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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)

DeeAnn M. Reeder,* Nicole S. Kosteczko, Thomas H. Kunz, and Eric P. Widmaier

Department of Biology, Boston University, Boston, MA 02215, USA

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Abstract

Baseline and stress-responsive glucocorticoid (GC) levels were characterized during the active period in free-ranging male and reproductive female little brown myotis (*Myotis lucifugus*). Bats were trapped and blood was sampled within 3 min of capture at two maternity sites during the summer and at one swarming site prior to hibernation in New England. Both GC hormones, cortisol and corticosterone, were detected, with cortisol accounting for an average of ~95% of total circulating GCs. Samples collected at the dusk emergence and after the first return from feeding showed significant seasonal differences across the active period (early pregnancy, mid-to-late pregnancy, lactation [and comparable mid-summer times for males], and pre-hibernation) within and between each sex. Elevated baseline values were found in mid-to-late pregnancy females at emergence, and in both males and females at the swarming site compared to other groups. Female GC values during mid-to-late pregnancy and during the pre-hibernation period were greater than those for males. Significantly higher GC levels following 15 min of restraint were exhibited by all animals in the summer and prior to hibernation. There was little variation between groups or sexes in the total GC levels reached following restraint. Taken together, these results suggest that: (1) GCs may be involved in the increased feeding and/or fat deposition characteristic of pregnancy and the pre-hibernation period, (2) GCs may be related to mating and to the generally increased levels of activity that occur during the pre-hibernation period, and (3) regardless of sex or reproductive condition, all animals maximally respond to restraint stress.

Keywords: Glucocorticoid; Stress; Seasonality; Free ranging; *Myotis lucifugus*; Pregnancy; Lactation; Pre-hibernation

1. Introduction

Animals live in a world of both predictable and unpredictable change. A variety of physiological mechanisms have evolved in response to these changes, including seasonal and stress-responsive shifts in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis and attendant secretion of the glucocorticoid (GC) hormones cortisol and corticosterone (McEwen and Wingfield, 2003; Romero, 2002). Seasonal changes in baseline and stress-induced GC levels have been determined for numerous free-ranging vertebrate species (Romero, 2002), but have rarely been documented in free-ranging mammals. The scarcity of baseline studies of GCs in mammals is largely due to methodological difficulties in trapping and obtaining blood samples within the ~3 min window necessary to avoid measuring the GC response to capture and sampling itself. Notable exceptions are studies of arctic ground squirrels (*Spermophilus parryii*) (Boonstra et al., 2001), yellow-pine chipmunks (*Tamias amoenus*) (Kenagy and Place, 2000; Place and Kenagy, 2000), and little brown myotis (Widmaier et al., 1994, 1997), in which baseline samples were collected within 3 min of capture. Because of the vital importance of GCs in modulating reproductive fertility, metabolic balance, and immune function (McEwen and Wingfield, 2003; Sapolsky et al., 2000), it is important to understand how baseline and stress-

* Corresponding author. Fax: 1-208-445-2436.
E-mail address: dreeder@bu.edu (D.M. Reeder).

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induced GCs vary across seasons and with respect to reproductive status in free-ranging mammals.

Pregnancy and lactation are the two most metabolically demanding periods of a mammal's life history (Wade and Schneider, 1992). Not surprisingly, these periods are associated with changes in the highly catabolic GC hormones. In laboratory mammals, GC levels generally increase during pregnancy in maternal plasma and then decrease following parturition and during lactation (e.g., Atkinson and Waddell, 1995). By contrast, stress-induced GC levels are decreased during pregnancy and particularly during lactation (Lightman et al., 2001; Stern et al., 1973). This phenomenon may reflect a selection pressure to minimize large fluctuations in GCs both in the fetus and in the neonate (in which GC enter via maternal milk, Lightman et al., 2001). High GC levels are known to negatively influence a number of developmental processes and subsequent health and survival (Wadhwa et al., 2001). In contrast to what has been determined from laboratory studies on how pregnancy and lactation affect the function of the HPA axis, and consequently baseline and stress-induced GC levels, these responses in free-ranging mammals are poorly understood.

For species that have evolved in seasonal environments, offspring survival is enhanced by timing the energetically expensive periods of pregnancy and lactation during periods of high resource availability. After rearing their offspring, some species conserve energy by hibernating during cold months, which adds an additional layer of complexity to the patterns of GC secretion. One such species is the little brown myotis (*Myotis lucifugus*), a small insectivorous bat (7–11 g) found throughout much of North America. Like other temperate bats, this species has food available for only part of the year, which imposes severe energetic constraints. This and other hibernating species survive by performing their most energetically demanding activities during the summer months and by utilizing a variety of energy conserving mechanisms. For little brown myotis, these mechanisms include a dissociated pattern of reproduction in which mating occurs in the fall when gonads are quiescent, followed by pregnancy and lactation in spring and summer (Gustafson, 1979), deposition of fat prior to hibernation (Kunz et al., 1998), and alterations in thermoregulatory balance (Humphrey and Cope, 1976; Studier and O'Farrell, 1972). By separating the behavioral and physiological components of reproduction, these bats have distributed the costs of reproduction over a period with vastly different metabolic states (fat storage vs fat depletion). How these different metabolic states affect baseline and stress-induced GC activity, however, is unclear. Gustafson and Belt (1981) found elevated non-baseline cortisol levels prior to hibernation in male little brown myotis held overnight in the laboratory, which suggests alterations in HPA activity at this time, but which must be assessed in true baseline samples.

The objectives of this study were to characterize baseline and stress-induced GCs throughout the active period in free-ranging male and reproductively active female *M. lucifugus*. We sought to describe seasonal patterns of cortisol and corticosterone in two baseline conditions: (1) dusk, following a prolonged (~14 h) daytime fasting period and (2) post-prandial, upon return from their first nightly (~2 h) feeding. Variations in stress responsiveness in males and females in different reproductive stages were assessed by restraining animals upon the return from feeding. We predicted that: (1) GC levels would be higher upon emergence following the daily fast than upon the return from feeding; (2) GC levels would be higher both at emergence and post-prandial in late pregnancy compared to early pregnancy or lactation; (3) GC levels would decrease during pregnancy and lactation after restraint stress; (4) baseline GC levels would increase in the pre-hibernatory fattening period (possibly compensated for by a decrease in stress-induced GC levels); and (5) GC levels in females would be greater than those in males, as is typical of most mammals that have been studied (e.g., in rats, Handa et al., 1994).

2. Methods

2.1. Animals

Myotis lucifugus is a seasonal breeder that mates in the fall prior to hibernation. Females store sperm during the winter hibernation until spring arousal, at which time ovulation and fertilization occur (Buchanan, 1987). Females then leave their hibernacula and migrate to maternity colonies containing several hundred to several thousand pregnant females (Humphrey and Cope, 1976). Gestation is approximately 60 days long, with birth of a single offspring occurring in mid-to-late June. The subsequent lactational period continues until pups are volant and weaned (in summer; Kurta et al., 1989). In males, spermatogenesis is suspended during hibernation and resumes upon spring arousal with a peak in late summer (Gustafson and Shemesh, 1976). Occasionally, a small number of males may be found associated with maternity colonies, but most males spend the summer in roosts separate from females. In both males and females, feeding typically occurs during the spring and summer in two discrete bouts, the first at dusk and the second in the early morning hours prior to dawn. By contrast, in the fall, when animals are mating in the vicinity of hibernacula, there is not a clear emergence nor return to the roost, as animals typically fly around the vicinity of the cave opening from dusk until dawn ("swarming"; Humphrey and Cope, 1976; Thomas et al., 1979).

A total of 142 adult female and 36 adult male *M. lucifugus* were captured in a harp trap (Kunz and Kurta, 1988) for blood collection during a total of 21 trap nights between June and September of 2002. Animals were trapped at two maternity colonies in southern New Hampshire and a swarming site in Vermont. The two maternity colonies were located in old barns that were approximately 5 km apart. Bats from these and other nearby maternity colonies are known to migrate to and swarm and hibernate at the Vermont locality. Little brown myotis display high site fidelity, especially for females and their maternity colonies, thus these two barns represented distinct populations. However, the animals in each of these barns were subject to nearly identical environmental conditions due to their similar structures and proximity. Insufficient data exist to compare hormone levels between the two maternity sites, but their close physical proximity and indistinguishable reproductive timing (births occurred at approximately the same time in each barn) argue that they are equivalent. At the maternity colonies, samples were collected in June and July upon emergence at dusk (~20:15 to 21:00 h) and from animals returning from feeding (22:30 to 01:00 h). Over 60% of the total daily food intake in *M. lucifugus* occurs in the first 2 h after emergence (Anthony and Kunz, 1977). Females sampled from these sites were classified as being either in early pregnancy, mid-to-late pregnancy (fetus with palpable skull), or lactating. At the swarming site, which was located at a cave approximately 100 km northeast of the maternity colonies, samples were collected on two nights in September beginning at emergence (~19:00 to 19:45 h) and continuing until 00:30 h. Samples were only collected from parous females (as indexed by nipple condition) and by males that were mature (as indexed by reduced testicular size, distended cauda epididymides, and associated reduction of pigmentation of the tunica vaginalis; Racey, 1988). Animals at this site were most likely from a variety of maternity colonies and other sites in the region, as the average migration for little brown myotis is 100 km (Humphrey and Cope, 1976). It is likely, but we cannot say for certain, that these animals experienced similar reproductive events and environmental conditions as the animals sampled at our maternity colonies in the summer. Animals at this site largely remained in the vicinity of the cave entrance and likely did not feed due to light rain and temperatures between 5 and 10 °C on the nights of capture.

2.2. Sampling

Animals were trapped at each site with harp traps (Kunz and Kurta, 1988), which allows for auditory detection of capture and for rapid retrieval. Upon hearing an animal enter the trap, a stopwatch was started and animals were quickly hand captured and brought to a staging area where a blood sample (20–100 μl) was collected until approximately 3 min after capture (average = 180 s ± 2.0 SE). In most cases, samples were collected by lancing the uropatagial vein with a 26 gauge needle and collecting blood into heparinized capillary tubes, although in a few cases samples were collected by decapitation to ensure sufficient blood was obtained from individual animals in which sample populations were limited (e.g., males). Capillary tubes were sealed with critoseal and centrifuged within approximately 15 min using a portable centrifuge (LW Scientific Zipocrit) powered by a portable solar battery (Solar World Solar Power Pak 8000). Approximately half of the animals sampled following return from feeding at the maternity colonies and during the collection period at the swarming site were not bled immediately, but rather placed into small restraint devices, consisting of short segments of PVC pipe with slits for wing exits on either side and partial closing at one end with a strip of Velcro. Blood samples were collected from these individuals as described above at the end of 15 min of restraint. After sample collection, reproductive condition, body mass, and length of forearm were determined. Despite efforts to minimize disturbance at sampling sites, it is important to note that both sampling at emergence and upon the return from feeding represent "baseline" rather than "basal" samples, as we do not know whether our presence or the presence of a trap had an effect on their physiology (Romero, 2002). Animals captured in the trap but not bled were marked each night with an all-weather non-toxic paint stick (LA-CO Industries) to ensure that they were not subsequently selected for a baseline bleed if caught again on the same night. Only one blood sample was collected from each animal during the study.

2.3. Hormone analysis and statistics

Cortisol and corticosterone were each determined in duplicate with 2–5 μl plasma using commercially available radioimmunoassay (RIA) kits (ICN Biochemicals, Irvine, CA) as previously described (Widmaier and Kunz, 1993). In those cases where little plasma was available, equal amounts of plasma from 2 to 3 samples (from the same sex, reproductive stage, and sampling condition) were pooled. Of the 178 usable samples collected, 84 were combined to create a total of 36 pooled samples. The remaining 94 samples were assayed directly.

Both radioimmunoassay kits employ antisera from rabbits inoculated with corticosterone or cortisol conjugated to 3-carboxymethyloxime:BSA. Parallelism with the standard curve for each hormone was determined for two pooled samples (see Fig. 1). Based upon these results and those from Widmaier and Kunz (1993), we concluded that an extraction step was not

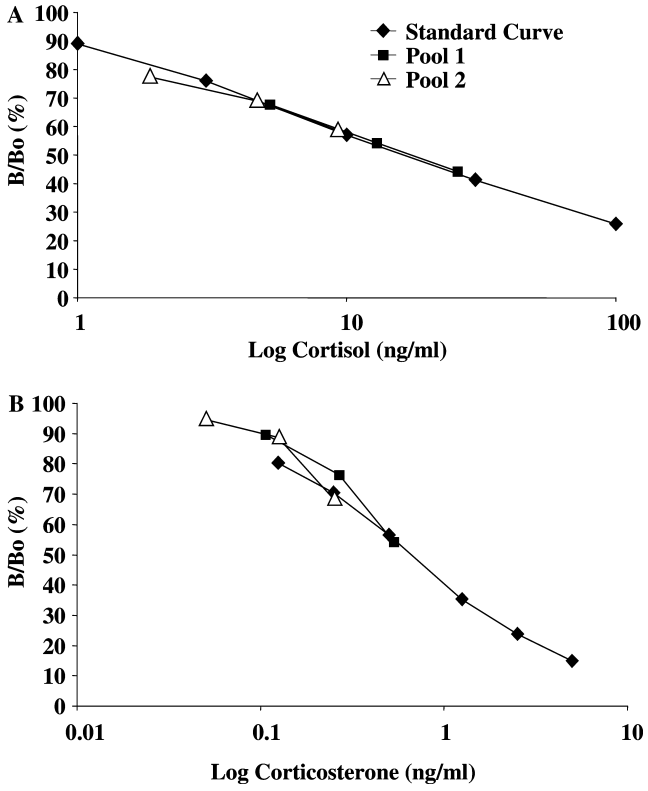

Fig. 1. Serial dilutions of two pooled *M. lucifugus* plasma samples demonstrate parallelism with the standard curve for both cortisol (A) and corticosterone (B).

necessary. Negligible cross-reactivity (<0.05%) of the corticosterone assay for cortisol and vice versa (3%) is reported for these kits by the manufacturer and was validated in our laboratory for little brown myotis plasma. Briefly, steroids were stripped from pooled *Myotis* plasma using double extraction with five volumes of dichloromethane, which extracted an average of 94.7 and 87.4% of radiolabeled cortisol and corticosterone, respectively. Purified cortisol or corticosterone solutions in ethanol were then added to steroid-stripped bat plasma samples, which were then subjected to the assay procedures. Cortisol added to bat plasma was not detected in the corticosterone assay and vice versa indicating no cross-reactivity. All samples were assayed in two assays per hormone. Intra-assay variabilities were 12.4 and 6.0% for cortisol and corticosterone, respectively. Interassay variabilities could not be calculated because all samples were analyzed in only two assays; however, this figure is typically 20% or less in our laboratory.

All data were analyzed non-parametrically, using either two-tailed Kruskal–Wallis (KW) tests or two-tailed Mann–Whitney *U* tests to detect differences between groups. All *p* values were corrected for multiple comparisons using the Bonferroni method where appropriate.

3. Results

Data within and across groups were variable, most likely due to the range of field conditions. Despite the variability in the data, however, a number of significant differences in GC levels were found between groups. Not surprisingly, plasma cortisol and corticosterone showed nearly identical profiles (see Figs. 2–4), and, with one notable exception (higher corticosterone levels following restraint in females prior to hibernation compared to pregnant and lactating females), yielded similar statistical results. Cortisol levels were much higher than corticosterone in both males and females across reproductive stages and pre- or post-prandial, with corticosterone accounting for an average of 4.77% (±2.29 SD) of total GC levels.

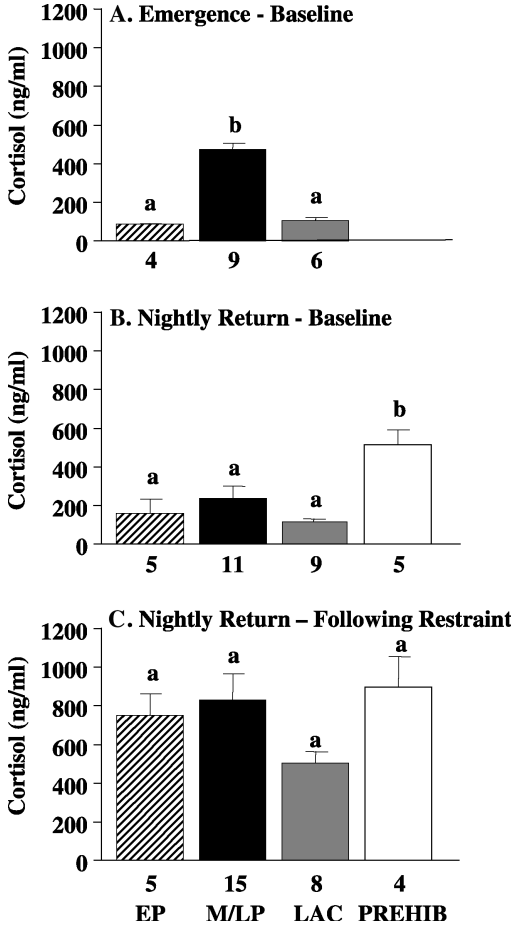

Fig. 2. Cortisol (means ± SE) in female *M. lucifugus* in different reproductive states (EP, early pregnancy; M/LP, mid-to-late pregnancy; LAC, lactating; and PREHIB, pre-hibernation) under different conditions [(A) Baseline bleed upon emergence at sunset after prolonged fasting; (B) baseline bleed 2–3 h later, upon return from the first feeding bout; and (C) following 15 min of restraint after returning from the first feeding bout]. There was no "emergence" condition in the pre-hibernation state (see text). Significant differences within each condition are indicated by different letters; significant differences between conditions are indicated in the text.

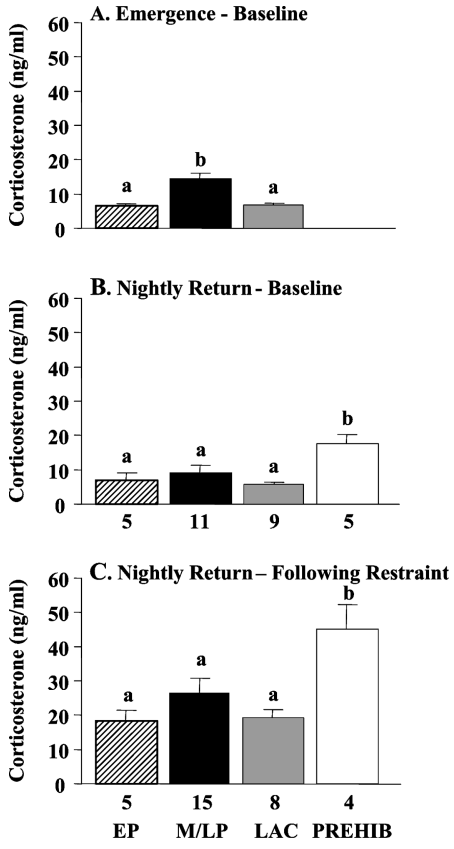

Fig. 3. Corticosterone (means ± SE) in female *M. lucifugus* in different reproductive states (EP, early pregnancy; M/LP, mid-to-late pregnancy; LAC, lactating; and PREHIB, pre-hibernation) under different conditions [(A) baseline bleed upon emergence at sunset after prolonged fasting; (B) baseline bleed 2–3 h later, upon return from the first feeding bout; and (C) following 15 min of restraint after returning from the first feeding bout]. There was no "emergence" condition in the pre-hibernation state (see text). Significant differences within each condition are indicated by different letters; significant differences between conditions are indicated in the text.

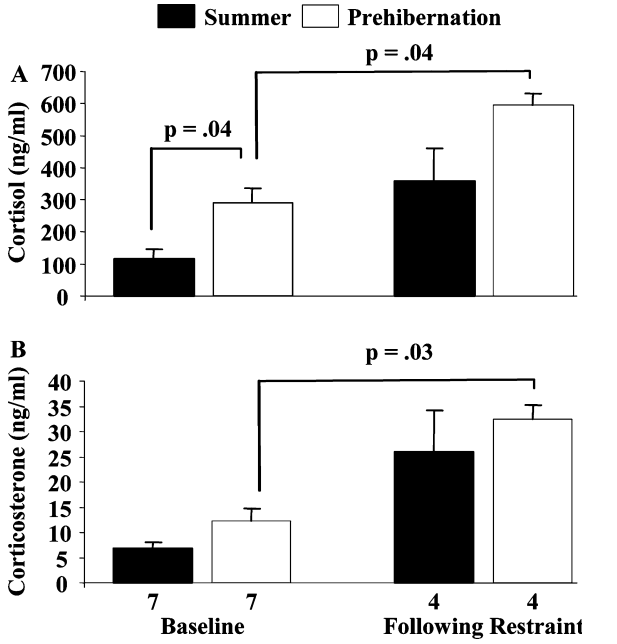

Fig. 4. Cortisol (A; means ± SE) and corticosterone (B) in male *M. lucifugus*. Samples were collected under baseline conditions (emergence and return from feeding combined) and following 15 min of restraint in early to mid summer and prior to hibernation. Significant differences for each hormone are indicated.

3.1. Females

GC levels at emergence varied significantly by reproductive stage (see Fig. 2A for cortisol and Fig. 3A for corticosterone; for cortisol, Kruskal–Wallis test—$X^2 = 13.5$, $df = 2$, $p = 0.001$; for corticosterone, $X^2 = 13.5$, $df = 2$, $p = 0.001$), with significantly higher levels of both cortisol and corticosterone upon emergence in mid-to-late pregnancy females relative to both early pregnancy and lactating females (cortisol: mid-to-late pregnancy vs early pregnancy, $U = 36$, $Z = -2.78$, $p = 0.01$; vs lactation, $U = 54$, $Z = -3.18$, $p = 0.003$; corticosterone: $U = 36$, $Z = -2.78$, $p = 0.01$; $U = 54$, $Z = -3.18$, $p = 0.003$). There were no significant differences in emergence values between females in early pregnancy and lactating females. Pregnant and lactating females showed the same baseline patterns of plasma GC levels upon return from feeding as they did at emergence, with elevated cortisol (Fig. 2B) and corticosterone (Fig. 3B) levels in mid-to-late pregnancy vs both early pregnancy and lactation, although these differences were not significant. Females in mid-to-late pregnancy had significantly lower GC levels upon return from feeding than they did at emergence (cortisol: $U = 87$, $Z = -2.85$, $p = 0.004$; corticosterone: $U = 80$, $Z = -2.34$, $p = 0.02$). However, there were no significant differences in emergence and return values for females in early pregnancy or lactation. Baseline GC levels in pre-hibernating females, which were collected at approximately the same time as the return samples in the summer (but which differed in that they were not necessarily post-prandial) were significantly greater than those of pregnant and lactating females (see Figs. 2B and 3B; cortisol: $X^2 = 11.74$, $df = 3$, $p = 0.01$; corticosterone: $X^2 = 8.95$, $df = 3$, $p = 0.03$).

Females in all groups exhibited elevated GCs following 15 min of restraint (Figs. 2C and 3C; pregnant and lactating females: cortisol: $U = 667$, $Z = -5.65$, $p < 0.0005$; corticosterone: $U = 666$, $Z = -5.63$, $p < 0.0005$; pre-hibernating females: cortisol: $U = 18$, $Z = -1.96$, $p = 0.05$; corticosterone: $U = 20$, $Z = -2.45$, $p = 0.01$), with total GC levels of up to 1440 ng/ml. Despite lower cortisol levels following restraint in lactating females relative to all other females (including those females in the pre-hibernation period, for which higher baseline GC levels were recorded) there were no significant differences between female groups in the plasma cortisol level following restraint (Fig. 2C, $X^2 = 5.22$, $df = 3$, $p = 0.16$). In contrast, the level of

corticosterone reached after restraint did differ between female groups, with significantly higher levels of corticosterone in pre-hibernation females compared to pregnant and lactating females (Fig. 3C, $X^2 = 9.4$, $df = 3$, $p = 0.02$).

3.2. Males

Capturing males in the summer was a rare event as they are not usually found in proximity to maternity colonies. However, a total of 11 samples or pooled samples were collected from adult males captured at maternity sites. Due to the small sample size available, data from both baseline conditions (emergence and return) from the summer were combined for the baseline values for male–male analyses (all values were roughly comparable; for cortisol, 68.9 and 88.8 ng/ml in the two returning males sampled and 129.5 ± 41.5 ng/ml in males at emergence [$N = 5$]; for corticosterone, 4.4 and 11.1 ng/ml in the two returning males sampled and 6.5 ± 1.5 ng/ml in males at emergence). As was the case for females, males sampled in the summer baseline conditions had lower GC levels than pre-hibernating males (Fig. 4), but this difference was only significant for cortisol ($U = 45$, $Z = -2.62$, $p = 0.04$; for corticosterone, $U = 39$, $Z = -1.85$, $p = 0.06$).

Both groups of males, those captured in the summer and those captured prior to hibernation, exhibited higher levels of GCs following 15 min of restraint (Fig. 4), which were significant for the pre-hibernation males but failed to be significant after correction for multiple comparisons for males sampled in the summer (summer males, cortisol: $U = 25, Z = -2.08, p = 0.08$; corticosterone: $U = 26$, $Z = -2.23$, $p = 0.07$; pre-hibernation males, cortisol: $U = 27$, $Z = -2.48$, $p = 0.04$; corticosterone: $U = 28$, $Z = -2.65$, $p = 0.03$). There were no statistically detectable differences between summer and pre-hibernation males in the plasma GC levels following restraint.

3.3. Males vs females

Sufficient data exist to assess sex differences from summer samples at emergence and following 15 min of restraint. When only the emergence data from Fig. 4 are considered, male GC levels at emergence (129.5 ± 41.5 ng/ml for cortisol and 6.5 ± 1.5 ng/ml for corticosterone) resembled those for early pregnancy and lactating females, but were significantly lower than those for mid-to-late pregnancy females (cortisol: $U = 44$, $Z = -2.87$, $p = 0.01$; corticosterone: $U = 42$, $Z = -2.6$, $p = 0.03$). During the summer, males had significantly lower levels of cortisol ($U = 91$, $Z = -1.99$, $p = 0.05$) but not corticosterone following 15 min of restraint than did pregnant or lactating females. Prior to hibernation, female GC levels were higher than male levels in both the baseline condition and following 15 min of restraint, but these differences were only significant for cortisol in the baseline condition ($U = 32$, $Z = -2.36$, $p = 0.02$).

4. Discussion

As predicted, clear seasonal patterns and sex differences were found in plasma GC levels in both baseline conditions. We had also predicted seasonal and sex differences in the plasma levels of both cortisol and corticosterone following restraint, but this was only found for corticosterone in females sampled prior to hibernation vs pregnant and lactating females. Our inability to detect differences between some groups may be due to variations in field conditions such as weather and nightly food intake, and also to insufficient power stemming in some cases from small sample sizes. This is especially the case for males, which we rarely trapped in the summer, and for animals sampled at the remote swarming site prior to hibernation.

The higher levels of GCs predicted at emergence compared to the return from feeding were found only for those females in mid-to-late pregnancy, which may reflect the orexogenic properties of GCs, as both food consumption (most of which is done in the 2 h between emergence and the first return) and body fat increase during pregnancy in *M. lucifugus* (Anthony and Kunz, 1977; Widmaier et al., 1997). Widmaier et al. (1997) also found higher levels of hypothalamic corticotropin-releasing hormone (CRH) and neuropeptide Y (NPY) as well as plasma leptin during late pregnancy in *M. lucifugus*, all suggesting a shift in the neuroendocrine control of energy balance at this time.

The predicted increase in GC levels in mid-to-late pregnancy and decrease in GC levels following parturition was evident in both baseline conditions, but was only significant for the emergence condition. In males, as expected, baseline GC levels during the summer were significantly less than those found in females during mid-to-late pregnancy. By contrast, the observation that GC levels in males during the summer were not significantly different from those of females during early pregnancy and lactation may be explained by the relatively low levels of testosterone found in males at this time (Gustafson and Shemesh, 1976), such that the generally suppressive effects of testosterone on GCs are not present (Handa et al., 1994; Viau, 2002). Further studies should attempt to replicate this finding with greater sample sizes and should explore the possible interrelationship between GCs and testosterone at this time.

In contrast to laboratory rats, which show slightly reduced GC levels during lactation at the peak of the circadian rhythm compared to cycling and post-parturient non-lactating females (Stern et al., 1973), GC levels in

lactating female *M. lucifugus* at the peak of their daily cycle were not distinguishable from those of females in early pregnancy or from those of males. It is possible, however, that GC levels are elevated during other parts of the circadian rhythm that would result in variations in total daily GC output, as observed in rodents (Stern et al., 1973). In laboratory rats, elevations of GCs attendant to chronic stress occur at the circadian trough and have unique metabolic consequences, resulting in fat deposition (Dallman et al., 2000). Clearly, future examination of circadian rhythm alterations in GC levels in relation to reproductive stage in *M. lucifugus* would be useful.

The marked behavioral transition that occurs from summer to fall, in which animals migrate to swarming sites and prepare for hibernation by depositing large quantities of fat (an increase of 32.9 and 29.6% in body mass for males and females, respectively; Kunz et al., 1998), is also accompanied by shifts in activity patterns and in baseline glucocorticoid profiles. In both male and female *M. lucifugus*, GC levels increased several fold, to levels in the same range as those observed upon emergence in mid-to-late pregnancy females. Although these pre-hibernating samples were collected at a time comparable to the return from feeding in the summer, they may represent a fasted condition due to rainfall and cold temperatures at the swarming site on the nights sampled. Additionally, at swarming sites, there is not a clear emergence nor return to the roost, as animals typically fly around the vicinity of the cave opening from dusk until dawn and may even fly within the cave during the day (Humphrey and Cope, 1976). Elevated GCs observed at this time have been reported prior to hibernation in male *M. lucifugus* sampled up to 24 h after capture (Gustafson and Belt, 1981) and in other species (e.g., free-ranging yellow-bellied marmots, *Marmota flaviventris*, Armitage, 1991; captive European ground squirrels, *Spermophilus citellus*, Shivatcheva et al., 1988; captive garden dormice, *Eliomys quercinus*, Boulouard, 1971; and captive European hedgehogs, *Erinaceus europaeus*, Saboureau et al., 1980; see also review in Gustafson and Belt, 1981), but unlike the present study, the samples collected in those studies were either not baseline samples or not from free-ranging animals.

Elevated GCs in the pre-hibernation period in mammals likely reflect and serve a variety of purposes. One function of GCs at this time appears to be the promotion of feeding via central control mechanisms (Dallman et al., 1995). Although generally known for their catabolic actions, GCs have a variety of anabolic effects in mammals (Dallman et al., 1995; Romero, 2002). For example, in laboratory rats, intracerebroventricular administration of GC promotes feeding behavior and gain in body mass (Green et al., 1992). Further evidence for a link between GCs, hyperphagia and increases in body fat comes from birds, where GCs are linked to parental hyperphagia (e.g., in ring doves, *Streptopelia risoria*, Koch et al., 2002) and to migratory fattening (characterized by hyperphagia and lipogenesis) in several species (e.g., yellow-rumped warblers, *Dendroica coronata*, Holberton, 1999). Physiological changes are known to occur in other systems during the pre-hibernatory fattening period in *M. lucifugus* (e.g., the dissociation between leptin and fattening, such that low levels of leptin are secreted despite high adipose tissue mass, Kronfeld-Schor et al., 2000), thus changes in HPA regulation are also likely. If GCs do indeed play a role in pre-hibernation fattening in *M. lucifugus*, then we would expect the increase in GCs observed during the pre-hibernation period to occur prior to fattening. As we did not collect samples during the period of greatest gain in body mass (mid-August), future studies should include sampling during this period.

The elevated baseline GCs during the pre-hibernation period may also be related to the generally higher levels of activity seen at the swarming sites (Humphrey and Cope, 1976) and the mating activity at this time. In fact, it has been suggested that seasonal rhythms in GC levels are related to the behavioral effects of GCs (Romero, 2002; Sapolsky et al., 2000). Higher GC levels are found during the mating season in a variety of mammals, both in the field and in captivity (Romero, 2002). However, the applicability of physiological data from most other species studied to *M. lucifugus* is questionable as most have associated patterns of reproduction, with mating and gonadal activity occurring simultaneously. Notwithstanding, in all species, a variety of energetically expensive behaviors are associated with mating, including male–male aggression, courtship, and copulation, which should theoretically influence energy balance and HPA regulation. Although there is little evidence for aggression in male *M. lucifugus*, the energy expended during the prolonged nightly activity period attendant to swarming and to courtship must be great for both males and females, as females actively solicit males as well (Thomas et al., 1979). In fact, both males and females reach maximum relative fat stores before mating and use up some of these fat stores prior to entering hibernation (Kunz et al., 1998). Perhaps high GCs at this time can centrally promote feeding (anabolic) while at the same time free up peripheral energy resources (catabolic) for mating. For many species, male sexual behavior is partially regulated by the activational effects of testosterone, which are typically related to decreased GC levels (e.g., breeding yellow-pine chipmunks, Handa et al., 1994; Place et al., 2002; Viau, 2002). By disassociating male sexual behavior and testosterone, such that testosterone levels are relatively low during the pre-hibernatory mating period (Gustafson and Shemesh, 1976), male *M. lucifugus* have theoretically eliminated this inhibitory influence. Low but detectable levels of progesterone (Buchanan and YoungLai, 1988) and presumably of estrogen in females

during the pre-hibernatory period may explain the higher levels of GCs in females vs males at this time, as estrogen can stimulate adrenal GC production and corticosteroid-binding globulin (CBG) synthesis (Coe et al., 1986) and may modulate glucocorticoid receptor-mediated negative feedback mechanisms in the hypothalamus (Handa et al., 1994).

Significantly higher GC levels following 15 min of restraint were evident in females in all conditions and in males sampled prior to hibernation, with non-significantly higher values in the males sampled during the summer. Despite the differences in baseline values between males and females and between bats sampled in the summer and those sampled prior to hibernation, a significant difference in GC levels following restraint was only detected for corticosterone (which was only 5.14% of total GC) in pre-hibernation females. Thus, the roughly equivalent stress GC values despite contrasting baseline values suggest differential responses to restraint stress on the part of each group. For example, the difference between the baseline and restraint stress values in the pre-hibernation phase in both sexes is much less than the difference between baseline and restraint stress in animals sampled in the summer, suggesting a dampened response to at least this one stressor during pre-hibernation, as we predicted. Alternatively, our failure to find any differences in restraint stress GC values between either sex and across all conditions may reflect our sampling regime or an HPA axis that is secreting at maximum levels, independent of baseline values. It is important to note that our failure to find differences in GC levels following restraint between the different groups does not necessarily indicate a lack of differences in HPA regulation between groups and across the season. The two distinct levels of GCs, baseline levels and elevated stress levels, are known to be regulated differentially, serve different functions, and act through different receptor populations, with GC type I receptors activated at baseline levels and type II receptors activated at higher levels (Munck and Náray-Fejes-Tóth, 1992; Sapolsky et al., 2000). Additionally, changes in either receptor levels or levels of CBG could theoretically enhance or buffer the effects of high GCs.

Cortisol was the major GC secreted in *M. lucifugus* and both cortisol and corticosterone showed similar plasma profiles across the sampling periods. Boonstra et al. (2001) found similarly low levels of corticosterone relative to cortisol in arctic ground squirrels, as did Kenagy and Place (2000) and Place and Kenagy (2000) in yellow-pine chipmunks. In these chipmunks, cortisol levels were 25- to 50-fold higher than corticosterone and both GCs varied seasonally in synchrony. In contrast, in golden-mantled ground squirrels (*Spermophilus saturatus*), where cortisol levels are roughly twice that of corticosterone, seasonal patterns of cortisol and corticosterone are not synchronous (Boswell et al., 1994). In the present study, we sampled GCs only during part of the circadian cycle. We previously reported a trend for greater levels of corticosterone vs cortisol at the trough of the circadian rhythm in *M. lucifugus* (Widmaier et al., 1994), suggesting a diurnal shift away from the 17-hydroxylation pathway. Thus, future studies on stress-induced steroid levels during different reproductive states would benefit from additional sampling times. The finding that pre-hibernation females sampled following restraint had significantly higher corticosterone (but not cortisol) levels compared to pregnant or lactating females was intriguing, but whether this reflects increased zona glomerulosa or fasciculata activity of the adrenal cortex is unknown because aldosterone levels were not measured in this study.

In addition to their behavioral and physiological uniqueness, *M. lucifugus* are also unique in that they can be readily captured from large free-ranging populations and easily and rapidly bled in the field, allowing for the proper assessment of baseline conditions that is so difficult to achieve in most other free-ranging mammals. *M. lucifugus* is only one of 102 species in the globally distributed genus *Myotis*, and only one of the 1111 species of bats (Simmons, in press); thus, the opportunities for comparative work with other species of bats are plentiful and promising. As shown in male arctic ground squirrels (*S. parryii*) and red squirrels (*Tamiasciurus hudsonicus*), even closely related species living in the same environment can have profoundly different responses to stress that are most likely related to differences in life histories (Boonstra and McColl, 2000). Because *M. lucifugus* is found in a wide range of environmental conditions throughout North America, they provide an excellent system for exploring not only interspecific, but also intraspecific variations in neuroendocrine relationships as they relate to the environment (e.g., as have been described for the HPA axis in arctic ground squirrels and dark-eyed juncos, *Junco hyemalis*, Hik et al., 2001; Holberton and Able, 2000). For example, low environmental temperatures, which typically covary with latitude and which typically result in decreased insect availability, result in longer gestation periods for a number of bat species (Racey, 1982; Speakman and Thomas, 2003) and greater reproductive synchrony at the highest latitudes (Schowalter et al., 1979). We predict that *M. lucifugus* would display variations in endocrine physiology related to the availability and predictability of resources in their different environments.

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