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# Arousal from torpor in the chilean mouse-opposum (*Thylamys elegans*): does non-shivering thermogenesis play a role?

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## Abstract

We examined the effect of norepinephrine injections on non-shivering thermogenesis (NST), rewarming rate, and metabolic cost during torpor arousal in warm- and cool-acclimated Chilean mouse-opposums, *Thylamys elegans*. Warm- and cool-acclimated animals did not display NST in response to NE injections. Values of  $VO_2$  (resting, after saline and NE injections) were not significantly different within treatments. Rewarming rates of warm-acclimated animals did not differ significantly from those in cool-acclimated animals. In contrast, the metabolic cost of torpor arousal was significantly affected by acclimation temperature. Warm-acclimated animals required more energy for arousal than cool-acclimated animals. Our study suggests that the main thermoregulatory mechanism during torpor arousal in this Chilean marsupial is shivering thermogenesis, and that its amount can be changed by thermal acclimation. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** *Thylamys elegans*; Marsupial; Thermal acclimation; Metabolic cost; Rewarming rate; Thermoregulation; Torpor arousal; Central Chile

## 1. Introduction

Heat production for thermoregulation in small placental mammals is derived from shivering thermogenesis (ST), ensuing from muscular contractions that produce metabolic heat, and from nonshivering thermogenesis (NST), which produces metabolic heat without muscular activity [3,16,43]. The brown adipose tissue (BAT), which is stimulated by norepinephrine (NE) elicits NST. The underlying mechanism that elicits NST in BAT is the uncoupling of proton conductance at the mitochondrial inner membrane [1,17,40]. Through this mechanism, energy is transformed almost directly to heat. According to [2,22], ST is activated when NST is insufficient to maintain body temperature ( $T_b$ ). In hibernating mammals, NST has been described as an important mechanism of heat production during arousal from hibernation and from torpor states [12,34]. Arousal is

considered the most critical period during torpor and hibernation [6,18,23,24,27,28].

In marsupials, the phenomenon of NST is controversial. Some authors have reported positive [25,33] and others negative results [5]. Further, morphological studies have failed to find evidence of the presence of BAT in many marsupial species [12,36] while others have reported BAT but without thermoregulatory properties [34]. This apparent lack of NST occurs in spite of the fact that many marsupials use torpor as an energy saving mechanism [41]. In addition, some authors have reported that marsupials have lower rewarming rates than eutherian mammals [15], whereas others have observed no differences between the two subclasses [8,10,39]. Consequently, the aim of this study was to test for the presence of NST and to determine the rewarming rate and metabolic cost of torpor arousal in the small marsupial *Thylamys elegans* (Didelphidae). The Chilean mouse-opposum inhabits the mediterranean environments of central Chile, is nocturnal and mainly insectivorous [30,35]. This species uses torpor when faced with low food availability and low temperatures [9].

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## 2. Material and methods

### 2.1. Animals and acclimation

Sixteen individuals of *T. elegans* (body mass =  $24.8 \pm 6.1$  g) were captured at Quebrada de la Plata, central Chile ( $70^\circ 56'W$ ;  $33^\circ 29'S$ , 500 m above sea level). Animals were captured with Sherman traps, transported to the laboratory, and maintained with Puppy Chow (Purina®), apples and water ad libitum during 1 week. To determine NST and the cost of arousal from torpor, two groups of eight individuals were-acclimated for 1 month at  $30^\circ C$  (= warm) and  $15^\circ C$  (= cool), respectively. Photoperiod was kept at LD = 12:12.

### 2.2. Non-shivering thermogenesis (NST)

After acclimation, we measured NST in both groups of animals according to [13]. Oxygen consumption ( $VO_2$ ) was measured in a computerized (Datacan V) open-flow respirometry system (Sable Systems) in metabolic chambers of 900 ml at an ambient temperature ( $T_a$ ) of  $25^\circ C$ . The metabolic chamber received dried air at a rate of  $900 \text{ ml min}^{-1}$ , from a mass flow controller (Sierra Instruments). Before and after the chamber, air was passed through  $CO_2$  absorbent granules of Baralyme [ $Ba(OH)_2$ ] and  $H_2O$  absorbent granules of Drierite [ $CaSO_4$ ], and was monitored every 5 s by an Applied Electrochemistry  $O_2$  analyzer model S-3A/I (Ametek). Both,  $CO_2$  and  $H_2O$  were removed before the mass flow meter. Ambient temperature was held within  $\pm 0.5^\circ C$  by maintaining the metabolic chamber in a water bath. Oxygen consumption values were calculated by Eq. 4a of [42]. All metabolic trials were completed between 08:00 and 16:00 h. Body mass ( $m_b$ ) was measured before and after each metabolic trial with an electronic balance. The experimental protocol was the following: (1)  $VO_2$  record during 45 min at resting (= RMR); (2) intramuscular injection of saline solution (0.9%) followed by a new  $VO_2$  record of 45 min; (3) intramuscular injection of norepinephrine bitartrate (Sigma®) solution and 45 min of  $VO_2$  record (= NST). Norepinephrine doses were calculated according to [43]. We used the same volume of saline and NE solutions. Values for each individual were obtained from an average of 150 samples of the  $VO_2$  records after NE perfusion [32].

### 2.3. Rewarming rate, thermal conductance and metabolic cost of torpor arousal

Nine animals (five warm-acclimated and four cool-acclimated) were fasted for 36 h to induce torpor in a climatic chamber at  $T_a = 12 \pm 2^\circ C$ . We measured  $T_b$  and  $VO_2$  simultaneously. We removed torpid animals

from the climatic chamber and inserted a Cu-Constantan thermocouple into the rectum, which was attached to the tail with cloth tape. Animals were then placed back in the metabolic chamber. Insertion of the thermocouple, which was completed in  $50 \pm 10$  s, was enough to induce arousal [27]. Body temperature was then recorded every min and  $VO_2$  was continuously recorded at  $T_a = 19^\circ C$ . We used this  $T_a$  because this temperature is similar to those in central Chile during March, the month of capture of our individuals (max =  $27.5 \pm 0.8^\circ C$ , min =  $9.6 \pm 1.1^\circ C$ , mean =  $18.5^\circ C$ ,  $n = 19$ , monthly temperatures between 1979–1998, Dirección Meteorológica de Chile, Pudahuel, Santiago). Each observation is an average of 600 samples (ca. 5 min) of  $VO_2$  records for each individual. Thus, if total rewarming takes, for instance 80 min, there will be  $80/5 = 16$  observation for this individual. We averaged both  $VO_2$  and  $T_b$  observations in the same time intervals in all treatments, for further comparisons. Thermal conductance was measured before and after acclimation in both groups by the ratio between oxygen consumption and thermal gradient:  $VO_2/T_b - T_a$ .

### 2.4. Statistics

Statistical analyses were performed with STATISTICA® [38]. Data were analyzed by one-way ANOVA and Student's *t*-test for independent samples. Results are reported as mean  $\pm 1$  SD. Slopes of rewarming rate and the metabolic cost of torpor arousal were analyzed with ANCOVA.

## 3. Results

Body masses of warm- and cool-acclimated individuals were not statistically different ( $32.7 \pm 6.5$  g vs.  $31.5 \pm 5.1$  g, respectively,  $t_{14} = 0.425$ ,  $P = 0.677$ ). Also, RMR at  $25^\circ C$  was not significantly affected by acclimation temperature ( $0.86 \pm 0.29 \text{ ml } O_2 \text{ g}^{-1} \text{ h}$  for warm-acclimated individuals and  $0.96 \pm 0.29 \text{ ml } O_2 \text{ g}^{-1} \text{ h}$  for cool-acclimated animals,  $t_{14} = 0.679$ ,  $P = 0.507$ ), and thermal conductance was not different between acclimation groups ( $0.0642 \pm 0.03$  for cool-acclimated individuals and  $0.059 \pm 0.02$  for warm-acclimated individuals,  $t_{14} = 0.5$ ,  $P = 0.63$ ). As shown in Fig. 1, warm- and cool-acclimated individuals did not react to NE injections. Values of  $VO_2$  (resting, after saline and NE injections) were not significantly different between treatments (one-way ANOVA,  $F_{2,21} = 2.927$ ,  $P = 0.08$  for warm acclimation and  $F_{2,21} = 0.186$ ,  $P = 0.831$  for cool-acclimated animals, see Fig. 1). During torpor, initial  $T_b$  was not significantly different between warm- and cool-acclimated animals ( $14.4 \pm 0.4$  and  $14.1 \pm 0.7^\circ C$ ,  $t_7 = 0.678$ ,  $P = 0.51$ ). Rewarming rates were not

different between acclimation groups ( $0.329 \pm 0.034^\circ\text{C min}^{-1}$  for warm-acclimated animals and  $0.328 \pm 0.044^\circ\text{C min}^{-1}$  for cool-acclimated animals). Nevertheless, the relationship between time course of  $\text{VO}_2$  and temperature (i.e. slope of plots in Fig. 3) during torpor arousal differed between treatments, being the slope in warm-acclimated animals larger than in cool-acclimated individuals (Fig. 3, ANCOVA,  $F_{1,18} = 55.594$ ,  $P < 0.001$ ). Further, maximum  $\text{VO}_2$  after torpor arousal was higher in warm-acclimated than in cool-acclimated animals ( $12.0 \pm 1.5$  and  $8.3 \pm 1.8 \text{ ml O}_2 \text{ g}^{-1} \text{ per h}$ , respectively,  $t_7 = 3.343$ ,  $P = 0.05$ , Figs. 2 and 3).

## 4. Discussion

### 4.1. Non-shivering thermogenesis

*T. elegans* did not show NST responses to NE injections, which is in agreement with what is usually known for other didelphids [5]. In general, adult marsupials do not perform NST, and in consequence they probably do not have BAT [12,36]. Alternatively, BAT may be present but it does not have thermoregulatory functions [21]. Any of these two alternatives raises the question of how *T. elegans* starts warming from torpor. In any event, we will try to answer this question by comparing the rewarming rate of *T. elegans* with that of other small mammals.

### 4.2. Rewarming rate

Rewarming rates of marsupials have been reported

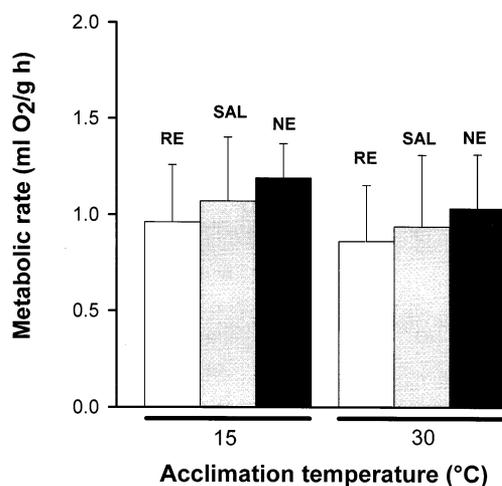


Fig. 1. Effects of saline (SAL) and norepinephrine (NE) injections on metabolic rate in warm and cool-acclimated *T. elegans* (mean  $\pm$  SD,  $n = 5$ ). Values of resting metabolic rate (RE) are indicated. Metabolic rate was measured at  $25^\circ\text{C}$ .

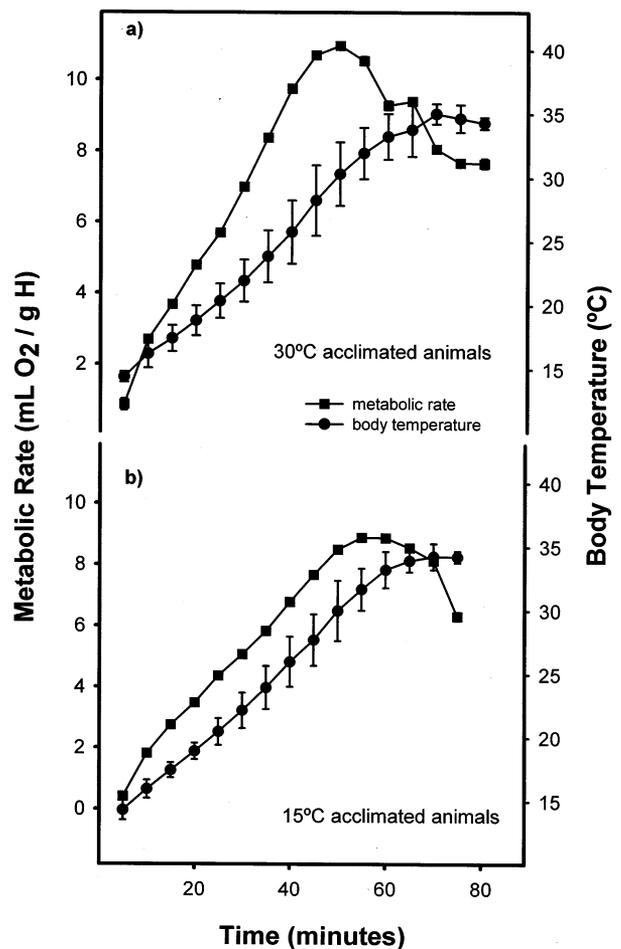


Fig. 2. Time course of metabolic rate and body temperature at  $T_a = 19^\circ\text{C}$  during torpor arousal in five warm-(a) and four cool-(b) acclimated individuals of *T. elegans*. Values are mean  $\pm$  SD. In many cases SD falls inside the symbols).

to be slower than [10] or similar to those of eutherians [4,8]. In comparison to other similarly sized marsupials measured at  $T_a = 18\text{--}23^\circ\text{C}$  [10,37] such as *Sminthopsis macroura* (28 g,  $0.7^\circ\text{C min}^{-1}$ ) and *Sminthopsis laniger* (27.4 g,  $0.75^\circ\text{C min}^{-1}$ ), rewarming rates of *T. elegans* were lower ( $0.329^\circ\text{C min}^{-1}$ ). A similar pattern is observed if we compare our findings with similarly sized rodents such as *Peromyscus leucopus* (23.9 g,  $0.42^\circ\text{C min}^{-1}$ , 10) and *Zapus princeps* (26 g,  $0.49^\circ\text{C min}^{-1}$ , 4). Further, our observed rewarming rates fall below what is predicted for a marsupial with the size of *T. elegans*. The expected rewarming rate for *T. elegans* ( $\log_{10}$  rewarming rate in  $^\circ\text{C min}^{-1} = 0.006 - 0.174 \log_{10} m_b$ , see Geiser and Baudinette, 10) is  $0.58^\circ\text{C min}^{-1}$  which is significantly higher (43.3%) than the observed value for *T. elegans* ( $t_{16} = 21.8$ ,  $P = 0.0001$ ). Thus, similarly sized marsupials can warm up faster than *T. elegans* [8,10,39], which may owe to the absence of NST in *T. elegans*.

#### 4.3. Effect of body mass, thermal conductance and ambient temperature

The main influences on energy cost and time-course of rewarming are body mass, thermal conductance and ambient temperature [8,10,14,24,29]. We controlled the first two by choosing a narrow gradient in acclimation temperature (15°C). Then, after just one month of thermal acclimation, the two groups of animals did not show significant differences in body size nor in thermal conductance ( $P = 0.67$  and  $P = 0.63$ , respectively, see Section 3). Certainly, different results can be obtained at different thermal conditions, but we selected 19°C to make our data comparable to published results (e.g. [4,10]) and to match field conditions as much as possible (see Section 2).

#### 4.4. Overshoot and metabolic cost of arousal

The relevance of metabolic cost during torpor arousal in marsupials has been addressed only recently [20,29]. Metabolic rate of *T. elegans* rises ca. tenfold during body rewarming from 15 to 35°C, at ambient temperature of 19°C. Such 10-fold increase is in agreement with previous findings [4,26,29]. We found that the maximum metabolic rate for torpor arousal of our warm-acclimated animals ( $12.0 \pm 1.5$  ml O<sub>2</sub> g<sup>-1</sup> per h) was higher than in other species of small marsupials such as *Sminthopsis macroura* (7.7 ml O<sub>2</sub> g<sup>-1</sup> per h) and *Sminthopsis laniger* (9.5 ml O<sub>2</sub> g<sup>-1</sup> per h, see [28]). However, values of arousal-maximum metabolism of our cool-acclimated *T. elegans* were similar to the above species (8.28 ml O<sub>2</sub> g<sup>-1</sup> per h, see Figs. 2 and 3).

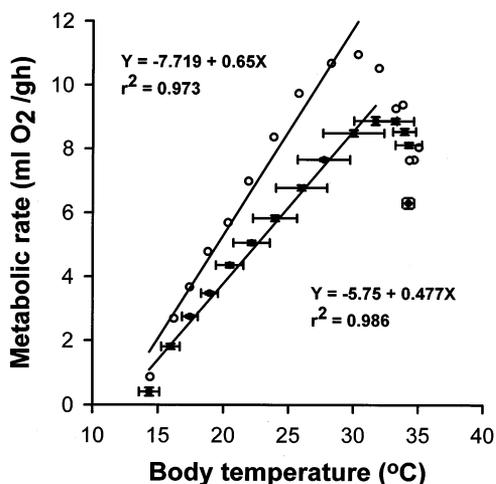


Fig. 3. Effect of acclimation temperature on rewarming rate from torpor in *T. elegans*. Four individuals were cool-acclimated (= black symbols) and five were warm-acclimated (= white symbols). Each data point represents mean  $\pm$  SD (in many cases SD falls inside the symbols). Regression analysis was performed between the minimum and maximum values of metabolic rate.

Studies documenting VO<sub>2</sub> and T<sub>b</sub> during torpor arousal in small mammals [4,11,19,26] often show an overshoot in VO<sub>2</sub> after euthermia is reached, which suggests the existence of a shivering mechanism by the end of arousal. Only our warm-acclimated animals showed such a response (Fig. 2a). The occurrence of this overshoot, and the different slopes detected in the relationship between VO<sub>2</sub> and T<sub>b</sub> (Fig. 3) indicate that acclimation to low temperature decreased the cost without influencing the rate of torpor arousal (see also [7,31]). Such finding is a novel feature previously unknown among heterothermic marsupials, which can be explained by several tissue and circulatory adjustments that make muscular contraction more efficient [38]. Based in this study, we suggest that shivering acclimation is an important mechanism for small hibernating mammals that face with low and fluctuating ambient temperatures (see also [33]). As with passive heating during thermal cycles in ambient temperature [29], physiological acclimation to low temperatures can minimize energy costs of torpor arousal, thus increasing reproductive output and survival in small mammals living in cold environments.

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