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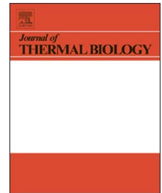
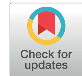


# Is there an effect of environmental temperature on the response to an antigen and the metabolic rate in pups of the rodent *Octodon degus*?



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A R T I C L E I N F O

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A B S T R A C T

Environmental temperature is a variable that influences all aspects of organisms, from physiological, e.g. immune function, and morphological traits to behavior. Recent studies have reported that environmental temperature modulates organisms' thermoregulatory capacity and immune response, suggesting that trade-offs must be made between thermoregulation and immune function. Despite this, studies that evaluate this trade-off in developing endotherms are scarce. The aim of this study was to evaluate the effects of environmental temperature experienced during development on the response to an antigen and its energetic costs in the precocial rodent *Octodon degus*. To accomplish this, we acclimated pups from birth to weaning at temperatures of 15 °C and 30 °C. At weaning, animals were inoculated with lipopolysaccharide (LPS) and cytokine interleukin-1β levels, sickness behavior, changes in body temperature and basal metabolic rate, and body mass were measured. Our results showed that environmental temperature influences cytokine levels, body temperature, and some aspects of sickness behavior. Specifically, acclimatization at 30 °C has a suppressive effect on the response to LPS, possibly due to a control to avoid overproduction of interleukin-1β. Body mass and basal metabolic rate were not affected by environmental temperature experienced during development, but inoculation with LPS affected both variables. Our results suggest that ambient temperature may be a key factor that affects the response to an antigen in pups of *O. degus;* however, no evidence of a trade-off between thermoregulation and immune function was found here.

## 1. Introduction

Ecoimmunological research focusing on trade-offs between the immune system and other fitness-related traits has received considerably more attention in recent decades (Bonneaud et al., 2003; Deerenberg et al., 1997; Cichon et al., 2002; Ilmonen et al., 2000; Nordling, 1998; Soler et al., 2003). These studies have shown that immune function is a costly trait in terms of energy and nutrients (Schmid-Hempel, 2011), and mounting an immune response negatively affects various components of fitness, including breeding effort (Bonneaud et al., 2003; Deerenberg et al., 1997; Ilmonen et al., 2000; Nordling, 1998), nestling growth rates (Soler et al., 2003) and thermoregulation (Cichon et al., 2002). Accordingly, it is assumed that in stressful conditions, typically accompanied by an increase in energy demand, the immune response is suppressed, thereby liberating resources that are reallocated to other costly traits such as thermoregulation (Ilmonen et al., 2003; Sheldon and Verhulst, 1996).

Thermoregulation is an important energetically expensive biological function (Konarzewski and Diamond, 1994). Studies have reported that animals acclimated at temperatures under the lower limit of the thermoneutral zone have higher metabolic rates than those acclimated at temperatures within the thermoneutral zone (Dawson et al., 1983; Hinsley et al., 1993; Maldonado et al., 2009; Repasky, 1991; Swanson, 1993; Wiersma et al., 2007; Withers and Williams, 1990). Hence, in endotherms, thermoregulation could be a major constraint to energy needed for immune function, and, thus, both functions could be compromised due to competition for common resources (Cichon et al., 2002). Therefore, it is expected that immune functionality is suppressed during the winter season or during conditions of low environmental temperatures (Cichon et al., 2002; Dabbert et al., 1997; Svensson et al.,

*Abbreviations:* mb, Body mass; Tb, Body temperature; LPS, Lipopolysaccharide; IL-1β, Interleukin-1β; BMR, Basal metabolic rate

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1998). While this reasoning is sound, studies evaluating the effects of environmental temperature on immune function have reported contradictory results (Burness et al., 2010; Cichon et al., 2002; Dabbert et al., 1997). For example, a negative effect of low temperatures on immune response was detected in the passerine *Cyanistes caeruleus* (Svensson et al., 1998), but not in the Bobwhite Quail (Dabbert et al., 1997).

In mammals, exposure to low environmental temperatures for periods of approximately 10 days negatively affects the immune response of adult mice (Cichon et al., 2002), while shorter exposures of about 24 h have no effect. This suggests that effects on the immune response depend on an individual's thermal history. In this sense, it would be expected that environmental temperatures experienced during development might impose a strong modulatory effect on the immune function of growing animals. Therefore, acclimation to two contrasting experimental ambient temperatures during development would provide an opportunity to explore potential trade-offs between immune function, thermoregulation, and growth.

To date, ecologists have predominantly focused on the immune system adaptive response as a measure of overall immunocompetence, but little is known about innate immunity (Bonneaud et al., 2003; Lee et al., 2006;Owen-Ashley and Wingfield, 2006). It has been proposed that the acute phase response (APR), belonging to the innate immune response, could be an important mediator of trade-offs between immunity and life-history traits (Bonneaud et al., 2003; Lee et al., 2006;Owen-Ashley and Wingfield, 2006) due to the very high costs involved in activating an APR (Bonneaud et al., 2003; Owen-Ashley and Wingfield, 2006). The APR refers to a global immune reaction to infection, tissue injury, trauma, neoplastic growth, or immunological disorders (Gordon and Koy, 1985; Gruys et al., 1999). During this reaction, macrophages and other immune cells synthesize pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor alpha (TNFβ; Baumann and Gauldie, 1994), and promote adaptive physiological and behavioral responses. Activation of the hypothalamic–pituitary–adrenal axis (HPA), synthesis of liver enzymes, and changes in core temperature are part of the physiological response. Sickness behaviors include crouching, piloerection, reductions in general activity and exploration, anorexia and increased lethargy to conserve energy (Aubert, 1999; Hart, 1988). These behaviors are required to control infection, collectively conserve heat, and promote fever (Oka, 2001); anorexia under these circumstances acts to reduce iron required for pathogen replication (Hart, 1988).

In order to explore putative trade-offs between traits, we evaluated the effects of environmental temperature experienced during development on the antigen response and basal metabolic rate of *Octodon degus* (Octodontidae; Hystricognathi). *Octodon degus* is a diurnal and precocial rodent (Fulk, 1976, Yáñez, 1976) naturally occurring in north-central Chile (Woods and Boraker, 1975). The social nature and communal breeding of this organisms results in an elevated risk of exposure to pathogens; thus, there are clear advantages to investing in immunity during the first days of life. We used lipopolysaccharide (LPS), the immunogenic component of the cell wall of Gram-negative bacteria, as the experimental antigen. It has been shown that LPS produces an inflammatory response in *O. degus* (Nemzek et al., 2008). Responding to LPS appears to be costly, thus providing an opportunity to study the trade-offs between the immune response and thermoregulatory abilities (Owen-Ashley and Wingfield, 2006).

## 2. Materials and methods

### 2.1. Animal capture and experimental setting

The experiments were run on the offspring of 29 females and 25 males of *Octodon degus*. The adults were captured in April and May of 2013 and 2014 in the Rinconada de Maipú (33°23′S, 70°31′W) in central Chile. Parental animals were maintained in laboratory conditions at an environmental temperature of 23 ± 1 °C and a photoperiod of 12 L: 12D. For breeding, females were monitored daily to determine when their vulva opened. Once opened, one female and one male were kept together for two weeks in rat cages (dimensions of cages were 58 × 36 × 30 cm) with a bedding of hardwood chips and water and food (commercial rabbit pellets) provided ad libitum. Once pregnancy had been confirmed, females were moved to individual cages and weighed every day. By the end of the gestation period (approximately three months) the cages were checked daily for the presence of pups. Subsequently, the date on which pup presence in the cage was detected was registered as the date of birth (day 0).

After parturition, each litter was randomly assigned to one of two experimental conditions, with one group being acclimated to 15 °C and the other to 30 °C. The temperature of 30 °C was selected because it is within the thermoneutral zone (Rosenmann, 1977) and therefore does not represent a challenge in terms of thermoregulation for adults of this species. Conversely, 15 °C was selected because it is below the thermoneutral zone and would likely be associated with increases in energy expenditure thus producing changes in thermoregulatory behavior (Nuñez-Villegas et al., 2014). The offspring were acclimated from day 0 until day 28, which is within the weaning period for this species (Reynolds and Wright, 1979). On day 28, pups of both groups were separated from the mother and transferred to a room with an environmental temperature of 23 ± 1 °C and photoperiod of 12 L: 12D. Both groups were inoculated on day 30 after birth. For each acclimation temperature, two pups were selected from each litter, resulting in two groups (15 °C and 30 °C) each with 16 pups and with equal male to female ratios. These two groups were then split again into experimental and control subgroups. One subgroup from each group was inoculated with lipopolysaccharide (LPS purified *Salmonella enterica*, Sigma; 500 μg/kg; Nemzek et al., 2003) while the other subgroup was injected with saline solution as a control (0.9% NaCl). In both cases, a final volume of 200 μl was used for the inoculations, and this was administered intraperitoneally. All inoculations were performed between 18:00 h and 19:00 h.

### 2.2. Body mass and body temperature

Using a digital scale ( ± 0.01 g), offspring body mass (mb) was measured before inoculation (0 h) and 24 h (24 h) after the immune challenge. Body temperature (Tb) was recorded in the abdominal area with a VeraTemp non-contact digital laser thermometer ( ± 0.2 °C) before inoculations (0 h) and 12, 15, 19, and 24 h after the immune challenge. These data allowed us to estimate Tb changes in response to inoculation with LPS.

### 2.3. Blood sampling and determination of Interleukin-1β (IL-1β) levels

After 24 h of inoculation with LPS or saline solution, blood samples (200 μl) were obtained from the lateral saphenous vein (UBC Animal Care Guidelines) for all pups. The total handling time measured from the initial restraint of an animal to the completion of the blood collection did not exceed 2 min. Blood samples were centrifuged at 15,000 rpm for 6 min, and plasma was separated and stored at −80 °C until subsequent assays were performed.

We used plasma samples to determine the levels of the pro-inflammatory cytokine IL-1β. The levels of IL-1β were measured using enzyme-linked immunosorbent assays (ELISA). Wells of a 96-well microtiter plate were coated overnight with monoclonal capture antibody (Life Technologies). Previous studies have reported cross-reactivity in the mouse ELISA kit (Becker et al., 2007). The plates were then blocked for one hour with PBS containing 1% BSA, 5% sucrose and 0.05% $NaN_3$. Serum samples were diluted in PBS, and placed in the wells in duplicate and incubated for 2 h at room temperature. Monoclonal antibody detection (Life Technologies) was subsequently added, and they were again incubated for a period of 2 h. After that, a solution (1:1 mixture of

$H_2O_2$ and tetramethylbenzidine) was added to each well and stopped with a solution of $H_2SO_4$ 2 N after 20 min. The absorbance of the plates was measured at 450 nm with a correction wavelength of 540 nm using an automated ELISA plate reader. All washings were done with PBS supplemented with Tween 20 (0.05%). Finally, the absorbance was used as a measure of the concentration of IL-1β, using a standard curve.

### 2.4. Sickness behavior data collection

The behavior of pups was recorded with a video camera (Handycam HDR CX220) mounted on a tripod in front of each cage. Video recordings were made over a period of 30 min after 15 h post-immune challenge. A record of the following behaviors was maintained: 1) crouching: characterized by a hunched posture with lowered head and hidden feet for a period of 60 s (number of periods in this position), 2) locomotion: movement from end to end of the cage (number of movements), and 3) eye closure: eyes closed for a period of 30 s (number of periods). These three behaviors have been reported during the acute phase response in pups of the species *Cavia porcelus*, another caviomorph rodent (Hennessy et al., 2004). Despite this, there are no previous records of sickness behavior in degus nor the exhibition of crouching behavior, eye closure, or decrease in locomotion as part of the inflammatory response in the young of this species.

### 2.5. Basal metabolic rate estimations (BMR)

The BMR of animals in the two acclimation groups was estimated by determining the rates of oxygen consumption ($VO_2$). Rates were measured 24 h before and after challenge with LPS or with the control. Oxygen consumption was measured using a computerized open-flow respirometry system (Sable Systems, Henderson, NV) calibrated with a known mix of oxygen (20%) and nitrogen (80%) that was certified by chromatography (INDURA, Chile). The measurements were performed in darkness in glass chambers, at an environmental temperature corresponding to the thermoneutral zone for this species (i.e., 30 ± 0.5 °C; Ronsenman, 1977). Metabolic chambers received dry air at a rate of 750 ml/min from a flow controller (Sierra Instruments). The air was dried before and after the chamber and monitored every 5 s using an oxygen analyzer 1FC-1B (Sable System). $CO_2$ was removed before entering the $O_2$ analyzer, and oxygen consumption was calculated using the Withers equation (1977, p 122): $VO_2 = [FR * 60 * (Fi\ O_2 - FeO_2)] / (1- Fi\ O_2)$, where FR is the flow rate in ml/min, and Fi and Fe are the fractions of $O_2$ concentration at the entrance and exit of the metabolic chamber, respectively.

### 2.6. Statistical analysis

The data were tested for normality prior to running any analyses. To meet the assumption of normality, body mass and basal metabolic rates were log-transformed. Additionally, the body temperature data were subjected to an inverse function transformation, and locomotion data were square root transformed. We analyzed the effects of environmental temperature and type of challenge (i.e., saline serum vs. LPS) on the dependent variables (i.e., response to antigen and BMR). The effect of environmental temperature on body mass prior to inoculation (0 h) was evaluated with an analysis of variance (ANOVA). After inoculation (24 h), the effect of environmental temperature and type of challenge on mb was assessed using a two-way analysis of variance (ANOVA). To evaluate specific differences *a posteriori Tukey* tests were used. Using a two-way repeated measures analysis of variance, the Tb of animals in the four treatments was compared over a period of 12 h after each inoculation; here, the effect of environmental temperature and type of challenge were the main factors, and time of inoculation was included as a random factor. An *a posteriori Tukey* test was used to assess specific differences in treatments.

The effect of environmental temperature and the type of challenge on IL-1β level was evaluated using a two-way analysis of variance (ANOVA). An *a posteriori Tukey* test was used to assess specific differences in IL-1β. Sickness behavior was analyzed using parametric and non-parametric analysis when ANOVA assumptions were not met. The effects of environmental temperature and the type of challenge on crouching and locomotion were evaluated using a two-way analysis of variance (ANOVA). Eye closure was evaluated by Aligned Rank Transform (ART; Wobbrock et al., 2011). This approach allows for factorial analysis of nonparametric data, including the interaction between factors. Here, a transformation is first performed that aligns the data of each effect; then the data are ranked. Then, the aligned and ranked data were analyzed using a factorial analysis of variance. An *a posteriori Tukey* test was again used to assess specific differences in sickness behavior.

The effect of environmental temperature on BMR prior to inoculation (0 h) was evaluated using an analysis of covariance with body mass at 0 h as the covariate. To evaluate the effect of type of challenge and environmental temperature on BMR, a two-way analysis of covariance was performed (ANCOVA) with body mass as the covariate. All statistical analyses were performed using Statistica for Windows program 7. The results are presented with the mean ± standard error.

## 3. Results

### 3.1. Changes in body mass (mb) and basal metabolic rate (BMR)

Initial mb (prior to inoculation, 0 h) was significantly affected by environmental temperature (ANOVA: $F_{1,18} = 7.16$; $p = 0.015$) where individuals reared at 15 °C had the lowest body mass (a *posteriori Tukey* test $p < 0.05$; Table 1). Challenge with LPS resulted in a significant loss of body mass (Table 2) with pups losing 2.7% of their initial weight (*a posteriori Tukey* test $p < 0.05$; Fig. 1). Neither environmental temperature nor the interaction between environmental temperature and type of challenge were found to affect body mass (Table 2).

BMR prior to inoculation (0 h) was not affected by environmental temperature (ANCOVA: $F_{1,30} = 0.004$; $p = 0.95$; Table 1). Significant effects on BMR due to the type of challenge were detected post inoculation (24 h); specifically, BMR increased in LPS challenged pups (*a posteriori* Tukey test, $p < 0.008$; Table 2; Fig. 2). Environmental temperature did not affect BMR (Table 2) nor did the interaction between

**Table 1**
Body mass (mb) and basal metabolic rate (BMR) measured pre (0 h) and post-inoculation (24 h) in offspring acclimated at 15 °C and 30 °C. Values are expressed as mean ± standard error.

| | Environmental temperature | | | |
|---|---|---|---|---|
| | 30 °C | | 15 °C | |
| | LPS (n = 8) | Saline (n = 8) | LPS (n = 8) | Saline (n = 8) |
| Mb pre-inoculation | 76.75 ± 6.01 | 73.37 ± 6.77 | 53.79 ± 2.59 | 52.07 ± 3.24 |
| Mb post-inoculation | 72.52 ± 6.1 | 71.79 ± 5.81 | 49.73 ± 1.04 | 57.6 ± 3.26 |
| BMR pre-inoculation | 81.56 ± 8.53 | 80.18 ± 11.4 | 56.16 ± 5.05 | 70.19 ± 8.55 |
| BMR postinoculation | 100.47 ± 7.76 | 78.17 ± 7.72 | 79.83 ± 5.37 | 69.87 ± 6.23 |

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**Table 2**
Results of the two-way analysis of variance (ANOVA).

| | **Challenge** (df), F, p | | | **Temperature** (df), F, p | | | **Temperature x challenge** (df), F, p | | |
|---|---|---|---|---|---|---|---|---|---|
| Mb | (1,29) | 8.49 | 0.007 | (1,29) | 3 | 0.09 | (1,29) | 2.73 | 0.11 |
| BMR | (1,27) | 12.17 | 0.002 | (1,27) | 0.00003 | 0.1 | (1,27) | 0.03 | 0.86 |
| IL−1b | (1,16) | 8.71 | **0.01** | (1,16) | 3 | 0.1 | (1,16) | 7.46 | 0.01 |
| Locomotion | (1,29) | 13.76 | 0.001 | (1.29) | 3.99 | 0.06 | (1,29) | 1.11 | 0.3 |
| Crouching | (1,29) | 10.23 | 0.003 | (1,29) | 2.92 | 0.09 | (1,29) | 0.26 | 0.4 |
| Eyes closed | (1,29) | 103.67 | < **0.0001** | (1,29) | 60.4 | < **0.0001** | (1,29) | 26.78 | 0.5 |

Analysis performed to evaluate changes in body mass and BMR, levels of IL-1b and sickness behavior of pups challenged with LPS and a saline solution and acclimated to two different environmental temperatures (30 °C and 15 °C). Significant values of the factorial ANOVA are shown in **bold**. d.f. = degree of freedom; F = F-value; p = p-value.

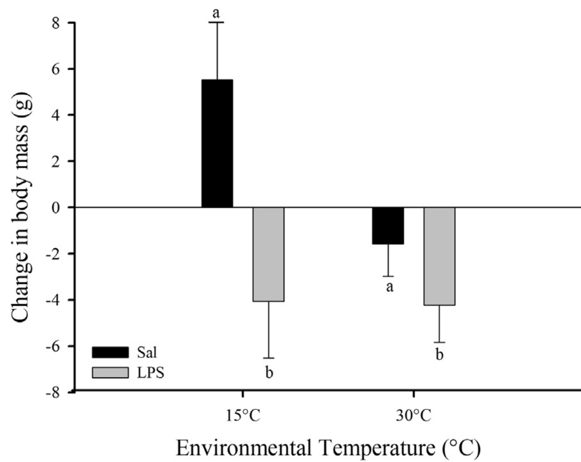


**Fig. 1.** Change in body mass of *O. degus* pups challenged with LSP or a saline serum and acclimated at one of two environmental temperatures (15 °C and 30 °C). Different letters denote significant differences between treatments.

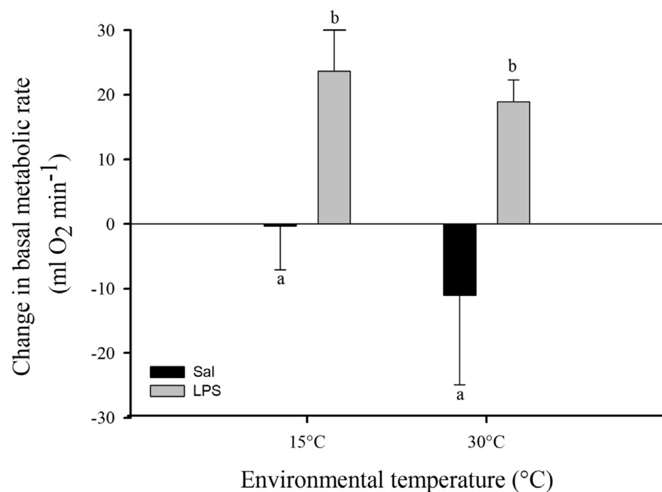


**Fig. 2.** Change in basal metabolic rate of offspring of *O. degus* challenged with LSP or saline serum and acclimated at one of two environmental temperatures (15 °C and 30 °C). Different letters denote significant differences between treatments.

type of challenge and environmental temperature (Table 2).

### 3.2. Sickness behavior, body temperature, and IL-1β levels

Comparison of body temperature at 0 h, 12 h, 15 h, 19 h and 24 h post-challenge showed a significant effect of post-inoculation time and of the interaction between post-inoculation time and type of challenge (Table 3). At 19 h post-challenge, the lowest body temperatures were detected in pups treated with LPS (a *posteriori Tukey* test p = 0.001; Fig. 3). There were no significant effects on body temperature due to

**Table 3**
Results of two-way repeated-measures analysis of variance (ANOVA) on body temperature.

| | **d.f.** | **F** | **p** |
|---|---|---|---|
| **Between effects** | | | |
| Temperature | 1,18 | 0.01 | 0.92 |
| Challenge | 1,18 | 1.9 | 0.19 |
| Challenge × Temperature | 1,18 | 1.1 | 0.31 |
| **Within effects** | | | |
| h.p.i | 4,72 | 17.7 | < **0.0001** |
| h.p.i × temperature | 4,72 | 1.8 | 0.14 |
| h.p.i × challenge | 4,72 | 2.7 | **0.03** |
| h.p.i. × challenge × temperature | 4,72 | 1.4 | 0.24 |

Analysis performed to evaluate changes in body temperature (°C) in pups challenged with LPS and saline solution at two different environmental temperatures (30 °C and 15 °C) and among different hours post-inoculation (h.p.i). The significant values of the factorial repeated-measures ANOVA are shown in **bold.** d.f. = degree of freedom; F = F-value; p = p-value.

the interaction between post-inoculation time and environmental temperature (Table 3) or due to the interaction between the three variables (Table 3).

A significant effect of type of challenge and of the interaction between environmental temperature and type of challenge on IL-1β levels was observed (Table 2). Specifically, higher levels of this cytokine were detected in LPS challenged pups acclimated to the lower temperature range (*a posteriori Tukey* test p < 0.05; Fig. 4). Environmental temperature did not affect IL-1β levels (Table 2).

Inoculation with LPS produced a significant reduction in locomotion (Table 2; Fig. 5a) although environmental temperature was not significantly affected (Table 2). The interaction between environmental temperature and type of challenge did not significantly affect this behavior (Table 2). While the crouching intervals of LPS challenged pups were observed to increase (Table 2; Fig. 5b), neither acclimation to environmental temperature (Table 2) nor the interaction between type of challenge and environmental temperature (Table 2) had significant effects on crouching behavior. Since eye closure events were not observed in several cases (and therefore are represented as zeros) non-parametric tests were used. The analysis of variance with a two-way ART procedure (see methodology) showed a significant effect of environmental temperature (Table 2) on eye closure; pups reared at 15 °C displayed this behavior more frequently than those reared at 30 °C. Similarly, type of challenge significantly affected the number of times pups performed this behavior (Table 2); specifically, pups treated with LPS closed their eyes more frequently. Finally, there was a significant effect of the interaction between type of challenge and environmental temperature (Table 2); eye closure intervals occurred most frequently in LPS challenged young acclimated to 15 °C (a *posteriori Tukey* test p < 0.05; Fig. 5c).

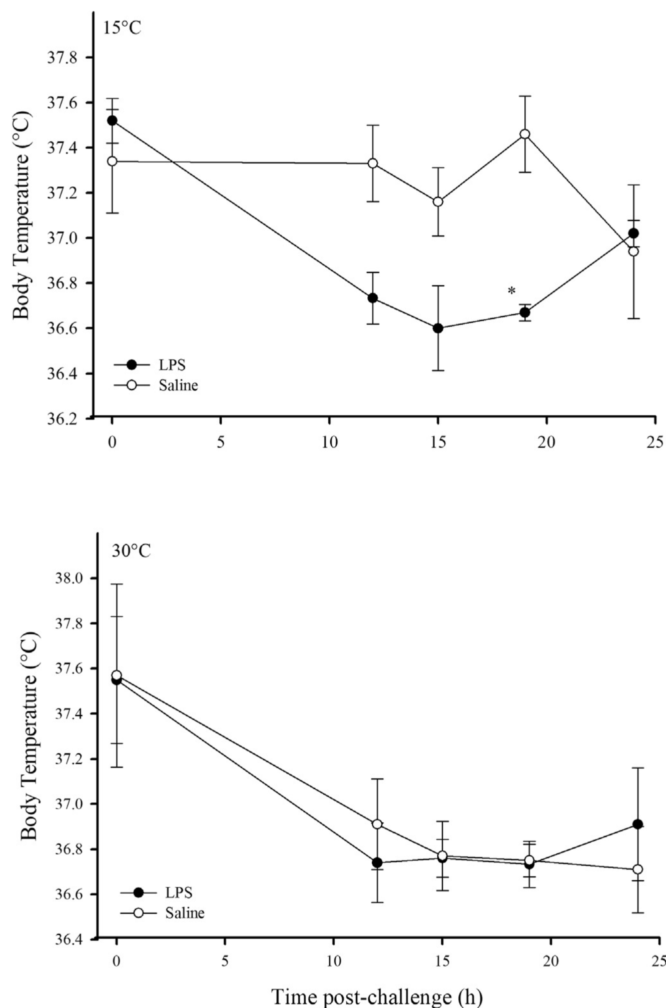


**Fig. 3.** Body temperature recorded at 0, 12, 15, 19 and 24 h after LPS challenge (closed circles) or saline inoculation (open circles) in pups of *O. degus*, acclimated at two different temperatures: 15 °C (upper panel) and 30 °C (lower panel). The inoculations began at 19:00 (0 h). Asterisk denotes a significant difference.

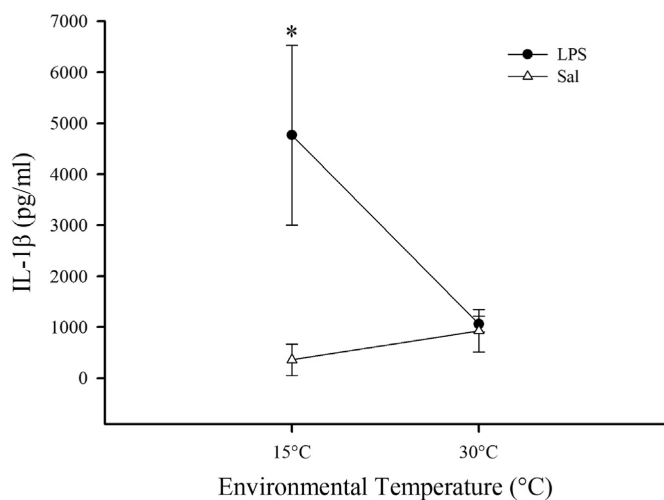


**Fig. 4.** IL-1β levels of *O. degus* pups challenged with LPS (closed circles) or treated with saline serum (open circles) and acclimated to one of two environmental temperatures (15 °C and 30 °C). Asterisk denotes a significant difference.

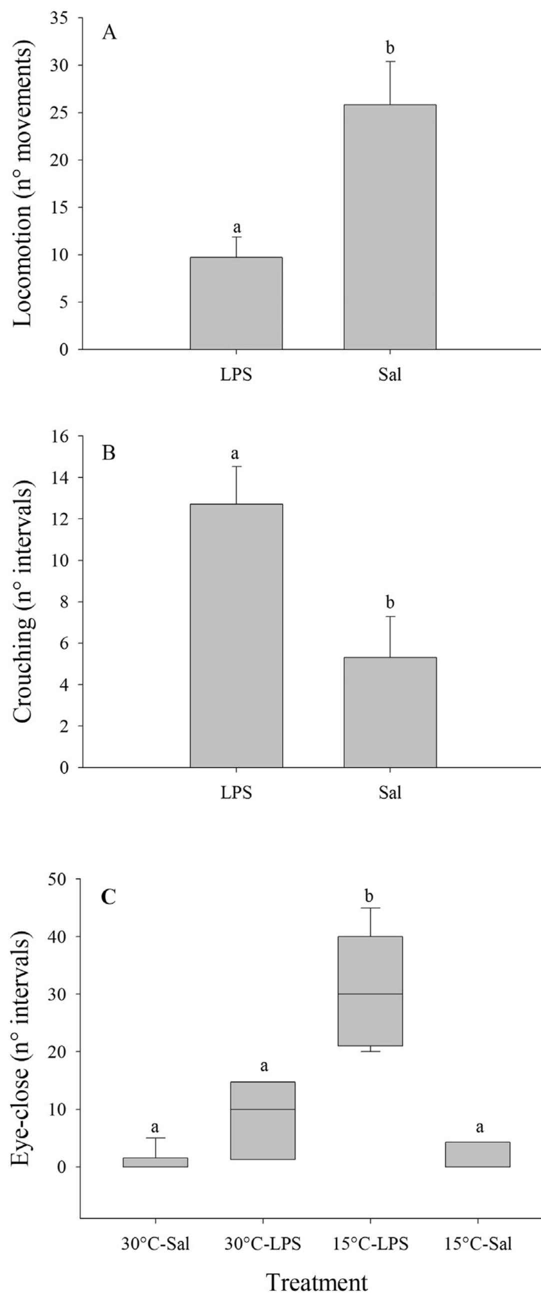


**Fig. 5.** Sickness behavior. A) locomotion, B) crouching and C) eyes closed. Records were made 15 h after treatment with LPS (black bars) or saline (gray bars) in pups of *O. degus* acclimated to 15 °C or 30 °C. Different letters denote significant differences between treatments.

## 4. Discussion

In this study, we evaluated the effect of exposure to contrasting environmental temperatures during development (15 °C and 30 °C) on the response to LPS in pups of *Octodon degus*. We hypothesized that there would be a trade-off between the cost of responding to an antigen and the cost of thermoregulation. Thus, we predicted a reduction in the magnitude of the response to an antigen in individuals acclimated to low environmental temperatures. Despite this, we found no evidence of such a reduction.

### *4.1. Changes in body mass and basal metabolic rate*

Environmental temperature was found to affect body mass prior to

the LPS challenge, with greatest reductions in mass observed in pups reared at 15 °C. After LPS injection, we expected pups reared at 30 °C to display greater reductions than pups reared at 15 °C, since the response to LPS is assumed to be energetically costly (Owen-Ashley and Wingfield, 2006), and only pups in good condition or with the greatest body masses should theoretically be able to mount a strong immune response (Burness et al., 2010). However, there was no significant effect on body mass following LPS inoculation of animals acclimated to either environmental temperature; reductions in mass following LPS injections in both treatments were similar (Fig. 1). Several studies have reported a reduction in body mass in adults and young animals challenged with an antigen (Brommer et al., 2004; Burness et al., 2010; Moreno-Rueda, 2011). This reduction may, in part, be attributable to the cost of the antigen response and the associated re-allocation of resources (Ots et al., 2001) or to a reduction in feeding rate as a consequence of sickness behavior (Bonneaud et al., 2003). We cannot rule out either of the two explanations, however, since we do not have data on feeding rates after pups were challenged with the antigen, nor do we have estimates of the energetic cost of the response to LPS.

Relative to the control group, the BMR of the group acclimated to 15 °C and challenged with LPS increased in 28.5% of the pups while the BMR of the group acclimated to 30 °C and challenged with LPS increased in 14.3% of the pups. In this vein, Demas et al. (1997) found that the resting metabolic rate of adult mice increases by 27% in response to keyhole limpet hemocyanin (KLH) inoculation. However, Pilorz et al. (2005) report that the metabolic rate of guinea pig pups does not change in response to the KLH inoculation. The increase in BMR in our study is well within the values previously reported for others rodent individuals (Demas et al., 1997; Schmid-Hempel, 2011), but the increases in BMR for both environmental temperature were not significantly different (Fig. 2). The absence of a significant effect of environmental temperature on the basal metabolic rate of LPS challenged animals may be explained in part by the duration of the period of acclimation. It has been reported that prolonged exposure to cold in adult mice results in suppression of the immune response due to adjustments in thermoregulatory capacity (Cichon et al., 2002). However, it is possible that the opposite might occur in pups where prolonged exposure to certain environmental conditions involves more acclimation and therefore reduced susceptibility to immune challenge (Thaxton, 1978). Thus, the acclimation from birth to weaning may involve the maintenance of a safe energy margin in case of unexpected energy demands (Diamond and Hammond, 1992).

### 4.2. IL-1β levels and changes in body temperature

Our results reveal that *degus* acclimated to 15 °C and challenged with LPS possessed the highest IL-1β levels in this study (Fig. 4). It has been documented that the overproduction of IL-1β is related to septic shock and death in animals, including humans (Natanson et al., 1989; Rees et al., 1990). Fairchild et al. (2000) report that at temperatures near 37 °C an explosion/suppression of the production of proinflammatory cytokines is observed so that individuals avoid prolonged exposure to the potentially cytotoxic effects of these cytokines. Thus, it is possible that exposure to 30 °C causes a reduction in the production of IL-1β to avoid decontrolled expression of this cytokine and accompanying cell injury, septic shock, and death of the animal (Muñoz et al., 1991; Tracy et al., 1990). Similar results have been observed in chicks of domestic chickens where temperatures around 32 °C produce a reduction in antibody levels (Thaxton, 1978). On the other hand, there are several studies reporting that low temperatures stimulate immune responses in both adult (Kaunisto et al., 2015; Zhang et al., 2015) and young individuals (Henken et al., 1983a; Thaxton et al., 1978), and conversely, high temperatures suppress immune responses. Consequently, Henken et al. (1983) propose that more extreme reduced environmental temperatures (below 15 °C) are necessary to cause a suppression of the immune response in developing organisms as there is a trade-off between maintaining normothermia and mounting an immune response.

Regardless of environmental temperature, the body temperature of pups challenged with LPS decreased while the control group (saline) displayed no changes in this respect throughout the study (Fig. 3). It is well documented that the febrile response (hyperthermia) is part of the APR (Owe-Ashley and Wingfield 2006). Here, pups of *Octodon degus* acclimated at both temperatures (15 °C and 30 °C) showed hypothermia. Hyper or hypothermia has been reported to result as a response to LPS in endotherms (Rudaya et al., 2005). In fact, it has been argued that both hyperthermia as well as hypothermia represent two different strategies to combat systemic inflammation, each having developed as an adaptive response to environmental conditions, specifically habitat productivity (Romanovsky and Szekely, 1998). In this sense, in geographic regions characterized by low habitat productivity (as is the case in central Chile), hypothermia represents an adaptive response against pathogens (Martin et al., 2008).

### 4.3. Sickness behavior

Our results show evidence of a stereotype behavior in response to a nonpathogenic antigen in *O. degus*. Specifically, inoculation with LPS resulted in an increase in crouching behavior and a decrease in locomotion. Behavioral changes following LPS inoculation, such as those recorded here, have been previously described in studies conducted in other precocial rodents (Hennessy et al., 2004) and birds (Burness et al., 2010). These studies have documented that individuals challenged with LPS showed a decrease in locomotion and an increase in resting (crouching) states (Hennessy et al., 2004). A decrease in activity and increased rest intervals may have an adaptive function if they allow individuals to overcome infection and therefore enhance their probability of survival (Hart, 1988; Wingfield, 2003). Both locomotion and crouch intervals were not affected by the interaction between environmental temperature and type of challenge. The only behavior affected by the interaction between treatments was the duration of eye closure; contrary to our expectations, LPS challenged pups acclimated at 15 °C engaged in this behavior for longer periods of time than pups challenged with LPS and acclimated to 30 °C.

In conclusion, our results suggest that environmental temperature, at least to some degree, affects the response to LPS by developing degus. In this regard, and contrary to our expectations, exposure to low temperatures appears to enhance the production of the cytokine IL-1β. Further, the increase in the levels of this cytokine is not related to an increase in BMR. It is possible that the dietary regime of animals in this study could mask effects of environmental temperature on rates of energy expenditure. For example, in chicks of *Riparia riparia* (Brzęk and Konarzewski, 2007), the costs of immune response have been found to be dependent on environmental factors that include food availability. The fact that food was provided ad libitum in this study could explain the absence of environmental temperature effects on most of the variables analyzed. Future research should consider the possible effect of diet quality and caloric intake on immune function and its possible interaction with other environmental variables.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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