RESEARCH ARTICLE

Environmental, Biological, and Social Factors Influencing Fecal Adrenal Steroid Concentrations in Female Japanese Macaques (*Macaca Fuscata*)

RAFAELA S. C. TAKESHITA[1]*, FRED B. BERCOVITCH[1], MICHAEL A. HUFFMAN[1], KEIKO MOURI[1],
CÉCILE GARCIA[2], LUCIE RIGAILL[1], AND KEIKO SHIMIZU[3]
[1]*Department of Ecology and Social Behavior, Primate Research Institute, Kyoto University, Inuyama, Aichi, Japan*
[2]*Laboratoire de Dynamique de l'Evolution Humaine, CNRS UPR 2147, Paris, France*
[3]*Faculty of Science, Department of Zoology, Okayama University of Science, Okayama City, Okayama, Japan*

The ability to determine hormonal profiles of primate populations using non-invasive techniques can help to monitor physical fitness, stress, and physiological responses to environmental changes. We investigated fecal glucocorticoids (fGC) and DHEAS concentrations in captive Japanese macaques (*Macaca fuscata*) in relation to environmental, biological, and social factors. The subjects were female Japanese monkeys from 4 months to 31 years old housed in captivity (27 in social groups and 12 in single cages). Fecal samples were collected from all females, and behavioral data from the social groups during the mating season and the following birth season. Hormonal concentrations were analyzed by enzyme immunoassay. Our results revealed that both fGC and fecal DHEAS concentrations are higher in females housed indoors in single cages than in those living outdoors in social groups. We also found that fGC concentrations were higher in the cycling females during the mating (winter) season than the lactating females in the birth (spring) season. Age was negatively associated to both fGC and fecal DHEAS levels, but the relationship between age and fecal DHEAS was more evident in females housed indoors in single cages than in females housed in outdoor social groups. We did not observe any association of dominance rank with either fecal DHEAS or fGC. This study showed that measurement of fecal DHEAS and fGC can be a good method to assess stress in Japanese macaques. These findings provide insights about the physiology of these two adrenal hormones in female Japanese macaques, which can be applied to wild populations and is fundamental for captive management and conservation biology. Am. J. Primatol.   © 2014 Wiley Periodicals, Inc.

Key words: glucocorticoids; dehydroepiandrosterone-sulfate; seasonality; stress; *Macaca fuscata*

INTRODUCTION

The hypothalamic–pituitary–adrenal (HPA) axis modulates individuals' physiological responses to social stress, promoting energy mobilization, and facilitating effective behavioral responses to challenges in the environment [Cavigelli, 1999]. When a stressor is perceived in the brain, the sympathetic response stimulates the secretion of epinephrine from the adrenal medulla, often followed by the release of cortisol from the adrenal cortex into the blood stream [Sapolsky, 2002].

For this reason, glucocorticoids (GC) have been used as an index of stress in nonhuman primates [Bayazit, 2009; Whitten et al., 1998]. Because fecal samples do not require direct contact with the animal, fecal glucocorticoids (fGC) have been widely applied for studying hormonal profiles in wild primates or populations reared in open enclosures. Although social stress is often examined as an influence on hormone levels, temperature stress is less often examined, yet can produce a physiological

response. An increase of fecal cortisol during winter in free-ranging female chacma baboons (*Papio hamadryas ursinus*) as an adaptation to cold stress has been documented [Weingrill et al., 2004], and Beehner & McCann [2008] found seasonal and altitudinal effects on glucocorticoid metabolites in wild gelada baboons (*Theropithecus gelada*). Reproductive state may also influence GC levels. Previous

studies report high cortisol levels in late gestation of ring-tailed lemurs (*Lemur catta*) [Cavigelli, 1999]. In lactating rhesus monkeys (*Macaca mulatta*), fGC rises as a response to stress related to perception of risk to infants [Hoffman et al., 2010; Maestripieri et al., 2008], while in lactating Japanese monkeys, fGC increases as a result of the suckling stimulus from their infants [Kondo et al., 2003].

Social factors can also affect hormonal secretion, and non-invasive analysis is the preferable method to measure hormones because it does not interfere with the individual response to the social environment. Ostner et al. [2002] described social correlates of fecal androgen secretion in male lemurs (*Eulemur fulvus rufus*). Beehner et al. [2005] demonstrated that the immigration of new alpha males increased fGC in female free-ranging chacma baboons (*P. h. ursinus*). Moreover, Barrett et al. [2002] associated fecal cortisol and testosterone with dominance rank and aggressive behavior in wild male Japanese macaques. Thus, many factors can potentially modify stress hormones [Honess & Marin, 2006].

Another adrenal hormone, dehydroepiandrosterone (DHEA) and its sulfated version (DHEAS) have been the focus of investigation as stress markers in humans [Du et al., 2011] and nonhuman primates [Goncharova et al., 2012; Maninger et al., 2010]. In mice, DHEA has been suggested to act as an anti-stressor [Hu et al., 2000]. This beneficial effect is thought to provide an antagonistic action to cortisol [Boudarene et al., 2002]. DHEA production and secretion also decrease throughout the aging process in humans [Baulieu, 1996] and nonhuman primates [Muehlenbein et al., 2003]. Some studies have revealed that DHEA and DHEAS increased memory [Flood & Roberts, 1988; Vallee et al., 2001] and enhanced the immune system [Khorram et al., 1997]. Increases in serum DHEAS concentrations have been observed in stressed rhesus monkeys (*Macaca mulatta*) [Goncharova et al., 2012; Maninger et al., 2010], and elevated serum DHEAS levels have been found in aggressive rhesus monkeys [Goncharova et al., 2010]. The relationship between DHEAS and cortisol has been used as an index to measure stress [Goodyer et al., 2000].

Nevertheless, the association between stress levels and DHEAS is not well elucidated due to the limited data in the literature. In Japanese macaques (*Macaca fuscata*), fecal samples have been used to monitor reproductive hormones [Bardi et al., 2003; Barrett et al., 2002; Fujita et al., 2004; Yoshida et al., 2001], social dominance, and cortisol levels [Barrett et al., 2002; MacIntosh et al., 2012], and seasonal impacts on steroid hormones [Muroyama et al., 2007; Takeshita et al., 2013], but few studies have been published regarding the possible impact of stress on glucocorticoids and DHEAS metabolite levels in this species. This study aimed to measure fGC and fecal DHEAS metabolites as possible indicators of stress in captive female Japanese macaques (*Macaca f. fuscata*). We examined environmental (housing condition and season), biological (chronological age), and social (dominance rank) factors as potential mediators of adrenal steroid levels.

We hypothesize that: (1) fGC and fecal DHEAS will show higher concentrations in individuals housed indoors in single cages due to stress, (2) age will be negatively correlated with fecal DHEAS, (3) adrenal hormone concentration will be higher in the winter mating season than in the spring birth season, and (4) fGC levels will be negatively associated with dominance rank.

METHODS

Subjects and Study Site

The subjects were 37 female Japanese macaques ranging in age from 4 months to 31 years. They were maintained at the Primate Research Institute, Kyoto University. Twelve females (age range = 2–31 years old, mean age = 10.5) lived in individual cages (D760 mm × W900 mm × H850 mm) in an indoor room with controlled light and temperature (18–33°C). They had visual, auditory, and olfactory exposure to other monkeys in the same room. Twenty-five females lived in two outdoor social groups (age range = 4 months to 27 years old, mean age = 6.9). Wakasa-5 troop (troop size grew from 32 to 41 individuals during the study period) lived in a 960 m² outdoor corral with metal climbing structures, one open-side 12 m² wooden-roofed shelter and two metal shelter boxes. PRI Arashiyama B troop (troop size grew from 56 to 62 individuals during the study period) lived in a 728 m² outdoor enclosure with metal and wooden climbing structures, a small pond, and nine circular plastic tunnels (900 mm length × 450 mm diameter) placed above ground secured by metal structures, that serve as shelter from adverse weather and cold, as well as resting platforms. One female (24-year-old) who had lived in Wakasa-5 troop since birth, was moved to an indoor single cage during the study period for management purposes. All the monkeys were fed monkey chow on a daily basis, sweet potato pieces three times a week and had ad libitum access to drinking water. Body weight was not associated with either fecal DHEAS (Spearman's rho: $R = 0$, $P = 1$, $N = 10$) or fGC levels (Spearman's rho: $R = -0.5$, $P = 0.22$, $N = 10$) and therefore was not included in further analyses.

The study site experiences strong seasonal changes in temperature. A thermo-recorder (OPUS II-WE2, Senecom, Inc., Saitama, Japan) obtains outdoor temperature every minute around the clock. Daily temperature in the outdoor enclosures varied between 1.1 and 31.8°C during the winter months (October to December) and between 15.8 and 28.9°C during the late spring months (May to June), two

periods that overlap with the mating and the birth season, respectively. The minimum daily temperature was significantly lower during winter ($9.09 \pm$ SD $5.22°C$) than during late spring ($17.78 \pm$ SD $1.23°C$; Mann–Whitney $U = 66$, $P < 0.0001$).

This research adhered to the American Society of Primatologists (ASP) principles for the ethical treatment of nonhuman primates. All manipulations of the subjects were approved (research clearance no. 2012-063-03) by the Primate Research Institute's Ethics Committee and conformed the PRI's Guidelines for Care and Use of Nonhuman Primates.

Sample Collection

The study was conducted during 3 months of the 2011 mating season (October to December) and 2 months of the following 2012 birth season (May–June). During the mating season, we collected a minimum of three fecal samples per subject over nonconsecutive days between 7:00 and 11:00 AM from all 37 subjects ($N = 112$). Seven adult females (5–14 years old) in the outdoor enclosures conceived during the mating season. Due to funding constraints, we were unable to analyze fecal endocrine metabolites for all females of the troop for both seasons, therefore our analysis is limited to two to three samples from each of these seven lactating females from the outdoor enclosure during the birth season ($N = 19$ samples). In addition, we collected five samples from one female who was transferred to an individual cage during the winter (two samples in the outdoor enclosure, one sample at 10, 24, and 31 days after she was moved to a single cage). Because this female was a special case, we did not include her data in the statistical analyses. Fecal samples were stored in labeled plastic bags and frozen at $-20°C$ approximately 1–3 h after collection. Then we heated approximately 2 g of each fecal sample in an electric oven. Dried samples were transferred to labeled Ziplock® plastic bags, and then frozen until extraction.

Behavioral Observations

Behavioral observations were conducted only with mature females (over 5 years old, $N = 14$) from the outdoor Wakasa-5 troop using focal animal and ad libitum sampling [Martin & Bateson, 1993], over the mating season (October to December). We recorded agonistic interactions (e.g., avoidances, supplantations, threats, chases, and bites) on an ad libitum basis. The dominance rank was established by the outcomes of agonistic and approach–avoidance interactions between two females using both focal and ad libitum data [Garcia et al., 2009]. The linearity of the dominance rank of the whole Wakasa-5 troop was tested by a randomization test developed by de Vries [1995].

Hormonal Assay

All samples were extracted and analyzed by enzyme immunoassay using commercially available kits for DHEAS and cortisol.

For DHEAS, we used a DHEAS ELISA kit developed for human serum (ALPCO Diagnostics, Salem, NH). The details of the assay methods have been previously described and validated for measurement of fecal DHEAS in Japanese macaques [Takeshita et al., 2013]. Standards, internal controls and fecal extracts were assayed in duplicate. The sensitivity of the kit is 0.005 μg/ml. The mean intra-assay coefficient of variation was 11.3% ($N = 40$), and the mean inter-assay coefficient of variation was 15.5% ($N = 4$).

For glucocorticoid measurements, we used an ELISA kit developed for quantitative analysis of cortisol concentrations in biological fluids (Oxford Biomedical Research, Oxford, USA). The assay methods were conducted as previously described [MacIntosh et al., 2012] with minor modifications. Briefly, 0.25 g of dried feces were diluted in 2.5 ml of extraction buffer (0.1 M, pH 7.0, 0.1% BSA with 0.05% Tween-20), homogenized for 24 h, and centrifuged (3,000 rpm, 0°C, 10 min). The supernatant of each sample was transferred to clean tubes and assayed in duplicate, following the instructions of the supplier. We used a curve-fitting program (LS-PLATE Manager 2004, Wako Pure Chemical Industries Ltd., Osaka, Japan) to calculate hormonal concentrations. The sensitivity of the kit is 0.04 ng/ml. Samples of the same individual were assayed in the same plate. The mean intra-assay coefficient of variation was 5.1% ($N = 40$) and the mean inter-assay coefficient of variation was 8.1% ($N = 3$).

Statistical Analysis

To test the significance of environmental and biological factors, we built two general linear mixed-effects model (GLMER using *lme4* package in R software (version 2.15.3)). The models were chosen based on Akaike Information Criterion (AIC), starting with all fixed factors, interactions, and random effects "monkey ID," "collection date," and "troop." The model that minimizes the AIC was chosen as the best [Burnham & Anderson, 2002]. To test age and housing condition, we included the main factors age, condition, and interaction, using samples from all individuals during the mating season. To examine seasonal influences, only mature females in the outdoor condition were included (10 females in the mating season and seven females in the birth season), because the indoor subjects were not exposed to drastic climatic differences over time. We constructed a GLMER model with season and age as fixed factors with no interaction. In addition, to investigate the direct influence of daily temperature on hormonal levels without reproductive state effect, we built two

GLMER models based on samples on the adult females in the mating season ($N = 10$). The first model includes fGC as dependent factor, and temperature as fixed factor. The second model includes fecal DHEAS as dependent factor, and age and temperature as fixed factors. Because the time lag between adrenal hormonal secretion and excretion in the feces in Japanese macaques is from 24 to 48 hr [Shimizu, 2005], in this model we used the average of the lowest daily temperature of the 2 days before each sample collection date. Due to non-normality, we excluded outliers as defined by Leys et al. [2013] and log-transformed the data prior to the analysis. In total, 14 samples were removed from the analysis. Most of the females that had outliers were housed indoors (8/14). Therefore, we had 80 data points for the first model (age and housing condition), 33 data points for the second model (season), and 19 data points for the third model (temperature).

In order to investigate possible influences of dominance hierarchy in adrenal hormones, we calculated the average of the samples per individual collected during the mating season and performed Spearman's rank correlation test between hormonal concentrations and relative rank position.

We report estimate $\pm$ SE for GLMER. Measures of central tendency report the mean $\pm$ SD. Sample sizes vary in the text depending upon the independent factor(s) analyzed for specific questions. For example, assessing the impact of age on endocrine concentrations included all females, while addressing the influence of dominance rank on endocrine concentrations only incorporated adult females. Figures show the original data, instead of log-transformed data. We tested for statistical significance using two-tailed tests, with an alpha level of $P < 0.05$.

RESULTS

Housing Condition

We compared fecal DHEAS and fGC between single caged individuals ($N = 12$) and socially living animals ($N = 25$). There was a significant housing condition effect for both fecal DHEAS (GLMER: $-1.25 \pm$ SE 0.34, $Z = -3.70$, $P < 0.001$) and fGC (GLMER: $-0.67 \pm$ SE 0.11, $Z = -6.17$, $P < 0.001$; see Fig. 1), with higher hormonal concentrations in females housed indoor in single cages. On the other hand, the fGC/DHEAS ratio did not differ between indoor ($11.09 \pm$ SD $18.77 \mu g/g$) and outdoor ($7.38 \pm$ SD $7.94 \mu g/g$; GLMER: $0.15 \pm$ SE 0.23, $Z = 0.67$, $P = 0.5$) conditions. Hormonal changes for the female who was transferred from the enclosure to a single cage are illustrated in Figure 2.

Season

The relationship between hormone concentrations and season is illustrated in Figure 3. fGC levels
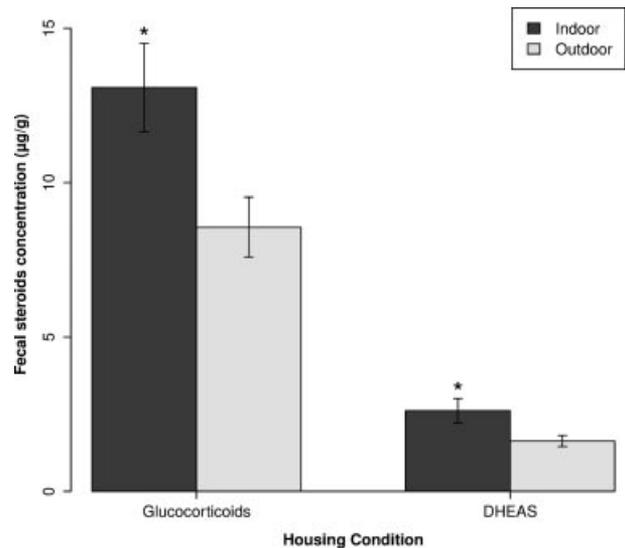

Fig. 1. Comparison of fecal glucocorticoids and fecal DHEAS concentrations among female Japanese macaques housed in indoor single cages ($N = 12$) and outdoor social groups ($N = 25$). *$P < 0.001$.

were significantly higher in cycling females during the mating season ($N = 10$) compared to lactating females in the birth season ($N = 7$) (GLMER: $-0.43 \pm$ SE 0.17, $Z = -2.52$, $P = 0.01$), but there was no significant difference in fecal DHEAS concentrations (GLMER: $0.03 \pm$ SE 0.17, $Z = 0.16$, $P = 0.9$) between seasons. Although reproductive hormones have been demonstrated to influence both GC and DHEAS, cold temperature stress has also been associated with
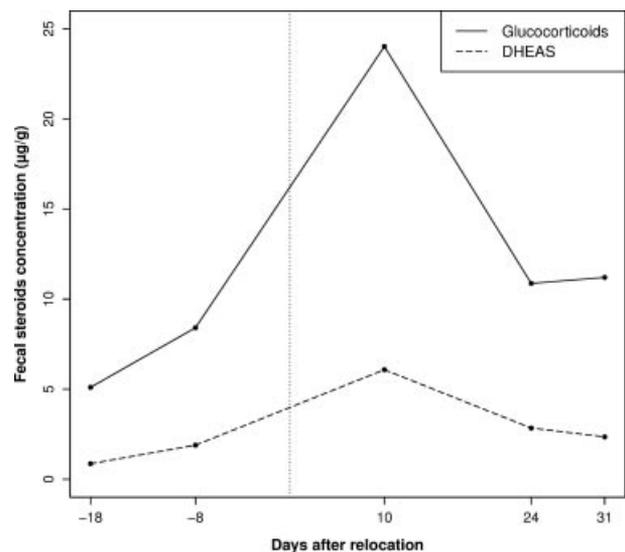

Fig. 2. Fecal glucocorticoids and fecal DHEAS metabolites changes in a 24-year-old female Japanese macaque with relocation. The vertical dotted line splits the graph in the two conditions. Left side represents housing in outdoor social groups and the right side represents the indoor single cage condition.
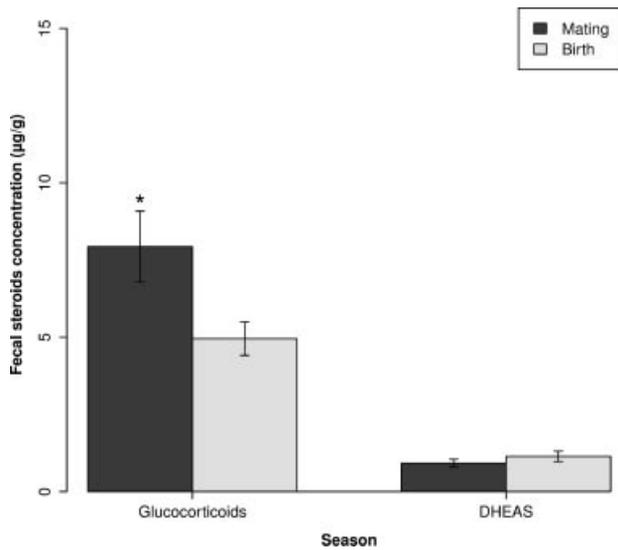
Fig. 3. Comparison of fecal glucocorticoids and fecal DHEAS concentrations among adult female Japanese macaques during the mating season ($N = 10$) and birth season ($N = 7$). *$P < 0.01$.

changes in stress hormone concentrations, so we examined the influence of cold temperature on both fGC and fecal DHEAS levels. Daily temperature had a significant negative impact on fGC (GLMER: $-0.3637 \pm$ SE 0.1706, $Z = -2.132$, $P = 0.033$), but not on fecal DHEAS (GLMER: 0.12035 $\pm$ SE 0.27598, $Z = 0.436$, $P = 0.663$), indicating that the lower the temperature, the higher the fGC levels (Fig. 4).
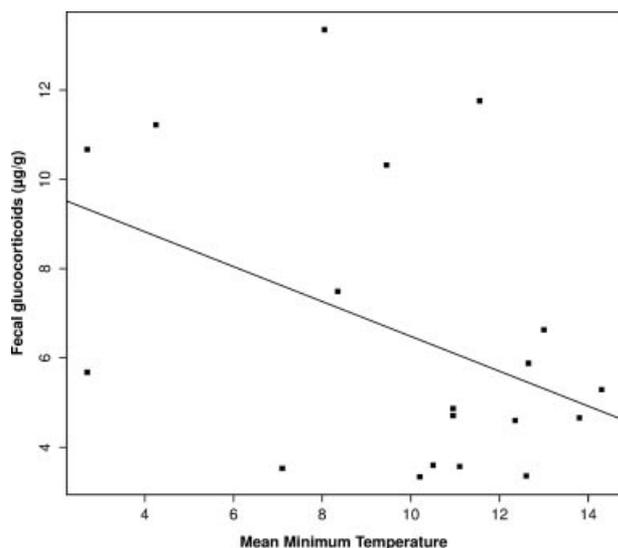

Fig. 4. Correlation between fecal glucocorticoid levels and atmospheric temperature among female Japanese macaques during the mating season ($N = 10$). Statistics were calculated based upon an average 2-day lag time between cold temperature readings and circulating hormone concentrations.

Chronological Age

Figure 5 shows that both adrenal steroids were negatively correlated with age ($N = 37$) (GLMER: fecal DHEAS: $-0.08 \pm$ SE 0.02, $Z = -4.61$, $P < 0.001$; fGC: -0.02 ± 0.01, $Z = -2.87$, $P = 0.004$), as well as the fGC/DHEAS ratio (GLMER: 0.04 ± 0.01, $Z = 3.01$, $P = 0.002$). There was an interaction between housing condition and age for fecal DHEAS (GLMER: $0.06 \pm$ SE 0.03, $Z = 2.066$, $P < 0.04$), suggesting that the correlation between age and fecal DHEAS was stronger in the indoor single cage condition.

Dominance Rank

The dominance hierarchy among all adult females housed in the Wakasa-5 troop was linear ($h' = 0.89$, $P < 0.001$, $N = 14$). However, due to logistical limitations, hormonal concentrations were measured for only seven mature females of this group. Nevertheless, there was no significant effect of relative rank order on either fGC (rho $= -0.32$, $P = 0.49$, $N = 7$) or fecal DHEAS concentrations (rho $= 0.43$, $P = 0.30$, $N = 7$) among adult female Japanese macaques. Dominance rank did not correlate significantly with rates of received aggression (rho $= 0.41$, $P = 0.30$, $N = 7$).

DISCUSSION
Housing Condition

The increased level of adrenal steroids observed in the indoor condition in our study contrasts somewhat with Muehlenbein et al. [2003], who reported no effect of cage size on either DHEAS or fGC in male rhesus monkeys (*Macaca mulatta*) and pig-tailed monkeys (*Macaca nemestrina*). However, the sample size reported in that study was small and not uniform across the three housing conditions (outdoor corrals, small outdoor enclosure, and individual cages), and the age-related hormonal changes might have masked the limited portion of the population sampled. Alternatively, species or gender differences may also explain differences in comparison with the previous report. In the current study, the higher levels of both fecal DHEAS and fGC observed in the indoor condition may be a reflection of stress caused by solitary housing, given the fact that this species naturally lives in social groups [Norikoshi & Koyama, 1975; Sugiyama, 1976]. Routine husbandry events (e.g., cage cleaning) and the periodic coming and going of caretakers in the room could also contribute to elevated stress hormones in monkeys housed in single cages [Honess & Marin, 2006]. The female who was transferred to an individual cage showed a drastic increase in both adrenal hormones 10 days after the transfer, which might indicate an acute stress response due to the relocation and separation from her social group. Moreover, even after a sharp reduction at day 24, fGC levels were still
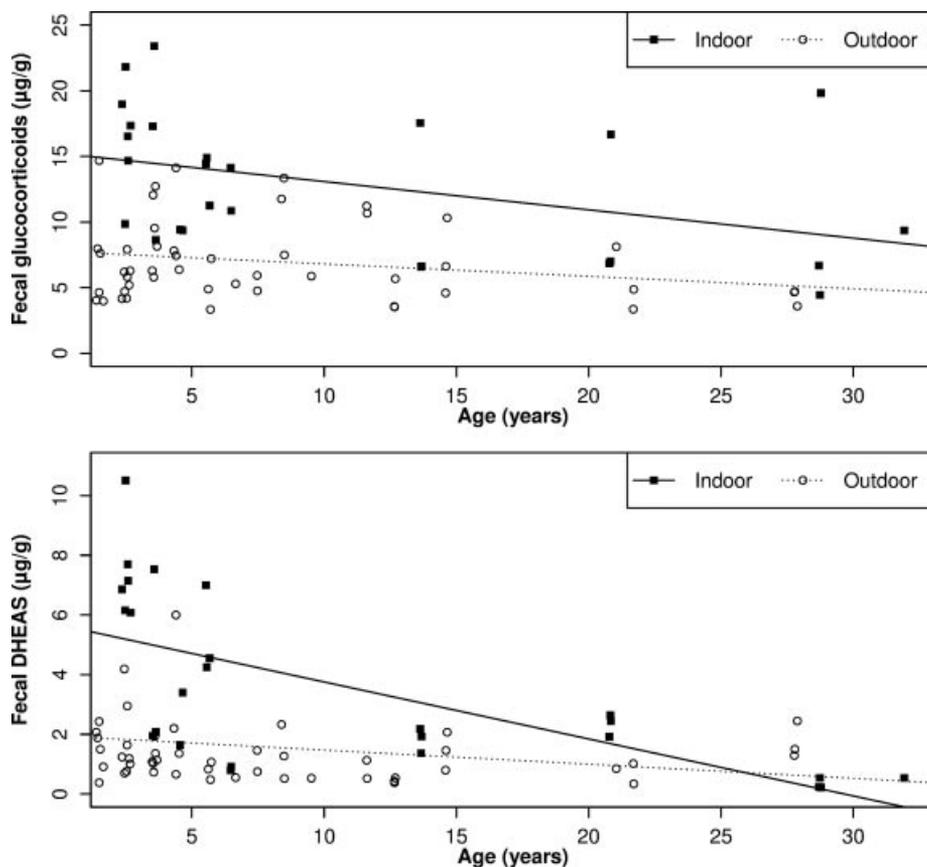
Fig. 5. Correlation between age and fecal glucocorticoids (top graph) and fecal DHEAS (bottom graph) concentrations among female Japanese macaques in an indoor (solid line) and outdoor (dashed line) conditions ($N = 37$). Note that the *y*-axis units are not identical between graphs.

elevated in comparison to the outdoor condition, suggesting an adrenal response in the resistance phase of stress [Selye, 1976]. Our results are in agreement with previous suggestions that solitary housing is stressful and that familiar companions ameliorate physiological stress responses [Coelho et al., 1991; Schapiro et al., 1993], reduce risk of diseases such as atherogenesis [Shively et al., 1989], and enhance recovery from distress [Gust et al., 1993]. A study with socially living rhesus monkeys showed that animals housed in outdoor enclosures have better coat quality than animals living in indoor enclosures [Steinmetz et al., 2006]. Schapiro et al. [1993] found that rhesus monkeys individually caged indoors show higher cortisol than those living in outdoor single cages, suggesting that indoor housing by itself has a negative influence on stress and it might have contributed to the high adrenal hormonal concentration in the single caged females.

Other factors that have been reported to account for fGC variation are body mass [Epel et al., 2000; Mustoe et al., 2012; Stalder et al., 2012], temperature [Huber et al., 2003; Weingrill et al., 2004], and physical activity [Girard & Garland, 2002; Rojas

Vega et al., 2006]. In our study, though, body weight was not correlated with hormonal concentrations. Temperature was negatively associated with fGC, as in previous studies [Huber et al., 2003; Weingrill et al., 2004]. Physical exercise also has been reported to increase GC levels. However, the social group was more able to do physical exercise than the individually caged females, and they were exposed to a lower temperature than the indoor group. Thus, the higher adrenal hormone levels in the indoor single caged females imply that housing condition might have a stronger impact over temperature and physical activity in Japanese macaques.

In humans, the relationship between DHEA and/or DHEAS and stress is inconsistent. Ritsner et al. [2004] found an elevation of the cortisol/DHEA ratio in schizophrenia patients, and Cruess et al. [1999] reported a cortisol/DHEAS increase in immunosuppressed men. Du et al. [2011], on the other hand, did not find an association between cortisol/DHEA and stress in bus drivers, although both DHEA and cortisol were significantly higher in stressed individuals. Similarly, in our study, fGC/DHEAS did not differ between housing conditions, despite the

significance found for both hormones separately. Possibly, the imbalance between those steroids occurs only under chronic stress conditions.

In nonhuman primates, Goncharova et al. [2010] found an increased cortisol/DHEAS ratio in depressed rhesus monkeys. A recent study also demonstrated that chronic moderate stress stimulated the production of DHEAS and reduced corticosteroid imbalance in old rhesus monkeys [Goncharova et al., 2012]. We suggest that initially, the body's response to stress is to increase production of both GC and DHEAS. When the stressor is removed, GC and DHEAS return to homeostasis. In the resistance phase of stress, both hormones are elevated, but the relative balance between the two steroids is maintained, unlike in the chronic phase [Selye, 1976]. Maninger et al. [2010] reported an increase in DHEAS concentrations in rhesus monkeys in response to both acute and chronic stress, but GC increased only during acute stress episodes. These findings may indicate that, under prolonged stress, the body secretes greater amounts of DHEAS and less GC, creating an imbalance in the ratio. Thus, either DHEAS or GC seems to increase in response to stress, but GC/DHEAS might be a possible indicator of chronic stress in primates.

Seasonal Impact

Our findings showed that season did not affect fecal DHEAS levels, in agreement with our previous study [Takeshita et al., 2013], although in some captive male squirrel monkeys (*Saimiri boliviensis boliviensis*) [Wiebe et al., 1988] and lemurs (*Microcebus murinus*) [Perret & Aujard, 2005], DHEAS levels appear to be elevated during the breeding (mating) season.

On the other hand, we found that cycling females (mating season) had the highest fGC concentrations, in comparison to lactating females in the birth season. These findings differ from previous studies in free-ranging rhesus monkeys from Cayo Santiago in that cortisol levels were significantly higher for lactating females than for cycling females [Hoffman et al., 2010; Maestripieri et al., 2008]. However, the daily temperature at Cayo Santiago does not change over the year [Rawlins & Kessler, 1985]. Unlike the previous studies in rhesus monkeys, our study shows that the daily temperature varied significantly between mating season and birth season and it seems to have a direct impact on fGC concentration. Another study in Japanese macaques demonstrated that lactating Japanese macaques had higher cortisol levels than non-lactating females [Kondo et al., 2003]. However, in that study, the samples for the lactating females were collected during the mating season, while the control group had samples taken during the birth season. Japanese macaques experience a mean minimum temperature of 9°C during the mating season, in contrast with an average minimum of 18°C during the birth season. Thus, the increased fGC levels during the winter mating season may reflect a response to cold stress. Our findings on the influence of temperature on fGC levels support this hypothesis. In female baboons (*P. h. ursinus*) [Weingrill et al., 2004] and in male and female red deer (*Cervus elaphus*) [Huber et al., 2003], minimum ambient temperature also had a strong effect on fecal cortisol levels, independent rank of reproductive state. Girard-Buttoz et al. [2009], in contrast, did not find cortisol variation between seasons in male long-tailed macaques (*Macaca fascicularis*), but there was no seasonal variation in temperature at the study site.

Chronological Age

The age effect on fecal DHEAS and fGC presented here is consistent with our previous study in female Japanese macaques [Takeshita et al., 2013]. In addition, we found that the age-related decline in DHEAS was stronger in the individuals housed indoor in single cages compared to those housed outdoor in social groups. Possibly, DHEAS is more strongly correlated with age under controlled, individually caged living conditions. DHEAS may not be a reliable biomarker of chronological age in a population living in social groups if concentrations of this adrenal steroid are impacted by social or environmental factors. A negative association between DHEAS and age has been reported from blood samples of rhesus monkeys [Downs et al., 2008; Kemnitz et al., 2000; Lane et al., 1997; Muehlenbein et al., 2003], baboons [Muehlenbein et al., 2003], and lemurs [Perret & Aujard, 2005]. An age-related decrease in fecal DHEAS has also been shown in wild chimpanzees (*Pan troglodytes schweinfurthii*) [Seraphin et al., 2008].

Erwin et al. [2004] found an age-related increase in basal plasma cortisol levels of fasted rhesus monkeys, but an inverse relationship in diabetic monkeys. In the current study, the amount of variance in fGC that was explained by age was small. Although our data show a stronger age effect on fecal DHEAS than fGC levels, neither can be considered as a reliable overall biomarker of chronological age.

Dominance Rank

In our study, dominance rank among females was not significantly correlated to either fGC or fecal DHEAS levels, nor were rates of received aggression associated with position in the dominance hierarchy. Many studies, however, have demonstrated an association between hypercortisolism and social subordination [Sapolsky et al., 1997; Virgin & Sapolsky, 1997], while others have shown that higher levels of cortisol occurred in dominant individuals [Muller & Wrangham, 2004]. Hoffman et al. [2010],

on the other hand, did not find any association between cortisol and social rank in female rhesus monkeys. Abbott et al. [2003] reported that hyper-cortisolism occurs among subordinate animals only in species in which low-rank status carries the highest rates of physical and/or psychological stressors, with few sources of social support, and in species that display high rates of aggression or subordination. Our findings conform to the pattern of no association between adrenal steroid concentrations and dominance rank under conditions of social stability. However, we cannot totally out rule the fact that our small sample size influenced this result. The association between social status and DHEAS concentrations in nonhuman primates is yet to be elucidated, but one study, on wintering geese (*Branta bernicla bernicla*), showed no obvious relationship connecting social status with DHEAS [Poisbleau et al., 2009].

In conclusion, we found that both fecal DHEAS and fGC concentrations were significantly higher in the indoor single cage condition, probably reflecting stress caused by a lack of social interactions, cage size or the lack of environmental variation as occurs outdoors. A negative association with age was found for both fecal DHEAS and fGC levels, and there was an interaction between age and housing condition for fecal DHEAS, but not for fGC. In the social outdoor groups, cycling females in the mating season showed higher fGC levels, than lactating females in the birth season, but fecal DHEAS did not differ across seasons. Similarly, temperature had a negative impact on fGC only. Dominance rank did not correlate with either fecal DHEAS or fGC concentrations. Our findings suggest that fecal DHEAS metabolites might be used along with fGC to measure the impact of environmental stress on primates. Additional studies of wild Japanese macaques and other species should give us more insights about the relationship between adrenal steroids and social factors in primates.

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