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Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird

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Abstract

Unpredictable events such as severe storms lead to an increase in circulating corticosterone (CORT) in breeding birds. This increase is often accompanied by elevations in foraging and irruptive behavior. We were interested in determining if acute food restriction (such as might occur during inclement weather) is a sufficient cue to elicit an increase in locomotor activity, increase CORT secretion, and/or decrease circulating levels of corticosterone binding globulin (CBG) in white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Male *Z.l. gambelii* were housed individually in environmental chambers on long days (LD 20:4) to simulate breeding season daylength. Birds were fed *ad libitum*, and on select days, food was removed 2 h after lights on (fasted treatment), or was removed and replaced (control). We analyzed CORT and CBG levels after 1, 2, 6, 22 (lights on), and 23 h under fasted and control conditions. We also measured activity during the 23-h experiment. Activity levels were increased under fasted conditions during the daytime relative to control conditions, but activity levels did not differ between treatments during the night. Fasting as little as 1, 2, and 6 h significantly increased total CORT levels above baseline (control), although after 22 h, total CORT levels under fasted conditions matched those under control conditions. Plasma CBG decreased after the 22-h fast, and remained low after the 23-h fast. This change was sufficient to significantly elevate free CORT levels in fasted birds relative to *ad libitum* food conditions, despite the lack of difference in total CORT levels.

Keywords: Activity; Corticosterone; Corticosterone binding globulin; Sparrows; Free corticosterone; Food restriction

Introduction

An increase in glucocorticoid secretion in response to a variety of unpredictable environmental stimuli has been well established among vertebrates (reviewed in Sapolsky et al., 2000). Acute elevation of glucocorticoids can lead to a series of behavioral and metabolic changes that can aid an animal in avoiding prolonged stress (reviewed in Wingfield et al., 1998). These changes include deactivation of territorial behavior and disintegration of social hierarchies, activation of locomotor activity associated with leaving the site of perturbation, and mobilization of stored energy reserves (Wingfield et al., 1998). Environmental perturbations may also be associated with prolonged stress, accompanied by sustained elevations in glucocorticoids that can lead to severe debilitation or death (Harvey et al., 1984).

Inclement weather is an environmental perturbation that is particularly relevant for seasonally breeding birds, as severe weather can interrupt breeding by reducing food resources, causing extreme temperature fluctuations, and/or damaging nest sites (Wingfield et al., 1983; Wingfield, 1984; Wingfield and Ramenofsky, 1997). Plasma levels of the steroid hormone corticosterone (CORT), the primary glucocorticoid in birds (Holmes and Phillips, 1976), have been shown to increase in the field during periods of inclement weather in several species of birds (Astheimer et al., 1995; Smith et al., 1994; Wingfield, 1985; Wingfield et al., 1983). A number of studies have also demonstrated increases in locomotor activity associated with moving

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away from the affected area during storms (Breuner and Hahn, in press; Lens, 1995; Wingfield and Ramenofsky, 1997). Similar patterns have been confirmed in captivity as well. Captive Gambel's white-crowned sparrows held on short days exhibited elevations in CORT as well as increased activity levels after short-term fasting (Richardson, 1997; S. Lynn, unpublished data).

Elevations in plasma levels of CORT, such as those seen during severe weather, are often used as an indicator of physiological "stress" (e.g., Holmes and Phillips, 1976). However, the physiological relevance of plasma levels of steroid hormones can depend upon additional factors, including circulating levels of hormone binding proteins and receptor levels at the target tissues (Breuner and Orchinik, in press; Breuner and Orchinik, 2002; Meaney et al., 1992).

The binding protein corticosteroid binding globulin (CBG) binds CORT with high affinity in circulation (e.g., Breuner and Orchinik, 2001; Hammond, 1995). Although the primary role of CBG is under debate, the Free Hormone Hypothesis suggests that unbound, or free, concentration of hormone in the plasma is biologically active. In other words, this hypothesis assumes that only free steroid is available to enter target tissues and bind to receptors (Ekins, 1990; Mendel, 1989). The presence of CBG, however, may regulate not only the availability or biological activity of CORT, but also the metabolic clearance rate, tissue-specific delivery to target cells, and/or binding of the CORT–CBG complex to binding globulin receptors on target cell membranes (Hammond et al., 1990a,b; Hryb et al., 1986; Hsu et al., 1986; Rosner, 1990). Certainly, these possible mechanisms of CBG action are not necessarily mutually exclusive. In this study, we focus on determining the possible role of CBG in regulating levels of free CORT during the acute stages of a stressor.

Plasma CBG levels are known to fluctuate over variable time scales. For example, CBG levels vary on a seasonal basis in birds (Assenmacher et al., 1975; Breuner and Orchinik, 2001; Daniel et al., 1981; Romero and Wingfield, 1998; Romero et al., 1998; Silverin, 1986; see also Deviche et al., 2001) mammals (Tinnikov and Oskina, 1994), and reptiles (Xavier, 1982). In mammals, plasma CBG levels have also been shown to fluctuate on a daily rhythm (Frairia et al., 1988; Hsu and Khun, 1988). Studies in rats indicate that CBG levels can also change under acute stress. Stressors such as tail shocks (Deak et al., 1999; Fleshner et al., 1995), restraint (Marti et al., 1997), social stress (Spencer et al., 1996), ether exposure, exercise, and fasting (Tinnikov, 1999; Woodward et al., 1991) all reduce plasma CBG, thereby increasing free CORT levels. To date, however, little is known about how short-term changes in CBG capacity may regulate free hormone levels in birds.

Wingfield et al. (1998) suggest that energy imbalances resulting from environmental stressors such as severe weather can trigger elevations in CORT secretion. We suggest that an energy imbalance that arises from acute, short-term fasting might have a similar effect on CBG levels in seasonally breeding birds. Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*) breed in the Arctic and boreal regions of North America (Cortopassi and Mewaldt, 1965), where unpredictable spring storms may be severe enough to completely obscure food supply at the outset of the breeding season (Hahn et al., 1995). In this study, we measured total levels of circulating CORT and CBG in captive male white-crowned sparrows exposed to short-term fasting, such as might occur during the initial stages of a severe storm. We then used these levels to calculate the amount of free CORT circulating in plasma for each male after fasting and during *ad libitum* food (control) conditions. We also measured activity levels of birds under fasted and control conditions over 23 h. We predicted that, compared to *ad libitum* food conditions, short-term fasting would lead to an elevation in activity levels, as well as increased total CORT, decreased specific binding of CORT for CBG, and consequently, elevated free CORT levels. Understanding such dynamics of free and bound CORT may be critical to understanding physiological and behavioral responses to transient stressors.

Methods

Animals and housing

Gambel's white-crowned sparrows were captured during fall migration in Sunnyside, Washington (46°1'N, 119° 5'W) in late September, 2000. They were maintained in outdoor aviaries at the University of Washington until use in this study. During this time, birds were provided *ad libitum* food and water, and experienced natural temperatures and light cycles.

On 22 December, 2000, 12 photosensitive males were moved into an animal room with adjustable light:dark conditions (L:D 20:4 for the entire experiment). Birds were photostimulated for 24 days before the experiment began. Ambient temperature was held at 20°C. Males occupied individual cages with *ad libitum* food (a mix of birdseed and chow) and water.

Experimental design

This study consisted of five individual experiments, which differed only in duration. For each experiment, an investigator entered the chamber at exactly 2 h after lights on. The investigator quickly replaced cage liners with clean paper (taking care to remove all visible seed from each cage) and either removed all the food cups from the chamber (fasted treatment), or removed all the food cups from the cages and then immediately replaced food cups before exiting the chamber (control treatment). Experiments lasted for 1, 2, 6, 22 (the next "morning"), and 23 h (Fig. 1). These sampling times were chosen to detect possible changes in physiology and behavior at two general phases of fasting:

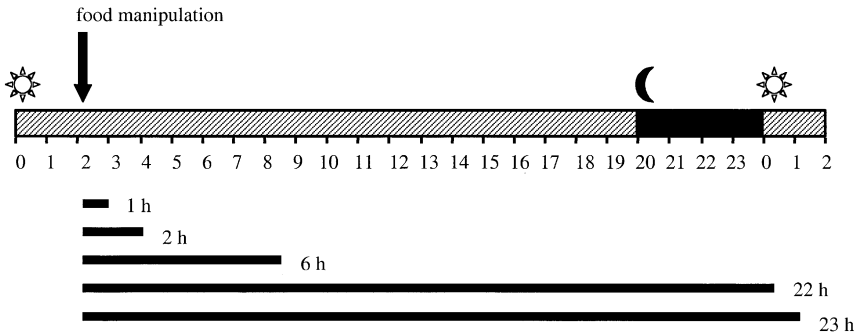

Fig. 1. Treatment regimen for captive male white-crowned sparrows ($n = 12$) held on long days (L:D 20:4). The period of lights on (day) is indicated by the hatched bar, and lights off (night) is indicated by the solid bar; numbers indicate the time of day. Food manipulation varied between experimental and control treatments: during the experimental condition, food cups were removed from the chamber, and during the control condition, food cups were removed and immediately replaced in cages. Solid lines indicate the length of time experimental or control conditions persisted for each experiment.

immediately following removal of food (1, 2, and 6 h) and the beginning of the second day of fasting (22 and 23 h). Each bird was used as its own control, and the order of experiments was completely randomized, with the exception of the 23-h experiment. The 23-h experiment (both fasted and control treatments) was conducted first due to limited availability of equipment for behavioral recording (see below).

Blood collection and behavioral recording

At the end of each experiment, we entered the chamber and collected a small blood sample (40–80 μl) from each bird to be analyzed for CORT and CBG levels. All samples were collected within 3 min of entering the chamber, to ensure that plasma CORT levels were not elevated due to capture stress (Wingfield et al., 1982). Blood samples were collected in heparinized microhematocrit capillary tubes following venipuncture of the alar vein (26-gauge needle). Plasma was separated by centrifugation, then stored at −20°C until assay.

In the 23-h experiment, 10 of the 12 cages were equipped with external infrared light detectors (Radio Shack). Beginning immediately after the investigator exited the chamber, we recorded the number of times an infrared light beam that bisected the cage was interrupted as birds hopped between perches. Data were recorded with a Macintosh computer running Labview, a program that converts analog signals into digital (Breuner et al., 1998; Wikelski et al., 1999).

Radioimmunoassay

Plasma CORT levels were measured using direct radioimmunoassay after extraction with redistilled dichloromethane as described in Wingfield et al., (1992). Recoveries for individual samples after extraction ranged from 76 to 97%. All samples were run in one assay to avoid problems of interassay variation. The limit of detectability for this assay was approximately 1.6 ng/ml. Intraassay variation was 9.49%.

Corticosteroid binding globulin assays

Protocols for experiments used to measure plasma CBG were based on methods used in Breuner and Orchinik (2001). Incubation time (2 h), temperature (4°C), rinse volume (9 ml ice-cold buffer), and plasma concentration (1:900 final dilution) were optimized to maximize specific binding. All assays contained 50 μl [^{3}H]CORT, 50 μl buffer (50 mM Tris) or unlabeled CORT, and 50 μl of diluted plasma. Nonspecific binding was determined using 1 μM unlabeled CORT. All samples were run in triplicate. Bound and free radioligand were separated using rapid vacuum filtration (Brandel Harvester) over glass fiber filters (Whatman GF/B, soaked in 25 mM Tris with 0.3% PEI for 1 h prior to filtering). After filtration, radioactivity bound to the filters was measured by standard liquid scintillation spectroscopy. Point sample analysis was run on individual plasma samples. Affinity (K_d) of CORT for CBG was estimated using pooled plasma in a separate equilibrium saturation binding experiment.

Plasma collection and preparation

Plasma collected to determine CORT level was also used to measure CBG capacity. To remove endogenous CORT, individual plasma samples were incubated with two volumes (vol / vol) of dextran-coated charcoal solution (0.1% dextran, 1% Norit A charcoal in 50 mM Tris) for 20 min at room temperature. Suspensions were centrifuged for 10 min at 4500 rpm, 4°C.

Individual point sample analysis

An estimate of CORT binding capacity in individual plasma samples was determined using 15 nM [^{3}H]CORT. Based on affinity estimates derived from equilibrium saturation analysis, this ligand concentration should occupy approximately 92% of total binding sites. To avoid interassay variation, CBG capacity was determined for all individuals in the same assay.

Equilibrium saturation analysis

Pooled plasma from captive, *ad libitum* fed *Z.l. gambelii* was incubated with 0.25 to 12 nM [^{3}H]CORT in the presence or absence of unlabeled CORT. Previous experiments have demonstrated that the affinity of CORT for CBG in *Z.l. gambelii* is similar among individuals, sexes, fasted and *ad libitum* fed birds, and captive and free-living birds (C. Breuner, unpublished data). Affinity estimates from these data were used in estimations of free CORT.

Free CORT analysis

Free CORT titers were estimated using the equation of Barsano and Baumann (1989):

$$H_{\text{free}} = 0.5 \times [H_{\text{total}} - B_{\max} - 1/K_a \pm \sqrt{(B_{\max} - H_{\text{total}} + 1/K_a)^2 - 4(H_{\text{total}}/K_a)}],$$

in which $K_a = 1/K_d$ (nM). We measured the affinity of CORT for CBG using equilibrium saturation analysis on pooled plasma samples. We measured CORT and CBG capacity in each individual (from blood samples taken at capture), thus allowing us to calculate free CORT estimations for each individual. Results are given as means ± standard errors for individuals in each treatment. Individual CBG capacity estimations represent approximately 92% of $B_{\max}$, so capacity values were increased to 100% for free CORT calculations.

Statistical analyses

Each experiment represents an independent sampling with each bird used as its own control. We log transformed total CORT, CBG $B_{\max}$, and free CORT data to stabilize variances. Because previously collected data indicated a significant increase in behavior and CORT secretion and a significant decrease in plasma CBG in fasted white-crowned sparrows held on short days (relative to controls) (S.E. Lynn, C.W. Breuener, J.C. Wingfield, unpublished data), we analyzed each experiment using one-tailed paired *t* tests.

We analyzed activity data for the 23-h experiment by organizing the total number of hops for each individual into 1-h bins. We then calculated the average hops for each individual during both lights on (daytime) and lights off (nighttime). Because each bird was used as a fasted subject and a control subject, we analyzed activity levels during lights on and during lights off using two paired *t* tests followed by a Bonferonni correction for multiple comparisons (Zar, 1996).

Results

Activity levels

Although activity levels immediately following treatment (food removal or removal and replacement) were similar, fasted birds had higher activity levels during the day than control birds (*t* test: $t_9 = 5.671$, $P = 0.0003$, Bonferonni critical value = 0.025; Fig. 2). There were no differences in activity levels between treatments at night ($t_9 = -0.757$, $P = 0.468$, Bonferonni critical value = 0.025; Fig. 2). Activity declined in both treatments as the day progressed, and activity levels were similar between fasted and control conditions prior to lights off. The following morn-

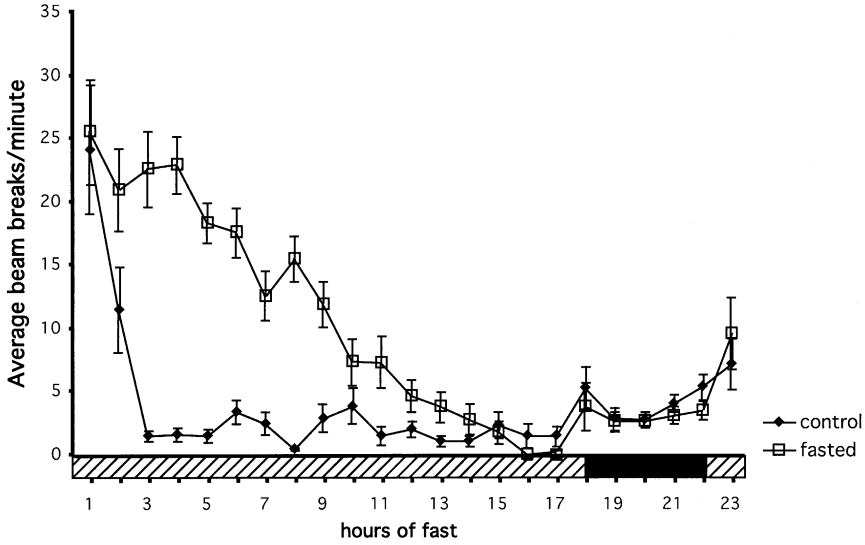

Fig. 2. Patterns of activity in captive male white-crowned sparrows ($n = 10$) held on long days during a 23-h fast (fasted) and *ad libitum* food conditions (control). Activity was measured by recording the number of times an individual broke an infrared light beam that bisected the cage. The period of lights on (day) is indicated by the hatched bar, and lights off (night) by the solid bar. We began recording activity immediately after the investigator exited the chamber; thus the elevated hops under both control and fasted conditions during the first hour reflect investigator disturbance. During the day, birds were more active under fasted conditions than under control conditions ($P = 0.0003$), and there were no differences in activity levels at night ($P = 0.4684$). Data are means ± SEM.

ing, activity levels under fasted and control conditions remained similar (Fig. 2).

Total CORT levels

Total CORT levels were higher under fasted conditions than control conditions in the 1-h (*t* test: $t_{11} = -1.803$, $P = 0.0494$), 2-h (*t* test: $t_{11} = -2.565$, $P = 0.0132$), and 6-h experiments (*t* test: $t_{11} = -3.160$, $P = 0.0046$; Fig. 3a). Total CORT levels did not differ between fasted and control conditions in the 22-h (*t* test: $t_{11} = -0.785$, $P = 0.225$) and 23-h (*t* test: $t_{11} = -0.277$, $P = 0.393$) experiments (Fig. 3a).

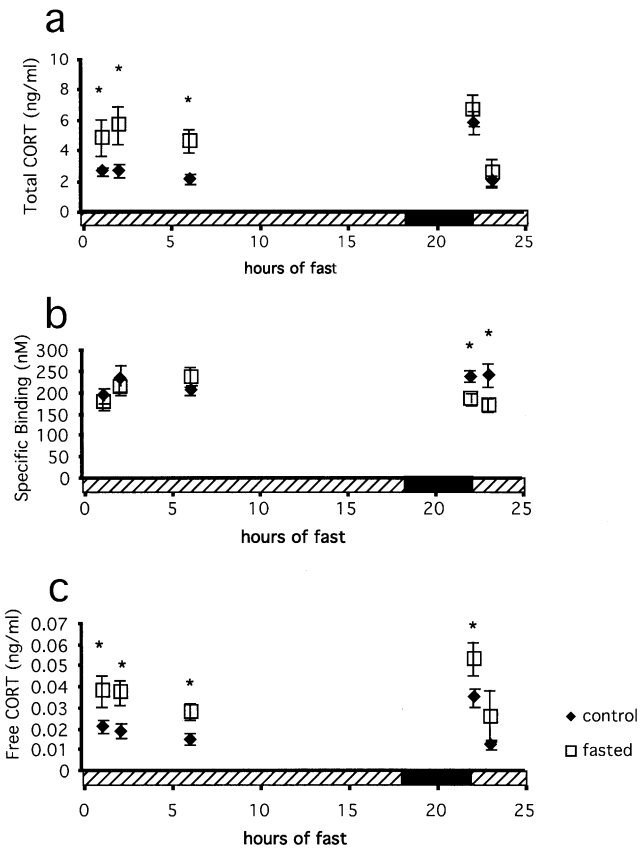

Fig. 3. (a) Total CORT levels measured by direct radioimmunoassay in captive male white-crowned sparrows ($n = 12$) under fasted and control conditions in five experiments. Birds held under fasted conditions for 1, 2, and 6 h had elevated total CORT compared to control conditions ($^*P < 0.05$). There were no differences in total CORT levels after 22 and 23 h. The period of lights on (day) is indicated by the hatched bar, and lights off (night) by the solid bar. (b) Specific binding of CORT by CBG in captive male white-crowned sparrows ($n = 12$) under fasted and control conditions in five experiments. There were no differences in specific binding after 1, 2, or 6 h of fasting, but specific binding decreased relative to control conditions after 22- and 23-h fasts ($^*P < 0.05$). (c) Free CORT levels calculated using the equation of Barsano and Baumann (1989) for captive male white-crowned sparrows ($n = 12$) under fasted and control conditions in five experiments. Free CORT levels were higher in fasted birds after 1, 2, 6, and 22 h ($^*P < 0.05$).

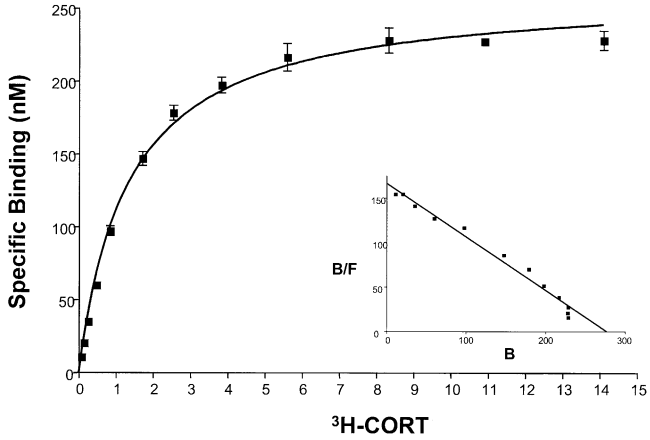

Fig. 4. Equilibrium saturation binding curve demonstrating specific binding of [^{3}H]CORT to Gambel's white-crowned sparrow plasma (4°C) as a function of increasing concentrations of radiolabeled CORT. Points represent means ± SEM. The inlay is a Scatchard–Rosenthal replot of the data.

Corticosterone binding globulin levels

Equilibrium saturation binding experiments demonstrated a single binding site for CORT in white-crowned sparrow plasma ($K_d = 1.36 \pm 0.08$ nM; Fig. 4). CBG capacity varied between treatments. Specific binding of CBG for CORT was not affected by fasting in the 1-h (*t* test: $t_{11} = 1.210$, $P = 0.126$), 2-h (*t* test: $t_{11} = 0.576$, $P = 0.288$), and 6-h experiments (*t* test: $t_{11} = -1.313$, $P = 0.108$; Fig. 3b). However, fasting resulted in a decrease in specific binding relative to control conditions in the 22-h (*t* test: $t_{11} = 3.728$, $P = 0.0020$) and 23-h (*t* test: $t_{11} = 2.048$, $P = 0.0326$) experiments (Fig. 3b).

Free CORT levels

Calculated levels of free CORT were higher under fasted conditions relative to *ad libitum* (control) conditions in the 1-h (*t* test: $t_{11} = -1.998$, $P = 0.0356$), 2-h (*t* test: $t_{11} = -2.474$, $P = 0.0155$), 6-h (*t* test: $t_{11} = -2.514$, $P = 0.0144$), and 22-h experiments (*t* test: $t_{11} = -2.564$, $P = 0.0141$; Fig. 3c). There was a strong trend for an elevation in free CORT under fasted conditions in the 23-h experiment (*t* test: $t_{11} = -1.695$, $P = 0.0591$), though this result was not significant (Fig. 3c).

Discussion

In response to fasting, activity levels and total CORT levels rose within 2 h, whereas CBG levels did not change until 22 h. Interestingly, although activity and total CORT levels were elevated after only 1 h of fasting, this effect was not evident after 22 and 23 h. Clearly, assuming that the Free Hormone Hypothesis holds, estimating the physiological impact of CORT in this study simply by monitoring the

levels of total circulating CORT is insufficient. The birds in this study exhibited elevated free CORT under longer-term fasted conditions as a direct result of decreases in CBG. The elevation in locomotor activity following food deprivation that we report here has been shown previously in captive birds (Astheimer et al., 1992; Richardson, 1997; S. Lynn, C. Breuner, J. Wingfield, unpublished data). In our study, although activity levels generally declined throughout the day in both groups, fasted birds were, on average, more active than controls during daylight hours (Fig. 2). The increased activity in fasted birds relative to controls that we report was apparently not a direct result of elevated free CORT *per se*, but was more likely due to increased food searching behavior. A second possibility is that activity levels were initially affected by increases in catecholamines that may have resulted from removal of food from the cages. Previous studies have demonstrated that feeding behavior increases under fed conditions after administration of catecholamines via intraventricular injection (in pigeons; Ravazio et al., 1990) or by infusion into the liver (in chickens; Denbow, 1994). The fact that increased activity levels did not persist into the second day of fasting may be an artifact of captivity (where food searching behavior may eventually become limited by spatial constraints.) However, additional data need to be collected. Effects of food deprivation on activity may be better evaluated in an aviary setting, where space is not as limited.

The CORT levels that we measured in the current study were well below maximal levels secreted by Gambel's white-crowned sparrows during an experimental stressor (i.e., capture and handling stress; Astheimer et al., 1994), clearly indicating that the birds were not maximally stressed. However, because the fasting plasma CORT concentrations that we report were significantly elevated over control levels, they may instead indicate a response to short-term energetic restriction that would lead to greater CORT secretion if more prolonged.

The possibility also exists that the levels of CORT secretion that we report are lower than what might be seen as a result of short-term energetic stress in the field. Typically, and particularly in birds held on long days, secretion of steroid hormones is dampened in the laboratory relative to secretion in the field (Romero and Wingfield, 1999). Additionally, because birds were housed in small cages with *ad libitum* food resources prior to fasting, their fat stores appeared to be near maximum capacity (even after the 23-h fast). Previous studies in other arctic breeding birds indicate that individuals with high levels of stored fat often have lower maximal levels of CORT secretion than leaner individuals during a stressor (Wingfield et al., 1994a,b). Similarly, negative correlations between body condition and maximal CORT levels achieved during a stress response have also been reported in nonavian vertebrates (Dunlap and Wingfield, 1995; Jessop et al., 2001). Based on these studies, we may likely have seen a more robust effect of food deprivation in leaner birds.

We have demonstrated that, in Gambel's white-crowned sparrows, an acute stressor is sufficient to decrease CBG capacity over a relatively short length of time. We have also demonstrated that the magnitude of this decrease is sufficient to alter circulating free CORT levels. Two intriguing questions remain, however: (1) what is the mechanism for the decrease in CBG? and (2) what is the physiological significance of acute elevations in free CORT?

Possible mechanisms for the decrease in CBG during fasting

CBG levels may have decreased during fasting for a variety of reasons. Two possibilities are that the decrease in binding protein occurred (1) directly as a result of energetic imbalance or (2) indirectly through fasting-induced elevations in CORT secretion.

Tinnikov (1999) suggested that in rats, decreased CBG during fasting stress may be independent of elevations in CORT. Instead, he suggested CBG may be involved in interactions with proteases activated to maintain basal catabolic metabolism, which result in degradation of the binding protein (Tinnikov, 1999). Hsu and Khun (1988) suggested that another possible mechanism for a decrease in CBG levels may be the reversible removal of CBG from the plasma (i.e., distribution between blood plasma and interstitial fluids, rather than removal by degradation), and this may also be promoted by fasting (Tinnikov, 1999). Thus, the decrease in CBG that we report in fasted birds relative to controls may have been a direct result of fasting, though further research is required to confirm the existence of such mechanisms in birds.

However, the possibility still remains that the decrease in CBG levels that we report occurred as an indirect result of fasting-induced increases in CORT. Evidence suggests that glucocorticoids themselves may function as modulators of CBG binding activity. For example, elevated levels of glucocorticoids inhibited synthesis and secretion of CBG by rat liver in vitro (Feldmen et al., 1979). An additional possible mechanism for a direct, CORT-induced decrease in CBG is via a gluconeogenic pathway. Glucocorticoids are effective promoters of gluconeogenesis (see Chester-Jones et al., 1972). Thus, a possible role for elevated glucocorticoids in the plasma could be promotion of CBG breakdown and subsequent use of the amino acids derived from this process as substrates for production of glucose. However, further support for this hypothesis is needed.

In the current study, our sampling times (1–6 and 22–23 h) make it difficult to determine whether or not the decline in CBG capacity was in fact a direct result of prolonged elevations of CORT or if it was more likely a result of increased energetic needs due to fasting.

Although our design does not allow us to draw direct comparisons across experiments, patterns of total CORT secretion under fed conditions appear to differ according to the time of day samples were taken for each experiment.

Under control conditions, baseline CORT levels appear highest at lights on (in the 22-h experiment) than at any other time point, and then drop again 1 h after lights on (23-h experiment). Fasted CORT levels also appear to track this pattern (Fig. 3a). Breuner et al. (1999) report a similar pattern in baseline CORT secretion in *Z. l. gambelii* held on both long days and short days: basal CORT levels peaked just prior to lights on and decreased 1 h after lights on. In our study, when the apparent diel peak in CORT was achieved (22-h experiment), CORT levels did not differ between fasted and control conditions. This diel peak in CORT secretion may have possibly overridden the fasting-induced elevations of CORT secretion seen in the 1-h, 2-h, and 6-h experiments. This idea is supported by evidence in rat studies, in which stress-induced elevations of CORT only occur at the trough of the daily rhythm of CORT secretion (Deak et al., 1999).

What is the physiological significance of acute elevations in free CORT?

In the field, stressors such as inclement weather correlate strongly with elevations in total plasma CORT. We have demonstrated that related stressors also increase total CORT and decrease CBG capacity in captive sparrows. Many studies suggest that elevations in CORT in response to environmental perturbations may orchestrate facultative behavioral and physiological responses to a variety of stressors. That the same environmental perturbations may also enhance the amount of biologically active CORT in circulation suggests that the importance of a stressor may be greater than commonly accepted. Thus, without measuring changes in CBG in response to stressors, we may underestimate the physiological impact of CORT as an indicator of stress in studies of environmental unpredictability.

Acknowledgments

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