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Glucocorticoids of bison bulls in relation to social status

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Abstract

A primary response to stress is an increase in circulating adrenal glucocorticoids (GC) such as cortisol. Two hypotheses propose differential stress responses to agonistic and aggressive interactions in social groups. If subordinate animals are subjected to social and psychological stressors leading to chronic GC elevation, the 'stress of subordination' hypothesis predicts that GCs will be higher in subordinates than dominants. Alternatively, if dominant animals are subject to physiological stressors (e.g., fight at higher rates than subordinates) or hierarchies are unstable, the 'stress of domination' hypothesis predicts higher GCs in dominant individuals. Both models predict that GC levels will peak during the breeding season. We tested these predictions in bison bulls (*Bison bison*) using fecal steroid analysis to characterize GC concentration and behavioral observations to determine dominance rank, copulatory success, and tending status of bulls. Fecal samples were collected during 2003 from adult bison bulls during pre-rut (June), rut (July–August), and post-rut (September). Matched sample data indicated that mean GC levels (ng/g feces) of bulls strongly peaked during the 4-week rut, doubling from pre-rut to rut and then declining again during post-rut. High ranked dominant bulls maintained higher GC levels than lower ranked subordinate bulls. Dominance rank was positively correlated with copulatory success and age, and dominant bulls were more likely to tend (guard) cows as they approached estrus. There was a positive correlation between GC level and copulatory success, with prime-aged bulls (≥7 years) obtaining the most copulations. GC levels were positively correlated with bull androgen levels determined in a previous study. These results support the 'stress of domination' hypothesis, indicating that dominant bison bulls pay a significant physiological price for high social status and the opportunity to mate.

Keywords: Fecal steroid; Cortisol; Glucocorticoids; Bison; Rut; Dominance; Stress

Introduction

Wild animals are subjected to a variety of environmental and behavioral stressors that threaten their survival and fitness. Physiologically, animals respond to stress with a series of neural and endocrine responses that divert energy from non-essential physiological processes (e.g., growth, digestion, reproduction) in order to resolve the stressful situation (Creel, 2001; Sapolsky, 2002). The neural stress response involves the release of catecholamines from the adrenal medulla and sympathetic nervous system that facilitates a state of arousal by mobilizing energy and elevating heart rate, blood pressure, and breathing (Abbott et al., 2003; Muller and Wrangham, 2004). The endocrine stress response starts with the activation of the hypothalamic–pituitary–adrenocortical axis and subsequent secretion of glucocorticoids (GCs; cortisol in large mammals) from the adrenal cortex, which alters metabolic pathways for the production of ATP to generate extra energy to deal with the stressful situation (Creel, 2001). Methodologically, the collective neural and endocrine response to stress may be measured by the increase in GC secretion. Despite the short-term advantages, there are indications that prolonged high GC levels can have harmful consequences, including loss of muscle mass, reproductive failure, immunosuppresion, and shorter life span (Creel, 2001; Abbott et al., 2003; Sands and Creel, 2004).

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The magnitude of the endocrine stress response depends on both psychological (social) and physiological stressors faced by individuals. A large body of experimental evidence has shown that unpredictability and loss of control (psychological stressors) are associated with a heightened stress response in social animals (reviewed by Muller and Wrangham, 2004). Early work with captive rodents and primates involved 'arena contests' in which unfamiliar individuals fought one another, resulting in losers (termed "subordinates") that were subject to greater harassment and less control and thus experienced elevated GC secretion (reviewed by Creel, 2001). Such 'winner–loser' experiments formed the basis for the 'stress of subordination hypothesis,' which predicts that GCs will be higher in subordinates than dominants. On the other hand, because GCs play a key role in mobilization of energy through gluconeogenesis (conversion of fatty acids and amino acids to glucose in the liver), physiological stressors involving changes in energy balance (e.g., food deprivation and acute exercise) also activate the stress response and increase GC secretion (Sapolsky, 2002; Muller and Wrangham, 2004). Agonistic and aggressive interactions among members of gregarious species can be a chronic source of stress (Sands and Creel, 2004). In species where dominance requires a high rate and intensity of fighting, high-ranking individuals may engage in energetically expensive aggressive displays and physical conflict that is associated with elevated GC levels (Goymann et al., 1999; Muller and Wrangham, 2004). The 'stress of domination' hypothesis predicts higher GCs in dominants than subordinates when dominants must fight more than subordinates or when hierarchies are unstable (Creel, 2001). Both the 'stress of subordination' and the 'stress of domination' hypotheses predict that male GC level will be highest at the time of year when aggression among males is highest.

The goal of this study was to test the hypotheses by relating GC level to dominance rank and its correlates in bison bulls. We used fecal steroid analysis to characterize GC levels in adult bull bison before, during, and after the rut (breeding season) and to examine differences in GC levels of known bulls differing in dominance rank, mating success, tending status, and age during the rut. If the 'stress of subordination hypothesis' holds true in bison, we predicted that low-ranking bulls would have higher levels of GC during the rut in relation to high-ranking bulls. In contrast, the 'stress of domination hypothesis' predicts that high-ranking bulls will exhibit higher levels of GC compared with subordinates. We also predict that a random sample of males will show that GCs are highest during the breeding season.

Review of bison reproductive biology

Bison exhibit male-dominance polygyny. Most breeding takes place from mid-July to mid-August, with the peak around 1 August (Lott, 1981; Meagher, 1986). During the rut, bulls move through the mixed herds seeking cows that are approaching estrus by sniffing the anogenital region and performing flehmen. When a cow interests a bull, he tends (guards) her by staying close alongside her until she comes into estrus then mates with her (Lott, 2002). During tending, bulls display by bellowing, scent-urination, pawing, rubbing, and wallowing. Tending bulls are frequently challenged by rival bulls surrounding the tending pair ('attending bulls'), and head-to-head fights are common. A tending bull will guard a cow until being displaced by a more dominant bull or copulating (Wolff, 1998). Copulations are quite brief, usually less than 10 s from mount to dismount, and most cows breed only once in a season (Lott, 1981). Immediately following a successful copulation, the cow arches her back, expels a small volume of clear or milky secretions from the vulva (presumably vaginal fluids and semen), and erects her tail (Lott, 1981; Berger, 1989; Berger and Cunningham, 1991; Komers et al., 1992b; Wolff, 1998). The 'tail-up' response is distinctive from tail elevation performed by bison in other contexts (e.g., defecation, urination, or agonistic interactions) in that the angle of the raised tail may be 135° or higher (if 180° is pointing straight up), and the duration of the tail-up averages 1–2 days. So reliable is this behavioral indicator of copulation that it can be used to infer copulations not directly observed (Berger, 1989; Berger and Cunningham, 1991; Wolff, 1998). After copulation, the bull may continue to guard the cow until he leaves to search for another female (Wolff, 1998).

Prior bison studies have indicated that the most dominant bulls (as measured by agonistic encounters) have the greatest breeding success (Lott, 1979; Berger and Cunningham, 1994; Wolff, 1998). In Montana bison, higher social standing of bulls was associated with higher rates of breeding (Lott, 1979: Tables 1–3). In South Dakota bison, the proportion of interactions won by prime-aged bulls (a measure of dominance) was positively correlated with copulatory success (Berger and Cunningham, 1994: Fig. 8.12). Finally, in a previous study of bison at Fort Niobrara, the number of copulations per bull was positively correlated with 'fighting ability,' a measure of dominance (Wolff, 1998: Fig. 6), and dominance rank of a given bull was positively correlated with the number of calves sired the following spring (Wolff, 1998). Dominance rank in Nebraska bison tended to peak in older bulls of 10–11 years (Wolff, 1998).

Materials and methods

Study site

The Fort Niobrara National Wildlife Refuge (77 km^2) is located along the Niobrara River near the town of Valentine in the Sandhills of north-central Nebraska (N 42° 53.65′, W 100° 28.47′). The topography of the refuge and surrounding region is flat or rolling hills of native grassland (mixed and sandhill prairie), providing excellent visibility for behavioral observations.

Established in 1912 as a sanctuary for bison, elk, and native birds, the refuge supports a population of plains bison (*Bison bison bison*) that is currently maintained at 350 head after the fall roundup and up to ~475 following calving. During the spring and summer, bison graze over about two-thirds of the refuge and are rotated among different pastures to avoid overgrazing. Unique among public bison herds, every adult in the Fort Niobrara herd is individually marked with unique brands. The composition of the herd in September 2003 was 126 calves, 68 yearlings, and 279 adults (≥2 years). The sex ratio of adults was 0.8:1 (123 bulls, 156 cows). Age classes of adults ranged to 17 years for bulls and 20 years for cows.

Behavioral observations

We conducted observations during the rut from 15 July through 13 August 2003, which bracketed peak rut for this species (Meagher, 1986). All observations were conducted from 4WD vehicles from <100 m (often within 50 m) of focal animals. We used 10× binoculars to read brand numbers. Two to three observers took shifts to maintain continuous surveillance of the herd during daylight hours (06:00 to 20:00 h). Some breeding occurs at night, and therefore nighttime rutting behavior (which we did not observe) may have influenced hormone levels from feces collected during the day. During the rut, the herd was maintained in the same grazing unit, and herd members tended to aggregate in one or several large groups that could be monitored by 4WD vehicle. Observers drove around all parts of the herd so as to account for every tending pair every 1–2 h. During these group observations, we identified all tending bulls and cows, recorded tail-ups by cows, documented copulations, and monitored bull interactions.

Agonistic interactions among bulls were opportunistically observed and recorded. Aggressive behavior patterns included supplanting, head-on threats, rush threats, broadside threats, nod threats, and overt fighting (Lott, 1979). For our operational definition, aggressive interactions between males were recorded whenever one male approached another to within 2 body lengths (<10 m) and either male turned away using at least 2 steps (Komers et al., 1992a,b). We assumed that the male turning away was displaced by the other one, and the male that raised its tail was considered the winner (Komers et al., 1992a). Dominance rank (DR) was calculated as the proportion of agonistic interactions won, in which DR of a given male is equal the number of interactions won divided by the number of interactions won and lost (Berger and Cunningham, 1994). By this measure, a DR of 0.5 is equivalent to an even win–loss record, whereas values >0.5 indicate higher rank.

A tending pair was recorded when a bull stood parallel to a cow and followed her movements closely, attempting to exclude competitors from the cow (Lott, 1974, 1981). Tending bulls were active in the rut for a mean (±SD) of 19 ± 6 days. A copulation was recorded either when directly observed from mount to dismount (observed copulation) or when a cow displayed tail-up and was tended by the same bull before and after this behavior was noted (inferred copulation). Copulation was only inferred if the tail-up was accompanied by one or more additional indications of copulation (e.g., swollen vulva, presence of vaginal secretions frequent squatting and urination by the cow). Whenever a cow was observed with tail-up, this was noted along with the angle of the tail (135°, 115°, 90°, or 45°, in which 0° would be pointing straight down and 180° is pointing straight up), whether the cow was being tended or not. Most cows exhibited estrus once during the 2003 rut (and copulated once), although 2 cows that copulated early in the rut came into a second estrus 3 weeks later. Tail-ups persisted anywhere from 1 h to 1 week (mean ± SD = 29 ± 42 h). Whenever fecal samples were collected, the current reproductive status of the bull (tending, not tending) was recorded.

Fecal steroid approach

Although reproductive physiology can be assessed by endocrine measurements, collection of blood samples (the classical approach to endocrinological studies) involves confinement or handling and may in itself be stressful and thus confound results (Möstl and Palme, 2002). Fecal sampling techniques have recently emerged as a non-invasive and convenient means of measuring hormones of free-ranging wildlife without stressing the animals (Kirkpatrick et al., 1991, 1992, 1992; Lasley and Kirkpatrick, 1991; Möstl and Palme, 2002). Glucocorticoid metabolites in feces accurately reflect adrenal activity and can be used to assess stress in wildlife (Morrow et al., 2002; Möstl and Palme, 2002; Möstl et al., 1999, 2002; Wasser et al., 2000). Fecal measures have the advantage of pooling short-term fluctuations in hormone secretion (Creel, 2001). Because hormones and metabolites in feces reflect hormone secretion over composite periods of time, they produce better GC estimates than a single blood measure, which changes quickly (Möstl and Palme, 2002), and thus may better represent individual daily hormonal levels than do blood samples (Pelletier et al., 2003). Following the procedure of previous studies (e.g., Mooring et al., 2004; Sands and Creel, 2004), we compared mean GC level with measures of average dominance status and its correlates throughout the rut.

Fecal glucocorticoid samples

Fecal samples were collected from June to September 2003 from adult bulls averaging 8.9 years of age (range: 3 to 17 years) during all daylight hours. We usually collected 1 sample per bull for each period (pre-rut, rut, and post-rut). Rut was defined as the period when most copulations occurred (beginning with the first copulation in mid-July through to mid-August), pre-rut incorporated the month prior to breeding (June), and post-rut was the month following peak breeding (September). Samples were collected from June 6–11 (pre-rut), July 14–August 13 (rut), and September 15–16 (post-rut). Samples were taken opportunistically from bulls only when defecation was observed from a known individual. Fresh fecal material was transferred to a 70 ml labeled polypropylene container with screw cap (Sarstedt, Inc., Newton, NC). Samples were placed immediately into an ice chest while in the field and later transferred to a freezer at −20°C for storage until shipped overnight on dry ice to San Diego.

At the laboratory, the large samples were lyophilized for 120 h in a Flexi-Dry microprocessor manifold lyophilizer (FTS Systems, Inc., Stone Ridge, NY) to reduce variability in water content. Vegetation was removed from the lyophilized samples by sifting through a mesh screen (2 × 1.5 mm). A 0.2 g sample of the sifted feces was added to a 16 × 150 mm borosilicate culture tube, wetted with distilled water (2 ml), and vortexed (2 min). Five milliliters of diethyl ether anhydrous (Mallinckrodt, Paris, KY) were added to each tube, vortexed (2 min), and flash frozen in a methanol:dry ice bath. The supernatant was poured into 12 × 75 mm culture tubes and allowed to evaporate in a water bath (37°C). The ether extract was resolubilized in 1 ml absolute ethanol.

Hormone assay

The fecal glucocorticoids were measured with a commercially available double-antibody 125 I radioimmunoassay (RIA) kit (MP Biomedicals, Costa Mesa, CA). The primary antibody in the RIA kit was raised against corticosterone and cross-reacts with a variety of fecal metabolites of both corticosterone and cortisol found in birds and mammals (Wasser et al., 2000). The kit protocol was followed with the exception that 10 μl of the ethanolic fecal extract, diluted 1:100 in phosphate-buffered saline 0.1 M pH 7.0 (PBS), was assayed in duplicate.

Validation of the assay was tested by comparing parallelism in a serial dilution of fecal extract with the corticosterone standard curve (r = 0.992). Extraction efficiency of added tritiated corticosterone to lyophilized and sifted feces (0.2 g) was 39.1 ± 17.72 (mean ± SD, N = 8). Assay sensitivity was 3.78 pg/tube (calculated as mean pg/tube at 90% B/BO, N = 9). Buffer blanks were below the assay sensitivity. Accuracy was determined as 108.85% + 5.88 (mean ± SD) by recovery of 6 known quantities of standard that were equivalent to 75% of the standards used in the standard curve (25–1000 ng/ml) added to a pool of fecal extract. A diluted fecal sample from a study male was used for this pool, which contained an immunoreactive content above the sensitivity of the assay. Inter-assay coefficients of variation (% SD/mean N = 8) were 7.9% based on duplicates of corticosterone controls provided in the kit with an immunoreactive content that yielded a %B/BO > 30% and 8.7% based on duplicates of a corticosterone control with an immunoreactive content that yielded a %B/BO > 65%. Intra-assay variation estimates (10 replicates of the same two controls in a single assay) were 4.3% for the high pool and 5.7% for the low pool. Results are presented as ng/g (equal to ng/g dry fecal weight).

High-pressure liquid chromatography

Following the protocol of Strier et al. (1999), reversed phase high-performance liquid chromatography (HPLC) was used to determine the number and relative proportions of immunoreactive steroid metabolites in fecal extract from bison. The system was composed of a dual HPLC pump (Beckman Instruments, Schaumberg, IL) which was connected to a diode array analyzer for UV detection. Pooled extract (100 μl from 9 bison samples) was first evaporated and then reconstituted in 30 μl of the mobile phase (40:60 acetonitrile:water), 20 μl was then injected into a reversed phase precolumn (5 μm, 4.6 mm × 4.5 cm, Beckman) and reversed phase HPLC column (ultrasphere, 5 μm, 4.6 × 25 cm, Beckman). The mobile phase remained constant, and fractions were collected at a rate of 1 ml/min for the first 25 min and then increased to 1.5 ml (50:50 acetonitrile:water) for an additional 15 min. The resultant fractions were evaporated to dryness, reconstituted in 500 μl assay buffer (PBS), and an aliquot (100 μl) of each assayed in duplicate in the immunoassay as described above.

Data analysis and animal welfare

Data were analyzed using the SPSS 11.5 statistical package for Windows (Norusis, 2002). Examination of standard deviations and Levene's test indicated that homogeneity of variance could not be assumed for several comparisons. We have dealt with this by using nonparametric tests based on ranked data: the Spearman rank order test for bivariate correlation and the Mann–Whitney test for comparison of central tendencies (Siegel and Castellan, 1988). These tests are robust to violations of homogeneity and are more conservative (have less power) than parametric counterparts. For comparison of GC for 39 matched sample bulls during pre-rut, rut, and post-rut, we used the repeated measures general linear model (GLM) with Bonferroni multiple comparisons. Data were log-transformed so that the assumption of sphericity was not violated, but analysis on raw data gave the same results. The level of significance was set at 0.05, and all tests were two-tailed. We have adhered to the guidelines for animal welfare specified in National Institutes Health *Guide for the Care and Use of Laboratory Animals* (NIH, 1985).

Results

High-pressure liquid chromatography

The majority of immunoreactivity in eluate from bison fecal samples was found with peaks eluting in fractions 4–7 (42% of total immunoreactivity) and 7–9 (36% of total immunoreactivity). On this HPLC system (Fig. 1), the retention times for cortisol, cortisone, and corticosterone were determined to be 4.3, 4.567, and 7.342 min, respectively, thus coinciding with 78% of the total immunoreactivity representing glucocorticoids. Two more polar fractions eluted at fractions 14–16 and 21–23 (17% and 6% of the total immunoreactivity). The retention times for 3 beta androstanediol and 3 alpha androstanediol are 15 and 23 min, respectively. The HPLC peaks illustrated in Fig. 1 correspond to cortisol and cortisone (I), corticosterone (II), 3 beta androstanediol (III), and 3 alpha androstanediol (IV).

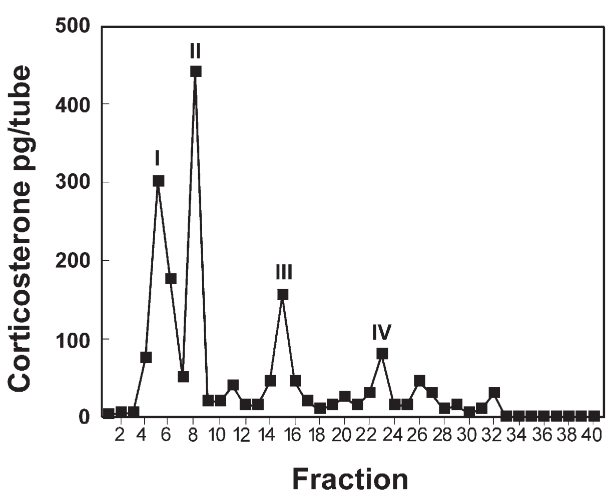

Fig. 1. Immunoreactive glucocorticoid metabolites after high-performance liquid chromatography (HPLC). Peak I corresponds to the retention times of cortisol (4.3 min) and cortisone (4.567 min), Peak II corresponds to the retention time of corticosterone (7.342 min), Peak III corresponds to the retention time of 3 beta androstanediol (15 min), and Peak IV corresponds to the retention time of 3 alpha androstanediol (23 min).

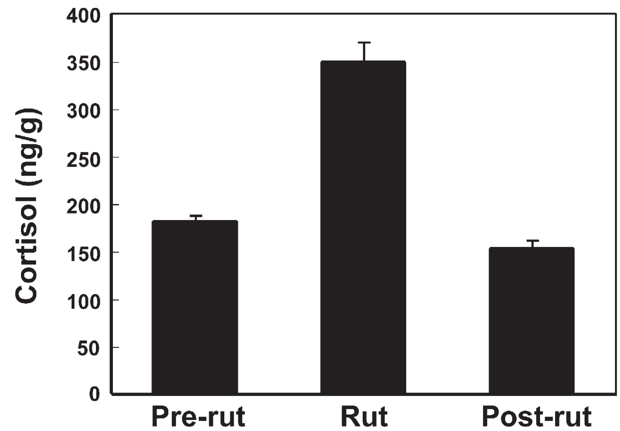

Fig. 2. Mean (±SEM) GC levels from fecal steroid analysis (ng/g feces) for bison bulls at Fort Niobrara NWR during pre-rut (June), rut (July–August), and post-rut (September). Matched samples involved 39 bulls for which fecal samples were collected from all 3 periods. Repeated measures GLM showed that GCs peaked during the rut, being significantly higher during rut compared with pre-rut or post-rut.

Seasonal levels of glucocorticoids

GC levels of bulls peaked during the rut (compared with pre-rut and post-rut) for matched samples of 39 bulls for which data were collected in all 3 periods (repeated measures GLM, log-transformed data: $F_{2,76} = 76.9$, $P = 0.0001$; Bonferroni multiple comparisons, $P = 0.0001$ for pre-rut vs. rut and rut vs. post-rut, $P = 0.01$ for pre-rut vs. post-rut; Fig. 2). The mean GC approximately doubled from pre-rut to rut and then declined by 43% during post-rut (Fig. 2).

Individual differences in glucocorticoids during the rut

Correlation analysis indicated a significant positive correlation between GC level and both dominance rank (Spearman: $N = 87$, $r_s = 0.26$, $P = 0.02$) and copulatory success ($N = 87$, $r_s = 0.24$, $P = 0.02$) of bulls. Because we were unable to observe all copulations (e.g., at night), bulls with a few copulations and high GC levels may have actually had additional copulations that went unrecorded. Dominance rank was positively correlated with number of copulations (Spearman: $N = 99$, $r_s = 0.46$, $P = 0.0001$; Fig. 3) and was highest among prime-aged bulls 7 years or older (Mann–Whitney: $N = 98$, $Z = 3.82$, $P = 0.0001$). There was also a positive correlation between GC level and age of bull (Spearman: $N = 86$, $r_s = 0.43$, $P = 0.0001$; Fig. 4), with prime-aged bulls having significantly higher GC levels compared with younger bulls (Mann–Whitney: $N = 86$, $Z = 4.22$, $P = 0.0001$). Bull age was positively correlated with number of copulations

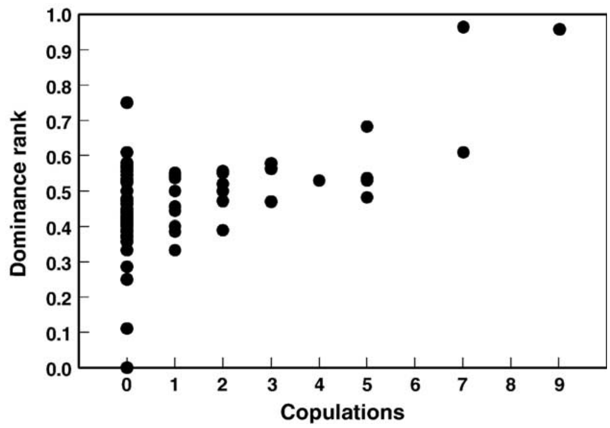

Fig. 3. Dominance rank (calculated from agonistic interactions) of bison bulls at Fort Niobrara NWR plotted against the number of observed copulations. Dominance rank was positively correlated with observed copulations.

observed (Spearman: N = 98, r = 0.22, P = 0.03), and prime-aged bulls had higher copulatory success compared with bulls less than 7 years old (Mann–Whitney: N = 98, Z = 3.24, P = 0.001). When GC levels were compared with androgens measured in the same bulls at the same time (reported in Mooring et al., 2004), GC and androgen levels were positively correlated (Spearman: N = 87, r_s = 0.73, P = 0.0001; Fig. 5).

During the rut, bulls that were actively tending cows at the time of fecal collection had higher levels of GC (mean ± SEM = 454.5 ± 47.5 ng/g) compared with bulls that were not tending at that time (280.0 ± 16.5 ng/g; Mann–Whitney: N = 85, Z = 3.71, P = 0.0001). Bulls that were not tending were either attending a tending bull and cow or not active in the rut at all. Tending bulls continued to sequentially tend cows throughout the time they were active in the rut (mean = 19 days), while non-tending bulls generally continued to not tend. Mann–Whitney comparisons revealed that tending bulls had a higher dominance rank, obtained more copulations, and were older (mean = 10.5 years) compared with non-tending

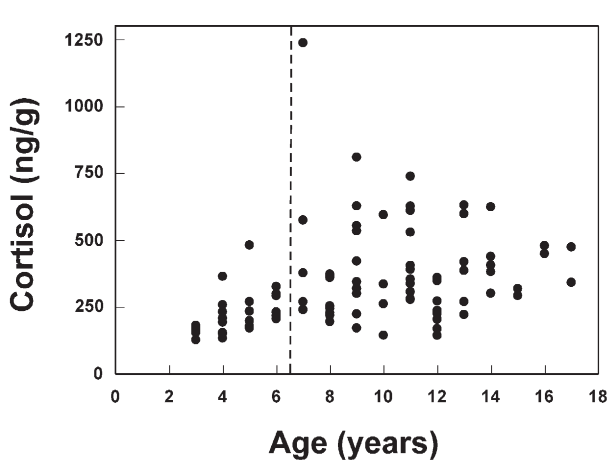

Fig. 4. GC levels from fecal steroid analysis (ng/g feces) of bison bulls at Fort Niobrara NWR according to age. Age of bull was positively correlated with GC level. Bulls ≥7 years (to right of dashed line) had significantly higher GCs compared with bulls <7 years.

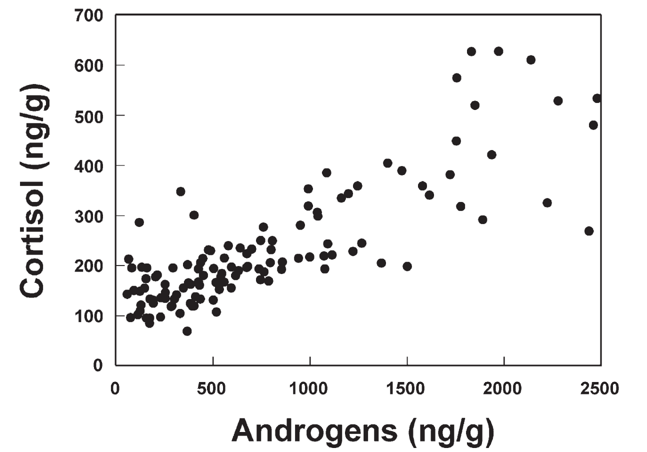

Fig. 5. GC and androgen levels from fecal steroid analysis (ng/g feces) of the same bison bulls at Fort Niobrara NWR during pre-rut, rut, and post-rut. GC level was positively correlated with androgen level.

bulls (dominance rank: N = 89, Z = 2.10, P = 0.04; copulations: N = 89, Z = 2.82, P = 0.005; age: N = 88, Z = 2.42, P = 0.01).

Discussion

Based on fecal steroid analysis, glucocorticoid (GC) levels of bison bulls at Fort Niobrara peaked during the rut in late July and early August, as predicted by both the ‘stress of subordination’ and ‘stress of domination’ hypotheses. GC levels doubled from pre-rut levels in June and then declined 43% during post-rut in September. The rut-related changes in GC of bulls were similar to seasonal changes in androgen levels previously reported in this herd (Mooring et al., 2004: Fig. 2), and indeed GC and androgen levels were positively correlated among samples taken from the same bulls during the same time period. Fecal androgen concentrations are positively correlated with aggressive and agonistic behavior (e.g., horning, chasing, biting, threats) in artiodactyls (Fletcher, 1978; Li et al., 2000, 2001; Patton et al., 2001). Our data are consistent with the view that prolonged aggressive interactions among rutting bull bison are energetically demanding and, combined with reduced feeding, are potent physiological stressors that increase GC secretion (Sapolsky, 2002; Muller and Wrangham, 2004; Sands and Creel, 2004). Such a stress response may be due to a combination of reduced nutritional intake, repeated aggressive interactions, and endurance competition over several weeks of intense rutting activity.

During the rut, GC level was positively correlated with the dominance rank, copulatory success, and age of bulls. High dominance rank was associated with more copulations, and prime-ages bulls of 7 years or older tended to have higher dominance rank than did younger bulls. Bulls that were actively tending cows on the day of fecal collection had higher GC levels compared with bulls that were not tending. Tending bulls had higher dominance rank and obtained more copulations compared with non-tenders. Non-tending bulls were either attending bulls (‘satellite’ bulls surrounding a tending pair) or had retired from rutting behavior altogether.

Because the average tending bull was in the rut for 19 days, we are confident that most of the bulls we sampled had been active in the rut for at least 1 day prior to collection. Following copulation or displacement, tending bulls usually moved directly to tend another cow. Assuming bull reproductive status at the time of fecal collection was representative of rutting activity when hormones were circulating in the blood 10–12 h earlier, these results indicate that bulls that were actively guarding cows from other bulls had elevated GC levels and were thus subjected to greater psychological or physiological stressors than non-tenders.

Our results are consistent with the 'stress of domination' hypothesis, which predicts that high-ranking individuals are more stressed than subordinates because they must engage in energetically expensive displays and fights and/or because the dominance hierarchy is constantly shifting (Creel, 2001). Recent field studies have provided support for the 'stress of domination' hypothesis in birds, fish, and mammals (Kotrschal et al., 1998; Overli et al., 1999; Creel, 2001; Barrett et al., 2002; Pravosudov et al., 2003; Sands and Creel, 2004).

Tending bulls in this and other bison populations are older and more dominant than non-tending bulls (Komers et al., 1992a; Wolff, 1998; Mooring et al., 2004), indicating that dominance rank tends to peak in mature bulls. Previously, we reported that older more dominant bulls maintained higher androgen levels and were more likely to tend cows and enjoy high mating success than younger more subordinate bulls (Mooring et al., 2004: Figs. 3 and 5). Our finding that both copulatory success and age of bulls were positively correlated with dominance rank and GC level suggests that older more dominant bulls are under greater stress compared with younger subordinate bulls. This indicates that higher-ranking bulls are challenged by a greater intensity of physiological or psychological stressors as they compete for mating privileges with estrus cows. To successfully tend and breed bison cows, bulls must outcompete any rivals that approach, either by exerting previously established dominance rank or by escalating aggressive behavior (threat displays, charging, fighting). Because social rank and fighting ability often increase with age, fecal and serum androgens have been found to be positively correlated with age in bovids and cervids (Oba et al., 1988; Ahmad et al., 1992; Ditchkoff et al., 2001; Pelletier et al., 2003).

Elevated GC level in dominant bulls could have been the result of greater psychological or physiological stressors than those experienced by subordinates. For example, if the dominance hierarchy was unstable and in a continuous state of flux during the rut, psychological stress would be implicated (Muller and Wrangham, 2004). On the other hand, if dominants experienced high energetic demands from frequent agonistic displays, physical conflict, and decreased feeding rate, physiological stress would be implied (Muller and Wrangham, 2004). Further work will be required to tease apart these possibilities and establish whether dominant bison bulls are challenged primarily by psychological or by physiological stressors during the rut. Either way, the results reported here support the 'stress of domination' hypothesis and show that dominant bison bulls pay a short-term physiological cost for their high rank and mating success. However, extended periods of high GC levels can have harmful consequences, such as reproductive failure, immunosuppresion, and shorter life span (Creel, 2001; Abbott et al., 2003; Sands and Creel, 2004). Thus, the weeks of elevated cortisol experienced by the average rutting bull probably have long-term deleterious effects that may abbreviate the number of years a bull can compete effectively for breeding rights.

Acknowledgments

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References

Abbott, D.H., Keverne, E.B., Bercovitch, F.B., Shively, C.A., Mendoza, S.P., Saltzman, W., Snowdon, C.T., Ziegler, T.E., Banjevic, M., Garland, T., Sapolsky, R.M., 2003. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. Horm. Behav. 43, 67–82.

Ahmad, M.M., Mughal, M.R., Bari, A., Khan, M.I., Shahab, M., 1992. Thyroid hormones and testosterone in sheep: age-related profiles of serum thyroxine triiodothyronine and testosterone in Kaghani Rambouillet and Kaghani × Rambouillet sheep. Asian-Australas. J. Anim. Sci. 5, 101–106.

Barrett, G.M., Shimizu, K., Bardi, M., Asaba, S., Mori, A., 2002. Endocrine correlates of rank, reproduction, and female-directed aggression in male Japanese macaques (*Macaca fuscata*). Horm. Behav. 42, 85–96.

Berger, J., 1989. Female reproductive potential and its apparent evaluation in male mammals. J. Mammal. 70, 347–358.

Berger, J., Cunningham, C., 1991. Bellows, copulations, and sexual selection in bison (*Bison bison*). Behav. Ecol. 2, 1–6.

Berger, J., Cunningham, C., 1994. Bison: Mating and Conservation in Small Populations. Columbia Univ. Press, New York.

Creel, S., 2001. Social dominance and stress hormones. TREE 16, 491–497.

Ditchkoff, S.S., Spicer, L.J., Masters, R.E., Lochmiller, R.L., 2001. Concentrations of insulin-like growth factor-I in adult male white-tailed deer (*Odocoileus virginianus*): associations with serum testosterone, morphometrics and age during and after the breeding season. Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol. 129A, 887–895.

Fletcher, T.J., 1978. The induction of male sexual behavior in red deer *Cervus elaphus* by the administration of testosterone to hinds and estradiol 17-beta to stags. Horm. Behav. 11, 74–88.

Goymann, W., Möstl, E., Van't Hof, T., East, M.L., Hofer, H., 1999. Noninvasive fecal monitoring of glucocorticoids in spotted hyenas, *Crocuta crocuta*. Gen. Comp. Endocrinol. 114, 340–348.

Kirkpatrick, J.F., Kincy, V., Bancroft, K., Shideler, S.E., Lasley, B.L., 1991. Oestrus cycle of the North American bison (*Bison bison*) characterized by urinary pregnanediol-3-glucuronide. J. Reprod. Fertil. 93, 541–547.

Kirkpatrick, J.F., Bancroft, K., Kincy, V., 1992. Pregnancy and ovulation detection in bison (*Bison bison*) assessed by means of urinary and fecal steroids. J. Wildl. Dis. 28, 590–597.

Kirkpatrick, J.F., Gudermuth, D.F., Flagan, R.L., McCarthy, J.C., Lasley, B.L., 1993. Remote monitoring of ovulation and pregnancy in Yellowstone bison. J. Wildl. Manage. 57, 407–412.

Komers, P.E., Messier, F., Gates, C.C., 1992a. Search or relax: the case of bachelor wood bison. Behav. Ecol. Sociobiol. 31, 195–203.

Komers, P.E., Roth, K., Zimmerli, R., 1992b. Interpreting social behaviour of wood bison using tail postures. Z. Saeugetierkd. 57, 343–350.

Kotrschal, K., Hirschenhauser, K., Möstl, E., 1998. The relationship between social stress and dominance is seasonal in greylag geese. Anim. Behav. 55, 171–176.

Lasley, B.L., Kirkpatrick, J.F., 1991. Monitoring ovarian function in captive and free-ranging wildlife by means of urinary and fecal steroids. J. Zoo Wildl. Med. 22, 23–31.

Li, C., Jiang, Z., Fang, J., Jiang, G., Ding, Y., Shen, H., Xu, A., 2000. Relationship between reproductive behavior and fecal steroid in milu (*Elaphurus davidianus*). Acta Theriol. Sin. 20, 88–100.

Li, C., Jiang, Z., Jiang, G., Fang, J., 2001. Seasonal changes of reproductive behavior and fecal steroid concentrations in Pere David's deer. Horm. Behav. 40, 518–525.

Lott, D.F., 1974. Sexual and aggressive behaviour of bison. In: Geist, V., Walther, F. (Eds.), The Behaviour of Ungulates in Relation to Management. IUCN, vol. 24, pp. 382–394.

Lott, D.F., 1979. Dominance relations and breeding rate in mature male American bison. Z. Tierpsychol. 49, 418–432.

Lott, D.F., 1981. Sexual behavior and intersexual strategies in American bison. Z. Tierpsychol. 56, 97–114.

Lott, D.F., 2002. American Bison: A Natural History. University of California Press, Berkeley.

Meagher, M., 1986. *Bison bison*. Mamm. Species 266, 1–8.

Mooring, M.S., Patton, M.L., Vance, V.A., Hall, B.M., Schaad, E.W., Fortin, S. S., Jella, J.E., McPeak, K.M., 2004. Fecal androgens of bison bulls during the rut. Horm. Behav. 46, 392–398.

Morrow, C.J., Kolver, E.S., Verkerk, G.A., Matthews, L.R., 2002. Fecal glucocorticoid metabolites as a measure of adrenal activity in dairy cattle. Gen. Comp. Endocrinol. 126, 229–241.

Möstl, E., Palme, R., 2002. Hormones as indicators of stress. Domest. Anim. Endocrinol. 23, 67–74.

Möstl, E., Messmann, S., Bagu, E., Robia, C., Palme, R., 1999. Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. J. Vet. Med., Ser. A 46, 621–631.

Möstl, E., Maggs, J.L., Schrotter, G., Besenfelder, U., Palme, R., 2002. Measurement of cortisol metabolites in faeces of ruminants. Vet. Res. Commun. 26, 127–139.

Muller, M.N., Wrangham, R.W., 2004. Dominance, cortisol and stress in wild chimpanzees (*Pan troglodytes schweinfurthii*). Behav. Ecol. Scoiobiol. 55, 332–340.

NIH, 1985. National Institutes of Health Guide for the Care and Use of Laboratory Animals. DHEW Publ., vol. 80-23. Office of Science and Health Reports, DRR/NIH, Bethesda, MD.

Norusis, M., 2002. SPSS 11.0 Guide to Data Analysis. Prentice-Hall, Upper Saddle River, NJ.

Oba, E., Define, R.M., Muniz, L.M.R., Ramos, A.D.A., 1988. Determination of the serum levels of FSH luteinizing hormone and testosterone in Nellore cattle at different ages using radioimmunoanalysis. Arq. Bras. Med. Vet. Zootec. 40, 25–34.

Overli, O., Olsen, R.E., Ringo, E., 1999. Dominance hierarchies in Artic charr, *Salvelinus alpinus* L.: differential cortisol profiles of dominant and subordinate individuals after handling stress. Aquacult. Res. 30, 259–264.

Patton, M.L., White, A.M., Swaisgood, R.R., Sproul, R.L., Fetter, G.A., Kennedy, J., Edwards, M.S., Rieches, R.G., Lance, V.A., 2001. Aggression control in a bachelor herd of fringe-eared oryx (*Oryx gazella callotis*), with melengestrol acetate: behavioral and endocrine observations. Zoo Biol. 20, 375–388.

Pelletier, F., Bauman, J., Festa-Bianchet, M., 2003. Fecal testosterone in bighorn sheep (*Ovis canadensis*): behavioural and endocrine correlates. Can. J. Zool. 81, 1678–1684.

Pravosudov, V.V., Mendoza, S.P., Clayton, N.S., 2003. The relationship between dominance, corticosterone, memory, and food caching in mountain chickadees (*Poecile gambeli*). Horm. Behav. 44, 93–102.

Sands, J., Creel, S., 2004. Social dominance, aggression and faecal glucocorticoid levels in a wild population of wolves, *Canis lupus*. Anim. Behav. 67, 387–396.

Sapolsky, R.M., 2002. Endocrinology of the stress response. In: Becker, J.B., Breedlove, M., Crews, D., McCarthy, M.M. (Eds.), Behavioral Endocrinology, 2nd ed. The MIT Press, Cambridge, MA, pp. 409–450.

Siegel, S., Castellan, N.J., 1988. Nonparametric Statistics for the Behavioral Sciences, 2nd ed. McGraw-Hill, New York.

Strier, K.B., Ziegler, T.E., Wittwer, D.J., 1999. Seasonal and social correlates of fecal testosterone and cortisol levels in wild male muriquis (*Brachyteles arachnoids*). Horm. Behav. 35, 125–134.

Wasser, S.K., Hunt, K.E., Brown, J.L., Cooper, K., Crockett, C.M., Bechert, U., Millspaugh, J.J., Larson, S., Monfort, S.L., 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. Gen. Comp. Endocrinol. 120, 260–275.

Wolff, J.O., 1998. Breeding strategies, mate choice, and reproductive success in American bison. Oikos 83, 529–544.