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Original investigation

# The effect of short-term food restriction on the metabolic cost of the acute phase response in the fish-eating *Myotis* (*Myotis vivesi*)

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ABSTRACT

Food restriction affects the activation of the immune system although the metabolic cost associated with mounting such a response has rarely been examined except in model animals. Wild animals are constantly exposed to variations in the availability of food resources and they need to balance their energy budget to fight against pathogens. We examined the effect of food restriction in the fish eating *Myotis* (*Myotis vivesi*), a species of bat that experiences periods in which foraging is limited due to ambient conditions. We tested the hypothesis that acute food restriction (~65% restriction for 1 night) would reduce the caloric response to lipopolysaccharidae (LPS) injection compared to bats fed *ad libitum*. We also measured a proxy for body temperature ($T_{skin}$) and expected reduced fever development when food intake was limited. Bats on the restricted diet had similar resting metabolic rate, total caloric cost and $T_{skin}$ after the LPS challenge than when fed *ad libitum*. However, there was a delay in the metabolic and pyrogenic responses when bats were on the restricted diet. The effect of acute food restriction in delaying the hyperthermia development in fish eating *Myotis* might be of importance for its capacity to fight pathogens. Similar to other bats, the fish eating *Myotis* can fast for several consecutive days by entering torpor and future work is warranted to understand the effect of long periods of food restriction on bat immune response.


## Introduction

Mounting an immune response is assumed to be energetically costly, because physiological processes associated with the activation of the immune system require a continuous input of energy to sustain optimal functionality (Nelson and Demas, 1996). Under natural conditions, when animals must invest in one particular process such as immune function, resources available to other vital processes might be limited (Demas et al., 2011; Norris and Evans, 2000; Ricklefs and Wikelski, 2002). Energetic trade-off relationships between the immune response and other physiological functions impose challenges to organism survival and fitness, particularly when animals confront climatic seasonality and fluctuation of food resources in time and space (French et al., 2009). In particular, variation in food availability affects immune functions in wild and laboratory animals (Berger, 2013).

The effects of food restriction on the immune system after animals are exposed to an immune challenge differ depending on the species examined. For example, 30% food restriction during long periods (2–4 weeks) decreased immunoglobulin production with respect to animals fed *ad libitum* in laboratory mice (*Mus musculus*; Książek and Konarzewski, 2012) and deer mice (*Peromyscus maniculatus*; Martin et al., 2006), but increased production in Siberian hamsters (*Phodopus sungorus*; Zysling et al., 2009). T-cell mediated immunity was reduced in Mongolian gerbils (*Meriones unguiculatus*) fasted for 3 days (Xu and Wang, 2010), while house sparrow nestlings (*Passer domesticus*) in which food ingestion was reduced by 40% for 2 days exhibited a reduced induced acute-phase protein response (Killpack et al., 2015).

In contrast to studies on the magnitude of an immune response, the effect of food restriction on the metabolic cost associated with

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mounting such a response has rarely been examined. For example, laboratory rats (*Rattus norvegicus*) administered lypopolisaccharidae (LPS) after 28 days of 30–40% food restriction, and little ringed plovers (*Charadrius dubius*; Gutiérrez et al., 2011) challenged with phytohemagglutinin after 3 days of similar levels of food restriction both exhibited significantly reduced metabolic rate increases during the acute response phase compared to individuals fed *ad libitum*. LPS is an endotoxin present in most gram-negative bacteria that stimulates the innate immune system through induction of the acute phase response (APR), provoking fever, weight loss, anorexia, and diminished activity (Bonneaud et al., 2003; Canale and Henry, 2011; Cutrera et al., 2010; Lee et al., 2005). The APR occurs in the first stage of infection and its main function is to minimize energy expenditure on non-essential organismal functions while limiting nutrient availability to pathogens, enhancing animal survival (Burness et al., 2010). At least in individuals not subjected to reduced food intake, activation of APR by LPS often involves an increase in resting metabolic rate in laboratory (Buchanan et al., 2003; MacDonald et al., 2012) and in wild animals (King and Swanson, 2013; Marais et al., 2011).

The activation of the APR might be particularly relevant for the survival of long-lived animals because they are more likely to be repeatedly exposed to, or exposed to a broader variety of, pathogens than short-lived animals, and therefore should invest more heavily in immune maintenance (Martin II et al., 2006). Bats have exceptionally long life spans that are on average 3.5 times longer than other eutherian mammals of similar size (Munshi-South and Wilkinson, 2010) and they have high mass specific field metabolic rates (Geiser and Coburn, 1999; Speakman and Król, 2010). Immune response in some bat species might be compromised as they lower their metabolic rate when food availability is limited (Bouma et al., 2010; Moore et al., 2011). We examined the effect of acute food restriction on the metabolic rate of the fish-eating *Myotis* (*Myotis vivesi;* Vespertilionidae) after activating APR with an administration of LPS. This bat feeds primarily on marine fish and crustaceans (Otálora-Ardila et al., 2013) and might not feed for one to several days presumably due to limiting foraging conditions (Salinas R. et al., 2014). We tested the hypothesis that acute food restriction (~65% restriction for 1 night) would reduce the caloric response to LPS injection compared to bats *fed ad libitum*. We also measured body temperature and expected no fever development when food intake was limited.

## Material and methods

### *Animal care and housing*

Individual fish-eating *Myotis* were captured in March 2014 on Partida Norte Island (28°52′30″N, 113°02′17″W), located in the midriff region of the Gulf of California, Mexico (Carreño and Helenes, 2002). Individuals were maintained in captivity for one week before experiments began. Bats were maintained in an outdoor flight cage ($3.4 \times 2.8 \times 1.8$ m) where they were fed with shrimp, salmon, and mealworms supplied *ad libitum* on several Petri dishes. Mean air ambient temperature was $29.3 \pm 1.6$ °C (mean ± s.e.m., here and thereafter) throughout the experiment.

### *Experimental procedures*

Seven adult, non-reproductive individuals (4 males, 3 females, $28.9 \pm 0.6$ g) were studied. Each bat was sequentially subjected to each of two feeding regimes one day before the onset of the data collection: unlimited (*ad libitum*) and restricted feeding. In both cases, the diet consisted of shrimp, salmon, mealworms, and water. For the *ad libitum* treatment, bats were maintained in the outdoor cage the night before the immune challenge and food was presented at 20:00 h in five Petri dishes to assure that it was not monopolized by some individuals. The amount of food consumed was estimated by subtracting the amount of food remaining in the dishes at 06:00 h from the amount provided the previous night. We calculated the amount of food consumed per individual during each pre-challenge night ($n = 6$) dividing total food consumed by the number of individuals. Bats on the *ad libitum* diet consumed $6.4 \pm 0.9$ g per individual on the pre-challenge night. This value is similar to the average amount of food consumed by bats ($6.7 \pm 0.6$ g per individual) during the period in which they were not managed for the experiments (34 nights). When bats were fed the restricted diet, they were placed in individual cages ($25 \times 15 \times 10$ cm) the night previous to the immune challenge. The restricted diet consisted of ~35% of the average food consumed *ad libitum*. Individual bats on the restricted diet consumed $2.2 \pm 0.1$ g when assigned to the PBS injection and $2.4 \pm 0.1$ g when assigned to the LPS injection. Body mass of each individual was measured at the beginning (20:00 h) and end (06:00 h) of each dietary treatment.

### *Immune challenge*

Each bat received a single injection of LPS in phosphate buffered saline (PBS) or an injection of PBS alone in separate trials at 07:00 h after each dietary treatment. Seven days after the completion of a round of data collection, each bat was injected with the alternative solution. The order in which bats received each solution was randomly assigned. As a result, each bat participated in four rounds of data collection: injection with either the LPS or PBS solution, followed by injection of the alternate solution while subjected to the *ad libitum* feeding diet and a subsequent series of two injections while subjected to the restricted diet. LPS doses consisted of a 1 mg mL$^{-1}$ solution of LPS (LPS L2630; Sigma, USA) diluted in 50 μL of PBS. Injections were administered sub-dermally to the dorsal thorax of the bats. Prior to injection, the skin surrounding the injection site was sterilized with ethanol.

LPS is pyrogenic (fever-inducing); therefore, we measured bat skin temperature ($T_{skin}$) using temperature-sensitive radiotransmitters (Holohil Systems, Ontario, Canada: model BD-2CT, 2.0 g) attached dorsally between the scapulae. We used R-1000 receivers (Communication Specialists Inc, California, USA) to record the pulse emission rate (number of beeps min$^{-1}$) produced by the radiotransmitters every two hours throughout experiment, and we used transmitter-specific calibration curves supplied by the manufacturer to determine $T_{skin}$. We recorded the pulse emission rate three times during 30 s for each 2-h period for each bat and used the average to assign $T_{skin}$. We calibrated radiotransmitters and found a mean difference of $0.24 \pm 0.17$ °C ($n = 35$) between water temperatures reconstructed with radio-transmitters and with a thermometer. We measured the net change in $T_{skin}$ due to the effect of LPS ($\Delta_{LPS\text{-}PBS}T_{skin}$) by subtracting the mean $T_{skin}$ value after the PBS injection from the mean $T_{skin}$ value after LPS injection for each 2 h period. We measured body mass of each individual 23, 13, and 1 h prior to, and 11.5 h following, injection.

### *Respirometry and experimental design*

We determined RMR by measuring $O_2$ consumption rate ($V_{O2}$) using flow-through respirometry during the resting phase of bats (07:00–19:30 h). We measured $V_{O2}$ one day before (Day –1), and on the day of LPS or PBS injection (Day 0). Bats were placed in individual 250-ml metabolic chambers for the measurements. To measure $V_{O2}$ rate, external air was drawn through three metabolic chambers (each containing a bat) and one empty reference chamber. Excurrent air from all chambers was sequentially sampled by precision gas analyzers (Field Metabolic System [FMS], Sable

Systems International). Air was drawn through each chamber at 400–500 mL $min^{-1}$ and flow rate was maintained by the mass-flow controller of the FMS. Recordings were obtained from 7:00 to 19:30 h. Excurrent air was sampled first for 5 min from the reference chamber and then sequentially from each of the three chambers containing bats for 30 min each time, followed by another 5 min from the reference chamber. By the end of trial each day, we had acquired 30 min recordings per bat corresponding to hours 1, 3, 5, 7, 9 and 11 after the bats were placed in the chamber.

Flow rate, chamber temperature, water vapor, and oxygen levels were recorded using ExpeData acquisition software (v. 1.7.2, Sable Systems International) at a frequency of 1 Hz. After lag correction, smoothing, and correction for water dilution effects on flow rate and apparent $O_2$ level, $V_{O2}$ was calculated by application of standard equations according to Lighton (2008). We identified the lowest 5 min mean $V_{O2}$ values within each 30 min sampling period as the bats' instantaneous resting oxygen consumption rate. Metabolic rates were expressed as ml $O_2$ $h^{-1}$.

We calculated the net metabolic cost of the injection response by subtracting the final pre-injection control values for $V_{O2}$ from each post-injection. Control-corrected $V_{O2}$ values were converted to their oxy-joules equivalents ($MR_{kj}$ in kJ $hr^{-1}$) according to the following equation from (Lighton, 2008) and assuming the respiratory exchange ratio ($RER = V_{CO_2}/V_{O_2}$; where $V_{CO2}$ is carbon dioxide production rate) was equal to 0.77, which was the average RER value observed in fasted *Myotis* fishing bats examined in a separate study (Welch et al., 2015):

$$MR_{kj} = V_{O2}x[16 + 5.164(RER)]$$

Following this, we fitted a spline function to these corrected post-injection measurements and calculated the area under the curve using the "rollapply" function in the "zoo" package (Zeiles and Grothendieck, 2005) in R. In a few instances, respirometric data for specific measurement periods on specific individuals were lost due to equipment failure. In these cases, missing data were replaced (to permit statistical analyses) with the immediately prior or subsequent measurement value.

### *Data analysis*

We performed three-way repeated measure analyses of variance (RM-ANOVA) to examine variations in RMR, body mass, chamber temperature and $T_{skin}$ related to dietary treatment, injection treatment, time after injection, and their interactions. For the body mass comparison, we included body mass changes at the end of the daytime period previous to injection (body $mass_{-13hour}$ − body $mass_{-23hour}$), at the end of the last feeding period previous to injection (body $mass_{1hour}$ − body $mass_{-13hour}$), and at the end of the experiment after injection (body $mass_{11.5hour}$ − body $mass_{1hour}$). For the chamber temperature, we considered only data

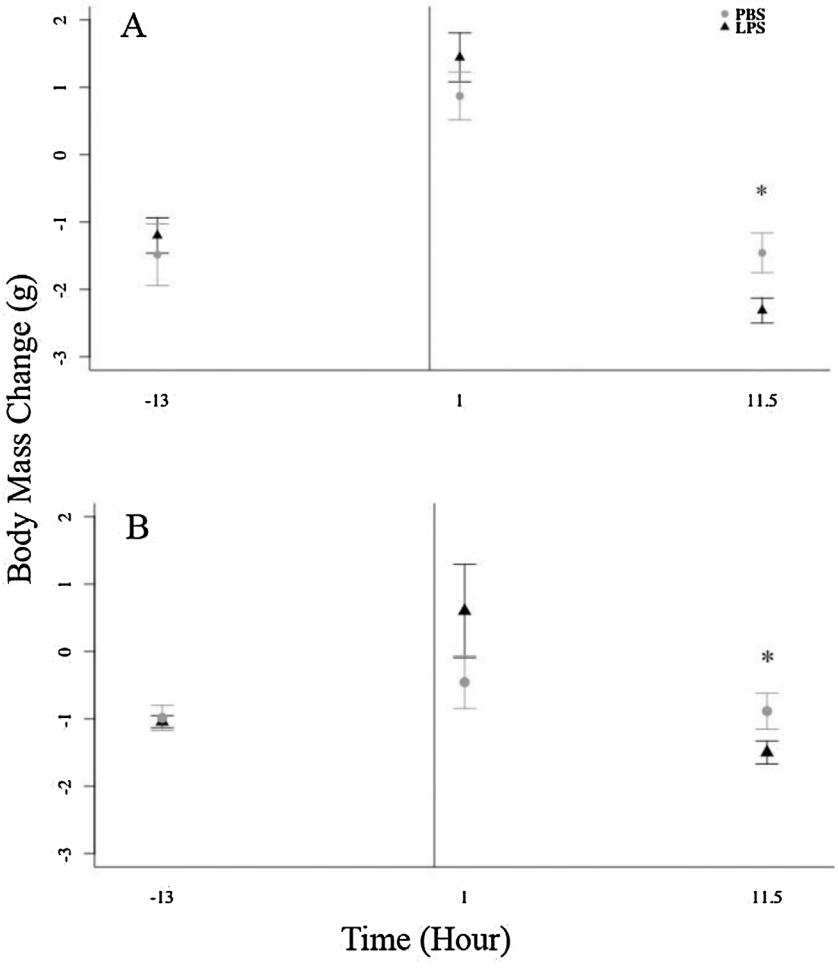


**Fig. 1.** Body mass changes (g) of fish-eating *Myotis* (*Myotis vivesi*) before and after the injection of lipopolysaccharide (LPS) or phosphate buffered saline (PBS). Individuals were fed feed *ad libitum* (A) or with restriction (B) on the night previous to injection. Values are mean ± s.e.m, $n = 7$ for each treatment. Vertical line indicates time of injection. * $p \leq 0.05$.

registered after the injection. We compared $\Delta_{LPS\text{-}PBS}T_{skin}$ between dietary treatments using two-way RM-ANOVA. We compared total caloric cost of injection with a two-way RM-ANOVA with dietary treatment and injection treatment as factors. When factors or their interactions were significant, we conducted planned orthogonal comparisons. We compared the total metabolic cost of PBS and LPS injection to zero using one-sample *t*-tests for each dietary treatment. Values are expressed as mean ± s.e.m. Results were considered significant at $p \leq 0.05$.

## Results

### *Body mass changes*

Body mass was significantly affected by dietary treatment ($F_{1,6} = 10.3$, $p = 0.01$), time after injection ($F_{2,12} = 12.2$, $p = 0.001$), and the interactions between dietary treatment and type of injection ($F_{1,6} = 21.9$, $p = 0.003$), between dietary treatment and time after injection ($F_{2,12} = 6.4$, $p = 0.02$), and between type of injection and time after injection ($F_{2,12} = 6.3$, $p = 0.001$). Body mass losses at the end of the daytime period previous to injection were not significantly different between bats assigned to the LPS or PBS treatments for bats on the *ad libitum* ($p = 0.3$) or restricted ($p = 0.7$) diets. On the feeding period before injection, bats on the restricted diet gained less body mass (0.6 ± 0.7 g) than when fed *ad libitum* (1.4 ± 0.3 g) but this difference was not significant ($p = 0.2$). At the end of the injection day, bats on the LPS treatment lost more body mass than bats on the PBS treatment when fed *ad libitum* ($p = 0.05$) or when subjected to dietary restriction ($p = 0.04$). Bats on the *ad libitum* diet lost more mass than bats on the restricted diet after the LPS treatment ($p = 0.02$) but there was no difference between dietary treatments after the PBS treatment ($p = 0.2$, Fig. 1). Only three bats in the restricted diet lost body mass on the feeding period prior to LPS injection, whereas all bats in the *ad libitum* diet gained body mass. Body mass of bats 1 h prior to LPS injection was higher when they were fed *ad libitum* (29.2 ± 0.6 g) than when diet was restricted (26.3 ± 0.6 g; $t_6 = 3.5$, $p = 0.01$).

### *Skin and chamber temperature*

Chamber temperature was significantly higher during the experiment with bats on the restricted diet (29.8 ± 0.1 °C; $F_{1,6} = 22.1$, $p = 0.003$) than on the *ad libitum* diet (28.9 ± 0.1 °C) but the effect of injection treatment was not significant ($F_{1,6} = 1.5$, $p = 0.2$). Chamber temperature varied significantly with time after injection ($F_{5,30} = 427.3$, $p < 0.0001$), with increasing values from the beginning (1 h after injection: 27.5 ± 0.1 °C) to end of the period (11 h after injection: 30.4 ± 0.1 °C). No factor interactions were significant.

Dietary treatment had no significant effect on $T_{skin}$ ($F_{1,6} = 0.5$, $p = 0.5$) but the effects of injection treatment ($F_{1,6} = 11.4$, $p = 0.01$), time after injection ($F_{10,60} = 16.2$, $P < 0.0001$) and the injection

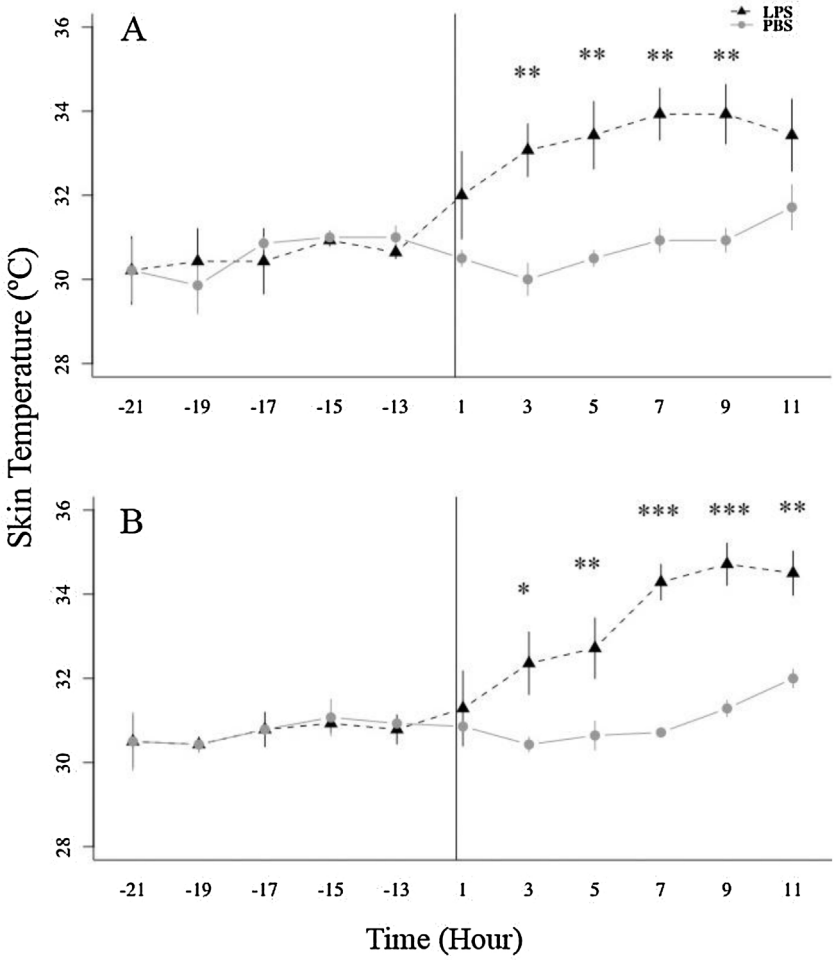


**Fig. 2.** Skin temperature (°C) of fish-eating *Myotis* (*Myotis vivesi*) before and after the injection of lipopolysaccharide (LPS) or phosphate buffered saline (PBS). Individuals were fed feed *ad libitum* (A) or with restriction (B) on the night previous to injections. Values are mean ± s.e.m, *n* = 7 for each treatment. Vertical line indicates time of injection. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

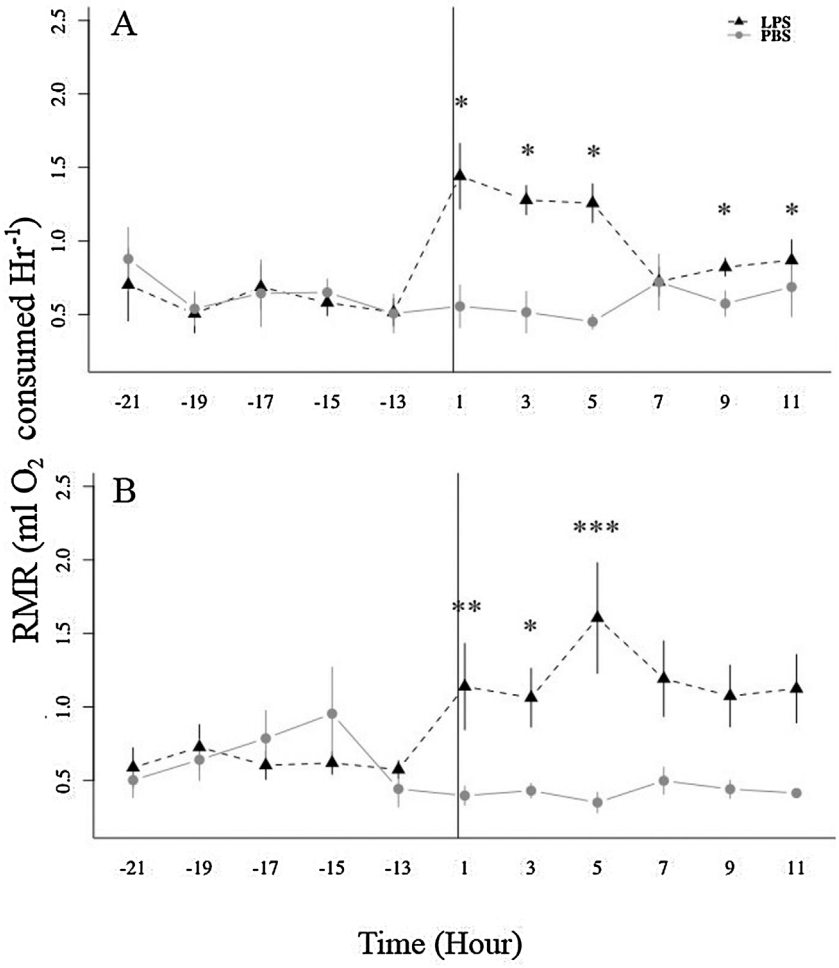


**Fig. 3.** Resting metabolic rate (RMR; ml $O_2$ consumed $Hr^{-1}$) of fish-eating *Myotis* (*Myotis vivesi*) before and after the injection of lipopolysaccharide (LPS) or phosphate buffered saline (PBS). Individuals were fed feed *ad libitum* (A) or with restriction (B) on the night previous to injection. Values are mean ± s.e.m, $n = 7$ for each treatment. Vertical line indicates time of injection. $^{*}p \leq 0.05$;$^{**}p \leq 0.01$; $^{***}p \leq 0.001$.

treatment-time interaction ($F_{10,60} = 15.7$, $p < 0.0001$) were significant. There were significant differences in $T_{skin}$ between bats injected PBS or LPS at 3 ($p = 0.002$), 5 (0.01) 7 ($p = 0.002$), and 9 ($p = 0.006$) h after injection when bats were fed *ad libitum*, and at hours 3 ($p = 0.03$), 5 (0.01), 7 ($p = 0.0001$), 9 ($p = 0.0003$), and 11 ($p = 0.006$) with bats on the restricted diet (Fig. 2). Mean $\Delta_{LPS\text{-}PBS}T_{skin}$ after injection ranged from 2.1 to 3.0 °C in the *ad libitum* diet and from −0.3 to 2.7 °C in the restricted diet. Dietary treatment had no significant effect on $\Delta_{LPS\text{-}PBS}T_{skin}$ ($F_{1,6} = 1.7$, $p = 0.2$) but the effects of time ($F_{5,30} = 3.8$, $p = 0.008$) and the diet-time interaction ($F_{5,30} = 3.5$, $p = 0.03$) on $\Delta_{LPS\text{-}PBS}T_{skin}$ were significant. Mean $\Delta_{LPS\text{-}PBS}T_{skin}$ was significantly higher in the *ad libitum* (2.1 ± 0.6 °C) than the restricted diet (-0.3 ± 0.3 °C) only 1 h after LPS injection ($p = 0.005$).

*Resting metabolic rate*

The effect of dietary treatment on post injection RMR was not significant ($F_{1,6} = 0.001$, $p = 0.9$), but the effects of injection treatment ($F_{1,6} = 6.1$, $p = 0.04$), time of injection ($F_{10,60} = 2.1$, $p = 0.03$), and the injection-time interaction ($F_{10,60} = 8.5$, $p < 0.0001$) all had significant effects on RMR following injection. RMR values were higher for LPS than PBS treatment in bats fed *ad libitum* only at 1 ($p = 0.01$), 3 ($p = 0.02$), and 5 ($p = 0.001$) h after the injection (Fig. 3). When bats were subjected to food restriction, RMR values were higher for LPS than PBS treatment only at 1 ($p = 0.04$), 3 ($p = 0.04$), 5 ($p = 0.02$), 9 ($p = 0.02$) and 11 ($p = 0.02$) h after the injection (Fig. 3).

*Total metabolic cost*

The overall metabolic cost of the response to injection was greater in bats injected with LPS compared to bats injected with PBS ($F_{1,6} = 13.9$, $P = 0.009$), but did not differ as a function of dietary treatment ($F_{1,6} = 0.005$, $P = 0.9$), nor was there a significant interaction of injection type and dietary treatment ($F_{1,6} = 0.7$, $P = 0.4$). There was no metabolic cost of the response to sham injection as the metabolic response after PBS administration was not significantly different from zero (*ad libitum* diet: 0.9 ± 1.0 kJ, $t_6 = 0.8$, $p = 0.4$; restricted diet: −0.2 ± 1.2 kJ, $t_6 = -0.1$, $p = 0.9$). However, the cost of the response to injection of LPS over the time frame examined was significantly different from zero (*ad libitum* diet: 6.5 ± 0.7 kJ, $t_6 = 9.2$, $p < 0.0001$; restricted diet: 7.7 ±1.9 kJ, $t_6 = 3.4$, $p = 0.009$).

## Discussion

In contrast to our predictions, one-night of food intake restriction did not affect the metabolic cost of the acute phase response in the fish eating *Myotis* but it delayed the metabolic response and the development of hyperthermia. In the following lines we discuss our findings with respect to what has been reported in other animals and its implications for batís ability to fight pathogens.

The strength of the metabolic response of fish eating *Myotis* to an LPS challenge was not affected by dietary treatment. LPS injection elicited a significant increase in metabolic rate even when food ingestion was restricted: individual values of RMR after LPS injection increased up to 4.2 ± 0.8 fold in the *ad libitum* diet and 4.4 ± 0.9 fold in the restricted diet with respect to RMR measured after PBS injection. The effect of food restriction on RMR after a LPS challenge has been examined previously only in laboratory rats (*Rattus norvegicus*; MacDonald et al., 2012). When the caloric intake of rats was reduced to 50% during 28 days, RMR after LPS injection did not increase, and it was lower than when rats were fed *ad libitum*. In contrast to the rat study, fish eating *Myotis* were on a restricted diet for only 1 night, resulting in a comparatively smaller difference in body mass between restricted and *ad libidum* treatment groups (∼10% lower in food restricted bats versus ∼22% lower in food restricted rats). While we found no significant difference in RMR values between dietary treatments, it appears that there was a delay in the metabolic response when bats were on the restricted diet. RMR peaked on average one hour after LPS injection when bats were fed *ad libitum* and five hours after injection when bats were fed a restricted diet. Additionally, RMR returned to pre-injection values by 7 h after LPS injection on average in bats fed *ad libitum*, but remained elevated at least 11 h following injection for bats on the restricted diet.

The total energetic cost of the immune response during the time period over which it was measured was similar in bats fed *ad libi*tum (6.5 kJ) and restricted diets (7.7 kJ), and amounted to 9.8–14.0% of the daily energy requirements of a bat the size of fish-eating *Myotis* (55–66 kJ day$^{-1}$; Speakman and Król, 2010). The elevated metabolic rate observed after LPS challenge paralleled an increase in body temperature and a greater body mass loss relative to bats injected with PBS. Bats in both dietary treatments increased their body temperature after the LPS injection with respect to the control treatment by 3.0 ± 0.7 °C in the *ad libitum* diet and by 2.7 ± 0.6 °C in the restricted diet. Although hyperthermia reached the same level in both dietary treatments, it was delayed in the restricted diet. In contrast to bats fed *ad libitum*, $\Delta_{\text{LPS-PBS}}T_{\text{skin}}$ one hour after injection was approximately zero when bats were fed the restricted diet. Peak $\Delta_{\text{LPS-PBS}}T_{\text{skin}}$ values (≥ 3 °C) in bats fed *ad libitum* were reached only 3 h after the injection but it took 7 h to reach this value for bats on the restricted diet. The effect of food restriction on the development of hyperthermia after an LPS challenge has been examined in a handful of studies mostly with model animals and it appears to be related to the duration and level of the restriction. Laboratory rodents with 50% food restriction for 28 days developed fever but with a delayed onset and sustained for a shorter period than when fed *ad libitum* (Radler et al., 2014). In another set of studies, fever was suppressed in laboratory rodents under 50% food restriction for 28 days (MacDonald et al., 2011, 2014) but they developed an attenuated febrile response after LPS injection when exposed to shorter periods of food restriction (14 days; MacDonald et al., 2011, 2014), to 25% food restriction (MacDonald et al., 2014), or when they were starved during 48 h (Inoue et al., 2008). In contrast to model animals, food restriction (40%) over a longer period (15 weeks) did not affect the pyrogenic response of grey mouse lemur (*Microcebus murinus*) over the 1st day after the LPS challenge (Canale and Henry, 2011). The physiological bases behind the delay in the pyrogenic response of food-restricted fish eating *Myotis* might result from the down-regulation of pro-inflammatory pathways and the intensification of anti-inflammatory pathways as suggested for laboratory rodents (MacDonald et al., 2011).

Bats subjected to both dietary treatments lost more body mass following LPS injection compared to when PBS was administered. However, body mass loss after LPS injection was 1.4 times in bats on the *ad libitum* diet (−2.3 ± 0.2 g or −7.9 ± 0.6%) than when fed the restricted diet (−1.5 ± 0.2 g, or −5.6 ± 0.5%). Higher body mass loss after an LPS challenge on animals fed *ad libitum* have been found in model animals and it seems to be related to the attenuation of the pyrogenic response when diet is restricted. For example, body mass loss was 2.5–4 times greater in laboratory mice and 3 times greater in laboratory rats fed *ad libitum* than when fed restricted diets that attenuated or cancelled the febrile response (MacDonald et al., 2011, 2012). Given that the total caloric cost after the LPS challenged was not significantly different between fishing *Myotis* on different diets, it is not clear to what extent the slightly lower body mass loss when fed the restricted diet was the result of the delayed pyrogenic response.

Short term restriction of food intake might compromise resistance to pathogens (Robertson and Mitchell, 2013). In particular, elevation of body temperature is associated with reduced disease duration and improved survival in most animals (Blatteis, 2003). Therefore, the effect of food restriction in delaying the hyperthermia development in the fish eating *Myotis* might be important for its capacity to fight pathogens. Our study simulated food restriction over a short period but individuals of fish-eating *Myotis* might remain torpid in their roosts for several consecutive days with no food ingestion (Salinas R. et al., 2014). Torpor reduces the number of circulating leukocytes and the production of cytokines, and probably compromises bat immune response (Bouma et al., 2010). For example, when mice are subjected to food restriction over long periods (∼20 weeks) they are more susceptible to infection by intact pathogens (Kristan, 2008). On the other hand, torpor is an energy saving mechanism that might be interrupted when animals are exposed to an infection. For example, torpor was absent when LPS was administered to grey mouse lemur (*Microcebus murinus*) maintained on *ad libitum* and restricted diets (40% caloric restriction) during several weeks (Canale and Henry, 2011). Interruption of torpor in hibernating bats as a result of pathogen infection might be detrimental for their survival as found in little brown *Myotis* (*M. lucifugus*) infected with *Pseudogymnoascus destructans* (Lilley et al., 2016). Torpor interruption during pathogen infections might also affect antioxidant activity. Antioxidant defenses increase in torpid bats (Filho et al., 2007) but frequent arousals from torpor due to infection might deplete antioxidants (Moore et al., 2013). Finally, recent work shows that in addition to quantity, quality of food is fundamental for immune response. For example, tree swallows (*Tachycineta bicolor*) have stronger immune response when fed diets supplemented with omega-3 long-chain polyunsaturated fatty acids (LCPUFA; Twininga et al., 2016). Aquatic prey had higher LCPUFA content than terrestrial preys and the strength of the immune response of the fish eating *Myotis* bats might be affected by the extent to which it preys on aquatic versus terrestrial items (Otálora-Ardila et al., 2013). Future work is needed to assess the effect of longer periods of food restriction (both in quantitative and qualitative terms) on bat immune response and in particular regarding the effect of pyrogenic infections on their use of torpor.

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