Mammalian Biology 82 (2017) 41–47

Contents lists available at ScienceDirect

Mammalian Biology

journal homepage: www.elsevier.com/locate/mambio

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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ARTICLE INFO

Article history:
Received 16 August 2016
Accepted 5 November 2016
Handled by Danilo Russo
Available online 8 November 2016

Keywords:
Caloric cost
Food restriction
Innate immune response
Lipopolysaccharidae
Resting metabolic rate

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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http://dx.doi.org/10.1016/j.mambio.2016.11.002
1616-5047/

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 ± 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 ± 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 ± 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 ± 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 ± 0.1 g when assigned to the PBS injection and 2.4 ± 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 ± 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