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Effect of exogenous corticosterone on respiration in a reptile

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Abstract

Release of glucocorticoids (GCs) enables organisms to meet energy requirements during stressful situations by regulating intermediary metabolism. In the absence of compensatory mechanisms, increased metabolic activity (e.g., protein catabolism, lipolysis, and gluconeogenesis) should translate to increases in whole animal metabolism, and therefore energy expenditures, by organisms. However, to our knowledge, no study has estimated the total energy cost of elevated plasma GCs in any organism. Here we evaluated the effect of exogenous corticosterone (CORT) on metabolism in captive western fence lizards (*Sceloporus occidentalis*) by conducting two experiments. In experiment I we determined the dynamics of plasma CORT concentrations resulting from CORT injections. In experiment II we frequently measured changes in respiration for 24 h before and after CORT injection. Injection of 0.025 μg/g (low CORT) and 0.40 μg/g CORT (high CORT) produced up to 26-fold increases in plasma CORT in lizards 3 h following injection compared to baseline levels. Plasma CORT concentrations returned to baseline levels 6 h after injection. CORT increased metabolism in both low and high CORT lizards (up to 121% and 245%, respectively) during the first 7.5 h after CORT injections compared to pre-treatment trials. Respiration returned to baseline rates 7.5 h after CORT administration. A surprising finding was that although high CORT males achieved higher plasma CORT concentrations than high CORT females, the metabolic response of high CORT females was 30% greater than high CORT males. Our results suggest that GC-induced changes in respiration may be important for understanding the overall energetic implications of stress.

Keywords: Stress; Bioenergetics; Metabolism; Lizard

1. Introduction

All organisms experience stress in their lifetimes and possess a suite of adaptations to cope with potentially harmful stimuli. Several investigators have proposed theoretical models that use bioenergetics as a unifying conceptual framework for understanding the effects of environmental stress on organisms (Wingfield et al., 1998; Wingfield and Ramenofsky, 1999; McEwen and Wingfield, 2003). Put simply, these models consider energy as a common currency acquired and utilized by all organisms for survival. Organisms must acquire a certain amount of energy from their environment to meet costs of existence (i.e., basic maintenance costs). When environmental conditions become stressful, organisms experience increased energy demands and must meet these demands by utilizing stored energy, acquiring additional energy from their environment, and/or conserving energy by altering non-essential activities. According to these models, when energy demands exceed the amount of energy available in the environment, an "emergency life-history stage" is initiated. During this life-history stage, release of glucocorticoids

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(GCs) is one of the primary mechanisms used to reestablish homeostasis.

Glucocorticoids have many effects in organisms, but one of their primary roles is the regulation of intermediary metabolism (i.e., sub-organismal metabolic processes affecting energy storage and utilization; Dallman and Bhatnagar, 2001). Release of GCs enables an organism to meet energy requirements during times of need by promoting the conversion of proteins and lipids to carbohydrates that are readily used by the organism (Mommsen et al., 1999; Schreck, 1993). In the absence of compensatory mechanisms to offset the additional costs of GC-induced increases in metabolic activity (e.g., protein catabolism, lipolysis, and gluconeogenesis), increases in intermediary metabolism could translate to increases in whole animal metabolism, and therefore energy expenditures, by organisms. Better understanding of the energy cost associated with an increase in circulating GCs could provide valuable insight into the ultimate costs of stress. However, relatively few studies have examined the influence of GCs on whole animal metabolism.

The studies that have examined the effect of GCs on whole animal metabolism have focused on birds and fish, and general consensus regarding the effect of GCs on metabolic rate remains unclear. Corticosterone (the primary GC associated with stress in birds and reptiles; hereafter CORT) increased metabolic rate in titmice (*Parus major*) and black headed gulls (*Larus ridibundus*), had no effect on metabolic rate in pigeons (*Columba livia*), and decreased nocturnal metabolic expenditures in chickens (*Gallus domesticus*), Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*), and pine siskens (*Carduelis pinus*) (Astheimer et al., 1992; Buttemer et al., 1991; Hissa et al., 1980; Hissa and Palokangas, 1970; Mitchell et al., 1986; Palokangas and Hissa, 1971). In fish, acute or chronic elevation in GCs generally results in increased metabolic rate (Wendelaar Bonga, 1997), but limited evidence sometimes supports (Barton and Schreck, 1987; Chan and Woo, 1978; DeBoeck et al., 2001; Morgan and Iwama, 1996) and sometimes contradicts this contention (Davis and Schreck, 1977). Some of the discrepancies among studies may be attributable to the use of pharmacological doses of exogenous GCs in some studies (Hissa and Palokangas, 1970; Palokangas and Hissa, 1971) versus physiological doses in other studies (Buttemer et al., 1991; Morgan and Iwama, 1996; Davis and Schreck, 1977). Discrepancies among studies may also relate to variations in procedures employed to estimate metabolism. In many cases, metabolic rate was determined at a single discrete time (e.g., 24 h after GCs were injected). However, changes in metabolism are dynamic and may not be captured with a discrete metabolic measurement. Furthermore, a small change in metabolism at a fixed point in time may not differ among experimental groups, but if this difference is maintained for extended periods of time, the total difference in energy expended could be profound. To fully appreciate the energy costs associated with stress, metabolic rate must be monitored over extended time periods. To our knowledge, no study has estimated the total energy cost of elevated plasma GCs in any organism.

In this study we sought to determine whether acute exposure to exogenous CORT increases whole animal respiration. To address this fundamental question we conducted two experiments to estimate the total energy cost of elevated plasma GCs in western fence lizards (*Sceloporus occidentalis*). In the first experiment we determined what dose concentrations of exogenous CORT were necessary to achieve physiological relevant plasma profiles. In the second experiment, we monitored metabolic rate (i.e., oxygen consumption and carbon dioxide production) of male and female lizards continually over a 24 h period after exposure to one of two concentrations of CORT (0.025 and 0.40 μg/g). We hypothesized that exposure to exogenous CORT would result in dose-dependent increases in plasma CORT and metabolism.

2. Materials and methods

2.1. Fence lizard natural history and husbandry

Sceloporus lizards belong to the family Phrynosomatidae which accounts for more than 30% of all lizards in the United States. Some *Sceloporus* serve as good study systems because their entire lifecycle is manageable in the laboratory, and a great deal is known about their ecology, physiology, performance, and life-history (e.g., Garland et al., 1990; Sinervo, 1990; Angilletta et al., 2002; Talent et al., 2002; Roe et al., 2005). The species used in this study, *S. occidentalis*, ranges from Mexico to Canada between the California coast and western Utah, USA. The parental stock originated from a population in the grasslands of the San Joaquin Valley, CA, USA. Most females reach sexual maturity under ad libitum feeding conditions in the laboratory within a year, and lay 3–6 clutches of 8–15 eggs per year. This population of western fence lizards does especially well under laboratory conditions and has been identified as a good candidate for use as a laboratory reptile model (Talent et al., 2002; Hopkins et al., 2005). Lizards from the San Joaquin Valley are also relatively large bodied (up to 25 g) making repeated blood sampling possible without harming the animal.

Adult western fence lizards were shipped to The Savannah River Ecology Laboratory (SREL) from a breeding colony at Oklahoma State University. A total of 159 lizards were used in this study (respirometry: $n = 96$; plasma CORT: $n = 101$). To reduce the number of lizards sacrificed during our studies 38 lizards used during the respirometry experiment were also used in the experiment to determine plasma CORT concentrations in lizards after CORT injection. Lizards used in both experiments were given at least two weeks to recover from the respirometry experiment and then were distributed among treatments in the plasma CORT experiment. Lizard husbandry was identical to Hopkins et al. (2005) with the following exceptions: a 10:14 (light: dark) photoperiod, a daytime temperature gradient of ~28–40 °C, and lizards were fed 4 crickets (~1.5 cm each) a day except on saturdays and sundays. Experimental procedures were approved by the University of Georgia IACUC (A2004-10049-0).

2.2. Plasma CORT concentrations resulting from injections

To determine the dynamics of plasma concentrations of CORT resulting from our injections, we collected blood samples from lizards ($n = 101$), pre-injection and 3, 6, and 12 h post-injection. Lizards were bled from the post-orbital sinus using heparinized micro-capillary tubes. Each treatment group consisted of 12–15 lizards per sex. Lizards were removed from their cage, bled within 1–3 min, exposed to their assigned treatment (control, vehicle, low CORT [0.025 μg/g] and high CORT [0.40 μg/g]) and then

returned to their cage. Lizards in the vehicle treatment were injected intraperitoneally with peanut oil while low CORT and high CORT lizards were injected intraperitoneally with CORT (Sigma, C2505) dissolved in peanut oil. Total injected volumes were always less than 160 μL. Capillary tubes were stored on ice for no longer than 1.5 h before plasma was separated using centrifugation. Plasma samples were then stored at −60 °C. Lizards were fasted for 48 h prior to blood collection but had continual access to water throughout the experiment. Because short periods between feeding intervals (weekends) were commonly experienced by our lizards, a 48 h fast was not a novel experience and should not have been stressful for the animals.

2.3. Respirometry

To determine the energy cost associated with exposure to exogenous CORT we followed methods similar to Hopkins et al. (2004) and Roe et al. (2005). Each animal was subjected to two sequential respiratory trials, a pre-treatment trial and a post-treatment trial. A total of 32 samples were collected over a 24 h period at 45 min intervals for each trial. The pre-treatment trial was used to determine each lizard's standard metabolic rate (SMR), which served as a baseline for comparison to the post-treatment trial. The post-treatment trial was used to determine increases in energy expenditure resulting from exposure to CORT. To ensure post-absorptivity, lizards were fasted for 48 h prior to collecting any respirometry measurements (Roe et al., 2005). At the end of fasting, each lizard was weighed and transferred to a glass respiratory chamber (600 ml) for its pre-treatment trial. Both oxygen consumption and carbon dioxide production were then continually monitored for a 24 h period by connecting each chamber to a computer-controlled, closed circuit respirometer (Micro Oxymax, Columbus Instruments). In this system both oxygen consumption (V_{O_2}) and carbon dioxide production (V_{CO_2}) are measured simultaneously. Incurrent air passed through columns of Drierite® to absorb water before passing into individual respirometry chambers. Air leaving respirometry chambers was dried again using magnesium perchlorate before passing into the oxygen sensor. Details of the respirometry system are discussed elsewhere (Hopkins et al., 1999, 2004). After 24 h lizards were removed from their chamber and then exposed to one of four treatments: control, vehicle, low CORT, or high CORT. Total injected volumes were always less than 140 μL. After injection, lizards were immediately returned to their chambers for post-treatment respirometry measurements. The total time for the transition from pre- to post-treatment trials was 1–3 min for each lizard. We measured metabolism in a total of 96 lizards, sexes were divided evenly among treatment groups ($N = 12$ females and 12 males per treatment).

All trials were initiated between 08:00 and 09:10 h. Lizards were housed in an environmental chamber held at 30 °C in total darkness throughout respiratory measurements. Individual respiratory chambers were also covered with paper to remove any external stimuli.

2.4. Respirometry calculations

From the respirometry data collected, SMR and post-treatment energetic expenditures were determined as follows. Because SMR is the metabolic rate of a resting, post-absorptive ectotherm at a specified temperature during the inactive phase of its circadian cycle (Bennett and Dawson, 1976), we removed peaks in respiration associated with activity and circadian rhythms from the pre-treatment data to estimate SMR. To accomplish this, SMR was estimated as the lowest quartile value of the pre-treatment trial. Other studies on reptiles have been successful at estimating SMR values using similar methods (Hopkins et al., 2004; DuRant et al., 2007). Once SMR was estimated, we then estimated the volume of gas exchanged that was required to support SMR as the integral of SMR (Fig. 1). In addition to estimating SMR requirements, we also estimated the total volume of O_2 consumed and CO_2 produced (V_{O_2} and V_{CO_2}, respectively) over the entire 24 h trial. Total V_{O_2} and V_{CO_2} were estimated as the integral (i.e., area under the curve) of respiration. V_{O_2} and V_{CO_2} above SMR (additional V_{O_2} and V_{CO_2}; energy allocated towards supporting circadian rhythms, spontaneous activity, and costs associated with elevated CORT) were estimated by subtracting the integral of SMR (from the pre-treatment trial) from total V_{O_2} and V_{CO_2} (from the post-treatment trial; Fig. 1). In statistical analyses we compared additional V_{O_2} and V_{CO_2} over the first 7.5 h of the pre- and post-treatment respirometry trials because this corresponded to the time in which plasma CORT concentrations were elevated (see Section 3). Using additional V_{O_2} and V_{CO_2} instead of total V_{O_2} and V_{CO_2} for analyses accounts for inter-individual variability in SMR.

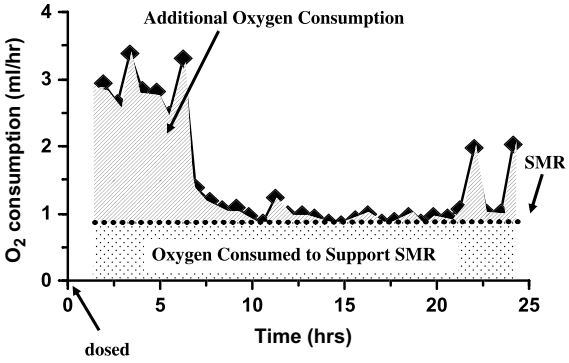

Fig. 1. A graphical representation of respiration components calculated for each lizard. SMR (dotted line) was estimated as the lowest quartile value of oxygen consumption rates. The integral of SMR (dotted region) represents oxygen consumed to support basic maintenance costs. The area above SMR (hatched region) represents oxygen consumed to support spontaneous activity, circadian rhythms, or increases in respiration attributable to corticosterone. Identical techniques were used to determine changes in carbon dioxide production.

2.5. Radioimmunoassay

Corticosterone was assayed by radioimmunoassay (RIA) following the methods of Wingfield et al. (1992). Briefly, samples were exposed to redistilled dichloromethane to extract CORT and then resuspended in phosphate buffer. Samples were compared to a standard curve, which contained known amounts of CORT, run with each assay. Small amounts of tritiated CORT were added to each sample to determine CORT recovery and the recovery was used to adjust final concentrations. Inter- and intra-assay variation were 15% and 6%, respectively, as determined by running a plasma standard in each assay.

2.6. Statistical analyses

For each statistical test, all data were $\log_{10}$ transformed prior to analyses to better achieve normal distribution and equal variance (Ryan-Joiners and Bartlett's test, respectively). All three and four-way interaction terms were included in initial models, however interactions with $p > 0.10$ were dropped from subsequent iterations. Metabolic parameters examined were not independent of one another, therefore we applied a sequential Bonferroni adjustment to account for multiple, non-independent comparisons and to maintain an experiment wide α of 0.05.

We conducted a repeated measures ANOVA (SAS proc mixed) to investigate the influence of CORT injection on circulating CORT plasma levels. In the model we considered treatment and sex as independent variables and time as the repeated variable.

We conducted two ANCOVAs (SAS proc mixed) to determine whether SMR (O_2 and CO_2) differed between treatment groups during the pre-treatment trial. In the model treatment and sex were considered independent variables with $\log_{10}$ mass as the covariate to control for the influence of body size on respiration (Andrews and Pough, 1985).

To examine the influence of CORT injections on additional V_{O_2} and V_{CO_2} during the first 7.5 h of the post-treatment trial compared to the pre-treatment trial, we conducted two repeated measures ANCOVA's

using a mixed model approach (SAS proc mixed). In the model we considered treatment and sex as independent variables, time as the repeated variable, and $\log_{10}$ transformed mass as the covariate.

3. Results

Both the low CORT and high CORT lizards had elevated plasma CORT concentrations 3 h after injection (treatment × time: $p = <0.001$; Table 1, Fig. 2). The interaction between sex and time also had a significant effect on plasma CORT concentrations of lizards ($p = 0.010$) with males exhibiting higher plasma CORT levels than females. Simple effects tests (LSMEANS/SLICE) were implemented to dissect significant two-way interactions and revealed that before injection there was no treatment × time interaction or sex × time interaction (for both variables $p > 0.53$). However, 3 h after injection there was a strong treatment × time interaction ($F = 9.72$, $p < 0.001$) but no sex - time interaction ($p = 0.14$). Six hours post-injection CORT concentrations no longer differed among treatments (treatment × time $p = 0.42$). However, there was a marginal sex × time interaction ($p = 0.06$) at 6 h post-injection which appeared to be driven by a slight elevation in plasma CORT concentrations of male control and vehicle lizards but not from females from the same groups. CORT levels returned to near-baseline levels 12 h post-injection and no longer differed among treatments or between sexes (treatment × time $p = 0.75$; sex × time $p = 0.15$).

Examination of the respirometry data revealed that lizards exhibited circadian fluctuations in respiration (Fig. 3). Respiration peaked in the afternoon, dropped to baseline over night, and then began to rise again the next day just prior to trial termination. These same circadian rhythms have recently been documented in lizards from the same population as the lizards used in our experiment (Roe et al., 2005). However, CORT-induced changes in respiration were easily distinguishable from respiration changes caused by diel activity patterns. SMR estimates for each individual transected the baseline respiration values observed overnight and were not influenced by diel activity patterns. After applying a Bonferroni sequential adjustment, we found that there was no effect of sex, treatment, or their interaction on SMR during the pre-treatment trial (O_2 and CO_2; $p > 0.0390$; adjusted $\alpha = 0.025$; Table 2).

When we examined the effects of exogenous CORT on additional V_{O_2} and V_{CO_2} we found that lizards had higher gas exchange rates in the post-treatment trial than pre-treatment trials (Figs. 3 and 4) but this was dependent upon treatment (time × treatment: $p < 0.001$ for both gases; Table 3), and sex ($p < 0.001$ for both gases). Males and females exposed to high CORT consumed 164% and 250% more O_2, respectively, during the post-treatment trial compared to the pre-treatment trial (Figs. 3 and 4). Similarly high CORT male and female lizards produced 157% and 245% more CO_2, respectively, after treatment with CORT. Low CORT males consumed 100% more O_2 and produced 121% more CO_2 during the post-treatment trial than in the pre-treatment trial, whereas low CORT females only consumed 14% more O_2 and produced 9% more CO_2 during the post-treatment trial.

Table 1
Results of repeated measures ANOVA for the effects of CORT injection on circulating CORT plasma concentrations in *Sceloporus occidentalis* pre- and 3, 6, and 12 h post-injection

Variable	Effect	Num df	*F*	*p*
Plasma CORT (ng/ml)	Trt	3	2.93	0.0414*
	Sex	1	0.29	0.6037
	Time	3	60.21	<0.0001*
	Trt × Sex	3	.82	0.6282
	Trt × Time	9	2.99	<0.0001*
	Time × Sex	3	3.57	0.0101*

An * denotes a significant effect based on an α of 0.05. The mixed procedure was performed using SAS. Sample size = 12–15 males and females for each treatment.
Controls: $N = 12$ females, 13 males; Vehicle: $N = 12$ females, 12 males; 0.025 μg/g: $N = 15$ females, 12 males; 0.04 μg/g $N = 13$ females, 12 males.

4. Discussion

The results of our study indicate that increases in plasma CORT do indeed increase whole-animal metabolism. We found that injection with CORT caused a dose-dependent increase in circulating CORT concentrations in lizard

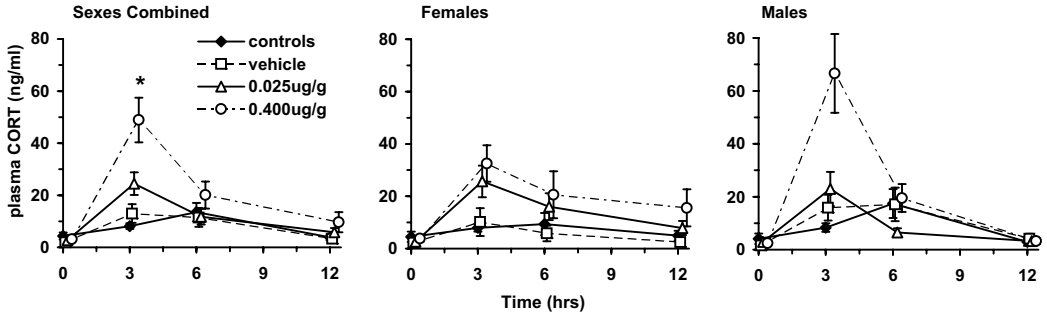

Fig. 2. Plasma corticosterone concentrations (ng/ml) of *Sceloporus occidentalis* before injection, and +3, +6, and +12 h after injection with varying concentrations of corticosterone. Error bars are ±1 standard error of the mean. $N = 12$–17 lizards per sex per treatment group for each time point. On the graph with sexes combined an * denotes when significant time × treatment effects were detected in post hoc analyses of the overall statistical model.

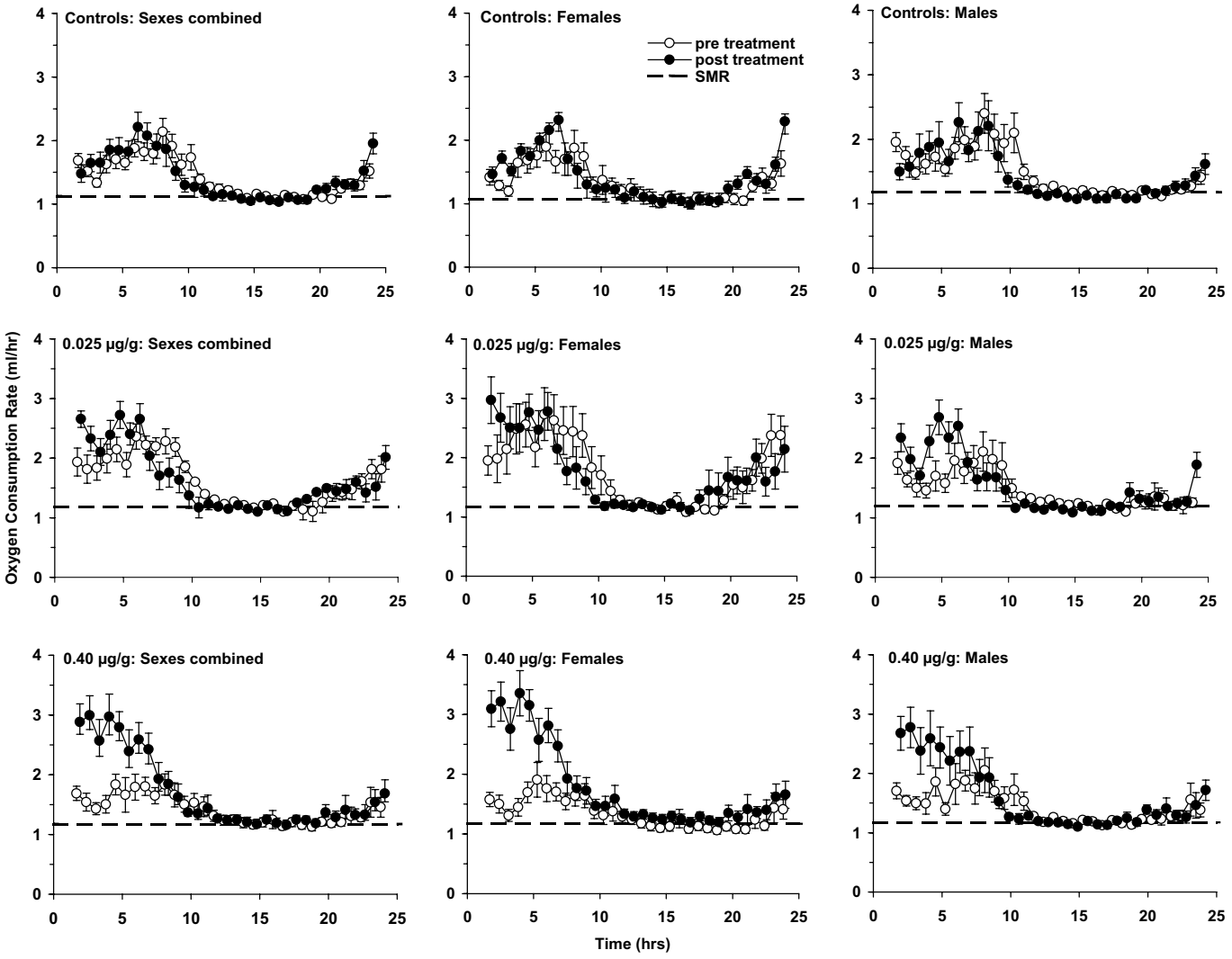

Fig. 3. Oxygen consumption rates (ml/h) of *Sceloporus occidentalis* before (open circles) and after (closed circles) injection with corticosterone. Dashed lines indicate the average SMR of lizards during the pre-treatment respirometry trial. Error bars are ±1 standard error of the mean. $N = 12$ lizards per sex per treatment group for both pre- and post-treatment trials.

plasma (Fig. 2). Concomitantly, energy expenditure increased in a dose-dependent manner after CORT injection (Figs. 3 and 4). Although males and females in each treatment group received the same dose of CORT, they responded differently to CORT injection both in plasma CORT levels and metabolic responsiveness. Whereas males appeared to achieve higher plasma CORT concentrations, females had a greater metabolic response.

Importantly, plasma CORT concentrations of lizards in our study were physiologically relevant, falling within the range of plasma CORT concentrations of wild *S. occidentalis* under baseline and stressed conditions. Plasma CORT concentrations of lizards, both males and females, did not differ prior to CORT injections (3.0 ± 0.5 ng/ml) and were comparable to baseline plasma CORT concentrations of wild *S. occidentalis* (~3–18 ng/ml; Dunlap, 1995; Dunlap and Schall, 1995; Dunlap and Wingfield, 1995). Increases in plasma CORT concentrations peaked 3 h post-injection and were significantly elevated compared to baseline concentrations (6- to 26-fold increases; Fig. 2) at this time. However, these effects were transient, with plasma CORT concentrations returning to near-baseline levels within 6 h of injection. The transient peak in CORT exhibited by our CORT treated animals is probably similar to that elicited by many types of acute stress. For example, Great Tits exposed to a simulated predator exhibited patterns of plasma CORT concentrations over time (Cockrem and Silverin, 2002) similar to those exhibited by our lizards after CORT injection. Our highest CORT concentration (0.40 µg/g) induced plasma CORT levels (48.9 ± 8.6 ng/ml) comparable to some of the highest plasma CORT concentrations noted in stressed wild *S. occidentalis* (~60 ng/ml; Dunlap, 1995; Dunlap and Schall, 1995; Dunlap and Wingfield, 1995) and our lowest CORT concentration achieved plasma CORT concentrations (24.5 ± 4.3 ng/ml) that were well within that range. Although males and females in the highest treatment group received the same dose of CORT, males achieved plasma CORT concentrations that

Table 2
Results of ANCOVA examining differences in SMR of *Sceloporus occidentalis* (log_{10} mass as a covariate) among treatment groups during the pre-treatment respirometry trial

Variable	Effect	Num df	Den df	*F*	*P*
SMR ($ml\ O_2\ h^{-1}$)	Trt	3	80	2.55	0.0617
	Sex	1	80	0.03	0.8581
	Trt × sex	3	80	2.32	0.0820
	log_{10} mass × trt	3	80	2.66	0.0538
	log_{10} mass × sex	1	80	0.05	0.8248
	log_{10} mass × trt × sex	3	80	2.39	0.0745
	log_{10} mass	1	80	2.27	0.1359
SMR (ml $CO_2\ h^{-1}$)	Trt	3	80	2.72	0.0501
	Sex	1	80	0.07	0.7850
	Trt × sex	3	80	2.87	0.0416
	log_{10} mass × trt	3	80	2.82	0.0442
	log_{10} mass × sex	1	80	0.04	0.8340
	log_{10} mass × trt × sex	3	80	2.92	0.0390
	log_{10} mass	1	80	2.27	0.1354

After applying a sequential Bonferroni adjustment to maintain an experimental wide α of 0.05 there was no effect of treatment, sex, or their interactions. The mixed procedure was performed using SAS. Sample size = 12 for each treatment for each sex for all variables.

Table 3
Results of repeated measures ANCOVA for the effects of exogenous CORT exposure on oxygen consumed and carbon dioxide produced above standard maintenance costs (log_{10} mass as a covariate) in *Sceloporus occidentalis* pre- and post-CORT injection

Variable	Effect	Num df	Den df	*F*	*p*
V_{O_2} (ml)	Trt	3	86	1.62	0.1916
	Sex	1	86	14.31	0.0003*
	Time	1	88	22.84	<0.0001*
	Trt × time	3	88	10.73	<0.0001*
	Trt × sex	3	86	2.25	0.0879
	Sex × time	1	88	1.11	0.2960
	Trt × sex × time	3	88	2.41	0.0722
	log_{10} mass	1	86	1.09	0.2983
	log_{10} mass × sex	1	86	13.25	0.0005*
V_{CO_2} (ml)	Trt	3	86	1.30	0.2785
	Sex	1	86	13.56	0.0004*
	Time	1	88	31.71	<0.0001*
	Trt × time	3	88	9.56	<0.0001*
	Trt × sex	3	86	2.70	0.0509
	Sex × time	1	88	0.00	0.9965
	Trt × sex × time	3	88	2.50	0.0650
	log_{10} mass	1	86	0.23	0.6296
	log_{10} mass × sex	1	86	12.59	0.0006*

An * denotes effects that were significant after application of a sequential Bonferroni adjustment. The mixed procedure was performed using SAS. Sample size = 12 for each treatment for each sex for all variables.

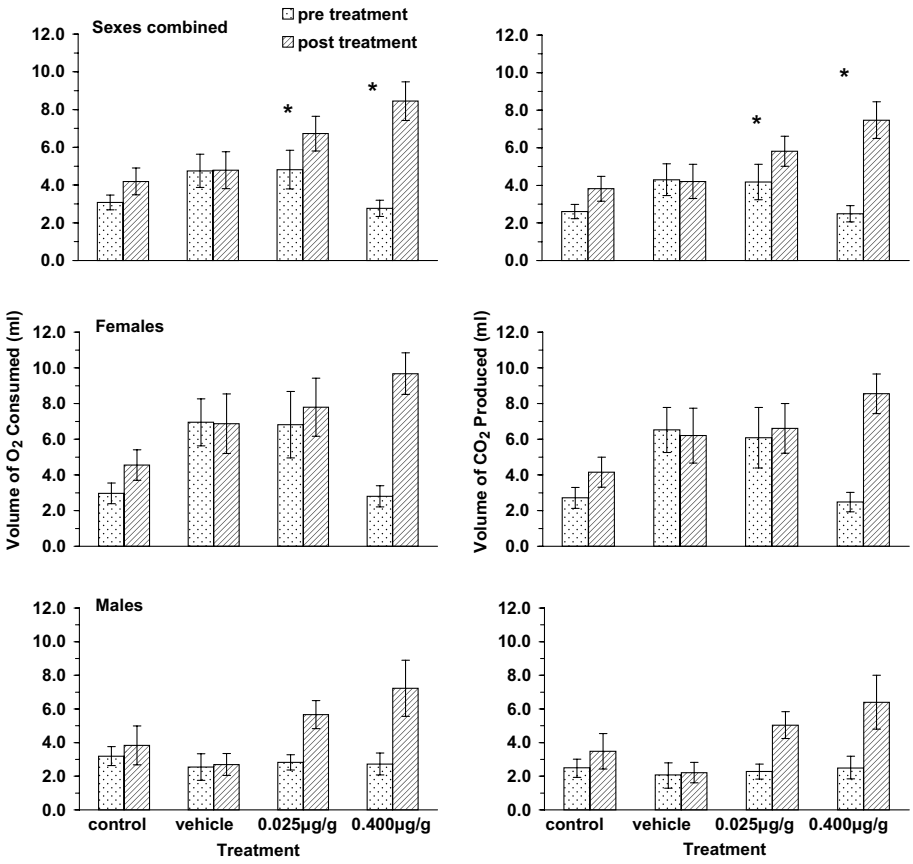

Fig. 4. Patterns of oxygen consumption (ml) and carbon dioxide production (ml) among western fence lizards (*Sceloporus occidentalis*) acutely exposed to varying concentrations of corticosterone. Oxygen consumed and carbon dioxide produced that was greater than maintenance costs (additional V_{O_2} and V_{CO_2}) over a 7.5 h period before and after CORT injection. Data are presented as means ± 1SE. On the graphs with sexes combined an * denotes for which treatments a significant treatment × time interaction was detected in post hoc analyses of the overall statistical model.

were twice as great as females (males: 66.6 ± 14.8 ng/ml; females: 32.5 ± 7.0 ng/ml; Fig. 2).

Similar to plasma CORT concentrations, respiration quickly increased in response to CORT injections, by 1.8 h after injection. Increases in respiration were also transient and returned to pre-injection levels approximately 7.5 h after injection (Fig. 3). While there was a clear dose-dependent metabolic response to plasma CORT concentrations in males, an increase in metabolism in females was only evident in high CORT females. However, the lack of dose-responsiveness in females may in part be attributable to outliers in our sample population. Three females in the vehicle and low CORT groups expended 40–151% more energy in pre-treatment trials than all other female lizards, perhaps obfuscating detection of elevated energy use post-treatment. In fact if the low CORT female with unusually high energy expenditure (151% greater) is eliminated from the data set, oxygen consumption increases in this group by 30% during the post-treatment trial compared to the pre-treatment trial.

A surprising result from our study is the difference in responses of males and females exposed to the highest CORT concentration. Although high CORT males achieved 105% higher plasma CORT concentrations than high CORT females, the metabolic response of these females was 30% greater than high CORT males (Figs. 3 and 4). These differences may be attributable to differences in the genetics or the internal milieu of males and females. For instance, there are differences in circulating testosterone (T) between males and females. Increases in testosterone have been shown to decrease CORT clearance rate by increasing plasma concentrations of binding proteins or increasing the plasma's binding capacity for CORT (Daniel and Assenmacher, 1974; Silverin, 1986; Klukowski et al., 1997; Jennings et al., 2000). If such sex-specific differences in the metabolic clearance of CORT occurred, it could also explain the lessened metabolic response in males because much of the CORT would have been bound in the plasma making less CORT available to bind to receptors.

Although CORT-induced increases in respiration were relatively brief (6–8 h) in both male and female lizards, when converted to energy equivalents (1 ml O_2 consumed = 19.8 J; Secor and Diamond, 1995) these increases represented a significant energy cost. Energy consumption in low CORT males and females increased by 14–100% and increased by 164–245% in high CORT males and females, respectively, following administration of CORT. This energy expense is equivalent to 3–11% of daily requirements to support SMR (SMR: 0.51–0.52 kJ) for low CORT male and female lizards and 17–27% for high CORT male and female lizards. Repeated and prolonged periods of elevated energy expenditure due to repeated acute or chronic stress could ultimately result in energetic trade-offs that have severe consequences for an individual. For example, in many reptiles, approximately 80% of their energy budget is comprised of maintenance costs whereas only 20% is allocated towards production of new tissue (Congdon et al., 1982, 2001). Therefore small increases in maintenance costs could result in proportionally large decreases in energy available for production.

In summary, our study represents the most comprehensive assessment of the effects of acute increases in CORT on energy expenditure to date. We demonstrated that physiologically relevant increases in CORT cause substantial increases in respiration. This is important because novel stressors are implicated as major factors contributing to population declines in vertebrates, including reptiles and amphibians (Gibbons et al., 2000; Stuart et al., 2004). Understanding the energetic impact of the stress response on vertebrates could provide insight into the consequences of stressors on traits which could ultimately translate into population-level effects (e.g., reproduction). Furthermore, our work provides support for the idea that energy deficits generated by stressors, resulting in the onset of an "emergency life-history stage", may be quantifiable and can provide a unifying framework for studying stress ecophysiology.

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