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The energetic costs of mounting an immune response in Pallas's long-tongued bat (*Glossophaga soricina*)

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Activation of immune response has been long assumed to be an energy-costly process but direct measures of changes in metabolic rate after eliciting immune response disputes the universality of this assertion. The acute phase response (APR) is the first line of defense of the vertebrate immune system against pathogens and is thought to be energetically costly. Measures of APR energetic cost in birds are higher than in rodents suggesting that this response is less expensive and important for mammals. However, large increase in metabolic rate after APR activation measured in a piscivorous bat species (*Myotis vivesi*) suggests that immune response is unusually costly for bats. Here we quantified the energetic cost and body mass change associated with APR in the nectarivorous Pallas's long-tongued bat *Glossophaga soricina* and compared with values previously measured for piscivorous bats and other vertebrates. APR activation implied an energy cost for *G. soricina* as indicated by a short-term decrease in body mass and an increase in resting metabolic rate (RMR). However, the increase in RMR was far from the large increase detected in piscivorous bats and it was similar to the highest values measured in birds. Caloric cost of APR represented only 2% of the total daily energy expenditure estimated for *G. soricina*. Overall our results suggest that the costs of APR for bats may vary interespecifically probably in relation to feeding habits. Measurement of the energy cost of vertebrate immune response is limited to a few species and further work is warranted to evaluate its significance for the animal´s energy budget.

The energetic costs of mounting an immune response in Pallas's long-tongued bat (*Glossophaga soricina*)

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Abstract

Activation of immune response has been long assumed to be an energy-costly process but direct measures of changes in metabolic rate after eliciting immune response disputes the universality of this assertion. The acute phase response (APR) is the first line of defense of the vertebrate immune system against pathogens and is thought to be energetically costly. Measures of APR energetic cost in birds are higher than in rodents suggesting that this response is less expensive and important for mammals. However, large increase in metabolic rate after APR activation measured in a piscivorous bat species (*Myotis vivesi*) suggests that immune response is unusually costly for bats. Here we quantified the energetic cost and body mass change associated with APR in the nectarivorous Pallas's long-tongued bat *Glossophaga soricina* and compared with values previously measured for piscivorous bats and other vertebrates. APR activation implied an energy cost for *G. soricina* as indicated by a short-term decrease in body mass and an increase in resting metabolic rate (RMR). However, the increase in RMR was far from the large increase detected in piscivorous bats and it was similar to the highest values measured in birds. Caloric cost of APR represented only 2% of the total daily energy expenditure estimated for *G. soricina*. Overall our results suggest that the costs of APR for bats may vary interespecifically probably in relation to feeding habits. Measurement of the energy cost of vertebrate immune response is limited to a few species and further work is warranted to evaluate its significance for the animal´s energy budget.

Introduction

Activation of immune response has been long assumed to be an energy-costly process leading to trade-offs with other important biological functions (Sheldon & Verhulst 1996). For example, the energy cost of immune response activation in vertebrates has been hypothesized to equal those of reproduction and growth (Lochmiller & Deerenberg 2000). However, recent direct measures of changes in metabolic rate after eliciting immune response appear to challenge this idea. For instance, resting metabolic rate (RMR) of several bird species increase only between 5 and 15% following activation of humoral and cell-mediated immunities (Hasselquist & Nilsson 2012). In particular, the acute phase response (APR) is thought to be the most energetically costly part associated with the activation of the immune system and hence, more prone to trade-off with other energetically expensive life-history traits (Lochmiller & Deerenberg 2000; Bonneaud et al. 2003; Lee 2006; but see King & Swanson 2013). The APR is the first line of defense of the immune system against pathogens, and involves leukocytosis, fever, increase RMR and a decrease in body mass (M_b), and is thought to be taxonomically conserved in vertebrates (Cray, Zaias & Altman, 2009). APR is experimentally triggered in vertebrate studies using lipopolysaccharide (LPS; Alexander & Rietschel 2001), an antigen that mimics a bacterial infection without actually getting the animal sick, and induces an inflammatory response by increasing the release of cytokines a few hours after being inoculated causing an energetically costly short-term response (Bonneaud et al. 2003; Demas et al. 2011). Short-term (<24 hours after LPS injection) APR metabolic cost has been measured in a handful of species with contrasting results. In birds, APR activation result in a large increase in RMR (~33–40%) in Pekin duck (*Anas platyrhynchos*; Marais, Maloney & Gray, 2011) and house sparrow (*Passer domesticus*; King & Swanson 2013), and a modest (~10%; Burness, Armstrong & Tilman-

Schindel, 2010) or null (Sköld-Chiriac et al. 2014) increase in zebra finch (*Taeniopygia guttata*). In mammals, modest (~10%) increments in MR have been measured in rodents (*Mus musculus* and *Rattus norvegicus*; Baze, Hunter & Hayes, 2011; MacDonald et al. 2012), but APR activation by fish-eating Myotis (*Myotis vivesi*; Otálora-Ardila et al. 2016) involves a considerable increase (up to 180%).

Strong immune response is assumed to be more likely in long-lived animals (Lochmiller & Deerenberg 2000), such as bats. Bats are one of the most diverse orders of vertebrates both in taxonomic and ecological terms and thus represent an exceptional model to test if APR is an energetically costly event. Although bats may share several features of the immune systems with other vertebrates, they do have marked qualitative and quantitative differences in their immune system (Baker, Schountz & Wang 2013). Also, it has been shown that the magnitude of bat immune response might vary as a function of physiological and ecological factors (Christe, Arlettaz & Vogel, 2000; Allen et al. 2009; Schneeberger, Czirják & Voigt, 2013b; Schneeberger, Czirják & Voigt, 2014; Strobel, Becker & Encarnação, 2015). In particular, recent examination of APR in bats shows that their response is far from being uniform. For example, APR triggered an increase in total leukocyte numbers and a decrease in M_b in the short-tailed fruit bat (*Carollia perspicillata*; Schneeberger, Czirják & Voigt, 2013a) and an increase in total leukocyte and neutrophill numbers and no change in M_b in the wrinkle-lipped bat (*Chaerephon plicatus*; Weise et al. 2017). APR induced a significant decrease in M_b and an increase in body temperature in the fish-eating Myotis (Otálora-Ardila et al. 2016), whereas the Pallas´s mastiff bat (*Molossus molossus*) did show a reduction in M_b but no change in total leukocyte numbers or body temperature (Stockmaier et al. 2015). The large increase in RMR reported in the fish-eating Myotis (Otálora-Ardila et al. 2016) is unusual among vertebrates but there is no available

67 information to evaluate if this feature is common to bats. Alternatively, and following the
68 findings reported for other aspects of bat APR, the metabolic cost of this response might vary
69 within this order with some species presenting changes in RMR in the same order than those
70 reported for other vertebrates.

71 In this study, we measured RMR and M_b of the nectarivorous Pallas's long-tongued bat
72 (*Glossophaga soricina*) (Pallas 1766; Phyllostomidae) before and after challenging its immune
73 system with an injection of LPS. With this protocol we aimed to quantify and describe the
74 magnitude of the energy costs associated with the APR in this plant-eating bat. We compared the
75 effect of the APR on RMR and M_b measured for this bat with changes determined in the Myotis
76 fishing bat and other vertebrates to test the hypothesis that the activation of this immune
77 response is unusually costly for bats. We also determined the total energy expenditure of the
78 Pallas's long-tongued bat after activating the APR to verify to what extent it jeopardizes its
79 energy budget.

80 **Materials & Methods**

81 *Animal capture and housing*

82 Adult non-reproductive individuals of *G. soricina* (9 males and 4 females; mean $M_b \pm 1$ S.D. =
83 10.3 ± 1.2 grams) were mist-netted at the entrance of the El Salitre cave, 3.6 km S of Los Ortices
84 (19°04'N, 103°43´W), Colima, Mexico, during late spring. Upon capture, bats were transported
85 to a nearby facility and kept in a 3 x 3 x 3 m flight cage exposed to natural conditions of
86 photoperiod and temperature during the whole period of experiments (30 days). During this
87 period, bats were fed a mixture of cereal, table sugar, powdered milk and banana diluted in
88 water, and maintained a stable body condition. Experiments started 2-3 days after capture and,
89 once finished, bats were released at the same site of capture. We followed American Society of

Mammologists guidelines to handle animals during the experiment (Sikes, Gannon & The Animal Care and Use Commitee of the American Society of Mammalogists, 2011). All protocols were performed under scientific collector license FAUT-0069 granted to LGHM by the Secretaría de Medio Ambiente y Recursos Naturales, Mexico.

Immune challenge

We challenged the immune system of bats by injecting 50 µL of a 0.56 mg ml^{-1} solution of LPS (L2630, Sigma-Aldrich, USA) in phosphate buffered saline (PBS; P4417, Sigma-Aldrich, USA). This is equivalent to mean dose (± 1 S.D.) of 2.84 ± 0.15 mg LPS kg^{-1}. LPS was injected subdermally and the skin surrounding the injection site was sterilized with ethanol prior and after the injection. Pilot experiments showed that this dose was high enough to elicit a sustained and significant response in RMR; lower doses did not cause a measurable response and higher doses elicited a blunted response. To control for possible effects of handling and injection, RMR was also measured separately in a group of bats injected with PBS.

Metabolic measurements

The energetic cost associated with mounting an immune response was indirectly assessed by measuring rates of oxygen consumption ($\dot{V}O_2$) in individual, fasted bats prior and after receiving LPS or PBS. Individual bats were ascribed randomly to each treatment and were tested only once; a given individual received LPS or PBS, but not both. Experiments started at 06:00-07:00 am, with measurements of pre-injection levels for two hours. Pilot experiments showed that bats usually settled down in the chamber after 1 hour; thus, this period was more than enough for RMR to achieve steady-states that could be used as standard for comparing the incremental responses associated with the administration of LPS or PBS (see results). After this period, we removed the bats from the respirometric chamber and injected them with either LPS

113 or PBS. After the injection, bats were placed back into the respirometric chamber, and $\dot{V}O_2$ were
114 continuously measured for 8-10 hours. The whole procedure of removing the bats in and out of
115 the chamber, and the injection with LPS or PBS, lasted less than five minutes.
116 We used open-flow respirometry to measure $\dot{V}O_2$ (Voigt and Cruz-Neto 2009). Bats were
117 weighed to the nearest 0.1 grams (Ohaus Precision Balance, USA), and placed in 300 ml
118 cylindrical metabolic chambers, fitted with inlet and outlet ports. A similar-sized, but empty,
119 respirometric chamber was used for baseline measurements. Air was pushed through all the
120 chambers by two sets of aquarium pumps. Prior to entering the chamber, the flow of air was
121 measured and maintained at 270-300 ml min^{-1} during the whole experiment. A tube containing
122 Drierite™ (W. A. Hammond Drierite, Xenia, Ohio, USA) absorbed the water from the incurrent
123 (before measuring the flow) and excurrent air. Experiment started by taking a 10-minute
124 baseline reading from the empty chamber and then 2 continuous reading of the excurrent air
125 form the experimental chamber that lasted for 60 minutes each, interspaced by a 10-minute
126 baseline reading between each run. After the second pre-injection run, bats were removed from
127 the chambers and injected either PBS or LPS as described above. During the injection procedure,
128 excurrent air from the empty chamber was monitored. After the bats were placed back in the
129 chamber, we recommenced the records of the excurrent air from their chambers. During this
130 period, we took continuous readings of the experimental chamber, interspaced by 10-minutes
131 reading of the empty chamber at every hour.
132 $\dot{V}O_2$ were measured from the excurrent air by a Sable System Field Metabolic System
133 (FMS, Sable Systems International, Las Vegas, USA). Data were recorded at a rate of one point
134 per second, and analyzed by the software Expedata 1.7.2 (Sable Systems International).
135 Readings from the O_2 channels were smoothed before the analysis, and $\dot{V}O_2$ were calculated

using equation 10.2 from Lighton (2008). We calibrated the O_2 sensors every other day by flowing gas from a commercial compressed gas tank containing 20.95% O_2 (Praxair, Danbury, CT, USA).

The mean temperature (T_a) of the chambers during the experiments was measured by placing a small temperature-record device (I-button, Maxim Corp, San Jose, CA, USA) at the bottom of the chamber. Mean T_a (± 1 S.D.) was 28.9 ± 0.8°C, with a variation of 2.1 ± 0.5°C, during the experiments. The mean value was slightly below the lower critical limit of the thermoneutral zone described for our focal species (31.4°C – Cruz-Neto & Abe 1997), and the maximum T_a attained during a given experiment (32.6°C) was below its upper critical temperature (35.2°C – Cruz-Neto & Abe 1997).

Data handling and analysis

Due to the small sample size for females (2 females in each treatment group), data were pooled for sex for analysis. M_b was measured before (M_{bi}) and after (M_{bf}) the experiments. Differences mean M_b, as well as in absolute ($M_{bf} – M_{bi}$) and relative [($M_{bf} – M_{bi} / M_{bi}$)] changes between groups were analyzed by t-tests.

$\dot{V}O_2$ fluctuated during the experiments due to random movements of the bats inside the chamber. To minimize such fluctuations, we used the nadir function to select the lowest and most constant 15 minutes trace, and an average of these values was used to characterize the $\dot{V}O_2$ for each hour time bin. These values of $\dot{V}O_2$ were then transformed to metabolic rate (MR) in kJ h^{-1}, by using the formula provided by Lighton (2008): MR = $\dot{V}O_2$ x [16 + 5.164 (RQ)]. Since we did not measured $\dot{V}CO_2$, and since our animals were in a fasted state, we assumed a RQ of 0.80 (Koteja 1996), and used the RMR values in all subsequent analyses.

We used a general linear model (RMR as dependent variable, time and treatment as fixed

159 factors) to test for a time by treatment effect on RMR, before and after the injection. Although
160 different individuals were used in the PBS and LPS treatments, we had repeated measurements
161 of RMR over time for each of these treatments. Thus, we also decided to include bat ID as a
162 random factor in these analyses. Finally, since M_b varied between groups, we carried out this
163 analysis using mean M_b as covariate. For the RMR data obtained after the injection, we carried
164 out this analysis using net values of RMR measured at each time bin. Net RMR was obtained by
165 discounting from the post-injection RMR, for each individual at each time bin, the lowest RMR
166 value obtained before the injection for that particular individual. In all of these analyses, a
167 Holm-Sidak post-hoc test was used for pairwise comparisons when the model identified
168 significant differences between means.

169 Two approaches have been used to estimate the energetic costs associated with mounting
170 an immune response in bats (Otálora-Ardila et al. 2016; Otálora-Ardila et al. 2017). One
171 approach estimates, for each individual, the percentage increase (PI) in RMR of LPS and PBS
172 injected bats, for each time bin, in relation to the RMR calculated for the same individuals before
173 the injection. To compare differences in PI between groups we used the same GLM approach as
174 described before. The other approach calculates an energy cost index (EC) associated with the
175 responses to LPS and PBS as being equal to the integral area under the curve that describes the
176 variation in net RMR after injection for each treatment. We calculated the area under the curve
177 using the trapezoid method (Tai 1994). Since M_b differed between groups, we expressed the
178 results on a mass-specific basis. A t-test was used to check for significant differences in mass-
179 corrected EC between the two treatment groups. All data expressed as ratios and percentage did
180 not meet the assumptions of normality and homogeneity of variances. To achieve such
181 assumption we therefore applied the arcsin square-root transformation to these raw data. All data

were presented, unless otherwise noted, as mean ± 1 s.e.m and a $p \leq 0.05$ was considered significant for all statistical analysis.

Results

Body mass changes

The M_{bi} and mean M_b of *G. soricina* did not vary between treatments (M_{bi}: $t_{11} = 0.63$, $p = 0.54$; M_b: $t_{11} = 0.99$, $p = 0.34$; Table 1). However, M_{bf} was significantly different ($t_{11} = 2.92$, $p = 0.03$). Bats injected with LPS lost more body mass than bats injected with PBS during the experiments, both in absolute ($t_{11} = 3.05$; $p = 0.01$) or in relative ($t_{11} = 3.70$; $p = 0.004$) terms.

Metabolic Rate

Metabolic rate recorded prior to injection varied with time ($F_{1, 23} = 19.4$, $p = 0.001$; Fig 1A) reaching the lowest value for both groups an hour before the injection. This value did not differ between the two treatment groups (PBS: 0.29 ± 0.04 kJ h^{-1}; LPS: 0.31 ± 0.06 kJ h^{-1}; $F_{1, 23} = 0.14$, $p = 0.71$). There was no significant treatment by time effect on pre-injection MR ($F_{1, 23} = 0.22$, $p = 0.65$). Thus, we used the RMR values obtained 1 hour before the injection as our standard for calculating the net RMR after injection.

After injection, the net RMR varied as a function of treatment ($F_{1, 95} = 5.5$, $p = 0.04$) and time ($F_{7, 95} = 22.38$, $p < 0.001$). The interaction term was significant ($F_{7, 95} = 13.89$, $p < 0.001$), with the increase evoked by LPS being higher than the increase evoked by PBS until 4 hours after the injection. After this period, no difference was observed between the net RMR of LPS and PBS injected bats ($p > 0.05$ for all pairwise comparisons).

The mean PI in RMR after injection (in relation to pre-injection RMR) varied as a function of the treatment ($F_{1, 95} = 6.1$ $p = 0.03$) and time ($F_{7, 95} = 23.7$, $p < 0.001$). The interaction term was also significant ($F_{7, 95} = 15.5$, $p < 0.001$; Fig 1B). One hour after the injection, RMR of

LPS treated bats increased by 67%, while the RMR of PBS treated group increased only by 14%. After this period, PI decreased steadily in the PBS treated group – two hours after injection the RMR already reached a value that was indistinguishable from the pre-injection RMR. For the LPS treated group, PI also decreased more slowly with time, and after 4 hours reached values that were, on average, 10% higher than pre-injection RMR. The EC associated with LPS injection was 0.72 ± 0.21 kJ or 0.10 ± 0.03 $kJ.g^{-1}$. Mass specific EC estimated for bats on the LPS treatment was significantly higher than the EC calculated for the PBS group (0.01 ± 0.01 $kJ.g^{-1}$) during the same time period ($t_{11} = 2.80$, $p = 0.01$).

Discussion

APR activation implied an energy cost for *Glossophaga soricina* as indicated by a short-term decrease in body mass and an increase in RMR. However, the increase in RMR after LPS injection for this species was far from the large increase detected in the fish-eating Myotis and it was similar to the highest values measured in birds. In the following sections we discuss our findings in relation to those reported for bats and other vertebrates.

Body mass loss (ΔM_b) of *G. soricina* challenged with LPS was nearly double that observed for individuals challenged only with PBS. We found a 11.2% decrease in body mass in LPS challenged individuals of *G. socirina* after 10 hours, a figure that was similar to changes reported for piscivorous (*M. vivesi*: 8% decrease; Otálora et al. 2016) and insectivorous bats (*M. molossus*: 7% decrease; Stockmaier et al., 2015), and *P. domesticus* (7% decrease; Bonneaud et al. 2003), but higher than those found for *M. musculus* (no change; Baze, Hunter & Hayes, 2011), *R. norvegicus* (4% decrease; MacDonald et al. 2012), and *P. domesticus* (~1.5% decrease; King & Swanson 2013). Although some of the difference in the magnitude of ΔM_b probably reflects differences in the dose used (see below), it mainly reflects the mobilization of nutrient

stores to cover the energetic costs associated with mounting an immune response and, thus, can be regarded as a universal component associated with the APR.

Pre-injection RMR did not differ between *G. soricina* assigned to the LPS or PBS treatments, but it decreased with time, with the lower values measured one hour after bats were placed in the respirometric chambers. Such a decrease was somewhat expected: manipulation of bats before placing them in the chamber usually leads to high metabolic rate at the beginning, which tends to decrease as bats settled down (Voigt & Cruz-Neto 2009). The average pre-injection RMR (pooled for both treatments) measured during the first (0.37 kJ h^{-1}) and second (0.32 kJ h^{-1}) hours before injection agrees well with basal metabolic rate measured for this species (0.35 kJ h^{-1}, range: 0.31 – 0.42 kJ h^{-1} - Cruz-Neto & Abe 1997). Mean RMR after LPS injection increased by 67% with respect to the mean value before injection. At a first glance, this increase in RMR seems to support the idea that mounting an innate immune response is indeed high. However, use of torpor by bats after PBS injection might overestimate the difference in RMR when compared with LPS-treated bats. Unfortunately, we cannot probe this idea because we did not record bat body temperatures, but *G. soricina* enters diurnal torpor only when food intake is restricted (Kelm and Helversen 2007). Food intake was not limited before injections and thus we assume that they remained normothermic during the experiments.

The increase in RMR after LPS found in *G. soricina* was lower than the increase in RMR reported in *M. vivesi* with respect to mean values after PBS injection (140 to 185%; Otálora-Ardila et al. 2016). There are a limited number of studies in mammals and birds that measured this cost using the same protocol (changes in RMR within a 24-hours period after LPS injection) as we did, and the results are quite diverse. Some studies reported no response in *M. musculus* (Baze, Hunter & Hayes, 2011) and *T. guttata* (Sköld-Chiriac et al. 2014), whereas other reported

increases in RMR of about 10% in *R. norvegicus* (MacDonald et al. 2012) and *T. guttata* (Burness, Armstrong & Tilman-Schindel) up to 33 and 40% in *P. domesticus* (King and Swanson 2013) and *Anas platyrhynchos* (Marais, Maloney & Gray, 2011), respectively. Unfortunately, our results are not strictly comparable with most of these studies for at least two reasons. First such comparison might be hampered by the differences in the mass-specific dose of LPS used in our study (2.84 mg LPS kg^{-1}) and these studies. For example, in birds, the mass-specific dose ranged from 0.1 mg LPS kg^{-1} in *A. platyrhynchos* (Marais, Maloney & Gray, 2011), 0.1 mg LPS kg^{-1} (Sköld-Chiriac at al. 2014) and 1 mg LPS kg^{-1} (Burness, Armstrong & Tilman-Schindel) in *T. guttata*, up to 5 mg LPS kg^{-1} in *P. domesticus* (King and Swanson 2013). In mammals the dose used varied between 0.05 mg LPS kg^{-1} in *R. norvegicus* (MacDonald et al. 2012) up to 1.75 mg LPS kg^{-1} in *M. vivesi* (Otálora-Ardila et al. 2016). Although high doses seem to elicit high responses (see also King & Swanson 2013), there are some discrepancies which could be due to the fact that the responses to LPS are highly variable, within and between species, even when the same lot and dose were used (Demas et al. 2011). It is noteworthy that even though we used a higher dose in *G. soricina*, the metabolic cost was higher in the fish-eating Myotis. Second, different authors measured RMR at different time bins after LPS injection, and handled the data in different ways to obtain a metric that could be used to assess whether, or not, LPS induced an increase in RMR. For example, in our study we measured RMR post-injection at hourly intervals up to 11 hours, and we calculated the factorial increment in RMR, after discounting the increment that was due to handling and injecting the bat (i.e. the increment due to PBS). This protocol was somewhat similar to the one used by Otálora-Ardila et al. (2016) for the fish-eating Myotis, and by Marais, Maloney & Gray (2011) for *A. platyrhynchos*,. Our approach does differ radically from the other studies where RMR was not measured continuously, but at a fixed, and

sometimes unique time bin, and the cost simply assessed by dividing RMR after LPS injection by RMR post-injection or by RMR measured at the same time after PBS injection at the same time bin. Difference in the dose used apart, given that results that considered the time course of variation in RMR after the injection and that also considered the effects of handling and injecting the animals in this cost returned results that showed high responses, it is likely that the way data was handled in the other studies might have underestimated the metabolic costs associated with APR.

Based on an allometric equation derived from field metabolic rate data for bats (kJ day^{-1} = $5.73M_b\,(g)^{0.79}$ – Speakman & Król 2010), we calculated that the daily energy expenditure for a 10.3 g *G. soricina* (mean M_{bi} of all individuals treated with LPS in our study – see Table 1*)* would be 35.3 kJ day^{-1}. Thus, the total cost associate with APR (0.72 kJ) represents 2% of the total daily energy expenditure of *G. soricina*. In contrast, Otálora-Ardila et al. (2016) did a similar calculation for the fish-eating Myotis, and found out that APR cost represents up to 12%-15% of its daily energy expenditure. Indeed, the overall mass-specific cost of the APR response for *G. soricina* (0.10 kJ g^{-1}) was ~half that estimated for for the fish-eating Myotis (0.23 kJ g^{-1}). Voigt, Kelm & Visser (2006) calculated that, on average, *G. commissarisi* (mean M_b = 8.7 g) consumes about 0.193 kJ per flower visit, and to cover its daily energy expenditure (45.7 kJ day^{-1}) it would need either to monopolize between 26 and 90 plants or visit roughly 236 flowers per night. If we applied the same calculation and assumption of Voigt, Kelm & Visser (2006) for *G. soricina,* then the results reveals that this species would need to visit 183 flowers to meet its daily energy expenditure. If we add the costs associated with APR to the total daily energy expenditure of *G. soricina*, then this species would need to monopolize one additional plant or visit 3-4 flowers to meet this extra cost. Whatever the case, and even if we consider that the

297 travel costs to visit these additional 3-4 flowers would certainly increase its daily energy
298 expenditure, it seems that the total costs associated with APR for *G. soricina* is trivial and would
299 not jeopardize its energy budget. However, APR activation decreased food intake in other
300 vertebrates (Aubert, Kelly & Dantzer, 1997, Vallés et al. 2000) and it might have a negative
301 impact on the foraging behavior of *G. soricina*, thus affecting its energy budget.

302 **Conclusions**

303 First direct measurements of the energy cost of APR activation in wild vertebrates were
304 conducted with birds and were higher than in laboratory rodents suggesting that this response is
305 less expensive and important for mammals (Marais, Maloney & Gray, 2011). Large increase in
306 RMR recently measured for the fish-eating Myotis (Otálora et al. 2016) and the results of our
307 study defy this idea. RMR increase in the Pallas's long-tongued was one third the highest
308 increase measured in the fish-eating Myotis but significantly higher than the increase measured
309 for laboratory rodents and similar to the highest increase reported for birds. Our finding suggests
310 that, similarly to other features of APR, its metabolic cost might vary among bats probably in
311 relation to ecological factors. For example, bats that include vertebrates in their diet have higher
312 leukocyte numbers than phytophagous and insectivorous species, and bacterial killing ability
313 (BKA) decreases with increasing roost permanence (Schneeberger, Czirják & Voigt, 2013b). In
314 addition to that, pace of life might also affect immune response as BKA is negatively correlated
315 with mass-adjusted basal metabolic rate (BMR) in birds (Tieleman et al., 2005). BMR in bats
316 varies with diet (Cruz-Neto & Jones, 2016 but its relation with immune response has not been
317 tested. Measurement of the energy cost of immune response in wild mammals is in its infancy
318 and further work is warranted to evaluate its significance for the animal´s energy budget.

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Figure 1(on next page)

Metabolic response of the Pallas's long-tongued bat *Glossophaga soricina* after LPS and PBS administration.

Figure 1 – Metabolic responses of the Pallas's long-tongued bat *Glossophaga soricina* after LPS and PBS administration. (A) Variation in resting metabolic rate (RMR) with time before and after LPS and PBS administration. (B) Variation in the percentage increase in MR, with respect to pre-injection levels, with time in the LPS and PBS treated groups.

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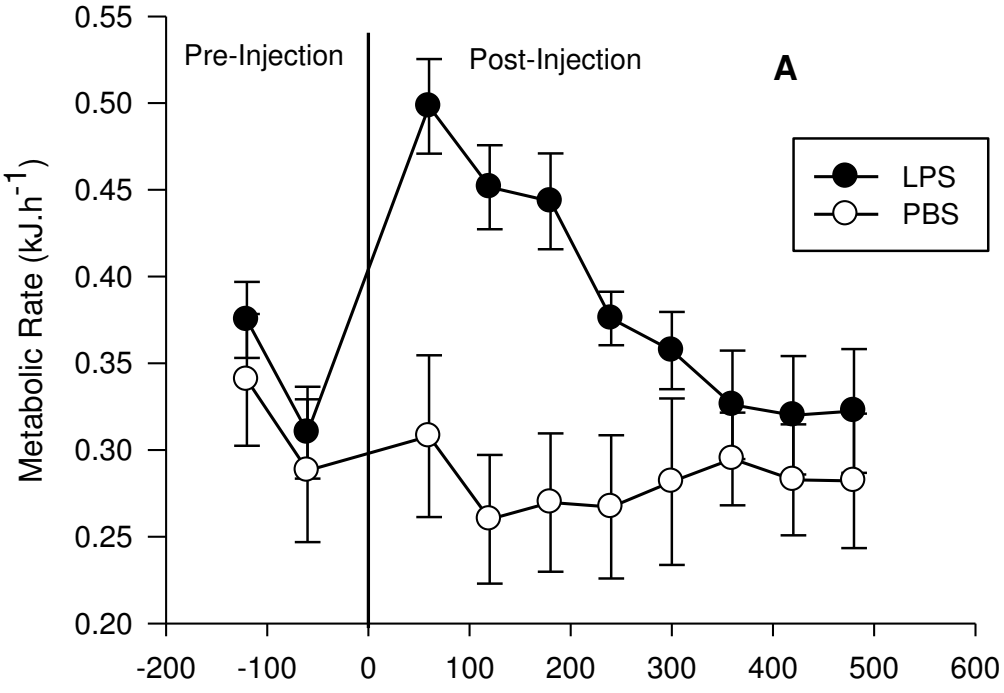

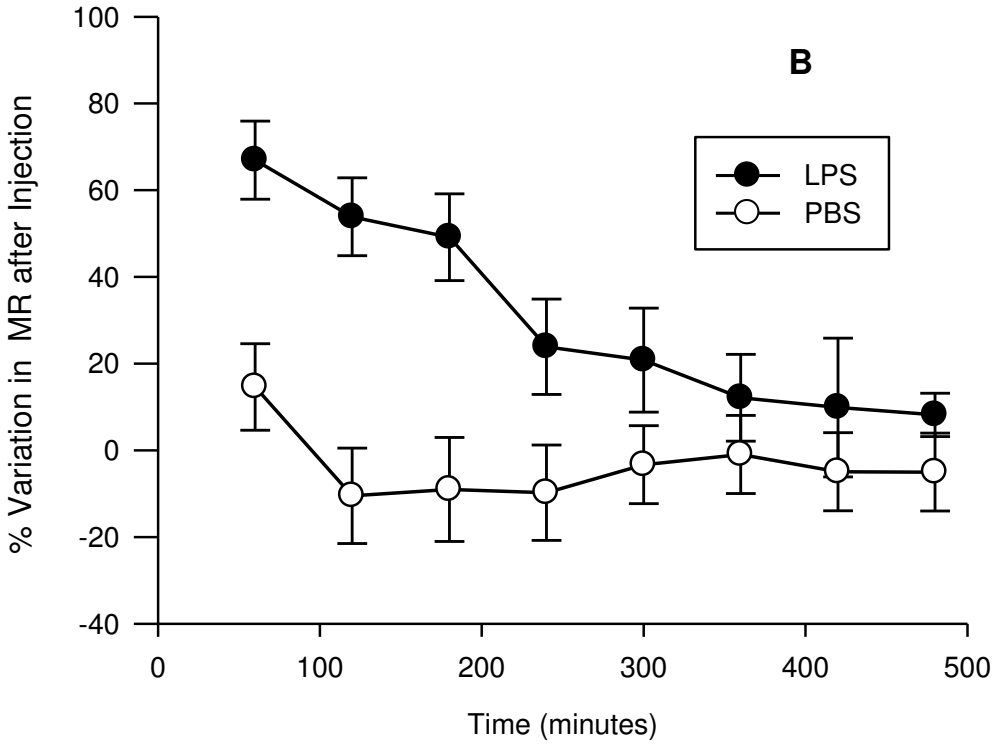

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Table 1(on next page)

Initial (M_{bi}), final (M_{bf}) and mean body mass (Mean M_b) of bats in the two treatment groups (LPS or PBS).

Table 1 – Initial (M_{bi}), final (M_{bf}) and mean body mass (Mean M_b) of bats in the two treatment groups (LPS or PBS). M_b values are in grams. AD = Absolute difference (M_{bf} – M_{bi}) and relative difference [(M_{bf} – M_{bi})/M_{bi}]. Values are presented as mean ± 1. s.e.m. Numbers in parenthesis denote range of observations.

1

2

Treatment	M_{bi}	M_{bf}	Mean M_b	AD	RD
LPS	10.05 ± 0.62	8.90 ± 0.50	9.47 ± 0.56	-1.15 ± 0.14	-0.11 ± 0.009
(n = 7)	(8.6 – 13.2)	(7.8 – 11.6)	(8.3 – 12.4)	(-0.60 – -1.62)	(-0.07 – -0.15)
PBS	10.61 ± 0.62	10.01 ± 0.66	10.31 ± 0.64	-0.60 ± 0.11	- 0.06 ± 0.01
(n = 6)	(8.8 – 12.8)	(7.9 – 12.0)	(8.35 – 12.4)	(-0.30 – -0.90)	(- 0.03 – - 0.10)