Social, state-dependent and environmental modulation of faecal corticosteroid levels in free-ranging female spotted hyenas†

Wolfgang Goymann[1,2]*, **Marion L. East**[1,3], **Bettina Wachter**[1,3,4], **Oliver P. Höner**[1,3,4], **Erich Möstl**[5], **Thomas J. Van't Hof**[2]¶ and **Heribert Hofer**[1,3]

[1]*Max-Planck-Institute for Behavioural Physiology, D-82319 Seewiesen Post Starnberg, Germany*
[2]*Research Centre for Ornithology of the Max-Planck-Society, Von-der-Tann-Strasse 7, D-82346 Andechs, Germany*
[3]*Institute for Zoo and Wildlife Research, Alfred-Kowalke-Strasse 17, D-10315 Berlin, Germany*
[4]*Ethological Station Hasli, University of Bern, CH-3032 Hinterkappelen, Switzerland*
[5]*Ludwig Boltzmann Institute for Veterinarian Endocrinology, Veterinarian University, Josef Baumanngasse 1, A-1210 Vienna, Austria*

Little is known about to what extent the sensitivity of the hypothalamic–pituitary–adrenal (HPA) axis may be state dependent and vary in the same species between environments. Here we tested whether the faecal corticosteroid concentrations of matrilineal adult female spotted hyenas are influenced by social and reproductive status in adjacent ecosystems and whether they vary between periods with and without social stress. Females in the Serengeti National Park frequently become socially subordinate intruders in other hyena territories by undertaking long-distance foraging trips to migratory herds, whereas in the Ngorongoro Crater they usually forage inside their own small territories on resident prey. The faecal corticosteroid concentrations in Serengeti females were significantly higher than in Ngorongoro females. Energy expenditure by lactation is exceptionally high in spotted hyenas and this may be reflected in their corticosteroid levels. The faecal corticosteroid levels in both populations were higher in lactating than in non-lactating females. During periods of social stability, faecal corticosteroid concentrations increased in non-lactating females but not in lactating females as social status declined. Lactating Serengeti females had significantly higher faecal corticosteroid concentrations during periods with acute severe social stress than during periods without, indicating that the HPA axis is sensitive to social stimuli even in lactating females. So far few studies have used non-invasive monitoring methods for assessing social stress in free-ranging animals. This study demonstrates for the first time, to the authors' knowledge, that corticosteroid concentrations may differ between periods with and without social stress for a free-ranging female mammal and that the modulating effect of social status may depend on reproductive status.

Keywords: stress; non-invasive monitoring; corticosteroids; lactation; spotted hyena; Serengeti

1. INTRODUCTION

Activation and tuning of the hypothalamic–pituitary–adrenal (HPA) axis modulates the release of corticosteroids and is an important component of the hormonal stress response (Munck *et al.* 1984; Sapolsky 1992*a*). Short-term elevation of corticosteroid levels may lead to adaptive behavioural and physiological processes (Wingfield *et al.* 1997, 1998), but chronic elevation of corticosteroids may cause reproductive failure and disease (Munck *et al.* 1984; Sapolsky 1992*a*; De Kloet *et al.* 1999). The sensitivity of the HPA axis may be adapted to environmental or life history states (Wingfield *et al.* 1995, 1998; Hofer & East 1998) and, thus, baseline concentrations of corticosteroids may differ between individuals and within individuals in different environmental or life history states. However, little is known about the effects of different

environments and life history states on the corticosteroid levels of most free-ranging mammalian species.

With recent developments in non-invasive hormone research (reviewed in Whitten *et al.* 1998; Goymann *et al.* 1999; Harper & Austad 2000), the levels of corticosteroids in free-ranging mammalian species can be measured with non-invasive methods (see, for example, Creel *et al.* 1997; Cavigelli 1999; Strier *et al.* 1999) that do not affect target levels (Hofer & East 1998). In this study we applied a non-invasive method that has been validated for spotted hyenas (*Crocuta crocuta*) by Goymann *et al.* (1999) in order to measure the levels of corticosteroids in the faeces of two populations, namely Serengeti and Ngorongoro hyenas, which live at similar population densities of 0.82 and 0.79 adults and subadults per square kilometre, respectively (Hofer & East 1993*a*; Höner 2001) and have the same social structure, but live in different environments.

In the Serengeti National Park, stable social groups (clans) of spotted hyenas with a mean of 45 adults and subadults defend large group territories (55.5 km²) with a communal den throughout the year (Hofer & East 1993*a*). Serengeti spotted hyenas are not migratory as previously thought (Kruuk 1972). All clan members forage inside their clan territory when large herds of migratory herbivores are present. However, when

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migratory herds are elsewhere, which is usually the case for three-quarters of the year (Hofer & East 1993a), the density of resident herbivores inside the territory is low and cannot sustain all clan members (Hofer & East 1993a). As a result, clan members travel individually or in small groups over long distances (40–70 km) in order to forage on the nearest large concentration of migratory herbivores as intruders in territories of other clans and then return to their home territory (the commuting system of Serengeti hyenas) (Hofer & East 1993a,b,c). As intruders, females are ranked lower than resident territory holders, irrespective of their social rank in the feeding hierarchy at home (Hofer & East 1993b). All female commuters are subject to this experience. Because the density of resident herbivores in the Ngorongoro Crater is more than one order of magnitude higher than in the Serengeti National Park (Hofer & East 1993a; Runyoro et al. 1995), spotted hyenas belonging to clans on the crater floor (Ngorongoro hyenas) live in small territories (26.6 km²) (Höner 2001) and usually feed on the herbivores inside their own clan territory. Thus, they live and feed in a well-known and predictable social environment in clans that, with a mean size of 21 adults and subadults, are smaller than in the Serengeti National Park (Höner 2001). As a consequence, food acquisition may be socially and energetically less demanding than in the Serengeti National Park. We thus predict that the corticosteroid levels of Serengeti hyenas should be higher than those of Ngorongoro hyenas.

Reproduction in mammals is energetically expensive for females (Harvey 1986; Gittleman & Thompson 1988). Lactation is often more costly than pregnancy and, in carnivores with a high investment in lactation (Oftedal & Gittleman 1989), lactation has been considered to be a good measure of parental investment (Oritz et al. 1984; Golla et al. 1999). However, little is known about the effect of lactation on the corticosteroid levels of most free-ranging mammalian species and how the effect of reproductive status is modulated by social factors. Energy expenditure by lactation is exceptionally high in spotted hyenas: the milk of female spotted hyenas has the highest protein content recorded for any terrestrial carnivore, a fat content that is only exceeded by that of Palaearctic bears and sea otters (*Enhydra lutris*), and a higher gross energy density than most carnivores (Hofer & East 1995, 1996). Furthermore, their period of lactation is prolonged (Mills 1990). Consequently, the predicted energy output of spotted hyenas during lactation is higher than that of any other terrestrial carnivore (Oftedal & Gittleman 1989). We therefore predict that such a tremendous maternal input should lead to differences in the corticosteroid concentrations of lactating and non-lactating females.

Subordinate animals in many social mammalian species in which most or all individuals attempt to reproduce (plural breeders sensu Mumme 1997) may experience reproductive failure due to stress from superior group members (Wasser & Barash 1983; Altmann et al. 1988; Wasser & Starling 1988; Dunbar 1989). It has been shown that, in free-ranging matrilineal baboons, the serum baseline corticosteroid concentrations of subordinate males are higher than those of dominants (Sapolsky 1982). However, this situation may change when the rate

of social interactions among group members declines. The serum cortisol concentrations of subordinate baboons dropped significantly during a severe drought, when all animals devoted virtually all their time to foraging and their rate of social interactions declined significantly (Sapolsky 1986).

In contrast to such closely knit primate societies, spotted hyenas live in a matrilineally organized fission–fusion society where attendance at the social centre of a clan, i.e. the communal den and, thus, the rate of interactions among females depends critically on reproductive status because lactating females are far more frequently at the den (in order to nurse their cubs) than non-lactating females (Hofer & East 1993c). Because female den attendance increases with social status (East & Hofer 1991), the rates of interaction are highest among high-ranking lactating females (East et al. 1993). Because the rate of social interactions may modulate the impact of social status on their corticosteroid levels (Sapolsky 1986) we predict that, in spotted hyenas, any effect of social status is likely to be dependent on their reproductive status. We thus predict that social status is more likely to affect the faecal corticosteroid levels of lactating than non-lactating females.

We also had the opportunity of assessing the sensitivity of the HPA axis of Serengeti hyenas to acute social stress by comparing the faecal corticosteroid levels of lactating females during periods with and without acute social stress.

2. METHODS

(a) *Study areas and animals*

The study was conducted in the Serengeti National Park and the Ngorongoro Crater in Tanzania, East Africa. In the Serengeti National Park, we studied clans that held territories in the centre of the park (Hofer & East 1993a). This region is a transition zone between the dry and wet season ranges of the migratory herds of ungulates that pass through the study area biannually (Sinclair & Norton-Griffiths 1979) and are the chief source of prey for Serengeti hyenas (Hofer & East 1993a). The clans studied in the Ngorongoro Crater defended territories on the crater floor. The Ngorongoro Crater is situated within the Ngorongoro Highlands at the eastern end of the Serengeti National Park plains. It is an almost self-contained grassland ecosystem that is linked to the adjacent Serengeti National Park plains by a series of game trails that descend from the slopes of the Ngorongoro Highlands.

Hyenas were individually recognized by their spot patterns (Frank 1986; Mills 1990; Hofer & East 1993a). Social status was determined from aggressive acts (direct approaches, pushes, stand overs, lunges, chases and bites) and submissive acts (retreats, displacements, cowering, tails between legs, ears back, head bobbing and head upside down) in dyadic interactions recorded *ad libitum* and during focal observations of individuals. The death or 'disappearance' of females and rank reversals altered the dominance hierarchy. A new rank was determined from dyadic interactions for all female clan members when such changes occurred. The rank of a female was thus known on the day it was sampled. Females were assigned a standardized rank for comparison of ranks across clans. Standardized ranks were calculated for all females after assigning the female with the highest social status the standardized rank 1 and the female with

the lowest social status the standardized rank -1. We considered only data from adult females (age > 2 years and mean 5.7 ± 0.2 years). Females were considered to be lactating (i) if they were seen suckling within a 14 day period before the sample was collected or (ii) if they were seen suckling the same litter on the day of sampling or after the sample had been collected. Females were considered to be non-lactating when they did not give birth within 110 days (Matthews 1939) following sampling and did not suckle cubs. Females were considered pregnant when giving birth within 110 days following sampling.

(b) *Collection of faeces and measurement of faecal corticosteroids*

One hundred and seventy-nine faecal samples collected during periods of social stability from 80 individually recognized adult females (25 non-lactating and 55 lactating females) in three clans in the Serengeti National Park and 47 faecal samples from 30 females (9 non-lactating and 21 lactating females) in five clans in the Ngorongoro Crater were obtained and included in the analysis. Twenty-eight further samples were collected during periods of social instability from lactating Serengeti females (see § 2c). Furthermore, 17 samples from 17 pregnant females were obtained in the Serengeti National Park and three samples from three pregnant females were obtained in the Ngorongoro Crater. We report the means for this reproductive state, but did not include them in the statistical model because the sample size in the Ngorongoro Crater was too small to be representative. Samples were collected in the Serengeti National Park between December 1990 and April 1999 and in the Ngorongoro Crater between June 1996 and February 2000. Faecal corticosteroids reflect the cumulative secretion and elimination of biologically active corticosteroids over a number of hours or days (Whitten *et al.* 1998). We measured faecal metabolites of cortisol with a method that has been described and validated previously (Goymann *et al.* 1999). The samples were collected immediately after an individual had defecated, chopped up and mixed thoroughly and a proportion of $1–5$ g was taken and stored within 1 h in liquid nitrogen or kept in a cool thermos for less than 3 h before storing the samples in liquid nitrogen at the field station. The samples were transported to Germany on dry ice and kept in an ultra-cold freezer (below $-70\,°C$) until further processing. The faecal samples were lyophilized and initially extracted with methanol and then with diethylether, as described in Goymann *et al.* (1999). The extracted samples were analysed with the enzyme immunoassay system described in Goymann *et al.* (1999) using a corticosterone antibody purchased from ICN-Biomedicals (Costa Mesa, CA, USA; no. 07–120116). This antibody is reported to cross-react with corticosterone (100%), desoxycorticosterone (0.34%), testosterone (0.10%), cortisol (0.05%), aldosterone (0.03%), progesterone (0.02%) and 12 other steroids tested ($< 0.01\%$).

Standard curves (range of standards $2.9–1500$ pg) and sample concentrations were calculated with IMMUNOFIT 3.0 (Beckman Inc., Fullerton, CA, USA) using a four-parameter logistic curve fit. The lower detection limit of the standard curve was determined as the first point outside the 95% confidence intervals for the zero standard and was 120 pg ml^{-1}. The assay accuracy was $97.6 \pm 4.5\%$ (mean $\pm$ s.e.m.) ($n = 32$) and the intra-assay coefficients of variation were $7.7 \pm 0.8\%$ for a high concentration pool ($n = 24$) and $7.5 \pm 1.0\%$ for a low concentration pool ($n = 24$). The interassay coefficients of variation were 5.0% for the high pool and 8.1% for the low pool. All samples

were assayed in duplicate and their concentrations are expressed as nanograms per gram of faecal dry matter.

(c) *Statistical analyses*

Statistical analyses were performed with SYSTAT 9.0 (SPSS Science Inc., Chicago, IL, USA), following Sokal & Rohlf (1995). The results are given as means $\pm$ s.e.m.s. The faecal corticosteroid data were not normally distributed and, thus, were transformed (using a reciprocial square-root transformation following Lamprecht (1992)) prior to parametric statistical analyses. As most individuals contributed more than one sample to the dataset we calculated the mean social status and mean faecal hormone concentrations for each individual and reproductive status category and then used these means for analysis. If an individual contributed samples for more than one category of reproductive status, we randomly selected one reproductive status category and then included only data for this category in the analysis in order to avoid pseudo-replication (Hurlbert 1984). We did not include samples from pregnant females, as the sample size for pregnant females was too low.

We applied a general linear model in order to test our predictions that faecal corticosteroid concentrations will be influenced by social status, reproductive status (non-lactating and lactating) and population (Serengeti and Ngorongoro) using samples collected from females during periods of social stability. We also investigated whether the impact of social status on faecal corticosteroid levels differed between individuals of different reproductive status and whether potential effects of social or reproductive status differed between populations.

We assessed the possible impact of acute social stress on faecal corticosteroid concentrations by comparing matched samples collected during periods with and without acute severe social stress from 18 lactating females in the Serengeti National Park using a Wilcoxon signed-ranks test. We calculated means if more than one sample was available for each female and period and used the means in the statistical test. Severe acute social stress was defined as intense conflict between adults. The respective samples ($n = 28$) stem from females known to have participated in severe fights within the previous 48 h or females with numerous severe wounds to their legs, feet, back, neck, face and ears that were acquired during periods of social instability amongst females. These samples were compared with samples ($n = 61$) from the same females during periods without such conflicts. The significance level was set at $\alpha = 0.05$. All p-values were two-tailed.

3. RESULTS

The faecal corticosteroid concentrations of the female hyenas varied significantly with reproductive status during periods of social stability in that lactating females had higher faecal corticosteroid concentrations (112.6 ± 35.5 ng g^{-1}) than non-lactating females (40.0 ± 9.3 ng g^{-1}) ($F_{1,103} = 22.287$ and $p < 0.00001$) (figure 1). The mean faecal corticosteroid concentration of pregnant females was 59.5 ± 17.3 ng g^{-1} ($n = 20$), but the data from pregnant females were not included in the analysis due to the small sample size in the Ngorongoro population (see § 2b). There was also a significant population effect in that the Serengeti hyenas had significantly higher faecal corticosteroid levels than the Ngorongoro hyenas ($F_{1,103} = 3.989$ and $p = 0.048$) (figure 2). The effect of social status on faecal corticosteroid levels depended on
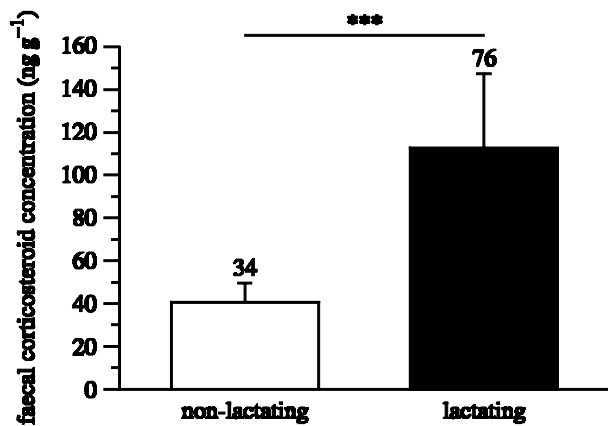
Figure 1. Mean (± s.e.m.) faecal corticosteroid levels of non-lactating and lactating female spotted hyenas. The numbers above bars represent the number of females (***$p < 0.00001$).
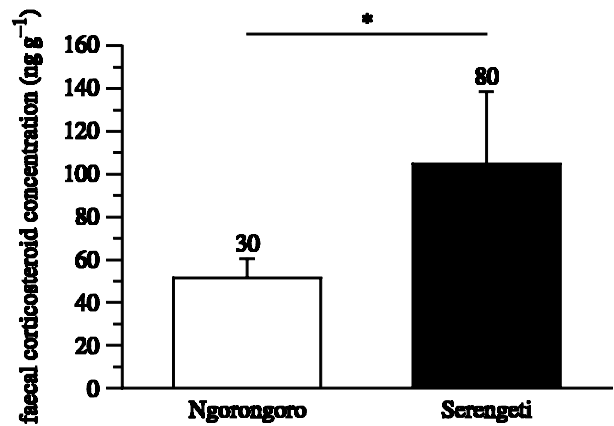

Figure 2. Mean (± s.e.m.) faecal corticosteroid levels of Serengeti and Ngorongoro spotted hyenas. The numbers above bars represent the number of females (*$p = 0.048$).

reproductive status. The faecal corticosteroid levels of non-lactating females declined as social status increased (figure 3a), whereas they were independent of social status in lactating females (figure 3b) (interaction of social and reproductive status, $F_{1,103} = 7.164$ and $p = 0.009$). Social status in itself showed no significant influence ($F_{1,103} = 1.828$ and n.s.). In addition, there were no effects of the interactions between social status and population ($F_{1,103} = 0.590$ and n.s.) and between reproductive status and population ($F_{1,103} = 0.010$ and n.s.).

The faecal corticosteroid concentrations of lactating Serengeti females were significantly higher during periods of severe acute social stress than during periods of social stability (Wilcoxon signed-ranks test, $z = 2.112$ and $p = 0.035$) (figure 4).

4. DISCUSSION

As predicted, the faecal corticosteroid concentrations of female spotted hyenas varied significantly with reproductive status. The faecal corticosteroid levels of non-lactating females increased as social status declined. Surprisingly, this was not the case in lactating females. Thus, in contrast to lactating females, non-lactating females conformed to expectations from other plural breeders (*sensu* Mumme 1997) such as baboons where subordinates typically have higher corticosteroid levels than dominants (Sapolsky 1982). Periods of acute social stress led to an increase in the faecal corticosteroid levels of lactating Serengeti females. In addition, as predicted, there was a significant difference between populations, with the Serengeti hyenas having higher faecal corticosteroid levels than the Ngorongoro hyenas. Because there is gene flow between the two hyena populations (Kruuk 1972; R. Albert, unpublished data) it is likely that the difference between the two populations is an environmentally determined conditional response rather than a genetically fixated response.

(a) *Social and reproductive status and corticosteroid levels*

Rates of social interactions and the occurrence of social conflicts may modulate the impact of social status on corticosteroid levels (Sapolsky 1986). In contrast to many

closely knit primate societies, the fission–fusion society of spotted hyenas may allow conflict management by avoiding superior competitors (East & Hofer 2001). Such tactics include the avoidance of locations where encounters with dominants are likely, visiting these sites when dominants are unlikely to be present or vacating these sites when dominants arrive (Hofer & East 2000). Yet there was a significant relationship between the social status and faecal corticosteroid levels of non-lactating females. Surprisingly, the relationship between social status and faecal corticosteroid levels was absent in lactating females, although the rate of social interactions was likely to be higher in lactating females as they regularly visit the communal den in order to nurse their cubs. The faecal corticosteroid levels of lactating females were generally higher than those of non-lactating females. These data corroborate our findings in blood plasma (Goymann 2000) and suggest that HPA axis activity was generally enhanced in lactating females and any effect of social status may have been muted by the effect of lactation (see below) during periods of social stability. It is important to recognize that the HPA axis of the lactating hyenas remained sensitive to severe acute social stress, as demonstrated for the Serengeti females, when the faecal corticosteroid levels of these females significantly rose in matched-pair comparisons during periods of intense social conflict.

There are few data available on the corticosteroid levels of free-ranging mammals in different reproductive states. Plasma corticosteroids have been found to be elevated during lactation in free-ranging degus (*Octodon degus*) (Kenagy *et al.* 1999), golden-mantled ground squirrels (*Spermophilus saturatus*) (Boswell *et al.* 1994) and yellow-pine chipmunks (*Tamias amoenus*) (Kenagy & Place 2000). So why is lactation accompanied by elevated corticosteroid levels in spotted hyenas?

First, the levels of social stress in lactating females may be higher than those in non-lactating females. Unlike other clan members, lactating females are forced to return to the social centre of the clan, i.e. the communal den, more frequently than non-lactating females in order to nurse their dependent cubs, which are stationed at the communal den. This is likely to elevate the rate of social interactions among lactating females in comparison with
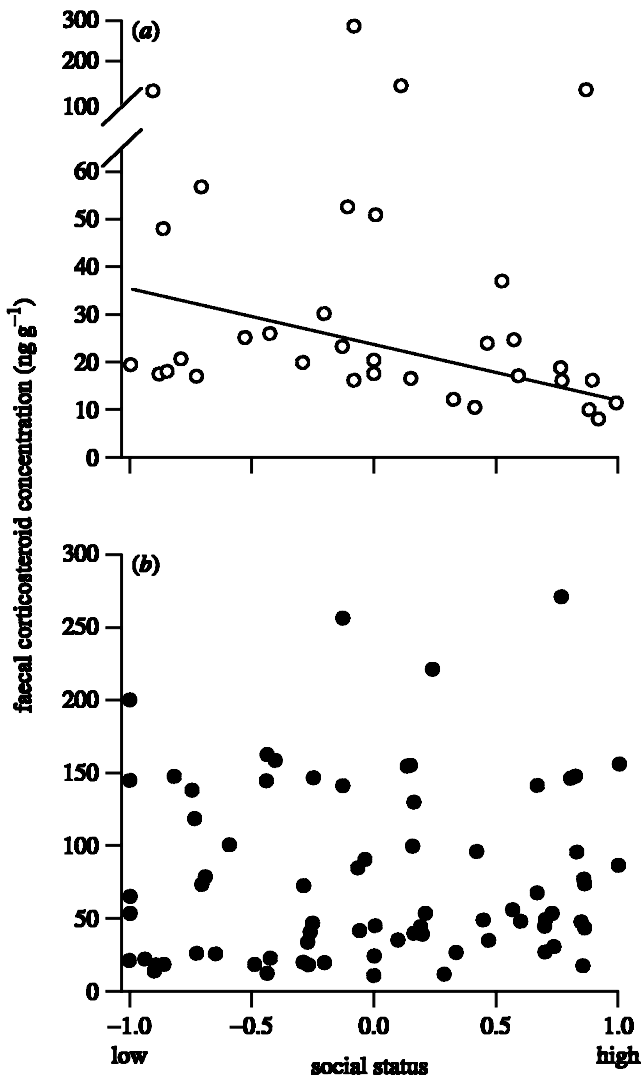
Figure 3. Interaction of social and reproductive status and faecal corticosteroid levels ($p = 0.009$) showing that high social status is associated with low faecal corticosteroid levels in non-lactating females (*a*), but not in lactating females (*b*).
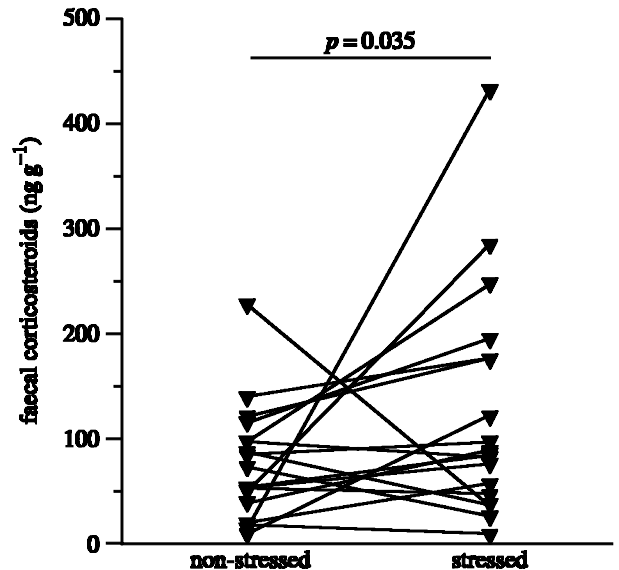

Figure 4. Pairwise comparison of the faecal corticosteroid levels of 18 lactating Serengeti female spotted hyenas during periods with and without severe acute social stress.

non-lactating females and, thus, lactating females may experience a higher degree of social stress.

Second, as outlined in § 1, reproduction is energetically expensive for hyena females. Since corticosteroids increase the metabolic rate, lactation may be a life history state that demands higher levels of these hormones. Thus, the energetic demands of lactation may overshadow any effect of social status on corticosteroid levels during periods of social stability. Corticosteroid concentrations may be subject to a trade-off between current reproduction, future reproduction and defence mechanisms that reduce the risk of mortality (Sibly & Calow 1989; Hofer & East 1998). High corticosteroid concentrations may carry costs (Munck *et al.* 1984; Sapolsky 1992*a*; De Kloet *et al.* 1999; Sapolsky *et al.* 2000) that may increase the risk of mortality and reduce the chances of future reproduction. The benefits of high corticosteroid concentrations during lactation may be an increased likelihood of survival of current offspring and the maintenance of body functions under conditions of heavy workload. A heavy physical workload leads to a change in the self-antigen repertoire (heat shock proteins), which increases the risk

of autoimmune diseases (reviewed in Råberg *et al.* 1998). The immunosuppressive effects of corticosteroids (reviewed in Besedovsky & Del Ray 1996; McEwen *et al.* 1997) may reduce the likelihood of such autoimmune diseases. Thus, if there is a trade-off between resistance to parasites and immunopathology, then the optimal solution to this trade-off may depend on the life history state of the organism (McNamara & Houston 1996; Wingfield *et al.* 1998; Jacobs & Wingfield 2000). Lactation in spotted hyenas is a life history state that is associated with a heavy physical workload (Hofer & East 1993*c*) and the increase in their corticosteroid levels may be an adaptation for reducing their risk of autoimmune disease (Råberg *et al.* 1998).

(b) *Population differences in corticosteroid levels*

The large territory sizes and low abundance of resident prey in the Serengeti National Park make life energetically more demanding than in the Ngorongoro Crater. In addition, Serengeti females regularly feed as socially subordinate intruders in the territories of other clans (commuting) (Hofer & East 1993*a,b,c*). As the movements of migratory herds across large areas are unpredictable, commuters are likely to intrude in hyena territories where they may know little about the resident territory holders. This stress affects all commuters and renders their lives less predictable than those of Ngorongoro females who feed mostly inside their territory in a predictable social environment. Serengeti mothers may suffer additional stress due to intense sibling competition (Hofer & East 1993*c*, 1997; Golla *et al.* 1999), which is absent in the Ngorongoro Crater (Wachter 2001).

(c) *Acute social stress in lactating females*

The plasma cortisol levels of baboons are elevated in all individuals during times of acute social stress, irrespective of social status (Alberts *et al.* 1992; Sapolsky 1992*b*). In the present study severe acute social stress led to a significant increase in the faecal corticosteroid

concentrations of individual lactating females. To the authors' knowledge, this is the first demonstration that changes in HPA axis activity due to severe acute social stress can be traced non-invasively in the faeces of individual free-ranging mammals. Furthermore, the increase in faecal corticosteroid concentration demonstrated that, despite their elevated levels of corticosteroids, lactating females were still able to respond to severe acute social stress with an increase in HPA axis activity. Thus, the function of the HPA axis was not compromised during lactation as it may be during chronic stress (Sapolsky 1992a). The HPA axis was more probably modulated (Wingfield *et al*. 1998) and adapted to the life history state of lactation.

(d) Conclusions

Life history theory is concerned with strategic decisions over an organism's lifetime (Stearns 1992). An organism can only acquire limited amounts of materials and energy and, thus, allocation of these limited resources to growth, reproduction or defence mechanisms will imply trade-offs (Sibly & Calow 1989). McNamara & Houston (1996) emphasized that the outcome of such trade-offs depends on the state of an organism (state-dependent life histories) and, thus, suggested that physiological systems should operate conditional responses. Because corticosteroid hormones imply costs and benefits for an individual, the level of HPA axis activity may be subject to such a trade-off (Hofer & East 1998; Wingfield *et al*. 1998). The present study linked the life history states of individual hyenas to their corticosteroid levels. Given the high costs of lactation, reproductive status was the major factor shaping the endocrine response of the HPA axis in female spotted hyenas in the Serengeti National Park and the Ngorongoro Crater. Large rather than small territory sizes, low rather than high abundance of resident prey and the commuting system in the Serengeti National Park make life socially less predictable and energetically more demanding than in the Ngorongoro Crater, even though the population densities were very similar in both populations. This was reflected in the higher faecal corticosteroid levels in the Serengeti females than in the Ngorongoro females. For the first time a study that uses non-invasive monitoring techniques demonstrates that, in free-ranging populations, lactation is associated with an elevated level of corticosteroids, that patterns of corticosteroid release might be conditional responses rather than genetically fixated responses to specific environmental changes and that corticosteroid levels may be elevated due to severe acute social stress.

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