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Corticosterone modulation of reproductive and immune systems trade-offs in female tree lizards: long-term corticosterone manipulations *via* injectable gelling material

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Summary

Physiological trade-offs arise because multiple processes compete for the same limiting resources. While competition for resources has been demonstrated between reproduction and immune function, the regulation of this competition remains unclear. Corticosterone (CORT) is a likely mediator due to its dual role in mobilizing energy stores throughout the body and regulating physiological responses to stressors. We manipulated CORT concentrations and resources in pre-reproductive and reproductive female tree lizards (*Urosaurus ornatus*) to test the hypothesis that CORT regulates the distribution of limiting resources between the reproductive and immune systems. To manipulate circulating concentrations of CORT we utilized a novel method of hormone implantation, in which a polymeric compound is mixed with hormone and injected in liquid form into the animal. After injection, the liquid quickly gels *in situ* forming a slow release hormone implant. This method of hormone delivery eliminated the need for substantial wounds to the animal or repeated handling required by other methods. In this study, the hormone-treated animals had plasma CORT concentrations comparable to high physiological concentrations. We found that CORT treatment suppressed immune function, but only when animals were energetically compromised. We assessed immune function by measuring the healing rate of a cutaneous biopsy. Healing was suppressed in all CORT-treated reproductive animals and in all CORT-treated animals (pre-reproductive and reproductive) undergoing food restriction, but CORT had no effect in *ad libitum* non-reproductive females. The context-dependent action of CORT renders its response adjustable to changing environmental conditions and may allow for the suppression of specific functions depending on resource availability.

Key words: polymer, *in situ* gelation, wound healing, context-dependent, resources, sex steroids, drug delivery.

Introduction

Corticosterone (CORT) is widely accepted as an energy-mobilizing hormone responsible for the breakdown and redistribution of energy stores in the body (Moore and Jessop, 2003; Romero, 2002). It also plays an important role during reproduction in many species where increased circulating concentrations facilitate the mobilization of energy stores to invest in offspring development (Comendant et al., 2003; Greenberg and Wingfield, 1987; Jessop et al., 2002; Romero, 2002; Wilson and Wingfield, 1992). Although the proximate mechanisms are unknown, trade-offs between reproduction and other physiological processes such as immune function and somatic growth are thought to arise because resources are often limiting (Taylor and DeNardo, 2005; Taylor et al., 2005). Thus, corticosterone provides a likely candidate for mediating physiological trade-offs between systems competing for the same limiting resources.

CORT action is not only important for routine organismal function, but also in facilitating physiological emergency states such as stress responses (Wingfield, 2003; Wingfield et al., 1998). Upon the application of a stressor, the hypothalamo–pituitary–adrenal (HPA) axis is activated, resulting in increased CORT concentrations that enable the animal to cope with or avoid the stressor (Saplosky, 1992). Stress-induced increases in CORT result in a suite of effects on organismal physiology. For example, chronic increases in CORT generally suppress immune function, but acute increases can enhance immune function, therefore CORT action is not necessarily direct (Dhabhar, 2000; Dhabhar and McEwen, 1997). These stress-induced effects on immune function are likely regulated *via* redistribution of leukocytes throughout the body (Dhabhar et al., 1996) and/or CORT receptors present on lymphatic tissues and leukocytes themselves (Cidlowski et al., 1996; Leonard and Song, 2002; Weyts et al., 1998; Wiegers et al., 1993). It has also been suggested that reproduction is itself a form of chronic stress, as many studies found that immune

function was suppressed during reproduction (Deerenberg et al., 1997; Hanssen et al., 2005). Because of the important role of CORT during a stress response, the energy mobilizing effects of CORT are often overlooked and it is probable that they play a role in the regulation of immunity during reproduction.

The mechanisms of physiological trade-offs between the reproductive and immune systems are still unclear. While some studies found evidence that sex steroid hormones serve this role, our research in tree lizards does not support this conclusion (Benten et al., 2002; Bilbo and Nelson, 2001; Duffy et al., 2000; Klein, 2000). Instead, we found that in tree lizards the decrease in immune function during reproduction only occurred when food resources were limited (French et al., 2007). Based on these findings and the known functions of CORT, it is a likely mediator of the physiological trade-offs between the reproductive and immune systems, and its action does not seem to be direct.

We tested three alternative hypotheses examining the effects of CORT on wound healing, an innate immune response, during reproduction; CORT action is either (i) direct, (ii) indirect or (iii) has no effect on wound healing during reproduction. To test these hypotheses we manipulated circulating CORT concentrations in both pre-reproductive and reproductive (vitellogenic) females in different energy states, and measured the affect on wound healing. First, if CORT has no effect on wound healing during reproduction, we would expect to find no differences in wound healing rate between CORT-treated and control animals. Second, CORT may act directly, in which case we would predict all CORT-treated animals to have suppressed wound healing regardless of diet or energy state. Lastly, CORT action may be indirect (e.g. dependent on energy state or resource availability), whereby only under energetically limiting conditions will CORT suppress wound healing to conserve resources. This last indirect hypothesis predicts that CORT treatment should result in suppressed wound healing only when resources are limiting, regardless of reproductive stage.

To measure immune function in this study we utilized cutaneous wound healing. Wound healing is an integrative response, involving multiple stages, and can occur in the absence of infection (as in the present study) (Martin, 1997; Stojadinovic et al., 2007; Werner et al., 2007). First, the acute inflammatory response involves the recruitment of neutrophils and monocytes that mediate inflammation at the wounded site. The following stages require the mobilization and or proliferation of platelets, granulocytes, cytokines, chemokines, fibroblasts and keratinocytes; eventually this is followed by a long granulation and re-modeling stage, all of which may be affected by glucocorticoids and stress (Martin, 1997; Stojadinovic et al., 2007; Werner et al., 2007). We used this measure previously in tree lizards and found it was stress sensitive: applying a stressor to animals significantly slows the rate of healing (French et al., 2006). In our studies involving tree lizards we have focused on the early, inflammatory stages of wound healing, to study primarily the immune components of the response. In addition, we chose this technique because it is biologically relevant to tree lizards as well as many other species that frequently incur wounds in their natural environment.

To experimentally manipulate circulating concentrations of CORT in this study we utilized a novel method of hormone implantation involving the solubilization of CORT in a polymeric drug delivery device formed through *in situ* gelation. *In situ* studies demonstrate that steroid hormones are released from the gelled material in a controlled, zero-order manner (Vernon et al., 2003; Vernon et al., 2004). Furthermore, pilot studies in our laboratory found that the implants achieve constant, sustained concentrations of plasma corticosterone for 2 weeks post-injection. This method of hormone delivery eliminated the need for substantial wounds to the animal required by other methods of hormone implantation (e.g. silastic implants) or repeated handling (e.g. injections, topical application), which results in added stress to the animal. The *in situ* gelling method also allowed more precision in attaining circulating concentrations of CORT within the physiological range of the species.

Materials and methods

Overview

To test the possible interaction between CORT and reproduction, we manipulated CORT concentrations in both pre-reproductive and vitellogenic females (see below for assessment of reproductive stage). We previously showed that limiting resources suppresses wound healing in vitellogenic females, but not pre-reproductive females (French and Moore, 2007). Therefore, in this study half of the animals were maintained on an *ad libitum* diet and the other half on a restricted diet to mimic the previously observed context in which trade-offs were present (in vitellogenic females). Because no previous trade-off was observed in pre-reproductive females they provided a valuable control for the reproductive (vitellogenic) condition.

We used a two-by-two experimental design testing the effects of resource availability and CORT in both reproductive stages where 33 pre-reproductive and 40 vitellogenic females were divided into four treatments. We attempted to get ten animals of each reproductive stage in each treatment: (1) CORT-treated (polymer injection) and *ad libitum* diet, (2) CORT-treated and restricted diet, (3) control (CORT-free polymer injection, blank) and *ad libitum* diet, (4) control (CORT-free polymer injection, blank) and restricted diet. *Ad libitum* animals were individually fed crickets every day until they stopped consuming them. These animals experienced mass gain over the course of the study. Restricted animals received one cricket two times per week. These animals had access to calories, but still showed a decrease in body mass and fat stores. *Ad libitum* and restricted treatments were previously used and are described elsewhere (French et al., 2007).

Upon capture, all animals were randomly assigned to one of the above treatment groups and received either a blank or a CORT-containing polymer injection (see below). All animals were then individually housed and placed on their dietary treatment for 2 days to enter the desired energy state and attain circulating CORT concentrations. After 2 days, all animals received a cutaneous biopsy. We terminated the study 5 days after biopsy (7 days post-capture), at which point we collected blood samples, re-measured cutaneous wounds, and measured follicle and fat body sizes. This period of time (5 days) was

chosen to assure that the animals sustained prolonged exposure to both dietary and CORT treatments, but did not change reproductive states over the course of the experiment. Throughout the course of the study we monitored female food consumption (mass) and body mass (every 3 days).

When analyzing CORT data, 5 of 18 pre-reproductive and 7 of 20 vitellogenic females treated with CORT had circulating CORT concentrations that were not elevated and so they were excluded from further analyses. In addition, we were not able to get sufficient plasma volumes for radioimmunoassay from four pre-reproductive and four vitellogenic females. All eight of these females were undergoing dietary restriction, and thus the lack of plasma is likely due to dietary treatment, especially because lizards acquire a large proportion of their water from food, and all lizards had *ad libitum* access to water throughout the study.

Animals and study site

Female tree lizards *Urosaurus ornatus* L. were used for this study. Pre-reproductive females were collected during the period April 2–7, 2006 and vitellogenic females were collected during April 25–30, 2006, due to strict seasonality in reproduction. Female reproductive stage was assessed by manually palpating the abdomen for the number and firmness of follicles/eggs and confirmed on the last day of the study by abdominal surgery. All lizards were collected within Tonto National Forest 16 km east of Superior, Arizona (Maricopa County), USA, just off of highway 60 (Latitude: 33.29°N, Longitude: 111.10°W). The site consists of large boulder fields in an upland Sonoran Desert scrub environment. Lizards were captured by noosing and placed individually in cloth bags for transportation back to Arizona State University. At the beginning of the experiment, all animals were similar in snout–vent length (*SVL*; 46.73±0.30 mm), and body mass (2.71±0.05 g), which were re-measured at the end of the study. *SVL* is typically used in reptilian species to assess body size and is measured from the tip of the animal's snout to their cloacal vent. They were housed individually in 26 cm×28 cm×50 cm polycarbonate terraria, in a room maintained on a 14 h:10 h L:D photoperiod at 27±0.5°C. A 25 W heat lamp was suspended over one end of the cage providing a thermogradient within the cage (29–40°C) and enabling the animals to behaviorally thermoregulate. Past results illustrate that animals in the field during the breeding season have an average preferred body temperature of 38°C (R. Knapp and M.C.M., unpublished data). Water was available to all animals *ad libitum*. All handling, care and procedures in this study were approved by the Arizona State University Institutional Animal Care and Use Committee under protocol # 03-678R.

Hormone delivery via in situ gelling materials

In preparation for the injections, 0.36 g of pentaerythritol tetrakis 3-merkaptopropionate (QT) (Sigma, St Louis, MO, USA) was loaded into each of several sterile syringes (Cole Parmer, Vernon Hills, IL, USA). Separate sterile syringes were loaded with 1.031 g of poly(ethylene glycol)diacrylate (PEGDA) Mn: 700 (Sigma). 0.464 g of sterile phosphate buffered saline (PBS) was loaded into each of a third set of syringes. All syringes were capped with sterile syringe caps (Fisher, Pittsburgh, PA, USA) for transportation and storage. PBS was prepared by mixing 1 l of deionized water with 3.33 g NaCl, 2.43 g monobasic sodium phosphate (Sigma), and 11.32 g dibasic sodium phosphate (Sigma). This mixture produces a PBS solution that is 100 mOsm, and buffers at a pH of 7.4. The material was titrated with 1 mol l^{-1} NaOH to a pH of 7.6.

0.05 g crystalline CORT (Sigma) was loaded directly into the PEGDA syringe. The PEGDA syringes were individually vortexed until all added hormone was solubilized and uniformly distributed in solution. Blank injections were prepared in the same fashion except that no CORT was suspended in the PEGDA.

Once the hormone had been added to the PEGDA, the PEGDA was mixed with the QT through a sterile syringe junction (Cole Parmer). The mixture of PEGDA and QT was mixed back and forth between the two syringes several times to ensure good mixing of the materials. After combination, the resulting mixture was drawn into one syringe and then joined to a second syringe, which contained the pH 7.6 PBS. The polymer solution and the PBS were mixed by hand for 30 s (moving the solution back and forth between the two syringes using the syringe junction), after which the mixture was loaded into one syringe and the junction was removed. A 23 g needle was attached to the syringe containing the solution and the material was injected approximately 50–70 s after the initiation of mixing. All animals were anesthetized and 0.05 ml of material was injected into the coelomic cavity of each animal. Injections were performed with the animals unconscious and unresponsive to stimuli using surface-induced deep hypothermia anesthesia, which is accomplished by packing the animal in crushed ice for approximately 10 min. Anesthesia was maintained by performing the injection with the animal resting on ice. All animals quickly recovered (within minutes) after removal from the ice. The injected compound probably gelled quickly since it was too viscous to inject by approximately 90–110 s post-mixing and was a solid in the syringe by approximately 3 min after the initiation of mixing. Specific methods and techniques involving this novel procedure have been described previously (Vernon et al., 2003; Vernon et al., 2004).

Biopsy procedure and wound measurements

After 2 days of hormone and dietary treatment, all pre-reproductive and vitellogenic lizards were anesthetized (see above) and received a cutaneous biopsy on the dorsal surface over the pelvis using a sterile 3.5 mm punch (Miltex Instrument Company, York, PA, USA). The punch was lightly twisted to create a circular cut through the skin. The circle of skin was removed using forceps, creating a cutaneous wound.

The wounds in all animals were photographed on the day of the biopsy procedure and again on day 5 after biopsy. To photograph the wounds, animals were captured and secured with restraints that had a metric ruler attached as a scale reference. Digital images were taken using a camera attached to a light microscope (Panasonic® GP-US502 Industrial Digital Signal Processing Color 3-CCD Camera). Images were then imported to an image analysis program (Image-Pro Plus, version 4.0, Media Cybernetics, Silver Springs, MD, USA). Handling and photographing times were kept under 3 min to limit stress to the

animal. At the end of the study, all digital photographs were randomized and then analyzed to assess wound size using the image analysis software, such that the investigator was blind to the treatment of the animal. Area was used to assess wound size.

Follicle and fat body measurements

To measure follicle sizes, animals were anesthetized (see above) and bilateral laparotomies were performed using techniques described (Crews, 1974; Moore, 1987). Briefly, the follicles of each female were extruded through a ventral incision and follicle diameters were measured using an ocular micrometer attached to the dissecting scope. Tree lizards store fat in two bilateral groups along the lower abdomen. These fat bodies were also extruded and scored in terms of size on a scale of 0–3 for each female.

Plasma samples and radioimmunoassay

Plasma samples were collected on the final day of the study (day 5 post-biopsy; day 7 post-capture) in all animals, between 10:00 h and 12:00 h. Upon capture, animals were bled immediately. No animal took longer than 30 s to capture and bleed. Blood samples were collected by rupturing the orbital sinus with a capillary tube. Plasma was separated from the blood *via* centrifugation and stored at –20°C until assayed. All samples from the study were analyzed within a single radioimmunoassay. Plasma samples were assayed for progesterone, testosterone, estradiol and CORT, using a previously described and established laboratory protocol (Moore, 1986). In brief, samples were extracted using 30% ethyl acetate/isooctane extractions. The 30% phase was separated, dried and resuspended in isooctane containing ethylene glycol. Individual hormones were separated from samples using columns packed with one layer made of a celite/water mixture and three layers made of a celite/glycol mixture. Different elutions of ethyl acetate/isooctane were added to the columns to separate out the different hormones (10% for progesterone; 20% for testosterone; 40% for estradiol; 50% for CORT). Separated samples were collected in vials, dried and resuspended in PBS buffer. Duplicate aliquots of these samples were then assayed for progesterone, testosterone, estradiol and CORT. The intra-assay coefficients of variation were 19.0% for progesterone, 9.3% for testosterone, 13.6% for estradiol and 6.6% for CORT.

Statistical analyses

The significance level for all statistical tests was P=0.05 unless otherwise stated. We conducted two-way ANOVAs to examine the effects of CORT and diet treatments on circulating hormone concentrations in pre-reproductive and vitellogenic groups separately, followed by Tukey's HSD corrected *post-hoc* comparisons to discern differences among the mean values. Hormone data were log-transformed to satisfy the assumption of equal variances. We used separate one-way ANOVAs (adjusted α) to assess effects of diet and CORT treatments on changes in body mass, *SVL*, follicle diameter, and fat body score over the course of the study in pre-reproductive and vitellogenic stages.

Lastly, wound healing data (percent wound healed over time) was arc-sin transformed to perform all statistical analyses. We analyzed wound healing separately in pre-reproductive and vitellogenic females using two-way ANOVAs examining effects of CORT and diet treatments on percentage of the wound healed over time. We performed Tukey's HSD *post-hoc* comparisons on wound healing data to assess differences. All statistical analyses were performed using JMP.IN version 5.1 analyses software (SAS Institute Inc., Cary, NC, USA).

Results

Hormone results

Implants were effective at elevating CORT concentrations in the majority of the CORT treated animals relative to blank treated control animals. Animals that did not respond to implants were excluded from analyses, as we were testing the effects of CORT and not the implants. As expected, in both pre-reproductive and vitellogenic females, CORT-treated animals had significantly elevated circulating CORT concentrations relative to untreated control animals (two-way ANOVA; $F_{\text{pre-reproductive}}$=12.90, d.f.=1,20, P<0.01; $F_{\text{vitellogenic}}$=12.78, d.f.=1,25, P<0.01; Fig. 1). There was no significant effect of feeding treatment and no interaction between feeding and CORT treatments on circulating CORT concentrations (all F<0.18, all P>0.67). Likewise, there were no significant differences in circulating testosterone or estradiol between treatments in either reproductive stage (all F<3.41, all P>0.08; Table 1). However, in both reproductive stages there was an effect of hormone treatment on circulating progesterone concentrations (all F>4.26, all P<0.05), such that progesterone

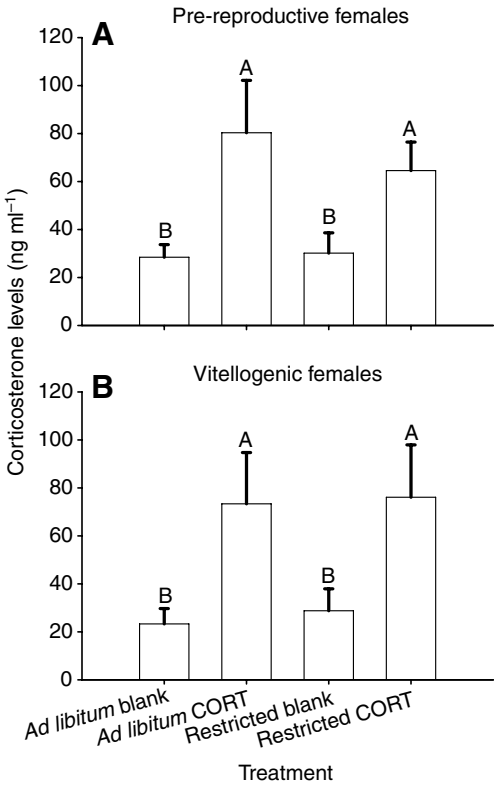

Fig. 1. Mean circulating CORT concentrations at day 5 post-wounding in pre-reproductive and vitellogenic females undergoing CORT and diet manipulations. See text for a description for treatment groups. Values are means ± s.e.m.; different letters denote statistically significant differences at $P \leqslant 0.05$.

Table 1. *Circulating steroid hormone concentrations, change in body mass, vitellogenic follicle diameter, and fat body score on day 7 of the study in both pre-reproductive and vitellogenic females on different diets and CORT treatments*

Diet	[Circulating steroid hormone] (ng ml^{-1})			Change in mass (g)	Follicle diameter (mm)	Fat body score (0–3)
	Progesterone	Testosterone	Estradiol			
Pre-reproductive						
Ad libitum						
Control	0.53±0.10	0.06±0.01	0.34±0.09	0.08±0.12	–	1.6±0.2
CORT-treated	1.99±0.63	0.10±0.03	0.48±0.11	0.19±0.21	–	1.8±0.1
Restricted						
Control	1.00±0.30	0.16±0.07	0.20±0.06	–0.11±0.03	–	1.1±0.1
CORT-treated	0.94±0.17	0.09±0.02	0.31±0.20	–0.14±0.06	–	1.5±0.2
Vitellogenic						
Ad libitum						
Control	0.68±0.20	0.36±0.19	0.70±0.40	0.50±0.12	2.77±0.57	1.8±0.1
CORT-treated	2.77±1.24	0.41±0.26	0.23±0.06	0.38±0.12	2.32±0.46	1.6±0.2
Restricted						
Control	1.38±0.71	0.09±0.03	0.10±0.03	–0.28±0.07	1.52±0.18	0.7±0.2
CORT-treated	3.04±1.26	0.12±0.02	0.13±0.02	–0.22±0.08	1.20±0.52	0.9±0.2

Values are means ± s.e.m. See text for description of dietary and CORT treatment groups.

concentrations were slightly elevated in CORT-treated animals (Table 1).

Size and mass results

Dietary treatments were effective at attaining different energy states as indicated by changes in body mass and fat body mass. Initial *SVL* (46.7±0.3 mm) and body mass (2.71±0.05 g) were similar across all treatment groups at the beginning of the study. Feeding manipulations significantly affected changes in body mass over the course of the study in both pre-reproductive and vitellogenic females (one-way ANOVA; $F_{\text{pre-reproductive}}$=4.06, d.f.=1,31, P=0.05; $F_{\text{vitellogenic}}$=43.62, d.f.=1,37, P<0.01; Table 1). In both reproductive stages, *ad libitum* females significantly gained body mass, while the restricted treatment groups significantly lost body mass. CORT treatment had no effect on change in body mass in either reproductive stage (all F<2.46, all P>0.13).

Feeding treatments also significantly affected follicle size, as measured by follicle diameter (mm), in vitellogenic females measured on day 7 of the experiment (one-way ANOVA; F=6.61, d.f.=1,37, P=0.01; Table 1). Follicles were significantly larger in animals on an *ad libitum* diet than in animals on a restricted diet. However, CORT treatment had no affect on follicle size (F=1.33, d.f.=1,30, P=0.26). As previously observed, females on an *ad libitum* diet had significantly larger fat bodies than females on a restricted diet (Table 1).

Wound healing results

In pre-reproductive females, both feeding and hormone treatments significantly affected wound healing rate (two-way ANOVA; F_{feeding}=8.92, d.f.=1,22, P=0.01; F_{hormone}=11.01, d.f.=1,22, P<0.01; Fig. 2A). There was also a significant interaction between the two treatments ($F_{\text{feeding}\times\text{hormone}}$=8.04, d.f.=1,22, P=0.01).

According to *post-hoc* comparisons, pre-reproductive females on a restricted diet treated with CORT had significantly slower healing relative to all other pre-reproductive treatment groups (Fig. 2A).

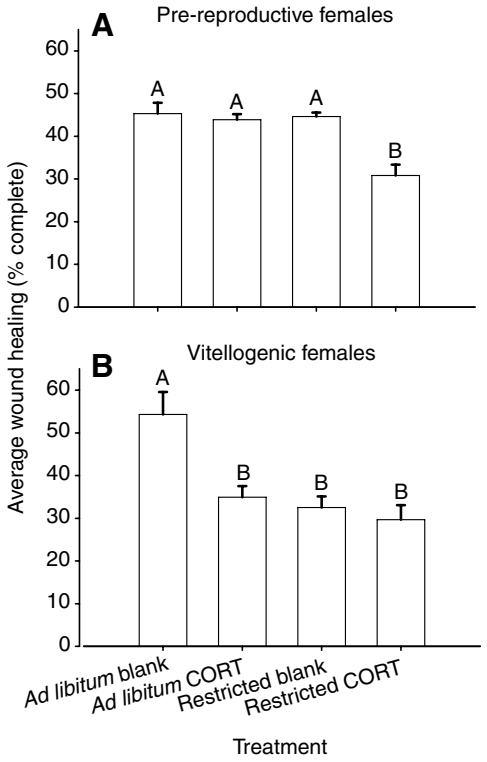

Fig. 2. Extent of wound healing at day 5 post-wounding in pre-reproductive and vitellogenic females undergoing CORT and diet manipulations. See text for a description for treatment groups. Values are means ± s.e.m.; different letters denote statistically significant differences at $P\leqslant$0.05.

In vitellogenic females, both feeding and hormone treatments

significantly affected wound healing rate (two-way ANOVA; $F_{feeding}$=10.03, d.f.=1,28, P<0.01; $F_{hormone}$=6.24, d.f.=1,28, P=0.02). There was, however, no interaction between treatments on healing in vitellogenic females (F=2.66, d.f.=1,28, P=0.11). According to *post-hoc* comparisons, healing was significantly slowed in vitellogenic females on the restricted diet relative to females on the *ad libitum* diet, regardless of hormone treatment (Fig. 2B). However, vitellogenic *ad libitum* females treated with CORT also showed slowed healing relative to their blank-implanted counterparts (Fig. 2B).

Discussion

The hypothesis that CORT suppresses wound healing is supported, but only indirectly, under energetically challenging conditions. When examining hormone-treated animals we found that CORT treatment did indeed alter immune function, and that this effect was not direct and instead depended on individual energy state. Thus in pre-reproductive animals only animals that were both on a restricted diet and CORT-treated had suppressed healing. In vitellogenic animals, CORT treatment suppressed healing rate regardless of food treatment (Fig. 2B). Presumably this occurred because vitellogenesis provokes a limited energy state despite *ad libitum* food availability. The costs of vitellogenesis are high, requiring substantial energy and resources (Burley et al., 1987; Challenger et al., 2001; Ojanen, 1983; Williams and Ternan, 1999). In fact, many studies have shown significant increases in metabolic rate associated with vitellogenesis (Angilletta and Sears, 2000; Nilsson and Raberg, 2001). Therefore, it seems likely that food-restricted and vitellogenic animals had increased sensitivity to CORT. This sort of sensitivity may be achieved by selective regulation of hormone receptors on immune cells and the cells controlling reproduction, even if the different cell types have the same hormone receptors, whereby under resource-limiting conditions immune cell receptors could be downregulated. Interestingly, in a previous study, CORT concentrations were inversely related to healing rate in stressed animals but not unstressed animals, again suggesting that it regulated wound healing in a context-dependent manner (French et al., 2006). Thus, we found support for the idea that CORT mediates trade-offs between the reproductive and immune system components based on individual energy balance.

In the absence of CORT treatment, these results are consistent with previous studies in tree lizards. We found that untreated vitellogenic animals on *ad libitum* food were able to sustain both reproduction and wound healing whereas animals maintained on a restricted diet were not. However, in untreated pre-reproductive animals, without the large draw on resources by reproduction, there was no immunosuppression regardless of organismal energy state.

CORT treatment was effective in attaining high circulating physiological concentrations. Previous *in situ* studies as well as pilot studies support the slow, time-release of corticosterone from the polymer (Vernon et al., 2003; Vernon et al., 2004). In addition, feeding treatments were also verified by significant increases (*ad libitum*) or decreases (restricted) in body mass depending on treatment. As previously observed, food intake significantly affected reproductive investment in vitellogenic females, whereby females on a restricted diet had significantly smaller follicles at the end of the study. This difference in follicle size suggests that reproduction is slowed or blocked in these animals. CORT treatment also affects mass and reproductive investment (Wilson and Wingfield, 1992), but did not significantly alter either component in this study. Additionally, circulating testosterone or estradiol concentrations did not differ between treatment groups in either reproductive stage. The hormone results of this study did not show an obvious interaction between sex steroids and wound healing, which has been suggested as a means of immunosuppression during reproduction (Casto et al., 2000; Duffy et al., 2000). However, circulating progesterone concentrations were elevated in CORT-treated reproductive females, for reasons that are yet unknown.

The novel method of hormone delivery used in this experiment provided a consistent delivery of CORT and successfully elevated circulating CORT to concentrations that were within the physiological range of the species. After an intra-coelomic injection, the implants were easily locatable, and non-degradable, so they retained their shape throughout the study. Successful treatment of the animals with the *in situ* gelling material required slightly elevated pH to promote rapid gelation. An initial group of six animals showed an LD-50 type response to the polymeric implants. This response was likely due to the 4-methoxyphenol (MEHQ) and 2,6-di-tert-butyl-p-cresol (BHT) gelation inhibitors that are solubilized in the polymers to prevent gelation during their manufacture and shipping. Further pilot studies determined that it was possible to protect animals from the toxic effects of these inhibitors by ensuring that the material gelled swiftly upon injection into the animal. The toxic effects of MEHQ and BHT are not expected to be an issue in larger animals, however they were problematic in tree lizards, due to their small body mass (<5 g), rendering them much more sensitive to toxins. In future studies, should such complications be encountered, it is possible to remove the MEHQ and BHT through an extraction *via* a silica-gel chromatography column or similar technique.

The results of this study emphasize the importance of CORT not as a stress hormone, but more accurately as an energetic hormone. It not only regulates the general release and uptake of energy throughout the body, but probably also plays a role in regulating the use of energy by multiple systems throughout the body, thereby mediating physiological trade-offs.

Conclusions

CORT is a likely mediator of physiological trade-offs between the reproductive and immune systems, as well as other systems. Its action appears to depend on energy availability. CORT suppresses wound healing only when the organism is in an energetically challenged state (e.g. investing in reproduction or other extreme resource restriction). This method of action creates a dynamic and adaptable response. If resources are limiting, then an animal can systematically suppress physiological processes, thereby conserving resources. However, if resources are readily available then no processes need be compromised. Tree lizards range throughout the Sonoran Desert, where available resources are unpredictable and often scarce. Facultative regulation in this environmental setting may be advantageous for suppressing physiological processes until the necessary resources become available.

Future studies should examine the specific mechanisms of CORT action on the immune system during reproduction, perhaps focusing on the regulation of CORT receptor expression on the lymphatic system and the effects of CORT on metabolism of specific energy stores throughout the body during reproduction. This type of context-dependent regulation may also account for discrepancies in previous results, where some studies find trade-offs and others do not.

Lastly, novel *in situ* gelling implants provide a promising new vehicle for hormone delivery. This method eliminates the need for surgeries, which can be stressful to the organism. It is also readily adaptable to fine-scale adjustments in delivery volume.

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