specific units, in spite of the fact that these authors reported M_b changes between seasons. As many authors have claimed, statistical comparisons made on mass-specific metabolism are unreliable (Packard and Boardman, 1999; Christians, 1999; Hayes, 2001), and there are several robust statistical procedures that control for M_b (e.g. residual analysis,

ANCOVA, multiple regression) (see Christians, 1999). Moreover, since V_{O_2} is measured on whole animals, in the absence of other options (e.g. when sample size is small), units of V_{O_2} per animal should be used (Hayes, 2001). For this reason, unfortunately, it is not possible to decide whether there were significant differences in MMR between seasons for the data presented by Holloway and Geiser (2001).

Compared with warm-acclimated mouse-opossums, cold-acclimated individuals presented higher BMR. This observation is not new, as many authors have demonstrated for both eutherians and metatherians (Dawson and Olson, 1988; Rose et al., 1999). An increase in BMR is interpreted as an increment in maintenance costs due to the enlargement of digestive organs (Konarzewsky and Diamond, 1995). This functional dependence is supported by our results because 71 % of the variance in BMR was significantly explained by the intestines and kidneys. Indeed, cold-acclimated individuals had significantly larger caecum, kidneys and liver than warm-acclimated animals. It is known that the kidneys and liver of vertebrates respond to cold acclimation with an increase in mass (Hammond and Wunder, 1995; Hammond et al., 2001). Similarly, the comparatively large caecum suggests that *T. elegans* may present some hindgut fermenter activity (Hume, 1999), and that under cold conditions (i.e. high energy demands) this activity is probably increased. This is not surprising since most mouse-opossums are described as hindgut fermenters (Hume, 1999).

An acclimation response of intestinal enzymes has been reported elsewhere in several vertebrate species, including marsupials (see Martínez del Río et al., 1995; Sabat et al., 1995, 1998). These changes, according to the adaptive modulation hypothesis (see Hume and Stevens, 1996), are responses to the intake of specific dietary substrates. In addition, conditions favoring hyperphagia, such as cold exposure, lead to increases in intestinal mass (Toloza et al., 1991; Karasov, 1996). These responses by the small intestine lead to an increase in total hydrolytic capacity, matching biochemical features with changes in the intake of dietary substrates. The null response of aminopeptidase-N specific activity (per mg protein), and the marked differences observed in total activity of aminopeptidase-N (after controlling for M_b), suggest that tissue growth (a quantitative response), rather than differential expression (a qualitative response), of enzymes was the acclimation response at the digestive level. Phenotypic plasticity in enzyme activity has been reported for *T. elegans* in particular (Sabat et al., 1995) as well as for mammals in general (Buddington, 1994; Sabat et al., 1999, and references therein). Similarly, the effect of acclimation on the size of the small intestine in vertebrates has received a great amount of interest, in particular diet acclimation (Piersma and Lindstrom, 1997) and thermal acclimation (Hammond and Wunder, 1995; Hammond et al., 2001). However, the effects of thermal acclimation on digestive enzyme activities are poorly known (e.g. Harada and Kano, 1976; Das and Das, 1982), and to the best of our knowledge no studies have been published dealing with this issue in marsupials.

So, how does *T. elegans* cope with cold periods without increasing thermogenic capacity? A partial answer to this question may be the use of torpor (Silva-Durán and Bozinovic, 1999); torpor could be used as an evasive strategy to avoid low T_a (e.g. Geiser, 1994). Still, interestingly, there are several species of eutherian mammals that hibernate or use torpor as an energy saving strategy, but at the same time are able to express a high phenotypic flexibility in thermogenic capacity (Heldmaier et al., 1982; Merritt et al., 2001). Moreover, the fact that the thermogenic capacity of *T. elegans* did not increase after cold acclimation does not imply that they cannot survive this T_a in euthermia, rather that *T. elegans* cannot increase its maximum capacity for heat production in excess of requirements.

A very different response was observed at the digestive level since acclimation induced changes in the mass of caecum, kidneys and small intestine. As expected, these changes were accompanied by significant differences in BMR. These changes suggest that cold-acclimated individuals can adjust for high-energy demands. However, the adjustment is mostly in tissue mass rather than specific enzyme activity, at least for aminopeptidase-N activity.

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