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Adrenal and white cell count responses to chronic stress in gestating and postpartum females of the viviparous skink *Egernia whitii* (Scincidae)

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Abstract

This study investigates the relationships between plasma corticosterone concentrations and white cell counts in captive females of the viviparous lizard *Egernia whitii* during two phases of the reproductive cycle. Gestating and postpartum females were captured in the field and held in the laboratory for 4 weeks. Plasma corticosterone and progesterone concentrations and white blood cell counts were examined in blood samples taken at capture and after 24 h, 1 week, and 4 weeks in captivity. At 24 h after capture, plasma corticosterone concentrations in both groups had increased significantly compared with initial values but then returned to initial concentrations after 1 week in captivity and remained low in the 4 week samples. Plasma progesterone concentrations remained elevated in the gestating females until the week 4 sample, just prior to parturition. The hormone data suggest that capture and captivity did not represent a significant long-term stressor to these animals. The increase in plasma corticosterone concentration was associated with heterophilia in the differential leucocyte count in both groups of females. Lymphocyte numbers decreased only in gestating females, suggesting that reproductive status may influence the interaction between adrenal activity and immune function.

Keywords: Adrenal; Captivity; Corticosterone; Immune system; Leucocytes; Lizard; Progesterone; Stress; Viviparous

1. Introduction

The effects of prolonged stress on immune function were first identified by Selye (1937) in his description of what he termed the General Adaptation Syndrome. It is now known that adrenal corticosteroids have profound effects on the immune system, to the extent that the immunosuppressive effects of corticosteroids are exploited clinically, while deviations in haematological parameters have been used as indicators of physiological stress (Dunlap, 1995).

Although studies of the effects of adrenal hormones on the reptilian immune system remain limited, reptiles exhibit seasonal patterns of variation in circulating white cell counts (e.g. Wojtaszek, 1992) and other measures of immune function that may reflect seasonal changes in circulating corticosteroids (Saad and El Ridi, 1988; Zapata et al., 1992). For example, in the lizard *Chalcides ocellatus*, increases in plasma corticosterone concentrations during the winter months were associated with lysis of splenic T and B lymphocytes, while injecting "summer" lizards with corticosterone resulted in similar reductions in lymphocyte numbers (Saad and El Ridi, 1988). Conversely, improved lymphocyte function during the winter months in the turtle *Mauremys caspica* was coincident with lower plasma corticosterone concentrations (Munoz et al., 2000).

In mammals there is considerable evidence that sex steroids also have profound influences on the immune system (Grossman, 1984). Broadly, it appears that progestagens and androgens reduce immune responses while oestrogens enhance them (Schuurs and Verheul, 1990). Indeed, it has been suggested that sexually dimorphic responses to stressors, mediated by sex steroids, may have

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evolved to maintain reproductive competence under stressful conditions (Handa et al., 1994). Thus it may be predicted that in viviparous species adrenal and immune responses to chronic stress would be attenuated in gestating, as compared with non-gestating, females to ensure that the significant investment in gestation is not threatened (Wingfield et al., 1998). Rooney and Guillette (2001), reviewing the evidence for stress-related immune depression in reptiles, pointed out the lack of direct studies of changes in immune parameters during a stress response. Indeed, to our knowledge, no-one has yet investigated the inter-relationships between adrenal and immune responses to a chronic stressor and their variations with reproductive state in females of a viviparous reptile. In this study we therefore compare the chronic stress responses of gestating and early postpartum females of the viviparous lizard *Egernia whitii*, and assess differences in white cell counts between these reproductive states in animals held in captivity for 4 weeks.

Egernia whitii is a medium sized viviparous skink distributed throughout eastern and northern Tasmania and most of the cool temperate south-eastern Australian mainland. Mating occurs in spring with a gestation period of about 12 weeks, resulting in the birth of one to five young (Milton, 1987). The relatively large body size (females 10–15 g) permits repeated blood sampling in volumes large enough to enable multiple hormone assays. Previous work (Jones and Bell, 2004) has demonstrated that males of *E. whitii* exhibit increased concentrations of plasma corticosterone in response to the chronic stress of captivity. In males brought into captivity, plasma corticosterone was elevated at 24 h and 1 week, but had decreased significantly in samples taken after 4 weeks in the laboratory. However changes in white cell parameters were not considered in that study, and the stress responses of female *E. whitii* have not yet been investigated. We hypothesise that adrenal responses to capture and captivity will be reflected in changes in plasma corticosterone concentrations and in the numbers and proportions of leucocytes in the blood. We further hypothesise that in females of this viviparous species such responses will differ between reproductive states.

2. Materials and methods

2.1. Animal collection and captivity

Nine female *Egernia whitii* were caught in January, 2000, late in gestation, and fourteen postpartum females were collected in February 2000, at Orford, Tasmania, Australia (42.32°S, 147.5°E). These groups are designated gestating and postpartum respectively throughout the text. Females caught in January were palpated to check their reproductive status: that they were gestating was confirmed when all females later gave birth in captivity. All females were bled immediately upon their capture in the field (at capture). Blood samples (approx. 100 μL) were taken from the suborbital blood sinus using a heparinized capillary tube: blood sampling was completed within 2–5 min of capture. The blood samples were placed on ice following collection; plasma was separated by centrifugation and stored at −20 °C until assayed for corticosterone and progesterone.

The animals were transported to the laboratory in cloth bags. In the laboratory, lizards were housed individually in plastic containers (30 × 20 × 10 cm). Each housing contained a basking site (a terracotta pot base) located under a 25 W light globe on a 13 h ON: 11 h OFF cycle. Lizards were provided with a retreat, and the cage substrate consisted of paper pellets to a depth of approximately 2 cm through which the lizards could burrow. Lizards were fed three times per week on a diet consisting predominantly of cat food supplemented by tenebrionid larvae (mealworms) and water was available ad lib. The room air temperature was maintained at 20 °C and fluorescent overhead lighting was set to 13 L: 11 D, mimicking the natural photoperiod.

Further blood samples were collected from each lizard at 24 h, 1 week, and 4 weeks following capture. Each blood sample was taken at the same time of day as that lizard was captured, in order to minimise any effect of diurnal hormone cycles. At the conclusion of the experiment, the animals were returned to their sites of capture. All procedures were approved by the University of Tasmania Animal Ethics Committee (Approval no. 99064).

2.2. Corticosterone assay

The assay procedure and its validation for *Egernia whitii* plasma are described in detail by Jones and Bell (2004). Corticosterone was extracted from plasma aliquots (25–50 μL) with 500 μL A.R. grade absolute ethanol: extraction efficiency was 100%. Duplicate aliquots of 200 μL ethanol extract were assayed by radioimmunoassay, using Endocrine Sciences anti-corticosterone antiserum (Cat. No. B3-1 63). The sensitivity of the assay is 0.5 ng/mL plasma, and the intra-assay and inter-assay coefficients of variation were 11.2% and 3.4%, respectively. All assays were done in duplicate.

2.3. Progesterone assay

Progesterone was assayed by radioimmunoassay as in Jones and Swain (1996). Briefly, progesterone is extracted from plasma samples into isooctane by column chromatography. Recovery of progesterone from plasma was 93%, and duplicate sub-samples of extract were assayed by radioimmunoassay. The progesterone assay has been previously validated for skink plasma (Jones and Swain, 1996; Edwards and Jones, 2001). The sensitivity of the assay is 0.5 ng/mL, and the inter- and intra-assay coefficients of variation were 12.1% and 8.4%, respectively.

2.4. White blood cell counts

Blood smears were made each time blood was collected. A small volume of blood was collected into an unheparinized capillary tube to avoid artefacts in stain and cell morphology caused by heparin (Hume, 1995). Blood smears were fixed and stained in the modified Wright's stain, Diff Quick™. The slide was scanned at 400× magnification until a large area of clearly stained cells in monolayer was found. The number of white blood cells in 10 (400×) consecutive fields of view was counted and a mean calculated. This mean, when multiplied by two, provides an estimate of the number of white blood cells $\times 10^9$/L blood, assuming that the ratio of white blood cells to red blood cells remains constant (Fudge, 1997). White blood cells types were identified under oil immersion at 1000× magnification. The number of each type of white blood cell encountered within the first 100 cells counted is referred to as the Differential Leucocyte Count (DLC) and is expressed as a percentage. All counts were carried out by a single operator (V. C.).

Table 1 summarises the characteristics employed in the identification of each white blood cell type in *Egernia whitii*. Cells were identified on the basis of descriptions in Campbell (1996) and the experience of B. G., a veterinarian familiar with reptilian blood cells. Small lymphocytes were distinguished from thrombocytes by the presence of a distinct crescent of cytoplasm and their smoothly rounded shape.

2.5. Statistical analyses

All data are expressed as the mean ± S.E. For plasma corticosterone and progesterone concentrations and white cell numbers, data were transformed if examination of standard deviations plotted against means indicated that the data were heteroscedastic and Cochran's test was then applied to check transformed data was homogeneous. Corticosterone concentrations were log transformed to stabilise variances while progesterone concentrations were stabilised using a square-root transformation. Of the white cell types, only small lymphocyte numbers required log transformation. Comparisons between reproductive states over time were conducted using one-way ANOVA.

For plasma corticosterone and progesterone concentrations, and each white cell type, a univariate repeated measures analysis was conducted to test for effects of reproductive stage and the length of time held captive. The model was as follows:

Between subjects
  Reproductive stage (gestating or postpartum)
  Lizard nested within reproductive stage
Within subjects
  Time in captivity
  Reproductive stage × Time in captivity
Residual
  Time in captivity × Lizard nested within reproductive stage

Repeated measures analysis of variance (ANOVA) was employed to determine whether the length of time that females of each reproductive status had been held captive was associated with significant variation in total white cell (TWC) counts. Standard deviations of mean total white blood cell counts displayed a significant linear relationship with means; however, application of a logarithmic transformation stabilised variances.

Within each reproductive state, post-hoc Student–Newman–Keuls tests were employed to contrast hormone concentrations and white cell numbers over time. Basophils and monocytes were seen too infrequently for count data to be analysed statistically.

For each of the parameters, (progesterone, corticosterone, and white cell counts) only females for which a complete data set (at capture, 24 h, 1 week, and 4 weeks samples) was available were included in the analysis. At 4 weeks, two of the gestating females had given birth: their plasma corticosterone concentrations, progesterone and white cell counts at 4 weeks were therefore not included in data analyses. Therefore, 8 gestating females and 14 postpartum females were included in the corticosterone and progesterone analysis. Further, the staining of some blood smears did not permit accurate counting and so data from a total of 6 gestating females and 11 postpartum females were used for white cell count analyses.

Table 1
White blood cell types identified in blood smears of *Egernia whitii* and their distinctive features as stained with Diff Quick™

	Size (μ)	Granules	Cytoplasm	Nucleus
Granulocytic leucocytes				
Heterophils	20	Red, coalescent	Clear	Lobulate
Eosinophils	20	Light red, dispersed throughout cell	Blue tinge	Lobulate
Basophils	15	Dark blue	Blue	Non-lobulate
Mononuclear leucocytes				
Small lymphocytes	7	Absent	Clear	Condensed, fills majority of cell
Large lymphocytes	10	Absent	Clear	Condensed, fills majority of cell
Monocytes	15	Absent	Red tinge	Large, ovoid, and indented

3. Results

3.1. Hormone concentrations

Plasma progesterone concentrations differed significantly between gestating and postpartum females (Fig. 1) (interaction between reproductive state and time in captivity: $F_{3,57}=6.4292$, $P<0.001$). Plasma progesterone concentrations were significantly higher in gestating than in postpartum females at capture ($F_{1,20}=32.92$, $P<0.001$), 24 h ($F_{1,20}=39.8$, $P<0.001$), and 1 week ($F_{1,20}=51.3$, $P<0.001$), but were not significantly different at 4 weeks. Plasma progesterone concentrations remained very low in postpartum females with post-hoc contrasts indicating that plasma progesterone concentrations of captive postpartum females did not vary significantly from initial values (Fig. 1).

Although mean concentrations of plasma corticosterone in gestating females appeared lower than postpartum females at capture, 10.6±3.27 and 18.2±3.22 ng/mL, respectively, this difference was not significant ($P=0.156$). However, there was a large degree of variability in the time zero samples, with gestating females having plasma corticosterone concentrations ranging from 1.6 to 19.9 ng/mL and postpartum females from 6.5 to 41.5 ng/mL.

Mean plasma corticosterone concentrations differed significantly between sampling times for both gestating and postpartum females ($F_{3,57}=17.3658$, $P<0.0001$). As illustrated in Fig. 2, the pattern of corticosterone over time was the same for both gestating and postpartum females ($F_{1,57}=1.5886$, $P=0.2228$). Post-hoc contrasts revealed that, for both gestating and postpartum females, plasma corticosterone concentrations were significantly elevated at 24 h post-capture but had returned to initial values by 1 week, and were not significantly elevated at 4 weeks.

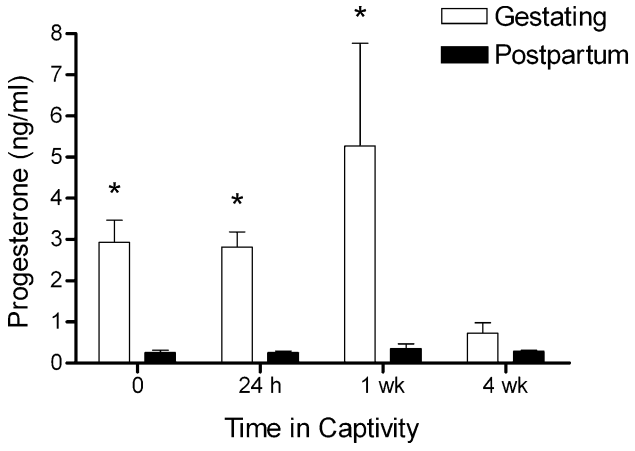

Fig. 1. Mean plasma progesterone concentrations in gestating ($n=8$) and postpartum female ($n=14$) *Egernia whitii*. *Times where gestating females have significantly higher mean plasma progesterone concentrations than postpartum females held captive for the same period. Vertical bars represent one standard error of the mean.

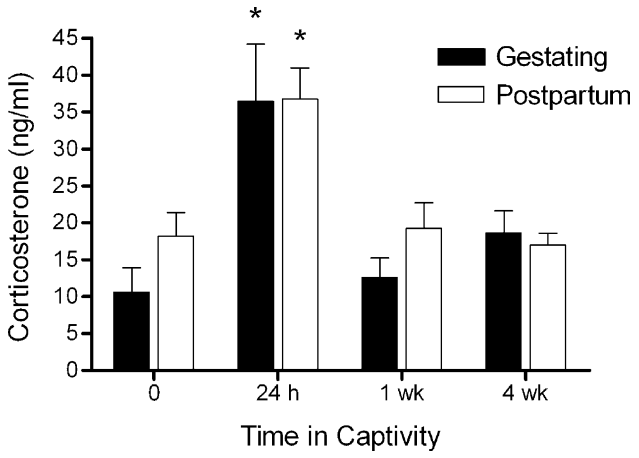

Fig. 2. Corticosterone concentrations throughout the 4 weeks of captivity in gestating ($n=8$) and postpartum ($n=14$) female *Egernia whitii*. *Value is significantly different from at capture. Vertical bars indicate the standard error of the mean.

3.2. Estimated total white blood cell counts

In animals of both reproductive states total white blood cell numbers did not change significantly throughout the 4 week period ($F_{1,15}=0.906$, $P=0.356$) (Fig. 3). As total numbers of white cells did not change over the period of captivity, differential leucocyte values are taken to represent changes in leucocyte numbers for each cell type.

3.3. Differential white cell counts

3.3.1. Lymphocytes

Gestating females had a significantly greater number of large lymphocytes ($F_{1,14}=17.643$, $P<0.01$) and small lymphocytes ($F_{1,14}=14.140$, $P<0.01$) than postpartum females at their time of capture.

There was a significant effect of time in captivity on large lymphocyte numbers ($F_{1,45}=19.6276$, $P<0.001$): post-hoc

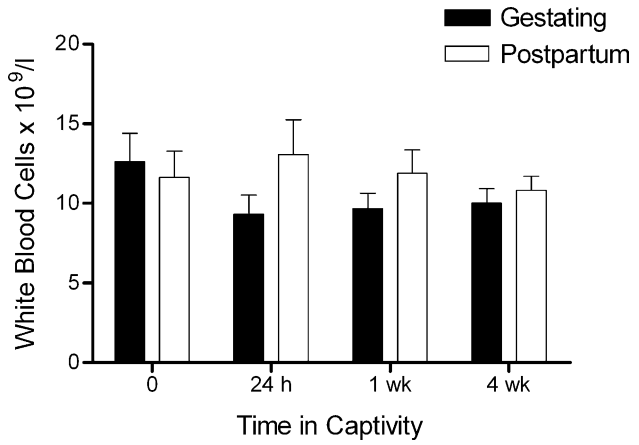

Fig. 3. Mean estimated number of white blood cells $\times 10^9$/l of blood as calculated from total white cell counts for gestating ($n=6$) and postpartum ($n=11$) *Egernia whitii* kept in captivity for 4 weeks. Vertical bars represent one standard error of the mean.

tests indicated that, in gestating females only, numbers of large lymphocytes were significantly lower at both 24 h and 4 weeks than at capture and at 1 week (Fig. 4). Similarly, only the gestating females showed any marked change in small lymphocytes with time held captive, with numbers significantly reduced at 24 h.

3.3.2. Granulocytic leucocytes

Gestating females had significantly fewer heterophils ($F_{1,15}=8.862$, $P<0.01$) and eosinophils ($F_{1,15}=11.969$, $P<0.01$) than postpartum females at the time of capture, while the percentage of heterophils in the differential leucocyte count increased significantly over the captive period for both gestating and postpartum females ($F_{3,45}=13.1042$, $P<0.001$) (Fig. 5). In gestating females, the percentage of heterophils was significantly lower at capture than at 24 h and 1 week; however, the percentage of heterophils was no longer elevated after 4 weeks. For postpartum females, the percentage of heterophils was significantly elevated at 24 h, 1 week, and 4 weeks in captivity.

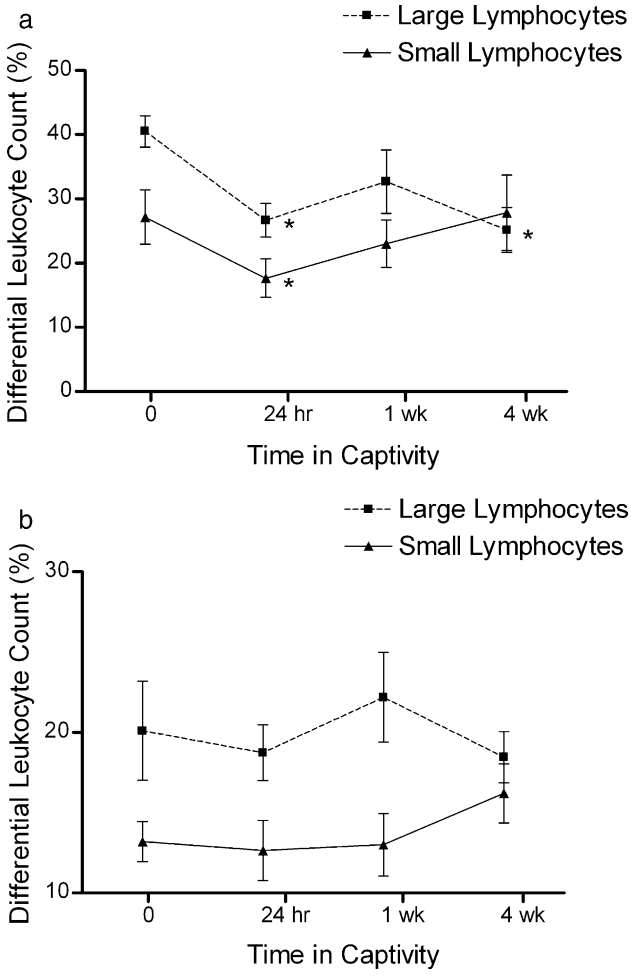

Fig. 4. Large lymphocyte and small lymphocyte differential leucocyte count profiles of (a) gestating ($n=6$) and (b) postpartum ($n=11$) female *Egernia whitii* in captivity for a period of 4 weeks. Vertical bars represent one standard error of the mean. *Value is significantly different from at capture ($P<0.05$).

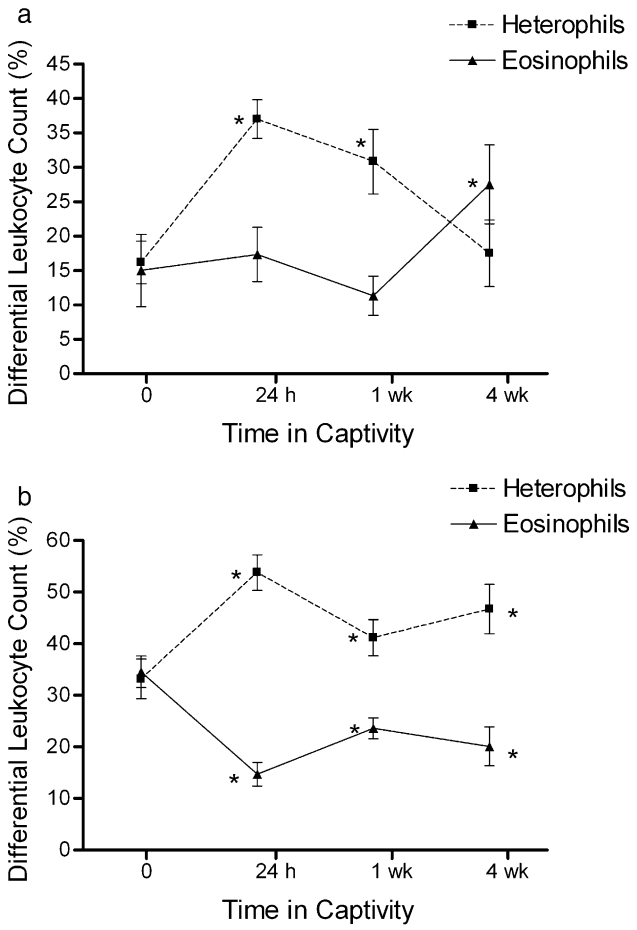

Fig. 5. Eosinophil and heterophil differential leucocyte count profiles of (a) gestating ($n=6$) and (b) postpartum ($n=11$) female *Egernia whitii* in captivity for a period of 4 weeks. Vertical bars represent one standard error of the mean. *Value is significantly different from at capture ($P<0.05$).

The repeated measures analysis revealed a significant interaction between time confined and reproductive status affecting eosinophil numbers ($F_{3,45}=8.1878$, $P<0.001$). Eosinophil numbers had declined significantly by 24 h in postpartum females and remained significantly lower than initial values at both 24 h and 1 week (Fig. 5). In gestating females, eosinophil numbers did not change significantly until 4 weeks in captivity, at which time they were significantly higher than initial values.

Comparison of the profiles of each granulocyte type in the white blood cell differentials of gestating and postpartum females demonstrates that there was an inverse relationship between the proportion of heterophils and eosinophils (Fig. 5).

4. Discussion

Gestating and postpartum females exhibited a similar adrenal response to chronic captivity with plasma cortico-

sterone concentrations significantly elevated at 24 h but returning to initial concentrations by 1 week, and again not differing significantly from baseline concentrations at 4 weeks. Previously, Jones and Bell (2004) investigated the effect of a 4 week captivity on plasma corticosterone concentrations in male *Egernia whitii* during the period of their reproductive quiescence. Plasma corticosterone concentrations did not rise significantly from capture to 24 h in captivity, although the authors note that there was a high level of variability in the sample taken at capture. In these males, plasma corticosterone concentrations remained elevated at 1 week, but had fallen significantly by 4 weeks, indicating a somewhat slower recovery in corticosterone concentrations than we observed in females of this species. This may reflect different responses between the sexes to isolation in captivity in this social species (Chapple, 2003).

Plasma progesterone concentrations were significantly higher in gestating *Egernia whitii* than postpartum females except at week 4 at which time females were very close to parturition. This confirms that endocrinological status differed between these groups of females, at least during the earlier stages of captivity: a fall in plasma progesterone concentrations some time before parturition has also been observed in other viviparous skinks, including *Niveoscincus metallicus* (Jones and Swain, 1996) and *Niveoscincus ocellatus* (Jones et al., 1997). The progesterone concentrations reported here are similar to those recorded previously in field-caught gestating *E. whitii* (Bell, 1997). Similarly, in females of the viviparous snake *Thamnophis sirtalis parietalis*, progesterone and oestrogen cycles over the weeks following hibernation did not differ between females subjected to confinement in the laboratory and those sampled in the field immediately upon capture (Whittier et al., 1987), while Weiss et al. (2002) found no effect of long-term captivity upon plasma progesterone, and other sex steroid concentrations in the oviparous *Sceloporus virgatus*, although they suggested that endocrine responses to captivity may vary with reproductive state.

However, contrary to our initial hypothesis, there was no difference in the pattern of plasma corticosterone concentrations between our gestating and postpartum females, and both groups of females exhibited higher plasma corticosterone concentrations than observed in males (Jones and Bell, 2004), especially at 24 h after capture (ca. 36 ng/mL in females compared with ca. 14 ng/mL in males). Similarly, pregnant females of the viviparous gecko *Hoplodactylus maculatus* exhibited no suppression of the stress response to captivity relative to vitellogenic females, with both groups having greater responses than males of that species (Cree et al., 2003). These results suggest that, as discussed extensively by Cree et al. (2003), there has been no selection for a reduced corticosterone response to stressors such as captivity during gestation in viviparous reptiles, implying that the stress of chronic confinement does not pose a threat to reproductive investment in these animals. We are currently exploring this hypothesis further, via experimental manipulations of the hypothalamo–pituitary–adrenal axis and examination of acute stress responses in female *Egernia whitii* (Cartledge and Jones, unpublished results). Interestingly, in contrast to these results for viviparous species, gravid female alligators do show a reduced response to (acute) capture stress compared with non-gravid females (Rooney and Guillette, 2001), and breeding green turtles also exhibit reduced adrenocortical function in response to environmental stressors (Jessop et al., 2000).

However this study focussed on potential inter-relationships between the stress response and the immune system. Studies of interrelationships between the stress response and the immune system of reptiles are very limited, and usually indirect (Rooney and Guillette, 2001). In *Egernia whitii*, blood smears indicated that the estimated total number of circulating white blood cells did not vary significantly throughout confinement in either gestating or postpartum females. This, combined with the continued good health of the animals, suggests that there was no inflammatory component to the differential cell counts, and that the changes were not a result of disease. The lack of change in total estimated white cell count contrasts with results from the crocodile *Crocodylus porosus* in which total white cell counts were significantly depressed 1 week after the onset of low temperature stress (Turton et al., 1997). Similarly, total white cell numbers had decreased in green turtles *Chelonia mydas* at 24 h after capture (Aguirre et al., 1995). The stability of total white cell numbers in this study is explained by the differential white cell counts: in gestating females, the decreases in lymphocyte numbers were countered by increases in heterophil numbers, while in postpartum females lymphocyte numbers were stable and the two granulocytes, heterophils and eosinophils, displayed an inverse relationship.

Lymphocyte counts were consistently higher in gestating females than postpartum females (ca. two-fold at their time of capture). This difference is unlikely to be seasonal as late gestation and postpartum females were caught within 6 weeks of each other. This result was unexpected because thymic involution, with a resultant reduction in lymphocyte numbers, is common during mammalian pregnancy (e.g., Millar et al., 1973; McLean et al., 1974) with this reduction thought to lessen or prevent immune rejection of the foetus, encouraging the pregnancy to term: as Lochmiller and Deerenberg (2000) point out, up-regulation of the maternal immune system is likely to have suppressive effects on foetal growth and development. However, in mammals higher plasma corticosteroids are consistently found during pregnancy (Keller-Wood and Wood, 2001). Again, this result differs from those for *Egernia whitii*, in which basal plasma corticosterone concentrations did not differ between gestating and postpartum females, which may explain why we did not see a decrease in lymphocyte count in late gestation. Indeed, there is very little information available on patterns of lymphocyte numbers during gestation in

reptiles. Impaired mitogenic function was noted in gestating females of the lizard *Chalcides ocellatus* particularly in late-stage pregnancy (Saad and El Deeb, 1990). While the number of circulating lymphocytes was not recorded in that study, thymic involution during gestation has been demonstrated in *C. ocellatus* (Saad, 1989). In contrast, gravid snakes of the oviparous species *Natrix natrix* were found to have lymphocyte numbers as high as those in males at the same time of year (Wojtaszek, 1992).

As well as lymphocyte numbers differing between reproductive states, the pattern of change in those numbers during the experimental period was different in gestating and postpartum females. In gestating females, both small and large lymphocyte numbers were significantly depressed at 24 h but had recovered by 1 week, only to be significantly lower again at 4 weeks. In contrast, lymphocyte numbers did not vary significantly over the experimental period in postpartum females. In mammals, stressful conditions and increased plasma corticosteroid concentrations have been repeatedly associated with a reduction in the number of circulating lymphocytes (e.g., Dhabar et al., 1995; Dhabar and McEwen, 1997; Baker et al., 1998). The small number of studies conducted in reptiles have produced similar results. For example, subcutaneous corticosterone implantation in juvenile *Alligator mississippiensis* resulted in significant reductions in the number of lymphocytes in the blood at 1 month after implantation (Morici et al., 1997). The lack of response in the postpartum females in our study warrants further investigation.

In both gestating and postpartum females, however, increases in plasma corticosterone concentration were coincident with increases in heterophil numbers and with either a decrease in eosinophil numbers (postpartum females), or no change in eosinophil numbers (gestating females). Heterophilia has been repeatedly associated with rises in plasma corticosteroid concentrations in mammals (e.g., Buddle et al., 1992; Dhabar et al., 1995) and a small number of studies in reptiles also support this relationship (e.g., Aguirre et al., 1995; Morici et al., 1997). Although plasma corticosterone concentrations had returned to baseline by 1 week in captivity for females of both reproductive states, heterophil numbers had not recovered by this time in either reproductive state and in postpartum females were still significantly higher than baseline even at 4 weeks. A lag in recovery time for neutrophils was also observed in mice exposed to a stressor, although that was a short term study in which mice restrained for 2 h showed a recovery in corticosterone concentrations by 3 h following the stress but neutrophil numbers remained significantly elevated at this time (Dhabar et al., 1995).

There is very little literature available on eosinophil responses to stress in reptiles: heterophil and lymphocyte counts are generally employed to gauge stress levels, so in most studies a full leucogram has not been reported. The very clear inverse relationship between eosinophil and heterophil numbers demonstrated in this study suggests a possible negative relationship with plasma corticosterone concentrations. Similarly, long-term corticosterone implantation in the alligator *Alligator mississippiensis* caused suppression of both circulating eosinophil and basophil numbers (Morici et al., 1997).

Thus, contrary to our initial hypothesis, adrenocortical responses to chronic captivity, as assessed by changes in plasma corticosterone concentrations, were very similar in gestating and postpartum females, although the patterns of change in white cell parameters over the 4 weeks of captivity did differ between reproductive states. Further studies should examine the responses of females in early gestation: however the confounding effect of season upon plasma corticosterone concentrations and upon leucocyte numbers (Zapata et al., 1992) would need to be considered in comparing such females with postpartum or vitellogenic females. This study also demonstrates that, in reptiles brought into captivity, variations in haematological parameters may continue after plasma corticosterone concentrations have returned to initial values. Indeed, Lance (1994) commented that steroids may not directly regulate the reptilian immune system. The observations reported here may reflect interrelationships between nutritional state and immunity (Lochmiller and Deerenberg, 2000), and require further investigation.

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References

Aguirre, A.A., Balazs, G.H., Spraker, T.R., Gross, T.S., 1995. Adrenal and hematological responses to stress in juvenile green turtles (*Chelonia mydas*) with and without fibropapillomas. Physiol. Zool. 68, 831–854.

Baker, M.L., Gemmell, E., Gemmell, R.T., 1998. Physiological changes in brushtail possums, *Trichosurus vulpecula*, transferred from the wild to captivity. J. Exp. Zool. 280, 203–212.

Bell, K., 1997. Corticosterone and the stress response in the lizard *Egernia whitii* (Scincidae). University of Tasmania, Unpublished Honours Thesis.

Buddle, B.M., Aldwell, F.E., Jowett, G., Thomson, A., Jackson, R., Paterson, B.M., 1992. Influences of stress of capture on haematological values and cellular immune responses in the Australian brushtail possum. N. Z. Vet. J. 40, 155–159.

Campbell, T.W., 1996. Clinical pathology. In: Mader, D.R. (Ed.), Reptile Medicine and Surgery. W. B. Saunders Co., Pennsylvania, pp. 248–257.

Chapple, D., 2003. Ecology, life-history, and behavior in the Australian Scincid genus *Egernia*, with comments on the evolution of complex sociality in lizards. Herpetol. Monogr. 17, 145–180.

Cree, A., Tyrrell, C.L., Preest, M.R., Thorburn, D., Guillette Jr., L.J., 2003. Protecting embryos from stress: corticosterone effects and the corticosterone response to capture and confinement during pregnancy in a live-bearing lizard (*Hoplodactylus maculatus*). Gen. Comp. Endocrinol. 134, 239–316.

Dhabar, F.S., McEwen, B.S., 1997. Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking. Brain Behav. Immun. 11, 286–306.

Dhabar, F.S., Miller, A.H., McEwen, B.S., Spencer, R.L., 1995. Effects of stress on immune cell distribution—dynamics and hormonal mechanisms. J. Immunol. 57, 1638–1644.

Dunlap, K.D., 1995. External and internal influences on indices of physiological stress: II. Seasonal ands size-related variations in blood composition in free-living lizards, *Sceloporus occidentalis*. J. Exp. Zool. 272, 85–94.

Edwards, A., Jones, S.M., 2001. Changes in plasma testosterone, estrogen, and progesterone concentrations throughout the annual reproductive cycle in male viviparous blue-tongued skinks, *Tiliqua nigrolutea*, in Tasmania. J. Herpetol. 35, 293–299.

Fudge, A.M., 1997. Avian clinical pathology—hematology and chemistry. In: Altman, R.B. (Ed.), Avian Medicine and Surgery. W. B. Saunders Co., Pennsylvania, pp. 142–157.

Grossman, C.J., 1984. Regulation of the immune system by sex steroids. Endocr. Rev. 5, 435–455.

Handa, R.J., Burgess, L.H., Kerr, J.E., O'Keefe, J.A., 1994. Gonadal steroid hormone receptors and sex differences in the hypothalamo–pituitary–adrenal axis. Horm. Behav. 28, 464–476.

Hume, A., 1995. Practical avian haematology. In: Gross, G.M. (Ed.), Australian Committee Association of Avian Veterinarians. Annual Conference Proceedings.

Jessop, T.S., Hamann, M., Read, M.A., Lipus, C.J., 2000. Evidence for a hormonal tactic maximizing green turtle reproduction in response to a pervasive ecological stressor. Gen. Comp. Endocrinol. 118, 407–417.

Jones, S.M., Bell, K., 2004. Plasma corticosterone concentrations in males of the skink *Egernia whitii* during acute and chronic confinement, and over a diel period. Comp. Biochem. Physiol., A 137, 105–113.

Jones, S.M., Swain, R., 1996. Annual reproductive cycle and annual cycles of reproductive hormones in plasma of female *Niveoscincus metallicus* (Scincidae) from Tasmania. J. Herpetol. 30, 140–146.

Jones, S.M., Wapstra, E., Swain, R., 1997. Asynchronous male and female gonadal cycles and plasma steroid concentrations in a viviparous lizard, *Niveoscincus ocellatus* (Scincidae), from Tasmania. Gen. Comp. Endocrinol. 108, 271–281.

Keller-Wood, M., Wood, C.E., 2001. Pituitary–adrenal physiology during pregnancy. Endocrinologist 11, 159–170.

Lance, V.A., 1994. Life in the slow lane: hormones, stress, and the immune system in reptiles. Perspect. Comput. Endocrinol., 529–534.

Lochmiller, R.L., Deerenberg, C., 2000. Trade-offs in evolutionary immunity: just what is the cost of immunity? Oikos 88, 87–98.

McLean, J.M., Mosley, J.G., Gibbs, A.C.C., 1974. Changes in the thymus, spleen and lymph nodes during pregnancy and lactation in the rat. J. Anat. 118, 223–229.

Millar, K.G., Mills, R.T., Baines, M.G., 1973. A study of the influence of pregnancy on the thymus gland of the mouse. Am. J. Obstet. Gynecol. 117, 913–919.

Milton, D.A., 1987. Reproduction in two closely related skinks, *Egernia modesta* and *Egernia whitii* (Lacertilia: Scincidae) in south-east Queensland. Aust. J. Zoology 35, 35–41.

Morici, L.A., Elsey, R.M., Lance, V.A., 1997. Effects of long-term corticosterone implants on growth and immune function in juvenile alligators, *Alligator mississippiensis*. J. Exp. Zool. 279, 156–162.

Munoz, F.J., Galvan, A., Lerma, M., De la Fuente, M., 2000. Seasonal changes in peripheral blood leucocyte function of the turtle *Mauremys caspica* and their relationship with corticosterone, 17β-estradiol and testosterone serum levels. Vet. Immunol. Immunopathol. 77, 27–42.

Rooney, A.A., Guillette Jr., L.J., 2001. Biotic and abiotic factors in crocodilian stress: the challenge of a modern environment. In: Grigg, G.C., Seebacher, F., Franklin, C.E. (Eds.), Crocodilian Biology and Evolution. Surrey Beatty and Sons, New South Wales, pp. 215–228.

Saad, A.-H., 1989. Pregnancy-related involution of the thymus in the viviparous lizard, *Chalcides ocellatus*. Thymus 14, 223–232.

Saad, A.-H., El Ridi, R., 1988. Endogenous corticosteroids mediate seasonal cyclic changes in immunity of lizards. Immunobiology 177, 390–403.

Saad, A.-H., El Deeb, S., 1990. Immunological changes during pregnancy in the viviparous lizard, *Chalcides ocellatus*. Vet. Immunol. Immunopathol. 25, 279–286.

Schuurs, A.H., Verheul, H.A.M., 1990. Effects of gender and sex steroids on the immune response. J. Steroid Biochem. 35, 157–172.

Selye, H., 1937. Studies on adaptation. Endocrinology 21, 169–188.

Turton, J.A., Ladds, P.W., Manolis, S.C., Webb, G.J.W., 1997. Relationship between blood corticosterone, immunoglobin, and haematological values in young crocodiles (*Crocodilus porosus*) to water temperature, clutch origin and body weight. Aust. Vet. J. 75, 114–119.

Weiss, S.L., Jennings, D.H., Moore, M.C., 2002. Effect of captivity in semi-natural enclosures on the reproductive endocrinology of female lizards. Gen. Comp. Endocrinol. 128, 238–246.

Whittier, J.M., Mason, R.T., Crews, D., 1987. Plasma steroid hormone levels of female red-sided garter snakes, *Thamnophis sirtalis parietalis*: relationship to mating and gestation. Gen. Comp. Endocrinol. 67, 33–43.

Wingfield, J.C., Maney, D.L., Breuner, C.W., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D., 1998. Ecological bases of hormone–behavior interactions: the emergency life history stage. Am. Zool. 38, 191–206.

Wojtaszek, J.S., 1992. Seasonal changes of circulating blood parameters in the grass snake *Natrix natrix* L. Comp. Biochem. Physiol., A 103, 461–471.

Zapata, A.G., Varas, A., Torroba, M., 1992. Seasonal variations in the immune system of lower vertebrates. Immunol. Today 13, 142–147.