The impact of age-class and social context on fecal glucocorticoid metabolite levels in free-ranging male giraffes

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Highlights:

- When in all male groups, sub-adult/juvenile bulls have the highest fGCM levels
- In the presence of females, older sexually active adult bulls have the highest fGCM levels
- Sexual activity leads to higher fGCM levels, but only in adult giraffe bulls.

Abstract:

One of the primary sources of perceived stress is the social environment of an animal and the interactions with conspecifics. An essential component of the response to a stressor is the activation of the hypothalamic–pituitary–adrenocortical axis, which results amongst others in a temporal increase in circulating glucocorticoid (GC) levels. Giraffes occur in a highly flexible fission-fusion social system and group compositions can change on a daily basis, with bulls establishing an age-related dominance hierarchy and showing a roaming strategy in the search for fertile females. The aim of this study was to non-invasively monitor the influence of different group compositions (mixed sex groups vs. all-male groups) on GC concentrations in free ranging giraffe bulls of different age classes. We collected fecal samples from free-ranging giraffe bulls for 12 months in a South African Private Game Reserve to examine age- and social context-related patterns of fecal GC metabolite (fGCM) concentrations. We found that fGCM levels in giraffe bulls are age-class dependent, as well as associated with changes in the social environment. Independently of the social setting, bulls of the youngest age class exhibited the highest fGCM levels compared to bulls of the other two older age-classes, with differences most pronounced when the bulls are associated in all-male groups. In contrast, an almost reversed picture appears when looking at the fGCM levels of sexually active individuals in mixed sex groups, where highest levels were found for the bulls in the oldest age-class, and the lowest for the bulls in the youngest age-class. The study stresses the importance to taking factors such as age-related status and social settings into account, when interpreting fGCM levels in free ranging giraffes.

Key words: Giraffe bulls, stress, non-invasive hormone monitoring, feces, glucocorticoids, South Africa

Abbreviations: fecal glucocorticoid metabolites (fGCM)

1. Introduction:

A stressor may be defined as the generic term for any stimulus that threatens, or appears to threaten the physiological balance of an individual (Möstl and Palme, 2002; Sapolsky, 2002). A stress response, in turn, is defined as a series of adaptive mechanisms that are aimed at protecting an individual and restoring homeostasis (Möstl and Palme, 2002). From a physiological perspective, an essential component of the response to stress is the activation of the hypothalamic–pituitary–adrenocortical axis (HPA), which results amongst others in a temporal increase in circulating glucocorticoid (GC) levels (Nelson, 2011). Furthermore, allostatic load represents the collective energetic costs to sustain homeostasis in everyday life and it increases concurrently with GC levels in the event of a stressful situation (McEwen and Wingfield, 2003). A first response to a stressor is an increase in catecholamine (epinephrine and norepinephrine) secretion. This rapid 'fight-or-flight response' causes changes in respiratory rate, cardiovascular tone and blood flow to the muscles and also increases blood glucose levels, which then delivers the needed energy for the response (Nelson, 2011). After this immediate response, the hypothalamic-pituitary-axis (HPA) is activated and glucocorticoids (GC) will be released to also facilitate hyperglycaemia for increased energy demands. This release is delayed over the course of minutes, and in some instances up to an hour (Sapolsky et al., 2000). Short-term increases in GC concentrations helps with the immediate survival of the individual, however, prolonged or chronic elevations of GCs may lead to diseases and reduced reproductive success (Sapolsky, 2002).

Stressful situations may arise from a variety of internal and external stimuli, such as environmental factors (e.g. extreme weather), social stimuli (e.g. mating or dominance conflicts), or physiological challenges (e.g. injuries or food scarcity) (Nelson, 2011; Touma et al., 2003; Touma and Palme, 2005). However, one of the primary sources for perceived stress is the social environment of an animal and its associated interaction with conspecifics (Creel et al., 2013). In this regard, the social status of an individual *per se* can also be seen as an influencing factor, especially in species where individuals frequently interact with a broader group of conspecifics (Creel et al., 2013). In a variety of primate species, e.g. olive baboons (*Papio anubis*, Sapolsky, 1982) and mandrills (*Mandrillus sphinx*, Setchell et al., 2010), subordinate individuals show comparatively higher GC levels due to increased aggression received from dominant individuals, or reduced access to resources ('stress of subordination' hypothesis; Abbott et al., 2003; Goymann and Wingfield, 2004). However, the opposite pattern with comparatively higher glucocorticoid levels in dominant individuals can also occur, as e.g. seen in male bison (*Bison bison*, Mooring et al., 2006). This pattern is likely explained by the higher energetic demands due to the challenges to maintain a high rank in the hierarchy, as well as increased mating activity and mate guarding ('cost of dominance' hypothesis; Abbott et al., 2003; Goymann and Wingfield, 2004). Finally, many studies could not demonstrate any relationship between social status and glucocorticoid levels (e.g. tufted capuchin (*Cebus apella nigritus*), Lynch et al., 2002; redfronted lemurs (*Eulemur fulvus rufus*), Ostner et al., 2008). These differences in the relationship between social status and GC levels presumably reflect the differences in the allostatic load of dominant and subordinate individuals in different species (Goymann and Wingfield, 2004). Allostatic load may decrease when social support is available and when individuals cooperate. However, it increases in times of social conflict, e.g. an unstable hierarchy, which mainly affects dominant individuals, or in times of food scarcity when access to resources is restricted, which affects subordinates mostly (Abbott et al., 2003; Goymann and Wingfield, 2004). Overall, it appears that comparatively higher GC levels are found in subordinates, when they live in societies in which they are frequently confronted with physical or psychological stressors and receive only few or no social support in these situations (Abbott et al., 2003).

Apart from the social environment, it has been shown that the presence of fertile females can also act as a distinct stressor leading to an increase in GC levels in males (e.g. African elephant (*Loxodonta africana*), (Rasmussen et al., 2008); bonobo (*Pan paniscus*), Surbeck et al., 2012). In most species, fertile females represent a limited resource to which not all males will have access. Thus, the presence of a fertile female can lead to an increased allostatic load, which might be greater in dominant individuals due to frequent mate guarding and intense mating activity itself (Creel et al., 2013; Goymann and Wingfield, 2004). However, this seems not to be the case in species with low male-male aggression (tufted capuchin, Lynch et al., 2002; redfronted lemurs, Ostner et al., 2008).

An ideal species to study the impact of the social environment on GC levels in males is the giraffe (*Giraffa camelorpadalis giraffa*). Giraffes occur in a highly flexible fission-fusion social system and group compositions may change on a daily basis (Carter et al., 2013a; Shorrocks and Croft, 2009). Bulls establish a dominance hierarchy with usually larger/older individuals being dominant over smaller/younger ones (Pratt and Anderson, 1985). Giraffes are non-seasonal breeders and males adopt a roaming strategy to search for fertile females (e.g. Dagg, 1971; Pratt and Anderson, 1982). Giraffe bulls are assumed to become sexually mature at an age of approximately three years (Hall-Martin et al., 1978). However, these age estimates are mainly based on observations from captive populations (Hall-Martin et al., 1978; Reuther, 1961), and therefore it is questionable if males of this age can be seen as mature in a free-ranging setting (Hall-Martin et al., 1978). Finally, respective group-specific assays have recently been established for monitoring adrenocortical activity in this species using a non-invasive approach (Bashaw et al., 2016). Thus, the aim of this study was to investigate the influence of different group compositions (mixed sex groups vs. all-male groups) on GC concentrations of giraffe bulls in different age classes.

2. Material and methods:

2.1 Study site and animals

The data collection took place in an approximately 70km^2 Game Reserve in Kwazulu-Natal, South Africa, which mainly comprises of acacia & marula woodland and mixed acacia woodland. The wet season is in summer (between April and September) with an average annual rainfall of 400-600 mm (Shannon et al., 2006). The study population comprised of 80 giraffes (27 males, 41 females, and 12 infants of unknown sex) and was observed over a period of 12 months (November 2014 – October 2015). The animals were identified by their individual pelage pattern, and assigned to respective age classes as described by Langman (1977), with infants younger than 3 months and still accompanied by their mothers, juveniles < 1.5 years, sub-adults ≤ 4 years, and adult fully grown individuals > 4 years. Juvenile and sub-adult bulls were subsequently combined and categorized as the youngest age-class (See supplemental material S1). Furthermore, adult bulls were initially classified into three age classes A, B and C as described by Pratt and Anderson (1982). However, an objective differentiation between B and C bulls was not possible, and therefore adult bulls were assigned into only two age classes, older mature bulls (Ad1 – formerly Class A) and younger mature bulls (Ad2 – formerly class B and C). Ad1 bulls showed a prominent bone structure on the forehead as described by Pratt and Anderson (1982) and were also the oldest and tallest. Ad2 bulls were younger and showed a less stout neck musculature and less pronounced bone structure on the forehead (See S1).

The study was conducted with the approval of the University of Pretoria Animal Use and Care Committee (Reference V053-14).

2.2 Social and Behavioral data

Collection of social and behavioral data took place on six days per week from dawn till dusk. The existing road network in the Game Reserve was used to locate giraffes, and individual males were followed up to four hours for focal animal sampling using focal and continuous recording (Martin and Bateson, 2007). Simultaneous observations took place if more than one male was initially present in the group or joined the group at a later stage. If any of the following behaviors were shown by an individual bull towards a female, this individual was considered to be sexually active: courtship behavior, investigation of the females’ anogenital region, urine testing, mating and mate guarding (Seeber et al., 2013). Consequently, a bull was considered sexually inactive if he did not show any of these behaviors, regardless of its social association.

We also distinguished between different social environments and thus giraffe groups were categorized as either ‘all-male groups’ (comprising only bulls) or ‘mixed sex groups’ (comprising all groups in which at least one female was present) (Wolf et al., submitted). Intra-sexual competition was determined for bulls and defined as being present if yielding from another bull, displacement of another bull, dominance gestures (i.e. an erect posture while standing still or a tensed and arched neck when moving), sparring (i.e. an individual swings its head against the body of a sparring partner with comparably soft blows) and fighting behavior was observed at least 3 times per day (Seeber et al., 2012).

2.3 Fecal sample collection, extraction, and assay

A total of 586 fecal samples were collected and analyzed for fecal glucocorticoid metabolite (fGCM) concentrations. To minimize bacterial metabolism of steroids post-defecation, fecal material was collected within 20 minutes post-defecation (Heistermann, 2010; Möstl and Palme, 2002). Upon collection, samples were placed in a polypropylene tube, stored on ice immediately, and frozen within six hours. All collected material were kept frozen until reaching the Endocrine Research Laboratory, University of Pretoria, South Africa.

In the lab, feces were freeze-dried, pulverized, and sieved through a thin metal strainer for removal of fibrous material (Fieß et al., 1999). For steroid extraction, 0.10 - 0.11 g of fecal powder was vortexed for 15 min. with 80% ethanol in water (3 ml). After centrifugation for 10 min. at 1500 g, the supernatants were aliquoted and stored at -20˚C until assay procedure (Seeber et al., 2013).

Fecal glucocorticoid metabolite (fGCM) concentrations were determined using an 11-oxo-etiocholanolone enzyme immunoassay (antibodies raised in rabbits against 5β-androstane-3α-ol-11-one-17-CMO:BSA; Möstl et al., 2002) which has been shown to reliably monitor fGCM alterations in a variety of mammals including giraffes (Bashaw et al., 2016; Palme, 2012 for review). The assay procedure followed established protocols (Ganswindt et al., 2002). Sensitivity of the assay at 90% binding was 0.6 ng/g fecal dry weight (DW). Inter-assay coefficients of variation, determined by repeated measurement of high- and low-value quality controls, ranged between 13.7% and 17.3%.

Intra-assay coefficients of variation was 4.8% for low and 3.3% for high quality controls. All analyses were performed at the Endocrine Research Laboratory, University of Pretoria, South Africa.

2.4 Statistical analyses

We included the results from 19 individual males (n = 5 Ad1 bulls, n = 6 Ad2 bulls and n = 8 young bulls) in the analysis. Between 11 and 62 samples were collected per individual and we calculated median fGCM levels for each individual in each of the different social settings prior comparison (see supplemental material S2). We used a Kruskal-Wallis (KW) One Way Analysis of Variance on Ranks to compare differences of individual median fGCM levels between bulls of different age classes (Ad1, Ad2, or young bulls). Testing was done within each social category (in all-male groups, in mixed sex groups, but sexually inactive, and in mixed sex groups sexually active), followed by *post hoc* pairwise analysis using a Mann-Whitney U-test. When comparing the individual median fGCM levels between the three social categories for animals in a specific age class, we used Friedman Repeated Measure on Ranks, followed by *post hoc* pairwise analysis using exact Wilcoxon signed rank test. To correct for multiple pairwise comparison we used the Bonferroni method.

To further investigate the fGCM pattern found between the different age classes within all-male groups, we divided the data set for each age class according to the occurrence of intra-sexual competition (yes vs no). A KW One Way Analysis of Variance on Ranks was then used, to compare differences of individual median fGCM levels between bulls of different age classes for both data subsets (animals showing intra-sexual competition and those who do not). To compare median fGCM levels of bulls of the same age class in the two different social conditions we used an exact Wilcoxon signed rank test (intra-sexual completion present or not).

In cases of pairwise multiple comparison procedures, we adjusted the α level by applying the Bonferroni method. All statistical analyses were done using R, version 3.0.2 (R Core Team, 2013).

3. Results

Independently of the social setting the median fGCM levels for Ad1 bulls were 0.87 µg/g dry weight (DW) (interquartile range (IQR): 0.633 – 1.215 µg/g DW), 0.92 µg/g DW (IQR: 0.699 – 1.208 µg/g DW) for Ad2 bulls, and 1.07 µg/g DW (IQR: 0.79 – 1.37 µg/g DW) for bulls of the youngest age-class, respectively.

When comparing fGCM levels of bulls in a particular social setting, we found significant differences in the all-male category between individual median fGCM levels of different age classes (Ad1 = 5, Ad2 = 6, young = 7 individuals, KW $\chi 2$ = 11.998, df = 2, p = 0.003; see figure 1). Ad1 bulls had a trend for lower fGCM levels compared to Ad2 bulls (p = 0.068) and had significantly lower fGCM levels compared to animals in the youngest age-class group (p = 0.005). Ad2 bulls also showed significantly lower fGCM levels compared to the youngest age-class individuals (p = 0.010). To further investigate the differences in fGCM levels found between the different age classes in the all-male category, we split the data set into animals showing either sexual competition or not. In this regard, older bulls (Ad1 54% and Ad2 45%, respectively) were observed more often being engaged in intra-sexual competition compared to males of the youngest age-class (37%). However, there was no significant difference (n = 17, KW $\chi 2$ = 15, df = 15, p = 0.451) in individual median fGCM levels over all males between the two social situations, with an overall individual fGCM level of 0.89 µg/g DW (IQR: 0.69 – 1.22 µg/g DW) for bulls showing intra-sexual competition compared to 0.99 µg/g DW (IQR: 0.74 – 1.32 µg/g DW) for males who did not show such behavior. Similarly, we also did not find significant differences in individual median fGCM levels within age classes between animals showing intra-sexual competition or not (Ad1 = 5 individuals, V = 9, p = 0.250; Ad2 = 6 individuals, V = 13, p-value = 0.6875; young = 6 individuals, V = 7, p-value = 0.5625). As with the general comparison of individual median fGCM levels between the age classes in the all-male category mentioned above, individual median fGCM levels between the age classes differed significantly within each social setting (KW $\chi 2$ =

10.773, df = 2, p = 0.005 without intra-sexual competition and KW χ2 = 11.230, df = 2, p = 0.004 with intra-sexual competition, respectively). Again the bulls from the youngest age class showed significantly higher fGCM levels than their adult conspecifics (intra-sexual competition present: Ad1 KW χ2 = 7.5, df = 1, p = 0.006 and Ad2 KW χ2 = 8.308, df = 1, p = 0.004; no intra-sexual competition present: Ad1 KW χ2 = 6.539, df = 1, p = 0.011 and Ad2 KW χ2 = 8.163, df = 1, p = 0.004).

When comparing individual median fGCM levels of sexually inactive bulls in mixed sex groups, no significant differences were found between the age classes (KW χ2 = 3.237, df = 2, p = 0.198; see figure 1). In contrast, a statistical trend in individual median fGCM levels between the age classes was found when comparing sexually active bulls in mixed sex groups (KW χ2 = 5.6772, df = 2, p = 0.059; see figure 1). Ad1 bulls in mixed sex groups being sexually active showed overall higher median fGCM levels compared to Ad2 bulls and bulls of the youngest age-class.

When comparing fGCM levels of bulls of a particular age class present in different social settings, we found significant differences in individual median fGCM levels for Ad1 bulls (N = 5, Friedman χ2 = 8.400, df = 2, p-value = 0.015, see figure 1), who showed a trend for higher fGCM levels when being in mixed-sex groups regardless of being sexually active (active: N = 5, 1.07 µg/g DW, IQR: 0.87 – 1.57 µg/g DW; inactive: 0.95 µg/g DW, IQR: 0.70 – 1.18 µg/g DW) compared to when seen in all-male groups (0.72 µg/g DW, IQR: 0.59 – 0.99 µg/g DW; p = 0.063). No significant differences in individual median fGCM levels for Ad2 bulls assigned to the three social categories were found (N = 6, Friedman χ2 = 4.333, df = 2, p-value = 0.115, see figure 1). When comparing fGCM levels of bulls of the youngest age-class present in the three different social settings, we found significant differences in the individual median fGCM levels (N = 8, Friedman χ2 = 9.333, df = 2, p-value = 0.009, see figure 1). When sexually active the bulls of the youngest age-class showed significantly lower median fGCM levels (0.78 µg/g DW, IQR: 0.66 – 1.02 µg/g DW) compared to when sexually inactive (1.07 µg/g DW, IQR: 0.80 – 1.32 µg/g DW; p = 0.019) as well as when in all-male groups (1.18 µg/g DW, IQR: 0.93 – 1.45 µg/g DW; p = 0.008).

4. Discussion

In giraffes the dominance status is most likely to be related to body conditions which usually increases with age, and our results show that fGCM levels in giraffe bulls are age-class dependent, with highest overall fGCM levels found in the juvenile/sub-adult individuals. These bulls are at a stage in their life history, in which they become sexually mature (Hall-Martin et al., 1978). It has already been shown, e.g. in humans, that glucocorticoid levels increase during the time of puberty (Kiess et al., 1995). Puberty is a time of increased hormonal activity and the increased glucocorticoid levels might reflect an overall higher activity of the HPA axis in preparation for the upcoming challenges in adult life (Gunnar et al., 2009; Kiess et al., 1995). Another possible factor leading to comparatively higher fGCM levels in younger individuals could be the challenges young bulls face in a newly developing social environment. After leaving their maternal unit, young giraffe bulls often congregate and challenge one another to establish their place in the hierarchy (Pratt and Anderson, 1985), and when reaching sexual maturation they might be targeted aggressively by older bulls. A variety of studies have shown that in species in which males establish a dominance hierarchy, the subordinate individuals have higher glucocorticoid levels than dominant individuals (e.g. olive baboons, Sapolsky, 1982). Subordinate olive baboons are frequently exposed to a variety of stressors often linked to physical aggression received to obtain a dominant position (Abbott et al., 2003). Similarly, the higher fGCM levels in the sub-adult/juvenile males in our study population might be due to the increased stress they experience when leaving their maternal group and establishing their place within the male community. However, aggression rates in our study population are generally low and an alternative explanation could be an increased metabolic demand in the young males due to rapid growth is occurring , as can be seen in immature male yellow baboons during the time of the pubertal growth spurt (Gesquiere et al., 2005).

An influence of social context on fGCM levels of juvenile/subadult giraffe bulls can be seen when comparing the fGCM levels of the different age classes within all-male groups. Here the highest fGCM levels are found in the youngest age-class bulls. All-male groups occur in a variety of mammalian species, but the reason for the liaison of males can differ from species to species. For example, male plains zebras (*Equus burchelli*) often congregate in bachelor groups to reduce predation risk (Rubenstein and Hack, 2004), whereas in African lions (*Panthera leo*), young males form a coalition to gain access to females (Bygott et al., 1979). In African elephants, all male groups give young bulls the opportunity to learn from older bulls about resource distributions or to find sparring partners (Chiyo et al., 2011). All-male groups in giraffes are thought to give the younger males similar opportunities as in elephants (Bercovitch and Berry, 2015; Coe, 1967). However, it has recently been questioned if male giraffes congregate with other young males in order to establish a dominance hierarchy, as no long-term relationships between bulls could be demonstrated thus far (Bercovitch and Berry, 2015). Nevertheless, even without a long-term linear hierarchy present, older individuals usually obtain a more dominant position than younger ones (Pratt and Anderson, 1985). Although observed agonistic behavior in our study was low and was even shown to be the lowest in the youngest age-class, this does not exclude the possible presence of subtle dominance behavior, which could in turn be indicated by the increased fGCM levels in the younger and subdominant individuals. Such relationships have been shown for a variety of primate species and is in line with the 'stress of subordination' hypotheses (Abbott et al., 2003; Goymann and Wingfield, 2004; Sapolsky et al., 2000).

Interestingly, the bulls of the youngest age-class showed significantly lower glucocorticoid levels when in mixed sex groups compared to all-male groups. These findings are in contrast to studies on other species, e.g. white-faced capuchin monkeys, where GC levels in sub-adult males increase in the presence of periovulatory females (Schoof et al., 2014). These juvenile/sub-adult bulls might not be fully mature yet and therefore their endocrine correlates do not seem to reflect sexual activity, which is further supported by previous findings, showing that the androgen levels of these bulls are also not higher in the presence of females (Wolf et al, submitted).

Younger adult bulls (Ad2), representing the in-between group with regards to age and dominance status, show the least variation in fGCM concentrations in relation to social context. When sexually inactive, fGCM concentrations of Ad2 bulls are also comparable with the respective steroid concentrations of sub-adult/juvenile and the oldest individuals. When sexually active, Ad2 bulls showed no significant differences in their fGCM levels compared to the sub-adult/juvenile individuals. A similar pattern can be found in white-faced capuchins (*Cebus capucinus*), where only the dominant males show significantly higher GC levels compared to subordinate adult and sub-adult males (Schoof et al., 2014). As in our study population, intra-sexual aggression is low in capuchins, indicating a low male competition and reproductive effort. However, the reproductive skew in this species suggests a higher male-male competition than expected by the low aggression rate and female mate choice might play a role as well (Schoof et al., 2014). Further genetic analyses will be needed to evaluate whether giraffes show a similar reproductive skew with only the dominant old giraffe bulls siring offspring or if the younger males follow alternative mating tactics.

Being at the top of the hierarchy may be stressful when the position has to be maintained continuously by aggressive encounters, as is seen in male chimpanzees (Muller and Wrangham, 2004). The metabolic demands for these displays are high and they are therefore suggested to be the cause of higher GC levels in dominant individuals. A similar pattern can be found in Alpine chamois (*Rupicabra rupicabra*), in this species territorial males show an increase in GC levels during the rutting period (Corlatti et al., 2012). However, the benefits of better mating opportunities have to be paid by higher energetic demands and parasite susceptibility (Corlatti et al., 2012). Interestingly, we found that the bulls of the oldest age class (Ad1), which are supposedly to be the most dominant individuals, showed the lowest overall fGCM levels and when part of all-male groups they showed significantly lower fGCM levels than the males of the youngest age-class. These bulls obtain a more dominant position due to their body size and age and often chase other adult bulls away, when entering a group with females (Pratt and Anderson, 1985). Although they were involved in more intra-sexual competition than the youngest age-class bulls, they were rarely observed to be engaged in physical aggression. This might indicate that at this stage of their life, their position is established within the population, resulting in comparatively lower fGCM levels. These findings would be in line with the ‘stress of subordination’ hypotheses, where more dominant individuals show lower GC levels than subordinate individuals due to less physical and social stressors (Abbott et al., 2003; Goymann and Wingfield, 2004). In contrast to female giraffes, males seem not to establish long-term relationships with each other and older bulls are observed more often solitary than the younger individuals (Carter et al., 2013b; Pratt and Anderson, 1985). All-male groups including old bulls might therefore form when younger bulls follow these bulls to learn about resource distribution (Bercovitch and Berry, 2015). The energetic costs for older bulls to restrain the younger ones from following them might be higher than to fight them off (Bercovitch and Berry, 2015) and as a result the older bulls might tolerate them around. However, further studies investigating paternity would be necessary to clarify if it is indeed only the oldest bulls who are siring the majority of offspring.

GC levels in the oldest age class (Ad1) seemed to be influenced by the presence of females and in relation to sexual activity. Compared to all male groups, we found higher fGCM levels when Ad1 bulls were associated with females, especially during times of sexual activity. Androgen levels in this age class were also elevated in the presence of females (Wolf et la., submitted), which may lead to the prediction that the reproductive effort in this age class is associated with physiological costs, and higher glucocorticoid levels might be involved in the regulation of the enhanced metabolic needs during courtship and mating (Creel et al., 2013). This could potentially be an expected pattern and consistent with findings in many other species where male glucocorticoid levels increase during times of mating activity (e.g. bison, Mooring et al., 2006; redfronted lemur, Ostner et al., 2008; chacma baboons, Kalbitzer et al., 2015). These increases in glucocorticoid levels are mainly due to the energetic costs of mate guarding and the mating activity itself (Higham et al., 2013; Muller and Wrangham, 2004). Similarly, when a giraffe bull locates a receptive female he will start mate guarding her and trying to restrict the access for other bulls to her (Bercovitch et al., 2006; Pratt and Anderson, 1982).

In summary, our study has shown that changes in glucocorticoid levels in wild giraffe bulls are age-class dependent, as well as associated with changes in the social environment. Independently of the social situation, the bulls of the youngest age-class exhibited the highest fGCM levels compared to bulls of the older age-classes, and these differences are most pronounced when the bulls are associated in all-male groups. These findings are in line with the ‘stress of subordination’ hypotheses and lead to the suggestion that the sub-adult/juvenile bulls still need to establish their position within the male community. In the oldest age class, the highest fGCM levels were found during times of sexual activity, suggesting an increased allostatic load due to mate guarding and mating activity. Further research will be needed to link the endocrine changes of these bulls with reproductive success. This study shows the importance to take factors like age-related status and social settings into account, when interpreting fGCM levels in free ranging giraffes.

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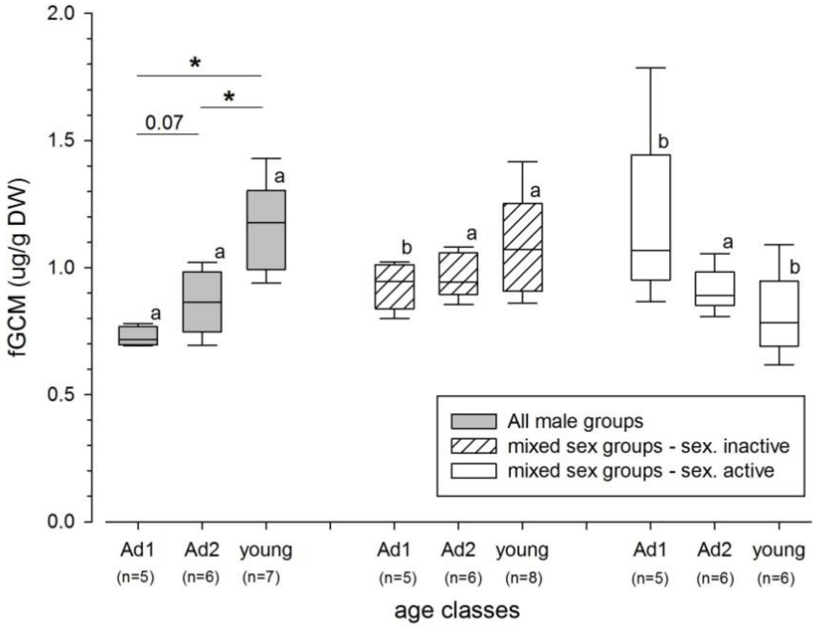

Fig. 1: Individual median fGCM levels of bulls of particular age classes (Ad1, Ad2, and young) in different social settings (in all male groups and mixed sex groups when either sexually inactive or sexually active). Significant differences between age classes within a specific social setting are indicated by asterisk. Different letters indicate significant differences between social settings within a particular age class.