Acute-Phase Immune Response Involves Fever, Sickness Behavior, and an Elevated Metabolic Rate in the Subterranean Rodent *Ctenomys talarum*

A. P. Cutrera*
F. Luna
R. R. Zenuto
Laboratorio de Ecología Fisiológica y del Comportamiento, Instituto de Investigaciones Marinas y Costeras (IIMyC), Universidad Nacional de Mar del Plata, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Correo Central 1245, Mar del Plata, Argentina

Accepted 11/29/2021; Electronically Published 2/11/2022

ABSTRACT

The acute-phase response (APR) is an induced innate response and may involve pronounced physiological and behavioral changes. One of the most common assays to study the APR involves the use of a lypopolysaccharide (LPS) from the cell wall of gram-negative bacteria. In this study, we determined the energetic costs of the APR in the subterranean rodent *Ctenomys talarum*, as well as the effects of the exposure to LPS on body temperature, body mass loss, and behavior in this species. Furthermore, we monitored levels of circulating endotoxin after LPS exposure. Our results suggest that in *C. talarum*, the APR is energetically costly, resulting in a 14% increase in metabolic rate. Animals exposed to LPS experienced a short-term thermal response, weight loss, and changes in their behavior that included more time spent resting and with their eyes totally or partially closed. However, the magnitude of the effects of LPS exposure varied between sexes and among animals. Also, there was a clear peak in circulating endotoxin levels in plasma 3 h postinjection (hpi) and a significant decrease of these levels 24 hpi, but peak endotoxin concentration values recorded were highly variable among animals. In light of these results, ecological determinants of immune function variation in tucotucos are discussed considering the roles of pace of life, habitat, and degree of pathogen exposure in these subterranean rodents.

Keywords: acute-phase response (APR), lypopolysaccharide (LPS), metabolic costs, life history.

*Corresponding author; email: acutrera@mdp.edu.ar.

Introduction

The field of ecoimmunology aims to understand the factors leading to immune function variation and disease susceptibility by examining the interaction between host physiology and disease ecology (Demas and Nelson 2012). Under this perspective, the study of trade-offs among competing fitness-related traits (Stearns 1989) is central (for a review, see Demas et al. 2012). This approach relies on two pivotal ideas: (1) that an animal's body has limited resources, which could be in the form of energy, food, or time (Hasselquist and Nilsson 2012; Lopes 2014), and (2) that activating and maintaining an immune response is costly and hence requires a reallocation of resources toward immunity at the expense of other body functions (Sheldon and Verhulst 1996; Schmid-Hempel 2011).

The energetic costs of immunity can be estimated directly by measuring the increase in metabolic rate associated with the activation and maintenance of an immune response (reviewed in Demas et al. 2012) or indirectly by looking at negative correlations between the magnitude of an immune response and life history traits, such as reproduction or growth (Lochmiller and Deerenberg 2000; Norris and Evans 2000). The different arms of immunity (innate vs. adaptive) are expected to have different costs and benefits and thus may vary differently with life history (reviewed by Lee 2006).

Innate immunity defenses include constitutive elements, such as cells (e.g., macrophages, granulocytes, and natural killers) and antimicrobial proteins that circulate at low levels in the blood, providing a rapid first-line of defense (Murphy and Weaver 2016). The metabolic costs of constitutive innate immune defenses are considered to be comparatively low (Klasing and Leshchinsky 1999; Lee 2006) mainly because they do not involve cell proliferation and development, which are part of the adaptive immune response (Lee et al. 2006). However, if the challenge is sufficiently strong, the highly costly systemic inflammatory response (or acute-phase response [APR]) may be elicited. This response is characterized by pronounced physiological and behavioral changes. The first include increased production of acute-phase proteins in the liver, as well as elevated body temperature (T_b; fever), while the second are grouped into a syndrome referred to as sickness behavior (Hart 1988; Dantzer and Kelley 2007) that involves anorexia (lack of appetite), analgesia (decreased pain sensitivity), anhedonia (lack of interest in pleasurable activities), and decreased locomotor activity. This sickness behavior syndrome is thought to

Physiological and Biochemical Zoology, volume 95, number 2, March/April 2022. https://doi.org/10.1086/718409

be adaptive, leading to heat conservation and hence promotion of fever, and decreasing blood levels of critical nutrients available for pathogen proliferation (Weinberg 1984; Hart 1988; Klasing and Leshchinsky 1999; Drakesmith and Prentice 2012), all of which may help to control infection. Sickness behavior may also entail individual isolation, contributing to reduced pathogen transmission among individuals and decreased social interactions, which may involve an energy cost to the sick animal (Moreno et al. 2021).

Although the metabolic costs associated with mounting an APR are considered extremely high, measurements of metabolic rate are not conclusive. One of the most used assays to study the APR involves the use of a lypopolysaccharide (LPS) from the cell wall of gram-negative bacteria (e.g., *Escherichia coli*). This antigen mimics a bacterial infection by increasing the release of proinflammatory cytokines, inducing a rapid systemic response by nonspecifically activating a wide array of immune cells only a few hours after exposure, but without actually infecting the animal with a pathogen. The metabolic costs of the APR elicited by LPS in endotherms have been measured mostly in birds (e.g., Burness et al. 2010; Marais et al. 2011; King and Swanson 2013; Sköld-Chiriac et al. 2014; Martin et al. 2017), and in a few mammals, both in standard laboratory models (*Mus musculus*: Baze et al. 2011; *Rattus norvegicus*: MacDonald et al. 2012) and in wild populations (fish-eating bat, *Myotis vivesi*: Otálora-Ardila et al. 2017; Pallas's long-tongued bat, *Glossophaga soricina*: Cabrera Martínez et al. 2018; degu, *Octodon degus*: Ramirez-Otarola et al. 2018) with contrasting results. While some mammal species showed a large increase in metabolic rate associated with the activation of APR shortly after exposure to LPS (e.g., fish-eating bats: Otálora-Ardila et al. 2017), others showed moderate (brown rat: MacDonald et al. 2012; degu: Ramirez-Otarola et al. 2018) to low (Pallas's long-tongued bat: Cabrera-Martínez et al. 2018) or null (mice: Baze et al. 2011) increase in their metabolic rate. Also, closely related species show marked variation in the strength of the APR symptoms, such as fever, anorexia, or levels of activity (e.g., *Peromyscus* sp.: Martin et al. 2008), suggesting a continuum of immunological strategies among *Peromyscus* species. As described by the authors, this may also apply to other taxa, according to their life history (Martin et al. 2008), emphasizing the need to explore this continuum in wild species distributed in diverse habitats and facing distinct challenges in terms of energy allocation toward immunity.

Ctenomys talarum (Talas tuco-tucos, Thomas 1898) is a South American subterranean rodent that lives solitarily in permanently sealed burrows, where most of their activities take place (Busch et al. 1989, 2000). The relatively low parasite richness of gastrointestinal helminths observed in this species (Rossin and Malizia 2002) and other subterranean rodents has been related to their solitary existence underground (Novikov et al. 2016). Besides limiting convective heat loss, the moist and stagnant conditions of the subterranean environment are hypothesized to have favored the low basal metabolic rate (BMR) observed in *C. talarum* (Busch 1989; Luna et al. 2009) and other subterranean rodents (McNab 1966). This low BMR has been associated, at least in part, with other singular traits of this species: tuco-tucos live "slow pace" reproductive lives; they have a delayed sexual maturity (6 and 9 mo for females and males, respectively; Busch et al. 1989); and females have a long gestation period (95 d) and give birth to altricial pups (Zenuto et al. 2002) only twice a year (Busch et al. 1989). For this species, the metabolic costs of mounting an antibody-mediated response to sheep red blood cells (SRBCs; Cutrera et al. 2010), as well as those of the local inflammatory response to phytohemagglutinin (PHA; Merlo et al. 2014*b*), were estimated using respirometry in captivity, suggesting that metabolic costs are variable among the different arms of immunity of tuco-tucos (Cutrera et al. 2010; Merlo et al. 2014*b*). Our goal in the present study was to determine the energetic costs of the APR in *C. talarum*, as well as the effects of the exposure to LPS on T_b, body mass loss, and behavior in this species. Furthermore, we monitored levels of circulating endotoxin after LPS exposure, to estimate how quickly they decrease, which is a measure of how rapid the inflammation could be resolved (Graham et al. 2005). We hypothesized that the antigen LPS would elicit a fever response, which is energetically costly. Sickness behavior, mainly in the form of reduced activity, can be associated with heat conservation and energy saving during the fever response. Times of peak and decrease of circulating endotoxin are expected to match those of fever and sickness behavior.

Material and Methods

Animal Capture and Housing Conditions

Adult *Ctenomys talarum* (tuco-tucos) of both sexes were caught with wire tube-shaped traps (diameter = 10 cm, length = 35 cm) set at fresh surface mounds, which are indicative of recent digging, inside the burrow. Captures took place during nonreproductive seasons (mid-February to mid-April 2017, 2018, 2019) and reproductive seasons (September to early December 2018, 2019) near the locality of Mar Azul, Buenos Aires Province, Argentina (37°34′S, 57°03′W). Only pregnant females were brought to the laboratory during the reproductive season; nursing females were immediately returned to their burrows after capture to avoid distressing the pups left in the nest. Animals were transported to the Laboratorio de Ecología Fisiológica y del Comportamiento, Instituto de Investigaciones Marinas y Costeras, Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de Mar del Plata, where they were weighed and placed in individual plastic boxes (45 cm × 32 cm × 26 cm tall) with a wire-mesh lid, wood shavings for bedding, and half terra-cotta flowerpot as refuge. Animals were fed daily with fresh food (mixed grass, lettuce, corn, sweet potatoes, and sunflower seeds) ad lib. to secure water provision since *C. talarum* does not drink free water. Photoperiod and temperature were automatically controlled (12L∶12D; 25°C ± 1°C). Tuco-tucos remained captive for the duration of the experimental assays (ca. 2 wk), after which they were released at the point of capture. We adhered to the 2012 revised International Guiding Principles for Biomedical Research Involving Animals developed by the Council for International Organizations of Medical Sciences and the International Council for Laboratory Animal Science, as well as the National Institutes of Health guidelines for the care and use of laboratory animals (publication 8023, revised 1978). All procedures were revised and approved by the local committee for animal use and care in research (Comité Institucional

para el Cuidado y Uso de Animales de Laboratorio, FCEyN UNMdP RD 467-17).

Immunization

Immediately after capture, all animals were randomly assigned to two groups: control (C) and immune challenged (IC). Animals remained in captivity for 10 d before receiving the injection because a previous study showed that this is the necessary time lapse for animals to acclimatize to captivity conditions and, hence, lower their stress levels (Vera et al. 2008). In all of the experimental procedures described below, animals of the IC group were weighed and injected subcutaneously in the lower abdomen with 1 mg/kg of animal body mass of LPS (from *Escherichia coli* O26 : B26, 1 mg/mL solution, Sigma L8274, Sigma Chemical, St. Louis, MO) and animals of the C group were weighed and injected with 1 uL/g of animal body mass of sterile saline solution. The LPS dose used in this study (1 mg/kg) was selected because it was within the spectrum most frequently used for rodents (e.g., Ross et al. 2001; Li and Blatteis 2004; Martin et al. 2008; Baze et al. 2011) and it was enough to elicit an APR response in tuco-tucos, without compromising their recovery.

Because it was impossible to use the same set of animals to measure all the variables involved in the response to LPS, given that some of the variables had to be measured at the same time after injection (e.g., behavior and metabolic rate) and that some measurements could be disruptive to the assessment of variables (e.g., blood collection for endotoxin detection and behavior recording/metabolic rate measurement), we decided to use different groups of animals for different purposes as follows (table 1). First, we characterized the change in T_b (thermal curve) and body mass in response to LPS, in order to study the temporal pattern of the immune response and define the time points to measure the rest of the variables. Changes in metabolic rate and T_b associated with the APR were recorded in the reproductive and nonreproductive seasons to assess possible trade-offs between immunity and reproduction. Sickness behavior was recorded during the reproductive season, when the energetic demands are more markedly different between sexes particularly due to the costs of gestation in *C. talarum* (Zenuto et al. 2002). Finally, change in levels of circulating LPS in time over a 24-h period was recorded to establish how rapidly the endotoxin was cleared in the organism and, hence, how quickly the inflammation could be resolved. Specifically, to study the temporal pattern of the APR response in *C. talarum*, changes in T_b and body mass elicited by LPS were recorded in 20 animals captured in the nonreproductive season of 2017 and assigned to one of the experimental groups: IC (5 females and 5 males) and C (5 females and 5 males; table 1). To measure T_b and metabolic rate in response to LPS, a total of 44 animals were captured during the reproductive seasons of 2017 and 2018 (IC = 6 males and 5 females, C = 8 males and 5 females) and the nonreproductive seasons of 2018 and 2019 (IC = 5 males and 5 females, C = 5 males and 5 females). In another set of experiments, we assessed the sickness behavior and the accompanying changes in T_b and body mass elicited by LPS exposure. To assess patterns of sickness behavior, a total of 52 animals (IC = 13 males and 15 females, C = 11 males and 13 females) were captured during the reproductive seasons of 2018 and 2019 (table 1). Finally, to monitor levels of circulating LPS, we used 10 tuco-tucos injected with LPS (5 males and 5 females) and three animals as negative controls (C group = 2 males and 1 female) captured during the nonreproductive season of 2019 (table 1).

Thermal Response Curve and Body Mass Change

To study the animals' T_b response to LPS over time, injections to animals in the C and IC groups were applied at 0800 hours and animals were placed in plastic boxes with no bedding or food inside a temperature test chamber (SEMEDIC, M5000) where ambient temperature (T_a) was maintained constant at 25°C, within the thermoneutral zone of *C. talarum* (Busch 1989). T_b was monitored just before the injections were applied and after, every hour over the next 12 h, as this is the time lapse within which the APR's thermal response occurs in other small mammal species (e.g., Ross et al. 2001; Martin et al. 2008). The rectal T_b was recorded using a YSI probe (93k73545-402) connected to a Cole-Parmer thermistor (8402–10; ±0.1°C; Vernon Hills, IL). Body mass was recorded just before LPS/saline injection to ensure appropriate LPS dosage using an electronic scale (FX-3000, AND; ±0.01 g) and after the 12-h period, when the trial ended.

Metabolic Rate

Metabolic rate measurements were performed a day before (day −1) the injection with LPS or saline (at 0800 hours) 1 and 6 hpi. We scheduled metabolic rate measurements on these two time points to estimate the cost of mounting an immune response against LPS in tuco-tucos (*a*) while the thermal response

Table 1: Sample sizes (*n*), year, season, and data collection time of each assay performed in this study

Assay	*n*	Year	Season	Time (hpi)
Thermal curve and body mass	20	2017	NR	0, 1, . . . , 12
Metabolism and T_b	44	2017–2019	NR, R	0, 1, 6
Sickness behavior	52	2018–2019	R	0, 1, 6, 24
Endotoxin clearance	10	2019	NR	0, 1, 24

Note. NR = nonreproductive; R = reproductive; hpi = hours postinjection; T_b = body temperature.

was developing before the peak response detected 1 hpi (according to the results of the thermal response curve, see below) and (*b*) around the time where signs of sickness behavior were recorded as preliminary data during the thermal response trial (6 hpi). Body mass and T_b were recorded immediately before and after measuring the metabolic rate. Animals spent a maximum of 90 min inside the metabolic chamber, but the metabolic rate of animals was measured during 60-min trials using a computerized positive-pressure open-flow respirometry system (Sable System, Las Vegas, NV) as previously done in *C. talarum* (Cutrera et al. 2010; Merlo et al. 2014*b*) to evaluate the energetic cost of an immune response in this species. Oxygen consumption ($\dot{V}_{O_2}$) was measured at 25°C (within the species thermoneutral zone; Busch 1989) in the morning (1 h before injection and 1 hpi) and in the afternoon (6 hpi), considering that *C. talarum* exhibits an arrhythmic pattern of daily activity (Luna et al. 2000). Animals were checked to ensure that they were inactive (in resting position, not moving or scratching the walls). The Perspex chamber received dry and CO_2-free air at 1,000 mL/min from a mass flow controller (Sierra Instruments, Monterey, CA).

Air passed through a CO_2-absorbent (self-indicating IQB, IQB Laboratories, Quilmes, Argentina) and water scrubber (Drierite, Hammond Drierite, Xenia, OH) before and after passing through the chamber. Excurrent air from the chamber was subsampled at 120 ± 10 mL/min and $\dot{V}_{O_2}$ was obtained from an oxygen analyzer FC-1B every second set by a ExpeData-PC program (Sable Systems). The baseline of the respirometry system was set in 20.95% of O_2 before the beginning of each experiment. The metabolic rate was calculated using equation (4*a*) in Withers (1977). The metabolic rate was measured as the 5-min lowest steady-state values of the last 30 min of a 90-min trial (Luna and Antenucci 2007*a*). The metabolic rate was reported at 0 h (preinjection trial, which could be considered as the resting metabolic rate [RMR]) and 1 and 6 hpi. For comparative purposes, data were expressed as mass-specific metabolic rate (mL O_2/g/h) and kilojoules per day. An equivalent of 20.08 J/mL O_2 was used to convert metabolic rate to energy values (Schmidt-Nielsen 1997).

Sickness Behavior

Each subject was placed into a Perspex box (45 cm × 30 cm × 30 cm) 30 min before beginning to record sickness behavior at 30 min, 5 h 30 min, and 23 h 30 min after inoculation with LPS or saline solution at 0800 hours. A trained observer recorded different behaviors using stopwatches, paper, and pencil during a 30-min trial. Once that time-lapse finished, the animal was returned to its home cage until the following trial. Fresh food was provided after the 6-h trial was completed. Body mass and T_b were recorded as described above, immediately before inoculation with LPS or saline (0 h) and 1, 6, and 24 hpi, to verify that the immune response against LPS was mounted. The Perspex cage was washed with tap water and odorless detergent, wiped with alcohol, and allowed to air-dry to ensure that no trace odors from a previous trial remained. The following behaviors were recorded in 30-min trials: (1) locomotion, the number of times that the entire body of the subject crossed lines dividing the floor of the cage in four equal rectangles; (2) resting, the number of 60-s periods in which the subject rested lying in a curled-up position; (3) eye-closing, the duration of time in seconds that the subject showed complete or half-closure of both eyes; (4) rearing, the number of times that the subject stood on the hind limbs, touching the wall of the cage; (5) grooming, the number of 3-s intervals during which the subject showed self-grooming of the coat; (6) scratching, the number of 5-s intervals during which the subject scratched the floor of the cage using its claws; and (7) coprophagy, the number of times the subject self-groomed the belly, curled the body, and obtained a fecal pellet from the anus (Martino et al. 2007). According to preliminary observations, other characteristics of the individuals were recorded to describe their sickness syndrome: (*a*) abdomen in contact with the floor, (*b*) nonlinear walking, (*c*) wet abdomen with urine, and (*d*) depressed hips. The first two variables were obtained during the 30-min behavioral trials, while the remaining two were recorded immediately after the trials were finished.

Endotoxin Detection

To monitor the presence and levels of circulating LPS, a blood sample was collected following the protocol for the species detailed in Vera et al. (2011). Blood was collected from the retro-orbital sinus (~200 uL) before injection (0800 hours) with LPS/saline and 3 and 24 hpi. Immediately after collection, blood was kept at 4°C until centrifuged at 3,000 rpm for 15 min, after which plasma was separated and stored at −20°C until analyses were performed. A Limulus amoebocyte lysate (LAL) endpoint chromogenic kit (Lonza QCL-1000, Walkersville, MD) was used following the manufacturer's instructions and details were provided in Martin et al. (2011) to measure levels of circulating LPS. This kit uses LAL and a synthetic chromogen to detect gram-negative bacterial endotoxin. Briefly, 50 uL of diluted samples (1:50 to 1:200) and standards were dispensed into a sterile 96-well flat-bottom plate with 50 uL of LAL added to each well. Ten minutes later, 100 uL of substrate solution was added; 6 min after that, 50 uL of stop reagent (10% SDS in ultrapure water) was added and the plate was read at 405 nm using a spectrophotometer (BioTek, ELx800, Winooski, VT) to determine the levels of endotoxin concentration (endotoxin units [EU]/mL). All incubations were performed at 37°C in an orbital shaker with heating (BIOMINT-BM081, Buenos Aires, Argentina). The linearity of the standard curve with the concentration range used to predict endotoxin values was verified at the beginning of each trial. Four endotoxin standards were used spanning the desired concentration range, along with a blank, in duplicates. The coefficient of correlation (r) for the individual mean Δ absorbance of the standards versus their respective endotoxin concentration ranged from 0.90 to 0.98. The intra- and interassay coefficients of variation were 6.63% and 7.09%, respectively.

Statistics

All tests were performed in Statistica (Statsoft, Tulsa, OK) using $a > 0.05$ to reject the null hypothesis. Throughout the text, results

are expressed as means ± SDs for T_b, body mass, and metabolic rate and means ± SEs for behavioral data. For the thermal curve data, a two-way repeated-measures ANOVA (rmANOVA) was used to evaluate the effect of treatment (IC vs. C) and sex on the change in T_b at different times after injection ($\Delta T_b = T_{bh} - T_{b0h}$, h = 1, 2, 3, … , 12). A two-way ANOVA was used to evaluate the hypothesis of no change in body mass between experimental groups (IC vs. C) and sex (percentage of change in body mass = (body mass t_{12h} − body mass t_{0h})/body mass t_{0h}). A three-way rmANOVA was used to evaluate the hypothesis of no differences in mass-specific metabolic rate of tuco-tucos between experimental groups (C vs. IC), sexes, and seasons (nonreproductive vs. reproductive) and among different times before or after injection (d − 1, 1 and 6 hpi). A three-way rmANOVA was used to evaluate the hypothesis of no differences in T_b of tuco-tucos between experimental groups (C vs. IC), sexes, and seasons (nonreproductive vs. reproductive), at 0 and 1 hpi (peak time of thermal response). To evaluate whether the magnitude of the fever response was positively correlated with the animal's metabolic rate, the effects of body mass on the thermal response and metabolic rate 1 and 6 h after LPS injection were first removed using the residuals of a linear regression of ΔT_b and metabolic rate 1 and 6 h after LPS injection on body mass. Residual ΔT_b measurements were correlated with residual metabolic rate measurements.

For the sickness behavior data, a two-way rmANOVA was used to evaluate the effect of treatment (IC vs. C) and sex on behavior at 1, 6, and 24 hpi. Parametrical conditions—normality and homoscedasticity—were achieved for resting behavior, while for the rest of behaviors homoscedasticity but not normality was met. Because of sample size, the robustness of the ANOVA test was enough to perform data testing, even when normality was not met (Underwood 1996). *z*-tests with Yates correction were used to compare the proportion of individuals that showed the characteristics associated with sickness syndrome as follows: (*a*) abdomen in contact with the floor, (*b*) nonlinear walking, (*c*) wet abdomen, and (*d*) depressed hips in at least one period assessed (1, 6, and 24 hpi). A two-way ANOVA was used to evaluate the effect of treatment (IC vs. C) and sex on the change in T_b at different times after injection ($\Delta T_b = T_{bh} - T_{b0h}$, h = 1, 6, or 24) in the group of animals used to assess sickness behavior. In this data set, the same test was used to evaluate the effect of treatment (IC vs. C) and sex on the change in body mass at different times after injection (percentage change in body mass = (body mass $t_{1, 6, \text{or } 24\text{ h}}$ − body mass t_{0h})/body mass t_{0h}). A rmANOVA was used to test the hypothesis of no change in endotoxin concentration between sexes and among different times before or after injection (0, 3, 24 h).

Results

Thermal Response Curve and Body Mass Change

T_b differed significantly between experimental groups (two-way rmANOVA, $F_{1, 19} = 5.889$, $P = 0.027$; fig. 1), between sexes ($F_{1, 19} = 5.067$, $P = 0.039$; fig. 1), and among times after injection ($F_{1, 19} = 7.192$, $P < 0.001$; fig. 1) in the 12-h trial. The interaction between time after injection and experimental group had a significant effect on T_b change ($F_{11, 19} = 10.271$, $P < 0.001$). The interactions between sex and experimental group ($F_{1, 19} = 0.764$, $P = 0.395$), time after injection and sex ($F_{1, 19} = 1.117$, $P = 0.351$), and among time after injection, experimental group, and sex ($F_{11, 19} = 0.722$, $P = 0.717$) did not have a significant effect on T_b change. Animals injected with LPS experienced a significant increase in their T_b compared with animals in the control group after an hour postinjection (Tukey, $P < 0.05$; fig. 1), and the T_b increase of males (1.96°C ± 0.40°C) lasted longer (6 h; fig. 1) than that of females (1.58°C ± 0.59°C, 1 h; fig. 1). In control animals, there were no significant differences in T_b among hours after injection (Tukey, $P > 0.05$; fig. 1).

Tuco-tucos' body mass change in the 12-h trial differed significantly between experimental groups (two-way ANOVA, $F_{1, 19} = 7.040$, $P = 0.017$) but not between sexes ($F_{1, 19} = 0.967$, $P = 0.34$), although the interaction between these factors was significant ($F_{1, 19} = 5.489$, $P = 0.032$). Males injected with LPS experienced a significantly greater body mass loss after 12 h of being immunized (10.87% ± 1.80%) compared with control males (Tukey, $P < 0.05$; fig. 2). Females injected with LPS did not experience a significantly greater body mass loss after 12 h of being exposed to the antigen (8.23% ± 1.99%) compared with control females (Tukey, $P > 0.05$; fig. 2).

Metabolic Rate

Mean mass-specific metabolic rate of tuco-tucos did not differ between experimental groups, seasons, or sexes (table 2,) but there was a significant effect of time after injection and the interaction between time after injection and experimental group was significant (table 2). Mean mass-specific metabolic rate of animals injected with LPS was 1.10 ± 0.13 mL O_2/g/h 1 h after injection (14.75% ± 17.83% increase compared with the metabolic rate recorded for these animals before injection; fig. 3) and 1.10 ± 0.20 mL O_2/g/h 6 h after injection (14.36% ± 14.67% increase calculated the same way; fig. 3), while the metabolic rate for control animals before injection was 1.01 ± 0.10 mL O_2/g/h and did not suffer a significant change 1 h (1.00 ± 0.12 mL O_2/g/h, −0.01% ± 0.13%) or 6 h (0.99 ± 0.12 mL O_2/g/h, −0.03% ± 0.14%) after injection (fig. 3). However, differences in mass-specific metabolic rate between treatments for each time recorded were not significant (Tukey, $P > 0.05$; fig. 3). The T_b of tuco-tucos differed significantly between experimental groups, seasons, and sexes (table 3), and the effect of time after injection and interactions between time after injection and (*a*) season, (*b*) experimental group, and (*c*) experimental group and sex were significant (table 3). Specifically, for this last significant interaction, females injected with LPS in the nonreproductive season experienced a significant increase in their T_b 1 hpi (38.24°C ± 0.28°C) compared with their T_b before the injection (36.50°C ± 0.43°C; Tukey, $P < 0.001$). In contrast, T_b of females injected with LPS in the reproductive season did not change with time after injection (0 hpi: 36.26°C ± 0.58°C, 1 hpi: 36.94°C ± 0.45°C; Tukey, $P = 0.123$). Residuals of the regression between body mass and ΔT_b did not correlate with residuals of the regression between body mass and increase in metabolic rate 1 hpi (Pearson $r = 0.229$, df = 20, $t = 1.028$,

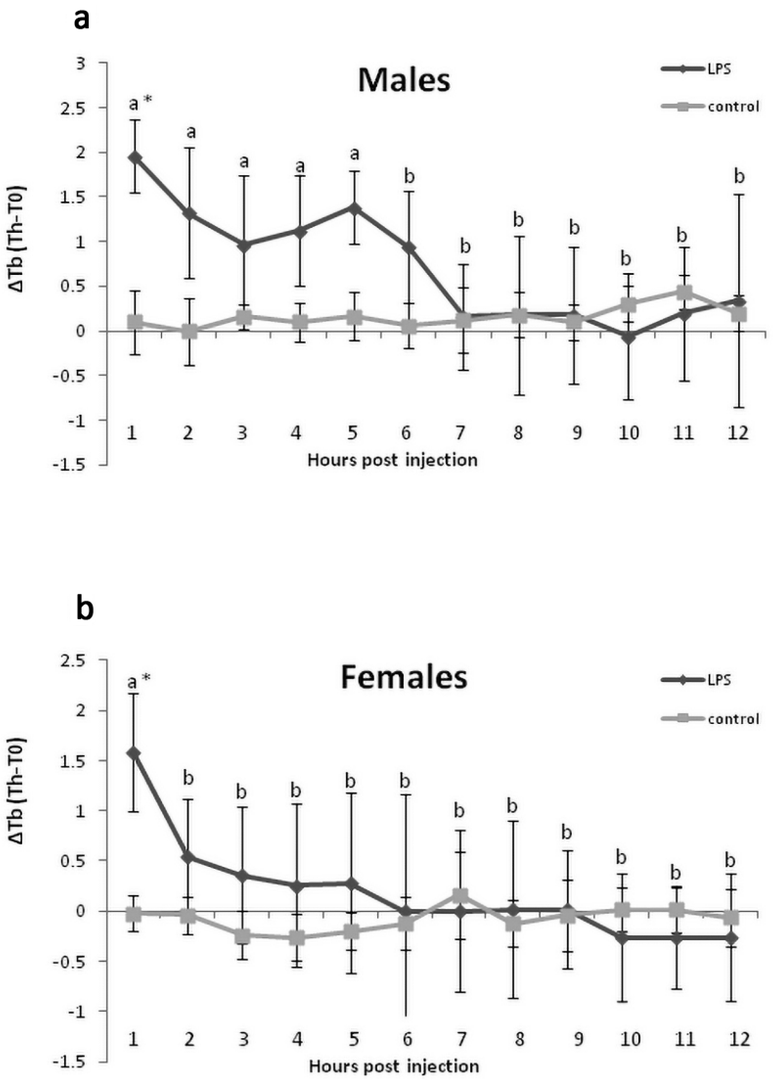

Figure 1. Effects of lipopolysaccharide (LPS) on body temperature change (ΔT_b), calculated as T_b at 1, 2, 3, ..., 12 h postinjection (hpi) minus T_b before injection (T_{b0}) in male (*a*) and female (*b*) *Ctenomys talarum*. Squares depict ΔT_b for control animals injected with saline solution; diamonds depict ΔT_b for animals injected with LPS. Different letters denote statistically significant differences among hpi within the same treatment (Tukey, $P < 0.05$). Asterisks denote statistically significant differences between treatments for the same hpi (Tukey, $P < 0.05$). Standard deviation bars are presented.

$P = 0.317$) or 6 hpi (Pearson $r = 0.216$, df $= 20$, $t = 0.963$, $P = 0.347$).

Sickness Behavior

Exposure of tuco-tucos to LPS produced a significant increase in resting behavior (fig. 4*a*; table 4) and in the time animals spent with their eyes totally or partially closed (fig. 4*b*; table 4). Although locomotion behavior appears to be affected by LPS, particularly in males (fig. 4*c*), differences were not significant between treatments (table 4). Similarly, rearing behavior seemed to occur more frequently in control than in LPS males (fig. 4*d*), but differences were not significant between treatments (table 4). LPS treatment did not affect scratching behavior (fig. 4*e*; table 4) or grooming behavior (fig. 4*f*; table 4). Finally, individuals engaged in coprophagy regardless of LPS or saline treatment (fig. 4*g*; table 4), but females did it in higher frequency than males (table 4). Individuals injected with LPS showed their abdomen wet in higher proportion than those injected with saline solution (LPS: 11/28, C: 0/24, *z*-test, $z = 3.118$, $P = 0.002$), but this was evident only in males (LPS: 9/13, C: 0/11, $z = 3.058$, $P = 0.002$) and not in females (LPS: 2/15, C: 0/13, $z = 0.628$, $P = 0.53$). A trend was detected in the proportion of individuals showing contact with the floor, both while resting or in movement, in relation to the challenge with LPS (LPS: 6/28, C: 0/24, $z = 1{,}945$, $P = 0.052$), but differences were not significant for

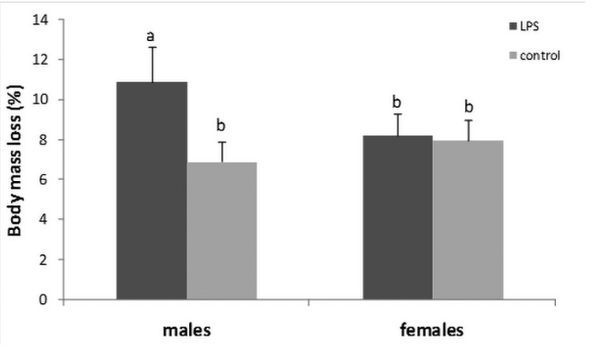

Figure 2. Effects of lypopolysaccharide (LPS) on body mass loss (%) at 12 h postinjection (hpi) in male and female *Ctenomys talarum*. Light gray bars depict body mass loss for control animals injected with saline solution; dark gray bars depict body mass loss for animals injected with LPS. Different letters denote statistically significant differences between treatments (Tukey, $P < 0.05$). Standard deviation bars are presented.

any of the sexes (females, LPS: 5/15, C: 0/13, $z = 1.801$, $P = 0.072$; males, LPS: 1/13, C: 0/11, $z = 0.008$, $P = 0.933$). Illness appearance, showing depressed hips, was found in LPS-treated individuals (LPS: 9/28, C: 0/24, $z = 2.686$, $P = 0.007$), but differences were not detected between sexes (females, LPS: 5/15, C: 0/13, $z = 1.801$, $P = 0.072$; males, LPS: 4/13, C: 0/11, $z = 1.462$, $P = 0.144$). Some tuco-tucos showed nonlinear locomotion, but no differences were detected between treatments (LPS: 4/28, C: 0/24, $z = 1.405$, $P = 0.160$) or sexes (females, LPS: 2/15, C: 0/13, $z = 0.628$, $P = 0.530$; males, LPS: 2/13, C: 0/11, $z = 0.619$, $P = 0.536$).

In this group of animals used to assess sickness behavior, change in T_b differed significantly between treatments (two-way rmANOVA, $F_{1,51} = 6.729$, $P = 0.013$) but not between sexes ($F_{1,51} = 0.538$, $P = 0.467$). T_b differed significantly among times after injection ($F_{2,51} = 6.837$, $P = 0.002$). The interaction between time after injection and treatment had a significant effect on T_b change ($F_{2,51} = 4.966$, $P = 0.009$). Furthermore, body mass loss differed significantly between treatments (two-way rmANOVA, $F_{1,51} = 18.759$, $P < 0.001$) but not between sexes ($F_{1,51} = 1.194$, $P = 0.280$). Change in body mass differed significantly among times after injection ($F_{2,51} = 4.848$, $P = 0.010$). The interaction between time after injection and treatment had a significant effect on body mass change ($F_{2,51} = 6.528$, $P = 0.002$). Females injected with LPS suffered a significant decrease in body mass 6 hpi compared with females in the control group (2.89% ± 2.05%; Tukey, $P < 0.05$), and the decrease in body mass of males was significant 24 hpi compared with those of the control group (4.89% ± 5.28%; Tukey, $P < 0.05$). In control animals, there were no significant differences in changes in body mass among hours after injection (Tukey, $P < 0.05$).

Endotoxin Detection

Control animals injected with saline solution had no detectable levels of gram-negative endotoxin. Mean levels of endotoxin concentration of animals injected with LPS differed significantly among times after injection (rmANOVA, $F_{2,9} = 15.21$, $P < 0.001$), but there was not a significant effect of sex ($F_{1,9} = 0.42$, $P = 0.53$) or the interaction between these factors ($F_{2,9} = 0.82$, $P = 0.46$). The endotoxin concentration measured 3 hpi with LPS (83.84 ± 54.32 EU/mL) was significantly greater than that measured before injection (1.14 ± 2.42 EU/mL; Tukey, $P < 0.05$; fig. 5). Endotoxin concentration levels decreased 24 hpi (19.43 ± 23.46 EU/mL), but they were not significantly different from levels measured before injection and 3 hpi ($P > 0.05$; fig. 5)

Discussion

Immunity requires energy to be activated and maintained and, although it grants pathogen and parasite resistance, it entails substantial costs. Costs might be metabolic, nutritional, or even related to collateral damage (e.g., oxidative stress, autoimmunity; reviewed in Hasselquist and Nilsson 2012). Our results suggest that in *Ctenomys talarum*, the APR is energetically costly, resulting in a 14% increase in metabolic rate compared with preinjection values. Animals exposed to LPS experienced a short-term thermal response, with an increase in T_b that peaked around 1 hpi; weight loss; and changes in their behavior that included more time spent resting and with their eyes totally or partially closed, as well as illness appearance and wet abdomen in contact with the floor. However, the magnitude of the effects of LPS exposure varied between sexes and among animals. More specifically, male *C. talarum* experienced a fever response that lasted longer than that of females and, furthermore, while males showed a significant decrease of weight 12 hpi, females did not. Individual variability made it difficult to detect significant differences between sexes in recorded

Table 2: Results of three-way repeated-measures ANOVA performed to evaluate the hypothesis of no differences in mass-specific metabolic rate of *Ctenomys talarum* (mL O_2/g/h) between experimental groups, sexes, and seasons and among different hours postinjection (hpi: 0, 1, 6; $n = 44$)

	df	*F*	*P*
Between effects:			
Treatment (LPS vs. control)	1	2.256	.142
Sex	1	2.484	.124
Season	1	1.598	.214
Treatment × sex	1	1.520	.226
Sex × season	1	.026	.873
Treatment × season	1	.081	.778
Treatment × sex × season	1	.662	.421
Within effects:			
hpi	2	4.864	.011*
hpi × treatment	2	8.331	.001*
hpi × sex	2	.245	.783
hpi × season	2	.124	.883
hpi × treatment × sex	2	1.009	.370
hpi × sex × season	2	.767	.468
hpi × treatment × season	2	.994	.375
hpi × treatment × sex × season	2	.451	.639

*Statistically significant.

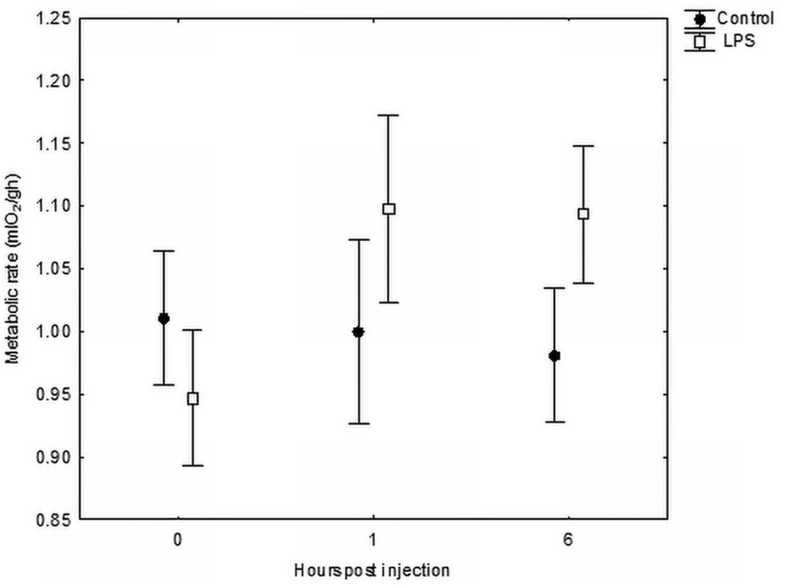

Figure 3. Effects of lypopolysaccharide (LPS) on specific metabolic rate at 0, 1, and 6 h postinjection in *Ctenomys talarum*. Circles depict metabolic rate for control animals injected with saline solution. Squares depict metabolic rate in animals injected with LPS. Vertical bars denote 0.95 confidence intervals.

behaviors. There was a clear peak in circulating endotoxin levels in plasma 3 hpi and a significant decrease of these levels 24 hpi, but peak endotoxin concentration values recorded were also highly variable among animals.

T_b and Body Mass Change

In response to LPS, *C. talarum* mounted an APR that involved an increase in T_b, with an average peak increase of 1.5°C–2°C verified only 1 h after exposure to the antigen. This rapid hyperthermic response in tuco-tucos was comparable in magnitude to that of other rodents, such as *Rattus norvegicus* (Romanovsky and Székely 1998), *Cavia porcellus* (Li and Blatteis 2004), *Peromyscus leucopus*, *Peromyscus maniculatus* (Martin et al. 2008), and *Mus musculus* (Rudaya et al. 2005), but it was greater than that of hamsters (*Mesocricetus auratus*; Blatteis 1983; Conn et al. 1990) and mice (*Peromyscus aztecus*; Martin et al. 2008) and lower than in fish-eating bats (*Myotis vivesi*; Otálora-Ardila et al. 2017).

The most common symptom of an APR is an increase in T_b, which is considered to have an adaptive value that contributes to eliminate invading pathogens (Romanovsky et al. 2005) and speed recovery from infection (Mackowiak 1994). However, during more severe responses, it is often observed that animals switch from hyperthermia to hypothermia (decreased T_b), and two contrasting hypotheses have been proposed to explain this. On the one hand, hypothermia is considered a symptom of septic shock and bad prognosis of infection (Clemmer et al. 1992). On the other hand, hypothermia may be adaptive, as a means to avoid a deleterious inflammatory response (Romanovsky et al. 2005; Liu et al. 2012). For example, closely related species of *Peromyscus* responded markedly different to LPS and, while some showed a strong fever response (*P. leucopus* and less so *P. maniculatus*), another species (*P. californicus*) showed a hypothermic response, suggesting that there may be ecological determinants of this variation (Martin et al. 2008). In *C. talarum*, out of a total of 126 animals that were used in this study, only four (~3%) showed a clear hypothermic response; all of them first showed an increase in T_b 1 hpi that was

Table 3: Results of three-way repeated-measures ANOVA performed to evaluate the hypothesis of no differences in body temperature of *Ctenomys talarum* between experimental groups, sexes, and seasons and among different hours postinjection (hpi: 0, 1; $n = 44$)

	df	*F*	*P*
Between effects:			
Treatment (LPS vs. control)	1	21.8	<.001*
Sex	1	5.3	.028*
Season	1	5.4	.026*
Treatment × sex	1	.8	.384
Sex × season	1	1.0	.335
Treatment × season	1	.8	.369
Treatment × sex × season	1	.5	.475
Within effects:			
hpi	1	47.2	<.001*
hpi × treatment	1	36.9	<.001*
hpi × sex	1	3.1	.088
hpi × season	1	12.6	.001*
hpi × treatment × sex	1	5.0	.031*
hpi × sex × season	1	2.0	.171
hpi × treatment × season	1	.7	.399
hpi × treatment × sex × season	1	2.6	.116

*Statistically significant.

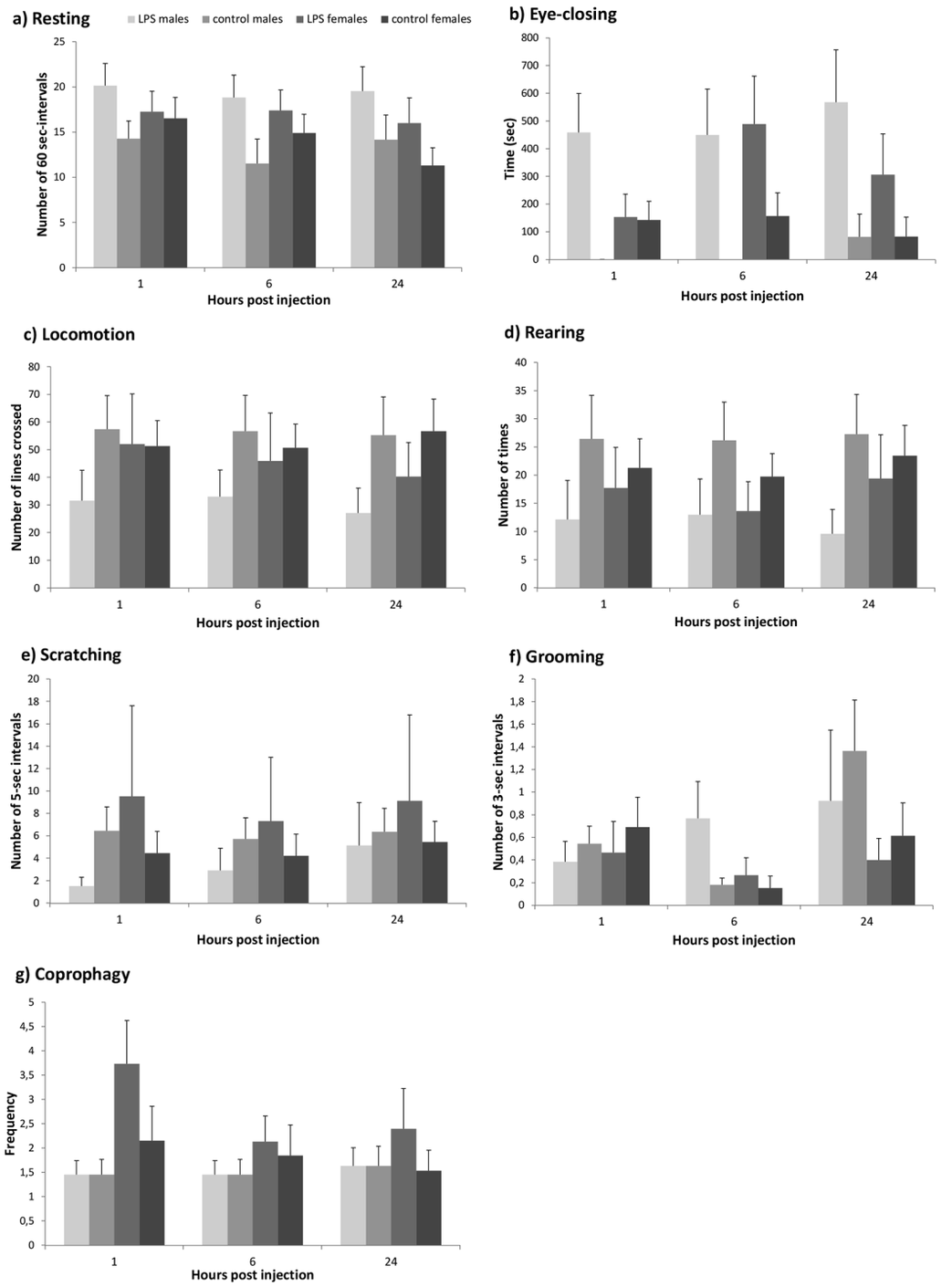

Figure 4. Effects of lypopolysaccharide (LPS) on time spent resting (*a*), with their eyes closed (*b*), locomotion (*c*), rearing (*d*), scratching (*e*), grooming (*f*), and coprophagy (*g*) at 1, 6, and 24 h postinjection in male and female *Ctenomys talarum*. From left to right, for each time after injection, bars depict the results of males injected with LPS, control males (injected with saline solution), females injected with LPS, and control females (injected with saline solution). Standard deviation bars are presented.

followed by a clear decrease in T_b. These four animals (one female and three males) died between 48 h and 1 wk after immunization. Thermoregulatory responses to LPS may also be dependent on the dose and T_a (Rudaya et al. 2005; Ramirez-Otarola et al. 2019). In our study, the LPS dose used (1 mg/kg) was selected because it was the most frequently used dose in the literature and it was enough to elicit an APR response in tuco-tucos, without compromising their recovery; furthermore, the T_a was maintained within the thermoneutral zone for all assays (i.e., 25°C; Busch 1989). Of note, the fever response was variable between sexes. Both sexes

Table 4: Results of two-way repeated-measures ANOVA performed to evaluate the hypothesis of no differences in behavior of *Ctenomys talarum* between experimental groups and sexes and among different hours postinjection (hpi: 1, 6, 24; $n = 52$)

	Treatment		Sex		hpi		Sex × treatment		hpi × sex		hpi × treatment		hpi × treatment × sex	
Behavior	F (df)	P	F (df)	P	F (df)	P	F (df)	P	F (df)	P	F (df)	P	F (df)	P
Resting	4.76 (1)	.034*	.18 (1)	.67	1.31 (2)	.27	.77 (1)	.38	1.69 (2)	.19	.33 (2)	.77	.58 (2)	.56
Eyes closing	11.17 (1)	.0016*	.15 (1)	.70	.96 (2)	.39	1.99 (1)	.16	1.67 (2)	.19	.77 (2)	.46	.79 (2)	.45
Locomotion	2.06 (1)	.16	.27 (1)	.60	.19 (2)	.83	.70 (1)	.41	.08 (2)	.92	.47 (2)	.62	.24 (2)	.78
Rearing	3.06 (1)	.08	.0004 (1)	.98	.26 (2)	.77	.87 (1)	.35	.67 (2)	.51	.07 (2)	.93	.21 (2)	.81
Scratching	.01 (1)	.91	.19 (1)	.66	.96 (2)	.39	.59 (1)	.44	.32 (2)	.73	.17 (2)	.84	.76 (2)	.47
Grooming	.06 (1)	.80	1.36 (1)	.24	2.25 (2)	.11	.05 (1)	.82	1.33 (2)	.27	1.22 (2)	.30	.29 (2)	.75
Coprophagy	.02 (1)	.88	6.66 (1)	.013*	.79 (2)	.45	3.43 (1)	.07	1.13 (2)	.32	.29 (2)	.74	.36 (2)	.69

*Statistically significant.

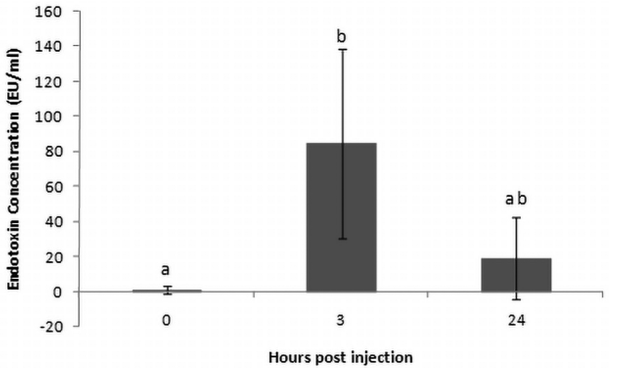

Figure 5. Concentration of endotoxin (endotoxin units[EU]/mL) in plasma of *Ctenomys talarum* at 0, 3, and 24 h postinjection (hpi) with lypopolisaccharide. Different letters denote statistically significant differences among hpi (Tukey, $P < 0.05$). Standard deviation bars are presented.

showed, on average, a peak increase in T_b only 60 min after exposure to LPS. However, male *C. talarum* experienced a fever response that lasted longer than that in females. This pattern of a longer fever response in male tuco-tucos over a 12-h period after exposure to LPS was also accompanied by a significant body weight loss (~10%) found only in males, probably reflecting the mobilization of energy reserves to cope with the energetic demands of fever, as has been documented in other mammals (Kozak et al. 1994; Stockmaier et al. 2015). As part of the characterization of sickness behavior, both T_b and body mass were recorded in the group of animals used for this purpose, before injection and 1, 6, and 24 hpi. In this group of animals, a difference between sexes in body mass loss was also evident, but not in the fever response. Both sexes exposed to LPS showed a significant increase in T_b compared with control animals. However, body mass loss 6 hpi was only significant for females compared with control animals, and this difference was only significant for males 24 hpi. Evidence in humans (Guerra-Silveira and Abad-Franch 2013), as well as in other animals (Roberts et al. 2004), indicates that infectious disease affects the sexes differently. These differences are thought to be driven by immunosupressive effects of sex hormones and glucocorticoids (Klein 2004; but see Bilbo and Nelson 2001). While for both sexes investment in sickness behaviors and fighting infection may lead to increased lifetime fitness by increasing survival, the magnitude of expression of these traits is plastic and may also vary with the costs and benefits that different social contexts pose on each sex (Lopes 2014). For *C. talarum*, it seems that when food was not provided over the 12-h trial, differences in the magnitude of fever and body mass loss between sexes were more evident. Animals that were monitored in their behavior were provided food ad lib. at the end of the trials (6 hpi). Before being fed, females showed a significantly greater body mass loss. However, even after food was made available, males showed a significantly greater body mass loss 24 hpi. This may indicate that males could have suffered a more severe lack of appetite, which is a known component of sickness behavior in other species (Kent et al. 1996; Ramirez-Otarola et al. 2019), although this variable was not recorded in the present study.

Metabolic Costs

The results of our study provide evidence that the APR in *C. talarum* is energetically costly: compared with preinjection, animals exposed to LPS showed a significant 14% increase in their metabolic rate only 1 hpi, and this increase was still verified 6 h after the exposure. The APR has been proposed to be the most energetically demanding component of the immune system (Lochmiller and Deerenberg 2000; Bonneaud et al. 2003; Lee 2006). However, this is not the case for *C. talarum*, in which previous studies have shown that mounting an antibody-mediated response against SRBCs represents a 20%–35% increase in metabolic rate, also compared with the preinjection condition, which is comparable to the cost of lactation for females of this species (Cutrera et al. 2010). Nonetheless, the APR is more energetically costly for *C. talarum* than the local inflammatory response triggered by PHA, which appears not to be associated with a significant elevation of metabolic rate (Merlo et al. 2014*b*), although it was negatively affected by food restriction (Merlo et al. 2016*a*) and parasitism (Merlo et al. 2016*b*, 2018). Furthermore, in comparison with other species of endotherms, the APR in tuco-tucos seems to incur in moderate to low energetic costs, comparable with those of other rodents (MacDonald et al. 2012; Ramirez-Otarola et al. 2018). This type of systemic inflammatory response represents a 0.7%–1.8% increase in the daily energetic expenditure (DEE) of captive female *C. talarum* and a 2.5%–3.5% increase for males in the same condition, depending on the season (table 5) and considering conservatively that the increase in metabolic rate is maintained for only 6 h after injection, which is the period in which metabolic rate was recorded in this study. It has been suggested that the DEE of free-living animals tends to be higher than the DEE estimated for those in laboratory conditions (Nagy 1989), so the fractional change due to the APR might even be lower in tuco-tucos in their natural habitat. In line with this, no differences in the magnitude of the energy allocated to the APR were found between the reproductive and nonreproductive seasons, which one would expect if APR costs were substantial. Interestingly, however, in the nonreproductive season, females injected with LPS experienced a significant increase in their T_b 1 hpi compared with their T_b before the injection, while this was not observed in the reproductive season. This suggests a possible trade-off between immunity and reproduction in the sex that faces higher energetic demands during reproduction. As a caveat, in this study, as well as in other laboratory-based studies in ecoimmunology, animals had ad lib. food availability, which could have masked potential energetic trade-offs under conditions where food resources were not limiting.

One of the reasons proposed to commonly explain the energetic costs associated with mounting an APR is that fever requires a great energetic investment. More specifically, a metabolic rate increase of 10% is expected for every 1°C rise in T_b (Kluger 1991). While this relation is often cited (e.g., Burness et al. 2010; Sköld-Chiriac et al. 2014, 2015) or even used to calculate the costs of the APR

Table 5: Daily energetic expenditure (kJ) for male and female *Ctenomys talarum*

	Nonbreeding female	Pregnant female	Nonbreeding male	Breeding male
Maintenance	57.07	57.07	57.07	57.07
Thermoregulation	45.57	45.57	45.57	45.57
Movements	8.73	8.73	8.73	8.73
Burrow construction (1 m)	5.66	5.66	5.66	5.66
Pregnancy	...	13.63	...	...
Total (kJ/d)	117.03	130.66	117.03	117.03
Acute-phase response	1.15	2.40	4.47	6.18
Total (kJ/d)	118.18	133.06	121.50	123.21
Antibody response	16.77	10.84	17.90	16.37
Total (kJ/d)	133.80	141.50	134.93	133.40

Note. During a cold day in the breeding and the nonbreeding season, faced with an immune challenge that elicits (*a*) an acute-phase response (APR) or (*b*) an antibody-mediated response in their first year of life (modified from Antenucci et al. 2007). APR costs (kJ) were calculated considering that the increase in metabolic rate lasts 6 h. Only pregnant females were considered in the breeding season, since lactating females were not used in this study. Data on energy costs associated with different processes were obtained from previous studies: cellular maintenance (Busch 1989; Luna et al. 2002), thermoregulation (Busch 1989), movements (Luna and Antenucci 2003), burrow construction (Luna et al. 2002), pregnancy (Zenuto et al. 2002), and antibody response (Cutrera et al. 2010).

(Martin et al. 2008), other determinants of the energetic costs of an increase in T_b might come into play, such as body size, fur coat density—and hence thermal insulation—or habitat (Hart and Hart 2019), and thus, empirically assessing the energetic costs of mounting such a response is necessary. For example, the T_b can influence an animal's ability to mount an immune response, influencing the maximum metabolic rate, which determines the metabolic limits of its energy budget (Książek et al. 2003). In *C. talarum*, the average 14% increase found in metabolic rate of *C. talarum* exposed to LPS was within what is expected for an endotherm (Kluger 1991), given that, on average, *C. talarum* experienced a 1.5°C–2°C rise in T_b as part of the APR. However, tuco-tucos that mounted stronger fever responses did not show a higher metabolic rate, which may also be attributed to the high variance in T_b found in this species 1 h after exposure to LPS.

As described above, the high humidity, low ventilation, and hypoxic conditions that characterize the subterranean environment are hypothesized to have favored the low RMR observed in *C. talarum* (Busch 1989) and other subterranean rodents (McNab 1966; Vleck 1979; Luna et al. 2009) as an adaptation to avoid overheating during digging (McNab 1966) or to cope with the costs of burrowing (Vleck 1979; Luna and Antenucci 2007*b*; Luna et al. 2009). These restrictions on the RMR of tuco-tucos may impose a limit on the energetic expenditure allocated to T_b rising and the APR in general (Lee 2006). However, in *C. talarum* the increase in metabolic rate associated with mounting an antibody response was greater (20%–35%; Cutrera et al. 2010), suggesting that tuco-tucos can cope with greater energetic costs of immunity if needed. It is also possible that, given the restrictions imposed on heat loss by the subterranean environment (where convective and evaporative heat loss are restricted; Buffenstein 2000) and by *C. talarum* physiology, elevating T_b by reducing heat loss may be more cost-efficient in this species (Baldo et al. 2015; Luna et al. 2020). Trade-offs between thermoregulation, RMR, and immune response have been documented in a few rodents (mice: Cichoń et al. 2002; Książek et al. 2003), including a fossorial species (degu: Ramirez-Otarola et al. 2018), and several bird species (e.g., Burness et al. 2010; King and Swanson 2013), and different mechanisms to reduce the costs of fever, such as seeking warmer environments, have been recorded (e.g., brown rats: MacDonald et al. 2012). Some of these mechanisms are discussed in the section that follows.

Sickness Behavior

Our results show evidence of a sickness behavior syndrome in response to LPS in *C. talarum*. In particular, individuals increased the time spent resting, with the eyes totally or partially closed. These results, together with a tendency toward decreased locomotion and rearing behavior, are in accordance with the proposed adaptive function of sickness behavior (Hart 1988). As described above, fever response involves substantial costs, primarily by increasing heat production through increased metabolic rate. Consequently, it is proposed that a suite of behaviors were selected that lead infected animals to remain calm and therefore conserve energy (Hart 1988; Hart and Hart 2019). Furthermore, reduced locomotion activity and motivation to seek food and water resources minimize muscle work and restrict nutrient intake—especially iron—that would be available for pathogens. Also, reduced activity contributes to decrease animals' exposure to cold environments and risk of predation, particularly important for a sick animal in a weak condition (Hart 1988). *Ctenomys talarum* live underground, but they gather food aboveground and later consume it in their burrows. Therefore, increased resting behavior allows tuco-tucos to spend more time in the burrow, which is a stable thermal environment that contributes to saving energy and also providing protection from predators (Busch et al. 2000).

Sickness behavior has been documented in several small mammal species. For example, adult *Octodon degus* showed reduced locomotion and increased crouching and eye-closed intervals (Ramirez-Otarola et al. 2019). Furthermore, studying five *Peromyscus* species, Martin et al. (2008) found decreased locomotion activity in most of them. Similarly, Moreau et al. (2008) found a decrease in locomotion and rearing activity in mice, while in Siberian hamsters

(*Phodopus sungorus*), LPS exposure induces anorexia and decreased nest-building behavior (Carlton and Demas 2015). Pups of *C. porcellus* injected with LPS showed crouching, eye-closing, and piloerection (Hennessy et al. 2004), and increased crouching behavior and reduced locomotion was also reported in pups of *O. degus* (Ramirez-Otarola et al. 2018). Sick animals often look dirty as a consequence of poor grooming behavior (Hart 2010). However, we found no differences in grooming activity between tuco-tucos injected with LPS and control animals. Neither did we observe differences in coprophagy, even when this behavior involves intense grooming. Nonetheless, both sexes showed illness appearance, particularly involving their depressed hips.

Fever may cause tissue damage to vital organs such as the heart, liver, or brain (Hart 1988; Kluger et al. 1996). Therefore, it is possible that in *C. talarum*, in which sweating or panting does not occur (Busch 1989), keeping the abdomen moist with urine (mainly observed in males) or in contact with the floor (more frequently in females) would help lose heat and regulate T_b in a safe range. In fact, conductance has been proposed as an important mechanism of heat loss in this species (Luna and Antenucci 2007*a*; Baldo et al. 2015; Luna et al. 2020), especially through the abdomen where fur is less dense (Cutrera and Antenucci 2004).

Finally, sickness behavior, added to the fever response and activation of the immune system, is an important strategy to eliminate a pathogen from the body (Hart 1988). Therefore, both sexes are expected to rely on this strategy to cope with an infection. However, differences in body mass in species showing sexual size dimorphism, or with different energy constraints associated with reproductive activity, may impose differences in the magnitude or type of behaviors affected. Despite this, we did not find clear differences between sexes in *C. talarum* in the extent of their sickness behavior, although males seemed to be more affected. This pattern followed increases in T_b and loss in body mass, which were evident after LPS treatment but without differences between the sexes. In addition, both parameters also showed high variability as well as behavior.

Endotoxin

A marked, although highly variable, increase in levels of circulating endotoxin (LPS) in plasma was detected 3 h after *C. talarum* was exposed to the antigen; levels of this antigen decreased significantly 24 hpi, although they remained higher than those before exposure. This is the first study that was able to monitor how rapidly the endotoxin levels circulating in plasma increase after applying an exogenous dose of LPS and then decrease, as a measure of the time it takes to resolve the systemic inflammation. Two previous studies attempted to use this assay in guinea pigs (*C. porcellus*; Hart 1988; Ross et al. 2001; Hart and Hart 2019) and house sparrows (*Passer domesticus*; Martin et al. 2011), but they did not find an effect of exogenous LPS on circulating endotoxin. In tuco-tucos, the peak of fever was found only 1 hpi, and we were able to detect an increase of circulating endotoxin levels 3 hpi. Animals usually showed a decrease in the febrile response 24 hpi, suggesting that the systemic inflammation was under resolution, matching the decreased endotoxin levels found at this moment, although the behavioral effects persisted. This is the first report of clearance speed of circulating endotoxin in a small mammal, and in this case, *C. talarum* is characterized by a low BMR, with the implications that this may have on different body functions and, more specifically, on the resolution of a systemic inflammation.

Ecological Determinants of Immune Function Variation in Tuco-Tucos

In light of the different costs of immunity arms, Lee (2006) proposed that "fast-living species" (those with high reproductive outputs and low survival rates) should rely more heavily on fast, nonspecific, inflammatory immunity, which is rapidly activated during injury or infection. In contrast, "slow-living species," with long life spans and high investment in offspring and future reproduction, should exhibit stronger adaptive immunity, and particularly antibody responses, which are slower but confer memory against repeated infections, that are more likely to occur in long-lived species. Supporting evidence comes from studies in wild birds (Tella et al. 2002; Martin et al. 2004, 2006) and rodents (Martin et al. 2008; Previtali et al. 2012), although it is not conclusive. Furthermore, other factors, such as levels of parasite exposure, have been proposed as ecological determinants of immune variation: species living in habitats with high parasitic exposure are expected to invest more heavily in immunity compared with species living in habitats where parasite exposure is lower (Piersma 1997; Bordes and Morand 2009).

Subterranean rodents represent an interesting opportunity to assess the relative importance of different ecological determinants of immune variation in natural populations of wild mammals. While their slow pace of life is expected to be associated with a higher investment toward more costly adaptive defenses, subterranean rodents live in habitats characterized by high prevalence but low diversity of parasite infections, which may facilitate innate immunity to reduce resource allocation toward more expensive adaptive immunity (Novikov et al. 2016). In fact, low polymorphism at major histocompatibility complex genes, involved in foreign antigen recognition and destruction (Klein et al. 1998), have been reported for different subterranean rodents (Nižetić et al. 1988; Kundu and Faulkes 2004), and in particular for ctenomyids (Hambuch and Lacey 2002; Cutrera and Lacey 2006; Cutrera and Mora 2017), relative to surface-dwelling rodents, presumably associated with low levels of parasite exposure in the subterranean habitat. In *C. talarum*, the APR elicited by LPS is not as energetically costly as in other small mammals, representing a 0.7%–3.5% increase in the DEE, while the antibody response against an artificial antigen (SRBC) represents an increase of 15% in the DEE (table 2). These results show that tuco-tucos allocate more resources toward a more expensive acquired immunity, which confers immunological memory against repeated infections that are expected to occur along their lives, providing support to the pace-of-life hypothesis (Lee 2006). As previously described, other elements related to overheating avoidance may also contribute to a comparatively lower metabolic allocation toward APR in tuco-tucos and possibly subterranean rodents in general. It is

interesting to note, however, that in *C. talarum* the three immune responses studied in great detail, such as the antibody-mediated response (Cutrera et al. 2010), local inflammation (Merlo et al. 2014*b*, 2014*a*), and the APR, as well as those recorded for specific purposes (bacterial killing capacity of the plasma, hemolysis and hemoagglutination by complement activation; see Merlo et al. 2016*a*), show moderate to low magnitudes of response compared with other small mammals, suggesting a possible role of the relatively lower parasite exposure in the evolution of immune strategies in this group of subterranean rodents. Our findings about the characteristics of the APR and the associated costs of this immune response in the subterranean rodent *C. talarum* emphasize the need to increase our knowledge of the continuum of immune strategies in nonmodel wild species facing natural environmental pressures.

Acknowledgments

We are grateful to the members of the Laboratorio de Ecología Fisiológica y del Comportamiento (IIMyC) for their support and assistance in the field. Financial support was provided by Consejo Nacional de Investigaciones Científicas y Técnicas (PIP 0292), Agencia Nacional de Promoción Científica (PICT 2349, 4271), and Universidad Nacional de Mar del Plata, Argentina (EXA 848/18).

Literature Cited

Antenucci C.D., R.R. Zenuto, F. Luna, A.P. Cutrera, P.P. Perissinotti, and C. Busch. 2007. Energy budget in subterranean rodents: insights from the tuco-tuco *Ctenomys talarum* (Rodentia: Ctenomyidae). Pp. 111–139 in D.A. Kelt, E.P. Lessa, J. Salazar-Bravo, and J.L. Patton, eds. The quintessential naturalist: honoring the life and legacy of Oliver P. Pearson. University of California Publications in Zoology. Vol. 134. University of California Press, Berkeley.

Baldo M.B., C.D. Antenucci, and F. Luna. 2015. Effect of ambient temperature on evaporative water loss in the subterranean rodent *Ctenomys talarum*. J Therm Biol 53:113–118.

Baze M.M., K. Hunter, and J.P. Hayes. 2011. Chronic hypoxia stimulates an enhanced response to immune challenge without evidence of an energetic trade-off. J Exp Biol 214:3255–3268.

Bilbo S.D. and R.J. Nelson. 2001. Sex steroid hormones enhance immune function in male and female Siberian hamsters. Am J Physiol 280:R207–R213.

Blatteis C.M. 1983. Thermal responses of hamsters to endotoxin. J Therm Biol 8:195–197.

Bonneaud C., J. Mazuc, G. Gonzalez, C. Haussy, O. Chastel, B. Faivre, and G. Sorci. 2003. Assessing the cost of mounting an immune response. Am Nat 161:367–379.

Bordes F. and S. Morand. 2009. Coevolution between multiple helminth infestations and basal immune investment in mammals: cumulative effects of polyparasitism? Parasitol Res 106:33–37.

Buffenstein R. 2000. Ecophysiological responses of subterranean rodents to underground habitats. Pp. 62–110 in E.A. Lacey, G.N. Cameron, and J.L. Patton, eds. Life underground: the biology of subterranean rodents. University of Chicago Press, Chicago.

Burness G., C. Armstrong, T. Fee, and E. Tilman-Schindel. 2010. Is there an energetic-based trade-off between thermoregulation and the acute phase response in zebra finches? J Exp Biol 213:1386–1394.

Busch C. 1989. Metabolic rate and thermoregulation in two species of tuco-tuco, *Ctenomys talarum* and *Ctenomys australis* (Caviomorpha, Octodontidae). Comp Biochem Physiol A 93: 345–347.

Busch C., C.D. Antenucci, J.C. del Valle, M.J. Kittlein, A.I. Malizia, and A.I. Vassallo. 2000. Population ecology of subterranean rodents. Pp. 183–226 in E.A. Lacey, G.N. Cameron, and J.L. Patton, eds. Life underground: the biology of subterranean rodents. University of Chicago Press, Chicago.

Busch C., A.I. Malizia, O.A. Scaglia, and O.A. Reig. 1989. Spatial distribution and attributes of a population of *Ctenomys talarum* (Rodentia: Octodontidae). J Mammal 70:204–208.

Cabrera-Martínez L.V., L.G. Herrera, and A.P. Cruz-Neto. 2018. The energetic cost of mounting an immune response for Pallas's long-tongued bat (*Glossophaga soricina*). PeerJ 6:e4627.

Carlton E.D. and G.E. Demas. 2015. Body mass affects seasonal variation in sickness intensity in a seasonally breeding rodent. J Exp Biol 218:1667–1676.

Cichoń M., M. Chadzińska, A. Książek, and M. Konarzewski. 2002. Delayed effects of cold stress on immune response in laboratory mice. Proc R Soc B 269:1493–1497.

Clemmer T.P., C.J. Fisher, R.C. Bone, G.J. Slotman, C.A. Metz, and F.O. Thomas. 1992. Hypothermia in the sepsis syndrome and clinical outcome. Crit Care Med 20:1395–1401.

Conn C.A., K.T. Borer, and M.J. Kluger. 1990. Body temperature rhythm and response to pyrogen in exercising and sedentary hamsters. Med Sci Sports Exerc 22:636–642.

Cutrera A.P. and C.D. Antenucci. 2004. Cambios en el pelaje del roedor subterráneo *Ctenomys talarum*: possible mecanismo térmico compensatorio. Rev Chil Hist Nat 77:235–242.

Cutrera A.P. and E.A. Lacey. 2006. Major histocompatibility complex variation in Talas tuco-tucos: the influence of demography on selection. J Mammal 87:706–716.

Cutrera A.P. and M.S. Mora. 2017. Selection on MHC in a context of historical demographic change in 2 closely distributed species of tuco-tucos (*Ctenomys australis* and *C. talarum*). J Hered 108:628–639.

Cutrera A.P., R.R. Zenuto, F. Luna, and C.D. Antenucci. 2010. Mounting a specific immune response increases energy expenditure of the subterranean rodent *Ctenomys talarum* (tuco-tuco): implications for intraspecific and interspecific variation in immunological traits. J Exp Biol 213:715–724.

Dantzer R. and K.W. Kelley. 2007. Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun 21:153–160.

Demas G.E., T. Greives, E. Chester, and S. French. 2012. The energetics of immunity: mechanisms mediating trade-offs in ecoimmunology. Pp. 259–296 in G.E. Demas and R.J. Nelson, eds. Ecoimmunology. Oxford University Press, Oxford.

Demas G.E. and R.J. Nelson. 2012. Introduction to ecoimmunology. Pp. 3–6 in G.E. Demas and R.J. Nelson, eds. Ecoimmunology. Oxford University Press, Oxford.

Drakesmith H. and A.M. Prentice. 2012. Hepcidin and the iron-infection axis. Science 338:768–772.

Graham A.L., J.E. Allen, and A.F. Read. 2005. Evolutionary causes and consequences of immunopathology. Annu Rev Ecol Evol Syst 36:373–397.

Guerra-Silveira F. and F. Abad-Franch. 2013. Sex bias in infectious disease epidemiology: patterns and processes. PLoS ONE 8:e62390.

Hambuch T.M. and E.A. Lacey. 2002. Enhanced selection for MHC diversity in social tuco-tucos. Evolution 56:841–845.

Hart B.L. 1988. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev 12:123–137.

———. 2010. Beyond fever: comparative perspectives on sickness behavior. Pp. 205–210. M.D. Breed and J. Moore, eds. Encyclopedia of animal behavior. Vol. 1. Academic Press, Boston.

Hart B.L. and L.A. Hart. 2019. Sickness behavior in animals: implications for health and wellness. Pp. 171–175 in Encyclopedia of animal behavior. Elsevier, Amsterdam.

Hasselquist D. and J.-Å. Nilsson. 2012. Physiological mechanisms mediating costs of immune responses: what can we learn from studies of birds? Anim Behav 83:1303–1312.

Hennessy M.B., T. Deak, P.A. Schiml-Webb, S.E. Wilson, T.M. Greenlee, and E. McCall. 2004. Responses of guinea pig pups during isolation in a novel environment may represent stress-induced sickness behaviors. Physiol Behav 81:5–13.

Kent S., J.L. Bret-Dibat, K.W. Kelley, and R. Dantzer. 1996. Mechanisms of sickness-induced decreases in food-motivated behavior. Neurosci Biobehav Rev 20:171–175.

King M.O. and D.L. Swanson. 2013. Activation of the immune system incurs energetic costs but has no effect on the thermogenic performance of house sparrows during acute cold challenge. J Exp Biol 216:2097–2102.

Klasing K.C. and T.V. Leshchinsky. 1999. Function, costs and benefits of the immune system during development and growth. Pp. 2817–2835 in N.J. Adams and R.H. Slotow, eds. Proceedings of the 22nd International Ornithology Congress. BirdLife South Africa, Johannesburg.

Klein J., A. Sato, S. Nagl, and C. O'hUigín. 1998. Molecular trans-species polymorphism. Annu Rev Ecol Syst 29:1–21.

Klein S.L. 2004. Hormonal and immunological mechanisms mediating sex differences in parasite infection. Parasite Immunol 26:247–264.

Kluger M.J. 1991. Fever: role of pyrogens and cryogens. Physiol Rev 71:93–127.

Kluger M.J., W. Kozak, C.A. Conn, L.R. Leon, and D. Soszynski. 1996. The adaptative value of fever. Infect Dis Clin N Am 10: 1–20.

Kozak W., C.A. Conn, and M.J. Kluger. 1994. Lipopolysaccharide induces fever and depresses locomotor activity in unrestrained mice. Am J Physiol 266:R125–R135.

Książek A., M. Konarzewski, M. Chadzińska, and M. Cichoń. 2003. Costs of immune response in cold-stressed laboratory mice selected for high and low basal metabolism rates. Proc R Soc B 270:2025–2031.

Kundu S. and C.G. Faulkes. 2004. Patterns of MHC selection in African mole-rats, family Bathyergidae: the effects of sociality and habitat. Proc R Soc B 271:273–278.

Lee K.A. 2006. Linking immune defenses and life history at the levels of the individual and the species. Integr Comp Biol 46:1000–1015.

Li Z. and C.M. Blatteis. 2004. Fever onset is linked to the appearance of lipopolysaccharide in the liver. J Endotoxin Res 10:39–53.

Liu E., K. Lewis, H. Al-Saffar, C.M. Krall, A. Singh, V.A. Kulchitsky, J.J. Corrigan, et al. 2012. Naturally occurring hypothermia is more advantageous than fever in severe forms of lipopolysaccharide- and *Escherichia coli*-induced systemic inflammation. Am J Physiol 302:R1372–R1383.

Lochmiller R.L. and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88:87–98.

Lopes P.C. 2014. When is it socially acceptable to feel sick? Proc R Soc B 281:20140218.

Luna F. and C.D. Antenucci. 2003. Daily movements and maximum speed in *Ctenomys talatum* (Rodentia: Ctenomyidae) in artificial enclosures. J Mammal 84:272–277.

———. 2007*a*. Energetics and thermoregulation during digging in the rodent tuco-tuco (*Ctenomys talarum*). Comp Biochem Physiol A 146:559–564.

———. 2007*b*. Energy and distribution in subterranean rodents: sympatry between two species of the genus *Ctenomys*. Comp Biochem Physiol A 147:948–954.

Luna F., C.D. Antenucci, and F. Bozinovic. 2009. Comparative energetics of the subterranean *Ctenomys* rodents: breaking patterns. Physiol Biochem Zool 82:226–235.

Luna F., C.D. Antenucci, and C. Busch. 2000. Ritmos de actividad locomotora y uso de las cuevas en condiciones seminaturales en *Ctenomys talarum* (Rodentia, Octodontidae). Rev Chil Hist Nat 73:39–47.

———. 2002. Digging energetics in the South American rodent *Ctenomys talarum* (Rodentia, Ctenomyidae). Can J Zool 80: 2144–2149.

Luna F., R. Šumbera, J. Okrouhlík, N. Mladěnková, and C.D. Antenucci. 2020. Evaporative water loss in seven species of fossorial rodents: does effect of degree of fossoriality and sociality exist? J Therm Biol 89:102564.

MacDonald L., D. Begg, R.S. Weisinger, and S. Kent. 2012. Calorie-restricted rats do not increase metabolic rate post-LPS, but do seek out warmer ambient temperatures to behaviourally induce a fever. Physiol Behav 107:762–772.

Mackowiak P.A. 1994. Fever: blessing or curse? a unifying hypothesis. Ann Int Med 120:1037.

Marais M., S.K. Maloney, and D.A. Gray. 2011. The metabolic cost of fever in Pekin ducks. J Therm Biol 6:116–120.

Martin L.B., D. Hasselquist, and M. Wikelski. 2006. Investment in immune defense is linked to pace of life in house sparrows. Oecologia 147:565–575.

Martin L.B., L. Kidd, A.L. Liebl, and C.A.C. Coon. 2011. Captivity induces hyper-inflammation in the house sparrow (*Passer domesticus*). J Exp Biol 214:2579–2585.

Martin L.B., H.J. Kilvitis, A.J. Brace, L. Cooper, M.F. Haussmann, A. Mutati, V. Fasanello, et al. 2017. Costs of immunity and their role in the range expansion of the house sparrow in Kenya. J Exp Biol 220:2228–2235.

Martin L.B., M. Pless, J. Svoboda, and M. Wikelski. 2004. Immune activity in temperate and tropical house sparrows: a common-garden experiment. Ecology 85:2323–2331.

Martin L.B., Z.M. Weil, and R.J. Nelson. 2008. Fever and sickness behaviour vary among congeneric rodents. Funct Ecol 22:68–77.

Martino N.S., R.R. Zenuto, and C. Busch. 2007. Nutritional responses to different diet quality in the subterranean rodent *Ctenomys talarum* (tuco-tucos). Comp Biochem Physiol A 147:974–982.

McNab B.K. 1966. The metabolism of fossorial rodents: a study of convergence. Ecology 47:712–733.

Merlo J.L., A.P. Cutrera, M.J. Kittlein, and R.R. Zenuto. 2018. Individual condition and inflammatory response to PHA in the subterranean rodent *Ctenomys talarum* (Talas tuco-tuco): a multivariate approach. Mamm Biol 90:47–54.

Merlo J.L., A.P. Cutrera, F. Luna, and R.R. Zenuto. 2014*a*. Inflammation in response to phytohemagglutinin injection in the Talas tuco-tuco (*Ctenomys talarum*): implications for the estimation of immunocompetence in natural populations of wild rodents. Can J Zool 92:689–697.

———. 2014*b*. PHA-induced inflammation is not energetically costly in the subterranean rodent *Ctenomys talarum* (tuco-tucos). Comp Biochem Physiol A 175:90–95.

———. 2016*a*. Food restriction affects inflammatory response and nutritional state in tuco-tucos (*Ctenomys talarum*): food restriction affects immunity in tuco-tucos. J Exp Zool A 325: 675–687.

———. 2016*b*. Parasite infection negatively affects PHA-triggered inflammation in the subterranean rodent *Ctenomys talarum*: parasites impair the PHA response of *C. talarum*. J Exp Zool A 325:132–141.

Moreau M., C. André, J.C. O'Connor, S.A. Dumich, J.A. Woods, K.W. Kelley, R. Dantzer, et al. 2008. Inoculation of *Bacillus Calmette-Guerin* to mice induces an acute episode of sickness behavior followed by chronic depressive-like behavior. Brain Behav Immun 22:1087–1095.

Moreno K.R., M. Weinberg, L. Harten, V.B. Salinas Ramos, V.B., M.L.G. Herrera, G.Á Czirják, and Y. Yovel. 2021. Sick bats stay home alone: fruit bats practice social distancing when faced with an immunological challenge. Ann N Y Acad Sci 1505:178–190.

Murphy K. and C. Weaver. 2016. Janeway's immunobiology. 9th ed. Taylor & Francis, New York.

Nagy K.A. 1989. Field bioenergetics: accuracy of models and methods. Physiol Zool 62:237–252.

Nižetić D., M. Stevanović, B. Soldatović, I. Savić, and R. Crkvenjakov. 1988. Limited polymorphism of both classes of MHC genes in four different species of the Balkan mole rat. Immunogenetics 28:91–98.

Norris K. and M.R. Evans. 2000. Ecological immunology: life history trade-offs and immune defense in birds. Behav Ecol 11:19–26.

Novikov E., D. Petrovski, V. Mak, E. Kondratuk, A. Krivopalov, and M. Moshkin. 2016. Variability of whipworm infection and humoral immune response in a wild population of mole voles (*Ellobius talpinus* Pall.). Parasitol Res 115:2925–2932.

Otálora-Ardila A., L.G. Herrera M., J.J. Flores-Martínez, and K.C. Welch. 2017. The effect of short-term food restriction on the metabolic cost of the acute phase response in the fish-eating *Myotis* (*Myotis vivesi*). Mamm Biol 82:41–47.

Piersma T. 1997. Do global patterns of habitat use and migration strategies co-evolve with relative investments in immunocompetence due to spatial variation in parasite pressure? Oikos 80:623.

Previtali M.A., R.S. Ostfeld, F. Keesing, A.E. Jolles, R. Hanselmann, and L.B. Martin. 2012. Relationship between pace of life and immune responses in wild rodents. Oikos 121:1483–1492.

Ramirez-Otarola N., J. Espinoza, A.M. Kalergis, and P. Sabat. 2018. Is there an effect of environmental temperature on the response to an antigen and the metabolic rate in pups of the rodent *Octodon degus*? J Therm Biol 71:17–23.

Ramirez-Otarola N., M. Sarria, D.S. Rivera, P. Sabat, and F. Bozinovic. 2019. Ecoimmunology in degus: interplay among diet, immune response, and oxidative stress. J Comp Physiol B 189:143–152.

Roberts M.L., K.L. Buchanan, and M.R. Evans. 2004. Testing the immunocompetence handicap hypothesis: a review of the evidence. Anim Behav 68:227–239.

Romanovsky A.A., M.C. Almeida, D.M. Aronoff, A.I. Ivanov, J.P. Konsman, A.A. Steiner, and V.F. Turek. 2005. Fever and hypothermia in systemic inflammation: recent discoveries and revisions. Front Biosci 10:2193–2216.

Romanovsky A.A. and M. Székely. 1998. Fever and hypothermia: two adaptive thermoregulatory responses to systemic inflammation. Med Hypotheses 50:219–226.

Ross G., T. Hübschle, A. Bickenbach, E. Zeisberger, R. Gerstberger, and J. Roth. 2001. Febrile and cortisol responses induced in guinea pigs by localized peripheral inflammatory stimulation. J Therm Biol 26:319–324.

Rossin A. and A.I. Malizia. 2002. Relationship between helminth parasites and demographic attributes of a population of the subterranean rodent *Ctenomys talarum* (Rodentia: Octodontidae). J Parasitol 88:1268–1270.

Rudaya A.Y., A.A. Steiner, J.R. Robbins, A.S. Dragic, and A.A. Romanovsky. 2005. Thermoregulatory responses to lipopolysaccharide in the mouse: dependence on the dose and ambient temperature. Am J Physiol 289:R1244–R1252.

Schmid-Hempel P. 2011. Ecological Immunology. Pp. 98–140 in P. Schmid-Hempel, ed. Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics. Oxford University Press, Oxford.

Schmidt-Nielsen K. 1997. Animal physiology: adaptation and environment. 5th ed. Cambridge University Press, Cambridge.

Sheldon B.C. and S. Verhulst. 1996. Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology. Trends Ecol Evol 11:317–321.

Sköld-Chiriac S., A. Nord, J.-Å. Nilsson, and D. Hasselquist. 2014. Physiological and behavioral responses to an acute-phase

response in zebra finches: immediate and short-term effects. Physiol Biochem Zool 87:288–298.

Sköld-Chiriac S., A. Nord, M. Tobler, J.-A. Nilsson, and D. Hasselquist. 2015. Body temperature changes during simulated bacterial infection in a songbird: fever at night and hypothermia during the day. J Exp Biol 218:2961–2969.

Stearns S.C. 1989. Trade-offs in life-history evolution. Funct Ecol 3:259.

Stockmaier S., D.K.N. Dechmann, R.A. Page, and M.T. O'Mara. 2015. No fever and leucocytosis in response to a lipopolysaccharide challenge in an insectivorous bat. Biol Lett 11:20150576.

Tella J.L., A. Scheuerlein, and R.E. Ricklefs. 2002. Is cell-mediated immunity related to the evolution of life-history strategies in birds? Proc R Soc B 269:1059–1066.

Underwood A.J. 1996. Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, Cambridge.

Vera F., C.D. Antenucci, and R.R. Zenuto. 2011. Cortisol and corticosterone exhibit different seasonal variation and responses to acute stress and captivity in tuco-tucos (*Ctenomys talarum*). Gen Comp Endocrinol 170:550–557.

Vera F., R.R. Zenuto, and C.D. Antenucci. 2008. Decreased glucose tolerance but normal blood glucose levels in the field in the caviomorph rodent *Ctenomys talarum*: the role of stress and physical activity. Comp Biochem Physiol A 151:232–238.

Vleck D. 1979. The energy cost of burrowing by the pocket gopher *Thomomys bottae*. Physiol Biochem Zool 52:122–136.

Weinberg E.D. 1984. Iron withholding: a defense against infection and neoplasia. Physiol Rev 64:65–102.

Withers P.C. 1977. Measurement of metabolic rate, $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, and evaporative water loss with a flow-through mask. J Appl Physiol 42:120–123.

Zenuto R.R., C.D. Antenucci, and C. Busch. 2002. Bioenergetics of reproduction and pup development in a subterranean rodent (*Ctenomys talarum*). Physiol Biochem Zool 75:469–478.