

Trade-Offs between the Reproductive and Immune Systems: Facultative Responses to Resources or Obligate Responses to Reproduction?

Susannah S. French,^{*} Dale F. DeNardo,[†] and Michael C. Moore[‡]

School of Life Sciences, Arizona State University, Tempe, Arizona
85287

Submitted October 26, 2006; Accepted February 20, 2007;
Electronically published May 11, 2007

ABSTRACT: A major challenge in biology is understanding how organisms partition limited resources among physiological processes. For example, offspring production and self-maintenance are important for fitness and survival, yet these critical processes often compete for resources. While physiological trade-offs between reproduction and immune function have been documented, their regulation remains unclear. Most current evidence suggests that physiological changes during specific reproductive states directly suppress various components of the immune system; however, some studies have not found this clear relationship. We performed two experiments in female tree lizards (*Urosaurus ornatus*) that demonstrate the presence of trade-offs between the reproductive and immune systems under controlled laboratory conditions. These results also support the hypothesis that these trade-offs are a facultative response to resource availability and are not obligatory responses to physiological changes during reproduction. We found that (1) experimentally increasing reproductive investment under limited resources resulted in suppressed immune function and (2) experimentally limiting resources resulted in immunosuppression but only during resource costly reproductive activities. There seems to be a critical balance of resources that is maintained between multiple processes, and changes in the balance between energy intake and output can have major consequences for immune function.

Keywords: energy, resources, follicle-stimulating hormone (FSH), wound healing, immunity, sex steroids.

^{*} Corresponding author; e-mail: ssfrench@asu.edu.

[†] E-mail: denardo@asu.edu.

[‡] E-mail: michael.moore@asu.edu.

Reproduction and self-maintenance are critical processes for animals, yet both require substantial resource investment (Lochmiller et al. 1993; Demas et al. 1997; Angilletta and Sears 2000; Lochmiller and Deerenberg 2000; Nilsson and Raberg 2001; Martin et al. 2003). Thus, it is not surprising that many studies have found support for trade-offs between the two processes, with immunity being suppressed during energetically taxing reproductive periods (Saad and Elridi 1984; Adamo et al. 2001). Furthermore, it is widely perceived in the literature that physiological changes that occur during reproduction (e.g., elevation in sex steroid concentrations) directly regulate immune function in a fixed relationship between the reproductive and immune systems. We term this the “obligate regulation hypothesis.” This hypothesis predicts that immune function will be suppressed in all reproductive animals regardless of energetic state. For example, sex steroids foster both sexual behavior and other physiological changes during reproduction directly via receptor-mediated effects but may also bind directly to receptors on immune cells, which could result in a direct and unavoidable coupling or trade-off.

However, immune suppression during reproduction is not always found or can vary depending on context. For example, wound healing rate is suppressed during vitellogenesis relative to other reproductive stages in female tree lizards (*Urosaurus ornatus*) in the field but not in the laboratory (French and Moore, forthcoming). Peters (2000) found similar context-dependent differences for effects of the reproductive hormone testosterone on humoral immunity in superb fairy wrens (*Malurus cyaneus*). Variation in energy resource availability is suspected to be the driving force behind this context-dependent relationship between the reproductive and immune systems, with functional trade-offs between the reproductive and immune systems occurring only when resources are limited. Given abundant resources, animals could maintain both reproduction and immunity. We term this context-dependent relationship the “facultative regulation hypothesis.” Fur-

thermore, this type of regulation is not mutually exclusive with a sex steroid hormone mechanism. Even if immune cells and the cells controlling reproduction have the same hormone receptors, receptors could be selectively regulated in the different tissues, whereby under resource-limiting conditions, immune cell receptors could be downregulated. While differential results within and among studies can be explained by the facultative regulation hypothesis, manipulative experiments that show direct competition for resources between the reproductive and immune systems have yet to be conducted.

We further explored the previous context-dependent results in tree lizards by conducting a pair of manipulative laboratory experiments with two main goals: (1) to demonstrate that there is a trade-off between reproductive and immune functions and (2) to determine whether the trade-off is obligatory or facultative. To experimentally demonstrate the presence of this trade-off, we artificially increased reproductive investment via injections of follicle-stimulating hormone (FSH) in female tree lizards. We predicted that if trade-offs are present, then artificially increasing reproductive investment would suppress immune function. FSH manipulations have been used in side-blotched lizards (*Uta stansburiana*), a species closely related to the tree lizard, to initiate vitellogenesis and increase reproductive investment (clutch mass) over a shortened period (Sinervo and Licht 1991a, 1991b; Sinervo and DeNardo 1996).

To test whether the trade-off between reproduction and immune function is obligatory or facultative (i.e., resource driven), we examined whether the trade-off was affected by varying food intake. The obligatory hypothesis predicts that all reproductively active animals should suppress immunity, whereas the facultative hypothesis predicts that immunosuppression will occur only in animals with limited access to resources.

Both experiments used the rate of cutaneous wound healing as an integrative measure of innate immune function (Martin 1997; Marucha et al. 1998; Padgett et al. 1998; Detillion et al. 2004; French et al. 2006). Wound healing has been used in this way for many years in both humans and various small mammal species (Martin 1997; Marucha et al. 1998; Padgett et al. 1998; Detillion et al. 2004). In tree lizards, wound healing is stress sensitive, such that restraint stress significantly alters the healing profile and reduces healing rate (French et al. 2006). In this study, we focus on the initial phase of the wound healing response, which involves the recruitment of neutrophils and monocytes that mediate inflammation at the wound site (Martin 1997). Most importantly, wound healing is biologically relevant; in a survey of tree lizards in the field, more than half exhibited a scar from some type of wounding (R. Knapp and M. C. Moore, 1993, unpublished data).

Methods

Animals and Study Site

Adult female *Urosaurus ornatus* were collected from March 22 to May 6, 2005, from our study site, located 10 miles east of Superior, Arizona (Maricopa County), adjacent to Highway 60, within the Tonto National Forest (33.29°N, 111.10°W). The site consists of large boulder fields in an upland Sonoran Desert scrub environment. At the experiment's start, all animals were similar in snout-vent length (SVL; 48.45 ± 2.94 mm) and mass (3.19 ± 0.58 g). Lizards were captured by noosing and placed individually in cloth bags for transportation back to Arizona State University. Animals were housed individually in $26 \times 28 \times 50$ -cm polycarbonate terraria in a room maintained on a 14L:10D photoperiod at $27^\circ \pm 0.5^\circ\text{C}$. 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. Water was available to all animals ad lib. All handling and procedures in this study were approved by the Arizona State University Institutional Animal Care and Use Committee, under protocol 03-678R.

Experiment 1: Reproductive Investment Manipulation Overview and Design. We manipulated reproductive investment by experimentally stimulating vitellogenesis (i.e., yolk production) using FSH injections. We captured 19 prereproductive females in early April 2005. Females were randomly divided into two groups. Each group received 0.02-mL intraperitoneal injections of either FSH (20 μg ovine FSH [Sigma-Aldrich] suspended in saline) or saline (0.9% sodium chloride). Animals were injected every 2 days for 19 days. On the day of the fifth injection, after the follicles of FSH-injected females reached a midvitellogenic size, which was assessed by manual palpation of the abdomen, all animals received a 3.5-mm cutaneous biopsy. This timing was necessary to ensure that manipulated females were actually investing resources in vitellogenesis while healing a cutaneous wound. On day 10 postbiopsy (day 19 of the experiment), we measured wound size, follicle diameter, body mass, and SVL, and we collected plasma samples from all animals.

Because the trade-off between reproduction and immune function may manifest itself only in an energy-limited environment, we did not want to provide an ad lib. diet to the females in this study. Therefore, all females in this study were fed a maintenance diet that allowed them to maintain a typical healthy body mass so we could determine what affect the additional demands of reproduction would have on an animal that had sufficient resources to maintain itself. This diet consisted of three crickets two times per week. All females in this experiment

ate all crickets given to them and therefore consumed the same amount of energy. Cricket intake was quantified as described in the next subsection. This dietary treatment provides a greater amount of food than the restricted diet used in the following study because in experiment 1, we were interested in the additive effects of reproductive investment on immune function, whereas in experiment 2, we were interested in how more severe resource limitation could affect immune function.

Experiment 2: Energy Intake Manipulation Overview and Design. We manipulated energy intake using dietary treatments. The only food source used in this study was crickets, and all crickets were in the same age class (second/third instar) and reared on the same diet (ground rat feed, lettuce). The specific treatments were as follows: (1) ad lib. access to food (daily unlimited access to crickets during feeding, where animals were individually fed crickets until they stopped consuming them): animals experienced mass gain over the course of the study; (2) restricted access to food (one cricket two times per week): animals had limited access to calories and showed a significant decrease in body mass and total fat stores (S. S. French and M. C. Moore, unpublished pilot data); and (3) no food (no access to crickets during the study): animals lost considerable mass and total fat stores (S. S. French and M. C. Moore, unpublished pilot data).

We captured 33 prereproductive females in late March 2005 and 30 early vitellogenic females in early May 2005 from the field. The two experiments were temporally separated because of the strong seasonality in reproductive activity in this population, and it is impossible to find appreciable numbers of mature nonreproductive females during the breeding season. Prereproductive females were randomly divided into the three dietary treatments. Females were placed on dietary treatments for a total of 20 days. After 10 days of treatment, all females received a 3.5-mm cutaneous biopsy and remained on the dietary treatment throughout wound healing (10 additional days). Four of the 14 ad lib. females ate fewer than 10 crickets and lost weight over the course of the experiment. Since the goal of this feeding treatment was the consumption of considerable calories in excess of energetic need and these four females were extreme outliers compared to the majority of the ad lib. females, which ate an average of 70.4 ± 9.5 crickets, these females were excluded from the analysis.

Vitellogenic females underwent similar treatments as prereproductive females, but vitellogenic females were randomly divided into only two dietary treatments—ad lib. and restricted—because the energetic demands of vitellogenesis could result in severe health concerns if females were fasted. Additionally, the duration of food restriction

before wounding was shortened from the 10 days used for prereproductive females to 4 days for vitellogenic females. This shortened duration was necessary to reduce the likelihood of ovulation during the study. Wound healing was still assessed 10 days after biopsy to remain consistent with the other studies. Despite the reduced study duration, 14 females ovulated before day 9 (halfway through wound healing). Because we were interested in examining healing during vitellogenic investment, we excluded these 14 females from the study.

We measured body mass and SVL on capture in both prereproductive and vitellogenic females. To quantify food consumption, the mass (g) of each cricket and the number of crickets given to each lizard were recorded every day. Crickets were unable to escape from the lizard cages and were supplied a food source (rat feed, which lizards did not consume) while there in order to maintain their body mass and nutritional value. Every 3 days, uneaten crickets were removed from the cages, and the mass (g) and number of these crickets were recorded and subtracted from the mass (g) and number initially added to the cage to calculate the amount consumed by each lizard. Lizard body mass was also measured every 3 days. At the end of the study, we measured wound size (area mm²), SVL, and follicle diameter, and we collected plasma samples for hormone analysis, as described in “Plasma Samples and Radioimmunoassay.”

Biopsy Procedure and Wound Measurements

Each lizard was anesthetized using surface-induced deep hypothermia, which is accomplished by packing the animal in crushed ice for 10 min, and anesthesia was maintained by performing the surgery with the animal resting on a bed of crushed ice. For this procedure and all others, post-surgery survivorship was 100%. Once anesthetized, the lizard received a cutaneous biopsy dorsal to the pelvis using a sterile 3.5-mm biopsy punch (Miltex Instrument, York, PA). The punch was lightly twisted to create a uniform circular cut through the skin. The resulting circle of skin was then removed using forceps, creating a controlled-size cutaneous wound.

The wounds in all animals were digitally photographed immediately after biopsy and again 10 days after biopsy, which is the middle of the healing profile, based on previous studies (French et al. 2006). In order to photograph the wounds, animals were secured to a plywood stage with Velcro straps. Since the first images were collected while the animals remained anesthetized for surgery and the second images were taken at the end of the study, restraint stress associated with imaging could not have influenced the results of the study. A metric ruler attached to the restraint straps provided a scale reference for size mea-

surements. Digital images were taken using a camera attached to a light microscope (Panasonic GP-US502 Industrial Digital Signal Processing Color 3-CCD). Images were then imported to an image analysis program (Image-Pro Plus, ver. 4.0, Media Cybernetics, Silver Springs, MD). At the end of the study, the digital photographs were randomized and then analyzed to assess wound size (i.e., area) using the image analysis software, such that the investigator was blind to the treatment of the animal.

Assessing Reproductive Stages and Measuring Follicles

Female reproductive stage was initially assessed by manually palpating the abdomen for the number and firmness of follicles/eggs. On the final day of each study, the reproductive stage and sizes of follicles, if present, were determined using abdominal surgery. In both studies, to measure follicle sizes, bilateral celiotomies were performed on the final day of the study using techniques described by Crews (1974) and Moore (1987). Briefly, the follicles of each anesthetized female were extruded through a small ventrolateral incision, the follicle sizes were measured using an ocular micrometer attached to the dissecting scope, and then the incision was sutured.

Plasma Samples and Radioimmunoassay

Blood samples were collected 10 days after biopsy (the final day of each study) in all animals, between 1300 and 1400 hours. Samples were collected before final wound imaging and follicle measurements, and it took no longer than 30 s to capture and bleed each animal. Blood samples were collected by rupturing the postorbital sinus with a capillary tube. Plasma was separated from the blood via centrifugation and stored at -20°C until assayed. All samples from the dietary manipulation study were analyzed within a single radioimmunoassay, as were all samples from the FSH manipulation study. Plasma samples were assayed using a previously described and established laboratory protocol (Moore 1986). In brief, samples were extracted using diethyl ether extractions. The ether phase was separated, dried, and resuspended in an ethyl acetate/isooctane mixture. Individual hormones were separated from samples by using columns packed with one layer of a celite/water mixture and three layers of a celite/glycol mixture. Different elutions of ethyl acetate/isooctane were added to the columns to separate different hormones (10% for progesterone, 20% for testosterone, 40% for estradiol, and 50% for corticosterone). The separate fractions were collected in vials, dried, and resuspended in phosphate buffer solution. Duplicates of these samples were then assayed for progesterone, testosterone, estradiol, and corticosterone. The intra-assay coefficients of variation for the

FSH manipulation study were 19.5% for progesterone, 3.3% for testosterone, 19.5% for estradiol, and 6.1% for corticosterone. The intra-assay coefficients of variation for the dietary manipulation study were 2.3% for testosterone, 10.3% for estradiol, and 17.6% for corticosterone.

Statistical Analyses

The significance level for all statistical tests was $\alpha = 0.05$ unless otherwise stated. For the energy intake manipulation study, prereproductive and vitellogenic replicate studies were analyzed separately for two main reasons. First, we performed the two studies at different times of the year, and second, the duration of the food restriction before biopsy in the two studies differed.

Wound healing data (percent wound healed over time) for all studies were arcsin transformed to perform all statistical analyses. For the reproduction manipulation study, a *t*-test was used to assess differences in wound healing and follicle size between FSH- and saline-injected females. For the intake manipulation study, we analyzed wound healing separately in prereproductive and vitellogenic females using a one-way ANOVA's examining effects of food intake treatments on percentage of the wound healed over time. We performed Tukey's HSD-corrected post hoc comparisons on wound healing data to assess differences.

We conducted separate *t*-tests (adjusted α) to compare circulating hormone levels in FSH- versus saline-injected females in the first study. For the second study, we conducted separate one-way ANOVAs to examine the effects of food intake treatment on circulating hormone concentrations in prereproductive and vitellogenic groups, 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 food intake on changes in body mass, SVL, and follicle diameter over the course of the study in prereproductive and vitellogenic stages. Last, to assess food intake over time in ad lib. females, we performed a repeated-measures ANOVA, which was Greenhouse-Geisser corrected for sphericity. All statistical analyses were performed using JMP.IN, version 5.1, analyses software (SAS Institute, Cary, NC).

Results

Experiment 1: Reproductive Investment Manipulation

Follicle Measurements and Wound Healing. As expected, FSH-treated females had significantly larger follicles than saline-injected controls ($t = 9.464$, $P < .0001$; fig. 1A), verifying that the FSH treatment was effective in increasing

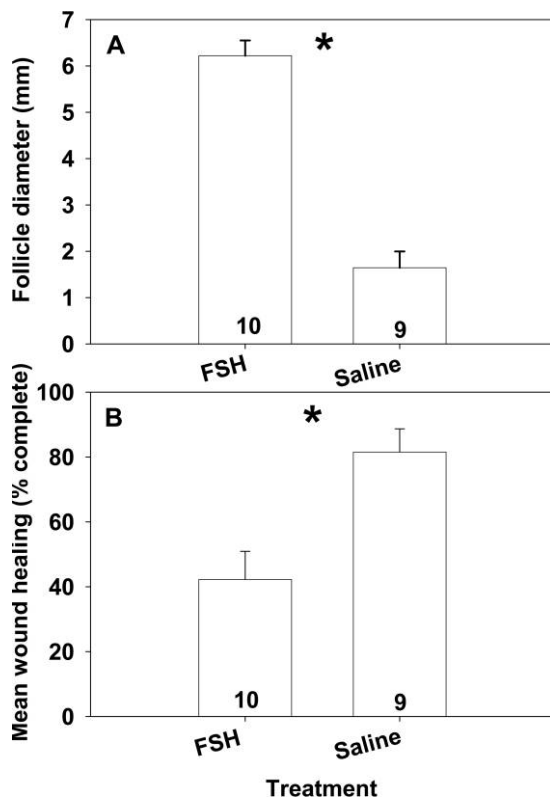


Figure 1: A, Average follicle diameter at day 10 postwounding in follicle-stimulating hormone (FSH)- and saline-treated females. B, Extent of wound healing at day 10 postwounding in FSH- and saline-treated females. Numbers at base of bars represent sample size, error bars represent standard error, and asterisks denote statistically significant differences at $P \leq .05$.

reproductive investment. The wounds of FSH-treated females were significantly less healed than those of saline-injected control females ($t = 3.470$, $P = .0033$; fig. 1B). When both treatments were combined, there was a significant inverse relationship between follicle diameter and healing ($F = 17.3122$, $df = 1, 17$, $P = .0007$). However, when separated by treatment, there was no significant relationship between wound healing and follicle diameter in either saline-injected control females ($F = 3.799$, $df = 1, 7$, $P = .0923$) or FSH-injected females ($F = 3.100$, $df = 1, 8$, $P = .3591$), suggesting that this relationship was mainly treatment driven.

Hormone Analyses. Circulating progesterone, estradiol, and corticosterone concentrations were all significantly greater in FSH relative to saline-injected animals (all $t > 3.164$, all $P < .0111$; table 1). However, there was no difference in circulating testosterone concentrations between treatments ($t = 0.694$, $P = .4972$; table 1).

Experiment 2: Energy Intake Manipulation

Prereproductive Females. Half of the females in the ad lib. treatment group entered vitellogenesis, whereas no females from other treatment groups entered vitellogenesis. There are many physiological differences between prereproductive and vitellogenic females, including resource demands and circulating hormone concentrations, and because of these differences, this treatment group had to be split so that prereproductive and vitellogenic females could be analyzed separately.

Body Mass and Intake. Feeding manipulation significantly affected change in body mass over the course of the experiment (one-way ANOVA; $F = 91.8743$, $df = 3, 25$, $P < .0001$; table 2), verifying that the diets did indeed affect organismal energy state. Both prereproductive and vitellogenic ad lib. females experienced a significantly greater change in mass relative to other groups, according to Tukey's HSD-corrected post hoc comparisons. Also, within the ad lib. treatment group, vitellogenic females gained significantly more mass than prereproductive females. The dissimilar changes in body mass between the ad lib. females is probably explained by differences in food intake, where reproductive stage significantly affected food intake over time in ad lib. animals (repeated-measures ANOVA; $F_{\text{stage}} = 11.1000$, $df = 1, 8$, $P = .0104$; fig. 2). Food intake also differed significantly over time in both groups ($F_{\text{time}} = 26.9532$, $df = 2.87, 22.97$, $P < .0001$, Greenhouse-Geisser corrected). Finally, there was not a significant time \times reproductive stage interaction ($F_{\text{stage} \times \text{time}} = 2.8446$, $df = 2.87, 22.97$, $P = .0620$, Greenhouse-Geisser corrected), so the effect of time on intake did not differ between the two reproductive stages; however, this may be the result of low power due to a small sample size.

Wound Healing and Follicle Measurements. There was no effect of feeding manipulation on healing ability in prereproductive females (one-way ANOVA; $F = 0.722$, $df = 2, 25$, $P = .548$; fig. 3A). However, there was a significant effect of feeding manipulation on follicle mass (one-way ANOVA; $F = 18.3071$, $df = 3, 25$, $P < .0001$). According to Tukey's HSD-corrected post hoc comparisons ($\alpha = 0.05$ level), ad lib. females that entered vitellogenesis had significantly greater follicle mass than other treatment groups. The same pattern holds true for follicle diameter, where dietary manipulation significantly affected follicle diameter (one-way ANOVA; $F = 40.0563$, $df = 3, 25$, $P < .0001$; fig. 4A) due to the progression of many ad lib.-fed females into vitellogenesis. Since follicle diameter and follicle mass were highly correlated ($P > .0001$), we employed only the less-invasive measure of follicle diameter in the vitellogenic and FSH experiments.

Table 1: Mean (\pm SE) circulating steroid hormone concentrations at day 10 postwounding in follicle-stimulating hormone (FSH)- and saline-treated females

| Treatment | Progesterone ^a (ng/mL) | Testosterone (ng/mL) | Estradiol ^a (ng/mL) | Corticosterone ^a (ng/mL) |
|------------|--------------------------------------|-------------------------|-----------------------------------|--|
| FSH (10) | 4.05 \pm .96 | 6.61 \pm 1.26 | 2.25 \pm .84 | 37.79 \pm 7.13 |
| Saline (9) | .47 \pm .13 | 5.77 \pm 1.34 | .13 \pm .03 | 5.90 \pm 1.14 |

^a Statistically significant differences at $P \leq .05$. See text for description of treatment groups.

Hormone Analyses. Estradiol (one-way ANOVA; $F = 4.127$, $df = 3, 25$, $P = .017$; table 2) and testosterone plasma concentrations ($F = 3.513$, $df = 3, 25$, $P = .030$; table 2) were both significantly greater in ad lib. females that entered vitellogenesis than in the other treatments. However, there was no difference in circulating corticosterone concentrations among females in different feeding manipulations ($F = 0.066$, $df = 3, 25$, $P = .977$; table 2).

Vitellogenic Females

Females that ovulated less than halfway through the course of the study were excluded from analyses (see "Methods"). Of the females included in the analyses, five out of nine ad lib. females ovulated during the second half of the study, while only one out of seven restricted females ovulated during this period. The six females that ovulated in the second half of the study were included in wound healing analyses but excluded from subsequent analyses. There are many physiological differences between vitellogenic and gravid females, such as differences in circulating hormone concentrations. Because of these differences, gravid female hormone data were not included in hormone analyses. Also, due to large differences in composition and structure, the eggs of gravid females cannot be compared to vitellogenic follicles.

Wound Healing and Follicle Measurements. Feeding manipulation significantly affected healing ability in vitellogenic females. Females on an ad lib. diet had significantly smaller wounds at day 10 than did females on a restricted diet (Welch ANOVA for unequal variances; $t = 4.225$, $P = .005$; fig. 3B). There was also a significant treatment effect on follicle diameter at the end of the study (Welch ANOVA for unequal variances; $t = 2.989$, $P = .022$; fig. 4B), where restricted females had significantly smaller follicles than ad lib. females. Last, females in the ad lib. group gained significantly more mass over the healing profile than did females in the restricted group ($t = 10.195$, $P < .001$; table 2).

Hormone Analyses. Circulating testosterone ($t = 12.124$, $P < .001$; table 2) and estradiol ($t = 2.617$, $P = .031$; table

2) concentrations were significantly greater in ad lib. females than restricted females. There were no significant differences in circulating corticosterone concentrations between the treatment groups (all $t = 2.053$, all $P = .074$; table 2).

Discussion

Reproductive Investment Manipulation: Is There Clear Evidence of a Trade-Off?

The FSH manipulation resulted in follicle development and wounds that were less healed (fig. 1), supporting the hypothesis that there is a physiological trade-off between reproductive and immune function. This is consistent with other manipulative studies that found experimentally increased reproductive effort results in both suppressed antibody and cell-mediated immune responses (Nordling et al. 1998; Verhulst et al. 2005). Similar reproductive manipulations revealed an associated increase in energy expenditure (Deerenberg et al. 1995), which may account for a subsequent decrease in immunity.

Plasma concentrations of estradiol and corticosterone were elevated in FSH-manipulated females (table 1) as expected during vitellogenesis and were within the natural ranges of vitellogenic tree lizards. These elevated levels of FSH directly could potentially mediate the observed suppression of wound healing. Corticosterone in particular is immunosuppressive under many circumstances (Dhabhar and McEwen 1999; Berger et al. 2005; Martin et al. 2005). Thus, from the results of this study alone, it is not possible to distinguish whether the resulting immunosuppression was driven by elevated circulating hormone concentrations or limited resources. This question is directly addressed in the following subsection.

Energy Intake Manipulation: Is the Trade-Off Obligatory or Facultative?

Overall, we found support for the facultative regulation hypothesis that investment into the reproductive and immune systems is under facultative control in response to energy resources. The obligate regulation hypothesis predicts that there should be no difference in wound healing

between animals in the same reproductive stage. Contrary to this prediction, we found a significant difference in healing rate between vitellogenic females on different food manipulations, such that under limited food conditions, healing was slowed (fig. 3B). Furthermore, we failed to detect a significant difference in wound healing between ad lib.-fed females that remained prereproductive and those that progressed to vitellogenesis (fig. 3A). This result is inconsistent with the obligate regulation hypothesis, which predicts a suppression of wound healing during vitellogenesis regardless of food resources. The results from this study are in agreement with our previous results in which a reproductive-immune systems trade-off was present in naturally reproductive wild females but not in females reared in a laboratory where food resources were plentiful (French and Moore, forthcoming), and support the contention that the previously context-based difference was energy based. Taken together, these results provide strong evidence to reject the obligate regulation hypothesis.

As expected for resource competition between the reproductive and immune systems, restricting food intake had a profound effect on wound healing rate only during costly reproductive stages (e.g., vitellogenesis; fig. 3). Conversely, given ample resources, reproductive animals were able to effectively mount an immune response that was comparable to that of their prereproductive counterparts. These results suggest that only under restricted resources do the high costs of reproduction limit resource availability to the immune system, supporting the facultative regulation hypothesis. If ample resources are available, females support the energy demands of both reproductive and immune function by increasing food intake (fig. 2).

The facultative regulation of this trade-off is also supported by the known resource costs associated with both reproduction and immune function and a large number of studies that demonstrate the importance of resource intake in mounting immune responses. For example, in pregnant ewes (*Ovis aries*), nutritional state and fat stores were significantly related to immunocompetence and re-

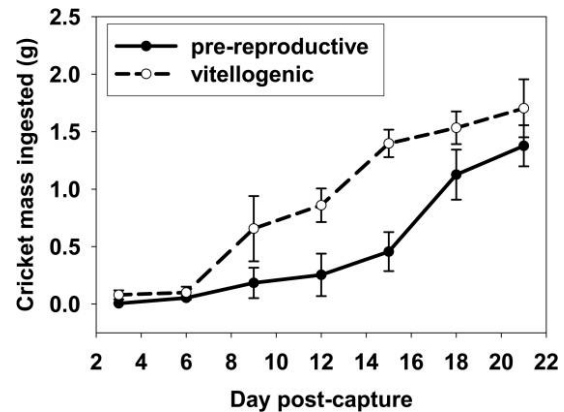


Figure 2: Mean food intake over time in females that were previtellogenic at time of capture but either remained previtellogenic (solid line) or progressed to vitellogenesis (dashed line) during the study. Reproductive stage had a significant effect on food intake. Error bars represent standard error.

sistance to intestinal parasite infection (Valderrabano et al. 2006). Moreover, differences in food intake in male Belding's ground squirrels (*Spermophilus beldingi*) lead to differences in circulating leukocyte numbers during reproduction (Bachman 2003). Additionally, limiting dietary protein intake in bobwhite quail (*Colinus virginianus*) and barn swallows (*Hirundo rustica*) negatively affects their cell-mediated immune response (Lochmiller et al. 1993; Saino et al. 1997). Similarly, several invertebrate studies have demonstrated that increasing food availability positively affects survival rate when an immune response is mounted (Moret and Schmid-Hempel 2000).

We found that vitellogenic females on an ad lib. diet had elevated circulating sex steroid hormone concentrations typical of the vitellogenic state (Lacy et al. 2002; Woodley and Moore 2002) and did not exhibit suppressed wound healing, rejecting the common idea that elevated sex steroids always suppress overall immune function (ta-

Table 2: Mean (\pm SE) circulating steroid hormone concentrations at day 10 postwounding in pre-reproductive and vitellogenic females maintained on varying diets

| | Testosterone ^a (ng/mL) | Estradiol ^a (ng/mL) | Corticosterone (ng/mL) | Change in mass ^a (g) |
|-------------------------|--------------------------------------|-----------------------------------|---------------------------|------------------------------------|
| Prereproductive: | | | | |
| Ad lib. prereproductive | .30 \pm .12 | .15 \pm .07 | 9.91 \pm 1.66 | 1.03 \pm .14 |
| Ad lib. vitellogenic | 20.15 \pm 15.03 | 1.69 \pm 1.01 | 11.64 \pm 3.88 | 1.85 \pm .16 |
| Restricted | .32 \pm .13 | .15 \pm .04 | 18.48 \pm 4.50 | -.29 \pm .06 |
| No food | .12 \pm .02 | .12 \pm .04 | 14.15 \pm 5.48 | -.30 \pm .07 |
| Vitellogenic: | | | | |
| Ad lib. | 39.54 \pm 11.45 | .69 \pm .37 | 21.29 \pm 1.04 | 1.04 \pm .24 |
| Restricted | .24 \pm .13 | .05 \pm .02 | 11.85 \pm 2.95 | -.49 \pm .09 |

^a Statistically significant differences at $P \leq .05$. See text for description of dietary treatment groups.

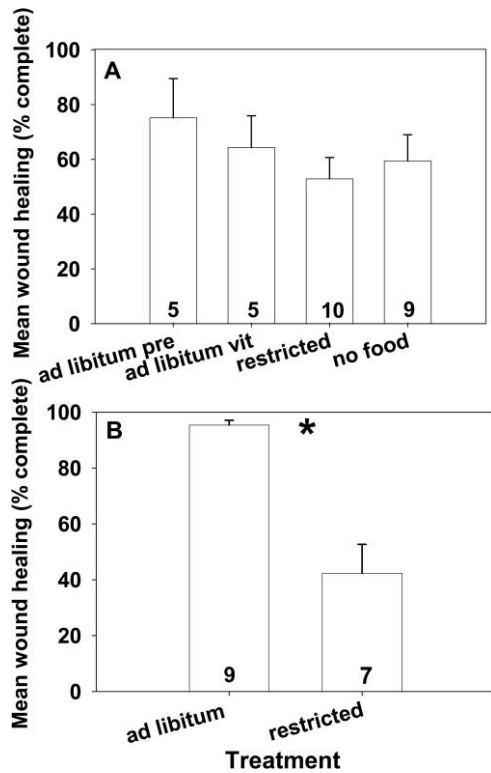


Figure 3: Extent of wound healing at day 10 postwounding in initially prereproductive (*pre*) females (A) and vitellogenic (*vit*) females (B) on varying diets. See text for a description of the treatment groups. Numbers at base of bars represent sample size, error bars represent standard error, and asterisks denote statistically significant differences at $P \leq .05$.

ble 2). Many studies have shown that high concentrations of circulating testosterone and estradiol regulate various aspects of immune function (e.g., cell-mediated and humoral responses; Casto et al. 2000; Duffy et al. 2000; Bilbo and Nelson 2001; Hughes and Randolph 2001; McMurray et al. 2001; Bilbo et al. 2003); however, our results suggest that this regulation is not necessarily absolute. This idea is further supported by a recent study in song sparrows (*Melospiza melodia*) that suggested that testosterone suppresses the immune system via indirect pathways, such as stress-mediated immunosuppression or resource allocation (Owen-Ashley et al. 2004). Furthermore, in our study, resource-limited vitellogenic females underwent immunosuppression yet had lower circulating sex steroid concentrations relative to their ad lib. counterparts, demonstrating that sex steroid concentrations are not the sole, or perhaps even primary, regulator of immune suppression (table 2). Changes in circulating hormone concentrations and differences in follicle size within vitellogenic females suggest that reproduction is either slowed or halted in food-restricted females. The females undergoing food re-

striction had relatively smaller follicles, with many follicles undergoing regression and reabsorption (fig. 4B). In addition, hormones that are normally elevated during vitellogenesis, estradiol and testosterone, were suppressed in food-restricted females, providing further evidence that reproduction, in addition to wound healing, is suppressed during resource-limiting conditions (table 2).

Last, within prereproductive females, half of the ad lib. treatment group entered vitellogenesis, while females from no other (restricted) treatment group entered vitellogenesis. These vitellogenic females had significantly larger follicles and elevated circulating sex steroid hormone concentrations at the end of the study than did the prereproductive females (fig. 4A; table 2), but they also ate significantly more food than the prereproductive females in their treatment group (fig. 2). Increased energy intake in vitellogenic relative to prereproductive females and the absence of healing rate differences between these females is consistent with the hypothesis that unlimited resources allow simultaneous vitellogenesis and rapid wound healing. Similarly, other studies have reported

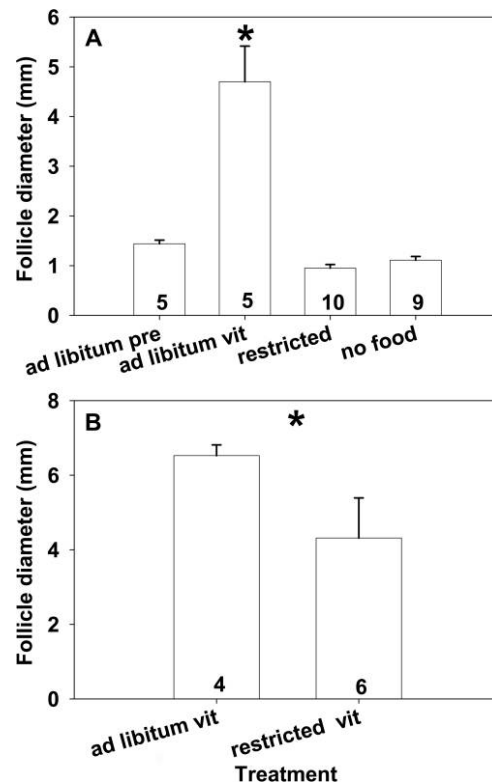


Figure 4: Average follicle diameter at day 10 postwounding in initially prereproductive (*pre*) females (A) and vitellogenic (*vit*) females (B) on varying diets. See text for a description of treatment groups. Numbers at base of bars represent sample size, error bars represent standard error, and asterisks denote statistically significant differences at $P \leq .05$.

increased energy intake in vitellogenic females relative to other reproductive stages, presumably to sustain the added costs of vitellogenesis (Lourdais et al. 2003; Gignac and Gregory 2005).

Conclusions

The results of these studies link the importance of resource availability to both reproduction and immune function and provide evidence for resource competition between the two processes. It is also clear from our second experiment that immunosuppression during reproduction is facultative and is not an obligatory response to physiological changes during reproduction. Both experiments emphasize the importance of energy and resources in the occurrence of trade-offs between the reproductive and immune systems. This energy conflict manifests only during resource-intensive reproductive times (i.e., vitellogenesis), during which limited resources have a profound effect on both immunity and reproduction. Furthermore, increased reproductive investment under limited resources elicited results consistent with the second study; namely, high reproductive costs combined with limited resources resulted in the suppression of cutaneous wound healing.

Based on the results of these two experiments, differences in species resource requirements, environmental resource availability, feeding regimes, and individual energy balance all have the capacity to influence trade-offs and produce disparate results, depending on experimental conditions, thereby providing a plausible explanation for the discrepancies of previous studies as to the occurrence of trade-offs between the reproductive and immune systems. For example, Williams et al. (1999) found no effect of antibody production on clutch number or size in European starlings (*Sturnus vulgaris*), whereas studies in collared flycatchers (*Ficedula albicollis*) found repeated evidence for reproduction-induced immunosuppression (Gustafsson et al. 1994, 1995; Nordling et al. 1998). While these differences may be phylogenetically based, it is equally plausible that the differences are a result of differing resource availability.

In our second experiment, reproductive females on a restricted diet had significantly reduced follicle sizes relative to their ad lib. conspecifics. These data suggest that reproductive investment is either slowed or halted entirely in restricted animals. Preliminarily, these results suggest that responding to an immune challenge while subject to limited resources may have the capacity to influence reproductive investment (i.e., the negative effects of energy trade-offs between the reproductive and immune systems may go in both directions). Previously, it was assumed that the consequences of slowed healing were limited to the increased likelihood of infection or parasites. However,

these results suggest another potentially significant cost of aggression or risky behavior—reduced reproductive fitness. Specifically, wounds resulting from an aggressive encounter or predation attempt may result in serious reproductive fitness consequences for the animal if resources are limited. This seems surprising for a short-lived animal such as the tree lizard. However, this type of facultative regulation allows animals to adjust their energetic investment into physiological processes to current environmental conditions. This dynamic response is especially advantageous for species such as the tree lizards that inhabit environments where resources are often scarce and unpredictable. Under these harsh conditions, animals can reduce investment or even shut down specific processes in order to survive until resources become available. These possibilities need to be further examined in future studies, as does the proximate control over the facultative regulation of trade-offs between the reproductive and immune systems.

Acknowledgments

Thanks to M. Feldner, C. Forester, T. Small, and E. Taylor for help in the field. Thanks to J. Brunner and G. Johnston for help with all parts of this study, from collecting lizards to radioimmunoassays. Thanks to J. Collins, K. Matt, and G. Walsberg for critical comments and feedback on the experiments and manuscript. Thanks to P.R.G. at Arizona State University for intellectual feedback on project ideas. Last, thanks to two anonymous reviewers for greatly improving the organization and overall quality of this manuscript.

Literature Cited

- Adamo, S. A., M. Jensen, and M. Younger. 2001. Changes in lifetime immunocompetence in male and female *Gryllus texensis* (formerly *G.-integer*): trade-offs between immunity and reproduction. *Animal Behaviour* 62:417–425.
- Angilletta, M. J., and M. W. Sears. 2000. The metabolic cost of reproduction in an oviparous lizard. *Functional Ecology* 14:39–45.
- Bachman, G. C. 2003. Food supplements modulate changes in leukocyte numbers in breeding male ground squirrels. *Journal of Experimental Biology* 206:2373–2380.
- Berger, S., L. B. Martin, M. Wikelski, L. M. Romero, E. K. V. Kalko, M. N. Vitousek, and T. Rodl. 2005. Corticosterone suppresses immune activity in territorial Galapagos marine iguanas during reproduction. *Hormones and Behavior* 47:419–429.
- Bilbo, S. D., and R. J. Nelson. 2001. Sex steroid hormones enhance immune function in male and female Siberian hamsters. *American Journal of Physiology* 280:R207–R213.
- Bilbo, S. D., F. S. Dhabhar, K. Viswanathan, A. Saul, and R. J. Nelson. 2003. Photoperiod affects the expression of sex and species differences in leukocyte number and leukocyte trafficking in congeneric hamsters. *Psychoneuroendocrinology* 28:1027–1043.
- Casto, J. M., I. M. Parker-Renga, E. D. Ketterson, and J. R. Nolan.

2000. Experimentally elevated testosterone in male dark-eyed juncos suppresses cell-mediated immune function of social mates and offspring. *American Zoologist* 40:967.
- Crews, D. 1974. Castration and androgen replacement on male facilitation of ovarian activity in lizard, *Anolis carolinensis*. *Journal of Comparative and Physiological Psychology* 87:963–969.
- Deerenberg, C., I. Pen, C. Dijkstra, B. J. Arkies, G. H. Visser, and S. Daan. 1995. Parental energy expenditure in relation to manipulated brood size in the European kestrel *Falco tinnunculus*. *Zoology* 99:39–48.
- Demas, G. E., V. Chefer, M. I. Talan, and R. J. Nelson. 1997. Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *American Journal of Physiology* 42:R1631–R1637.
- Detillion, C. E., T. K. S. Craft, E. R. Glasper, B. J. Prendergast, and A. C. DeVries. 2004. Social facilitation of wound healing. *Psychoneuroendocrinology* 29:1004–1011.
- Dhabhar, F. S., and B. S. McEwen. 1999. Enhancing versus suppressive effects of stress hormones on skin immune function. *Proceedings of the National Academy of Sciences of the USA* 96:1059–1064.
- Duffy, D. L., G. E. Bentley, D. L. Drazen, and G. F. Ball. 2000. Effects of testosterone on cell-mediated and humoral immunity in non-breeding adult European starlings. *Behavioral Ecology* 11: 654–662.
- French, S. S., and M. C. Moore. Forthcoming. Immune function varies with reproductive stage and context in female and male tree lizards, *Urosaurus ornatus*. *General and Comparative Endocrinology*.
- French, S. S., K. S. Matt, and M. C. Moore. 2006. The effects of stress on wound healing in male tree lizards (*Urosaurus ornatus*). *General and Comparative Endocrinology* 145:128–132.
- Gignac, A., and P. T. Gregory. 2005. The effects of body size, age, and food intake during pregnancy on reproductive traits of a viviparous snake, *Thamnophis ordinoides*. *Ecoscience* 12:236–243.
- Gustafsson, L., D. Nordling, M. S. Andersson, B. C. Sheldon, and A. Qvarnstrom. 1994. Infectious diseases, reproductive effort and the cost of reproduction in birds. *Philosophical Transactions of the Royal Society B: Biological Sciences* 346:323–331.
- Gustafsson, L., A. Qvarnstrom, and B. C. Sheldon. 1995. Trade-offs between life-history traits and a secondary sexual character in male collared flycatchers. *Nature* 375:311–313.
- Hughes, V. L., and S. E. Randolph. 2001. Testosterone increases the transmission potential of tick-borne parasites. *Parasitology* 123: 365–371.
- Lacy, E. L., M. A. Sheridan, and M. C. Moore. 2002. Sex differences in lipid metabolism during reproduction in free-living tree lizards (*Urosaurus ornatus*). *General and Comparative Endocrinology* 128: 180–192.
- Lochmiller, R. L., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* 88:87–98.
- Lochmiller, R. L., M. R. Vestey, and S. T. McMurry. 1993. Selected immune responses of adult cotton rats (*Sigmodon hispidus*) to dietary restriction. *Comparative Biochemistry and Physiology A* 104:593–599.
- Lourdais, O., X. Bonnet, R. Shine, and E. N. Taylor. 2003. When does a reproducing female viper (*Vipera aspis*) “decide” on her litter size? *Journal of Zoology (London)* 259:123–129.
- Martin, L. B., A. Scheuerlein, and M. Wikelski. 2003. Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proceedings of the Royal Society B: Biological Sciences* 270:153–158.
- Martin, L. B., J. Gilliam, P. Han, K. Lee, and M. Wikelski. 2005. Corticosterone suppresses cutaneous immune function in temperate but not tropical house sparrows, *Passer domesticus*. *General and Comparative Endocrinology* 140:126–135.
- Martin, P. 1997. Wound healing: aiming for perfect skin regeneration. *Science* 276:75–81.
- Marucha, P. T., J. K. Kiecolt-Glaser, and M. Favagehi. 1998. Mucosal wound healing is impaired by examination stress. *Psychosomatic Medicine* 60:362–365.
- McMurray, R. W., S. Suwannaroj, K. Ndebele, and J. K. Jenkins. 2001. Differential effects of sex steroids on T and B cells: modulation of cell cycle phase distribution, apoptosis and bcl-2 protein levels. *Pathobiology* 69:44–58.
- Moore, M. C. 1986. Elevated testosterone levels during nonbreeding-season territoriality in a fall-breeding lizard *Sceloporus jarrovi*. *Journal of Comparative Physiology A* 158:159–163.
- . 1987. Castration affects territorial and sexual behavior of free-living male lizards, *Sceloporus jarrovi*. *Animal Behavior* 35: 1193–1199.
- Moret, Y., and P. Schmid-Hempel. 2000. Survival for immunity: the price of immune system activation for bumblebee workers. *Science* 290:1166–1168.
- Nilsson, J. A., and L. Raberg. 2001. The resting metabolic cost of egg laying and nestling feeding in great tits. *Oecologia (Berlin)* 128: 187–192.
- Nordling, D., M. Andersson, S. Zohari, and L. Gustafsson. 1998. Reproductive effort reduces specific immune response and parasite resistance. *Proceedings of the Royal Society B: Biological Sciences* 265:1291–1298.
- Owen-Ashley, N. T., D. Hasselquist, and J. C. Wingfield. 2004. Androgens and the immunocompetence handicap hypothesis: unraveling direct and indirect pathways of immunosuppression in song sparrows. *American Naturalist* 164:490–505.
- Padgett, D. A., P. T. Marucha, and J. F. Sheridan. 1998. Restraint stress slows cutaneous wound healing in mice. *Brain Behavior and Immunity* 12:64–73.
- Peters, A. 2000. Testosterone treatment is immunosuppressive in superb fairy-wrens, yet free-living males with high testosterone are more immunocompetent. *Proceedings of the Royal Society B: Biological Sciences* 267:883–889.
- Saad, A. H., and R. Elridi. 1984. Corticosteroids and seasonal changes in reptilian immune responses. *Developmental and Comparative Immunology* 8:945.
- Saino, N., S. Calza, and A. P. Møller. 1997. Immunocompetence of nestling barn swallows in relation to brood size and parental effort. *Journal of Animal Ecology* 66:827–836.
- Sinervo, B., and D. F. DeNardo. 1996. Costs of reproduction in the wild: path analysis of natural selection and experimental tests of causation. *Evolution* 50:1299–1313.
- Sinervo, B., and P. Licht. 1991a. Hormonal and physiological control of clutch size, egg size, and egg shape in side-blotched lizards (*Uta stansburiana*): constraints on the evolution of lizard life histories. *Journal of Experimental Zoology* 257:252–264.
- . 1991b. Proximate constraints on the evolution of egg size, number, and total clutch mass in lizards. *Science* 252:1300–1302.
- Valderrabano, J., C. Gomez-Rincon, and J. Uriarte. 2006. Effect of nutritional status and fat reserves on the periparturient immune

- response to *Haemonchus contortus* infection in sheep. *Veterinary Parasitology* 141:122–131.
- Verhulst, S., B. Riedstra, and P. Wiersma. 2005. Brood size and immunity costs in zebra finches *Taeniopygia guttata*. *Journal of Avian Biology* 36:22–30.
- Williams, T. D., J. K. Christians, J. J. Aiken, and M. Evanson. 1999. Enhanced immune function does not depress reproductive output. *Proceedings of the Royal Society B: Biological Sciences* 266:753–757.
- Woodley, S. K., and M. C. Moore. 2002. Plasma corticosterone response to an acute stressor varies according to reproductive condition in female tree lizards (*Urosaurus ornatus*). *General and Comparative Endocrinology* 128:143–148.

Associate Editor: Elizabeth Adkins-Regan
Editor: Monica A. Geber