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Assessing the energetic costs and trade-offs of a PHA-induced inflammation in the subterranean rodent *Ctenomys talarum*: Immune response in growing tuco-tucos

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ABSTRACT

A traditional approach used to assess whether immune defense is costly is to explore the existence of trade-offs between immunity and other functions; however, quantitative studies of the energetic costs associated with the activation of the immune system are scarce. We assessed the magnitude of a PHA-triggered immune response and the associated energetic costs in 60-day old *Ctenomys talarum*. We expected that the magnitude of the macroscopic inflammatory response to PHA is lower in young tuco-tucos compared with that of adults, given the allocation of substantial energy to growth, and that the magnitude of the inflammation is lower in male pups compared to females, due to the higher investment in growth of the larger sex. Concomitantly, we expected that the pups challenged with PHA show an increase in oxygen consumption compared to control animals and that a positive association exists between magnitude of the PHA-induced inflammation and oxygen consumption. Contrary to what was expected, young tuco-tucos mounted a higher inflammatory response compared with adults and there were no differences in the magnitude of this response between sexes. The inflammatory response induced by a PHA injection did not represent a significant energetic cost for young tuco-tucos. There were no differences in oxygen consumption between PHA-injected and control animals, and tuco-tucos that mounted a higher inflammatory response to PHA did not show higher oxygen consumption. Energy expenditure, however, is not the only physiological cost involved in trade-offs between immune response and various functions of the organism, and other currencies are discussed.

1. Introduction

Immune activation and maintenance are not only expected to represent fitness benefits in the form of resistance against pathogen infections but also expected to represent substantial costs (Lochmiller and Deerenberg, 2000). These processes may use resources that the host could need for other essential functions, such as reproduction, growth or dispersal (Sheldon and Verhulst, 1996; Lochmiller and Deerenberg, 2000; Norris and Evans, 2000; Lee, 2006; Phillips et al., 2010). Briefly, the vertebrate immune system comprises the innate and the adaptive branches. Innate immunity encompasses the macrophage–phagocyte system and the adaptive immune system represents the second line of defense and is generally divided into a cell-mediated component – which fights primarily against intracellular pathogens such as viruses (Janeway et al., 2004) – and a humoral component – which recognizes and destroys extracellular parasites and pathogens through the concerted action of B- and Th2-cells that differentiate and produce antibodies (Janeway et al., 2004). The costs of the induced antibody and cell-mediated immune responses are considered to be substantially higher than those associated with the development of macrophages, natural killer cells and neutrophils responsible for innate immunity (Janeway et al., 2004). A traditional approach used to assess whether immune defense is costly is to explore the existence of trade-offs between immunity and other functions; i.e., ectoparasites were reported to have a profound negative effect on individual reproductive success and on body mass in female Columbian ground squirrels (Neuhaus, 2003), while an increase in parental effort weakens the antibody-mediated immune response against experimentally injected antigens in zebra finches and collared flycatchers (Deerenberg et al., 1997; Nordling et al., 1998). However, quantitative studies of the energetic costs associated with the activation of the immune system are scarce (Derting and Compton, 2003; Martin et al., 2003; Pilorz et al., 2005).

In general, nursing young mammals can optimize energy allocation to growth and thermoregulation versus other costly processes, such as

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immunity; the outcome of such balance may vary across different developmental stages, depending on which processes are prioritized (growth, sexual maturation, and development of immune defenses). For example, parasitized pups of *Mus musculus* grow faster, reaching the reproductive condition earlier and, probably, modulating the effects of parasites (Kristan, 2002). Moreover, in sexually dimorphic species, members of the larger sex invest less in immune response since they may have to allocate more resources and energy towards growth (Fargallo et al., 2002; Chin et al., 2005). Besides the fact that negative correlations between immune responsiveness and growth can result from the allocation of limited resources between these two processes (Fair et al., 1999), immune response of developing young animals is expected to be lower compared with that of adults due to immaturity of the immune system (Palacios et al., 2009). This is the assumption even for precocial pups (Hõrak et al., 1999; Klasing and Leschinsky, 1999). In particular, in newborn mammals, the adaptive immune system is functionally immature and neonates rely on their own innate immune system as well as on passive immunity acquired through maternal sources (Cahill et al., 1999). Although much of the immune response at this early phase of development involves the innate system, this period is characterized by immaturity of the neutrophil function and production (Carr, 2000). Furthermore, a markedly reduced release of proinflammatory signals in mononuclear cells of human infants exposed to LPS (Levy, 2005) also exemplifies that the innate response undergoes a period of maturation in early life too.

In this study, we examined the magnitude of the inflammatory response and associated energetic costs in pups of *Ctenomys talarum* (tuco-tucos), which are subterranean rodents, distributed in coastal grasslands and sand dunes in Buenos Aires Province, Argentina, that only leave their burrows to forage and at the time of dispersal (Busch et al., 1989). Despite these rodents are solitary, mothers share the burrows with the nurslings until weaning, which occurs 45 days after birth (Zenuto et al., 2002). Tuco-tucos are polygynous and males, the larger sex, do not exert parental care (Zenuto et al., 2001, 2002). Pups of tuco-tucos are rather altricial with respect to developmental stage at birth (Zenuto et al., 2002; Cutrera et al., 2003). Adult body temperature is reached at 30 days of age, although it stabilizes when pups are 45 days old, at the time when weaning occurs. Pups achieve adult resting metabolic rates (RMR) approximately two months after they are born, when they are also ready to leave the maternal burrow (Zenuto et al., 2002); however, body mass of both sexes continues to increase after this age and even after sexual maturity, with females reaching an asymptotic size earlier than males (Malizia and Busch, 1991). With respect to immunocompetence, substantial energetic costs associated with a humoral response against a novel antigen were reported in adult *C. talarum* (Cutrera et al., 2010) while no significant increase in oxygen consumption ($\dot{V}O_2$) was verified in tuco-tucos challenged with phytohemagglutinin (PHA, Merlo et al. unpublished results). PHA is a vegetal lectin expected to trigger both the innate immune system (inflammation) and the cell-mediated (T-lymphocytes) arm of the adaptive immune system (Goto et al., 1978), although the relative importance of these two systems in the inflammatory reaction differs between species (Martin et al., 2006). Tuco-tucos in particular develop a macroscopic inflammatory response to PHA that starts 6 h after immunization and lasts 48 h. However, the peak of infiltrating leukocytes in the zone occurs at 12 h post-injection, and it is composed mainly by neutrophils (innate-arm cells; Merlo, 2011). The aim of this study was to assess the magnitude of a PHA-triggered immune response and the energetic costs of this process in 60-day old *C. talarum*. We consider this as a particularly interesting stage of development because even though young tuco-tucos have reached certain adult characteristics of their metabolism (body temperature, maximum metabolic rate, Zenuto et al., 2002, Cutrera et al., 2003, Luna et al., unpublished results) they are still growing in body size and they most likely face novel immune challenges associated with the process of leaving the maternal burrow (i.e. increased aggression with siblings and mother, and with other conspecifics in neighboring burrows, higher predation risk and parasite exposure; Zenuto et al., 2001; Kittlein et al., 2001). In terms of trade-offs, given that the adult values of maximum metabolic rate are achieved at 10 days of age (Luna et al., unpublished results) if the response to PHA is energetically costly for young *C. talarum*, we expect that 1) the magnitude of the macroscopic inflammatory response to PHA is lower in young tuco-tucos compared with that of adults, given the allocation of substantial energy and resources to growth and, possibly, the immaturity of the immune system; and 2) the magnitude of the inflammation are lower in male pups compared to females, due to the higher investment in growth of the larger sex. In terms of quantifying the costs associated with immunity, if the response to PHA is energetically costly for young *C. talarum*, we expect that 3) the pups challenged with PHA show an increase in oxygen consumption compared to control animals and 4) a positive association exists between magnitude of the PHA-induced inflammation and percentage increase of oxygen consumption.

2. Material and methods

2.1. Animal capture and captivity conditions

Pregnant females were captured using live wire-tube traps in the sand dunes of Mar de Cobo, Argentina (37° 45 S; 57° 56′ W) between July and November of 2011 and 2012. Captured individuals were carried to the laboratory and housed individually in plastic boxes ($0.30 \times 0.40 \times 0.25$ m). These boxes, containing wood shavings for bedding and nesting material and half a terra cotta flowerpot as refuge, were covered with dark fabrics. Females tended to use these flowerpots as shelters and they remained most of the time inside them. Animal room temperature was maintained at 24 ± 1 °C, and photoperiod was light/dark 12:12 (lights turned on at 7 a.m.). Relative ambient humidity ranged from 50% to 70%. Animals were fed with mixed grasses, sweet potato, lettuce, corn and sunflower seeds ad libitum. Water was not provided since *C. talarum* does not drink free water.

2.2. PHA challenge

Of 20 pregnant females, twelve gave birth to a total of 30 pups. Three litters were born in 2011 and 9 in 2012, with an average of 2.76 ± 1.09 pups/litter (range: 1–5). Except for one litter of only one pup, we were able to use one or two "pairs" of pups per litter for the experimental design ($n = 14$ pairs). One pup of the pair was assigned to the control group (group "C") and the other one to the immune-challenged group (group "IC"). In this way, all of the litters had at least one pup injected with PBS and one pup injected with PHA.

In order to compare the magnitude of inflammation between young and adults of *C. talarum*, the results obtained in this study were compared with those obtained for adult tuco-tucos by Merlo et al. (unpublished results). Briefly, adults of both sexes were captured during 2012 in the same study population (17 females and 14 males) and injected with PHA using the same methodology described below for pups. Adult animals were maintained in captivity under the same conditions as pups and inflammation were measured 24 h after the injection.

In litters of 1, 3 or 5 pups, 1 pup was used to assess at what point in time the PHA-induced inflammation peaks, in order to determine when to measure oxygen consumption. Prior to injection, the thickness of each hind foot was measured with a digital micrometer (Insize, Sao Paulo, Brazil) to the nearest 0.01 mm. Pups ($n = 9$) were injected subcutaneously in the instep of the left hind foot with sterile phosphate-buffered saline solution (PBS; 0.3 μL/g of mass) and on the right hind foot with phytohemagglutinin (*Phaseolus vulgaris* PHA-Sigma L-8754 solution dissolved in PBS, 3 mg/mL; 0.3 μL/g of mass) using a 30G needle, as described by Merlo (2011). Measurements were repeated twice and averaged. At time intervals of 6, 12, 24, 48 and 72 h (±30 min) post-injection, swelling measurements were

performed. The PHA or PBS response was calculated as the difference between pre- and post-injection thickness divided by initial foot thickness (response = (post–pre)/pre; Gouy de Bellocq et al., 2006; Xu and Wang, 2010).

Oxygen consumption ($\dot{V}O_2$) of pups was measured when they were 60 days old, as described in the next section. After that, the thickness of the right hind foot was measured with a digital micrometer (Insize®) to the nearest 0.01 mm and the area was treated with an antiseptic solution (PERVINOX®, Phoenix laboratories). Following this procedure, pups were injected subcutaneously in the instep of the right hind foot with either sterile PBS (group C: 0.3 μL/g of mass) or PHA (group IC: PHA-Sigma L-8754 solution dissolved in PBS, 3 mg/mL; 0.3 μL/g of mass) using a 30G needle. In light of the results obtained regarding the inflammation response in time for pups, the $\dot{V}O_2$ was measured for a second time 12 h after injection, as described below; 24 h post-injection, thickness of the right hind foot was measured again to calculate the injection-induced swelling, following Merlo (2011). Inflammatory response was calculated as the difference between pre- and post-injection thickness is divided by initial thickness ((response = (post–pre)/pre); Xu and Wang, 2010).

2.3. Oxygen consumption measurements

Oxygen consumption ($\dot{V}O_2$) of pups was measured in 30–45-min trials using a computerized positive pressure open-flow respirometry system (Sable System, Las Vegas) at 60 days of pups' age, following Luna et al. (2009, 2012). $\dot{V}O_2$ was measured in the morning or in the afternoon, since *C. talarum* exhibits an arrhythmic pattern of daily activity (Luna et al., 2000; Cutrera et al., 2006). The chamber system consisted of a transparent acrylic cylinder (1.8 L). It received dry and CO_2 free air at 1400 mL/min from a mass flow controller (Sierra Instruments, Monterey, CA, USA). Air passed through CO_2-absorbent (IQB) and water scrubber (Silica Gel) before and after passing through the chamber. Excurrent air from the metabolic chamber was subsampled at 130 ± 10 mL/min and oxygen consumption was obtained from an Oxygen Analyzer FC-1B every 5 s set by an ExpeData PC program (Sable System, Las Vegas, NV, USA). Animals were allowed to habituate to the respirometry chamber, at least 30 min before trials. The baseline of the respirometry system was set in 20.95% of oxygen before the beginning of each experiment, also the chamber required 6 min to stabilize before the $\dot{V}O_2$ measurement started and 2 min after to compute a final baseline. Oxygen consumption values were measured using the following equation (Withers, 1977):

$$\dot{V}O_2 = FR(FiO_2 - FeO_2)/(1 - FiO_2)$$

where $\dot{V}O_2$: oxygen consumption rate (mL/h), FR: air flow rate (mL/min), Fi O_2: fractional O_2 concentration in the incurrent air flow (0.2095), Fe O_2: fractional O_2 concentration in excurrent airflow. Minimal $\dot{V}O_2$ was measured as the 5 min lowest steady-state values of the trial, and the lack of animal's activity during this period was confirmed by direct observation (Baldo et al., 2014) For comparative purposes, $\dot{V}O_2$ data were expressed as mass-specific (mL O_2/gh). All results are presented as means ± S.D.

2.4. Statistics

A two-way ANOVA was used to test the hypothesis that the magnitude of the inflammation did not differ between young and adult *C. talarum* or between sexes. A two-way ANOVA was also used to test the hypothesis that the magnitude of the inflammation in pups did not differ between treatments (PBS- vs. PHA-injected animals) or sexes. A one-way repeated-measures ANOVA was used to test the hypothesis that PHA-induced inflammation did not vary among different times post-injection and between treatments (PBS vs. PHA). A two-way ANOVA was used to test if the difference in body weight (Δ_{bw}) between 0 and 12 h post injection, calculated as (body weight at 12 h − body weight at 0 h) / body weight at 0 h, was the same between treatments (PHA vs. PBS) or sexes. A two-way repeated measures ANCOVA (with body weight as the covariate) was used to test the hypothesis that oxygen consumption did not differ between treatments (PBS- vs PHA-injected animals) or sexes. Scatterplots of $\dot{V}O_2$ versus body mass were built in order to detect possible outliers. If a possible outlier was identified from these plots, the modified Z-score was calculated; any number in a data set with the absolute value of modified Z-score exceeding 3.5 may be considered an outlier (Barnett and Lewis, 1994). We also evaluated the association between the magnitude of the PHA-induced inflammation and the oxygen consumption in pups. In order to do this, the effects of body mass on thickness measurements and $\dot{V}O_2$ 12 h post PHA-injection were first removed using the residuals of a linear regression between thickness measurement 24 h post injection and body mass and a linear regression between $\dot{V}O_2$ 12 h post PHA-injection and body mass. Values of body mass, $\dot{V}O_2$ and inflammation were log-transformed to achieve linearity. Residual thickness measurements were correlated with residual $\dot{V}O_2$ measurements using a Pearson product–moment correlation. Throughout the text, results are expressed as mean ± standard deviation.

3. Results

Sixty-day old tuco-tucos mounted a significantly larger macroscopic inflammatory response in the PHA-injected foot compared with the foot injected with PBS (one-way repeated-measures ANOVA; $F_{1,16} = 66.11$, $p < 0.001$; Fig. 1). The peak of the magnitude of the inflammatory response to PHA was detected 24 h after the inoculation of the antigen (PHA-injected foot thickness at 24 h = 4.37 ± 0.58 mm, inflammation ratio = 0.68 ± 0.17; PBS-injected foot thickness at 24 h = 2.74 ± 0.24 mm, inflammation ratio = 0.02 ± 0.05; one-way repeated-measures ANOVA; $F_{4,16} = 22.39$, $p < 0.001$, Scheffé post hoc test $p < 0.05$, Fig. 1).

Contrary to what was expected, the magnitude of the PHA-induced inflammation was higher in young tuco-tucos (foot thickness at 0 h: 2.51 ± 0.28 mm; foot thickness at 24 h: 4.05 ± 0.85 mm; inflammation ratio = 0.61 ± 0.28) compared with adults (foot thickness at 0 h: 3.04 ± 0.32 mm; foot thickness at 24 h: 4.31 ± 0.58 mm; inflammation ratio = 0.42 ± 0.19; two-way ANOVA; $F_{1,41} = 5.97$, $p = 0.018$; Fig. 2) but there was not a significant effect of sex (two-way ANOVA; $F_{1,41} = 0.059$, $p = 0.809$; Fig. 2) or a significant interaction between these two factors (two-way ANOVA; $F_{1,41} = 0.403$, $p = 0.529$). Although 60-day-old tuco-tucos injected with PHA showed a significantly larger inflammation (foot thickness at 0 h: 2.51 ± 0.28 mm; foot thickness at 24 h: 4.05 ± 0.85 mm; inflammation ratio = 0.61 ± 0.28) than those of the control group (foot thickness at 0 h: 2.47 ± 0.29 mm; foot thickness at 24 h:

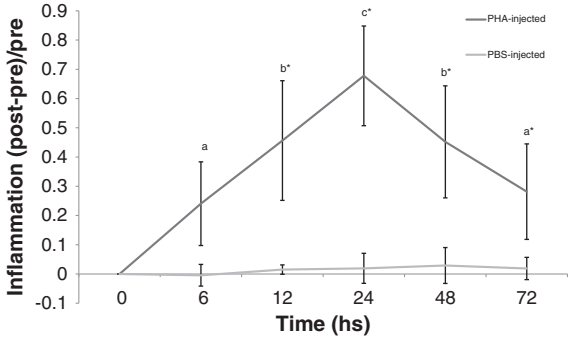

Fig. 1. Mean ± SD inflammatory response in 60-day old tuco-tucos injected with PHA in the right hind foot (darker line) and PBS in the left hind foot (lighter line) at 6, 12, 24, 48 and 72 hour post injection. Asterisks denote significant differences between PHA- and PBS-injected feet (one-way repeated measures ANOVA, Scheffé $p < 0.05$). Different letters represent significant differences in the magnitude of the PHA-induced inflammatory response among times post injection (one-way repeated measures ANOVA, Scheffé $p < 0.05$).

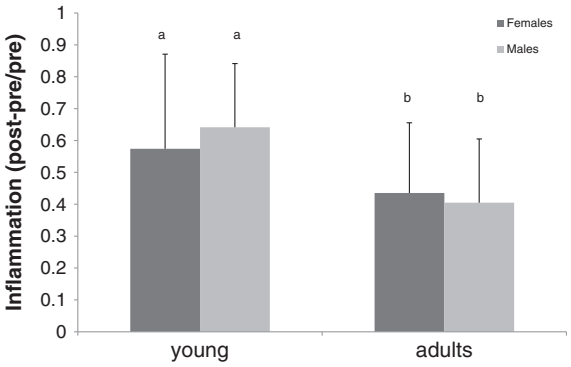

Fig. 2. Mean + SD values of inflammation measured 24 hour post-injection in young (60 days old) and adult tuco-tucos of both sexes injected with PHA. Different letters represent significant differences (two-way ANOVA, $p < 0.05$).

2.53 ± 0.22 mm; inflammation ratio $= 0.03 \pm 0.08$; two-way ANOVA; $F_{1,13} = 45.71$, $p < 0.001$; Fig. 3), there were no differences in the magnitude of the inflammation between males and females (two-way ANOVA; $F_{1,13} = 0.02$, $p = 0.9$; Fig. 3) and there was not a significant interaction between these factors (two-way ANOVA; $F_{1,13} = 0.45$, $p = 0.51$).

With respect to the effect of PHA injection on body weight, Δ_{bw} did not differ between treatments (two-way ANOVA; $F_{1,13} = 0.03$, $p = 0.869$) or sexes (two-way ANOVA; $F_{1,13} = 0.002$, $p = 0.962$). The interaction between these factors was not significant either (two-way ANOVA; $F_{1,13} = 0.09$, $p = 0.765$). Regarding the costs of the response to PHA, no significant differences in oxygen consumption were detected between young tuco-tucos injected with PHA and those injected with PBS (Tables 1 and 2). The association between $\dot{V}O_2$ 12 h post PHA-injection and body mass was not significant ($r = 0.09$, $F_{1,9} = 0.0745$, $p = 0.791$), as well as the association between inflammation 24 h post PHA-injection and log body mass ($r = 0.004$, $F_{1,9} = 0.0001$, $p = 0.991$). No outliers were detected in the scatterplots of $\dot{V}O_2$ data versus body mass, after calculating the modified Z-scores (all Z-scores < 3, Fig. 4). Finally, there was not a significant association between the magnitude of the inflammation 24 h post injection with PHA and $\dot{V}O_2$ 12 h post injection with this antigen (Pearson product–moment correlation; $r = 0.393$, $p = 0.236$, $n = 11$).

4. Discussion

Our findings suggest that the inflammatory response induced by a PHA injection does not represent a significant energetic cost for young tuco-tucos. In particular, and contrary to what was expected, young tuco-tucos mounted a higher inflammatory response to PHA compared with adults and there were no differences in the magnitude of this response between sexes, despite the marked sexual dimorphism in body size observed in *C. talarum*. Further, there were no differences in oxygen consumption or body weight between young tuco-tucos injected with PHA and control animals (injected with PBS). Concomitantly, tuco-tucos that mounted a higher inflammatory response to PHA did not show higher oxygen consumption.

Even in the absence of developmental constraints associated with immaturity of the immune system, other studies have found that young animals have a lower ability to respond to immune challenges compared to adults, probably associated with resource reallocation from immunity to other pressing costly activities, such as growth (e.g., Lozano and Lank, 2003). However, we found the opposite picture for *C. talarum*: young tuco-tucos mounted a significantly higher inflammatory response to PHA compared with that of adults. During this stage of development, young tuco-tucos still share the maternal burrow with their siblings and their mother, and the frequency of injuries increases around this age as they get close to the moment when they leave the maternal burrow, associated with mother–pups aggression and fights among siblings as well as with interactions with predators (Zenuto et al., 2002). This may lead young tuco-tucos to mature an innate immune system earlier than observed for model species such as mice or rats (reviewed by Marshall-Clarke et al., 2000), which allows them to rely on a rather less costly, more immediate, although less specific response, such as that induced by PHA. In fact, this response has been proved in bird species to have lower effects on growth parameters (i.e. fluctuating asymmetry) compared to a humoral response (Fair et al., 1999; Amat et al., 2007). On the other hand, older tuco-tucos are more parasitized than young animals (Rossin et al., 2002, 2010) and also have to balance immunity against other costly activities, such as reproduction and territory defense during the breeding season (Cutrera et al., 2010), all of which could impact negatively on the magnitude of the inflammatory response to a non-pathogenic antigen like PHA in adults compared to young animals (Verststeegh et al., 2012). Most results about the development of immune function and the associated costs come from studies using mice and rats as study models (Marshall-Clarke et al., 2000). Our findings stress the importance of studying the implications of an immune challenge in a wider array of

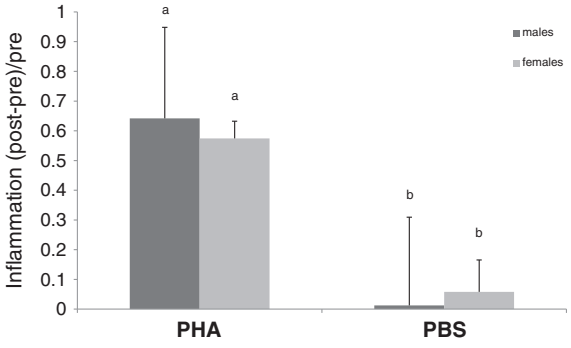

Fig. 3. Mean + SD values of inflammation in PHA- and PBS-injected young tuco-tucos (60 days old) of both sexes 24 hour post-injection. Different letters represent significant differences (two-way ANOVA, $p < 0.05$).

Table 1
Mean values of body mass and metabolic rate ($\dot{V}O_2$) recorded in 60-day old *Ctenomys talarum* of both sexes before (0 h) and after (12 h) injection with PHA or PBS. Standard deviation values are given between parentheses.

Treatment	Sex	Time (h)	Body mass (g)	$\dot{V}O_2$ (mL/ h)
PHA	Male	0	61.21 (7.55)	77.20 (14.88)
		12	62.53 (6.21)	99.48 (25.5)
	Female	0	55.90 (5.78)	72.74 (8.73)
		12	57.80 (6.93)	82.48 (16.13)
PBS	Male	0	61.20 (8.62)	78.79 (23.1)
		12	63.56 (10.21)	86.29 (19.8)
	Female	0	57.92 (3.91)	74.68 (18.43)
		12	59.79 (6.47)	93.38 (33.25)

Table 2
Results of the two-way repeated measures ANCOVA (with animal weight as the covariate) used to test the hypothesis of no differences in oxygen consumption (mL/h) between treatments (PHA vs. PBS) and sexes in 60-day-old tuco-tucos.

Effect	df	F	p
Weight 0 h	1	0.444	0.512
Weight 12 h	1	2.439	0.133
Treatment	1	0.000	0.991
Sex	1	0.020	0.888
Treatment × sex	1	0.627	0.437
Time (within effect)	1	0.138	0.714
Time × weight 0	1	2.119	0.160
Time × weight 12	1	3.555	0.073
Time × treatment	1	0.385	0.541
Time × sex	1	1.083	0.309
Time × treatment × sex	1	1.366	0.255

species, particularly in wild animals, whose immune responses have been modeled by natural selective pressures associated with their particular habits and environments.

Due to the fact that both sexes continue to grow after sexual maturity, but females reach an asymptotic size earlier than males (Malizia and Busch, 1991), we expected male tuco-tucos to mount a weaker response to PHA compared to females. However, no differences were detected in the magnitude of the response to PHA between sexes. While the importance of body size for future recruitment has been well documented for several groups of vertebrates (Klasing and Leschinsky, 1999; Norris and Evans, 2000; Mc Elligott et al., 2001), the evidence is not as clear for *C. talarum*. Further research is needed to establish the role of male body size in female mate choice to assess the long-term consequences of an experimentally-induced allocation to immunity during the development of tuco-tucos of both sexes to contribute to our understanding of the ultimate costs and benefits of immunocompetence in young members of this species.

Assessing the costs of resistance to infection may also contribute to understand the existence and maintenance of diversity in host resistance in natural populations (Gillespie, 1975; Antonovics and Thrall, 1994; Auld et al., 2013). As pointed out by Klasing (2004), generalizations regarding the energetic costs of resistance are difficult due to the diversity of types of immune challenges used, their intensities and their duration, as well as the different arms of the immune system that are triggered by these challenges. According to our results, energetic costs associated with a PHA-induced inflammatory response in young *C. talarum* seem to be low, relative to previous studies conducted in birds. Specifically, a 19% increase in RMR was observed in adult little ringed plovers injected with PHA (*Charadrius dubius*, Gutierrez et al., 2011), while a 29–32% increase in RMR was reported in PHA-challenged house sparrows (*Passer domesticus*, Martin et al., 2003, 2006). In contrast, little effect of the PHA challenge was reported on the RMR of great tits (*Parus major*, Nilsson et al., 2007), and white-footed mice (*Peromyscus leucopus*, Derting and Compton, 2003) and even a 20–25% decrease in RMR was observed in PHA-injected tree sparrows (*Passer montanus*, Lee et al., 2005). Although some of these differences may be associated with methodological issues (see Lee et al., 2005; Nilsson et al., 2007 for discussion), more studies on the energetic costs of the cell-mediated immune response triggered by PHA, particularly on small mammals, are needed to elucidate the implications of this immune response. Young tuco-tucos used in this study were maintained in captivity from the moment they were born until the experiments were finished; they were fed ad libitum and kept in plastic boxes where digging was not possible. Also, ambient temperature of the animal room was set within thermoneutrality. Therefore, one of the questions that remain unanswered is how this PHA-induced immune response would impact the energetic budget of a wild tuco-tuco in natural conditions, at the moment when they leave their natal burrow. In their natural habitat, dispersing tuco-tucos have to face other energy demands associated with, for example, thermoregulation, burrow construction and maintenance, encounters with conspecifics and also movements below and above ground, assumed to be high as proposed in Antinuchi et al. (2007). Our findings suggest that the magnitude of PHA-induced swelling is not associated with $\dot{V}O_2$ in tuco-tucos. Given that the energetic costs associated with this response might be very low and that variance seems large among individuals, our ability to detect significant results may have been reduced. Therefore, future studies will be aimed to assess the role of maternal effects, pups' characteristics and litter size on modeling such as variability in *C. talarum*. Moreover, even if the costs associated with this type of immune response were too small to be detected by our study, and the impact of the PHA challenge on the energy budget of young tuco-tucos may seem low in the short term, it could still influence their performance at this crucial stage of development or even be significant in the long term, as previously reported in eiders (*Somateria mollisima*, Hanssen et al., 2004). Energy expenditure, however, is not the only physiological cost that may affect trade-offs between immune response and other functions of the organism, and specific nutrients, such as proteins, may also mediate such trade-offs (Zuk and Stoehr, 2002; Brommer, 2004; Klasing, 2004). Protein resources are particularly limiting in young growing mammals and hence their influence on the balance between immunity and growth may be important (Wikelski and Ricklefs, 2001). Finally, the requirement of specific micronutrients that induce increased immunocompetence (see Brommer, 2004) in the face of an immune challenge may also be hampered in captive conditions, where food was provided ad libitum. Therefore, field studies of the impact of an immune challenge in tuco-tucos in natural conditions are crucial to further our understanding of the costs of infections in these small mammals.

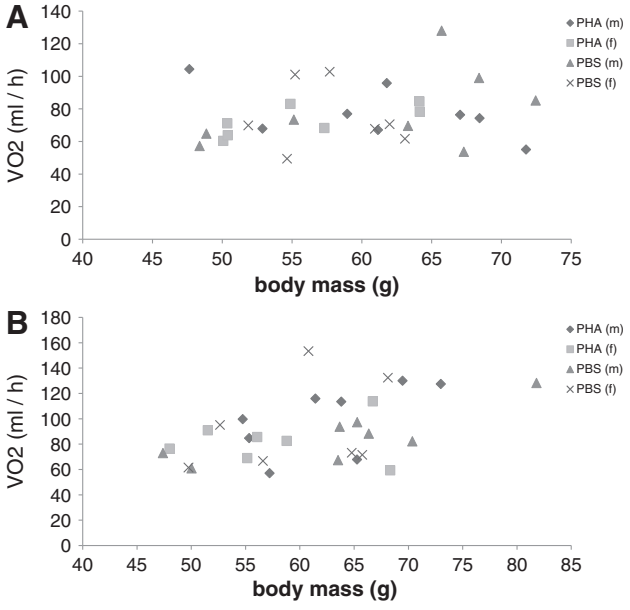

Fig. 4. Scatterplots of $\dot{V}O_2$ versus body mass values measured in males (m) and females (f) at 0 h (4A) and 12 h (4B) post-injection with PHA or PBS.

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