



Compensating for a stressful pregnancy? Glucocorticoid treatment during gravidity reduces metabolic rate in female fence lizards post-parturition

K.J. MacLeod^{a,b,c,*}, T. Langkilde^b, J.J. Heppner^{a,d}, C.A.F. Howey^{b,e}, K. Sprayberry^b, C. Tylan^b, M.J. Sheriff^{a,f}

^a Department of Ecosystem Science and Management, Pennsylvania State University, Forest Resources Building, University Park, PA 16802, USA

^b Department of Biology, Pennsylvania State University, Mueller Laboratory, University Park, PA 16802, USA

^c Department of Biology, Lund University, Sölvegatan 37, Lund 223 62, Sweden

^d Department of Biology, University of Nevada, Reno, NV 89557, USA

^e Department of Biology, University of Scranton, Loyola Science Center, Scranton, PA 18510, USA

^f Biology Department, University of Massachusetts Dartmouth, Dartmouth, MA 02747, USA

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ABSTRACT

Reproduction is a critical part of an animal's life history, but one which incurs significant costs to survival and future reproductive potential. These physiological consequences are likely to be influenced by context – for example, if an individual is subject to environmental stressors, physiological and behavioral changes associated with reproduction may be altered. Glucocorticoids, hormones produced as part of the physiological response to stressors, may alter how reproduction affects female physiology and behavior, and therefore the outcomes of reproductive trade-offs. Glucocorticoids prioritize immediate survival over reproduction, for example through changes in immune function, metabolic rate, and foraging, which may reduce energy expenditure or increase energy gain. However, we previously found that female eastern fence lizards (*Sceloporus undulatus*) experiencing elevated glucocorticoid levels during gestation were nevertheless able to maintain reproductive output and body condition. Here we investigate compensatory mechanisms by which eastern fence lizard females may maintain reproduction under experimental increases in a glucocorticoid, corticosterone (CORT). We found that, although CORT-treated females had similar immune function and behavior, they had reduced metabolic rates 3–5 days post-parturition compared to control females. Given that CORT-treated females spent a similar time basking and had equal food intake compared to control females, we suggest that the reduced metabolic rate is a mechanism by which CORT-treated females maintain their energy balance and reduce the energetic costs of gestation during periods of stress. This study suggests that physiological responses to reproduction may be context-dependent and could act to minimize costs of reproduction in situations where CORT is elevated (such as during periods of environmental stress).

1. Introduction

A central tenet of life history theory is the all-important trade-off between reproduction and maintenance, with resources allocated to one being unavailable for the other (Stearns, 1989). The allocation outcomes of reproductive trade-offs (i.e., to what extent reproduction results in immunosuppression, changes in body condition, etc.) are likely to show a degree of plasticity (Warne et al., 2012) and may depend on external factors. For example, experimentally increased reproductive investment leads to immunosuppression in the tree lizard (*Urosaurus ornatus*), but only in food-limited conditions (French et al., 2007a; French and Moore,

2008; French et al., 2007b). A similar pattern is seen in the cowpea seed beetle (*Callosobruchus maculatus*): the trade-off between reproduction and survival is completely mitigated by high food availability during egg production, suggesting that nutritional state influences this trade-off (Tatar and Carey, 1995). Although food limitation clearly influences reproductive trade-offs, little is known about how other environmental stressors may alter the costs of reproduction through effects on physiology and behavior. This is crucial information to obtain given the ubiquity of stressors in the natural environment, including conspecific aggression (Creel et al., 2013) and predation risk (Clinchy et al., 2013).

Vertebrates, in general, cope with environmental stressors via a

* Corresponding author at: Department of Biology, Lund University, Sölvegatan 37, Lund 223 62, Sweden.

E-mail address: kirsty.macleod@biol.lu.se (K.J. MacLeod).

physiological response characterized by activation of the hypothalamic-pituitary-adrenal (HPA) axis and subsequent release of glucocorticoid hormones, among other factors (Sapolsky et al., 2002). Glucocorticoids at baseline levels are important regulators of energy homeostasis (McEwen and Wingfield, 2003), while stressor-induced increases in glucocorticoids function to promote immediate survival and the restoration of physiological homeostasis. However, this may come at the expense of other non-emergency and energetically costly processes such as growth, immune function, and reproduction (Wingfield et al., 1998; Wingfield and Romero, 2011; Crespi et al., 2013; Sapolsky et al., 2000). Although just one component of the complex vertebrate response to exogenous stressors (MacDougall-Shackleton et al., 2019), glucocorticoids have provided a useful window into the physiological outcomes of stressor exposure. Glucocorticoids at elevated levels have been linked with reduced body weight and condition (De Vos et al., 1995; Klein, 2015); changes in food intake (Osborne, 2015; Cote et al., 2006); altered rates of metabolism (Haase et al., 2016; DuRant et al., 2008); and, suppressed immune function (McCormick et al., 2014).

Given their key role in mediating resource allocation, elevated glucocorticoid levels may reorganize female reproductive strategies and alter reproductive trade-offs during periods of stress (Lancaster et al., 2008). Although increases in glucocorticoid levels are generally thought to reduce reproductive investment (MacLeod et al., 2018a, 2018b; Sheriff et al., 2009) and lower female body condition (Busch et al., 2008), we previously found that gravid eastern fence lizards (*Sceloporus undulatus*) with experimentally increased glucocorticoids had similar reproductive output as control females, while also maintaining their own body condition (Ensminger et al., 2018). While increased glucocorticoid levels have the potential to alter the physiological outcomes or short-term “costs” of reproduction - for example, by exacerbating immunosuppressive effects of reproduction (Franchimont, 2004; Tuckermann et al., 2005) and/or elevating energy use through increasing metabolic rate (Meylan et al., 2010; Wack et al., 2012; Jimeno et al., 2017; Preest and Cree, 2008; Haase et al., 2016), they may also provide a mechanism by which immediate energetic costs of reproduction are minimized. Indeed, some studies have shown that elevated glucocorticoid levels reduced (Miles et al., 2007; Hand and Hardewig, 2002) or had no effect (Buttemer et al., 1991; Buehler et al., 2012; Spencer and Verhulst, 2008; Wikelski et al., 1999) on resting metabolic rates. Metabolic downregulation reduces energy use at a cellular level, and may be an additional or alternate means by which reproductive females compensate for periods of increased energy demand (Johnston et al., 2007). Glucocorticoids at high levels may also promote other mechanisms that allow females to more effectively balance current reproduction with future fitness. Behavioral strategies that increase energy intake or reduce energy expenditure may compensate for increased energetic demand during reproduction. For example, in the common lizard (*Zootoca vivipara*), daily increases in glucocorticoid levels significantly increased food consumption (Cote et al., 2006). Elevated glucocorticoid levels can also affect other behaviors that might influence physiological and biochemical systems, and therefore whole-animal performance, such as heat-seeking behavior as in the New Zealand common gecko, *Hoplodactylus maculatus* (Preest and Cree, 2008).

The relationship between glucocorticoid levels and reproduction is complex and highly variable between species - for example, gravid tree lizards (*Urosaurus ornatus*) have higher baseline levels of CORT as compared to vitellogenic females, but the magnitude of stress response-induced CORT is lower for gravid individuals, suggesting a dampened stress response late in reproduction (Woodley and Moore, 2002). However, the same pattern is not seen in other species, such as the viviparous gecko *Hoplodactylus maculatus* (Cree et al., 2003). It is important to consider the influence of stressor-induced increases in glucocorticoid levels in the context of reproduction to allow us to better interpret and understand stressor and glucocorticoid effects in ecological systems. While the effects of glucocorticoid elevation during reproduction on offspring are increasingly well-studied (e.g. Ensminger

et al., 2018), persistent effects on reproductive and post-reproductive females have remained understudied.

Here we test the response of female eastern fence lizards to experimental increases of glucocorticoids at ecologically relevant levels during gravidity (Ensminger et al., 2018). While researchers often test the hypothesis that stress-induced increases in glucocorticoids reduce reproductive investment (MacLeod et al., 2018a, 2018b; Sheriff et al., 2009), we examine potential mechanisms that would allow females to conserve energy balance in the face of an external stressor. We test the hypothesis that glucocorticoid-treated females are able to overcome the normally energetically costly effects of elevated glucocorticoid levels (i.e., reproductive suppression) by increasing energy intake or reducing other energetically expensive processes. We predict females will i) not upregulate their metabolic rate in response to glucocorticoid treatment, given the large energetic costs associated with elevated metabolism; ii) reduce investment in immunity; iii) increase food intake; and, iv) alter thermoregulatory behavior to increase energy savings. As animals face increasing sources and levels of environmental stressors due to anthropogenic factors (Wong and Candolin, 2014; Sih et al., 2011), it is important to understand how females will balance the energetic costs of reproduction as they face additional environmental stressors.

2. Methods

The eastern fence lizard (*Sceloporus undulatus*) is a small, diurnal lizard found across the East and South-east of the United States of America (Powell et al., 2016). These lizards are subject to a variety of stressors in their environment, the physiological effects of which have been well-studied, allowing us to mimic natural glucocorticoid responses to stressors under controlled laboratory conditions (e.g., Trompeter and Langkilde, 2011; McCormick et al., 2017). We captured female fence lizards from 3 populations in southern Alabama in April and May of 2017. Upon capture, lizards were weighed (± 0.01 g), and measured (snout-vent length [SVL] ± 1 mm). Gravidity was determined by abdominal palpation (Graham et al., 2012) and non-gravid females were released. Gravid females were transported to Pennsylvania in cooled containers over a maximum of 2 days to keep the stress of transport to a minimum (the glucocorticoid response is attenuated at reduced temperatures in fence lizards; Racic et al., 2020). At Penn State University, females were housed individually in plastic tubs ($46 \times 40 \times 30$ cm) in a temperature-controlled room (21 ± 1 °C) from capture until laying (a mean duration of 35.5 ± 16.7 days). Housing tubs contained a plastic perch/shelter and a water bowl. When females were close to laying (eggs become shelled and can be clearly felt during palpation; Graham et al., 2012), we provided moist sand in which females could lay eggs. A 60-W incandescent light bulb was suspended over one end of each housing tub which elevated available temperatures above the perch to approximately 30 °C for 8 h a day and provided a thermal gradient (approx. 21–32 °C) within the tub for thermoregulation. Overhead lights were maintained on a 12:12 light:dark schedule (0700–1900 h). Live crickets (*Acheta domestica*) dusted with nutritional supplements (Zoo Med Reptivite with D3 and Zoo Med Repti Calcium without D3) were provided three days a week, and water was available ad libitum. All research presented here adheres to the Guidelines for the Use of Animals in Research and the Institutional Guidelines of the Pennsylvania State University (IACUC #35780 to T.L.), and animal collection was permitted by the Alabama Department of Conservation and Natural Resources.

2.1. Glucocorticoid manipulation

Gravid females were assigned randomly to either the control or experimental treatment group. From capture until laying, females in the experimental group ($N = 18$) received a daily transdermal application of a corticosterone solution (hereafter CORT, the primary glucocorticoid in reptiles; (Meylan and Clobert, 2005), which was corrected for each

individual's body weight (0.2 $\mu\text{L/g}$ lizard of 4 mg CORT [$\geq 92\%$, Sigma C2505, Saint Louis, MO] in 1 mL of sesame seed oil vehicle, resulting in doses of 0.8 μg CORT/g body mass). Females in the control group ($N = 17$) received a dose of the sesame seed oil vehicle only. Due to variation in the time females spent in treatment before laying, some received more doses than others, with females receiving a mean of 8 doses (range 2–21). This variation was controlled for in statistical models (see “Statistical Analysis”). The solution was applied with a pipette to the middle of the back between 1930 and 2030 h when lizards were less active, in order to minimize disturbance. Handling the lizards was not required for this treatment. A dosage of 0.8 μg CORT/g body mass has been shown to result in circulating plasma CORT concentrations approximating plasma CORT concentrations in lizards 30 min after exposure to a number of ecologically relevant stressors (McCormick et al., 2017; Owen et al., 2018b; Trompeter and Langkilde, 2011; Graham et al., 2012). A time-series experiment showed that circulating CORT levels were significantly higher 30 min after dosing but returned to baseline levels 90 min after dosing (MacLeod et al., 2018a). This procedure, as well as being relatively non-invasive (which allowed us to avoid any potential confounds with handling stress, stress of dosing by injection, etc.) has previously been shown to successfully mimic the short-term increase in CORT experienced by free-living lizards who encounter ecologically relevant stressors (McCormick et al., 2017; Owen et al., 2018a, 2018b), rather than the sustained release of hormone implants (e.g. Breuner, 2008; Crossin et al., 2016).

Housing tubs were checked multiple times daily for signs of egg-laying (e.g., freshly dug sand, the presence of laying holes, mass loss/shape change in females). Upon laying, eggs were immediately counted and transferred to plastic containers filled with moist vermiculite (approximately 200 kpa) and sealed with plastic wrap (Warner et al., 2009; Langkilde and Freidenfelds, 2010). Containers were kept in temperature-controlled incubators ($30 \pm 1^\circ\text{C}$) and rotated every other day to avoid position effects until hatching (after approximately 45 days). Females were weighed using an electronic balance (± 0.01 g) immediately after discovery of eggs within the housing tub ($N = 17$ control females, $N = 18$ CORT-treated females) in order to obtain post-partum mass.

To minimize additional disturbances to gravid females during gestation and egg-formation, physiological performance and behavioral trials were only conducted after egg-laying, shortly after glucocorticoid treatment ceased (sensu Meylan et al., 2010). Physiological effects of increased CORT during gestation have previously been shown to persist for short periods (<1 week) post-laying in this (Sprayberry et al., 2019) and other lizard species (Meylan et al., 2010). Given that our physiological measurements were taken within similar time frames, we are confident that differences between treatment groups are likely to represent real treatment effects as in these studies, and if anything are likely to be conservative (i.e. effects may be stronger during gestation).

To test effects of CORT treatment during gestation on immune function, females were injected with phytohemagglutinin (PHA) within 24 h of laying, and immune response was measured an additional 24 h later (see “Immune Function”). After PHA measurement, a subset of lizards was rested for 48 h before undergoing a respirometry trial (3–5 days post-laying: see “Metabolic Rate”). Following the respirometry trial, lizards were paired in a new housing tub with a female of the opposite treatment. *S. undulatus* females are not typically aggressive to conspecifics (Vinegar, 1975). Pairs were matched according to lay date (within 7 days of one another). We also attempted to match them as closely as possible for mass, though matching for lay date was prioritized to minimize variation in how long it had been since treatment ended for each female (max difference in mass = 4 g, mean difference = 1.65 g). A colored dot of non-toxic nail polish was placed on each female's back to indicate treatment (Pure ICE, New York, NY, USA). Paired housing tubs ($46 \times 40 \times 30$ cm) contained a wooden perch (10 cm height), plastic shelter, and water bowl. The perch was placed directly beneath the heat source on one side of the tub. Behavioral trials began the day after

females were paired and ran for 9 days (beginning 2–13 days post laying: see “Behavioral trials”). Following the completion of behavioral trials, lizards were weighed to allow for calculation of changes in mass and body condition (± 0.01 g), and then re-housed in groups until they were returned to their capture site (within 3 months of initial capture).

2.2. Metabolic rate

We measured the resting metabolic rate (V) in a subset of lizards (10 control, 10 CORT-treated) 3–5 days post-parturition (mean 3.5 days) sensu (Meylan et al., 2010). Variation in days since laying was due to logistical constraints limiting the number of lizards able to be tested per day. Prior to running metabolic trials, each lizard was fasted for at least 24 h (Waldschmidt et al., 1987). Trials took place during the morning active period in a temperature-controlled room (set to the same temperature range as in the lizard housing rooms). We used stop-flow respirometry (Lighton, 2018) with a Field Metabolic System (FMS, Sable Systems, Las Vegas, NV). Each lizard was placed in an air-tight respirometry chamber (500-mL), and multiple chambers were connected to the Field Metabolic System via a Respirometer Multiplexer (RM-8-1, Sable Systems, Las Vegas, NV). Animals were allowed one hour to recover from handling and acclimate to respirometry chambers prior to metabolic trials (Watson and Burggren, 2016). Following the acclimation period, each chamber was flushed for five minutes to bring O_2 and CO_2 levels back to baseline. Each chamber was closed for 5 h, at which time O_2 was consumed and CO_2 produced by the organism. This closed period was followed by a five-minute flush period where O_2 and CO_2 levels were measured, allowing calculation of O_2 depletion and CO_2 production. Temperatures were kept constant throughout each trial (mean of 1.3°C fluctuation in within-trial temperature from start to end of trial) but varied slightly between trials (range of temperatures at start of trial, $27.3 - 32.2^\circ\text{C}$). We accounted for temperature within statistical models (see Statistical Analysis below). Note that the mean temperature did not differ between the two treatments (CORT-treated mean $30.82^\circ\text{C} \pm 0.91$, control mean $30.15^\circ\text{C} \pm 1.08$; Mann Whitney U test $W = 32.5$, $P = 0.20$). Flow rate for each trial was set to 150 mL/min. Data were analysed using ExpeData Software (Sable Systems, Las Vegas, NV) where we calculated $V \text{ O}_2$ and $V \text{ CO}_2$ (mL/min) for each individual. We also calculated the respiratory quotient (RQ), the ratio of $V \text{ CO}_2$ and $V \text{ O}_2$, which reflects the relative catabolism of fat, carbohydrate, and protein (Schutz, 1995).

2.3. Immune function

Cell-mediated immune response was measured in 14 control females and 15 CORT-treated females, using the phytohemagglutinin (PHA) skin test, which stimulates a swelling response by inducing T-lymphocyte localization (Tylan and Langkilde, 2017). This test has been shown to be effective in lizards as a measure of immune response (Tylan and Langkilde, 2017). Within 24 h of laying, we measured the female's left or right (determined randomly) hind foot thickness in triplicate using a pressure sensitive micrometer accurate to 0.01 mm (Mitutoyo 7301 thickness gauge: Mitutoyo America Corporation, Illinois USA). Subsequently, the same foot was subcutaneously injected with 10 μL of 2 mg/mL PHA-L (Sigma, L2769) using a 31-gauge hypodermic needle and 0.3 mL syringe. The lizard was then returned to its housing tub. Twenty-four hours after injection, we remeasured the injected foot in triplicate. The researcher measuring the swelling was blind to the lizard's treatment. We calculated the mean pre- and post-injection foot thicknesses and the difference between the two means as a measure of the swelling response over the 24-h period. Note that the PHA skin test is commonly performed without including a vehicle control injection, as this decreases the coefficient of variation due to measuring inaccuracies, as well as reducing the amount of handling time and stress on the animal (Smits et al., 1999). The amount of swelling due to the injection of the vehicle control alone is not predicted to be a confounding variable, since it is usually

very minimal (Tylan and Langkilde, 2017) as fluid injected into the subcutaneous space is absorbed quickly in all but the most cardiovascularly debilitated of animals (Caccialanza et al., 2018; Heard, 2001).

2.4. Behavioral trials

Trials to determine CORT effects on lizard thermoregulatory behavior (specifically, location relative to a heat source) and food consumption took place for 9 days beginning at 0900, following the females being moved into new paired housing tubs. Thermoregulatory behavior observations took place daily and food consumption trials took place on days 2, 4, 6, 8 and 10.

2.4.1. Thermoregulatory behavior

To assess thermoregulatory behavior, lizard position relative to the heat source was recorded at four time points (every 30 min). At each timepoint, a lizard's location in the enclosure was determined using a 12-cell grid system marked on the base of the tub with non-toxic marker (3 by 4 cells approx. 11.5×13 cm each; A-C, 1-4). The mean temperature of each grid cell was determined by measuring the temperature of each cell using a thermocouple thermometer (Fluke Thermocouple Thermometer 51/52 II) across three replicates of the housing tubs, identical in housing set up (but empty of lizards). Each grid cell and perch position was given a heat score ranging from 0 to 4: the top of the perch (closest to the heat source) was given a heat score of 0 (mean temp \pm s.d. 31.3 ± 0.94 °C), the base and side of the perch a heat score of 1 (mean temp 28.8 ± 0.95 °C), and grids further from the heat source were given increasing heat scores (up to 4; heat zone 2 mean temp, 25.8 ± 1.04 °C; heat zone 3, 23.9 ± 0.85 °C; heat zone 4, 21.5 ± 0.29 °C). Lizards found under the shelter were given a score of 4. Heat scores were averaged (mean) for each female per day for analysis. To avoid disturbance, there was minimal movement in the area of active space/heat trials by observers.

2.4.2. Food consumption

Food consumption trials (days 2, 4, 6, 8, and 10) began after thermoregulatory behavior observations had been completed for the day (to avoid influencing lizard space use through the disruption of feeding). Lizards were not fed between each food consumption trial. All enclosure furnishings were removed from housing tubs to allow full visibility of crickets. We placed one cricket in the housing tub, equally distant from both lizards, to alert them to the presence of food. Another four crickets were then quickly added to the housing tub. The number of crickets each lizard ate of the 5 presented trial crickets was recorded. After all trial crickets were eaten, lizards were fed to satiation (at least 3 crickets consumed per lizard) to reduce potential differences in hunger or motivation to feed in the subsequent trial (i.e. neither female was beginning the next food consumption trial having not eaten in the previous trial). Furnishings were replaced in the housing tub after food competition was complete.

2.5. Statistical analysis

Pre-treatment measures (lizard mass, snout-vent length, clutch size, stage of gravidity at the start of treatment) were compared using *t*-tests or, where data were non-parametric, Mann-Whitney *U* tests to determine that there were no random differences between groups initially (all tests were $P > 0.25$).

Despite the lack of difference between treatment group females initially in stage of gravidity, CORT treatment appeared to lengthen gestation, potentially due to stress-related egg retention – as a result, females in the CORT treatment received more doses than those in the control treatment (CORT 10.17 ± 1.14 doses, control 6.3 ± 0.89 doses, $P = 0.02$). We therefore include dose number as a covariate in all analyses determining effects on post-treatment metrics.

To determine whether CORT treatment affected female body

condition immediately after laying, and after trials ended, body condition (residuals of the correlation between log mass and log SVL) was set as the dependent variable in two linear mixed models (LMMs: 1, post-laying condition; 2, post-trial condition). Treatment (CORT or control group) was specified as an independent variable. Lizard pair identity and site of origin were set as random terms in both models to account for non-independence of paired females and lizards originating from the same sites of capture. In the model testing treatment effects on post-trial condition, the number of doses the female received was additionally included as an independent variable to account for differences in the length of treatment, as well as an interaction term of dose number \times treatment as a previous study showed that duration of treatment influenced CORT effects (MacLeod et al., 2018a). Treatment effects on mass change (difference in mass [g] between the beginning and end of trials) were tested using the same model structure. All statistical tests were completed using the statistical software R (R Core Team, 2020).

Metabolic rate was analysed using linear mixed models, with models testing either rate of O_2 consumption ($V O_2$) or CO_2 production ($V CO_2$) as dependent variables, with treatment (CORT/control), mass, dose number, dose number \times treatment, and the average temperature of the metabolic chamber during the trial included as independent variables. Because females were tested over a range of days since laying (3–5 days) we initially tested whether number of days since laying predicted $V O_2$ / CO_2 using the same model structure. Days since laying was not important in either case and so was not included in further analyses ($V O_2$ $X^2_{1,19} = 1.61$, $P = 0.20$; $V CO_2$ $X^2_{1,19} = 1.45$, $P = 0.23$). As in all other models, maternal site of capture was included as a random term – we also included trial ID to account for potential non-independence of data from separate trials.

We confirmed that the injection of PHA had indeed caused swelling by comparing foot thickness before and after injection using a paired *t*-test. PHA swelling response was also analysed using a LMM in which the difference between pre- and post-swelling injection hind foot thickness (mm) was specified as a dependent variable, and treatment (CORT/control), body condition, dose number, and initial hindfoot thickness were set as independent variables. Again, an interaction term of dose number \times treatment was included, and site of capture was set as a random term.

To determine whether increased CORT levels during gravidity influenced thermoregulatory behavior, the mean heat score for each individual (across all days of observation) was set as a dependent variable in a LMM, with the following set as independent variables: treatment (stress/control group), body condition, dose number, dose number \times treatment. To test whether increased CORT during gravidity influenced food consumption, we first tested whether CORT-treated and control females differed overall in the proportion of crickets they ate of the total provided across all trials using a paired Wilcoxon signed rank test. We then analysed whether body mass relative to partner mass (focal individual mass – partner mass) and dose number influenced the proportion of total crickets eaten by the focal individuals (CORT-treated females) using a binomial GLMM with site set as a random term (one row per pair). We used a focal individual approach because the proportion of crickets eaten by the focal female is inversely proportional to that of the control female, so within-pair data shows negative covariance, and including both in a model results in pseudoreplication.

Means are reported with 1 standard deviation unless specified otherwise. Effect sizes (partial η^2 , calculated from model *F* statistics) are also presented where treatment groups were compared.

3. Results

Our treatment groups consisted of 17 control female fence lizards, and 18 females that underwent CORT treatment. The two treatment groups did not initially differ in starting mass (mass at capture: $T_{1,34} = 0.02$, $P = 0.98$), SVL ($T_{1,34} = 0.60$, $P = 0.55$), estimated gravidity at start of treatment (Mann Whitney *U* test $W = 164$, $P = 0.26$), or clutch size

($T_{1,35} = 0.16$, $P = 0.87$). We also found no differences in post-treatment metrics of reproductive output: CORT treatment did not influence total clutch mass ($T_{1,32,9} = -0.17$, $P = 0.86$), or relative clutch mass (the ratio of clutch mass to pre-laying female body mass: Mann Whitney U test $W = 157$, $P = 0.91$).

3.1. Female body condition

Female lizards did not differ in body condition at the beginning or end of trials based on whether they had received CORT or control treatment throughout gravidity (pre-trial condition, effect of treatment $X^2_{1,34} = 1.32$, $P = 0.25$, $\eta^2 = 0.07$; post-trial condition, effect of treatment $X^2_{1,34} = 0.71$, $P = 0.40$, $\eta^2 = 0.05$). The number of doses a female received did not influence post-trial condition ($X^2_{1,34} = 1 \times 10^{-4}$, $P = 0.99$, $\eta^2 < 0.001$). Treatment effects also did not differ depending on the number of doses received (interaction $X^2_{1,32} = 0.64$, $P = 0.43$, $\eta^2 = 0.04$). Treatment groups also did not differ in mass change between the start and end of trials (treatment $X^2_{1,34} = 0.49$, $P = 0.48$, $\eta^2 = 0.02$). The number of doses a female received did not predict mass change alone ($X^2_{1,34} = 2.69$, $P = 0.11$, $\eta^2 = 0.08$), or in interaction with treatment ($X^2_{1,32} = 0.14$, $P = 0.71$, $\eta^2 < 0.01$).

3.2. Metabolic rate

Treatment during gravidity was associated with a reduced resting metabolic rate 3-5 days after treatment ceased: the rate of O_2 consumption was significantly lower in CORT-treated individuals ($N = 10$) relative to individuals that received the control treatment ($N = 10$) ($T_{1,16} = -2.81$, $P = 0.005$, $\eta^2 = 0.45$; Fig. 1; Table 1). The rate of CO_2 production was lower, but not significantly, in CORT-treated relative to control individuals ($T_{1,16} = -1.58$, $P = 0.09$, $\eta^2 = 0.16$; Fig. 1; Table 1). Temperature had a slight positive effect on the rate of O_2 consumption ($T_{1,17} = 1.68$, $P = 0.09$, $\eta^2 = 0.22$) but no effect on CO_2 production ($T_{1,17} = 1.36$, $P = 0.16$, $\eta^2 = 0.18$). The effects of CORT treatment on metabolic rate were consistent across the range of temperatures (Fig. 1).

The length of treatment (the number of doses a female had received) was negatively associated with a female's rate of CO_2 production: the more doses lizards received, the lower their rate of CO_2 production ($T_{1,16} = -1.81$, $P = 0.05$, $\eta^2 = 0.22$). There was no effect of length of treatment on O_2 consumption ($T_{1,16} = -1.20$, $P = 0.19$, $\eta^2 = 0.18$). However, there was a trend for a stronger effect of CORT treatment on O_2 consumption when treated for longer (dose number \times treatment, O_2 consumption: $X^2_{1,12} = 3.38$, $P = 0.07$); this was not the case for CO_2 production ($X^2_{1,12} = 0.14$, $P = 0.71$, $\eta^2 = 0.01$). Due to the apparent influence of this wide range of treatment lengths (3-21 doses), we repeated the analysis after removing outliers at each extreme, restricting the dataset to a narrower range (6-15 doses, $N = 8$ CORT females, $N = 8$ control females). In this dataset, we did not see the same effects of treatment length on O_2 consumption (dose number \times treatment $X^2_{1,12} = 2.41$, $P = 0.12$, $\eta^2 = 0.06$) nor CO_2 production ($X^2_{1,12} = 0.39$, $P = 0.53$, $\eta^2 = 0.04$). However, the effects of female CORT treatment remained strong in this dataset, for both O_2 consumption ($T_{1,12} = -2.84$, $P = 0.005$, $\eta^2 = 0.37$) and CO_2 production ($T_{1,12} = -2.33$, $P = 0.02$, $\eta^2 = 0.29$).

Body mass and body condition did not influence either the rate of O_2 consumed or CO_2 produced in the full dataset (O_2 consumed, mass: $X^2_{1,19} = 0.0002$, $P = 0.98$; condition $X^2_{1,19} = 0.16$, $P = 0.69$, η^2 (both) < 0.01 ; CO_2 produced, mass: $X^2_{1,19} = 0.02$, $P = 0.90$; condition $X^2_{1,19} = 0.63$, $P = 0.43$, η^2 (both) < 0.01). The average respiratory quotient was 0.71 (in the whole group, as well as in each treatment group; Table 1), which closely approximates results found in other studies of reptiles (Bennett and Dawson, 1976; Litzgus and Hopkins, 2003; Roe et al., 2005).

3.3. Immune function

The PHA test did induce swelling across treatment groups (paired t -

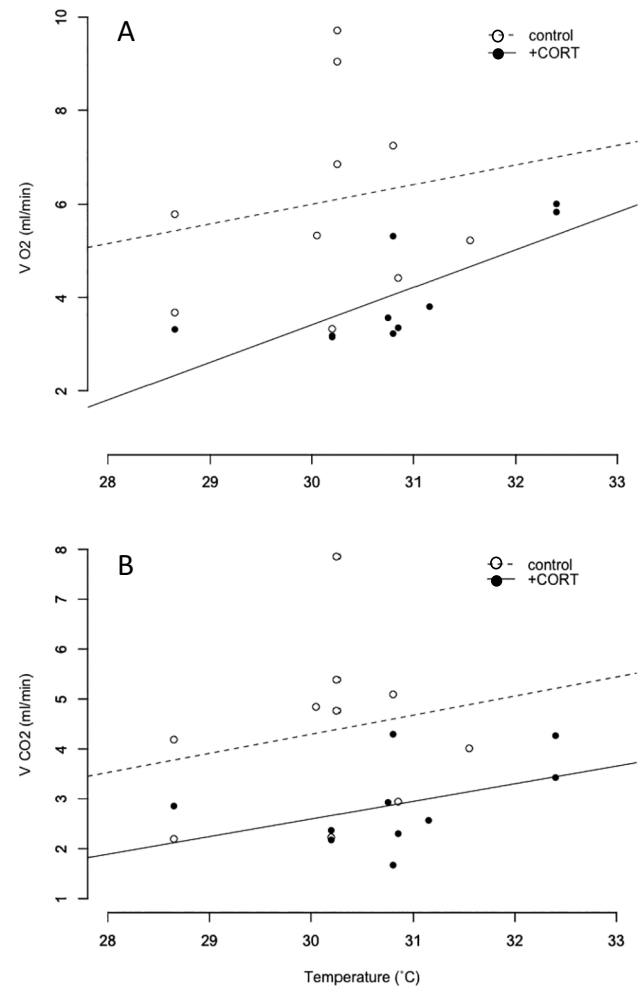


Fig. 1. (A) O_2 consumed was significantly reduced ($P = 0.005$) and (B) CO_2 produced was non-significantly reduced ($P = 0.09$) in CORT-treated females compared to control females across a range of temperatures. Circles represent individual data points – open circles are control individuals, and filled circles are CORT-treated individuals.

test of thickness before and 24 h after injection: $T = -6.07$, $P < 0.001$). Treatment during gravidity had no effect on the PHA swelling response post-parturition ($X^2_{1,28} = 0.07$, $P = 0.79$, $\eta^2 < 0.01$; Table 1). There was also no effect of body condition ($X^2_{1,28} = 0.15$, $p = 0.70$, $\eta^2 < 0.01$) or initial hind foot thickness ($X^2_{1,28} = 2.46$, $P = 0.12$, $\eta^2 = 0.08$) on this immune measure. The number of doses a female received did not predict immune response alone ($X^2_{1,28} = 0.37$, $P = 0.54$, $\eta^2 = 0.01$) or in interaction with treatment ($X^2_{1,27} = 2.81$, $P = 0.09$, $\eta^2 = 0.09$).

3.4. Behavioral trials

3.4.1. Thermoregulatory behavior

CORT-treated females did not differ from control females in their mean heat score over the observation period ($X^2_{1,34} = 0.87$, $P = 0.35$, $\eta^2 = 0.05$; Table 1) indicating that average temperature and proximity of females to the heat source did not differ between treatments. Neither body condition nor dose number influenced heat score (condition: $X^2_{1,34} = 1.29$, $P = 0.29$, $\eta^2 = 0.06$; dose number: $X^2_{1,34} = 1.25$, $P = 0.26$, $\eta^2 = 0.04$). Dose number also did not influence treatment effects on average heat score (dose number \times treatment $X^2_{1,33} = 2.74$, $P = 0.10$, $\eta^2 = 0.17$).

3.4.2. Food consumption

Lizards that had received CORT treatment during gravidity

Table 1

Corticosterone (CORT) treatment effects on gravid female fence lizards, measured shortly post-parturition.

	CORT females	Control females	Test statistic	P
Total clutch mass	2.54 ± 0.19 (N = 18)	2.49 ± 0.19 (N = 17)	T _{1,32.9} = -0.17	0.86
Post-trial condition	0.02 ± 0.04 (N = 18)	-0.02 ± 0.05 (N = 17)	X ² _{1,34} = 0.71	0.40
Mass change	0.64 ± 0.23 (N = 18)	0.69 ± 0.23 (N = 17)	X ² _{1,34} = 0.49	0.48
Immune response (PHA swelling, mm)	0.09 ± 0.02 (N = 15)	0.09 ± 0.02 (N = 14)	X ² _{1,28} = 0.07	0.79
Metabolic trials				
O ₂ consumption (mL/min)	4.07 ± 0.37 (10)	6.06 ± 0.68 (10)	T _{1,16} = -2.81	0.005**
CO ₂ production (mL/min)	2.89 ± 0.28 (10)	4.35 ± 0.53 (10)	T _{1,16} = -1.58	0.09
Respiratory quotient	0.711 ± 0.03	0.713 ± 0.03	T _{1,16} = 0.06	0.95
Behavioral trials				
Heat score	1.81 ± 0.08 (N = 18)	1.82 ± 0.10 (N = 17)	X ² _{1,34} = 0.87	0.35
Proportion total crickets eaten	0.54 ± 0.06	0.48 ± 0.06	Wilcoxon V = 103.5	0.45

Mean ± 1 standard error results from all tests, including model or other test statistic (detailed in text). * signifies significance at the $\alpha = 0.05$ level; · signifies significance at the $\alpha = 0.10$ level.

subsequently did not differ from control females in the proportion of total crickets that they ate (Wilcoxon signed rank $V = 103.5$, $P = 0.45$, Cohen's $d = 0.41$; Table 1). In the focal group (CORT-treated females), dose number and relative body mass did not influence the proportion of total crickets consumed (dose number: $X^2_{1,34} = 0.28$, $P = 0.60$, $\eta^2 = 0.01$; relative body mass: $X^2_{1,34} = 1.97$, $P = 0.16$, $\eta^2 = 0.12$).

4. Discussion

Here we show that female fence lizards exposed to experimentally elevated CORT levels during gravidity did not have reduced body condition or reproductive output (as measured by relative clutch mass). We found that shortly after parturition, CORT-treated females had lower metabolic rate, but immune response, thermoregulatory behavior, and food consumption did not differ between treatment groups. These results could indicate that CORT-treated females were able to maintain their reproductive output, post-parturition body condition, and immune function by reducing their metabolic rate, even after the period of CORT-treatment had ended (measured 3–5 days post-treatment). This may be a potential compensatory strategy for coping with additional environmental stressors experienced during gravidity. Further work is needed, however, to determine whether the patterns we observed immediately post-parturition also reflect physiological and behavioral parameters during gravidity, as well as how glucocorticoid increases at earlier, potentially more energetically costly, stages of gestation (i.e. vitellogenesis) influence the same parameters.

4.1. CORT treatment reduced metabolic rate

Females treated with CORT during gravidity (i.e., post-vitellogenic reproduction) showed reduced resting metabolic rates shortly post-parturition compared to control females (significantly reduced O₂ consumption and biologically significant trend toward reduced CO₂ production). Although other studies have found effects of stressor exposure on respiratory quotient (e.g. McGregor et al. 1994), we did not see differences in RQ between treatments. Our findings are contrary to conventional predictions that elevated glucocorticoids and environmental stressors result in increased metabolic rates, which is frequently cited as evidence of a “cost” of exposure to stressors (Haase et al., 2016). For example, a comparable study in common lizards (*Lacerta vivipara*; study

also used transdermal application of CORT) found that elevated glucocorticoid levels resulted in an increase in metabolic rate (Meylan et al., 2010). The different result that we obtained with a similar study design may provide informative insights into the actions of glucocorticoids at different levels. The transdermal CORT treatment used in Meylan et al. (2010) resulted in increases in plasma CORT up to 282 ng/mL, substantially higher than levels reached in this study (22 ng/mL; MacLeod et al., 2018a). Indeed, the baseline CORT levels in Meylan et al. (2010) approximate the “stressed” levels in our study, suggesting underlying physiological differences between these species that likely influenced metabolic responses. Alternatively, the metabolic response to short-term, large increases in circulating glucocorticoids (as in the common lizards) is likely to be different to the response to more persistent, low-level increases (as in our study of fence lizards) – a rapid increase in metabolic rate is likely to be adaptive in the former scenario but not the latter. More work is needed to investigate how metabolic response varies based on glucocorticoid dosage – and indeed, how variation in the degree of “stress” translates into variation in glucocorticoid increase.

A lower metabolic rate reduces energy expenditure, which may be adaptive under periods of social or food stress, as in side-blotched lizards (*Uta stansburiana*) which showed a reduction in resting metabolic rate as a result of increased corticosterone levels (Miles et al., 2007). By reducing energy use through downregulation of metabolism in this species, females experiencing increased CORT exposure may be able to save energy, allowing for the maintenance of reproductive output and body condition (Ensminger et al., 2018). This strategy is not necessarily cost-free – metabolic downregulation can result in reduced growth, for example (Auer et al., 2015; Kozłowski, 1992) – but may nevertheless be an important strategy in relatively short-lived species, in which current reproductive output is likely to take precedence over future growth.

Some limitations of our study design mean that further work is necessary to more robustly test the potential for a reduction in metabolic rate to act as a compensatory strategy and clarify patterns of causality. First, it is possible that metabolic rate changes could be driven by variation in behavior associated with our CORT treatment, such as food consumption, rather than a direct effect of CORT. Although we found no influence of treatment on behavior post-parturition, assessment of CORT effects on behavior during gravidity would illuminate the potential for changes in metabolic rate to be a consequence of behavior, rather than endocrinological change. Additionally, we saw a reduced metabolic rate and equal food consumption, but CORT-treated females did not show an increase in body condition as might be expected. As we did not measure metabolic rate during gravidity, when it is likely to be higher (Angilletta and Sears, 2000), we cannot say conclusively how long metabolism was downregulated in the CORT-treated group; if this was a late-gravidity or post-parturition effect, there may have been insufficient time for subsequent differences in body condition. Mounting an immune response can lead to changes in metabolic rate (e.g. Ardia et al., 2012); however, as all individuals were subject to the same PHA test regime, and there were no differences in immune response between treatments, the differences we see in metabolic rate are not likely the result of other physiological tests. No differences between treatment groups in respiratory quotient is not surprising given how rapidly respiratory quotient shifts, including in response to stressors (e.g. McGregor et al. 1994). Again, measuring changes during gestation would be valuable to determine whether CORT changes the primary substrate of catabolism. Whether the observed reduction in metabolism of CORT-treated females affects their future reproduction and survival would also be important for determining the adaptive potential of this apparent compensatory strategy in natural systems.

4.2. CORT treatment does not reduce immune function

There was no difference between CORT-treated and control females in their response to the PHA skin test post-parturition, a test of cell-mediated immune response, suggesting that elevated CORT did not

translate into increased immune costs in this study. This is contrary to our initial predictions, based on previous findings showing that both reproduction (French et al., 2007a; French et al., 2007b; French and Moore, 2008; Tylan et al., 2020) and CORT elevation (Tuckermann et al., 2005; Morici et al., 1997; Berger et al., 2005; Thomas and Woodley, 2015) are immunosuppressive. Our results could, however, potentially provide support for energy reallocation via metabolic downregulation allowing the maintenance of immune response. It is possible that we may have seen suppressed immune function had we tested females earlier in reproduction, when energetic investment is higher (i.e. during vitellogenesis, the period in which a previous study in this species demonstrated reduced immune function: Tylan et al., 2020); or, using different tests. For example, in the common lizard (*Zootoca vivipara*), elevated CORT does not affect the PHA swelling response (tested one day after parturition/end of treatment, as in our study) but does induce an increased antibody response to diphtheria (Meylan et al., 2010). Alternatively, given that evidence for high costs of the immune response is mixed (Sheldon and Verhulst, 1996; Owens and Wilson, 1999), it is possible that the energetic costs of mounting this kind of immune response are sufficiently low as to not have been affected by CORT treatment. However, this is not likely in this case; CORT treatment in different populations of the same species results in a decrease in swelling response to a PHA test, indicating that there is a cost to maintaining immune function at least in some populations of fence lizards (Sprayberry et al., 2019), with the primary difference between our study population and that which was affected by CORT being the presence of fire ants at our study sites. As fire ant interactions are known stressors of fence lizards (Graham et al., 2016), this may have altered the physiological response to CORT and other stress hormones in lizards from these sites. We suggest that the lack of effect of our CORT treatment on immune response provides further tentative evidence that CORT-treated females were able to balance trade-offs in energy allocation (e.g. by reducing metabolic rate) in order to maintain their immune response despite having elevated CORT levels.

4.3. Thermoregulatory behavior and food consumption

Contrary to our prediction, CORT-treated lizards did not show changes in either thermoregulatory behavior or food consumption relative to control females in the post-parturition period. In particular, we predicted that we would see an increase in food consumption in CORT-treated females as a possible mechanism allowing the maintenance of body condition and reproductive output that has been previously shown in this species (Ensminger et al., 2018), and which we also see in this study. CORT treatment has been shown to positively affect food consumption in other species (Dallman et al., 2004): for example, CORT-treated common lizards (*Zootoca vivipara*) increased food intake relative to control individuals (Cote et al., 2006). In the same study, however, CORT treatment was also associated with an increase in activity levels, which may have necessitated increased food intake to meet the increase in energy requirements (Cote et al., 2006). Although we did not explicitly measure activity levels, a lack of difference in thermoregulatory behavior suggests that CORT-treated and control females were unlikely to have been active at different rates, which may have resulted in no need for increased food intake. There was no difference in mass change over the trial period between groups, indicating the CORT-treated and control females were putting on the same amount of mass per food item. Given that the resting metabolic rate was reduced in CORT-treated females, CORT-treated females may be allocating energy toward other processes, such as maintenance of immune response and reproductive output in terms of clutch mass of live offspring produced, instead of an increase in body mass/condition.

The regular provision of food to our lizards may have masked any energetic trade-offs (French et al., 2007a; French et al., 2007b) and prevented any differences in activity translating to differences in consumption. Similarly, the lack of observed differences in

thermoregulatory behavior may be caused by laboratory conditions providing more consistently favorable temperatures than would be expected in the wild. However, this is unlikely, as the average temperature of cells in heat zones 1-4 were below the preferred body temperature of 35 °C for this species, which is highly consistent across its range (Angilletta, 2001). Consequently, to advance our understanding of reproductive trade-offs, it is necessary to study trade-offs in the wild, where individuals face both the ecological and physiological costs of reproduction. Of course, it is possible that our behavioral observation protocol, which was replicated over 10 days, was too long after the period of CORT treatment for us to see any effects, given the relative plasticity of many behaviors in response to changes in the environment (Mery and Burns, 2010). As before, testing differences in behavior during the period of gravidity would also be valuable in future studies, as it is possible that post-parturition, behavior patterns changed and do not perfectly reflect changes induced by pre-parturition glucocorticoid elevation.

4.4. Conclusions

In concurrence with previous work (Ensminger et al., 2018), we found that female eastern fence lizards which experienced experimentally elevated CORT during late-stage gravidity (mimicking exposure to natural environmental stressors) did not show a reduction in body condition or reproductive output (relative clutch mass) relative to control females. This is surprising given that elevated levels of glucocorticoids such as CORT have long been thought to suppress reproduction to promote immediate survival (Sapolsky et al., 2000; Wingfield et al., 1998), and that strong links between elevated CORT and reduced reproduction and condition have been demonstrated in a number of species (Sheriff et al., 2009; MacLeod et al., 2018a, 2018b; Busch et al., 2008). Here we tested a number of hypotheses for possible mechanisms by which reproductive output may be maintained under elevated CORT. First, we show that CORT-treatment was associated with metabolic downregulation post-parturition (by approximately 30%), suggesting decreased energy use during gravidity or at least, a substantial reduction in energy use post-parturition which could also function as a compensatory measure. The pathways by which glucocorticoids influence metabolism are many and complex, for example altering the activity of enzymes involved in fatty acid synthesis and promoting gluconeogenesis (Wang, 2005). More work is needed to determine specific cellular mechanisms or effects in lizards. Second, we show that the maintenance of reproductive output and body condition in CORT-treated females did not come at a cost to immune response, which was also maintained in CORT-treated females. Third, we show that CORT treatment did not influence thermoregulatory behavior or energy intake via food consumption post-parturition. We suggest that under scenarios where CORT is elevated, reducing metabolic rate but maintaining food intake at normal levels leads to increased net energy, which allows females to maintain reproduction, body condition, and immune function during and/or after stressor exposure. The latter may be especially pertinent in this population, as a primary stressor is the invasive red imported fire ant (*Solenopsis invicta*) which attacks lizards by stinging them (Langkilde, 2009), potentially selecting for maintained immune response particularly in the skin (the site of our immune response test; McCormick and Langkilde, 2014). More broadly, the costs of reproduction are most apparent in natural environments (Koivula et al., 2003; Huber et al., 1999; Lambin and Yoccoz, 2001; Neuhaus, 2000; French and Moore, 2008). Consequently, to advance our understanding of reproductive trade-offs, it is necessary to study trade-offs in the wild, where individuals face both the ecological and physiological costs of reproduction.

Credit authorship contribution statement

KJM, TL, & MJS conceived the study; all authors contributed to

experimental protocols; KJM and JH conducted fieldwork; KJM, JH, CT, CFH, and KS conducted lab work; KJM performed statistical analyses; KJM wrote the manuscript, and all authors contributed to editing.

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