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THE STRESS RESPONSE TO ENVIRONMENTAL CHANGE IN CAPTIVE CHEETAHS (*ACINONYX JUBATUS*)

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Abstract: The captive North American cheetah (*Acinonyx jubatus*) population is not self-sustaining because of high prevalences of unusual diseases and poor reproductive success. Cheetahs are commonly moved between zoos for breeding purposes to maintain genetic diversity within the captive population, and movement may exacerbate infertility and disease. Fecal corticoids were analyzed by radioimmunoassay to measure the stress response of cheetahs to movement between facilities. Fecal samples were collected from 15 cheetahs for 14 days before movement and for at least 30 days after movement. For each cheetah, premovement fecal corticoid concentrations were used to determine baseline and then compared with trends in postmovement concentrations. In general, postmovement corticoid concentrations either increased ($n = 8$), did not change ($n = 2$), or decreased ($n = 5$). Although individual animal differences occurred, corticoid concentrations increased for most animals moved on-exhibit and decreased in animals moved off-exhibit. Animals moving on-exhibit had an 18-times greater risk of having corticoids elevated more than two standard deviations above baseline for 30 days after movement compared with animals that moved off-exhibit. In addition, greater day-to-day variation in corticoids occurred in animals moved on-exhibit. In general, animals with initially low baseline corticoid concentrations had a greater postmovement corticoid response than cheetahs with initially high baseline levels. These results indicate that some cheetahs have a prolonged stress response when moved between facilities, and the magnitude and character of this response is influenced by the exhibit environment.

Key words: *Acinonyx jubatus*, cheetah, corticoids, fecal steroids, movement, stress.

INTRODUCTION

Cheetahs (*Acinonyx jubatus*) are highly endangered, with less than 10,000 animals estimated to exist worldwide.[7] To complement in situ conservation efforts, captive breeding programs have been instituted. The American Zoo and Aquarium Association (AZA) Cheetah Species Survival Plan (SSP) was formed to manage these captive breeding programs. To achieve the SSP goal of maintaining genetic diversity, animals are commonly moved between zoos for breeding. However, captive cheetah numbers continue to decline, in part because of poor health from diseases such as feline infectious peritonitis (FIP) and venoocclusive disease (VOD) that occur when animals are moved to facilities with greater public exposure (Munson, unpubl. data).[10] Wild cheetahs in general are spared the diseases common in captive populations (Munson, unpubl. data), despite a similar genetic background.[8,10,11] The occurrence of these unusual diseases in only captive animals and exacerbation after movement suggests an environmental effect. Chronic stress has been suspected to be an important contributing factor, because baseline corticoids were significantly higher and adrenal cortices larger in captive than wild cheetahs.[16]

The objective of this study was to determine whether moving captive cheetahs to new environments results in a prolonged stress response measurable by elevated corticoid concentrations. Cortisol is the preferred hormone to measure in cheetahs because it can reflect both acute and chronic stress, and because the majority of cortisol metabolites are excreted in feces, which can be obtained noninvasively.[1,4,13,14] Furthermore, fecal corticoids represent an average, pooled value for the previous 12–24 hr, whereas serum cortisol fluctuates diurnally and in a pulsatory pattern throughout the day.[3,18] Therefore, fecal corticoid analyses would be the optimal method to measure chronic stress as a result of movement in captive cheetahs.

MATERIALS AND METHODS

Animals

Fifteen adult cheetahs, including nine males and six females housed in AZA-accredited facilities and moved between facilities for Cheetah SSP breeding recommendations, were studied. Animals ranged from 5–12 yr of age. All animals were exposed to natural variations in photoperiod and were fed a commercial ground meat–based diet. Animals were defined as ''on-exhibit'' if they were on display for public viewing or ''off-exhibit'' if they were housed away from public viewing. Off-exhibit fa-

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cilities were 0.1 to 0.2 ha outdoor enclosures. On-exhibit facilities also were outdoors and ranged from 0.1 to 0.6 ha in size. Four post-movement animals were placed in a 0.1 ha outdoor off-exhibit quarantine facility for 4 wk and then moved to another off-exhibit enclosure at the same facility.

Fecal samples were collected for 14 days before movement between sites during a period of consistent management. No animals were moved or anesthetized during the premovement collection period. After movement, fecal samples from each animal were collected every day (as available) for 1 mo and then every other day for up to 3 mo. Approximately 15 g of feces were collected and stored frozen at −20°C immediately after collection. For animals housed together, a few drops of food dye or cracked corn were added to the diet of one cheetah to differentiate an individual cheetah's feces. Once collection was complete, samples were shipped on dry ice by overnight express mail to the laboratory.

Fecal extraction

Fecal samples were lyophilized and pulverized, and the steroids were extracted using established methodology developed for cat feces.[1,4] Approximately 0.20 g of the dried fecal sample was boiled in 90% ethanol for 20 min. After centrifugation (1,500 rpm, 20 min), the supernatant was recovered and the pellet resuspended in an additional 5 ml of 90% ethanol, vortexed for 1 min, and recentrifuged. The ethanol supernatants were combined, dried under air, and resuspended in 1 ml of methanol before diluting (1:20) in phosphate buffer for radioimmunoassay (RIA).

Radioimmunoassay

Concentrations of cortisol metabolite immunoactivity in fecal extracts were quantified using a double antibody ^{125}I-corticosterone RIA (ICN Pharmaceuticals Inc., Costa Mesa, California 92626, USA) validated previously for cheetahs.[15] Fifty microliters of fecal extract (diluted 1:20 in phosphate buffer) was added to 50 μl of steroid diluent (from the assay kit) and assayed in duplicate. Assay sensitivity was previously determined to be 12.5 ng/ml and all inter- and intraassay coefficients of variation were <10%.[15] All data are expressed on a per gram dry weight basis.

Data analysis

The average premovement fecal corticoid metabolite concentration was calculated for each animal. For the basis of this study, the term ''baseline'' will be used to refer to the premovement average corticoid concentration. Statistical analyses were only performed on data obtained during the first 30 days after movement because fecal samples were not available for all study animals beyond 30 days. Because concentrations differed, all values were normalized as a percent change in response relative to baseline to allow comparisons among animals, with the baseline designated as 100%. Because of the relatively large daily variation in fecal corticoid concentrations, trends in postmovement steroid concentrations were evaluated using best fit polynomial curves ($y = ax^3 + bx^2 + cx + d$; ''trendlines'') calculated for postmovement data for each animal (Excel 97, Microsoft Corp., Redmond, Washington 98052-6399, USA). To evaluate the magnitude of the response, three case definitions were delineated: a postmovement trendline remaining at least two standard deviations above baseline for 30 days, a postmovement trendline remaining above baseline for 30 days, and a postmovement trendline remaining above baseline for 15 days. The change in the standard deviation (Δ SD) from pre- to postmovement was calculated to quantify the amount of day-to-day variation that was not evident in the trendlines. Because cheetahs were moved to facilities with different degrees of public exposure that could influence the magnitude and character of the stress response, odds ratios and Fisher exact 95% confidence intervals were calculated for the different types of public exposure (Epi Info 2000, version 1.1.2, Centers for Disease Control and Prevention, Atlanta, Georgia 30333, USA). The Mann–Whitney *U*-test was used to determine whether the magnitude of premovement baseline affected postmovement responses and whether the change in standard deviation correlated with postmovement exhibit type (SPSS for Windows, 8.0.0 Standard version, 1997, Chicago, Illinois 60606, USA).

RESULTS

Three general patterns were observed in postmovement corticoid responses when compared with baseline: increased corticoid concentrations ($n = 8$; Fig. 1a), no apparent change in corticoid concentration ($n = 2$; Fig. 1b), or a general decrease in corticoid concentration ($n = 5$; Fig. 1c). Of the seven animals that did not have an increase in corticoids after movement, four animals had a single peak in corticoids immediately after movement (Fig. 1c). Eleven cheetahs were moved directly to their final exhibit location, (quarantine on site) including all seven cheetahs moved to on-exhibit enclosures. All four cheetahs that were quarantined had an acute stress response corresponding with movement out of quarantine (Fig. 1c). In some

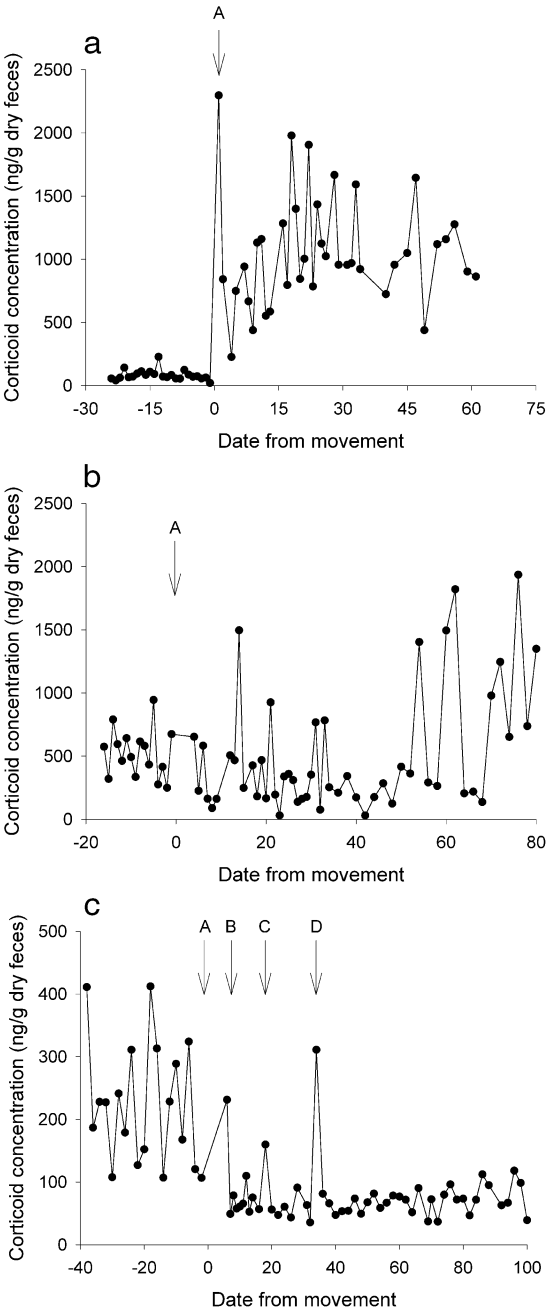

Figure 1. Representative profiles of fecal corticoid concentrations in cheetah after movement between zoos. The day that the cheetah was moved is marked with an arrow (A). All negative dates correspond to premovement collection days, and all positive values correspond to postmovement collection days. Each point represents an individual fecal sample. Profiles represent cheetahs that had a marked increase (**a**), no change (**b**), and a general decrease (**c**) in corticoid concentrations. In Figure 1**b**, note that there was no peak immediately after movement; however, no fecal samples were available until day 4 after

cheetahs, there were marked daily fluctuations in corticoid concentrations before movement (Fig. 1b, c), whereas others showed little daily variation from the mean (Fig. 1a). Marked variations in day-to-day corticoid concentrations also were noted after movement (Fig. 1a, b). Changes in daily variation after movement (expressed as Δ SD) correlated ($P = 0.002$) with exhibit type, with greater variation occurring when animals were moved on-exhibit (Table 1; Fig. 1a). Although some animals had no apparent change in overall corticoid concentration after movement (based on trendlines), daily corticoid fluctuations differed between pre- and postmovement time periods (based on Δ SD) (Fig. 1b).

Different magnitudes and durations of response were detected from trendline analyses (Table 1). Seven of 15 cheetahs had corticoid concentrations above baseline for 15 days after movement, and six of these animals continued to show elevated corticoid concentrations through 30 days after movement. Of these six, four had markedly elevated corticoid concentrations at least two SD above baseline throughout the 30-day period. An additional cheetah also had elevated corticoids after movement but for less than 15 days. A representative trendline for a postmovement stress response is shown in Figure 2. Six cheetahs with elevated corticoids and fecal samples available for 60 days had persistent elevation (Fig. 1a). Four of these six cheetahs had fecal samples available for 90 or more days after movement, and three of these four cheetahs continued to have persistent elevations in corticoids through at least 90 days.

Overall, marked differences in baseline premovement corticoid concentrations were observed between individual cheetahs (Table 1). Cheetahs with low baseline corticoid concentrations were more likely to have elevated corticoids for 15 days ($P < 0.01$), 30 days ($P = 0.088$) or for two SD above baseline for 30 days ($P = 0.04$) after movement (Table 1).

In general, cheetahs moving to on-exhibit enclosures showed a higher risk of having increased corticoids after movement irrespective of the premovement exhibit type (Table 2). This risk was greatest in cheetahs moving from off-exhibit enclosures. Despite these trends, animals moved to the same

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movement. In Figure 1**c**, peaks occurred in the first available sample post-movement (B), on the day after immobilization (C), and the day after movement out of quarantine (D).

Table 1. Baseline (average) corticoid concentration before movement, change in standard deviation (ΔSD) from pre to postmovement, exhibit type, and case definitions based on postmovement corticoid patterns for cheetahs moved between facilities. Exhibit type was defined as ''On''-exhibit if animals were on display for public viewing or ''Off''-exhibit if they were housed away from public viewing.

Cheetah	Premovement average (mean ± SD)[a]	ΔSD[a,c]	Exhibit		Corticoid concentration		
			Pre-movement	Post-movement	>Two SD above baseline for 30 days	>Baseline for 30 days	>Baseline for 15 days
1	59.52 ± 56.10	104.19	On	On			X
2	71.48 ± 41.98[b]	132.22	On	Off	X	X	X
3	82.43 ± 49.88	398.74	Off	On	X	X	X
4	93.48 ± 28.77	293.28	Off	On	X	X	X
5	123.51 ± 31.67	32.57	On	Off		X	X
6	132.31 ± 74.16	−55.59	On	Off			
7	146.17 ± 59.98	350.11	Off	On	X	X	X
8	176.97 ± 61.10	9.05	On	Off			
9	179.29 ± 40.07	−35.14	Off	Off			
10	233.02 ± 44.01	−52.75	On	Off			
11	329.48 ± 73.26	−200.73	On	Off			
12	385.69 ± 54.90	184.79	On	On		X	X
13	524.20 ± 36.32	466.41	On	On			
14	574.51 ± 68.52	194.63	Off	On			
15	888.67 ± 96.11	−404.67	On	Off			

[a] Expressed as ng/g dry feces.
[b] This cheetah developed feline infectious peritonitis and died during the sample collection period.
[c] Calculated as the postmovement SD minus the premovement SD.

facility differed in the magnitude and daily variation of postmovement corticoids (Fig. 3).

DISCUSSION

This study is the first to document long-term adrenal responses to movement in cheetahs. Although there was marked variation in individual animal responses before and after movement, changes in corticoid concentrations after movement could still be detected. Three general patterns of response were noted: 1) an increase in corticoids after movement, 2) no change in corticoids after movement, or 3) a decrease in corticoids after movement. Six of seven animals that had increased postmovement corticoids had a prolonged (greater than 60 day) stress response Although the magnitude and nature of the stress response differed by animal and facility, corticoid concentrations generally increased if animals were moved on-exhibit and decreased if moved off-exhibit.

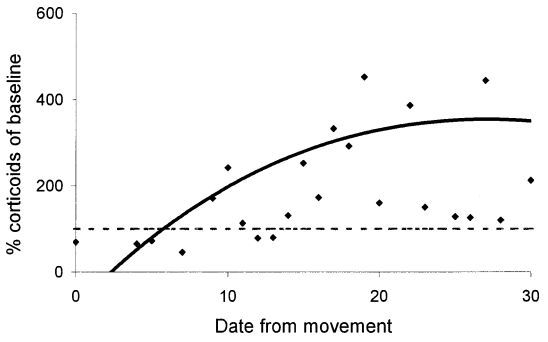

Figure 2. Example of a trendline (solid line) for postmovement corticoid concentrations in a cheetah. The dashed line at 100% represents the baseline (premovement average). All values are represented as a percentage of baseline. This animal's corticoid concentration remained above baseline for at least 30 days.

The increased corticoids in cheetahs moved on-exhibit was not surprising because cheetahs in the wild are solitary, have large home ranges, and avoid human contact.[7] Intensive handling, a necessary part of the movement procedure, also could account for some of the initial elevated corticoid concentrations. Acute stress responses were seen as single peaks, such as in the cheetah after immobilization and movement out of quarantine. However, prolonged elevations are more likely a chronic stress response to environmental change, as evidenced by the increased risk of elevated corticoids in animals moved on-exhibit. All animals experienced changes in animal-care staff and management practices after movement, but these variables differed for each animal, precluding statistical anal-

Table 2. Relative risk for increased corticoid concentrations in cheetahs moved on public exhibit. Exhibit type was defined as ''On''-exhibit if animals were on display for public viewing or ''Off''-exhibit if they were housed away from public viewing.

Premovement to postmovement exhibit type	Number of animals	Odds ratios (95% confidence intervals)		
		Corticoids > two standard deviation above baseline for 30 days	Corticoids above baseline for 30 days	Corticoids above baseline for 15 days
Off → On	4	18 (0.50–1096)	7.5 (0.29–470)	7.5 (0.29–470)
On or Off → On	7	5.25 (0.27–314)	4.0 (0.30–64)	7.5 (0.52–127)

ysis of these risk factors. Other possible variables such as transit time, mode of transport, and proximity of the enclosure to other large carnivores (including other cheetahs) were also not included in the risk analyses because of insufficient information and sample size.

Most animals had an elevation in corticoids when moved on-exhibit regardless of premovement location. Those animals that moved from an off-exhibit facility were at an even greater risk of having elevated postmovement corticoids. Three of the four cheetahs with the highest and most prolonged postmovement stress response moved from off- to on-exhibit. The fourth animal moved on- to off-exhibit but subsequently developed FIP and died during the study period. This disease likely contributed to the prolonged corticoid elevation in this animal. FIP has occurred in several other captive cheetahs after movement (Munson, unpubl. data), suggesting that the stress of movement alters immune function, exacerbating immune-mediated diseases such as FIP.

Overall, animals with lower baseline levels were more likely to have an increased postmovement response. In contrast, animals with higher baseline corticoids tended not to increase their corticoid concentrations significantly after movement. The adrenal cortical output of these latter animals may already have been at maximal production, precluding further stimulation.

The marked day-to-day variation characterized by high peaks and deep troughs that occurred both before and after movement in individuals may be attributed to the production–secretion cycle of a stimulated adrenal gland. When an animal is subjected to a stressor, the cells of the adrenal cortex discharge their secretory granules into the bloodstream leading to corticoid peaks.[12] The gland then becomes depleted of corticoids and enters a period of resistance while steroids are synthesized.[12] This period of resistance may correspond to the low corticoid concentrations immediately following peaks in the cheetahs with high day-to-day variation. The amount of daily corticoid variation within individual animals provides further information on the character of the stress responses that was not evident in the trendlines. Therefore, quantifying this daily variation in addition to absolute corticoid levels should be part of a stress response analysis.

Adrenal cortical stress responses to environment

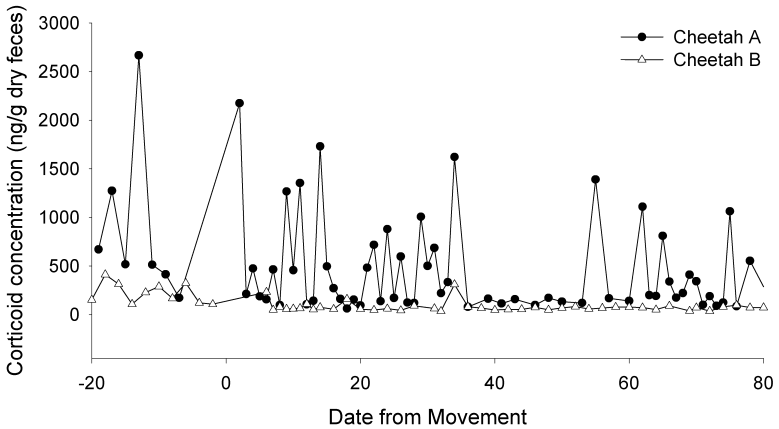

Figure 3. Comparison of corticoid profiles from two cheetahs moved from on-exhibit to the same off-exhibit facility. Dissimilarities in corticoids levels reflect individual animal differences in response to the same environment.

have been shown previously in captive leopard cats (*Felis bengalensis*).[2] When these small cats were placed into cages near large predators such as lions, they had marked elevations in circulating cortisol as well as increased pacing. When the enclosures were modified with hiding places, cortisol concentrations and stereotypic pacing decreased whereas exploratory behavior increased. Another study on clouded leopards (*Neofelis nebulosa*) found that animals on public display or housed in visual contact with other large predators had higher average fecal corticoid concentrations than animals off display or without predator visibility.[18] These results emphasize the importance of creating the appropriate environmental conditions to alleviate environmental stressors.

Although there was a trend in the adrenal response with respect to the type of postmovement exhibit, there were still variations among individuals, indicating diverse abilities to adapt to change. Cheetahs are known to differ in behavioral reactions to novel situations in captivity,[19] and animal disposition likely accounts for this variation. Future studies should compare how individual temperament affects the postmovement corticoid response, although these studies would be confounded by the complexity of behavioral responses in cheetahs.[19] Further studies should also aim to identify management practices that reduce the stress response to movement. Factors to consider include maintaining consistent management, moving cheetahs with familiar objects, and adapting cheetahs to the transport cage. In addition, administration of drugs such as long-acting neuroleptics[5] to reduce anxiety may be warranted in some situations.

Stress responses to movement and translocation have been documented in other species. Red deer (*Cervus elaphus*) had significantly increased cortisol concentrations in response to road transport during a short-term study.[17] A study of wild black rhinoceroses (*Diceros bicornis*) documented significantly elevated serum cortisol concentrations after translocation and persistent hypercortisolemia after confinement for extended periods (up to 80 days) in an enclosure.[6] Similar to the cheetah, black rhinoceroses also have high prevalences of unusual diseases which may, in part, be associated with a prolonged stress response to confinement.[9]

In summary, cheetahs moved from off- to on-exhibit facilities can have prolonged stress responses. This persistent hypercortisolemia may contribute to postmovement health and reproductive problems. The decrease in corticoids when animals were moved off-exhibit advocates use of off-exhibit enclosures to reduce stress in cheetahs. Individual animal temperament should be considered in interzoo movement because some animals continued to have elevated corticoids even after moving off-exhibit. The risks disclosed by this study should be weighed against the benefits of moving individual cheetahs to maintain genetic diversity in the captive population.

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