

Baseline and Stress-Induced Glucocorticoids During Reproduction in the Variable Flying Fox, *Pteropus hypomelanus* (Chiroptera: Pteropodidae)

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ABSTRACT Baseline and stress-responsive glucocorticoid (GC) levels were assessed during early pregnancy, late pregnancy, and lactation in female variable flying foxes (*Pteropus hypomelanus*) and in males over the same time period. Animals were maintained in a breeding colony in captivity. High levels of both cortisol and corticosterone were detected, with total plasma GC levels being among the highest documented in vertebrates (up to 3000 ng/ml in individual animals, with cortisol being the primary GC, accounting for $\approx 78\%$ of total GCs), and significantly greater in males than in females. Plasma levels of cortisol and corticosterone showed nearly identical profiles within each sex, with the exception of females in late pregnancy, in which corticosterone, but not cortisol, increased significantly. Baseline levels of plasma cortisol were highest in September (when pups were between 1 and 2 months of age) in both sexes, which may be related to the approaching onset of the mating period. There was a continuum in the magnitude of the response to stress (handling and sampling) over time in females, with the greatest stress response in early pregnancy, a dampened response during late pregnancy, and no significant stress response during lactation. Surprisingly, males failed to exhibit elevated GCs after this stress, but did have significant stress-induced hyperglycemia and suppression of plasma testosterone levels. This may be due to their high (perhaps maximal) baseline levels, which suggests that being in a breeding group was chronically stressful for males. *J. Exp. Zool.* 301A:682–690, 2004. © 2004 Wiley-Liss, Inc.

INTRODUCTION

Pregnancy and lactation are the two most metabolically demanding periods of a mammal's life history (Wade and Schneider, '92). Not surprisingly, these periods are typically associated with changes in the highly catabolic glucocorticoid hormones, cortisol and corticosterone. In laboratory mammals, baseline plasma glucocorticoid levels generally increase during pregnancy and decrease following parturition and during lactation (Atkinson and Waddell, '95). In contrast, in some species stress-induced glucocorticoid levels are dampened during pregnancy and especially during lactation (Stern et al., '73; Johnstone et al., 2000; Lightman et al., 2001; Walker et al., 2001). This phenomenon may reflect a selection pressure to minimize large fluctuations in glucocorticoids in the fetus and in the neonate (in which GC enter via maternal milk, Lightman et al., 2001), as high glucocorticoid levels negatively influence a number of developmental processes and subsequent health and survival (Wadhwa et al., 2001). These processes have been documented in a number of

common laboratory mammals (see Lightman et al., 2001; Altemus et al., '95). However, a variety of other mammalian taxa are routinely housed in captive populations either for research or for educational purposes. Many of these other taxa may prove to be excellent models for understanding the relationships between glucocorticoids and reproductive physiology. One such taxonomic group is bats, which are physiologically, ecologically, and behaviorally unique among vertebrates, as they are the only truly volant mammals. Most bat species live in highly seasonal environments (Racey, '82; Racey and Entwistle, 2000; Heideman, 2000) and give birth yearly to a single, large offspring, weighing between 12 and 43% of maternal body mass (Kurta and Kunz, '87; Kunz

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and Hood, 2000; Barclay and Harder, 2003). Because they often must fly great distances in search of food during both pregnancy and lactation (with the pup attached in some cases), their energetic needs are greater than those of most mammals and are tightly linked to their environment (Racey and Entwistle, 2000).

Despite the fact that bats account for approximately one fifth of all mammalian species, with a total of 1,111 species currently recognized (Simmons, 2004), relatively little is known about their glucocorticoid physiology. Baseline and stress glucocorticoid levels are known from a handful of bats sampled at singular time points in captivity (see review by Kwiecinski and Damassa, 2000). Seasonal glucocorticoid patterns are known for male little brown myotis (*Myotis lucifugus*; Gustafson and Belt, '81) and greater Asiatic yellow house bats (*Scotophilus heathi*; Krishna et al., '98) captured in the field but sampled in the laboratory. Lastly, baseline and stress responsive glucocorticoid levels are known from male and female free-ranging little brown myotis sampled during the active period in the field (Reeder et al., 2004).

Previous research in our laboratory (Widmaier and Kunz, '93; Widmaier et al., '94) has documented exceptionally high levels of glucocorticoids in several species of captive bats, most especially in *Pteropus hypomelanus*, the variable flying fox. This species is a relatively large (425-450 g with a wingspan of approximately 1 m) frugivorous Old World bat found patchily distributed throughout southeast Asia (Jones and Kunz, 2000; Simmons, 2004). *Pteropus hypomelanus* is highly social and can travel large distances to forage each night (up to 6-8 km; Mickleburgh et al., '92). In this species, baseline glucocorticoid levels averaged 1269 ± 207 (SE) ng/ml for cortisol and 590 ± 154 ng/ml for corticosterone (Widmaier and Kunz, '93). These values are among the highest recorded in mammals, and exceed those measured in any other bat species. Despite these high baseline levels, *P. hypomelanus* is still capable of a robust response to restraint stress, with significant elevations in plasma adrenocorticotrophic hormone (ACTH), glucocorticoids, and glucose levels (Widmaier and Kunz, '93; Widmaier et al., '94). Baseline glucose levels are in the normal mammalian range, suggesting that these captive bats are not chronically stressed. In the wild, this species is relatively tolerant of disturbance and has no known predators, but, like many of the 64 other species of *Pteropus* (some of which are critically

endangered), it is currently threatened by hunting. Due largely to habitat destruction, bat-human interactions are becoming increasingly common and it is apparent that several large species of *Pteropus*, including *P. hypomelanus*, are natural reservoirs for several recently documented and serious emergent diseases (Chua et al., 2001, 2002).

Thus, from both a theoretical perspective, in which *P. hypomelanus* is an excellent model for examining glucocorticoid physiology, and from a practical perspective, in which a greater understanding of their physiology is important for management reasons, it is important to further our understanding of basic hormone profiles and of the response to handling and sampling in this species. Although the basic characteristics of the response to a strong stressor (restraint) is known in this species (Widmaier and Kunz, '93; Widmaier et al., '94), what is unknown is how the glucocorticoid response to stress varies by reproductive stage. Therefore, the objective of this study was to describe changes in the glucocorticoid response to stress during early vs. late pregnancy and after the birth of infants in female *P. hypomelanus*. Potential changes in both glucocorticoid activity and plasma testosterone levels in males across this same time period were also assessed. We hypothesized that females would exhibit peak baseline glucocorticoid levels during late pregnancy, and a dampened stress response during late pregnancy and especially during lactation. For males, we hypothesized no differences in baseline or stress-responsive glucocorticoid levels across the periods of female gestation and lactation, and we predicted that all males would exhibit a significant increase in glucocorticoids in response to stress. We have previously documented decreased testosterone levels following stress in the little golden-mantled flying fox (*Pteropus pumilus*; Reeder et al., 2002), thus we predicted a decrease in plasma testosterone levels in response to stress in *P. hypomelanus* males. Preliminary data from an earlier study (Widmaier and Kunz, '93) strongly suggested that total glucocorticoid levels would be higher in male *P. hypomelanus* than in females, a pattern that is unusual for mammals.

MATERIALS AND METHODS

Study species

Subjects included six male and six female *Pteropus hypomelanus* living in the same breeding group in captivity at the Lube Bat Conservancy

in Gainesville, Florida. The breeding group, which also included an additional eight females, was housed in an octagonal, double-wire enclosure, measuring approximately 11 m in diameter and 2 m high. The enclosure contained an outdoor portion that encircled a smaller inside roost ("the nighthouse"; 3 m in diameter and temperature controlled). Enclosures were designed to maximize the ability of bats to feed, rest, and fly freely. All subjects were wild-born, captured from Indonesia in 1990, and had been housed in captivity for 10 years. They were all reproductively mature at the onset of the study and each was easily identified by numbered thumb-bands and uniquely coded transponders (Trovan, Santa Barbara, CA) implanted beneath the skin in the mid-scapular region. Animals were fed a mixture of fresh fruits and vegetables and monkey chow (Purina) daily at 1500 h; water was available *ad libitum*.

Sampling protocol

The breeding group was formed in November, 2000 and sample collection began once pregnancy was detected by palpation. Samples were collected from females in mid-March, 2001 (early pregnancy), in either mid-May or mid-July, 2001 (late pregnancy; birth of a single pup to each dam occurred between mid-July and mid-August), and again in mid-September, 2001 (when pups were between one and two months of age). Males were sampled at each of these time points. Data from males in mid-May and mid-July, which did not differ, were averaged for this analysis in order to correspond to the average sampling time of late pregnancy in females. In the mid-September postpartum bleed, pups remained with their mothers during all procedures. Samples at each time point were collected in both baseline and stress conditions. Prior to sampling, animals were locked inside of their nighthouse after feeding, which is a routine procedure for these animals during cold months and should not have generated undue stress. At approximately 1800 h (3 hrs postprandial), bats were quickly hand captured and brought to a centrally located staging area outside of their pen. They were manually restrained and blood samples (0.6 ml) were collected by venipuncture within three minutes of capture from either a small wing vein or a vein in the forearm and placed into EDTA containing microtubes in an ice-water bath. After sampling, animals were either placed in the outside portion of their enclosure or in a smaller pen located adjacent to it. These

smaller, triangular shaped pens (4.4 × 4.4 × 6.25 m × 2 m high) are routinely used for short term (minutes to hours) housing. To look for changes in stress reactivity during pregnancy and after the birth of infants (and during the same time of season in males), approximately 60 minutes (mean ± SE: 63 min 25 sec ± 54 sec) after collection of the initial handling and baseline sample, animals were hand caught and a second sample (0.4 ml; the stress condition) was quickly collected, this time under anesthesia (to minimize the effects of repeated sampling; we used 5% isoflurane gas mixed in oxygen until the animal relaxed, then maintained it on 2.5% isoflurane gas until the blood sample was obtained). Isoflurane gas is routinely and safely used for anesthesia with these animals, who rapidly recover from its effects. A total of 90 baseline and stress reactivity samples were collected. Sample collection averaged 2 min 24 sec (SE = 7 sec) from the time of initial handling. Samples were collected rapidly to avoid measuring the response to the second sampling procedure itself (Widmaier and Kunz, '93; Widmaier et al., '94), and plasma was stored at -20°C until assay.

Hormone and glucose assays

Pteropus hypomelanus has detectable levels of both glucocorticoid hormones, cortisol and corticosterone. Thus, each hormone was separately assayed directly in a volume of 5 µl plasma each using commercially available radioimmunoassay (RIA) kits (ICN, CostaMeca, CA), as previously described and validated for this species (Widmaier and Kunz, '93; Widmaier et al., '94). Additional testing in our laboratory has confirmed negligible cross reactivity of the cortisol assay for corticosterone and vice-versa for *Pteropus* plasma (DM Reeder, unpublished data). Samples were run in a total of 6 assays for cortisol and 4 for corticosterone.

Testosterone was determined in 15 µl of non-extracted plasma using a commercially available RIA kit (ICN). Parallelism with the standard curve was determined for three pooled *Pteropus* plasma samples (see Fig. 1) and accuracy (observed vs. expected values for pooled plasma spiked with known amounts of testosterone) was determined to be 115.4 ± 11.7 SD percent. The least detectable dose was 0.15 ng/ml.

Glucose levels for a limited number of samples from males were measured using the glucose oxidase method (Fisher Scientific, Fairlawn, NJ: Oxidase/Trinder reagents).

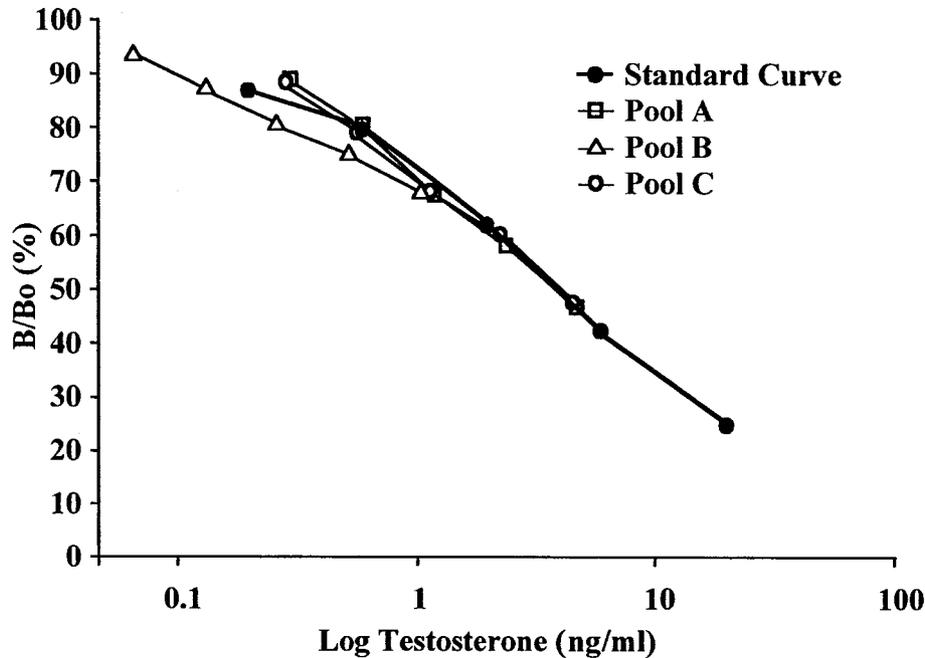


Fig. 1. Parallelism of three pooled plasma samples with the testosterone standard curve. Plasma was obtained from 4–6 different animals and used to generate each of the three pools prior to testosterone radioimmunoassay.

Statistics

Cortisol levels, corticosterone levels, and total glucocorticoid levels (cortisol + corticosterone) were each analyzed with a 2 (sex) X 2 (baseline or stress) X 3 (time) repeated measures ANOVA. Testosterone was analyzed with a 2 (baseline or stress) X 3 (time) repeated measures ANOVA. Homogeneity of variance between groups was confirmed using Levine's test prior to analysis. Power for the ANOVAs was calculated using SPSS 9.0 (SPSS, Inc.).

Significant interactions were explored a priori with t-tests; *P* values were corrected for multiple comparisons using the Bonferroni method where appropriate. Sufficient samples for glucocorticoid analysis from all time points were not available for one male, thus the glucocorticoid analysis was completed with data from five males. Baseline and stress-responsive glucose levels for males were averaged across the March and September sampling periods and were analyzed with t-tests.

RESULTS

Glucocorticoids

As previously described by our laboratory (Widmaier and Kunz, '93; Widmaier et al., '94); total plasma glucocorticoids in *P. hypomelanus* are

among the highest documented for mammals, with total glucocorticoid levels in some individuals of up to 3000 ng/ml measured in this study. Cortisol levels were higher than corticosterone at all times, with corticosterone accounting for $22 \pm 1\%$ (mean \pm SE) of total glucocorticoids. With one notable exception (corticosterone values in the period corresponding to late pregnancy in females), cortisol and corticosterone displayed nearly identical profiles (Fig. 2). Despite the fact that the collection of baseline samples from all of the subjects at each time period took approximately one hour, there was no "order effect" or relationship between time of sampling and baseline plasma glucocorticoid levels (r^2 for a regression of total glucocorticoids and time of sample collection relative to time of first entry into the pen ranged from 0.0015 to 0.28, *P* ranged from 0.175 to 0.911).

Significant changes over time in both cortisol and corticosterone were observed in both sexes (cortisol: $F_{(2,18)} = 9.99$, *P* = 0.001; corticosterone: $F_{(2,18)} = 8.82$, *P* = 0.002). For females, baseline cortisol levels were not distinguishable during early vs. late pregnancy, but significantly rose above late pregnancy values in the post-partum lactational period (Fig. 2A: $t = -4.079$, *P* = 0.03). In contrast, baseline corticosterone values peaked during late pregnancy, and were significantly

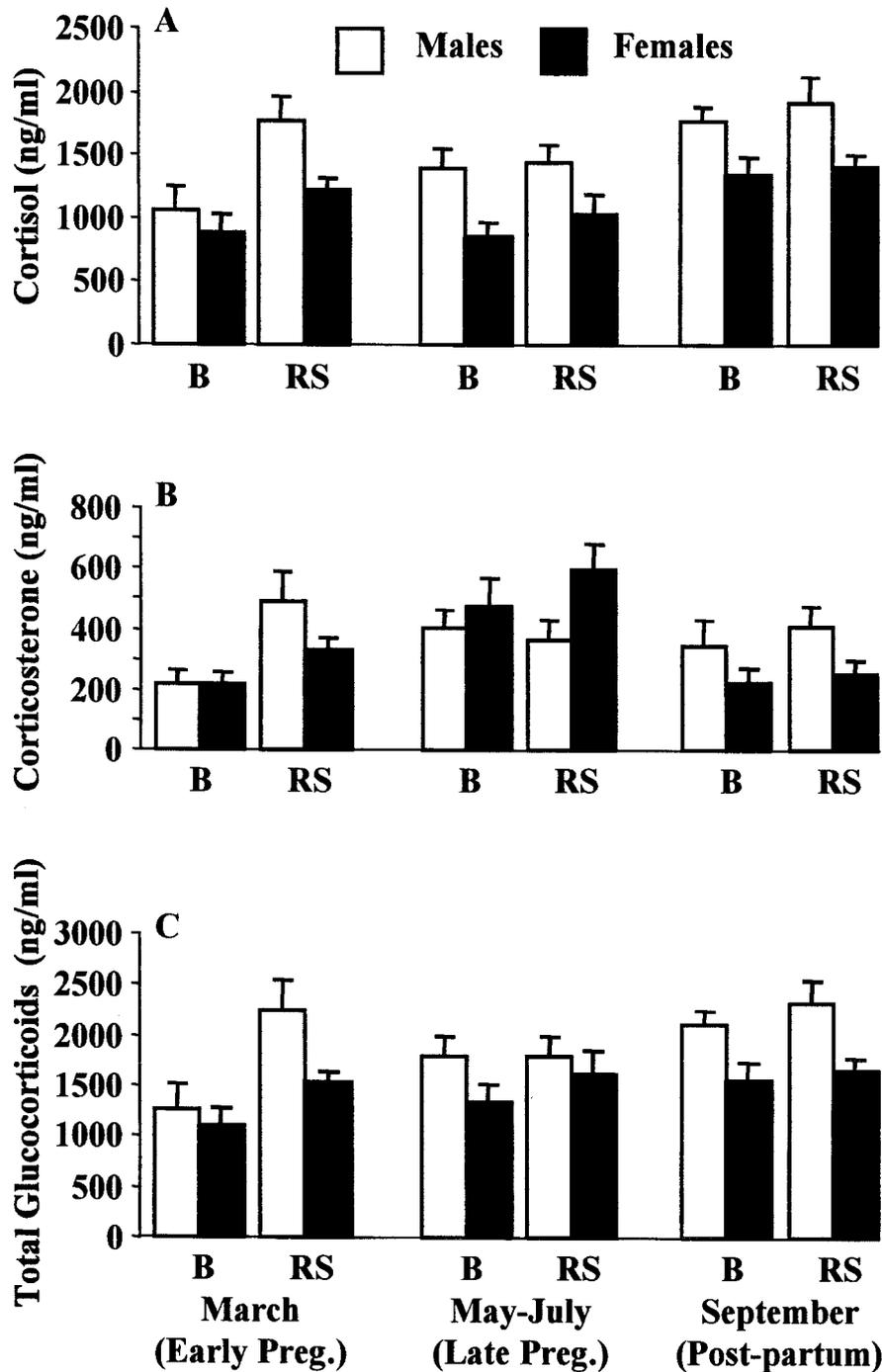


Fig. 2. Mean \pm SE levels of cortisol (2A), corticosterone (2B), and total glucocorticoids (2C) in male and female *Pteropus hypomelanus* during the month of March (corresponding to the females' first trimester of pregnancy), May and July (an average of samples collected from these times for males; for females, one sample from these dates correspond-

ing to the last trimester was analyzed), and in September, when pups were between one and two months of age. Samples were collected immediately upon capture (baseline = "B") and again 60 minutes later (the response to handling and sampling = "RS"). Significant differences are discussed in the text.

greater than levels seen during lactation (Fig. 2B: $t = 3.961$, $P = 0.03$). These elevated corticosterone levels in late pregnancy, without a similar elevation in cortisol during this same period, reflect a

dramatic shift in the percentage of total glucocorticoids accounted for by corticosterone. During early pregnancy and lactation, corticosterone is a relatively small proportion of total glucocorticoids

measured (21% and 15%, respectively). During late pregnancy however, the relative proportion of corticosterone significantly increased to 36% of total glucocorticoids (vs. early pregnancy: $t = -5.082$, $P = 0.008$; vs. lactation: $t = 7.231$, $P = 0.003$). When considered in combination, the different patterns of baseline cortisol and corticosterone somewhat cancel each other, for a net effect of no significant differences in total glucocorticoid levels over time in females (Fig. 2C: $F_{(1,5)} = 1.017$, $P = 0.396$). In males, baseline cortisol (and consequently total glucocorticoids) rose steadily over time. As was the case for females, the highest cortisol levels were measured in September (the post-partum period for females), with these values being significantly greater than those from March and from May/July (September vs. March: $t = -6.114$, $P = 0.012$; vs. May/July: $t = -4.078$, $P = 0.03$). For corticosterone, males showed a similar seasonal profile over time as females, with slightly elevated baseline levels in the May/July period corresponding to late pregnancy in females, but this difference was not significant after correction for multiple comparisons.

Cortisol levels and consequently total glucocorticoid levels were always greater in males than in females (cortisol: $F_{(1,9)} = 10.713$, $P = 0.01$; total glucocorticoids: $F_{(1,9)} = 7.529$, $P = 0.023$). Despite elevated corticosterone values for females in late pregnancy relative to males sampled at the same time, there were no significant sex differences at any time in plasma corticosterone levels ($F_{(1,9)} = 0.103$, $P = 0.76$). Our inability to detect differences in corticosterone between the sexes is likely the result of low statistical power stemming from small sample sizes and high variability (Power [1-B] = .06 for the between subjects main effect of sex; Power [1-B] = .06 for the condition (stress)*-sex interaction).

Significantly higher plasma glucocorticoid levels in response to the handling and blood sampling that occurred 60 min prior were seen in several conditions. For females, cortisol and consequently total glucocorticoid levels were significantly elevated over baseline in early pregnancy and late pregnancy, but not during lactation (cortisol: early pregnancy: $t = -4.009$, $P = 0.01$; late pregnancy: $t = -2.543$, $P = 0.05$; lactation: $t = -0.92$, $P = 0.4$; total glucocorticoids: early pregnancy: $t = -3.589$, $P = 0.016$; late pregnancy: $t = -3.079$, $P = 0.027$; lactation: $t = -1.157$, $P = 0.3$). Cortisol levels appeared to be elevated in response to stress in males sampled in March (but not in May/July or in

September); but there were no significant differences in plasma cortisol for males at any time point ($F_{(1,4)} = 4.207$, $P = 0.11$). Despite the lack of a significant cortisol response to stress in males, glucose levels were significantly higher after stress, indicating stress-induced hyperglycemia (baseline glucose = 87.2 ± 6.6 (SE), stress = 117.7 ± 5.1 (SE) mg/dl; $t = -5.274$, $P = 0.006$). Plasma corticosterone levels displayed roughly similar patterns to those of cortisol, but, as described above, were not statistically different between the sexes. An analysis of corticosterone levels in males and females combined indicated a significant stress response in the period corresponding to early pregnancy (March), but not at other times (early pregnancy: $t = -2.908$, $P = 0.016$; late pregnancy (May/July): $t = -0.974$, $P = 0.353$; lactation (September): $t = -2.050$, $P = 0.068$).

Differences in the magnitude of the stress response between the sexes and over time were assessed by comparing: 1) peak levels of glucocorticoids reached 60 minutes following initial handling and sampling, and 2) the absolute difference between baseline and stress levels. Despite the fact that baseline cortisol levels (and consequently total glucocorticoid levels) were highest during the period of lactation (September) in both males and females, there were no significant differences over time in peak levels of cortisol (or total glucocorticoids) in response to stress in either sex. Thus, significant differences over time in the absolute difference between stress and baseline levels of cortisol (and consequently of total glucocorticoids) exist for both sexes, with a much greater absolute difference between stress and baseline levels during the period of early pregnancy (March) in both sexes (cortisol: early pregnancy vs. period of late pregnancy (May/July): $t = 3.094$, $P = 0.022$; vs. lactation period (September): $t = 2.327$, $P = 0.041$; total glucocorticoids: early pregnancy vs. period of late pregnancy (May/July): $t = 2.677$, $P = 0.046$; vs. lactation period (September): $t = 2.332$, $P = 0.042$). For corticosterone, there were no significant differences in the peak response to stress over time for males (and no significant changes over time in baseline levels). However, females had significantly higher corticosterone levels following stress during late pregnancy than at other times (late pregnancy vs. early pregnancy: $t = -5.255$, $P = 0.009$; vs. lactation: $t = -4.966$, $P = 0.008$). Elevated peak corticosterone levels in females during late pregnancy mirror the higher baseline corticosterone levels at this time,

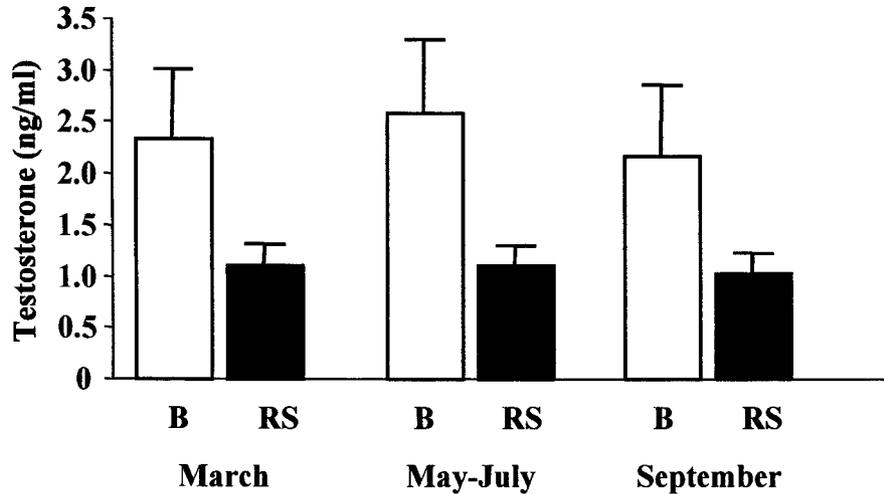


Fig. 3. Mean \pm SE levels of testosterone in male *Pteropus hypomelanus* sampled during the month of March (corresponding to the females' first trimester of pregnancy), May and July (an average of samples collected from these times for males, corresponding to the females' last trimester), and in September, when pups were between one and two months of

age. Samples were collected immediately upon capture (baseline = "B") and again 60 minutes later (the response to handling and sampling = "RS"). Testosterone did not change over time but significantly declined in response to handling and sampling.

consequently there were no differences over time in the absolute difference between stress and baseline corticosterone levels for females. Among males, there were no significant differences over time in the absolute difference between stress and baseline levels of corticosterone.

Testosterone

There were no changes over time in testosterone levels in males (Fig. 3; $F_{(2,10)} = .289$, $P = 0.65$), but testosterone significantly declined at all time points in response to prior handling and blood sampling ($F_{(1,5)} = 6.94$, $P = 0.046$).

DISCUSSION

Several interesting differences in both baseline and stress-responsive levels of glucocorticoid hormones over time and between sexes were observed in this species. Our data generally support only some of our predictions and some of our results are surprising and novel. For female *P. hypomelanus* we had predicted: 1) peak baseline glucocorticoid levels during late pregnancy (relative to early pregnancy and lactation) and, 2) a dampened stress response during late pregnancy and especially during lactation. As expected, there was a continuum in the magnitude of the stress response over time for females. Animals sampled in early pregnancy exhibited the greatest stress response (as indicated by the absolute difference between stress and baseline values), which was signifi-

cantly greater than their response during late pregnancy, and during lactation they failed to mount a significant glucocorticoid response at all to the stressor. These data concur with data from studies of laboratory mammals, in which the response to stress is dampened in late pregnancy and lactation (Stern et al., '73; Johnstone et al., 2000; Lightman et al., 2001; Walker et al., 2001). It should be noted, however, that the stressor used in the present study (handling and blood sampling one hour earlier) is a relatively mild stress; it is possible that a more prolonged or a more rigorous stressor would have resulted in increased corticoid levels even in the lactating animals.

We failed to demonstrate the typical mammalian pattern of elevated total glucocorticoids in late pregnancy. This may be due to our sampling regime, with "late pregnancy" samples collected between 4 and 68 days prior to parturition, resulting in high variability and the potential to miss the peak glucocorticoid levels common at the end of pregnancy. If this were the case however, we might expect a relationship between glucocorticoid levels and the date relative to parturition, and this was not so. Clearly, future studies should include more intensive sampling during late pregnancy. What is most interesting about the longitudinal glucocorticoid data in females is the different profile exhibited by cortisol and corticosterone, with peak baseline cortisol levels during lactation, and peak corticosterone levels during late pregnancy. Corticosterone, therefore, but not

cortisol, peaked as we predicted during late pregnancy, resulting in a much higher percentage of corticosterone relative to total glucocorticoids than in females at other time points, and in males at all time points. Among the mammals in which it has been studied, it is relatively rare to make both cortisol and corticosterone in significant amounts, and this relative increase in the proportion of corticosterone secreted during late pregnancy has not been described in any other species. In sheep, cortisol, corticosterone, and aldosterone increase in late pregnancy, and are necessary for maintaining the normal increase in maternal plasma volume (Jensen et al., 2002). It could be that selection for increased aldosterone in this species is driving the increase in corticosterone, which is its precursor in the corticoid biochemical pathway. There have been no studies to date of aldosterone function in any bat. Further sampling is clearly needed to determine if this finding of increased corticosterone but not cortisol is generally characteristic of late pregnancy in this species, or whether cortisol peaks at a different point in late pregnancy. For males, we had predicted: 1) no differences in baseline or stress-responsive glucocorticoid levels across the periods of female gestation and lactation, 2) that all males would exhibit a significant increase in glucocorticoids and decrease in testosterone in response to stress, and 3) that males would have higher total glucocorticoid levels than females. As was the case with females, these predictions were only partly met. Males did in fact have significantly higher cortisol (and consequently higher total glucocorticoid) levels than females overall. The reasons for this sexual dimorphism are unclear. It may be that males are generally more glucocorticoid resistant than females, that their levels of binding protein are higher, that their glucocorticoid receptor levels are less numerous or less sensitive to negative feedback, and/or, that being in a breeding group is chronically stressful to males. Further testing is needed to assess the first three possibilities, but behavioral evidence suggests that males did find being in a breeding group stressful, as aggression between males but not between females was common in this group.

The failure of male *P. hypomelanus* to mount a significant stress response may be related to their elevated baseline glucocorticoid values. If it is the case that males are stressed, thus resulting in higher baseline values on average, they may already have maximal glucocorticoid output and therefore be unable to mount a stress response.

Alternatively, males may have failed to mount a stress response because they were habituated to handling and sampling. This is not likely however, because the animals in this study were not often handled, being sampled for this study and once per year for their annual physical. Although behavioral responses to stress are not always good indicators of whether or not a significant physiological response is mounted in response to stress, that these males resisted capture and were aggressive to their handlers suggests that they are not habituated to this procedure. Additionally, that plasma glucose levels were significantly elevated and testosterone levels clearly suppressed in response to handling indicates that these males perceived and responded to this stressor. As to be expected given the pulsatile nature of gonadotropin releasing hormone (GnRH; Anthony, 2000), which presumably results in pulsatile testosterone secretion, variability in baseline testosterone values was somewhat higher in the baseline condition than in the response to stress condition. The significant stress-induced suppression of testosterone levels appears not to be mediated by increases in glucocorticoids, but could result from the central effects of stress, perhaps acting on GnRH.

As was the case with females, male *P. hypomelanus* had their highest levels of cortisol (and consequently total glucocorticoids) in September, when pups were between 1 and 2 months of age. We suspect that this reflects a seasonal rhythm in glucocorticoid levels leading up to the mating period. Unlike primates, in which HPA activation can occur when a member of one's social group is captured and handled (Mendoza and Mason, '97), we saw no evidence for "order effects" or glucocorticoid responses to the capture of other animals within the breeding group. This suggests that these animals are relatively resistant to perturbations that do not directly affect them. We believe that this species is an excellent model system for further exploration of changes in stress reactivity across different reproductive conditions and across the annual reproductive cycle. Because of their exceptionally high levels of glucocorticoids, and their variability in stress reactivity, *P. hypomelanus* is a valuable animal model for exploring glucocorticoid resistance. Finally, the unusual pattern of increased corticosterone but not cortisol during late pregnancy may suggest changes in aldosteronogenesis, or shifts in the enzymatic pathways responsible for cholesterol metabolism in the adrenal cortex.

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LITERATURE CITED

- Altemus M, Deuster PA, Galliven E, Carter CS, Gold PW. 1995. Suppression of hypothalamic-pituitary-adrenal axis responses to stress in lactating women. *J Clin Endocrinol Metab* 80:2954–2959.
- Anthony ELP. 2000. Endocrinology of reproduction in bats: Central control. In Crichton EG, Krutzsch PH, editors. *Reproductive Biology of Bats*. London: Academic Press. p. 1–26.
- Atkinson HC, Waddell BJ. 1995. The hypothalamic-pituitary-adrenal axis in rat pregnancy and lactation: circadian variation and interrelationship of plasma adrenocorticotropin and corticosterone. *Endocrinol* 136:512–520.
- Barclay RMR, Harder LD. 2003. Life histories of bats: life in the slow lane. In Kunz TH, Fenton MB, editors. *Bat Ecology*. Chicago: The University of Chicago Press. p. 209–253.
- Chua KB, Koh CL, Hooi PS, Wee KF, Khong JH, Chua BH, Chan YP, Lim ME, Lam SK. 2002. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect* 4: 145–151.
- Chua, KB, Wang L-F, Lam SK, Cramer G, Yu M, Wise T, Boyle D, Hyatt AD, Eaton BT. 2001. Tioman virus, a novel paramyxovirus isolated from fruit bats in Malaysia. *Virology* 283:215–229.
- Gustafson AW, Belt WD. 1981. The adrenal cortex during activity and hibernation in the male little brown bat, *Myotis lucifugus lucifugus*: annual rhythm of plasma cortisol levels. *Gen Comp Endocrinol* 44:269–278.
- Heideman PD. 2000. Environmental regulation of reproduction. In Crichton EG, Krutzsch PH, editors. *Reproductive Biology of Bats*. London: Academic Press. p. 469–499.
- Jensen E, Wood C, Keller-Wood M. 2002. The normal increase in adrenal secretion during pregnancy contributes to maternal volume expansion and fetal homeostasis. *J Soc Gynecol Investig* 9:362–371.
- Johnstone HA, Wigger A, Douglas AJ, Neumann ID, Landgraf R, Seckl JR, Russell JA. 2000. Attenuation of hypothalamic-pituitary-adrenal axis stress responses in late pregnancy: changes in feedforward and feedback mechanisms. *J Neuroendocrinol* 12:811–822.
- Jones DP, Kunz TH. 2000. *Pteropus hypomelanus*. *Mammalian Species* 639:1–6.
- Krishna A, Singh K, Doval J, Chanda D. 1998. Changes in circulating insulin and corticosterone concentrations during different reproductive phases and their relationships to body weight and androstenedione concentration of male *Scotophilus heathi*. *J Exp Zool* 281:201–206.
- Kunz TH, Hood WR. 2000. Parental care and postnatal growth in the Chiroptera. In Crichton EG, Krutzsch PH, editors. *Reproductive Biology of Bats*. London: Academic Press. p. 415–468.
- Kurta A, Kunz TH. 1987. Size of bats and birth and maternal investment during pregnancy. *Symposium Zool Soc Lond* 57:79–106.
- Kwiecinski GG, Damassa DA. 2000. Peripheral endocrines in bat reproduction. In Crichton EG, Krutzsch PH, editors. *Reproductive Biology of Bats*. London: Academic Press. p. 65–89.
- Lightman SL, Windle RJ, Wood SA, Kershaw YM, Shanks N, Ingram CD. 2001. Peripartum plasticity within the hypothalamo-pituitary-adrenal axis. *Prog Brain Res* 133: 111–129.
- Mendoza SP, Mason WA. 1997. Attachment relationships in New World primates. *Ann NY Acad Sci* 807:203–209.
- Mickleburgh SP, Racey PA, Hutson A, editors. 1992. *Old World Fruit Bats: An Action Plan for the Family Pteropodidae*. Gland, Switzerland: IUCN.
- Racey PA. 1982. Ecology of Bat Reproduction. In Kunz TH, editor. *Ecology of Bats*. New York: Plenum Press. p. 57–104.
- Racey PA, Entwistle AC. 2000. Life-history and reproductive strategies of bats. In Crichton EG, Krutzsch PH, editors. *Reproductive Biology of Bats*. London: Academic Press. p. 363–414.
- Reeder DM, Widmaier EP, Kunz TH. 2002. Stress responsiveness in the solitary little golden-mantled flying fox (*Pteropus pumilus*): glucocorticoids, testosterone, and behavior. *Horm Behav* 41:486.
- Reeder DM, Kosteczko NS, Kunz, TH, Widmaier, EP. 2004. Changes in baseline and stress-induced glucocorticoid levels during the active period in free-ranging male and female little brown myotis, *Myotis lucifugus* (Chiroptera: Vespertilionidae). *Gen Comp Endocrinol* 136:260–269.
- Simmons NB. Order Chiroptera. 2004. In Wilson DE, Reeder DM, editors. *Mammal Species of the World. A Taxonomic and Geographic Reference* (3rd edition). Washington, DC: Smithsonian Institution Press (in press).
- Stern JM, Goldman L, Levine S. 1973. Pituitary-adrenal responsiveness during lactation in rats. *Neuroendocrinol* 12:179–191.
- Wade GN, Schneider JE. 1992. Metabolic fuels and reproduction in female mammals. *Neurosci Biobehav Rev* 16: 235–272.
- Wadhwa PD, Sandman CA, Garite TJ. 2001. The neurobiology of stress and human pregnancy: implications for prematurity and development of the fetal central nervous system. *Prog Brain Res* 133:131–142.
- Walker CD, Toufexis DJ, Burlet A. 2001. Hypothalamic and limbic expression of CRF and vasopressin during lactation: implications for the control of ACTH secretion and stress hyporesponsiveness. *Prog Brain Res* 133:99–110.
- Widmaier EP, Harmer TL, Sulak AM, Kunz TH. 1994. Further characterization of the pituitary-adrenocortical responses to stress in Chiroptera. *J Exp Zool* 269: 442–449.
- Widmaier EP, Kunz TH. 1993. Basal, diurnal, and stress-induced levels of glucose and glucocorticoids in captive bats. *J Exp Zool* 265:533–540.