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Seasonal Changes in Plasma Glucocorticosteroids of Free-Living Female Yellow-Pine Chipmunks: Effects of Reproduction and Capture and Handling

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We measured plasma levels of cortisol and corticosterone in female yellow-pine chipmunks (*Tamias amoenus*) while observing seasonal reproductive and life-history events by live-trapping a natural population during the active (nonhibernating) season. Both glucocorticosteroids (GCs) varied significantly from March through September, starting with minimal values at the time of mating (cortisol ~900 ng/ml, corticosterone ~50 ng/ml), rising to a peak by late lactation (cortisol ~1600 ng/ml, corticosterone ~175 ng/ml), and then declining prior to hibernation. Following their emergence from natal burrows, young of the year had GC levels indistinguishable from those of adults. Body mass also varied significantly over the season, increasing after mating and again after parturition to a peak in lactation, after which it declined steadily until hibernation. In addition to the use of standard trapping to describe seasonal hormonal patterns, we also trapped chipmunks using a special protocol to examine the effects of capture and handling on GCs; we obtained an initial (basal) blood sample immediately, within 1–3 min of observing a capture, and then a second sample 30 min after holding the animal in the trap. Chipmunks consistently increased GCs above the initial (basal) level during the 30 min after capture and initial handling; these significant increases in GCs ranged approximately 70–130% for cortisol and 50–190% for corticosterone, depending on season and reproductive state. GC levels at 30 min after capture and handling were similar to those obtained from samples drawn from our standard trapping and blood sampling. We conclude that although capture and handling increase the absolute level of plasma GC hormones, that effect does not obscure natural patterns of seasonal variation in GCs. Overall, our observations suggest an important role of adrenocortical activity in the energy balance of these free-living rodents in two different contexts: (1) the seasonal regulation of physiological state, including body mass, energy reserves, and reproductive function, and (2) an acute response to stimulatory events, encompassing physiological stress, as represented here by capture and handling. © 2000 Academic Press

Key Words: glucocorticosteroids; cortisol; corticosterone; seasonality; reproduction; lactation; capture and handling effects; stress; field endocrinology; body mass; chipmunk; *Tamias amoenus*.

Small mammals that breed seasonally in temperate latitudes undergo profound changes in physiological state, energy balance, and reproductive condition during the year. This is emphasized in hibernating species and in females in particular, due to the heavy energetic demand associated with lactation (Kenagy, 1987). Hormonal variations accompany these changes of state in response to seasonal environmental cycles (Bronson, 1989; Wingfield and Kenagy, 1991). These regulated seasonal changes preserve the homeostatic integrity of the animal's behavior and physiology over a variety of

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environmental conditions, while also inducing the necessary changes in body condition and reproductive state. The glucocorticosteroid (GC) hormones of the hypothalamo-pituitary–adrenal (HPA) axis include cortisol and corticosterone and are involved in the general homeostasis of energy, as well as in acute and chronic responses to stress (Norris, 1997). They also act more specifically in the mammary tissue to prepare for and maintain lactation (Voogt *et al.,* 1969; Walker *et al.,* 1992). Within their role in the regulation of energy balance in mammals (King, 1988), GCs produce a variety of anabolic effects (Berdanier, 1989), e.g., acting directly in the hypothalamus to promote feeding behavior (Green *et al.,* 1992).

GCs can either inhibit or stimulate reproductive physiology, depending on the timing of the annual cycle of a species (Brann and Mahesh, 1991). Both acute and chronic stresses, including socially mediated behavioral stress, are associated with the activation of GC hormone secretion in mammals. This response may have inhibitory influences on reproductive physiology and endocrinology and thereby on the status of individuals within a population of a social species (Selye, 1976; Sapolsky, 1987). A specific interaction of reproduction with a stress response has been observed in rats, in which the stress-related increase in plasma corticosterone is suppressed during lactation (Lightman and Young, 1989).

We have undertaken a study of the patterns of adrenocortical hormone activity in free-living female yellow-pine chipmunks (*Tamias amoenus*), which are seasonal breeders and hibernators (Kenagy and Barnes, 1988). We wanted to understand how the HPA axis of these animals operates under natural environmental conditions over the year and what additional effects on plasma GC levels result from capture and handling. We expected that the natural seasonality of plasma GC hormone concentrations in yellow-pine chipmunks would be interesting because of the potential role of GCs in regulation of energy balance and body mass and because these chipmunks display a single, conspicuous period of lactation centered in the 7-month above-ground activity season (Kenagy and Barnes, 1988).

Our study focused on assessing the level of plasma GCs in individual female chipmunks live-trapped in the field. We wanted to define and interpret the hormonal variation that occurs in relation to season and physiological state, particularly reproduction. Past studies of hormonal levels of free-living animals have suffered from the problem that capture and handling might have effects that could obscure natural or basal levels of hormones. We therefore also investigated the short-term (''acute'') effect of capture and handling on plasma GC concentration. Among sciurid rodents seasonal variation in reproductive steroid hormones of free-living animals has been reported in ground squirrels, *Spermophilus* (e.g., Holekamp and Talamantes, 1991), and seasonal GC patterns have been described for marmots, *Marmota* (Armitage, 1991) and ground squirrels (Boswell *et al.,* 1994). We are unaware of published articles on seasonality of GC hormones in small sciurids such as chipmunks, *Tamias* spp., and on the effects of capture and handling on these hormone levels in rodents in general.

METHODS

Animals. We studied individually marked chipmunks by live-trapping, examination, and release from Autumn 1995 through Summer 1998 at a previously described study site (Kenagy *et al.,* 1989). This main site is characterized as montane forest (elevation 670 m) and is located in Chelan County, Washington. We trapped an area of 3.8 ha with a 14×14 grid of 196 Sherman live traps on 15-m centers. We also investigated chipmunks at a nearby high-altitude site (1570 m) that lies within about 7 km of the main site and at which we established another grid of identical dimensions.

Sampling. Following initial observations and trapping in Fall 1995, we conducted a study to assess seasonal activity, reproductive condition, and plasma GC hormones in spring through late summer at the main site. We trapped 37 days from late March through September of 1996, 29 days from April through September of 1997, and 15 days from April through early June of 1998. Trapping at the high-altitude site was limited by late snow melt and consisted of 8 days in August through mid-September of 1996, 6 days in late May through mid-August of 1997, and 7 days in late June through mid-August of 1998. The numbers of individual females trapped (adults, young of year) at the

main site were as follows—1996 30, 32; 1997 29, 10; and 1998 19, 0. (We did no postweaning trapping in 1998.) Numbers of individual female chipmunks captured at the high-altitude site were 1996 32, 31; 1997 33, 35; 1998 25, 9. We report plasma hormone titers and body mass combined over short calendar intervals (1–2 weeks) and specified for a single median date. Most samples were collected from females bled only once or twice over the course of 7 months; one animal was bled six times and two individuals were bled seven times. No juvenile was sampled more than three times. For the study of the 30-min effect of capture and handling on plasma GC concentrations (see below) about 2/3 of the animals were sampled only once and 1/3 twice, and only one individual was sampled three times.

Traps were generally baited in the early morning with a birdseed mix and collected about 2 h later; we took traps to a central area for processing. The chipmunks were weighed with a Pesola spring balance to the nearest gram, while held in a bag, and then examined for reproductive condition and other external information. We interpreted a swollen or open vulva as indication of actual or potential mating activity and enlarged and lengthened teats (from which we often gently expressed milk manually) as indication of lactation. Animals were lightly anesthetized (10–20 s exposure) with ethyl ether inhalant before we took a blood sample from the infraorbital sinus. Blood was sampled within about 3 min of removing the animal from its trap. Despite rapid handling of individuals under routine conditions, we recognize that animals were typically held in the traps for 1–3 h before being sampled for blood, and therefore it is unlikely that GC concentrations were basal.

To measure basal GC levels and examine the adrenocortical response to capture and handling, we used a novel trapping protocol on dates separate from our routine trapping of the grid. We did this at the main (lower elevation) study site in 1996 on 13 days, before lactation (April 11–19), during lactation (May 22–30), and after lactation (July 15–24 and September 26 and 27), and in 1998 on 5 days during lactation (May 19–June 4). We ran the same protocol at the high-altitude site in 1998 on 4 days during lactation (June 24–July 1). By sitting quietly and directly observing a limited number of traps, each worker could quickly obtain a blood sample within 1 to 3 min of capture from animals that were observed entering the traps. We followed each of these initial samples with a second blood sample after allowing the animal to rest for 30 min in its trap. A tight cluster of up to 40 specially placed traps for each of one to three observers was continuously monitored, beginning in the early morning and continuing no later than noon. As soon as a trap door closed, the observer noted the time, ran to obtain the trap, and began processing the animal in the usual manner. As long as a blood sample was removed within 3 min of capture, we returned the animal to the trap for 30 min and then took a second blood sample. Of the 47 cases on which we report data, 36 (77%) were obtained within 2 min of capture, leaving 23% obtained 2–3 min after capture. To assess the effects of reproduction on the adrenocortical response, we conducted these tests both during and after the reproductive season.

Blood samples of about 200μl were taken for routine seasonal sampling on the grids and samples of 100μl for the two samples at 30-min intervals for assessment of the adrenocortical response to capture. Blood was stored in a cooler for several hours and centrifuged. Plasma was stored in 200-μl plastic containers at −20°C until analysis.

Hormone radioimmunoassays. Cortisol was measured using a solid-phase radioimmunoassay kit with ^{125}I purchased from Incstar (Stillwater, MN). Plasma samples were diluted fivefold with the serum blank provided and aliquoted in 10-μl amounts into duplicate assay tubes. Cross-reaction of the cortisol antibody with corticosterone is $<0.4\%$, as reported by the manufacturer. A dilution curve of chipmunk plasma was parallel to the standard curve of the kit. Cortisol was not detected in charcoal-stripped chipmunk plasma. Intra- and interassay variations were 5.6 and 5.9%, respectively.

Corticosterone was measured by direct radioimmunoassay, as described by Boswell *et al.* (1994) and Wingfield *et al.* (1992). Plasma samples of 20μl were extracted with 4 ml of dichloromethane, dried under nitrogen, reconstituted in 550μl of phosphate-buffered saline, and aliquoted into duplicate assay tubes of 200μl each and one recovery tube of 100μl. Reconstituted extracts were incubated with a primary antibody to corticosterone (B21–42; Endocrine Sciences, Tarzana, CA) and [1,2,6,7-^{3}H]corticosterone (NEN Research

Products, Boston, MA), and dextran-coated charcoal was used to separate bound and free-labeled hormone. Cross-reaction of the corticosterone antibody with cortisol was 5.2%, as reported by the manufacturer. Intra- and interassay variations were 8.9 and 17.9%, respectively.

Statistics. Results were analyzed with a commercial statistical program (Statview; SAS, Cary, NC) using analysis of variance. Because most of the seasonal trapping samples (seven different periods, by month, in 1996) were from animals that were bled only once or twice, we used an ANOVA design without repeated measures for hormones. Likewise we represented body mass in 1996 over the course of 7 months by entering the first value obtained for any individual in a given month, also applied to an ANOVA without repeated measures. This approach preserves strong sample sizes across seasons and is a practical compromise to the randomness of captures within a large natural population and the rare incidence of consistently repeated sampling of the same individuals. A repeated-measures ANOVA was used for the 0- to 30-min comparisons of hormones within the same adults in which blood sampling time was the repeated measure and season was examined as a covariate. The Fisher PLSD was used as a post hoc test. A paired *t* test was used to compare 0- and 30-min samples of both hormones in juveniles. We used Pearson product-moment correlation to examine the relationship between body mass and plasma glucocorticoids in individual females.

RESULTS

Individuals in the population showed a single bout of reproduction (one litter) following emergence from hibernation, and all females captured during March–May showed indications of breeding. Hibernation was initiated during the previous November and continued until March. When we captured the first females in late March, they showed external signs of readiness to mate (open or swollen vulvas). After a gestation of just less than a month, females began giving birth in late April and early May. We continued to observe lactation (extended nipples from which milk could often be expressed) in the population through the end of June. The first young emerged in early June, and the population remained active throughout the period of trapping (September).

Body mass of adult females showed a cycle of significant variation ($F(6, 92) = 16.7$, $P < 0.001$) over the 7-month active season from March through September (Fig. 1). Body mass of adults was at a minimum when they emerged from hibernation; mass increased steadily over gestation to a peak, dropped following parturition, and increased to a peak during lactation, finally showing a slow decline over the remainder of the active season. Juveniles also varied significantly in body mass over the 4 months after their postnatal emergence ($F(3, 79) = 33.0$, $P < 0.001$), showing rapid postnatal growth followed by a slower increase over the latter half of summer, but not achieving statistical congruence with adults ($P > 0.05$) until late September (Fig. 1).

We found seasonally significant variation in the plasma titers of both cortisol ($F(6,55) = 24.0$, $P < 0.0001$) and corticosterone ($F(6,55) = 17.3$, $P < 0.0001$). Levels of cortisol were generally an order of magnitude higher than those of corticosterone (Fig. 2). Following low values at the start of the season, during mating and gestation, corticosterone rose significantly during lactation and dropped precipitously thereafter; cortisol also rose during lactation and then declined gradually over the rest of the season. Whereas both May and June levels of corticosterone, during lactation, were significantly greater than those of April (Fisher PLSD, $P < 0.0001$), cortisol did not rise significantly above the April level until June (Fisher PLSD, $P < 0.0001$). The peaks of both glucocorticoids in June, in late lactation, were significantly greater than the levels in all other months (Fisher PLSD, $P < 0.0001$). Summer values of both GCs in the female young of year did not differ significantly from those of adults (corticosterone $F(1,54) = 0.09$, $P = 0.77$; cortisol $F(1,54) = 0.39$, $P = 0.53$) (Fig. 2).

The general form of the seasonal cycle of body mass resembles the form of the seasonal cycle of plasma GCs—low at first, then high, and finally low again (Figs. 1 and 2). This relationship is borne out by a low but significant level of correlation in individual chipmunks across the whole active season between body mass and cortisol ($r = 0.34$, $P < 0.01$, $n = 62$) and corticosterone ($r = 0.43$, $P < 0.001$, $n = 62$). However,

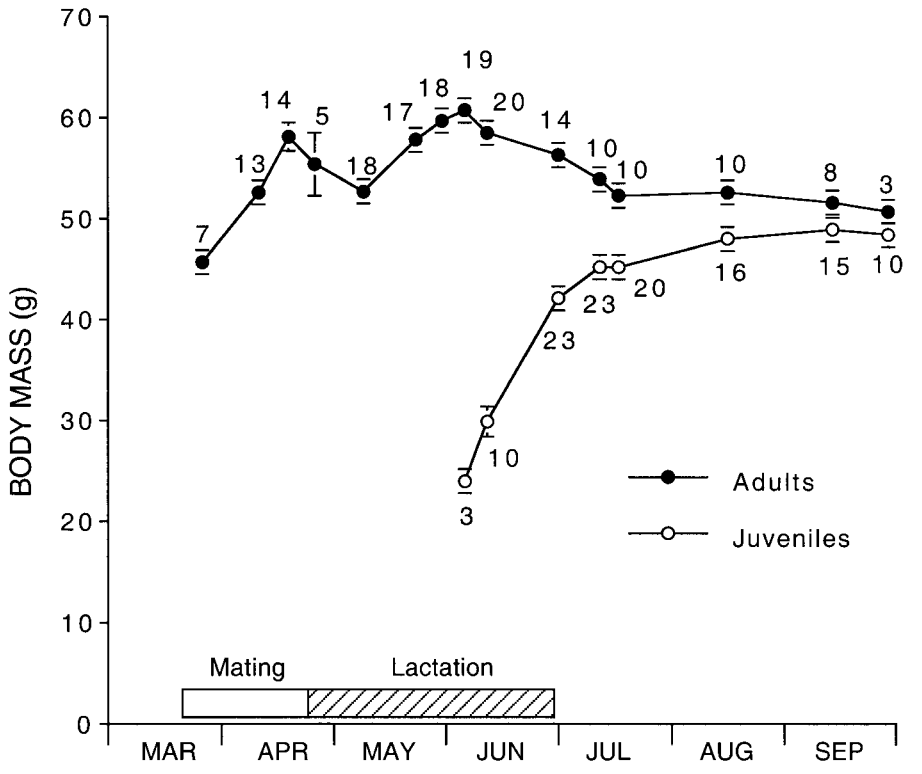

FIG. 1. Seasonal patterns of body mass of free-living female chipmunks in 1996, according to age class. Values are means ± SEM, and sample sizes are indicated. Intervals of mating and lactation for the population as a whole are indicated by horizontal bars, as obtained by observation of individuals in 1996 trapping and extrapolations based on the individual duration of gestation (~4 weeks) and lactation (~7–8 weeks) (Kenagy and Barnes, 1988).

when examined within shorter segments of the season (prelactation, $n = 20$; lactation, $n = 17$; postlactation, $n = 25$) no significant correlations were found between body mass and levels of either of the GCs ($P > 0.1$).

Our alternative method of sampling blood immediately upon direct observation of capture allowed us to determine initial, or "basal," levels of GCs. Basal corticosterone level varied significantly over the three periods (Fig. 3; $F(2,17) = 9.357$, $P = 0.002$), with lactation level greater than those of prelactation (Fisher PLSD, $P = 0.001$) and postlactation (Fisher PLSD, $P = 0.006$). On the other hand, basal cortisol did not vary significantly over the periods of prelactation, lactation, and postlactation (Fig. 3; $F(2,17) = 1.068$, $P = 0.37$). Overall variation, including basal values and those obtained 30 min later, over the three phases of the season was not significant for cortisol ($F(2, 17) = 0.47$, $P = 0.64$), but it was significant for corticosterone ($F(2, 17) = 12.1$, $P = 0.005$), for which the levels for lactation were significantly greater than those of prelactation (Fisher PLSD, $P = 0.0002$) and postlactation (Fisher PLSD, $P = 0.004$). We note that the frequency and timing of sampling differed over the active season between our two methods, with sampling over 7 months for standard trapping and over only three limited periods (prelactation, lactation, and postlactation) for the immediate blood sampling method. Furthermore, the lactation samples for the immediate sampling method were obtained in May, rather than in June, when the levels of both GCs had peaked in samples obtained during standard trapping (Fig. 2). We believe this is responsible for differences in statistical outcome, notably in the case of seasonality of basal GCs, for which changes were not as robust and consistently demonstrable in the immediate sampling data set (Fig. 3) as in the standard trapping (Fig. 2).

Capture and handling had an effect of increasing plasma GCs above the initial ("basal") level. After 30 min of resting in the trap following capture and handling for blood sampling, female chipmunks had

significantly higher plasma GC concentrations, and these effects were generally repeatable before, during, and after lactation (Fig. 3; cortisol $F(1, 17) = 40.54$, $P = 0.0001$; corticosterone $F(1,17) = 27.21$, $P = 0.0001$). The 30-min increases were significant at all three phases of the season for corticosterone ($P = 0.0002$, 0.04, and 0.003, respectively) and for cortisol at two of the three times (before and after but not during lactation, $P = 0.005$, 0.07, and 0.002, respectively).

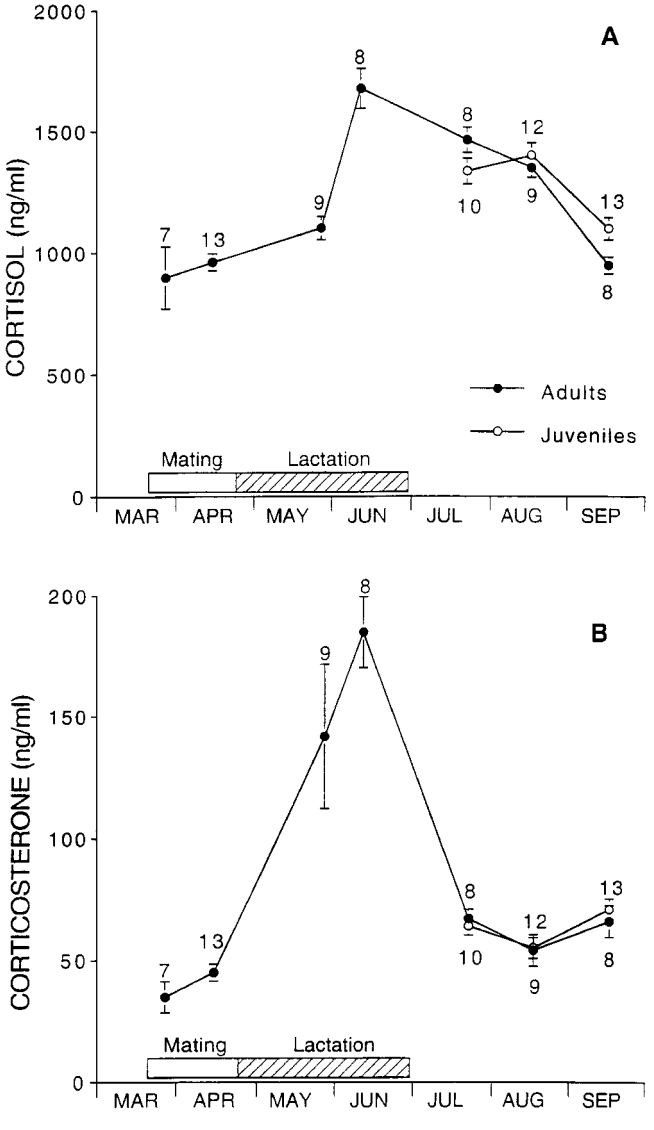

FIG. 2. Seasonal patterns of the plasma hormones (A) cortisol and (B) corticosterone of free-living female chipmunks in 1996, according to age class. Data are for chipmunks captured in a natural population by standard trapping (see Methods). Symbols and reproductive season as in Fig. 1.

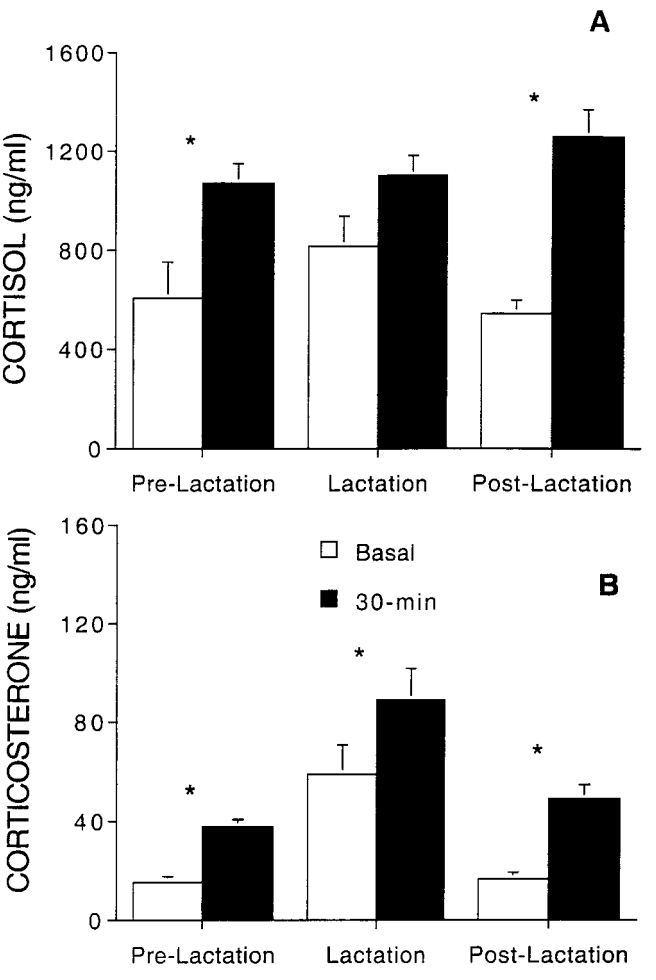

FIG. 3. Plasma concentrations of (A) cortisol and (B) corticosterone in adult female chipmunks at three stages of the seasonal cycle in 1996, first within 3 min after capture and handling (white) and then after being held in the trap for 30 min (black) (see Methods). Prelactation values ($n = 8$) during pregnancy, in April; lactation values ($n = 8$) in May; and postlactation values ($n = 4$) in July. Asterisk above pair of columns indicates significant difference between basal and 30 min.

Due to the exceptional nonsignificance ($P = 0.07$) of the apparent increase in plasma cortisol over 30 min following capture and handling during lactation in 1996 (Fig. 3), we reexamined this effect with more thorough sampling and at two different sites in 1998—the usual study grid and a nearby high-altitude site where the active season was delayed by about a month and otherwise somewhat compressed. The reason for the additional test at high altitude was to determine if lactating females under more stringent environmental

conditions might show a suppression of their short-term adrenocortical response. In 1998 we found consistently significant increases in cortisol of lactating females over the 30-min interval following capture and handling at both sites (Fig. 4; $P = 0.003$ at main site and $P = 0.009$ at high altitude; overall variation $F(1,14) = 32.0$, $P < 0.0001$). We conclude that lactating female chipmunks are generally able to sustain their ability to increase circulating levels of cortisol, as they do also for corticosterone, in response to capture and handling.

Young-of-year females, measured in July 1996 at the same time as the adult females shown in Fig. 3, also showed a robust and significant adrenocortical response to capture and handling, which is interesting in light of the differences in age and body mass (cf. Fig. 1). Mean levels of cortisol in 11 juveniles in July were 708 ng/ml (SD = 358) at capture and 1251 ng/ml (SD = 135) after 30 min, a significant increase of 77% ($P < 0.0001$). Mean corticosterone levels for the same individuals were 22.7 ng/ml (SD = 14.6) at capture and 49.5 ng/ml (SD = 15.6) after 30 min, a significant increase of 118% ($P < 0.0001$). These results for juveniles are statistically indistinguishable (cortisol $F(1, 13) = 0.50$, $P = 0.49$; corticosterone $F(1, 13) = 0.25$, $P = 0.62$) from those of the postlactation adult females at the same time (Fig. 3).

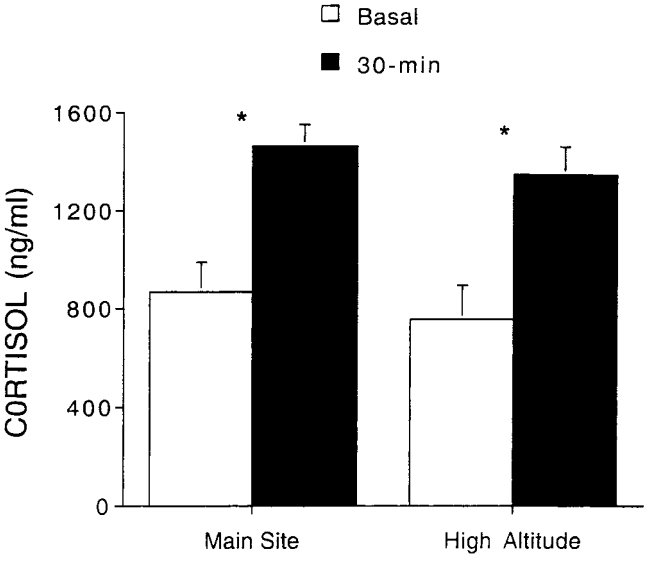

FIG. 4. Plasma cortisol of lactating chipmunks in 1998, first within 3 min after capture and handling (white) and then after being held in the trap for 30 min (black) (see Methods), at the main study area, at 670 m elevation (May, $n = 8$), and a nearby high-altitude site, at 1570 m elevation (June, $n = 8$). Asterisk above columns indicates significant difference between basal and 30 min.

DISCUSSION

Female yellow-pine chipmunks, *T. amoenus*, show pronounced seasonal cycles of plasma cortisol and corticosterone concentration that are similar in form but about an order of magnitude higher for the former than for the latter. These GC hormones are low at the beginning of the active season following hibernation; they rise to a peak by the time of late lactation and then return to a low level before hibernation. When we obtained blood samples immediately, within 3 min of capture, basal GC levels were lower than those we measured in animals trapped by our usual procedure (1 to 3 h waiting in the trap before blood sampling), whereas the levels we measured after holding the animals for 30 min after capture and handling were more similar to our standard field hormone levels. Our study is the first to report data collected in both ways in a field investigation of rodents. It is clear that standard field trapping (with blood sampling typically delayed one to several hours following capture) is adequate to demonstrate seasonal changes in circulating hormone levels and that such natural seasonal patterns are not obscured by adrenocortical activity associated with capture and handling.

Cortisol appears to be the principal GC in the plasma of *T. amoenus*, whereas corticosterone occurs at a much lower concentration. However, the form of their seasonal cycles of concentration is roughly similar, with differences mainly in phasing, e.g., during lactation. In the golden-mantled ground squirrel *Spermophilus saturatus*, in which both cortisol and corticosterone are also present in the plasma, the forms of the seasonal cycles of concentration are not parallel, but fluctuate in opposite directions (Boswell *et al.*, 1994). The different functions of multiple GC hormones within the same species are not understood at present, and one of the issues that requires definition is the relative amount of free and bound hormone (Rosner, 1990). For example, do corticosterone and cortisol bind to corticosteroid-binding globulin equally well? Additionally, do the GCs differ in their binding affinities for

the glucocorticosteroid receptors? The possibility of difference in function related to two different classes of brain receptors for GCs, one for anabolism and one for catabolism (Devenport *et al.,* 1989) may be part of the complexity of dual GC hormones. We believe that measuring and characterizing plasma binding globulin and GC receptors will be important in future investigations of these systems containing two different plasma GCs.

Seasonally cyclic variation in plasma GCs has been documented previously in two other hibernating sciurid rodents in the field, the golden-mantled ground squirrel *S. saturatus* (Boswell *et al*., 1994) and the yellow-bellied marmot *Marmota flaviventris* (Armitage, 1991). The form of the cycles of these other species differs somewhat from that of the chipmunks, and we suggest that these different patterns reflect differences in seasonal timing of reproduction, hibernation, and regulation of body mass cycles and their amplitudes. The European ground squirrel, *Spermophilus citellus*, studied in the laboratory, showed low cortisol levels following hibernation, followed by an increase during and after reproduction (Shivatcheva *et al.,* 1988). We believe that these general seasonal patterns of change in GC hormones are associated with regulation of seasonal changes in energy balance (King, 1988; Berdanier, 1989; Green *et al.,* 1992); GC hormones may also play a special role in the seasonality of fat metabolism (Lamberts *et al.,* 1975). Ground-dwelling sciurids in particular show great changes in energy metabolism between different seasonal physiological states, e.g., hibernation, reproduction, and molt (Kenagy *et al*., 1989). The adrenal glands of chipmunks, *T. amoenus*, undergo seasonal changes in size under natural conditions (Sheppard, 1968), and the patterns of change in absolute adrenal mass are consistent with patterns of plasma GCs of this species.

Seasonal regulation of energy balance in hibernating rodents includes regulation of body mass changes (Mrosovsky, 1990). The annual cycles of the hibernating sciurids mentioned in the previous paragraph have strong endogenous components, yet the animals are able to make adjustments in response to the environment (Davis, 1976; Kenagy, 1981). We found that plasma GC levels were positively correlated with body mass across the active season in yellow-pine chipmunks, but correlations of mass with hormone level did not exist within the samples at specific times of the year. This means that the seasonal cycling of the chipmunks is correlated with GC endocrinology, but that hormonal variation is not likely to play a strong role in individual variation within a season. In addition to the energy demands of reproduction, and the interesting association of peak levels of GCs with the energy-demanding time of late lactation, it is also noteworthy that *T. amoenus* has two bouts of molt during the active season, distributed from pregnancy through prehibernation (Broadbooks, 1968). Thus, it is important to consider the timing and regulation of molt along with reproduction if one is to unravel the complex interactions of the adrenocortical system with general seasonal changes of physiological state and energy mobilization. Regarding the generation of the seasonal GC hormone cycles, we cannot yet say what features of the neuroendocrine system are responsible, e.g., changes in CRF release, ACTH receptors in the adrenals, negative feedback, or others.

Our specialized sampling protocol designed to reveal basal plasma levels of GCs allowed us to demonstrate that the combination of capture and handling (removal of animal from trap and taking a blood sample) induces a significant rise above basal levels of both corticosterone and cortisol over the course of 30 min. In our female chipmunks this effect occurred across the entire active season: before, during, and after lactation in adults and in juveniles following their emergence. The significant 30-min increases in cortisol ranged from about 70 to 130% (Figs. 3 and 4), and the corticosterone increases ranged from about 50 to 190% (Fig. 3).

It is noteworthy that during the time of heavy energy demands associated with lactation and the seasonal peak in plasma GCs female chipmunks were still able to show a further rise in GC levels upon capture and handling. The highest levels of both cortisol and corticosterone that we found in female *T. amoenus* occurred during lactation. Lactation is a time of specialized action of GCs in female mammals (Bronson, 1989). Lactation is maintained by GC secretions that are promoted by ACTH release from the hypothalamus, which in turn is stimulated by suckling (Lightman, 1992). Basal GC levels are elevated during lactation in rats, while a further rise in GCs in response to stress is suppressed (Lightman, 1992). Our result in

lactating chipmunks, with significantly greater levels of corticosterone and cortisol 30 min after capture and handling than the basal levels, is contrary to the concept of suppression of the HPA stress response during lactation. It appears that female chipmunks living under natural conditions do not lose the ability to respond to acute physiological stress while lactating, as do laboratory rats.

Acute (short-term) rises in circulating levels of GCs are well known as a key feature of the ''physiological stress response'' (Selye, 1976; Sapolsky 1987). We believe that the consistent and robust responses shown by chipmunks in the 30-min period following the combined effects of capture and handling represent a physiological response that coincides with that of acute stress. It appears that the kind of data collection protocol we used can demonstrate these sorts of responses in rodents in general (Romero *et al.,* 1997), although the protocol does not allow distinction of the relative magnitude of the effect of capture and the effect of handling. Other research with mammals on the physiological stress response is dependent, in turn, on various sampling schemes, as well as the endocrinology and behavior of the particular species studied. Both acute and chronic (long-term) effects of capture and immobilization procedures (e.g., anesthetic darting and netting), as well as challenges by exogenous adrenocorticotropic hormone, have demonstrated changes in GC secretions in a variety of free-living carnivores (van Jaarsveld and Skinner, 1992; Brown *et al.,* 1993; de Villiers *et al.*, 1995) and artiodactyls (Brown *et al.,* 1991; Hastings *et al.,* 1992). In free-living birds (Astheimer *et al.,* 1994; Wingfield, 1994) and lizards (Dunlap and Wingfield, 1994) an increase in plasma GCs also is variously accompanied by different means of capture and handling

The GC hormones and their association with stress have been invoked to explain population regulation in certain small mammals, particularly murid (including ''microtine'') rodents and small semelparous marsupials, such as *Antechinus* spp. (Christian, 1980; Lee and McDonald, 1985). High levels of GCs, adrenal hypertrophy, immunological failure, and increased mortality rates are the suite of correlated characteristics that suggest the operation of a population regulation mechanism. On the other hand, social ties, including pair bonding (in monogamous species), may have either positive or negative influences on plasma GC levels in various murid rodent species (for review see de Vries *et al.,* 1995). The most powerful way to resolve the conflicting suggestions in the literature may well be to conduct correlational and experimental field studies involving detection of social relations of individuals and density relations within a population. The natural seasonal endocrine patterns of the adrenocortical system that we measured in female yellow-pine chipmunks, *T. amoenus*, members of the squirrel family (Sciuridae), do not provide any evidence for population regulation or mediation of social relations in this species, which is a promiscuous breeder. The chipmunks have low levels of GCs both at the time of mating and then again later during the summer period of increased population size associated with recruitment of the single annual cohort of juveniles. They also do not show strong fluctuations of population size from year to year (Broadbooks, 1958, 1970; Kenagy and Barnes, 1988), as do small mammals for which stress and GC hormones have been implicated in population regulation (Christian, 1980; Lee and McDonald, 1985). Armitage (1991) reported a lack of relationship between population density of marmots (sciurid rodents) and their plasma GC concentrations. More recent studies of murid (microtine) rodents suggest that GCs are less likely to be involved in population regulation, as originally proposed, or else that their role is much more complicated than originally conceived (Boonstra and Boag, 1992). We conclude that GC hormones do not appear to play a role in promotion of mortality and population regulation in chipmunks and other sciurid rodents.

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