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RESEARCH ARTICLE

Urinary Corticosteroid Excretion Patterns in the Okapi (*Okapia johnstoni*)

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Stress is known to alter a variety of biological processes, including behavior and reproduction. It is therefore important to understand the stress levels of animals in captivity, especially those for whom captive breeding is a priority, such as the okapi. Levels of stress hormones can be measured from samples collected noninvasively, such as urine or feces, which are preferable with nondomestic species for whom drawing blood might in itself be a considerable stressor. To understand the excretion of cortisol in urine in the okapi, four (1.3) animals were subject to three injections: saline, 200 IU of an adrenocorticotropic hormone (ACTH) analogue, and 300 IU of the analogue. Their 24-hr urinary corticosteroid levels were compared with 4 baseline days. Injection with the ACTH analogue significantly increased the urinary corticosteroid levels compared with saline injections and baseline. Eight (3.5) okapi were then observed for 24 hr per day for 5 days to determine their normal patterns of corticosteroid production. The mean corticosteroid levels varied significantly by individual. A significant circadian pattern in urinary corticosteroid was apparent independent of individual or gender, with cortisol rising during the daylight hours and decreasing again at night. Zoo Biol 0:1–13, 2008.

Keywords: ungulate; behavior; circadian pattern; cortisol; giraffid

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INTRODUCTION

There is little doubt that stress can negatively impact conservation efforts. Prolonged, repeated, acute, or simply well-timed stressors can disrupt reproduction, impair immune function, impact metabolism, interfere with cognitive function, and alter behavior [Nelson, 2005; Fitzgerald et al., 1997; Ziegler et al., 1995; Thompson, 1989; Sapolsky, 1985]. Unfortunately, our ability to reliably identify "stressed" organisms is limited, naturally limiting our ability to mitigate that stress before it becomes harmful or disruptive. This is particularly true for captive animals, which may exist in environments and social milieu very different from those in which they evolved. In spite of efforts to mimic natural surroundings, captive animals are often placed in situations that may be inherently stressful. For example, animals may have limited opportunity to practice natural behaviors, have inadequate sensory stimulation, be handled by unfamiliar humans, be held in inappropriate housing conditions, or be in unusual proximity to predators or unwelcome social partners. These conditions may be suboptimal in a manner that is difficult to detect yet elicits a potentially harmful stress response [Breazile, 1987; Moberg, 1985].

The most common way to assess a stress response is to combine behavioral assessments with an analysis of adrenal activity [e.g. Cavigelli, 1999; Carlstead et al., 1993a; Barnett et al., 1984]. During a stressful event, increase in corticotropin-releasing hormone leads to an increase in adrenocorticotropic hormone (ACTH), which in turn results in the secretion of corticosteroids from the adrenal gland [Axelrod and Reisine, 1984]. Researchers have demonstrated a relationship between events such as novelty, capture, translocation, handling, and anesthesia with changes in the corticosteroid levels in a variety of species [e.g. Laws et al., 2007; Rukstalis and French, 2005; Wielebnowski et al., 2002; Whitten et al., 1998; Ziegler et al., 1995; Carlstead et al., 1993b; Crockett et al., 1993; Colborn et al., 1991; Mitchell et al., 1988; Barnett et al., 1984]. Hennessy et al. [1979] and others have demonstrated that the level of increase in corticosteroid secretion is roughly correlated with the perceived intensity of the stressor.

Corticosteroids can be measured in blood, saliva, urine, feces, and even hair [e.g. blood: Lyons et al., 1995; Mitchell et al., 1988; Barnett et al., 1984; saliva: Ekkel et al., 1996; Dathe et al., 1992; Vining et al., 1983; urine: Rukstalis and French, 2005; Brown et al., 1995; Crockett et al., 1993; feces: Laws et al., 2007; Whitten et al., 1998; Brown et al., 1995; Carlstead et al., 1993a; hair: Koren et al., 2002]. Of these, samples that can be collected noninvasively, such as urine and feces, are particularly appropriate for studying nondomestic species, which are typically unaccustomed to the handling required for drawing blood.

Okapi are solitary, forest-dwelling animals native to the Ituri Forest in Zaire [Hart and Hart, 1988]. In captivity, it is virtually impossible to mimic not only the size of their territories, but the density and the variety of the rain forest habitat. Okapi have difficulty surviving transportation out of the wild and transition to captivity [Van Bruggen, 2001; Lukas, 1997], which may indicate that this species is sensitive to stress. Additionally, reproductive success within the captive okapi population has been suboptimal and the causes for the failures are not always apparent [Leus, 2004; Loskutoff, 1997; Van Puijenbroeck and Leus, 1996; Raphael, 1988; Raphael et al., 1986; Benirschke, 1978; Rabb, 1978]. However, studies of females of a number of other species indicate that a variety of stressors can disrupt

normal reproduction [e.g. Karsch et al., 2002; Johnson et al., 1991; Edwards et al., 1987; Hagino, 1972].

Unfortunately, studies of stress in nondomestic ungulates are limited and the methodology to study stress and the stress response in general is unclear [see Schwarzenberger, 2004; Christensen et al., 2003; Saltz and White, 1991; Loskutoff et al., 1987]. A systematic and controlled approach would do much to clarify the techniques for use in okapi and a number of other species. This study was designed to determine whether the urinary cortisol levels can be used to study stress in okapi. The specific goals of the study were to confirm that okapi excrete detectable levels of corticosteroids, to test whether the corticosteroid levels increase in response to an ACTH challenge, and to evaluate the circadian pattern of urinary corticosteroid excretion in the okapi.

METHODS

Subjects

The subjects were nine (3.6) okapi, ranging in age from 3 to 22 years (Table 1). All but the two oldest animals were born at the Dallas Zoo. Both of the older animals had been housed at the Dallas Zoo for more than 10 years. The reproductive status of all animals was known during the study periods. Animals were housed individually within the same barn and had olfactory and visual access to at least one conspecific from indoor and outdoor holding areas. Barn stalls measured 5.9×6.0 m and 4.5×6.0 m, with solid stainless steel walls up to $\sim$1.5 m and interwoven metal mesh to the ceiling. Animals were regularly shifted between stalls and were familiar with all holding areas. Over the 3-year duration of the study, animals were exposed to a 14:10-hr light:dark cycle, with light onset at 07:00. Outdoor access was provided daily, but limited during inclement weather. Feed consisted of a mixed diet of grain (Mazouri) and produce (carrots, yams, onions, kale, and banana), fed twice daily at approximately 0:800 and 15:00 hr, with alfalfa hay and water available ad libitum. Fresh browse was offered regularly and oatmeal was fed daily. Animals were weighed weekly and participated in operant conditioning once or twice a week.

TABLE 1. Subjects and treatments

Sex	Animal	Age (years)[a]	Study section(s)	Order of injections[b]
Females	Bambesa	19	ACTH, circadian rhythm	200 IU, saline, 300 IU
	Kamili	17	ACTH	Saline, 200 IU, 300 IU
	Kwanini[c]	10	Circadian rhythm	
	Safarani	8	ACTH, circadian rhythm	200 IU, 300 IU, saline
	Mira	3	Circadian rhythm	
	Zenzelle	3	Circadian rhythm	
Males	Fons	22	Circadian rhythm	
	El-Jawar	5	ACTH, circadian rhythm	Saline, 200 IU, 300 IU
	Jamal	5	Circadian rhythm	

ACTH, adrenocorticotropic hormone.
[a]All animals were born at the Dallas Zoo except Bambesa and Fons, who each arrived in Dallas at 4 years of age.
[b]For the ACTH study.
[c]This female was pregnant during the study and was not included in the behavioral analysis.

During the course of the study, routine husbandry practices were maintained, no veterinary procedures were performed, and no changes in primary staff occurred.

ACTH Challenge

Four (1.3) animals were exposed to ACTH to determine whether ACTH exposure would result in measurable cortisol in urine samples (Table 1). Observers monitored each okapi continuously (24 hr per day) for the 4 days prior to treatment. Urine was collected mid-stream into a stainless steel or plastic collection cup or immediately aspirated off the ground. Samples were labeled with the individual's identity, date, and time of urination, and immediately frozen. Samples aspirated from the ground which were cloudy with sediment were centrifuged at 1,800 rpm for 10 min and supernatant was used for analysis. On the day of treatment, each animal was given one of the three intra-muscular injections: control (saline) injection, 200 IU of the ACTH analogue Cortrosyn (Cosyntropin; Organon Inc., Ben Venue Laboratories, Inc., Bedford, OH), or 300 IU of Cortrosyn. All injections were administered at approximately 15:00 hr and the order of injections was varied among animals (Table 1). Each injection was then followed by another 4 days of 24-hr urine collection. All urine samples were stored at $-20^{\circ}C$ until assayed at UNOmaha's Endocrine Bioservices Laboratory.

Circadian Patterns of Cortisol Secretion

Urine samples were collected 24 hr a day for 5 consecutive days from eight (3.5) animals to determine normative patterns of cortisol secretion (Table 1). One of the females was 3 months pregnant during data collection. Behavioral data on each study animal were collected at 15-min intervals. Behavior was recorded in two sections: (1) at every scan, one of three mutually exclusive behaviors (stand, lie, and locomotion) was recorded, and (2) one of six additional behaviors (eat, drink, ruminate, explore, self-maintain, and eliminate) could be recorded as well (see Table 3). To compensate for individual differences for the analysis of circadian patterns, cortisol measurements were normalized by subtracting out the overall mean cortisol level for that individual. Both behavior and normalized cortisol were averaged across 2-hr blocks for analysis. Behavioral data were not available for the pregnant female.

Enzyme Immunoassay

Urinary cortisol levels were measured in all samples using an enzyme immunoassay described previously [Smith and French, 1997] and modified for use in okapi. The cortisol antibody (produced by Coralie Munro, University of California, Davis) was highly specific for cortisol, with cross-reactivities of less than 1.0% with corticosterone and other glucocorticoid metabolites. The standard curve ranged from 7.8 to 1,000 pg/well, and all standards, samples, and quality controls were assayed in duplicate. Urine samples were run neat or diluted in distilled–deionized water from 1:2 to 1:10. Assay of serial dilutions of a pool of okapi urine produced a displacement curve that was parallel to the standard curve throughout the entire range of the standard curve. Intra-assay coefficient of variation (CV) based on duplicate sample agreement was 3.7%. Inter-assay CV based on repeated assay of an okapi urine pool was 17.0%. To account for variability in fluid intake and output, cortisol values were corrected for urinary creatinine concentration. For creatinine

analysis, urine samples were diluted 1:80 in water, and a modified Jaffe end-point assay was used to quantify creatinine [Smith and French, 1997]. Samples with creatinine levels less than 0.1 mg/ml were not included in the analysis because of potential water contamination (this occurred in 29 samples (9%) in the ACTH study only).

RESULTS

ACTH Challenge

The ACTH challenge resulted in increased levels of urinary cortisol excretion in all animals. On an average, the first urine was collected 56 ± 8 min following injection. All injections with the ACTH analogue Cortrosyn resulted in significantly elevated cortisol levels in the first urine excreted (mean = 93.84 ng/mg Cr) compared with preinjection levels from the same time on the day prior to injection (mean = 44.52 ng/mg Cr) (paired t-test, $t(7) = 5.16$, $P < 0.001$). There was no evidence of a dose response to the two levels of Cortrosyn (200 IU mean = 120.63 ng/mg Cr; 300 IU mean = 67.05 ng/mg Cr; paired t-test, $t(3) = 1.23$, $P = 0.15$). Cortisol concentrations in the first urine sample following saline injections were inconsistent, with one animal's level increasing considerably, whereas another's level actually decreased (paired t-test to the same time on the prior day, $t(3) = 0.95$, $P = 0.2$). These differences may have been related to the order of injection: the two animals whose cortisol levels increased with saline were the two animals for whom saline was the first injection (see Table 1).

Peak cortisol was defined as the maximum level recorded in the 12 hr following injection. On an average, the urinary cortisol levels peaked at 2.8 ± 0.5 hr after injection, but the timing of that was highly variable for all three injection types. The peak urinary cortisol levels were elevated following treatment with Cortrosyn compared with saline injections and with the maximum cortisol level recorded for the baseline period of the 4 days prior to any injections (Fig. 1; analysis of variance (ANOVA), $F(3, 12) = 5.62$, $P = 0.01$).

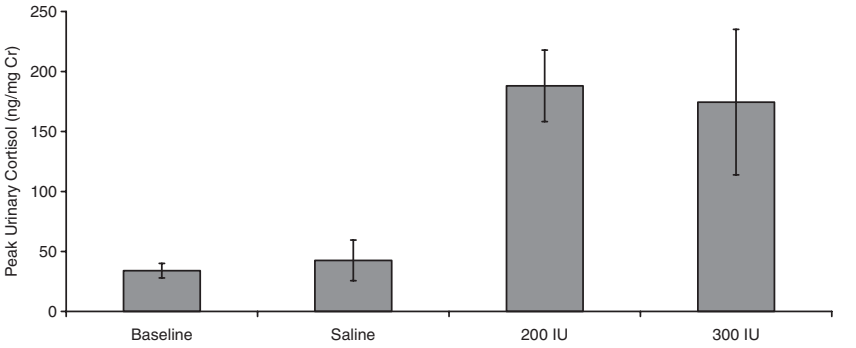

Fig. 1. Average peak urinary cortisol by treatment. Means are averages over all four (1.3) okapi of a single peak cortisol measurement for each okapi for that treatment. Injection with the ACTH analogue Cortrosyn resulted in significantly elevated cortisol compared with saline injections or the baseline period of the 4 days prior to the first injection (ANOVA, $F(3, 12) = 5.62$, $P = 0.01$). ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance.

Individual and Circadian Variation in Cortisol Excretion

The average cortisol level varied significantly by individual (Table 2, ANOVA, $F(7, 325) = 17.33$, $P < 0.001$). The lowest mean cortisol level was found in the pregnant female, Kwanini. Some of the individual variation can be explained by sex, with nonpregnant females showing significantly higher cortisol levels than males (ANOVA, $F(2, 330) = 38.33$, $P < 0.001$). This result is partially driven by the oldest female, Bambesa, whose mean cortisol levels were particularly high (Table 2). Interestingly, however, age was not a driving factor (correlation between age and mean cortisol = 0.07). Bambesa, the oldest female, had the highest mean cortisol, whereas Fons, the oldest male, had the lowest mean cortisol except for the pregnant female.

A circadian pattern in urinary cortisol excretion was apparent among all animals (Fig. 2, Table 2). To account for the individual variation, the circadian data were normalized as the difference from the individual's overall mean before analysis. There were significant differences between times of day independent of individual or sex (ANOVA, $F(11, 321) = 7.5$, $P < 0.001$). Cortisol was highest during the 10:00–12:00 time block and lowest during the 00:00–02:00 block. By post hoc tests, the midnight (00:00–02:00) block was significantly lower than the daytime blocks (08:00–20:00) and the midday (10:00–12:00) was significantly greater than the nighttime blocks (20:00–08:00) ($P \leq 0.02$ in all cases). The fact that this result was independent of individual indicates that although absolute cortisol varies between individuals, all individuals show a similar daily pattern (Fig. 2, Table 2).

TABLE 2. Individual circadian patterns

Sex	Animal	Mean cortisol[a]	Maximum cortisol[a]	Time of maximum[b]	Minimum cortisol[a]	Time of minimum[b]	Rate of change[c]
Females	Bambesa (age 19)	12.8 ± 1.1	25.1	10:00	4.6	00:00	2.1
	Safarani (age 8)	9.7 ± 1.2	23.1	10:00	3.7	00:00	1.9
	Mira (age 3)	6.2 ± 0.8	14.3	10:00	2.2	04:00	2.0
	Zenzelle (age 3)	9.71 ± 0.8	17.4	12:00	3.3	00:00	1.2
Pregnant female	Kwanini (age 10)	4.0 ± 0.3	6.2	08:00	1.4	00:00	0.6
Males	Fons (age 22)	5.3 ± 0.3	9.5	10:00	3.5	06:00	1.5
	El-Jawar (age 5)	9.5 ± 0.8	13.1	12:00	4.2	00:00	0.8
	Jamal (age 5)	5.8 ± 0.5	8.4	08:00	2.9	22:00	0.6
Average		7.9 ± 0.3	11.2	10:00	3.3	00:00	0.8

[a]Cortisol levels are in ng cortisol/mg creatinine.
[b]Beginning of the 2-hr time block.
[c]Rate of change calculated as (max cortisol−min cortisol)/(time of max−time of min), with time determined in hours; therefore, rate is (ng cortisol/mg creatinine)/hr.

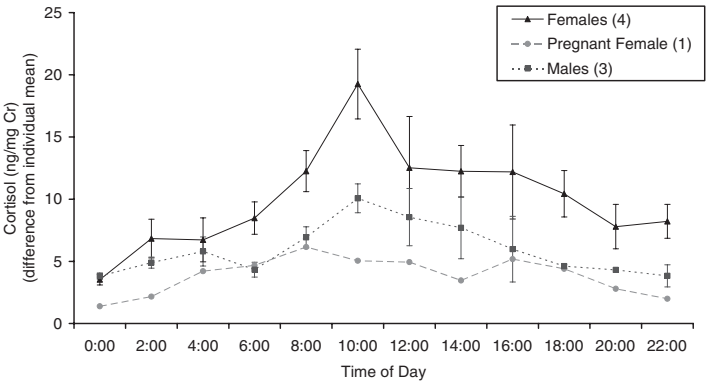

Fig. 2. Circadian pattern in the cortisol levels by sex. The cortisol levels were normalized to individual means and averaged for individuals in 2-hr time blocks. A two-way ANOVA (time of day, sex) found a significant circadian rhythm (time of day: $F(11, 297) = 5.8$, $P < 0.001$), but no difference between sexes (sex: $F(2, 297) = 1.32$, $P = 0.27$; sex/time-of-day interaction: $F(22, 297) = 1.57$, $P = 0.052$). ANOVA, analysis of variance.

TABLE 3. Behavioral patterns

		Correlation with cortisol	
Behavior	M/F differ significantly ($P < 0.05$)?	Males	Females
Standing	M > F 21:00–14:00, F > M 14:00–21:00	0.56	0.69[a]
Lying	No difference	−0.66[a]	**−0.88**[b]
Locomotion	M > F 13:00–18:00, M = F rest of time	0.48	**0.91**[b]
Eating	M > F 19:00–22:00, F > M 12:00–19:00[c]	0.30	**0.75**[b]
Drinking	No difference	0.42	0.01
Ruminating	M > F 07:00–21:00, M = F rest of time	−0.65[a]	**−0.78**[b]
Exploring[d]	M > F overall, esp. 09:00–13:00	**0.83**[b]	0.61[a]
Self-maintaining[e]	No difference	−0.30	−0.18
Eliminating[f]	No difference	−0.10	−0.39

[a] $P < 0.05$ but > 0.006, which is required by Bonferonni adjustment for many comparisons. Therefore, these did not quite reach significance.
[b] r Value is significant ($P < 0.006$).
[c] Males and females were fed at the same time; therefore, these differences are not the result of different feeding schedules.
[d] Investigate/explore environment, includes licking and sniffing nonfood items such as walls and fences.
[e] Self-groom, chew on self, lick, rub, or scratch self.
[f] Urinate or defecate.

Behavioral Patterns

Many of the behaviors showed clear circadian patterns of their own. The okapi lay down and ruminated more at night, whereas they walked, explored, and ate more during the day. When this circadian pattern was taken into account, there were clear differences between male and female behavior (Table 3). For instance, males walked more than females between 13:00 and 20:00 (two-way ANOVA(sex, time of day),

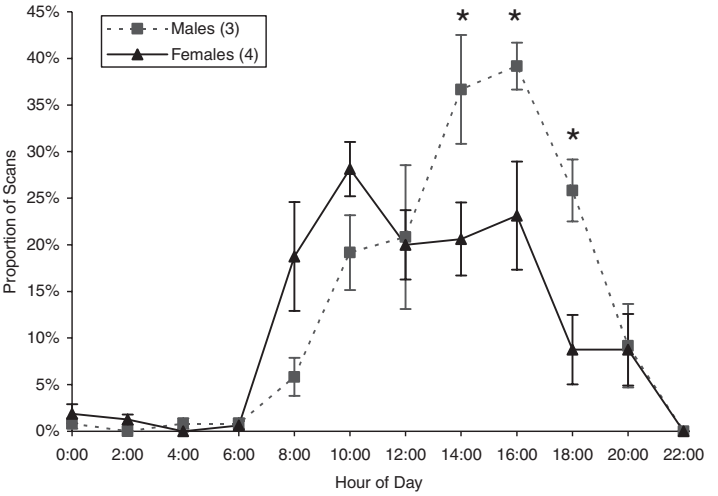

Fig. 3. Sex difference in circadian pattern of walking. Proportion of 15-min scan samples marked as locomotion, averaged over 2-hr time blocks for all individuals. * indicates 14:00–18:00 levels significantly different between sexes ($P < 0.05$). See Table 3 for definitions and correlation of these trends to the cortisol pattern shown in Figure 2.

$F(11, 60)$(interaction) = 2.41, $P = 0.015$; Fig. 3). The rest of the time, males and females walked about the same amount. The two sexes even ate differently: the females tended to eat in the afternoon, whereas the males ate in the early evening (Table 3). In addition, males explored more than females irrespective of time of day (M: 7.2 ± 2%; F: 4.3 ± 1%; two-way ANOVA(sex, time of day), $F(1, 60)$(sex) = 5.09, $P = 0.03$).

Sex differences were also apparent in the correlation between cortisol and behavior (Table 3). For males, only exploring was significantly positively correlated with cortisol. Ruminating and lying were slightly, but not significantly, negatively correlated with cortisol in males. The cortisol levels in females, on the other hand, were highly correlated with their behavior. Locomotion and eating were significantly positively correlated with cortisol in females, whereas lying and ruminating were significantly negatively correlated. Standing and exploring were slightly but not significantly positively correlated with cortisol in the females.

DISCUSSION

Assay Validation

One of the objectives of this study was to validate a noninvasive method for determining the cortisol levels in okapi. In this study, the glucocorticoid levels in urine increased measurably in response to a stimulation of the hypothalamic–pituitary–adrenal (HPA) axis. Urine is therefore a good substrate for detecting biologically significant elevations in cortisol in okapi. To our knowledge, this is

the first time a biologically and immunologically validated assay for urinary cortisol metabolites has been published for a giraffid [for fecal assays, see Christensen et al., 2003; Schwarzenberger, 2004].

Cortisol metabolites have been successfully measured in the urine of many other species, including domestic ruminants [Palme et al., 1996], deer [Saltz and White, 1991], elephants [Brown et al., 1995], and many species of carnivores and primates [e.g. Constable et al., 2006; Owen et al., 2004; Bahr et al., 1998, 2000; Ziegler et al., 1995; Carlstead et al., 1992]. Many of these have used radioimmunoassays instead of enzyme immunoassays [e.g. Constable et al., 2006; Owen et al., 2004; Bahr et al., 1998; Palme et al., 1996; Brown et al., 1995; Carlstead et al., 1992; Saltz and White, 1991]. However, enzyme immunoassays are becoming more popular because they do not require the use of radioactive substances [e.g. Bahr et al., 2000; Ziegler et al., 1995]. The ability to noninvasively measure the hormonal stress levels in nondomestic animals using nonradioactive methods is quickly becoming a necessary tool in the understanding of animal welfare. This study demonstrates that these methods can be used in giraffids as well.

Several researchers have recently begun using fecal samples to determine cortisol excretion profiles, instead of urinary samples, in part because fecal samples are easier to collect than urine samples [e.g. Christensen et al., 2003; Schwarzenberger, 2004; Möstl et al., 2002; Wasser et al., 2000]. Fecal corticosteroids are a good method for measuring the overall stress levels and for recognizing chronic stress in animals [Touma and Palme, 2005]. However, the excretory dynamics of fecal corticosteroid concentrations, especially in slow digesters such as ruminants, cannot highlight responses to specific, especially short-term, stressors. In these species, then, urinary corticosteroids may be a better measure of acute stress reactions. The rapid rise in urinary cortisol and subsequent return to baseline in response to the ACTH challenge suggest that urinary cortisol can document short-term stress responses in okapi.

Concentrations of urinary cortisol reflect the integration of corticosteroid concentrations over a longer period than the plasma levels. Plasma glucocorticoids can be overly sensitive to extremely short-term fluctuations in the HPA function [see Touma and Palme, 2005]. As many nondomestic animals are unaccustomed to the handling required to collect plasma, the sensitivity of this biological substrate to short-term fluctuations means that plasma measurements may reflect the stress of handling more than the animal's natural stress level. We suggest that urinary corticosteroid measurements therefore provide a useful intermediate between plasma and feces in estimating the HPA function.

Patterns of Cortisol Secretion in Okapi

The second objective of this study was to understand the normal patterns of cortisol secretion in okapi. Our results demonstrated a significant circadian pattern in the normal okapi cortisol levels, with cortisol being higher during the more active, daytime hours and lower during the more sedentary nighttime hours. This pattern is common in diurnal species, with nocturnal species showing the opposite pattern [e.g. Touma and Palme, 2005; Suzuki et al., 2003; Thun et al., 1981]. Our results also showed significant individual differences among okapi. Previous studies have demonstrated individual differences as well as diurnal and seasonal patterns in the

corticosteroid levels in a variety of species [e.g. Peel et al., 2005; Oki and Atkinson, 2004; Huber et al., 2003; Suzuki et al., 2003; Thun et al., 1981; reviews: Touma and Palme, 2005; Romero, 2002].

These results mean that individual and time of day are important when evaluating changes in the cortisol levels and that the frequency of sample collection can impact results. Researchers investigating the response of okapi or other ungulate species to stressors, therefore, would be well advised to normalize for time of day to each individual's baseline. Although the fecal corticosteroid levels average a longer period of time, the same caveats are likely to apply.

In evaluating individual differences, two individuals stood out from the group: the 19-year-old female with very high mean cortisol levels and the pregnant female with very low mean levels. Age overall was not a controlling factor, however, as the oldest male (22 years) did not have cortisol levels equivalent to the oldest female (19 years). It is not clear whether the pregnancy had an effect on the pregnant female's cortisol level: other research has not found an impact of pregnancy on the cortisol levels in okapi [Christensen et al., 2003], but a comparison of data from Dallas Zoo studies suggests that it may [unpublished data]. Pregnancy has been shown to have an effect on the corticosteroid levels in some species [e.g. bats: Reeder et al., 2003; tamarins: Bales et al., 2005] but not in others [e.g. elephants: Meyer et al., 2004]. In tamarins, the cortisol levels decreased in early pregnancy and increased in late pregnancy [Bales et al., 2005]. A similar phenomenon may be reflected in this study, where the okapi with the lowest cortisol level was in her first trimester of pregnancy.

Understanding how cortisol levels in okapi can be measured, as well as their normal patterns of variation, allows us to ask many more interesting questions about the impact of specific stressors on okapi. For instance, the impact of construction noise, various social situations, and different management decisions on the okapi's cortisol levels can now be quantified. The sex differences in behavioral patterns are particularly interesting and warrant further investigation. As field research has demonstrated that male home ranges are larger than female home ranges [Hart and Hart, 1989], gender differences in walking behavior and its relationship to the stress levels are perhaps to be expected. It is not clear, however, how much of the correlation between behavior and cortisol levels is an artifact of matching circadian rhythms vs. an actual connection between the hormones and the behavior. However, the ability to reliably measure the cortisol levels puts us a significant step closer to reliably identifying important physical and psychosocial stressors in captive and (potentially) wild okapi.

CONCLUSIONS

1. This paper validates an enzyme immunoassay for measuring corticosteroid metabolites in the urine of okapi.
2. Both male and female okapi show a great deal of individual variation in the cortisol levels, which cannot be explained by age. Some of this variation can be explained by sex.
3. Both male and female okapi show a circadian pattern in cortisol excretion.
4. Cortisol excretion is correlated with some behavioral patterns.

5. Both behavioral patterns and their correlations with the cortisol levels differ between males and females.

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