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RESEARCH ARTICLE

Energy expenditure across immune challenge severities in a lizard: consequences for innate immunity, locomotor performance and oxidative status

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ABSTRACT

Reptiles, like other vertebrates, rely on immunity to defend themselves from infection. The energetic cost of an immune response is liable to scale with infection severity, prompting constraints on other self-maintenance traits if immune prioritization exceeds energy budget. In this study, adult male side-blotched lizards (*Uta stansburiana*) were injected with saline (control) or high (20 µg g^{-1} body mass) or low (10 µg g^{-1} body mass) concentrations of lipopolysaccharide (LPS) to simulate bacterial infections of discrete severities. The costs and consequences of the immune response were assessed through comparisons of change in resting metabolic rate (RMR), energy metabolites (glucose, glycerol, triglycerides), innate immunity (bactericidal ability), sprint speed and oxidative status (antioxidant capacity, reactive oxygen metabolites). High-LPS lizards had the lowest glucose levels and greatest sprint reductions, while their RMR and bactericidal ability were similar to those of control lizards. Low-LPS lizards had elevated RMR and bactericidal ability, but glucose levels and sprint speed changes between those of high-LPS and control lizards. Levels of glycerol, triglycerides, reactive oxygen metabolites and antioxidant capacity did not differ by treatment. Taken together, energy expenditure for the immune response varies in a non-linear fashion with challenge severity, posing consequences for performance and self-maintenance processes in a reptile.

KEY WORDS: Antioxidant capacity, Bactericidal ability, Energy metabolites, Lipopolysaccharide, Sprint speed, Reactive oxygen metabolites, Resting metabolic rate, *Uta stansburiana*

INTRODUCTION

Immunity is an important aspect of survival for reptiles and other taxa (McKean and Lazzaro, 2011; Wobeser, 2013). Yet, immune defense and cellular repair are energetically costly (Demas et al., 2011, 2012; Hasselquist and Nilsson, 2012), requiring intricate coordination between the immune system and other physiological systems (reviewed in Zimmerman et al., 2010; Zimmerman, 2020). Assuming investment increases proportionally with the severity of an immune challenge, energy deficits may manifest when stronger responses are mounted, conflicting with investment elsewhere (Lochmiller and Deerenberg, 2000; Ardia et al., 2011). The extent to which competing physiological systems are concurrently regulated may ultimately determine survival outcomes (Graham et al., 2011; Meylan et al., 2013). Despite growing evidence of immune prioritization in reptiles (Neuman-Lee and French, 2014; Smith et al., 2017; Hudson et al., 2021), whether infection severity differentially impacts their energy budget and competing self-maintenance processes has not been resolved.

Energy expenditure during costly physiological processes, such as an immune response, is mediated by metabolic changes (Ganeshan and Chawla, 2014). For reptiles, shifts in metabolic activity (e.g. O_2 consumption) and energy metabolites (e.g. glucose, triglycerides, glycerol) likely mediate immune activation and maintenance (Price, 2017; Lind et al., 2020; Hudson et al., 2020a). Energetic adjustments for the immune response in animals have so far been shown to vary by species (Martin et al., 2003; Merlo et al., 2014), sex (Brace et al., 2015), immune challenge type (Cox et al., 2015; Smith et al., 2017) and infection severity (Armour et al., 2020). Yet, energy strategy for reptilian immune activation is not well understood, prompting the question of how infection severity interacts with energetic state and self-maintenance.

Prioritizing energy for an immune response could conflict with systems that are necessary for meeting ongoing ecological challenges, particularly those involving locomotor performance (e.g. fleeing, foraging; Le Galliard et al., 2004). Differences in locomotor capacities are accrued by long-term muscle development and maintenance costs, as well as the daily cost of muscle use (Atherton and Smith, 2012; Lailvaux et al., 2018). For reptiles, locomotor performance can decrease while mounting an immune response (e.g. sprint speed; Zamora-Camacho et al., 2014), and immunocompetence can diminish with frequent and intense exercise (Husak et al., 2016, 2017; Wang et al., 2019). These reciprocal outcomes suggest the energetic costs of investment can constrain expression of competing self-maintenance traits. The costs associated with immune challenge severity may in turn impact the degree to which locomotor performance becomes limited.

Mounting an immune response may impact cellular processes that not only are relevant to immediate survival but also contribute to longevity (Buttemer et al., 2010; van de Crommenacker et al., 2010). Pathways that destroy pathogens (e.g. acute phase response; Cray et al., 2009) are often augmented by the production of reactive oxygen species (reviewed in West et al., 2011; Nathan and Cunningham-Bussel, 2013). Although prooxidants are regularly generated for essential cellular functions (Halliwell and Gutteridge, 2015), excessive amounts can lead to oxidative stress, particularly when there is an imbalance with antioxidant defenses (Sorci and Faivre, 2008; Sies et al., 2017). Oxidative stress can cause severe damage to DNA, proteins and lipids if prolonged (i.e. oxidative damage), potentially compromising multiple self-maintenance processes that contribute to long-term survival (Metcalfe and

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Alonso-Alvarez, 2010). The potential for oxidative stress has recently been shown to scale with immune challenge severity (Armour et al., 2020), yet whether the oxidative outcomes of immune activity translate to reptiles is unclear.

The present study tested whether immune challenge severity alters energy use, innate immunity, locomotor performance and oxidative status in side-blotched lizards (*Uta stansburiana*). Lizards were subjected to discrete concentrations (control, low or high) of *Escherichia coli*-derived lipopolysaccharides (LPS) and monitored for changes in metabolic activity (O_2 consumption), energy metabolites (glucose, triglycerides, glycerol), bactericidal ability, sprint capacity and oxidative status (antioxidant capacity, reactive oxygen metabolites). We hypothesized that increasing immune challenge severity would proportionally increase metabolic activity, but also decrease energy metabolites, sprint capacity and oxidative status.

MATERIALS AND METHODS

Animals and overview

Adult male side-blotched lizards, *Uta stansburiana* Baird and Girard 1852 (n=53), were captured with a snare pole from Washington County, UT, USA, on 16–17 April 2019. During collection, lizards were inspected and kept if there were no open wounds or severe mite infections present. The following day, all lizards were transported to Utah State University in Logan, UT, USA, where each lizard was housed in a plastic terrarium (48×18×23 cm) with paper substrate, a refuge and a water dish. Terraria were stored on shelves inside programmable environmental chambers (DR-36VL, Percival Scientific) that included internal temperature and humidity controllers and a lighting system of vertically mounted fluorescent lamps. Environmental controllers were set to maintain a relative humidity of 60% and nycthemeral temperatures of 36±1°C during a 12 h light period (08:00–20:00 h) and 20±1°C during a 12 h dark period (20:00–08:00 h). All terraria were positioned similarly throughout the chambers (0.3±0.05 m from nearest light source).

Lizards were assigned an individual identity and measured for body mass (mean±s.e.m. 4.54±0.07 g) with a digital scale (±0.1 g, model MS500, Pesola, Schindellegi, Switzerland) and snout–vent length (49.56±0.29 mm) with a metric ruler. Body mass and snout–vent length metrics were controlled for in an interspersion assignment of one of three groups: control (n=17), low LPS (n=16) or high LPS (n=20). All groups were of similar body mass ($F_{2,50}$=1.85, P=0.168) and snout–vent length ($F_{2,50}$=0.92, P=0.406) as a result. Lizards were given 3 days to acclimate and achieve physiological baseline prior to undergoing the 7 day experiment (Table 1; Obernier and Baldwin, 2006). All procedures described below were permitted under Utah State Department of Wildlife Resources (COR #1COLL8382) and approved by the Utah State University Institutional Animal Care and Use Committees (protocol #2529).

Table 1 . Timeline of procedures for experiment

Time	Experimental procedure
Before day 1	Acclimation period (food provision/restriction)
Day 1	Sprint trials/metabolic trials
Day 2	Food provision/restriction
Day 3	Lipopolysaccharide injections
Day 4	Food provision/restriction
Day 5	Sprint trials/metabolic trials
Day 6	Blood sampling/food provision

Feeding regime

Lizards were fed *ad libitum* during the acclimation period and switched to a controlled, restrictive feeding regimen at the start of the experiment (e.g. Lailvaux et al., 2012; Husak et al., 2016). Lizards from each treatment were fed pre-weighed pin-head crickets (Fluker Farms, Port Allen, LA, USA) on days 2 and 4 (Table 1). Rather than limiting the number of crickets allotted to each lizard, the total mass of all crickets was instead limited to 0.16 g per feeding period (mean±s.e.m. 0.129±0.001 g). Uneaten crickets were removed and weighed on days 3 and 5 to calculate the net difference in pre- and post-feeding mass (i.e. food intake mass). When no crickets were eaten, food intake mass was recorded as zero for the sampling occasion. The cumulative mass of crickets allotted throughout the study did not differ among lizards ($F_{2,50}$=0.208, P=0.813, n=53), nor did food intake mass ($F_{2,50}$=0.57, P=0.569, n=53).

LPS injections

To simulate an infection, lizards received an intraperitoneal injection of LPS (serotype 0127:B8, Sigma-Aldrich, St Louis, MO, USA) diluted in phosphate-buffered saline (PBS) at either low (10 µg LPS/20 µl PBS) or high (20 µg LPS/20 µl PBS) concentrations. Control lizards received an intraperitoneal injection of only PBS (0 µg LPS/20 µl PBS). All dosages were mass-adjusted (20 µl g^{-1} body mass) based on previous research with this species (Smith et al., 2017). Lizards were returned to their individual enclosures immediately after injection.

Metabolic measurements

Resting metabolic rate (RMR) was measured using closed-flow respirometry (Lighton, 2008) between 23:00 h and 07:00 h on days 1 and 5, approximately 48 h before and after injection (Table 1). Food was restricted on days 2 and 4, approximately 24 h prior to sampling, to limit confounding effects of digestion (Burton et al., 2011). Lizards were then transferred from their terraria to one of 15, 700 ml glass respirometry chambers (RC-3, Sable Systems, Las Vegas, NV, USA) housed in an incubator without light at 36±0.6°C (Heratherm IMH180, Thermo Scientific, Waltham, MA, USA). Metabolic chambers were flushed for 1 h and supplied with dry, CO_2-free air (i.e. air scrubbed with Drierite® and Ascarite®) at 500 ml min^{-1} using a mass flow system and pump (MFS, Sable Systems) and two 8-channel multiplexers calibrated for closed-flow operation (RM-8, Sable Systems). Air exiting each chamber was dried, sampled by a calibrated carbon dioxide analyzer (CA-10, Sable Systems), scrubbed, and then sampled again by a calibrated oxygen analyzer (Oxzilla, Sable Systems). Sampling and data collection were automated using Expedata software (v.1.9.1, Sable Systems) so that each chamber was sampled for O_2 consumption every hour for each trial. Volumetrics of O_2 consumption were averaged across measures and calculated as a rate over time (ml h^{-1}). Under the assumption that metabolic rates scale as a ¾ power of body size (Kleiber, 1947; Brown et al., 2004), O_2 consumption (ml O_2 h^{-1}) was mass-adjusted for each lizard using their body mass$^{0.75}$ (Smith et al., 2017). Absolute change in RMR (post-injection measure−pre-injection measure) was calculated to account for individual variation while comparing treatment effects among groups.

Sprint speed measurements

Sprint speed was quantified on a 2 m racetrack (9 cm wide) to provide an indicator of locomotor capacity (Irschick and Garland, 2001). Pre-injection sprint trials took place between 09:00 h and

11:00 h on day 1, approximately 60 h prior to injections (Table 1). Post-injection sprint trials took place at the same time on day 5, approximately 36 h after injections. The racetrack included a synthetic carpet substrate and a visible refuge to promote a natural, direct locomotor response to simulated predation (Zani et al., 2009; Tulli et al., 2012; Wagner and Zani, 2017). For each set of trials, lizards were removed from the environmental chambers across four groups (N=13–14 lizards) and raced in a random order. Cloacal temperatures (mean±s.e.m. 31.35±0.22°C, range 26.3–35.2°C) were measured immediately before racing each lizard using a 1 mm diameter thermocouple (model TP870, Extech Instruments, Waltham, MA, USA) and thermometer (model 561, Fluke, Everett, WA, USA). Pre- and post-injection sprint speeds were not related to body temperature, nor were there temperature differences among treatment groups (P>0.05 in all cases).

At the onset of each trial, lizards either immediately fled from the observer or required tactile stimulation by hand. Successive time intervals for when lizards traveled a distance of 0.5 m were collected using markers spanning the racetrack. Times were recorded at 24 frames s^{-1} (41.66 ms between frames) with a video camera (Canon Vixia HF R600) and analyzed using QuickTime Player (v.7, Apple). Trials were run in duplicate to allow lizards to acclimate to the racetrack. The fastest time from both trials was used to calculate maximal sprint speed (m s^{-1}). Lizards that did not successfully complete a trial on either day were excluded from analysis (n=5). As sprint speed was previously validated to be moderately repeatable in this species (n=10, P<0.001, R=0.786, 95% confidence interval 0.475–0.903), absolute change in speed (post-injection measure−pre-injection measure) was calculated within individuals to compare among groups.

Blood sampling

Retro-orbital blood samples were collected on day 6, approximately 60 h after LPS treatment (Table 1). Each sample was collected within 3 min between 09:00 h and 11:00 h to control for restraint stress and circadian differences in physiological activity (Tylan et al., 2020). Blood was centrifuged at 3900 ***g*** for 10 min to separate plasma, which was then isolated and stored at −80°C until assays were performed. Afterwards, each lizard was weighed again to account for potential changes in body mass during the study. As there were no treatment differences in body mass change ($F_{2,50}$=0.09, P=0.914), this metric was excluded from subsequent analyses. Lizards were allotted 3 days to recover prior to returning them to their respective points of capture.

Energy metabolite measurements

Glucose concentrations were measured in blood plasma (1 µl) on an Accu-Chek Aviva Plus (Roche Diagnostics, Indianapolis, IN, USA), which has been validated for use in several vertebrates (Stoot et al., 2014). Glycerol and triglyceride concentrations were measured using sequential enzymatic color endpoint assays (F6428, T2449 and G7793, Sigma-Aldrich). Both the manufacturer's protocol and a dilution protocol were followed for use with a 96-well plate (see Guglielmo et al., 2002; Fokidis et al., 2011, 2012). Glycerol reagent was added to blood plasma (5 µl) and incubated for 5 min at 37°C. Absorbance was measured on a spectrophotometer at 505 nm (xMark, Bio-Rad Benchmark, Hercules, CA, USA) to calculate glycerol, an indicator of endogenous triglyceride catabolism. A lipase reagent was then added to dissociate fatty acids from their glycerol backbones (i.e. triglyceride dissociation) and the plate was again incubated for 5 min at 37°C. Absorbance of triglycerides was measured at 505 nm. Intra-plate variation was 5.57% for glycerol and 3.46% for triglycerides.

Immune measurements

Bactericidal ability was quantified with a validated volume of blood plasma (6 µl) to assess the relative abundance of circulating immune components (Neuman-Lee and French, 2014). Using the protocol outlined in French and Neuman-Lee (2012), a 1:2 plasma dilution was combined with CO_2-independent medium (Gibco # 18-045-088, ThermoFisher Scientific, Grand Island, NY, USA), 4 nmol l^{-1} L-glutamine, 10^4 colony-producing units of *E. coli* (EPower™ Microorganisms #483-581-1, ATCC 8739, MicroBioLogics, St Cloud, MN, USA) and agar broth in a 96-well microplate. Included were both positive (i.e. media and bacteria with no plasma) and negative (i.e. media and no plasma or bacteria) controls to account for potential growth and ensure there was no contamination. The plate was incubated at 37°C for a 12 h period, at which point absorbance per well was measured with a microplate reader at 300 nm (xMark, Bio-Rad Benchmark). Bactericidal ability was then calculated as [1−(absorbance of sample/absorbance of positive controls)×100]. Each sample was run in duplicate to generate an average percentage score of bactericidal ability. Average intra-plate variation was 3.85% and inter-plate variation was 3.94%.

Oxidative measurements

Two types of colorimetric assays were used on blood plasma to measure both reactive oxygen metabolites and the capacity to bind to and clear those metabolites (Vassalle et al., 2004; Vassalle, 2008). Reactive oxygen metabolites were measured using a d-ROMs test that detects variable levels of hydroperoxides (MC435, Diacron International), which signal lipid and protein oxidative damage. Following Lucas and French (2012), 5 µl of plasma was diluted into 100 µl of the provided acidic buffered solution and 'end-point mode' manufacturer instructions were followed thereafter using 96-well microplates and a spectrophotometer reading at 505 nm (xMark, Bio-Rad Benchmark). Measures of reactive oxygen metabolites (mg H_2O_2 dl^{-1}) were acquired with average intra-plate variation at 2.92% and inter-plate variation at 4.54%.

Total non-enzymatic antioxidant capacity was measured using an OXY-Adsorbent test (MC002, Diacron International), which determines the effectiveness of the blood antioxidant barrier by quantifying tolerance of the oxidant action of hypochlorous acid (HClO). Here, 2 µl of plasma was diluted in 100 µl of distilled water, and the manufacturer's instructions were followed thereafter using 96-well microplates with a spectrophotometer reading at 505 nm (xMark, Bio-Rad Benchmark). Measures of total antioxidant capacity (mol HClO ml^{-1}) were acquired with an average intra-plate variation at 2.65% and inter-plate variation at 2.85%.

Statistical treatment of data

One-way analysis of variance (ANOVA) models were run to compare LPS treatment effects on RMR change, glucose, triglycerides, glycerol, bactericidal ability, sprint speed change, reactive oxygen metabolites and antioxidant capacity. The Tukey method was used for multiple comparisons of significant treatment differences. Residual distributions of models were assessed for normality and homogeneity of variance was compared across groups when appropriate. All analyses and visual representation of data were performed in R statistical software (v.3.5.1, http://www.R-project.org/) using the following packages: 'car' (v.2.1-6, https://CRAN.R-project.org/package=car), 'plyr' (v.1.8.6; Wickham, 2011), 'reshape2' (v.1.2, https://CRAN.R-project.org/package=reshape2),

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'rcompanion' (v.2.3.21; https://CRAN.R-project.org/package=rcompanion), 'ggplot2' (v.3.1.0, Wickham, 2016) and 'ggsignif' (v.0.6.0, https://CRAN.R-project.org/package=ggsignif).

RESULTS

RMR

Mass-adjusted RMR significantly differed across simulated infection severities ($F_{2,50}$=9.418, P=0.0003, n=53; Fig. 1). Low-LPS lizards exhibited increased rates relative to control lizards (P=0.0002) and high-LPS lizards (P=0.023), but high-LPS and control lizards did not differ (P=0.191).

Energy metabolites

Glucose levels significantly differed with simulated infection severity ($F_{2,46}$=5.558, P=0.007, n=51; Fig. 2), such that levels were lower in high-LPS lizards relative to control lizards (P=0.005). Low-LPS lizards had no relative differences in glucose levels, as they were between those of high-LPS (P=0.566) and control lizards (P=0.107). No differences in triglycerides ($F_{2,44}$=0.516, P=0.600, n=47) or glycerol ($F_{2,44}$=2.028, P=0.144, n=47) were found.

Innate immune function

Bactericidal ability significantly differed with simulated infection severity ($F_{2,49}$=13.94, P<0.0005, n=52; Fig. 3), whereby low-LPS lizards had greater percentage scores than high-LPS (P=0.007) and control lizards (P<0.0005). High-LPS lizards had marginally non-significant differences in bactericidal ability compared with control lizards (P=0.057).

Sprint performance

Sprint speed significantly changed with simulated infection severity ($F_{2,45}$=4.506, P=0.016, n=48; Fig. 4). High-LPS lizards decreased speed relative to control lizards (P=0.019), but not low-LPS lizards (P=0.102). Low-LPS lizards did not perform differently from control lizards (P=0.674).

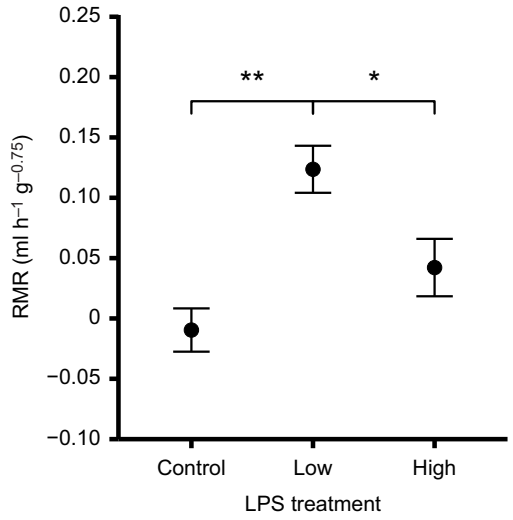

Fig. 1. Mass-adjusted resting metabolic rate (RMR) in adult male side-blotched lizards following lipopolysaccharide (LPS) treatment. Data are shown for lizards in the low LPS (10 µg g^{-1} body mass), high LPS (20 µg g^{-1} body mass) and control treatment groups ($F_{2,50}$=9.418, P=0.0003, n=53). Points with error bars represent group mean concentrations with two-sided 95% confidence intervals. Asterisks represent the degree of significance between comparisons (*P<0.05, **P<0.005), whereas a lack thereof indicates no significant relationship.

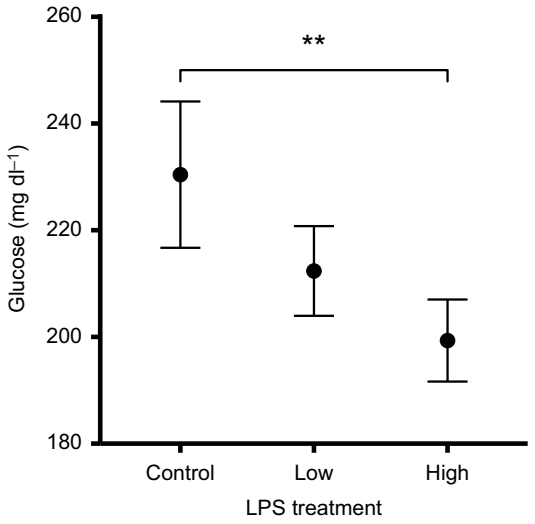

Fig. 2. Circulating glucose levels in adult male side-blotched lizards following LPS treatment. Data are shown for lizards in the three treatment groups ($F_{2,46}$=5.558, P=0.007, n=51). Points with error bars represent group mean concentrations with two-sided 95% confidence intervals. Asterisks represent the degree of significance between comparisons (**P<0.005), whereas a lack thereof indicates no significant relationship.

Oxidative status

There were no differences in antioxidant capacity ($F_{2,29}$=2.383, P=0.110, n=33) and reactive oxygen metabolites ($F_{2,27}$=0.009, P=0.991, n=30) across simulated infection severities.

DISCUSSION

Immunity is vital to survival, yet the intrinsic costs of the immune response pose constraints on the energy budget for self-maintenance processes (McKean and Lazzaro, 2011). This study demonstrates that simulated infection severity elicits differences in energy expenditure, innate immunity and locomotor performance for side-blotched lizards, *U. stansburiana*. Greater metabolic activity and glucose usage following LPS challenge partially reflected the scaling of energetic costs necessary to mount an effective immune response. Discrepancies in bactericidal ability and sprint speed revealed a complex relationship between immunological and locomotor performance during/after recovery. These findings collectively provide support that immune challenge severity can prompt differences in energetic state and competing traits linked to survival in a reptile.

The LPS challenges in this study led to increased RMR, as recently shown in other reptiles (e.g. snakes; Lind et al., 2020). Yet, significantly greater metabolic upregulation was only detected in low-LPS lizards. The concentration of an LPS challenge can have varying effects on the timing of physiological changes in other taxa (e.g. mammals; Vedder et al., 1999; Bison et al., 2008), suggesting that peak responses could differ by challenge severity (Zamora-Camacho, 2018). Previous work assessing the metabolic costs of lower LPS concentrations (2.5–5.0 µg g^{-1} body mass) in this species did not detect metabolic change within a shorter, 24 h period (Smith et al., 2017). Metabolic upregulation while mounting an immune response to a more intense challenge could therefore occur sooner than lesser challenges to minimize damaging outcomes. In this case, the comparatively mild metabolic changes detected in high-LPS lizards could be an artifact of sampling period; that is, metabolic rate could have peaked earlier in time.

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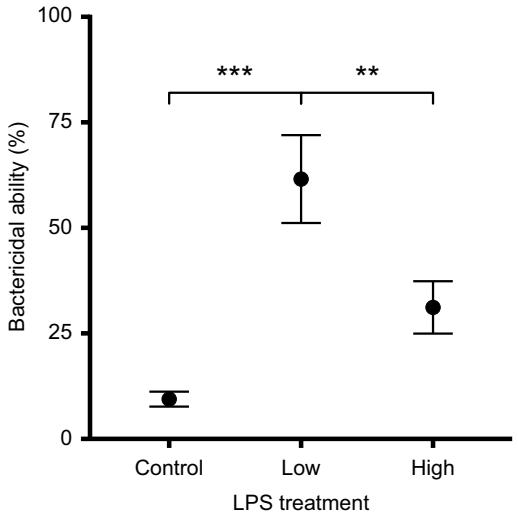

Fig. 3. Bactericidal ability in adult male side-blotched lizards following LPS treatment. Data are shown for lizards in the three treatment groups ($F_{2,49}$=13.94, P<0.0005, n=52). Points with error bars represent group mean concentrations with two-sided 95% confidence intervals. Asterisks represent the degree of significance between comparisons (**P<0.005, ***P<0.0005), whereas a lack thereof indicates no significant relationship.

High LPS concentrations also led to significantly lower circulating glucose following recovery, but no differences in glycerol and triglycerides. As glucose has previously been shown to be associated with immune activation and maintenance in *U. stansburiana* (Hudson et al., 2020b), decreased glucose levels could indicate greater energy expenditure with increased challenge severity, even though they were not reflective of RMR comparisons. If immune prioritization occurred with greater simulated infection severity, metabolic responses could have peaked sooner, at which point energy metabolites (i.e. glucose) were necessarily exhausted. Here, glycerol and triglycerides did not seem necessary for recovery. For other taxa responding to discrete LPS

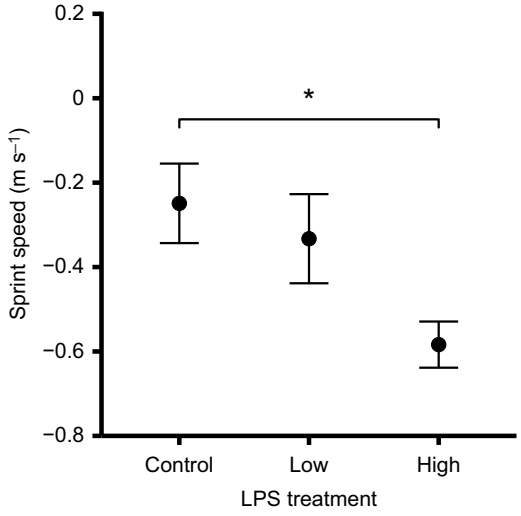

Fig. 4. Sprint speed in adult male side-blotched lizards following LPS treatment. Data are shown for lizards in the three treatment groups ($F_{2,45}$=4.506, P=0.016, n=48). Points with error bars represent group mean concentrations with two-sided 95% confidence intervals. Asterisks represent the degree of significance between comparisons (*P<0.05), whereas a lack thereof indicates no significant relationship.

concentrations, glycerol levels have similarly been shown to remain unchanged, while circulating triglycerides decrease (Shini et al., 2008; Armour et al., 2020). If glucose levels were even lower in this species, perhaps from a more severe infection, triglycerides may then be used as an alternative energy source (e.g. fatty acid oxidation; Price, 2017). Nonetheless, only certain metabolites seem to be relevant across the challenge severities and recovery period considered here.

Differences in bactericidal ability for *U. stansburiana* reflect immune activity during each challenge and/or immunological state after recovery (Tan and Kagan, 2014). Given that low-LPS lizards demonstrated greater bactericidal ability, the immune response mounted was likely successful in fighting a simulated infection, allowing immunocompetence and other energetically demanding processes to be maintained. Here, activation of the immune system could have occurred sooner, leading to fewer components (e.g. natural antibodies, complement, antimicrobial peptides) remaining in circulation when bactericidal ability was tested (Neuman-Lee and French, 2014). Greater immune investment with increased challenge severity would be consistent with lower circulating glucose among high-LPS lizards and, potentially, with their patterns of metabolic activity following recovery.

Locomotor adjustments during recovery were dependent upon challenge severity, as only high-LPS lizards had decreased sprint speeds. Low-LPS lizards were instead capable of mounting an immune response without compromising sprint performance. Locomotor constraints may be attributed to the costs of immune prioritization, as previously shown for lizards challenged with lower LPS dosages (Zamora-Camacho et al., 2014). Energy and nutrients required for maintaining the muscular system could become limited during an immune response, such that the mechanical power responsible for burst locomotor performance decreased (Gleeson and Harrison, 1988; Farley, 1997; Hudson et al., 2021). In this study, however, sprint speed changes were not related to energy metabolite levels nor change in body mass, indicating that other resources or factors could be more relevant to recovery.

Sprint performance may also be attributed to motivational state (Foster et al., 2015), which can characteristically change during recovery. Sickness behaviors that accompany the acute phase response to LPS in reptiles (e.g. lethargy) may reduce motivation to perform at maximal capacity during exercise (Adelman and Martin, 2009; Rakus et al., 2017; Hart and Hart, 2019). Depressions in motivational state may be compounded by pain associated with the inflammatory processes of the acute phase response (Gao et al., 2007; Ashley et al., 2012; Gregory et al., 2013). Lizards with greater LPS infections, and in turn stronger responses, could be further deterred from exercise. Sprint reductions may be co-opted to conserve resource expenditure for the immune response and prevent additional discomfort from rapid muscle contractions (Lopes et al., 2014). Devoting less energy to locomotion could be beneficial if recovering lizards are in refuges and are less susceptible to the risks of daily ecological challenges (e.g. predation, competition; Huey and Pianka, 1981; Brodie et al., 1991). Multiple behavioral responses to LPS should therefore be considered in future work to test motivational differences among recovering reptiles (Todd et al., 2016; Klinck et al., 2017).

The lack of a detectable change in both reactive oxygen metabolites and antioxidant capacity indicates that *U. stansburiana* did not experience immediate oxidative stress during LPS recovery (Costantini, 2016, 2019). Despite LPS challenges incurring oxidative costs in other taxa at lower dosages (Baylor and Butler, 2019; Paardekooper et al., 2019; Armour et al., 2020), lizards may

instead maintain oxidative resistance across a wider range of infection severities. Shifts in oxidative status could have occurred during recovery, albeit transiently throughout the inflammatory processes of immunity (Sebastiano et al., 2018; Fritze et al., 2019). During the acute phase response, prooxidants could be mitigated by a proportional release of molecules and enzymes with anti-inflammatory (Thomsen et al., 2013; Belcher et al., 2018) and antioxidant properties (Puertollano et al., 2011; Surai et al., 2019). Oxidative stress from LPS recovery could also have a delayed onset or manifest from more severe challenges, such that any changes in the oxidative markers were undetected within the sampling period and/or LPS concentrations tested (Costantini and Møller, 2009). Preventative mechanisms maintaining oxidative resistance across challenge severities should therefore be tested in future studies (Costantini, 2019).

In conclusion, the induced reptilian immune response appears to depend upon LPS concentration, resulting in adjustments to both energy expenditure and locomotor performance. Energetic strategies for handling differing severities of an immune challenge do not seem to compromise oxidative status, evidenced by the fact that reactive oxygen metabolites and antioxidant capacity were not affected by LPS overall. Immune prioritization could be a principal strategy across challenge severities for reptiles, whereby energy is increasingly diverted from other self-maintenance processes for upregulation. The costs of performance may only manifest at critical thresholds as a result. Yet, the reptile immune response is presumably more complex than presented in this study (reviewed in Zimmerman et al., 2010; Zimmerman, 2020). Further disentangling of the costs of immunity should provide insight into the proximate and ultimate mechanisms by which reptiles respond to the diversity of immune challenges in the natural world.

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The authors declare no competing or financial interests.

Author contributions
Conceptualization: S.F.; Methodology: S.B.H., E.V., M.K.; Formal analysis: S.B.H.; Investigation: S.B.H.; Resources: S.F.; Data curation: S.B.H.; Writing - original draft: S.B.H.; Writing - review & editing: S.B.H., E.V., M.K., S.F.; Visualization: S.B.H.; Supervision: S.F.; Project administration: S.B.H.; Funding acquisition: S.F.

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