Thermal acclimation and non-shivering thermogenesis in three species of South American rodents: a comparison between arid and mesic habitats

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Non-hibernating mammals that live in seasonal and arid environments change their non-shivering thermogenesis (NST) capacity to maintain homeothermy. Metabolic capabilities of animals, such as NST, are considered adaptive plastic traits because they have a broad range of possible phenotypes with different ambient temperatures (i.e. the reaction norm). Consequently, we determined the reaction norm for NST in Octodon degus (Bennett, 1832; \( m_b = 189 \) g) and Phyllotis darwini (Waterhouse, 1837; \( m_b = 61 \) g) from the mesic habitat of central Chile, and in Phyllotis xanthopygus (Waterhouse, 1837; \( m_b = 67 \) g) from the high Andean plains of northern Chile, an arid and seasonal habitat. Octodon degus showed a 22% increase in NST with thermal acclimation, whereas P. xanthopygus showed a 112% increase, and P. darwini showed a 117% increase in NST, being the largest change observed. These results are in agreement with our hypothesis of evolutionary inertia, which states that observed metabolic plasticity in Phyllotis species is consequence of their high Andean origin, in spite of the fact the actual habitat of P. darwini is the less seasonal central valley of Chile.

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Keywords: non-shivering thermogenesis; thermal acclimation; seasonality; Andes range; Phyllotis; Octodon

Introduction

Production of heat for thermoregulation in resting animals is derived from shivering and non-shivering thermogenesis (NST). Brown adipose tissue is stimulated by sympathetic fibers through norepinephrine eliciting NST (Cannon & Nedergaard, 1985), thereby transforming chemical energy resources directly into heat. Maximum metabolic rate is composed of resting metabolic rate (RMR), shivering thermogenesis, and NST (Bozinovic & Rosenmann, 1989; Bozinovic et al., 1990; Wunder & Gettinger, 1996). Higher values of NST, as well as MMR, are associated with acclimation to cool and short days in small mammals (Heldmaier & Steinlechner, 1981). An increase in thermogenic capability is important in preparing small mammals for cool conditions in...
seasonal environments because it may allow an increase in survival. According to Degen (1997), reduced RMR and high capacity for NST allow rodents a low metabolic heat production during activity and permit them to increase heat production quickly under cool-to-cold conditions. Thus, thermogenic capacity can change quickly through modifications of NST in response to environmental cues and can be the main mechanism of adaptive phenotypic plasticity applied to metabolic traits (sensu Stearns, 1992).

In arid environments, conspicuous variables such as temperature, photoperiod and food availability exhibit great fluctuations at both daily and seasonal basis (Degen, 1997; Kronfeld-Schor et al., 2000). Because of this, energy supply and demand vary greatly during the life of an animal, and individuals must be able to cope with these variations in order to maintain a positive energy balance (Mysterud & Ims, 1998; Williams & Ternan, 1999). Thus, species inhabiting unpredictable habitats might change their capacity for NST in response to thermal acclimation to a larger extent than species inhabiting more stable habitats. Therefore, observed capabilities of changes in metabolic traits of animals could be a consequence of either habitat adaptation or phylogenetic inertia.

To differentiate between these two hypotheses, we examined the capacity of NST to change under thermal acclimation in Phyllotis xanthopygus Waterhouse, 1837 (Muridae: Sigmodontinae) from the high Andean plateau of northern Chile (a seasonal habitat), the degu, Octodon degus Bennett, 1832 (Hystricognatha: Octodontidae) from a more stable Mediterranean habitat, the central valley of Chile, and the sympatric ‘leaf’-eared mouse, Phyllotis darwin Waterhouse, 1837, from the same habitat as O. degus but phylogenetically more closely related to P. xanthopygus.

The biogeographic origin of the genera Phyllotis and Octodon are markedly different. The family Octodontidae originated from tropical zones of South America during the Oligocene (Reig, 1986; Contreras et al., 1987), whereas the tribe Phyllotini (Sigmodontinae) originated at the Pliocene, during major uplift of Andes ranges in the present high-altitude plains of northern Chile, Peru, and Bolivia, the current distribution of P. xanthopygus (Reig, 1986; Engel et al., 1998).

If these species have lived in their present habitats long enough to permit evolutionary adaptation to determine their current metabolic capabilities, we would expect a larger percentage increase in NST after thermal acclimation in P. xanthopygus than in the other two species. Furthermore, O. degus and P. darwini should exhibit similar slopes in reaction norms (i.e. similar differences in NST after thermal acclimation). Conversely, if phylogenetic inertia was more important than present-day abiotic selective pressures on NST, we expect more metabolic similarities between species of Phyllotis than between P. darwini and O. degus in spite of the overlap in use of habitat by the latter two species.

Here, we use the term reaction norm as ‘the set of phenotypes expressed by a single genotype across a range of environmental conditions’ (Stearns, 1992, p. 41). In our study, we use ‘phenotype’ to refer to NST, the ‘genotype’ to the individual species, and the range of environmental conditions to our two acclimation temperatures. We suggest a flexible reaction norm (Stearns, 1989) because capacity of NST increases and decreases during the life of the individuals (i.e. each season).

Materials and methods

Animals and acclimation

Ten individuals of P. xanthopygus were captured in Chiuchiu (northern Chile) at 2529 m a.s.l. (28°18’S; 68°38’W, mean annual temperature and precipitation = 12·2°C and 5·4 mm, respectively). Because of the Humboldt current and the Pacific anticyclone, the Chilean Atacama desert is unusually dry which provoke large daily thermal amplitudes (Marquet et al., 1998), conditions that change over 3500 m due to
exponential increase of rainfall and seasonality (Marquet et al., 1998). At 2500 m a.s.l.,
desertic conditions are still strong, but the effect of seasonality begins to take place
(see Marquet et al., 1998, figs 2–4). Thus, *P. xanthopygus* experiences a rather singular
desert environment since it is influenced by thermal fluctuations in both seasonal and
daily basis.

Twelve individuals of *O. degus* and 11 of *P. darwini* were captured at Quebrada de la
Plata at 800 m a.s.l. (33°31′S; 70°50′W, mean annual temperature and precipita-
tion = 14.72°C and 281 mm respectively). The area has a mediterranean climate char-
acterized by warm dry summers and cold wet winters and is located within the
biogeographical zone known as matorral (Gajardo, 1994). All animals were captured in
Autumn using Sherman traps and transported to the laboratory in Santiago where they
were maintained on rabbit food pellets and water *ad lib* for 1 week. Thereafter,
individuals of each species were randomly divided into two groups. Half the individuals
were acclimated for 1 month at 30°C (warm) and the other half at 15°C (cool) ± 2°C
(S.D.). Both groups were kept with a photoperiod of 12L:12D.

**Non-shivering thermogenesis**

Once animals were acclimated, we measured NST in both groups of animals (Haim,
1996). Oxygen consumption (VO$_2$) was measured using a computerized (Datacan V,
Sable Systems, Henderson, NV, U.S.A.) open-flow respirometry system in metabolic
chambers of 900 ml at an ambient temperature (T$_a$) of 25°C, which is into the
thermoneutral zone for these three species (see Rosenmann, 1977 for *O. degus* and
Bozinovic et al., 1988 for both *Phyllotis* species). The metabolic chamber received dried
air at a rate of 1000 ml min$^{-1}$ from mass flow controllers (Sierra Instruments, Mon-
terey, CA, U.S.A.) to ensure adequate mixing of air in the chamber. Both before and
after the chambers, CO$_2$ and H$_2$O were absorbed using granules of Baralyme (Ba(OH)$_2$
) and Drierite (CaSO$_4$), respectively. Oxygen was determined every 5 s by an Applied
Electrochemistry O$_2$ analyser model S-3A/I (Ametek, Pittsburgh, PA, U.S.A.). Ambient
temperature was held constant (± 0.5°C) by maintaining the metabolic chamber in an
incubator. Oxygen consumption was calculated using equation 4a of Withers (1977).
All metabolic trials were completed between 0800 and 1600 hours. Body mass (m$_b$) and
rectal temperature (T$_b$) were measured before and after each metabolic trial with an
electronic balance and a Cole-Parmer thermocouple, respectively.

The experimental protocol used on each animal was following: (1) VO$_2$ record of 45
min at rest (= RMR); (2) intramuscular perfusion of saline solution (= injection
control) followed by a new VO$_2$ record of 45 min, and; (3) an equal volume of
norepinephrine solution was injected intramuscularly and VO$_2$ recorded for 45 min
(= NST). Doses of norepinephrine and saline were calculated according to the
equation of Wunder & Gettinger (1996). We used the same volume of saline and
norepinephrine. Whole NST (including any RMR component, *sensu* Wunder & Gettin-
ger, 1996) for each individual was obtained from the average of 200 samples recorded
just after norepinephrine perfusion (ca. 17 min).

**Body mass control**

To allow for interspecific comparisons it was necessary to adjust for body size ef-
ects. We did not have enough data to perform an ANCOVA and determine the scaling
of NST and body size from our own results (Packard & Boardman, 1999). However,
according to Wunder & Gettinger (1996, p. 137), the interspecific relationship
between NST (per gram) and body mass is $NST = am_b^c$, where $c = -0.49$ in 23°C
acclimated animals ($n = 39$, $r = 0.98$) and $c = -0.51$ in 5°C acclimated animals
(n = 41, r = 0.96). Therefore c ≈ -0.5 gives an accurate approximation to the exponent for the NST allometry. This relationship can be expressed on a per animal basis by multiplying this expression by m_w, which yields NST = am_w^0.5. Thus, by dividing each whole-animal NST measurement by the square root of body mass we removed the scaling effect among the different species. Resting metabolic rate was compared between acclimation groups (intraspecific comparisons) and not between species. Then, there was not necessary to correct by body mass and comparisons were done with data divided by body mass. For intra-species and intra groups comparisons between saline injection and norepinephrine injections (to account for presence of NST in animals) respectively, we used values divided by body mass (i.e. VO_2 per gram).

Statistics

Statistical analyses were performed using Statistica® (1997). Intragroup saline/NST comparisons for each acclimation temperature were done with t-tests for dependent samples, and RMR comparisons between acclimation groups were performed with t-tests for independent samples (Sokal & Rohlf, 1995). Data of NST among species were analysed by a two-way fixed model analysis of variance (ANOVA, Sokal & Rohlf, 1995; Statistica®, 1997). Normality of data was tested with the Shapiro–Wilk test. Factors were species (with three levels: O. degus, P. xanthopygus and P. darwini) and acclimation temperature (two levels: 15°C and 30°C acclimation temperatures), and the dependent variable was NST. All factors were considered fixed (ANOVA, model I).

Results

Non-significant differences were found in m_b between acclimation groups in each species (O. degus: t_w = 1.02, p = 0.3; P. xanthopygus: t_b = 0.99, p = 0.35; and P. darwini: t_b = 0.31, p = 0.76, Table 1). Both m_b and VO_2 data were normally distributed (W = 0.96, p < 0.36). Comparisons between VO_2 after saline and norepinephrine injections (that account for NST presence) in each acclimation group were significant for O. degus both cool (t_b = 7.0; p = 0.0004) and warm (t_b = 5.9; p = 0.0041) acclimated, as for P. xanthopygus (t_b = 9.9; p = 0.0006) and P. darwini (t_b = 4.7; p = 0.006) cool acclimated. The same comparisons showed non-significant differences for P. xanthopygus (t_b = 2.14; p = 0.1) and P. darwini (t_b = 0.88; p = 0.43) both warm-acclimated, which suggest absence of NST in these species when 30°C acclimated. In addition, there was not significant differences between RMR and saline metabolic rate in any species (p > 0.05, Student’s t-test) showing that saline injection did not raise metabolism. Comparisons between acclimation groups in RMR (see Table 1) were significant only for P. darwini (O. degus: t_w = 1.48, p = 0.17; P. xanthopygus: t_w = 1.81, p = 0.10; and P. darwini: t_w = 5.95, p = 0.0002). Two-way ANOVA with standardized data by m_b^0.5 showed significant effects in species (F_w = 14.4, p < 0.0001), acclimation (F_w = 75.8, p < 0.0001) and interaction (F_w = 3.4, p < 0.049; Table 2, Fig. 1, non-standardized). Differences in NST due to acclimation (cool mean - warm mean in Table 1) were 82.9 ml O_2 h^-1 (22.2% of warm mean) in O. degus, 140.4 ml O_2 h^-1 in P. xanthopygus (112.5% of warm mean) and 126.9 ml O_2 h^-1 (117.4% of warm mean) in P. darwini. Post-hoc comparison showed significant differences in NST between acclimation groups (cool vs. warm acclimation) in all species (O. degus p = 0.03, P. xanthopygus p = 0.0002 and P. darwini p = 0.001, Tukey test), but among species there was significant differences only between O. degus warm acclimated contrasted with Phyllotis species (p = 0.033, Tukey test, see Fig. 1).
Table 1. Body masses, VO$_2$ after resting (RMR), saline and norepinephrine injection (NST) in the three species of rodent studied. RMR was divided by $m_b$ and NST standardized to $m_b^{0.5}$ (see Materials and methods). Statistical analyses in the text.

<table>
<thead>
<tr>
<th>Species</th>
<th>Octodon degus</th>
<th>Phyllotis xanthopygus</th>
<th>Phyllotis darwini</th>
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<tr>
<td>Acclimation (°C)</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$m_b$ (g)</td>
<td>182.7 ± 17.1</td>
<td>195.5 ± 26.4</td>
<td>65.1 ± 15.5</td>
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<td>Saline metabolism</td>
<td>1.64 ± 0.22</td>
<td>1.33 ± 0.22</td>
<td>2.22 ± 0.62</td>
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<td>(mLO$_2$/g h)</td>
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<td>1.22 ± 0.24</td>
<td>1.89 ± 0.35</td>
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<tr>
<td>RMR (mLO$_2$/g h)</td>
<td>1.64 ± 0.22</td>
<td>1.33 ± 0.22</td>
<td>2.22 ± 0.62</td>
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<tr>
<td>NST/m$_b^{0.5}$</td>
<td>36.5 ± 6.0</td>
<td>27.9 ± 2.7</td>
<td>33.3 ± 4.5</td>
</tr>
<tr>
<td>(mLO$_2$/g$^{0.5}$ h)</td>
<td>36.5 ± 6.0</td>
<td>27.9 ± 2.7</td>
<td>33.3 ± 4.5</td>
</tr>
</tbody>
</table>
Discussion

Interspecific NST comparisons

After we eliminated the effects of body mass on NST, we observed a significant interaction between species and acclimation (ANOVA, see Table 2 and Fig. 1). Post-hoc comparison corroborated the inter-specific difference by showing that NST of *Octodon degus* was significantly different to *Phyllotis* species when warm acclimated but no differences were found among these three species when cool acclimated (see Fig. 1). These results alone support the hypothesis of phylogenetic inertia suggesting that *Octodon degus* is less plastic than both *Phyllotis* species in NST capacity. Furthermore, our data shows two additional evidences that support this hypothesis: (1) the small differences in NST between acclimation groups of *Octodon degus* (ca. 22%) compared with great

<table>
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<th>Source</th>
<th>df. effect</th>
<th>MS effect</th>
<th>df. error</th>
<th>MS error</th>
<th>F</th>
<th>p-value</th>
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<td>324.2</td>
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<td>22.5</td>
<td>14.4</td>
<td>&lt;0.0001*</td>
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<td>22.5</td>
<td>75.8</td>
<td>&lt;0.0001*</td>
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<tr>
<td>Species × Acclimation</td>
<td>2</td>
<td>75.7</td>
<td>28</td>
<td>22.5</td>
<td>3.36</td>
<td>0.049*</td>
</tr>
</tbody>
</table>
changes of *Phyllotis* species (ca. 113% for *P. xanthopygus* and ca. 117% for *P. darwini*) and (2) the virtual absence of NST capacity observed only in the warm acclimated *Phyllotis* individuals.

It is well known that small field mammals display seasonal acclimatization in MMR (Rosenmann *et al.*, 1975; Feist & Rosenmann, 1976; Bozinovic *et al.*, 1990) and NST (Zegers & Merritt, 1988a,b; Merritt & Zegers, 1991; Merritt, 1995; Kronfeld-Schor *et al.*, 2000) but studies describing inter-specific differences in change capacity under laboratory acclimation are less common (but see Heimer & Morrison, 1978; Klaus *et al.*, 1988). Moreover, most of the knowledge on NST acclimation are from laboratory species (see Jansky, 1973; Heldmaier *et al.*, 1985; for reviews). Among acclimation studies, Heimer & Morrison (1978) compared the change in MMR after thermal acclimation in two species of rodents. These authors found that the species with the wider geographic distribution (*Peromyscus maniculatus*) exhibited a higher metabolic plasticity in comparison to the more narrowly distributed species (*Microtus pennsylvanicus*). On the other hand, similar seasonal patterns in NST have been observed in sympatric species of rodents, which show different strategies of seasonal adaptation (i.e. behavioral changes, *m*ₜ, thermal conductance, and brown adipose tissue; Klaus *et al.*, 1988; Zegers & Merritt, 1988b). Field (i.e. acclimatization, Bozinovic *et al.*, 1990) and laboratory (i.e. acclimation, Nespolo & Rosenmann, 1997) studies reported that the Andean mouse, *Abrothrix andinus* (a murid species inhabiting an environment similar to that of *P. xanthopygus*), can change its termogenic capacity as much as 50% between warm and cold seasons, and that this change can occur within 2 weeks after the beginning of thermal acclimation. Our results show higher metabolic plasticity in *P. xanthopygus* than reported by these authors, and this plasticity may stem from the different evolutionary origin of tribe Philotini.

Non-shivering thermogenesis is not the unique strategy for acclimation in small non-hibernating mammals. Changes in thermal conductance and shivering thermogenesis have been reported to be important in seasonal acclimation (Bozinovic *et al.*, 1990; Nespolo *et al.*, 1999). In this work, shivering thermogenesis and thermal conductance were not measured. In any event, there is no reason to suspect that these variables changes more than NST in one species than in another (Lilly & Wunder, 1979; Böckler & Heldmaier, 1983).

**Plasticity of metabolic traits**

Metabolic variables such as RMR, MMR and NST have been reported as distinctive traits of species (e.g. Rosenmann & Morrison, 1975; Tomasi *et al.*, 1987; Bozinovic & Rosenmann, 1988; Bozinovic, 1992; Haim & Izhaki, 1995). However, many authors have found a high degree of plasticity on these variables (Heimer & Morrison, 1978; Heldmaier *et al.*, 1982; Bozinovic *et al.*, 1990; Nespolo & Rosenmann, 1997; Nespolo *et al.*, 1999). For comparative purposes, Haim & Izhaki (1995) reported NST values ranging from 0.95 to 6.72 ml O₂/g·h for rodents inhabiting mesic and arid habitats, including a NST value of 3.4 ml O₂/g·h in *Apodemus flavicollis*. However Klaus *et al.* (1988) demonstrated that this species is able to change its NST capacity by more than 70% through thermal acclimation (5–8.5 ml O₂/g·h). Tomasi *et al.* (1987) compared NST values for *Peromyscus maniculatus* (5.43 ml O₂/g·h) with those of *Sorex vagrans* (13–12 ml O₂/g·h), however Zegers & Merritt (1988a) reported a seasonal change that ranged from 4.5 to 10.5 ml O₂/g·h for *P. maniculatus*, and Merritt (1995) reported a seasonal change in NST ranging from 8.02 to 14.6 ml O₂/g·h for a congener of *S. vagrans*, *Sorex cinereus*. Based on these examples, as well as from many other studies (Feist & Rosenmann, 1976; Zegers & Merritt, 1988b; Klaus *et al.*, 1988; Hayes, 1989; Merritt & Zegers, 1991; McDevitt & Speakman, 1996; Haim, 1996), it is quite clear that fixed values of NST give little information because of the high variations of this feature.
between seasons or after thermal acclimation. In fact, even fossorial species which live in a fairly constant ambient would show variations in NST capacity (Goldman et al., 1999). To avoid these problems, measurements should be done on individuals acclimated to the same conditions (e.g. Wunder & Gettinger, 1996), or by comparing reaction norms. This procedure may yield a better insight about physiological performance in seasonal environments.

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