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Concentrations of faecal glucocorticoid metabolites in physically injured free-ranging African elephants *Loxodonta africana*

André Ganswindt, Stefanie Münscher, Michelle Henley, Rupert Palme, Peter Thompson & Henk Bertschinger

Free-ranging African elephants *Loxodonta africana* use their front feet frequently during the process of foraging and this could be the reason for the high prevalence of physical injuries to these parts of the body. Although the occurrence of severe lameness caused by foot lesions in adult elephants has already been investigated and the clinical and pathological findings have been reported, the effect of foot injuries on glucocorticoid levels as a potential physiological stress response has not been examined. Given the practical difficulties involved in monitoring unpredictable events in free-ranging animals, like the occurrence of foot injuries in elephants, it is not surprising that information regarding the endocrine correlates of physical injury is still limited for elephants. In our study we investigated the effects of foot injuries on concentrations of faecal glucocorticoid metabolites (GCM), body condition score (BCS) and reproductive behaviour in two GPS/radio-collared elephant bulls in the Kruger National Park, South Africa. We monitored the bulls aged 40+ (Bull 1) and 30+ (Bull 2) 2–3 times per week for 13 months starting in June 2007 and frequently collected faecal samples for non-invasive hormone monitoring. Faecal samples were lyophilised, extracted and assayed with an enzyme immunoassay which detects GCM with a 3 α -hydroxy-11-oxo-structure. Both bulls acquired foot injuries (right-front), which caused temporary lameness, but the effect of injury on GCM concentration differed between bulls ($P < 0.001$). In Bull 1 the injury lasted ± 250 days and was associated with an up to four-fold increase in GCM concentrations ($P < 0.001$) and his BCS reduced from 'good' to 'very thin' by the end of the injury period. In Bull 2 the injury lasted 65 days and was associated with a smaller increase in GCM concentrations ($P = 0.03$) together with a reduced loss in condition when compared to Bull 1. Following recovery, the condition of both bulls improved progressively and faecal GCM returned to baseline concentrations. Collectively, the data clearly underlined the value of non-invasive hormone measurements as a tool to provide information on the level of stress experienced by elephants. Thus, monitoring GCM levels could help improve the assessment of an elephant's state of health.

Key words: African elephant, cortisol, enzyme immunoassay, injury, Kruger National Park, *Loxodonta africana*, non-invasive, stress

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Adrenal glucocorticoids play an important role in homeostasis and, together with adrenal medullary catecholamines, they are part of the physiological stress response (Burchfield 1979, O'Connor et al. 2000, Möstl & Palme 2002, Sapolsky 2002). Stress involves changes in behaviour as well as neuro-endocrine and autonomic function, and is dependent on the central nervous system that assesses whether a stimulus or a group of stimuli (stressor or stressors) represents a significant challenge to an organism (Selye 1936, Burchfield 1979, O'Connor et al. 2000, Sapolsky 2002). The stress response is a series of adaptive mechanisms that are aimed at protecting the organism and restoring homeostasis (Burchfield 1979, O'Connor et al. 2000, Sapolsky 2002, Bomholt et al. 2004). The effects of a stressor can be monitored by measuring behavioural responses, which tends to be more subjective (Pretorius 2004, Weary et al. 2006), or by measuring physiological responses. An essential component of the physiological response to stress is the activation of the hypothalamic-pituitary-adrenal axis (HPA axis), which results amongst others in an increase in glucocorticoid secretion (O'Connor et al. 2000, Möstl & Palme 2002, Sapolsky et al. 2002, Touma & Palme 2005).

During situations like courtship, copulation or predatory behaviour, circulating glucocorticoids can improve fitness by mobilising energy or mediating physiological and behavioural changes, albeit on a temporary basis (Möstl & Palme 2002, Touma & Palme 2005). However, chronic stress can have negative consequences on health because the related prolonged periods of elevated glucocorticoid levels may decrease fitness by causing immunosuppression and atrophy of tissues (Munck et al. 1984, Möstl & Palme 2002). Furthermore, the occurrence of stress is also well known to have disruptive effects, for example on reproduction, cognition and behaviour of vertebrates (Liptrap 1993, Möstl & Palme 2002, Sapolsky 2002). In this regard, the measurement of glucocorticoids can be a valuable tool for studies in animal welfare or wildlife conservation.

Generally, adrenocortical activity can be monitored using either invasive or non-invasive methods. Given the practical difficulties involved with blood sampling from animals in the wild, invasive methods are mostly limited to studies on domestic animals. Additionally, the stress associated with animal-handling during sample collection can elevate glucocorticoid levels, thereby confounding the results (Möstl & Palme 2002, Touma & Palme

2005). By using blood, saliva or even urine as sample material, changes in adrenal endocrine activity, due to circadian rhythm, also need to be taken into account (Möstl & Palme 2002, Touma & Palme 2005, Kelling 2008, Brown et al. 2010). Like other diurnally active mammals, African elephants *Loxodonta africana* demonstrate a circadian rhythm in cortisol secretion, with a peak in the morning (Kelling 2008, Brown et al., 2010) followed by a second smaller peak in the afternoon (Kelling 2008). Faecal samples, however, are less affected by episodic fluctuations or the pulsatility of hormone secretion, because faecal hormone metabolite levels rather reflect the cumulative production rate of hormones over a certain period of time (Touma & Palme 2005). Furthermore, faeces can be easily obtained while avoiding direct contact with a potentially dangerous animal. Therefore, repeated sampling of the same individual is possible and feedback free (Ganswindt et al. 2003, Touma & Palme 2005). The collection of faeces as sample material for the analysis of adrenal endocrine activity is, therefore, a reliable alternative to hormone measurements in blood or other excreta.

In recent years, a large number of reliable non-invasive methods for assessing adrenocortical activity have been established in a variety of different mammalian species including the African elephant (Ganswindt et al. 2003, Touma & Palme 2005, Schwarzenberger 2007). For African elephants in zoos as well as in the wild, these methods have been shown to be practical and enable long-term monitoring of environmental and social factors impacting on adrenocortical activity without interfering with the result (Ganswindt et al. 2005a,b, Rasmussen et al. 2008, Ganswindt et al. 2010). In this regard, several studies demonstrated that extrinsic and intrinsic factors like translocation, seasonal variation, intra-group competition or sexual state can affect glucocorticoid levels in African elephants (Foley et al. 2001, Ganswindt et al. 2005a,b, Viljoen et al. 2008a,b, Ganswindt et al. 2010, De Nys et al. 2010). For various reasons, the relationship between adrenal activity and physical injuries is less documented. Ethical reasons prohibit any kind of experimental approach and the probability to be able to monitor such an event coincidentally is very low, especially in free-ranging animals. Ganswindt et al. (2005b) mentioned a clear increase in faecal glucocorticoid metabolite levels found in two free-ranging East African elephant bulls following severe physical injury (i.e. a broken

leg and gunshot wounds). However, the sample set of these two cases were extremely limited and recovery processes could not be documented, because both injuries were fatal. A recent study on injuries of free ranging African elephants in Kenya showed, that adult elephant bulls had the highest prevalence of injury and that the forelegs were the most susceptible body part to injury (Obanda et al. 2008). The front feet are frequently used during the foraging process and may well be the reason for the high prevalence of injuries to these parts of the body in elephants (Obanda et al. 2008). However, in the study by Obanda et al. (2008) the effect of the recorded injuries on glucocorticoid levels as a potential physiological stress response was not investigated.

In this article, we aim to provide detailed information regarding the physical and endocrinological response to the putative stressful occurrence of physical injury in free-ranging African elephant bulls in the Kruger National Park. Such information, especially on the timing and intensity of the stress-related physiological response will help to better assess the impact of temporary physical injuries and, thereby, the welfare of captive and free-ranging elephants.

Material and methods

Study area and animals

The study area comprised approximately 5,500 km² with an average annual precipitation between 450 and 500 mm and was located in the northern part of Kruger National Park (KNP), South Africa, (Venter et al. 2003, Ganswindt et al. 2010). The area consisted of predominately open shrub to fairly dense bush savannah and partly open to moderately dense tree savannah (Venter et al. 2003, Ganswindt 2008). The total elephant population within the KNP is estimated at around 12,500 individuals and

is the largest free-ranging elephant population in South Africa (van Aarde et al. 2008).

During the course of a study that focussed on reproductive patterns of elephants, we noted temporary foot injuries in two of the study animals (Bulls 1 and 2). We determined the age of the study animals on the basis of physical appearance (i.e. facial shape, tusk size and general appearance; Ganswindt et al. 2005b) and estimated the ages of Bulls 1 and 2 to be 40+ and 30+ years old, respectively.

Observations and sample collection

We monitored the study animals continually for 13 months between June 2007 and the end of June 2008, and located them via GPS/radio-tracking. The Transboundary Elephant Research Programme of Save the Elephants fitted the collars in December 2006 as part of an ongoing project in the KNP. We observed the animals regularly (i.e. 2-3 times per week) for at least half an hour per session using *ad libitum* sampling (Altmann 1974). We estimated body condition visually using a scoring system of six categories (Table 1) described by Poole (1989). Observations further included recording of current position, associations and group composition, presence and degree of physical musth signs, as well as the presence of foot injuries (see below).

In order to monitor faecal glucocorticoid metabolites (GCM), we collected approximately 50 g of faeces from each elephant bull during each observation session shortly after the animal had defecated and moved away. Using a disposable rubber glove we removed a sample from the middle of a bolus to avoid cross-contamination with urine or contamination with other faecal samples in the area. Subsequently, we transferred the sample into a glass vial, immediately placed it on ice, froze it within one hour of collection (-20°C) and stored it until analysis.

Table 1. Body condition score (BCS) system for elephant bulls.

BCS	Visual indicators for body condition score estimation
Emaciated (1)	Ribs, shoulder blades, pelvic bone and backbone clearly protrude.
Very thin (2)	Shoulder blades, pelvic bone and backbone protrude.
Thin (3)	Shoulder blades, pelvic bone and backbone are noticeable.
Good (4)	Slight sinking in front of the pelvic bone is noticeable and the backbone and shoulder blades protrude slightly.
Fat (5)	No signs of shoulder blades, pelvic bone or backbone.
Very fat (6)	No signs of shoulder blades, pelvic bone or backbone and fat hangs from the belly.

Foot injuries

We regarded a foot injury as present if a penetration wound or swelling was visible, or if there were signs of lameness such as an abnormal gait (favouring the leg) or abnormal position of the foot for an extended period while resting (Fig. 1A). After we had classified each observation and had matched faecal sample as either from a bull with injury present (1) or injury absent (2), we defined the onset of an injury period as the date when signs of an injury were first recognised, and the end of an injury as the date when such signs were recorded for the last time.

Extraction and analysis of faecal glucocorticoid metabolites (GCM)

We lyophilised and pulverised the faecal samples and sifted them using a mesh strainer to remove fibrous material (Ganswindt et al. 2005a). We then extracted approximately 0.05 g of the faecal powder with 3 ml 80% ethanol in water. After vortexing for 15 minutes, we centrifuged the mixture for 10 minutes at 3,300 g, and transferred the supernatant

to a microcentrifuge tube for hormone analysis (Ganswindt et al. 2010).

We analysed immunoreactive GCM in diluted extracts (1:50 or 1:100 in aqueous buffer) with an 11-oxoetiocholanolone enzyme immunoassay (EIA) first described by Möstl et al. (2002). This EIA detects GCM with a 3 α -hydroxy-11-oxo-structure and was successfully validated for monitoring adrenocortical activity in elephants (Ganswindt et al. 2003). The sensitivity of the assay, as determined at 90% binding, was 3 pg/well and intra- and inter-assay coefficients of variation, determined by repeated measurements of high and low concentration quality controls, ranged between 4.2 and 13.8%.

Data analysis

We determined an individual baseline level of GCM for each of the bulls by calculating the mean hormone concentration from all samples when no signs of physical injury were recorded. According to the procedures described by Ganswindt et al. (2005a), an increase of 50% above the individual steroid baseline was used to define a threshold level above which glucocorticoid levels were considered to be elevated. We defined the onset of a period of elevated GCM as the first date when at least two consecutive samples exceeded the threshold and, accordingly, the end of such a period as the date when glucocorticoid levels fell and remained below the threshold for at least two consecutive samples. Consequently, the duration of a period of elevated GCM concentrations was determined by calculating the interval between the onset and the end of a period of elevated GCM levels.

We used a multiple linear regression model to estimate the effect of physical injury on adrenocortical activity. In this regard, we used faecal GCM concentration as the continuous dependent variable and injury as a binary independent variable. The unit of interest was the individual observation. To account for clustering of observations within animals, 'Bull' was modelled as a fixed effect. To account for the serial autocorrelation of measurements, we included a first-order autoregressive disturbance term with the autocorrelation parameter (ρ) estimated based on a single-lag regression of residuals. Due to the fact that season and reproductive state also affect glucocorticoid levels in African elephants (Foley et al. 2001, Ganswindt et al. 2005a, Viljoen et al. 2008b, Ganswindt et al. 2010), both factors were additionally entered as binary independent variables. We categorised sam-

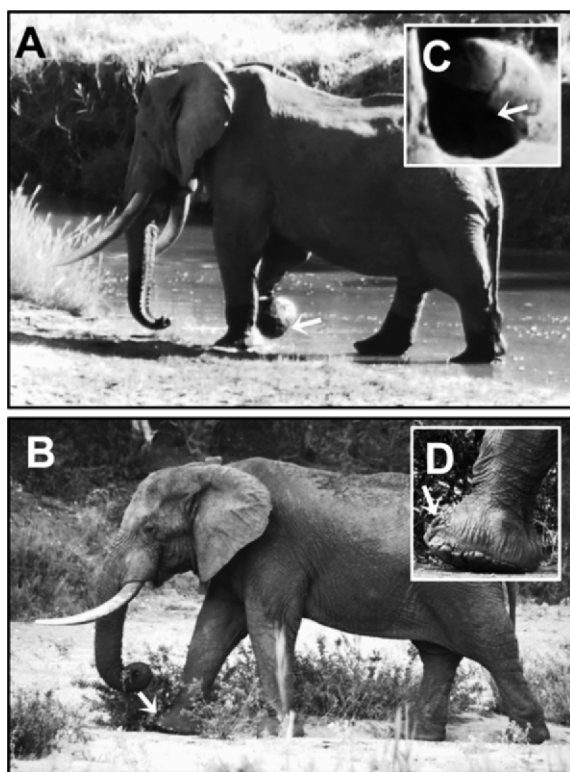


Figure 1. African elephant Bull 1 (A) and 2 (B) showing an injury of the right front foot. Inserts show details of the injured foot of the respective animal (C & D). Photo: A. Ganswindt & S. Münscher, Kruger National Park.

ples by season into two groups (i.e. dry and wet season) according to Viljoen et al. (2008b). The categorisation was verified using rainfall data recorded throughout the study period at three different stations (i.e. Shingwedzi, Mooiplaas and Letaba) across the study area (dry season: 52–80 mm of rain; wet season had 378–588 mm of rain). We categorised samples as those collected from musth or non-musth bulls according to the occurrence of common physical signs of musth (i.e. temporal gland swelling (TG), temporal gland secretion (TGS) and urine dribbling (UD; Ganswindt et al. 2005a,b). We defined an animal as reproductively active (in musth) when at least two consecutive samples were collected from an elephant exhibiting signs of TG/TGS and UD. We included two-way interactions of injury with bull, season and reproductive state in the model and retained them if significant at $P \leq 0.05$. We used Stata 10.1 (StataCorp, College Station, Texas, USA) for all statistical analyses.

Results

Bull 1 was already injured prior to commencement of our study in June 2007. The injury was first noticed on 15 February 2007 (J. Oelofse, pers. comm.). The bull was immobilised the following day and the wound was inspected. The bull had

ulcerative pododermatitis on the right front foot (see Fig. 1C). The area of the lesion measured 150×75 mm and the ulcer penetrated the entire depth of the sole. Some granulation was present, indicating that the injury was not fresh. As a result, the veterinarian decided not to treat the lesion (P. Buss, pers. comm.). From the onset of our study, we observed the injury in Bull 1 regularly for 130 days, from 12 June 2007 until 20 October 2007 (see Fig. 1A). Bull 2 developed an injury in the middle of the toe area above the sole of the foot (see Fig. 1B and D). We initially recorded the injury of Bull 2 on 26 August 2007 and recognised it for the last time on 30 October 2007, giving a total of 65 days of injury.

Bull 1 showed two periods of elevated GCM concentrations (Fig. 2A): a longer more pronounced (up to a four-fold increase) one associated with the foot injury lasting 133 days and a shorter period with lower concentrations. In February 2007 the consulting veterinarian reported a generally good body condition for Bull 1 (P. Buss, pers. comm.). In June 2007, the BCS had decreased to three (see Fig. 2A) and it deteriorated to two from mid-September until the end of November 2007. At the beginning of December 2007 the bull started gaining condition until reaching a BCS of four at the beginning of February 2008. The additional 26-day period of elevated GCM concentrations that occurred at the beginning of March, thereafter ending abruptly, overlapped with the beginning of

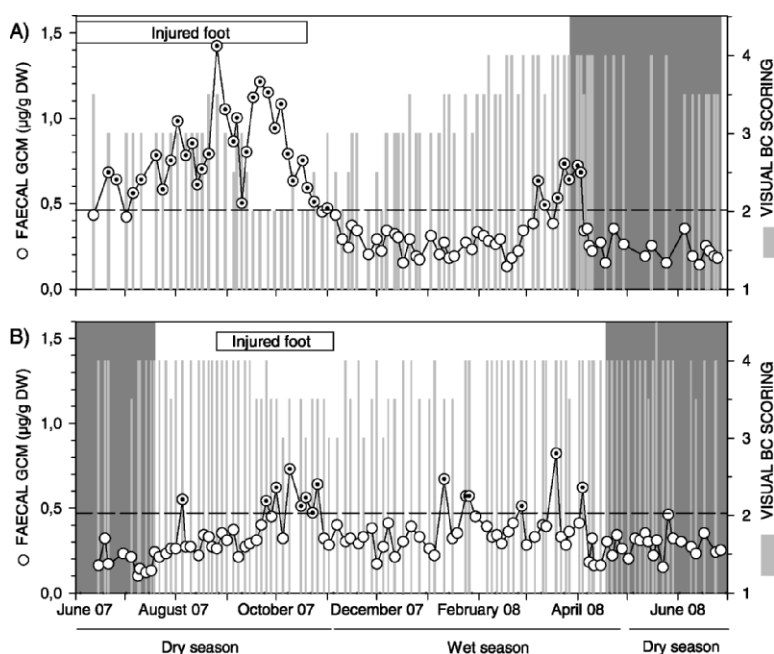


Figure 2. Profiles of faecal glucocorticoid metabolite (GCM; ○) immunoreactivity throughout a 13-month period in the two adult male African elephants, Bull 1 (A) and Bull 2 (B). The respective dashed line indicates the individual threshold for elevated GCM levels and values exceeding the respective individual GCM baseline are indicated with a dot. Light grey bars indicate visual body condition score (BCS), dark grey areas indicate periods of musth, and white boxes at the top of the subfigures indicate when physical injury was observed.

Table 2. Effect of injury on concentrations of faecal GCM in the two African elephant bulls, adjusted for musth and season (N = 194) showing results of a multiple regression model with serial autocorrelation parameter.

Variable	Level	b*	SE(b)	95% C.I.	P-value
Injury	injured vs not injured (Bull 1)	0.578	0.065	0.450, 0.705	< 0.001
	injured vs not injured (Bull 2)	0.148	0.068	0.014, 0.281	0.030
Bull	Bull 1 vs Bull 2 (injured)	0.385	0.045	0.297, 0.473	< 0.001
	Bull 1 vs Bull 2 (not injured)	-0.017	0.027	-0.070, 0.036	0.528
Musth	musth vs non-musth	-0.004	0.044	-0.090, 0.082	0.920
Season	wet vs dry	0.093	0.047	0.001, 0.185	0.048
Injury and bull interaction		-	-	-	< 0.001

* Estimated regression coefficient.

the musth period of Bull 1. We observed signs of musth from April until the end of June 2008.

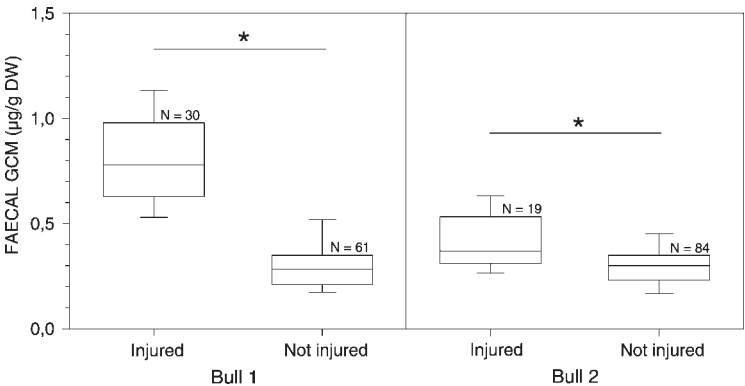
We recorded the injury of the 30+ year old Bull 2 during the end of dry season from the end of August until the end of October 2007 (see Fig. 2B). A period of moderate (i.e. up to two-fold) elevated GCM concentrations, which lasted 17 days, occurred at the end of the injury period. An additional two-day period of elevated GCM concentrations as well as four single measurements above threshold also occurred between January and the beginning of April 2008. We initially assessed the BCS of Bull 2 as four, but it declined from mid-September 2007 and onwards. Thereafter, the bull gained condition from mid-November and onwards. By mid-January 2008 he had reached his pre-injury BCS, remaining fairly constant until the end of the study. In Bull 2, we observed musth signs twice; the first time from June until mid-July 2007 and a second time from the end of April 2008 until the end of the study period.

We used 194 observations (91 for Bull 1 and 103 for Bull 2) for the multiple regression analysis to estimate the effect of injury on faecal GCM concentration (Table 2). The autocorrelation param-

eter (ρ) was estimated to be 0.797. Because we observed the injuries only during non-musth periods and almost exclusively during the dry season, neither the injury and musth nor the injury and season interactions could be fitted. However, as the injury and bull interaction was highly significant ($P < 0.001$; see Table 2) we present the coefficients for injury and bull separately for bull and injury status, respectively. In both Bull 1 and Bull 2, the difference in faecal GCM concentrations between 'injured' and 'not injured' was significant ($P < 0.001$ and $P = 0.030$ respectively; see Table 2), although the effect of injury was much greater in Bull 1 than in Bull 2. In their non-injured state we found no significant difference in faecal GCM concentrations between the two bulls ($P = 0.528$). Adjusted for injury, we found a significant increase in faecal GCM concentrations during the wet season compared to the dry season ($P = 0.048$) and no significant difference between musth and non-musth periods ($P = 0.920$).

Box plots of GCM concentrations by bull and injury status (Fig. 3) showed that the median GCM concentration of Bull 1 when injured (0.78 $\mu\text{g/g}$) was

Figure 3. Box plots of grouped concentrations of GCM in the faeces of the two adult male African elephants (Bull 1 and Bull 2), which were temporarily injured. The boxes show the median value and the upper and lower quartile values, while the whiskers show the 10th and 90th percentiles of the values. Asterisks indicate statistically significant differences between groups, determined using the multiple linear regression model.



2.5 times higher than when no signs of physical injury were recorded (0.29 µg/g). The median GCM concentration of Bull 2 was also higher (0.37 µg/g) during injury than when the bull was not injured (0.30 µg/g; see Fig. 3).

Discussion

A study by Keet et al. (1997) has already investigated the occurrence of severe lameness caused by foot lesions (ulcerative pododermatitis) in adult elephant bulls in the Letaba Land System of the KNP. The number of foot injuries recorded in this study, as well as in other reports, challenges the view that lameness is a relatively rare clinical entity in free-ranging African elephants (Keet et al. 1997, Obanda et al. 2008). Furthermore, their findings indicate that the two cases highlighted in our study could represent typical examples of temporary moderate foot injuries in wild African elephants. In both cases, the stress that we documented was caused by temporary injuries of the right front foot, resulting in lameness, which influenced the movement of the animals. Each period of injury was associated with significant elevations in faecal glucocorticoid metabolites, which amounted to a four-fold increase in Bull 1. By comparison, an intramuscular injection of adrenocorticotrophic hormone (ACTH) caused a 3-4 fold elevation of faecal GCM concentrations in an African elephant bull (Ganswindt et al. 2003). Furthermore, translocation as a putative stressful event resulted in 4-5 fold increases in faecal GCM concentrations in elephants (Laws et al. 2007, Viljoen et al. 2008a), whereas fatal injuries detected in wild African elephants produced 5-10 fold elevations in adrenal glucocorticoid output (Ganswindt et al. 2005b). The changes in GCM concentrations described in these various studies are at least partly comparable with the results of our study, underlining the importance of foot injuries in elephants as potential stressors. It should be noted, however, that our results cannot be extrapolated to all bulls with foot injuries. The nature or severity of foot injuries will vary as to the degree of lameness and pain. Accordingly, the response in terms of faecal GCM concentrations will vary from bull to bull (Dingemanse et al. 2010).

In our study, each respective period of injury was associated with a decline in body condition and we can assume that the reduction in body condition was caused to some extent by decreased food intake

(Poole 1989). Therefore, a confounding effect of lower food intake on GCM levels in our two study animals might be possible. However, in cattle food intake was described to influence excretion rate of steroids into the faeces, but not concentrations of faecal steroid metabolites (e.g. Rabiee et al. 2001). Our data further demonstrate that, in both cases, the elevated glucocorticoid concentrations were more pronounced at the end of each respective period of injury, which may have been caused by the progress of injury (Voigtländer et al. 2006). It may also have been a reflection of body condition loss which in itself may be stressful (Kitaysky et al. 1999, Cabezas et al. 2007). The fact that the injury of Bull 2, which lasted 65 days, was associated with an overall lower increase in GCM concentrations compared to the injury of Bull 1, which lasted in excess of 250 days, could indicate that the intensity of the stress-induced response is influenced by the duration of the injury (e.g. Voigtländer et al. 2006). On the other hand, the differences in BCS as well as differences in the severity of injury associated inflammatory processes and degree of pain could have been responsible for the differences observed (Kitaysky et al. 1999, Merl et al. 2000, Voigtländer et al. 2006).

Previous studies have demonstrated that season also affects glucocorticoid concentrations in African elephants with significantly elevated glucocorticoid metabolite levels during the dry season (Foley et al. 2001, Rasmussen et al. 2008, Viljoen et al. 2008b, Ganswindt et al. 2010). Interestingly, in both study animals we found that the respective ends of the injury periods with their more pronounced elevations in GCM coincided with the end of the dry season (for Bull 1 during September-October and for Bull 2 during October). Therefore, a synergistic effect between injury and seasonal change may have occurred. The previously described seasonal differences in faecal glucocorticoid metabolite concentrations of African elephants, determined by using the same or similar enzyme immunoassay systems, only revealed differences of 20-110% between regular seasons (Foley et al. 2001, Viljoen et al. 2008b). In addition, an analysis of seasonal related differences in faecal GCM concentrations in the remaining elephant bulls of the study group revealed only a median maximum of 23% elevation during the dry season (data not shown). Therefore, it is, at least in the case of Bull 1, most likely that the detected elevation in GCM concentrations was predominately caused by the injury itself. This

conclusion is supported by the results of the multiple regression model, indicating that injury was associated with an increase in GCM concentrations after adjusting for season. Interestingly, the effect of season on GCM concentrations after adjustment for injury in these two bulls was small but still significant ($P = 0.048$), with an increase during the wet season. This finding, however, may have been influenced by the occurrence of musth because both bulls showed musth predominantly during the dry season. Previous studies provide evidence for a delayed suppressive effect of musth on adrenocortical endocrine function (Ganswindt et al. 2003, 2005a, 2010).

In addition to the periods of elevated GCM levels, which were associated with the observed foot injury in the two study animals, we also detected short periods of elevated GCM concentrations as well as single measurements above threshold which do not seem to be related to injury. Although any kind of perceived or real endangerment could be the reason for these additional increases in GCM levels, it is noticeable that all these cases, except one single measurement of Bull 2, occurred during the wet season. During this time of the year, there is a higher probability of intra-sexual competition due to a more frequent occurrence of musth bulls (Ganswindt et al. 2010), which may be an explanation for the additional GCM elevations found.

Due to the fact that the decline in BCS that was observed in both bulls also started during the end of the dry season, we can assume an accumulative effect of injury and the more challenging seasonal conditions. Codron et al. (2006), for example, showed that for elephants in the southern KNP the percentage of faecal nitrogen, which is known as a useful indicator of nutritional status, increased from the dry to the wet season. Additionally, Poole (1989) showed that injured East African elephants in musth lost more condition than bulls that were not injured.

Sexual activity in adult African elephant bulls is mostly associated with the occurrence of musth, a state or condition which refers to a set of physical, physiological and behavioural characteristics (Poole 1987, Ganswindt et al. 2005a). Around 30-35 years of age, musth commonly stabilises to an annual episode that occurs at roughly the same time each year (Poole 1987, Rasmussen et al. 2008). Previous studies indicate that good nutrition and body condition are a necessary requirement for the successful expression of musth and that musth bulls

in poor condition usually cease their musth behaviour (Poole 1989). Our findings corroborate these observations, as Bull 1 failed to come into musth in 2007 despite having experienced regular annual musth cycles since 2002 (M. Henley & S. Henley, pers. comm.). The case of Bull 1 shows the value of a combined approach where behavioural observations and non-invasive hormone monitoring can help to explain temporal variations in the occurrence of important behaviours, such as musth.

Our study clearly underlines the importance of non-invasive hormone measurements as a powerful tool to provide information about an animal's endocrine status. The provided data on the intensity and variability of the physical and endocrine responses to injury could also help to improve the assessment as well as the prognosis of the condition in African elephants. Finally, the generated information regarding elephants should help to initiate further studies which examine endocrine responses to putative stressful circumstances in other mammals.

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