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Low rank and primiparity increase fecal glucocorticoid metabolites across gestation in wild geladas

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ABSTRACT

Integrative behavioral ecology requires accurate and non-invasive measures of hormone mediators for the study of wild animal populations. Biologically sensitive assay systems for the measurement of hormones and their metabolites need to be validated for the species and sample medium (e.g. urine, feces, saliva) of interest. Where more than one assay is available for hormone (metabolite) measurement, antibody selection is useful in identifying the assay that tracks changes in an individuaĺs endocrine activity best, i.e., the most biologically sensitive assay. This is particularly important when measuring how glucocorticoids (GCs) respond to the subtle, additive effects of acute stressors during a predictable metabolic challenge, such as gestation. Here, we validate a group-specific enzyme immunoassay, measuring immunoreactive 11β-hydroxyetiocholanolone, for use in a wild primate, geladas (*Theropithecus gelada*). This group-specific assay produced values correlated with those from a previously validated double-antibody, corticosterone ^{125}I radioimmunoassay. However, the results with the group-specific assay showed a stronger response to an ACTH challenge and identified greater variation in gelada immunoreactive fecal glucocorticoid metabolites (iGCMs) compared with the corticosterone assay, indicating a higher biological sensitivity for assessing adrenocortical activity. We then used the group-specific assay to: (1) determine the normative pattern of iGCM levels across gelada gestation, and (2) identify the ecological, social, and individual factors that influence GC output for pregnant females. Using a general additive mixed model, we found that higher iGCM levels were associated with low rank (compared to high rank) and first time mothers (compared to multiparous mothers). This study highlights the importance of assay selection and the efficacy of group-specific assays for hormonal research in non-invasively collected samples. Additionally, in geladas, our results identify some of the factors that increase GC output over and above the already-elevated GC concentrations associated with gestation. In the burgeoning field of maternal stress, these factors can be examined to identify the effects that GC elevations may have on offspring development.

1. Introduction

Often overly simplified as “stress hormones”, glucocorticoids are commonly used to monitor the impact of environmental and metabolic challenges on individuals (Keay et al., 2006). In response to challenging stimuli, vertebrates activate their hypothalamic-pituitaryadrenal (HPA) axis, stimulating the adrenal cortex to secrete glucocorticoids (GCs), a class of steroid hormones that increase with energetic challenges (Sapolsky et al., 2000). These hormones travel through the bloodstream and eventually filter into downstream excreta such as saliva, urine, or feces. In cases where hormone concentrations in these downstream products are proportional to concentrations in the bloodstream (Sheriff et al., 2010), these sources have provided researchers with a non-invasive window into the metabolic demands that accompany energetic challenges (Behringer and Deschner, 2017; Palme, 2019).

Because GCs modulate an organism’s energy balance from moment to moment, the term “metabolic hormones” (e.g., Dantzer et al., 2016) has been suggested as a more appropriate term than "stress hormones" (MacDougall-Shackleton et al., 2019). That is, the same physiological meditators produced by the HPA axis during a stress response also serve to mediate routine metabolic functions (e.g., digestion, migration, reproduction, and circadian/circannual rhythms (Romero et al., 2009; Wingfield, 2013)). More energy-demanding periods will be associated with higher levels of GC secretion than less energy-demanding periods –

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whether this constitutes an unexpected encounter with a predator or a routine annual migration event. Therefore, higher GC secretion should not necessarily be equated with a stress response (MacDougall-Shackleton et al., 2019).

Under this theoretical umbrella, variation in GC secretion across unexpected/acute challenges (e.g., a predator encounter) produces an elevation in GCs, termed "reactive homeostasis" (Romero et al., 2009), that constitutes the classic stress response, prioritizing the release of energy for immediate use to help the body cope with the stressor. Variation in GC secretion across predictable/long-term challenges (e.g., annual migration) also produces an elevation in GCs, termed "predictive homeostasis" (Romero et al., 2009), but this metabolic response is predictable, and the HPA axis should have evolved to easily incorporate these circadian or circannual elevations. For example, prior to departure for migration, red knots (*Calidris canutus*) have elevated concentrations of GCs (i.e., corticosterone) that prepare these birds for the behavioral and energetic challenges necessary to complete an arduous flight (Piersma et al., 2000).

One predictable challenge for mammalian females is gestation. Although there is species-specific variation in the amount of energy allocated across and within different pregnancies, gestation stimulates a predictable and necessary rise in GCs for mammalian females (Edwards and Boonstra, 2018; Saltzman and Maestripieri, 2011). Through anti-inflammatory actions, an elevation in GCs during early gestation helps prevent the mother's body from rejecting the embryo (Korgun et al., 2012). Moreover, during mid- to late-gestation, GCs are necessary for the proper development of the placenta, ensuring sufficient fetal placental blood flow (Jensen et al., 2004) and the maturation of numerous fetal organs, such as the gut, liver, and lungs (Korgun et al., 2012). In late stages of gestation, GCs help initiate parturition (Edwards and Boonstra, 2018) before quickly declining postpartum.

More important for ontogenetic and evolutionary studies, maternal GCs during gestation can have permanent organizational effects on offspring by altering their developmental trajectories. GCs are often an accurate predictor of energetic state (Sapolsky et al., 2000). Thus, a fetus can use maternal GCs to predict current (and possibly future) maternal energetic investment, allowing them to adjust their own energetic allocations through developmental plasticity (Sheriff et al., 2017). However, mothers may use signals, such as GCs, to decrease investment in their current offspring possibly to increase their future reproductive success (Kuijper and Johnstone, 2018; Lu et al., 2019; Wells, 2007). From rodents to humans, mothers with higher levels of GCs produce offspring that exhibit differences in their development and stress reactivity, including (1) low birth weight (laboratory rats: Belkacemi et al., 2011; Mairesse et al., 2007; humans: Thayer et al., 2012), (2) accelerated postnatal growth (North American red squirrels (*Tamiasciurus hudsonicus*): Dantzer et al., 2013; Assamese macaques (*Macaca assamensis*): Berghänel et al., 2016), and (3) greater HPA axis reactivity (laboratory rats: Tazumi et al., 2005; humans: Thayer and Kuzawa, 2015, 2014). Studies in wild populations indicate that the effects of maternal GCs may be adaptive for offspring, either in preparing them for the immediate external environment they will soon experience (e.g., "predictive adaptive response", PAR) (Gluckman et al., 2005) or in preparing them for a life-long reduction in somatic resources (Nettle and Bateson, 2015). Further tests of these maternal effects require that we first identify which offspring are exposed to higher-than-average GCs while still in utero.

To do this, we need to separate the rise in GCs due to predictive homeostasis from additional increases in GCs due to reactive homeostasis. It is established that gestation is a predictable metabolic challenge for female mammals (Crespi and Semeniuk, 2004), resulting in elevated levels of free GCs across pregnancy (Edwards and Boonstra, 2018). Further increases in GCs in response to less predictable challenges, such as ecological and social factors, can be subtle, especially when measured in non-invasive samples such as feces. Thus, to detect deviations in GC or GC metabolite (GCM) levels from the normative trajectory across gestation, it is essential to identify an assay system with a high biological sensitivity that enables smaller-scale and subtle differences in hormone (metabolite) concentrations to be detected (Braga Goncalves et al., 2016; Möstl et al., 2005; Palme, 2019; Shutt et al., 2012).

Here, we validate and investigate the detection of such subtle differences with a group-specific assay for use in a wild primate, geladas *(Theropithecus gelada)*. Non-human primates can help bridge the research on maternal stress in rodents and humans due to their long developmental periods, complex social groups, and close relatedness to humans. Additionally, primates face a variety of ecological and social challenges that have been found to increase GCs (Beehner and Bergman, 2017). In particular, geladas are an ideal primate taxon for research on maternal stress. First, geladas live in a highly-seasonal environment that can generate large seasonal differences in GCs. For example, geladas experience cold-stress (Beehner and McCann, 2008; Tinsley Johnson et al., 2018) and significant variation in food availability (Jarvey et al., 2018). Second, geladas live in complex, multilevel groups (Snyder-Mackler et al., 2012), with each core reproductive group (hereafter, "unit") experiencing a different set of social conditions that could affect maternal GC levels. Units vary in size from 1 to 12 adult females (Snyder-Mackler et al., 2012). Small and large groups are differentially exposed to male takeovers (i.e., the replacement of the dominant male in the unit by a new male, often resulting in infanticide (Beehner and Bergman, 2008) or spontaneous abortion (Roberts et al., 2012)), and females within each unit form strict dominance hierarchies that affect competitive behavioral interactions (le Roux et al., 2011). Third, the Simien Mountains Gelada Research Project (SMGRP) has more than a decade of long-term demographic, behavioral, and hormonal data from one gelada population, providing a longitudinal dataset for future investigations into maternal GC effects on offspring development.

In previous studies, differences in wild gelada fecal immunoreactive glucocorticoid metabolites (iGCMs) have been successfully quantified in response to temperature with a commercial double-antibody, corticosterone ^{125}I radioimmunoassay (RIA) (Beehner and McCann, 2008; Tinsley Johnson et al., 2018); however, this assay appears less ideal for investigating more subtle, small-scale differences in excreted gelada iGCMs. For comparison, in closely related baboons (*Papio* spp.) this corticosterone assay indicated iGCM levels ranging from 28-143 ng/g in response to seasonal changes (Gesquiere et al., 2011; Gesquire *pers. comm.*) and from 45-89 ng/g across gestation (Beehner et al., 2006). By contrast, in geladas, the same assay indicated iGCM levels ranging from 30-50n/g in response to seasonal changes (Beehner and McCann, 2008) and from 20-35 ng/g across gestation. In other words, baboon iGCMs detected by the corticosterone antibody exhibit a much greater response to a well-known environmental stressor and the energetic challenge of gestation, compared to geladas.

While this comparison may reflect true species differences in the magnitude of the stress response to the same type of challenge between geladas and baboons, it may also indicate that, in geladas, the corticosterone assay has limited biological sensitivity for tracking changes in adreonocortical activity via fecal iGCM measurements. This may be because assays principally designed to measure glucocorticoids in blood (which the corticosterone RIA has been developed for) are specific for the bioactive hormone(s) (e.g., cortisol and corticosterone) and show usually only a limited degree of cross-reactivity with their metabolites found in excreta (Palme, 2019; Möstl et al., 2005). To overcome this limitation, researchers have developedso-called group-specific assays, specifically designed to measure groups of excreted glucocorticoid metabolites (Möstl et al., 2005; Möstl and Palme, 2002). These assays detect a variety of fecal GC metabolites which usually lead to a stronger signal and thus to enhanced biological sensitivity compared to more specific assays (e.g., Bashaw et al., 2016; Braga Goncalves et al., 2016; Shutt et al., 2012). Although the corticosterone RIA previously used for geladas (see above) has also been successfully applied to measure fecal

iGCMs in numerous other species (e.g., Wasser et al., 2000) and may thus have some group-specific properties, it is assumed to have low cross-reactivities with 5α/5ß-reduced metabolites of cortisol (Möstl et al., 2005), thus likely reducing its efficacy as a group-specific assay for many mammal species in which cortisol is the primary GC secreted by the adrenal gland.

We therefore aimed to determine if a group-specific enzyme immunoassay (EIA) measuring immunoreactive 11β-hydroxyetiocholanolone was successful in detecting subtle differences in gelada iGCMs. This assay was first used to quantify iGCMs of graylag geese (Frigerio et al., 2004) and provides a group-specific measurement of 5-reduced 3α,11ß-dihydroxylated cortisol metabolites which are abundant in the feces of vertebrates (Heistermann et al., 2006; Möstl and Palme, 2002). Measurement of these metabolites has been validated in a variety of taxa (Braga Goncalves et al., 2016; Ganswindt et al., 2003; Palme and Möstl, 1997), including all major primate groups (i.e., lemurs: Fichtel et al., 2007; Hämäläinen et al., 2014; platyrrhines (New World monkeys): Rimbach et al., 2013; Wheeler et al., 2013; Cercopithecoid monkeys: Heistermann et al., 2006; and great apes: Shutt et al., 2012; Weingrill et al., 2011) using ACTH challenge tests and/or responses to natural or experimental challenges.

We first validated the aforementioned EIA using fecal samples collected during an ACTH challenge conducted in 2005 (Beehner and McCann, 2008). During an ACTH challenge, ACTH (adrenocorticotropic hormone) is injected into a subject, stimulating the production of glucocorticoids from the adrenal cortex (Wasser et al., 2000). Thus, an animal injected with ACTH produces high levels of GCs in the blood, which should be reflected by high iGCM concentrations in excreta. Gelada fecal samples were collected post-injection to determine peak iGCM levels in response to the ACTH challenge. We assayed these samples in 2018 with both the group-specific EIA and the corticosterone RIA to compare the biological sensitivity of the two assays, predicting that the group-specific assay would reveal a greater percent increase from baseline to peak iGCMs. We then performed both assays to measure iGCMs in samples collected before and after conception in wild geladas, again predicting that the group-specific assay would reveal a greater increase post-conception.

After successful biological and analytical validation of the group-specific assay for assessing iGCMs in gelada feces, we used it to establish the normative profile for iGCMs across gelada gestation for the wild population. We then examined several ecological, social, and individual factors that could further increase iGCMs across gestation. Because geladas live in a highly seasonal environment but give birth throughout the year with only a moderate birth peak (Tinsley Johnson et al., 2018), we hypothesized that food availability and temperature will affect GCs during pregnancy. Specifically, we predicted that low green grass availability (as predicted by rainfall (Jarvey et al., 2018)) will result in higher iGCMs and that low temperatures will result in higher iGCMs.

In regard to social factors, we predicted that during gestation, low-ranking (compared to high-ranking) females will have higher iGCM concentrations due to increased levels of received aggression. Across reproductive states, social rank has been associated with iGCMs in various primates, though often with mixed results (reviewed in: Beehner and Bergman, 2017). For example, in wild chacma baboons (*Papio ursinus*), low-ranking and socially-isolated females had the highest iGCM levels (Engh et al., 2006). However, in this same study, during periods of rank instability, high-ranking females experienced increased iGCM levels (Engh et al., 2006).

Additionally, we predict that females in large and small units (compared to mid-sized units) will have higher iGCM concentrations during gestation because of increased feeding competition (for large units) and increased infanticide risk (for small units). In general, the relationship between GCs and group size across primates has been mixed. However, there is evidence that larger groups experience greater feeding competition (Janson and Goldsmith, 1995), even in folivorous species (Chapman and Chapman, 2000), and smaller groups tend to be more vulnerable to social factors such as infanticide (Chapman and Pavelka, 2005) and predation risk (Janson and Goldsmith, 1995). In captive rhesus macaques (*Macaca mulatta*), increasing population density correlated with increasing GCs (Dettmer et al., 2014), but in wild sifakas (*Propithecus verreauxi*) and red colobus (*Procolobus rufomitratus*), there was no relationship between group size and iGCMs (Rudolph et al., 2019; Snaith et al., 2008).

Lastly, individual characteristics of each mother could influence their GC secretion. We predict that first-time (primiparous) mothers (compared to experienced, multiparous mothers) and mothers that give birth to male offspring (compared to female offspring) will have higher iGCMs during gestation. Reproduction may be more taxing to new mothers, as they may not have reached their full adult body size (Mas-Rivera and Bercovitch, 2008); thus, they have fewer fat reserves and are fueling their own growth and gestation at the same time. Male primates tend to be born at heavier birth weights than females, particularly in sexually dimorphic species (Smith and Leigh, 1998), potentially incurring higher energetic costs in male-carrying mothers.

2. Materials and methods

2.1. Study site and subjects

2.1.1. Captive geladas

Data for the hormone validation were collected from three zoo-housed geladas at the Bronx Zoo (1 male, 2 females). An ACTH challenge had been administered to these captive geladas in 2005 as part of an earlier iGCM validation (Beehner and McCann, 2008). Fecal samples from this validation remained frozen (−20 °C) for the past 14 years. We thawed these samples to conduct the iGCM validation presented here (see 2.2), assaying them in 2018 with both the new group-specific 11ß-hydroxyetiocholanolone assay and with the previously validated corticosterone assay.

2.1.2. Wild geladas

Data for assessing GC output during gelada gestation were collected from a population of wild geladas living in the Simien Mountains National Park of Ethiopia (13°15′N, 38°00′E, elevation 3250 m a.s.l) from 2007 to 2014. We used fecal samples collected from 46 adult females from 20 different reproductive units across a total of 57 successful pregnancies for this analysis (see below). All subjects were individually recognized and fully habituated to observers on foot.

2.2. Validation of the 11ß-hydroxyetiocholanolone assay

2.2.1. Sample collection, extraction, and storage

Samples from captive and wild geladas were processed the same way. In brief, we collected fecal samples opportunistically from known individuals within minutes of defecation. Sample collection, extraction, and storage all followed protocols previously validated for use in geladas (Beehner and McCann, 2008). Specifically, approximately 0.5 g of wet feces was added to 3.0 ml of a methanol (MeOH):acetone solution (4:1, v:v) and homogenized for 1 min using a battery-powered vortexer.

Subsequently, we extracted hormones from fecal samples by filtering 2.5 ml of the fecal homogenate through a 0.2 μm polytetrafluoroethylene (PTFE) syringeless filter, and the filter was subsequently washed with an additional 0.7 ml of MeOH:acetone (4:1). We then added 7 ml of distilled water to the filtered homogenate, capped and mixed the solution. We loaded the aqueous extract onto a reverse-phase C_{18} solid-phase extraction cartridge (Sep-Pak Plus, Waters Corporation, Milford, MA). Prior to loading, we prepped Sep-Pak cartridges according to the manufacturer's instructions (with 2 ml methanol followed by 5 ml distilled water). After loading, we washed the cartridge with 2 ml of a sodium azide solution (0.1%). We then stored all cartridges in separate sealed bags containing ~2 g of silica gel. Cartridges were allowed to dry for 3 days, then they were frozen at −20 °C until

shipment at room temperature to the University of Michigan where samples were again frozen and stored at −20 °C until hormone metabolites were eluted from the cartridge. For elution, we thawed cartridges and then used 2.5 ml of 100% MeOH to elute hormone metabolites from the cartriges. All eluates were then stored at −20 °C until immunoassay analysis. Fecal hormone values are expressed as ng/g of dry feces with the exact weight of dry fecal matter determined using a portable scale (to ± 0.001 g).

2.2.2. 11β-Hydroxyetiocholanolone assay

We analyzed GCMs in our samples using a group-specific EIA for the measurement of immunoreactive 11β-hydroxyetiocholanolone (Frigerio et al., 2004). Briefly, samples (diluted 1:80 in assay buffer) and standards (range: 3.9 to 250.0 pg/well) were added to each plate in duplicate (50 µl/well), followed by the addition of 50 µl of biotin-labeled hormone and 50 µl of antibody to each well. Plates were incubated for at least 18 h at 4°C. Plates were then washed and 150 µl of streptavidin-peroxidase was added to each well, incubated for one hour, and after washing the plates again, 100 µl of TMB substrate solution was added to each well. The reaction was stopped after approximately 40 min with the addition of 50 µl of sulfuric acid to each well, and the plate was then read in a plate photometer at a wavelength of 450 nm. The 50% intercept was about 13 pg/well, the sensitivity was 1.9 pg/well (calculated as two standard deviations from the mean of the zeros (Möstl et al., 2005)), and cross-reactivities for this assay are presented elsewhere (Frigerio et al., 2004). All samples from the ACTH challenge in captivity were run in the same microtiter plate to avoid the effects of inter-assay variation.

2.2.2.1. Analytical validation. The group-specific 11ß-hydroxyetiocholanolone assay was validated in gelada samples with respect to parallelism, accuracy, and precision. First, we determined parallelism by modeling the percent binding from the concentrations of a serial dilution of a gelada fecal extract pool (mixed-sex) and of the assay standard curve. There was no significant interaction between the concentrations and the type of sample (fecal pool vs. standard) (ANOVA: $t = 0.030$, $p = 0.976$), indicating that the slopes of these lines are not significantly different. Second, we determined the accuracy of the assay by spiking each standard with a diluted aliquot of the gelada fecal extract mixed-sex pool. Mean recovery was 105.1 ± 5.4%, indicating accuracy of our fecal measurements. Lastly, we determined the precision of the assay using two methods: (1) we ran 4 different samples 4 times on the same plate (i.e., intraassay CV), and (2) we ran low (70% binding) and high concentration (15% binding) mixed-sex fecal extract pools on all plates (i.e., interassay CV). Our intraassay CV was 7.5%, while our interassay CV was 17.4% (low concentration pool) and 15.3% (high concentration pool) ($N = 20$ plates). Additionally, to control for a potential drift across the assay plates, high and low concentrated fecal extract pool controls were run twice for each plate (one set of duplicates at the start and one set at the end). Average intraassay CVs for these high and low concentration pools were 7.1% and 14.2%, respectively ($N = 20$ plates), indicating the absence of assay drift.

2.2.3. Biological validation

An ACTH challenge was conducted on three captive geladas at the Bronx Zoo (1 male, 2 females; Beehner and McCann, 2008) to validate the 11ß-hydroxyetiocholanolone EIA. On the day of injection, subjects were individually transferred to a "restraint" chute where the male received 27.6 IU and females received 18.4 IU (administered i.m.) of ACTH suspended in 16% gelatin to provide a prolonged adrenal corticoid release (H.P. ACTHAR gel; Questcor Pharmaceuticals, Inc., Union City, CA). Subjects were not anesthetized prior to injection and routinely enter the transfer chute as part of normal husbandry practices (Beehner and McCann, 2008). To document the peak in adrenocortical activity and return of iGCM concentrations to baseline, fecal samples were collected starting 16 h post-injection until 140 h post-injection. Unfortunately, fecal samples collected prior to injection did not have sufficient fecal matter left over from the previous study for extraction in the current study. Thus, for each animal we determined baseline iGCM levels using samples collected more than 40 h post-injection. Peak iGCM levels were recorded as the highest value during the first 40 h post-injection, based on the finding that in mammals, including primates, iGCM peak excretion following activation of the HPA axis usually occurs within the first two days (Fichtel et al., 2007; Heistermann et al., 2006; Wasser et al., 2000). On average, 7 fecal samples were collected from each individual (range: 6–10 samples; total: 22 samples).

2.2.4. RIA comparison

Previously, we had validated gelada iGCMs using a double-antibody, corticosterone ^{125}I radioimmunoassay (RIA) kit (MP Biomedicals, Orangeburg, NY), (Beehner and McCann, 2008). In order to assess whether the newly established group-specific 11ß-hydroxyetiocholanolone EIA showed a higher biological sensitivity for tracking iGCM changes compared to the previously used corticosterone RIA, we analyzed, in parallel, all samples in both the EIA and RIA. Based on the data produced, we then assessed whether the group-specific assay is more sensitive (i.e. shows a greater response) in picking up the predicted iGCM rise in response to the ACTH challenge and conception.

2.3. Analyzing iGCMs across gelada gestation

To establish the gestation period, we back-calculated from the day of birth. Previous research on this population of wild geladas estimated gestation to be 182 days (Roberts et al., 2017). Therefore, using existing demographic data on all adult females in the population, we estimated their date of conception as 182 days before parturition. We identified individuals with at least one fecal sample collected during the period of interest for this study: from 3 months prior to conception until 3 months post-parturition. Samples collected in the 3 months prior to conception were used to establish cycling iGCM values; samples collected during the 6 months of gestation were used to determine the normative pattern of iGCMs during pregnancy; and samples collected in the 3 months after parturition were used to identify the decrease in iGCMs after parturition.

A total of 535 samples were collected from 46 mothers across 57 successful pregnancies. On average, 9.4 ± 7.1 (range 1–30) samples were collected during each pregnancy. Samples were binned into 14-day periods starting from the date of conception (time 0). Each 14-day period contained samples from an average of 16 different pregnancies (range: 11–21). Because takeovers alter the reproduction of female geladas (Roberts et al., 2012), we did not include fecal samples from any female that experienced a takeover at any point from 6 months before conception until parturition.

2.4. Potential factors affecting iGCM output during gestation

Since GC output can be influenced by a variety of ecological, social and intrinsic individual factors (see below; Table 1), we examined their impact on iGCM levels in our pregnant females. Here, we used only samples collected between conception and parturition. For statistical models, a total of 275 samples were analyzed from 35 mothers across 44 pregnancies. On average, 6.3 ± 5.1 (range 1–22) samples were used from each pregnancy.

2.4.1. Ecological factors

Ecological conditions during gestation, such as cold temperatures (Beehner and McCann, 2008; Tinsley Johnson et al., 2018) or limited green grass availability, can impose higher metabolic costs in geladas. Previously, average minimum daily temperatures across the previous

Table 1
The potential factors affecting iGCMs during gestation and the number of pregnancies falling within each type of factor. *These factors were calculated for each pregnancy during each month. Rank and unit size sometimes changed over the course of pregnancy, which is why the total numbers add to more than 44 pregnancies.

Factor Type	Factor	Type	# of pregnancies
Ecological	Minimum temperature (°C)	Mean minimum daily temperature across the previous 30 days	44
	Rainfall (mm)	Cumulative precipitation across the previous 30 days	44
Social	Unit size*	Large	12
		Medium	14
		Small	21
	Dominance rank*	High	30
		Low	15
Individual	Parity	Primiparity	9
		Multiparity	35
	Fetal sex	Male	20
		Female	24

30 days reliably predicted gelada female iGCM concentrations, with low temperatures predicting higher iGCMs, indicating thermoregulatory costs (Tinsley Johnson et al., 2018). Green grass availability is significantly predicted by cumulative rainfall over the past 30 days (Jarvey et al., 2018), and while it was not a reliable predictor of gelada female iGCMs previously (Tinsley Johnson et al., 2018), pregnant females may be more vulnerable to food stress than all females taken together.

2.4.2. Social factors

Geladas live in reproductive units of various sizes (range 1–12 related, adult females (le Roux et al., 2011)). The majority of social interactions for females occur within the unit, including agonistic interactions that result in a linear dominance hierarchy (le Roux et al., 2011) and affiliative interactions that result in close female social bonds (Tinsley Johnson et al., 2014). As such, the number of female competitors or bond partners may have a strong influence on female GCs across pregnancy. We, therefore, considered the number of adult females in each unit as a potential factor affecting maternal iGCM output. As the number of adult females in a unit changes frequently due to maturations and deaths we binned units into group sizes to have more consistency across a period of time (i.e., gestation). Similar to previous analyses, units were binned into small (1–4 adult female), medium (5–7 adult females), and large (8+ adult females) (Tinsley Johnson, unpub. data, https://doi.org/10.1101/348383).

Dominance rank was assigned to each adult female using Elo-ratings (Albers and de Vries, 2001; Neumann et al., 2011), described specifically for geladas in Tinsley Johnson et al. (2014). Elo-ratings were calculated for each female, in each unit, during each month, and then converted to ordinal rank. Within each unit we then divided females evenly into high-ranking or low-ranking bins. If there was an uneven number of females, we arbitrarily put the middle female in the higher-ranking group.

There may be an interaction between unit size and dominance rank, such that being low-ranking in a small unit is different from being low-ranking in a large unit. However, we did not test for an interaction here due to sample size.

2.4.3. Individual factors

Primiparous mothers likely have worse energetic status given their young age and small size. In other primates, primiparous mothers exhibited higher GCs during lactation (Dettmer et al., 2015). In our dataset many females' exact parity was unknown, therefore, we divided females into primiparous or multiparous females to examine the effects of maternal experience on iGCM output.

Finally, we also examined whether fetal sex affected female GCM levels during gestation (Brown, 2001).

2.5. Data analyses

All analyses were conducted in R (R Core Team, 2017). First, we determined the relationship between iGCM values generated by the two assays, i.e., the group-specific 11ß-hydroxyetiocholanolone EIA and the corticosterone RIA, by using Spearman correlation tests for both the captive ACTH-challenge dataset and the wild gestation dataset.

Second, to examine the biological sensitivity of each assay we used Mann-Whitney U tests to compare baseline and peak iGCMs for the ACTH-challenge dataset and cycling and early gestation iGCMs for the wild gestation dataset (independent samples from 34 pregnancies). We repeated this comparison in 11 animals for which matched fecal samples were available during the cycling and early pregnancy stage, using the Wilcoxon signed rank test, on the iGCM values from the group-specific 11ß-hydroxyetiocholanolone EIA. We also compared the x-fold change from baseline to peak between the two assays in the ACTH-challenge dataset.

Third, using the group-specific 11ß-hydroxyetiocholanolone EIA, we established a normative pattern of iGCM levels across gelada gestation by grouping samples into 14-day periods. We first averaged iGCM concentrations within each pregnancy for each time period and then we calculated the mean and standard error across pregnancies for each time period.

2.5.1. Data imputation

Due to missing daily values of minimum temperature and rainfall, we could not calculate two predictor variables, mean minimum temperature and cumulative rainfall for the past 30 days, for 10% and 24% of data points, respectively. Rather than remove these days, which would reduce our gestation dataset and potentially introduce biases (especially as these data were missing at particular times of the year associated with holidays and excessive rainfall), we filled in the missing daily ecological values using multiple data imputation conducted in the R 'mice' package (van Buuren and Groothuis-Oudshoorn, 2011). Data imputation is a statistical procedure that predicts missing data points by using existing covariate and distributional information. Rather than imputing each data point once, multiple imputation selects a set of plausible values for each missing data point, creating multiple imputed datasets and thus ensuring that specific imputed values do not skew the analyses (Nakagawa and Freckleton, 2011).

We used the daily ecological dataset collected by the SMGRP from Jan 2006 to Aug 2019 to impute the missing data points. Daily minimum temperature was missing for 24% of days from Jan 2006 to Aug 2019. Minimum temperature (logged) was imputed via predictive mean matching, with the predictors year (factor), month (factor), and day (numeric). Due to the highly seasonal rains in the Simien Mountains National Park, zeros were inserted for all missing rainfall values during the dry season (Nov-Mar). In the wet season (Apr-Oct), a cumulative amount of rainfall is recorded when days are missed, so this amount was divided over the previous missing days (up to 7). After these manual adjustments, rainfall in the wet season from 2006 to 2019 was missing 7% of daily data points, and these were imputed via predictive mean matching with the predictors year (factor), month (factor), and day (numeric). For both daily minimum temperatures and daily rainfall amounts, data imputation was performed five times (the default of the 'mice' package), resulting in five datasets with imputed data (Schafer and Olsen, 1998). From these imputed datasets, we calculated mean minimum temperature and cumulative rainfall for the past 30 days for each of the data points in our existing dataset on maternal iGCMs during gestation. We thus had five gestation datasets, each with their own imputed data, on which to perform our analyses, as described below.

2.5.2. Modeling GCMs across gestation

To determine which factors best predicted iGCMs across gestation, we used General Additive Mixed Models (GAMMs) with the 'mgcv' package in R (Wood, 2004). These models allow us to flexibly model non-linear relationships. In this case, we know that there is a relationship between the time since conception and iGCM concentrations, but it is not necessarily linear. A GAMM combines multiple functions to create smooth terms that best fit the data. GAMMs can also test for the main effect of factors as well as interactions between factors and smooth terms. For example, rank may influence the relationship between iGCMs and time since conception.

We modeled log GCs across each of our five datasets (with the imputed ecological data). All models used a log-link function and included maternal ID and year of birth as random effects. Predictors included cumulative rainfall, mean minimum temperature, unit size, dominance rank, parity, and fetal sex. We created a full model including all predictors, a smooth function with days since conception, and interactions between the smooth function of days since conception and the categorical predictors named above. We then tested the full model against all combinations of simpler models (n = 324 models for each dataset) using an information criterion approach with the 'bbmle' package (Bolker and Development Core Team, 2019) specifically Akaike's Information Criterion (AICc) which penalizes for the number of parameters in the model (Anderson and Burnham, 2002).

Across all five datasets, the same three models came out on top, though none carried more than 14% of the AICc weight and together these three models did not carry more than 35% of the AICc weight. Because our objective here is to identify reliable predictors of GCs during gestation, we decided to conduct model averaging over all models within a cumulative weight of 95% (for each dataset (Nakagawa and Freckleton, 2011)). Model averaging minimizes the effect of uninformative parameters as those parameters receive a coefficient of 0 for each model in which they do not appear (Anderson and Burnham, 2002; Symonds and Moussalli, 2011). We used the 'Mu-MIn' package (Barton, 2019) in R to conduct model averaging and to calculate a sum of AICc weights for each predictor variable based on the models that included that predictor (Arnold, 2010). The averaged model provides more accurate estimates of a predictor's effect size while the sum of AICc weights indicates the probability that a predictor is included in the best model (Symonds and Moussalli, 2011).

After calculating an averaged model and the sum of weights for each predictor in each dataset, we pooled across the imputed datasets (Nakagawa and Freckleton, 2011) according to Rubin's rules (Rubin, 1987) which take into consideration the error due to imputed values. We also calculated 85% confidence intervals for each predictor's estimate (Arnold, 2010). These pooled results are presented below.

3. Results

3.1. EIA validation

The iGCM values that we generated with our previous corticosterone assay were significantly and moderately strongly correlated with the iGCM values obtained with the group-specific 11ß-hydroxyetiocholanolone EIA for both the ACTH-challenge dataset (Spearman correlation = 0.63, p = 0.002) and the wild gestation dataset (Spearman correlation = 0.32, p = < 0.001). However, the group-specific assay revealed on average about 50-fold higher absolute iGCM concentrations and greater variation across conditions with a larger increase in iGCMs for both datasets (Figs. 1 and 2). Specifically, in response to the ACTH challenge, the group specific assay exhibited an average 2.7-fold increase from baseline iGCMs to peak levels compared to only an average 1.7-fold increase measured for the corticosterone assay (Fig. 1). Importantly, the corticosterone measurement conducted here yielded a similar percent increase in iGCM values compared with the corticosterone analysis conducted in 2005 (Beehner and McCann, 2008), indicating no effect of storage time on the frozen fecal samples and their iGCMs. In the wild animal dataset, the group-specific assay revealed a significant increase of 32% from cycling to early gestation iGCM levels (Mann-Whitney *U* test, W = 333, p = 0.0023) while the corticosterone assay did not yield a significant difference (Mann-Whitney *U* test, W = 612, p = 0.683) and, if anything, exhibited a decrease in values (Fig. 2). For 11 individuals, matching samples collected during cycling and early gestation revealed the same result in the group-specific assay of higher iGCMs in early gestation (Wilcoxon signed rank test, V = 1, p = 0.002) as well as highlighting the variation between females in their GC output in response to conception (Fig. 2c).

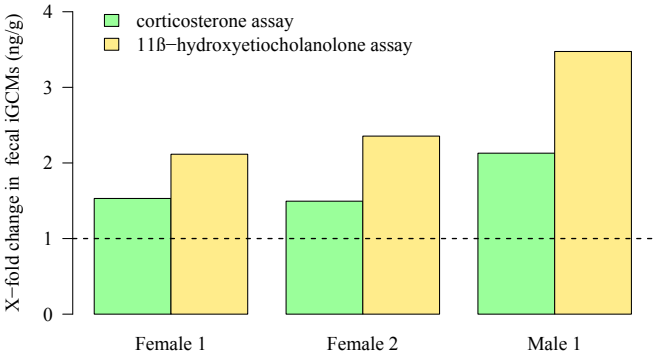

Fig. 1. X-fold change in iGCM concentrations from baseline to peak values for the exact same fecal samples per individual gelada experiencing an ACTH challenge. The green bars show the iGCM change measured by the corticosterone assay, the yellow bars show the iGCM change measured by the group-specific 11ß-hydroxyetiocholanolone assay. The horizontal dotted line shows the value equal to baseline (i.e., an X-fold change of 1).

Because the group-specific 11ß-hydroxyetiocholanolone assay (compared to the corticosterone assay) was found to have a greater ability to detect smaller-scale changes in gelada iGCMs, we only report results from the 11ß-hydroxyetiocholanolone assay for the remainder of the manuscript.

3.2. Normative patterns of iGCMs across gestation

A composite normative profile of iGCMs across gelada gestation demonstrates an increase in GCs immediately after conception with elevated levels being maintained for the first 10–12 weeks of gestation. In mid-gestation, iGCM levels decline slightly until increasing again shortly before parturition. iGCMs then decline within a month after birth to non-pregnant cycling levels (Fig. 3).

3.3. Factors affecting iGCMs across gestation

All five datasets had the same top 3 models. However, together these top models contributed less than 35% of AICc weight. Therefore, for each dataset, we calculated an averaged model over all models within a cumulative AICc weight of 95%. We then pooled the results of these averaged models from the five datasets.

The pooled model for predicting iGCMs during gestation included all predictors as independent main effects (Table 2). The sum of AICc weights for low rank, male fetus, and primiparity were above 70%, indicating that these predictors are highly likely to be included in the best model. Minimum temperature, rainfall, and unit size had low sums of AICc weights (< 25%), indicating that they were only included in unlikely models and thus less likely to be included in the best model.

According to the pooled model, low-ranking females exhibited 20% higher iGCMs during gestation compared to high-ranking females, and primiparous females had 13% higher iGCMs compared to multiparous females (Fig. 4; Table 2). However, the estimate for primiparity is less reliable as the 85% confidence interval overlaps 0 (Fig. 4). The

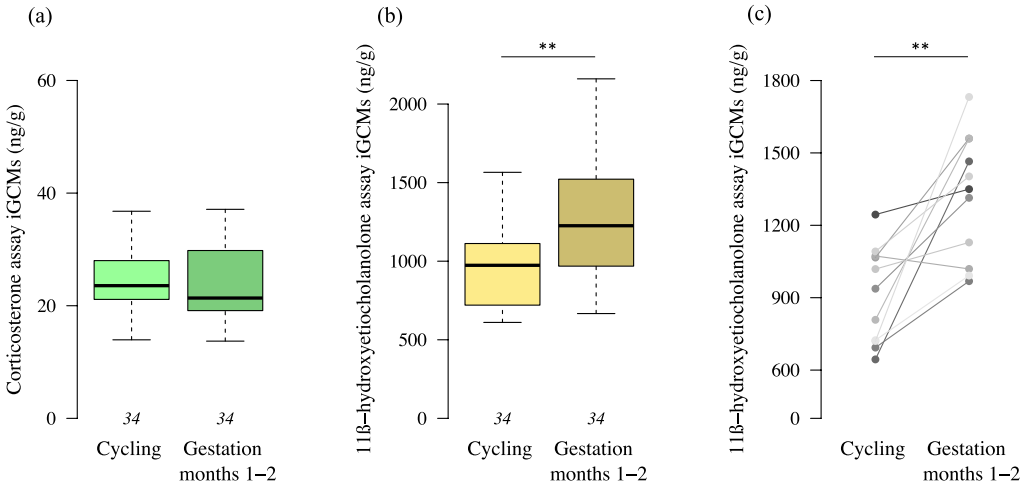

Fig. 2. Boxplot of iGCMs (ng/g) surrounding conception for successful pregnancies in wild geladas. Cycling iGCMs are from the 3 months prior to conception and gestation iGCMs are from the first 2 months of gestation. The italicized numbers below the plot indicate sample size (number of pregnancies). The first panel in green (a), indicates iGCMs measured with the corticosterone assay. No difference was detected between cycling and early gestation. The second panel in yellow (b), indicates iGCMs measured with the group-specific 11ß-hydroxyetiocholanolone assay. Here we see a significant increase from cycling to early gestation. The third panel (c) details matched iGCMs for females with samples from both cycling and early gestation (measured with the 11ß-hydroxyetiocholanolone assay) for 11 individuals (c). ** $p < 0.01$.

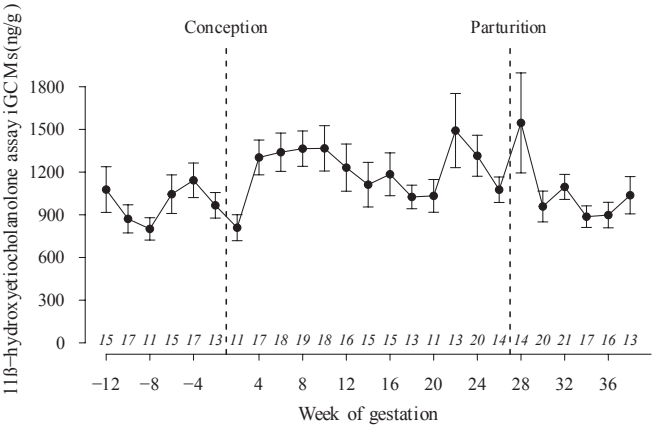

Fig. 3. Composite profile for iGCM concentrations (mean ± SEM) for successful pregnancies in geladas, starting 3 months prior to conception and extending 3 months postpartum. The dotted vertical lines indicate timing of conception and parturition. The italicized numbers indicate sample size (number of pregnancies contributing to the respective data point).

Table 2
Pooled model results for all main effects across the five datasets, including the average sum of AICc weights (SW). The last column reports the range of summed AICc weights across the five datasets. Variables are listed in order of decreasing importance (SW).

Main effects	Estimate	SE	Avg. SW	SW Range
Low rank	0.18	0.08	0.98	0.98–0.98
Male fetus	−0.02	0.09	0.84	0.84–0.85
Primiparity	0.12	0.09	0.71	0.71–0.75
Min temp	0.00	0.03	0.21	0.21–0.27
Rainfall	0.01	0.03	0.20	0.20–0.22
Medium unit	0.02	0.08	0.20	0.20–0.20
Large unit	0.00	0.09	0.20	0.20–0.20

estimates for minimum temperature, rainfall, unit size, and fetal sex all indicate small effect sizes and their 85% confidence intervals overlap 0, indicating their unreliability as predictors (Fig. 4).

The pooled model only included hierarchical rank, fetal sex, and parity as interactions with the smooth terms for days since conception (Table 3). The interactions with rank and fetal sex were highly likely (> 75%) to be included in the best model, while the interaction with parity was moderately likely to be included (48%).

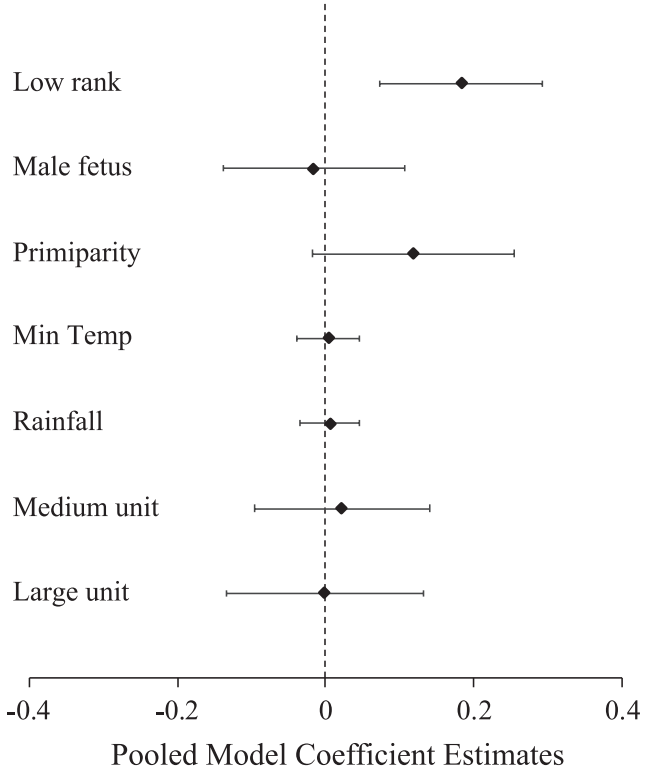

Fig. 4. A coefficient plot showing the size of the coefficient for each of the main effects in the pooled model (across the five datasets), and the 85% confidence interval for each estimate. Coefficients are on the log scale. Points falling to the right of the line indicate higher iGCMs while points falling to the left indicate lower iGCMs for that predictor.

Table 3
The interactions between smooth terms for the days since conception and factors included in the pooled model, with the average and range of summed AICc weights (SW) across the five datasets.

Smooth terms	Avg. SW	SW Range
s(Days since conception) × Rank	0.91	0.89–0.98
s(Days since conception) × Fetal sex	0.77	0.77–0.78
s(Days since conception) × Parity	0.48	0.46–0.51

4. Discussion

To guide future hypothesis testing in the burgeoning field of maternal stress, we need to be able to accurately measure subtle differences in GC output across individuals. Moreover, to really understand fitness outcomes for offspring exposed to different levels of maternal GC concentrations, these analyses need to be conducted in wild animal populations using non-invasive methods. Here, we have shown that a group-specific enzyme immunoassay for the measurement of cortisol metabolites with a 3α, 11β-dihydroxy structure is better at detecting differences in iGCM concentrations in gelada fecal samples than a previously used corticosterone assay (Beehner and McCann, 2008). This is shown in particular by a stronger iGCM response to the ACTH challenge measured by the group-specific assay, with the magnitude of the response being within the range of those reported for numerous other species (see e.g. Touma and Palme, 2005 for a review of ACTH studies). Our results thus confirm findings from previous studies on various other primate and non-primate species, demonstrating that a group-specific assay for the measurement of major cortisol metabolites has enhanced biological sensitivity for detecting changes in adrenocortical activity when compared to a more specific cortisol or corticosterone assay (e.g., Bashaw et al., 2016; Braga Goncalves et al., 2016; Shutt et al., 2012). This is because group-specific antibodies detect a variety of fecal GC metabolites compared to more specific antibodies designed to measure the bioactive circulating hormone with little cross-reactivity with other metabolites (Möstl et al., 2005; Palme, 2019).

Since the two assay systems compared here are likely measuring different GC metabolites, it is not completely surprising that the correlation between the two measurements for the gestation dataset was relatively low, though significant. This may be partly due to potentially different excretion delay times of the metabolites picked up by the two assays (e.g. Braga Goncalves et al., 2016). In addition, there is the potential that group-specific assays cross-react with gonadal metabolites (Möstl et al., 2005), mainly those derived from androgens (Ganswindt et al., 2003; Heistermann et al., 2006), which, if present, may affect comparability of iGCM measurements derived from different assays. Future characterization of the metabolites measured by the two assays would be needed to verify to what extent this might have been the case regarding our iGCM data sets.

Overall, the higher biological sensitivity of the group-specific 11β-hydroxyetiocholanolone EIA validated here enabled us to detect smaller-scale differences in GC output of our female geladas, making this assay superior over the more specific corticosterone RIA used previously for monitoring iGCM excretion in geladas (Beehner and McCann, 2008). Additionally, establishing an EIA system for measuring iGCMs is beneficial for other laboratories, as EIAs do not require sophisticated equipment and permits for handling radioactive material.

Using the newly validated 11ß-hydroxyetiocholanolone EIA, we characterized the normative pattern of GC output across gestation in a population of wild geladas. The iGCM pattern generated here for wild geladas revealed the typical mammalian pattern for GC output during gestation (e.g., Edwards and Boonstra, 2018), increasing after conception and peaking near parturition (Fig. 3). In primates, GC concentrations typically decline after parturition, though there is variation in how quickly they return to cycling levels (Saltzman and Maestripieri, 2011). Here, we found that gelada female iGCM concentrations decline to cycling levels by one month post-partum. In wild yellow baboons, iGCMs decline throughout lactation (Gesquiere et al., 2018), though it is unclear how quickly they return to cycling levels (Beehner et al., 2006). GCs also play a role in ovulation (Tetsuka, 2007) and have been found to fluctuate with the estrous cycle. For example, in Asian elephants circulating cortisol peaks just prior to ovulation and has low levels during the luteal phase (Fanson et al., 2014). Our data on iGCMs in female geladas during the 12 weeks prior to conception show considerable variation that may be cyclical, though it does not match with the known cycle length in this population of 33 days (Roberts et al., 2017). As we obtain more samples, we will be able to further investigate the fluctuations of iGCMs during the estrous cycle and continue to update their normative pattern across gelada gestation. In the future we will also examine differences between individuals in their GC output in response to conception (Fig. 2c) and other stressors, which may reflect adaptive versus non-adaptive GC responses (Cockrem, 2013) and contribute to variables related to offspring developmental plasticity (c.f. Berghänel et al., 2016).

Here we used GAMMs to determine which factors were associated with elevated iGCM levels in pregnant geladas. Our pooled model did not identify unit size as an informative predictor for maternal iGCM concentrations during gestation, supporting previous findings in other wild primates, though they did not consider gestation specifically (sifakas: Rudolph et al., 2019; red colobus: Snaith et al., 2008; geladas: (Tinsley Johnson, unpub. data, https://doi.org/10.1101/348383)). To our knowledge, only one primate study, on captive rhesus macaques, identified a positive relationship between group size and GCs, with adults in the high-density environment having hair cortisol levels over twice as high as adults in the low-density environment (Dettmer et al., 2014). With a larger dataset in the future, we will examine interactions between rank and group size (i.e., unit size) because these factors may have additive effects on maternal GCs. Although our pooled model identified fetal sex as an informative predictor due to its sum of AICc weights (Table 2), sex did not have a reliable or large effect on maternal iGCMs (Fig. 4). Geladas are sexually dimorphic and there is evidence that males grow faster in utero in some sexually dimorphic species (Brown, 2001). We do not have data on fetal growth in geladas, but data on post-birth growth indicate that gelada males do not grow faster than females, they simply grow for longer (Lu et al., 2016). If gelada males and females have similar growth rates in utero, that may explain why fetal sex was not a reliable predictor of maternal iGCMs.

Our pooled model indicated that low-ranking females exhibited iGCM concentrations significantly higher than those of high-ranking females (Table 2; Fig. 4) and that rank affected the pattern that iGCMs exhibited over the course of gestation (Table 3). This result makes sense for geladas both socially and ecologically. Low-ranking gelada females receive more aggression and are the victims of food theft, particularly during the dry season (Jarvey et al., 2016). Thus, both social and ecological stressors may be contributing to higher iGCMs in low-ranking geladas.

Many wild primate studies report no association between female dominance rank and GC concentrations (reviewed in: Beehner and Bergman, 2017). Of the studies that did find an association, all of them report low rank in females to be associated with higher GC concentrations (Beehner and Bergman, 2017; Cavigelli and Caruso, 2015), with the exception of ring-tailed lemurs (*Lemur catta*) (Cavigelli, 1999; Cavigelli et al., 2003), where the opposite was found. In particular, of the studies examining female dominance rank during energetically taxing periods (e.g., pregnancy and lactation), three found no effect on GCs (golden lion tamarins (*Leontopithecus rosalia*): Bales et al., 2005; mandrills (*Mandrillus sphinx*): Setchell et al., 2008; Assamese macaques: Berghänel et al., 2016), and five found the same result as ours – where low-ranking females exhibited higher GC levels in comparison to high-ranking females (chimpanzees (*Pan troglodytes*): Emery Thompson et al., 2010; Murray et al., 2018; blue monkeys (*Cercopithecus mitis*):

Foerster et al., 2011; mantled howler monkeys (*Alouatta palliata*): Gómez-Espinosa et al., 2014; rhesus macaques: Maestripieri and Georgiev, 2016). In ring-tailed lemurs, high rank is thought to be associated with higher GCs due to the amount of aggression high-ranking females engage in Cavigelli (1999). The lack of an effect of rank in tamarins and mandrills was attributed to the relatively low rates of aggression and high degrees of social support in these species. In chimpanzees and howlers, agonistic interactions best predicted GC levels, whereas in blue monkeys GCs were predicted by food accessibility. Whether low rank in geladas affects GCs via aggression, food access, or both, remains to be determined.

Our pooled model also indicated that primiparous females trended towards higher iGCMs than multiparous females (Table 2; Fig. 4), suggesting that carrying a pregnancy is more energetically-taxing for first-time mothers. Additionally, parity likely affected the pattern that iGCMs follow across gestation (Table 3). Although gelada females reach 97.2% of their full shoulder-rump length (Lu et al., 2016) by the time they first give birth (mean 6.06 ± 0.60 years old (Roberts et al., 2017)), they do not reach full adult shoulder-rump length until 7.72 years of age (Lu et al., 2016). Furthermore, linear growth is likely finished before mass growth. For example, in wild yellow baboons body mass growth rates suggest that first birth occurs when only 83% of adult body mass has been attained (Altmann and Alberts, 2005), suggesting that primiparous females, compared to multiparous females, are more energetically challenged because they are still fueling their own development. Studies on captive primates, such as rhesus macaques, generally report higher GC concentrations in primiparous females, although a study on southern pig-tailed macaques (*Macaca nemestrina*) and another in vervet monkeys (*Chlorocebus aethiops*) found no effect of parity on GCs (Grant et al., 2017; Petrullo and Lu, 2019). In rhesus macaques, hair samples representing late gestation and early lactation (Dettmer et al., 2015) and breastmilk samples (Hinde et al., 2015) revealed higher GCs in first-time mothers compared to mothers with at least one prior birth. Additionally, primiparous rhesus macaque mothers took longer to recuperate their body condition post-parturition, suggesting that they were in worse condition to begin with (Mas-Rivera and Bercovitch, 2008). However, the few studies that have been conducted in wild primates report no effect of parity on GC concentrations during pregnancy (yellow baboons: Altmann et al., 2004; Nguyen et al., 2008)), but see (golden lion tamarins: Bales et al., 2005). With further research in geladas, we can see if the trend towards higher iGCMs in pregnant primiparous females gets stronger or weaker. Additionally, we can test parity as a continuous predictor (e.g., the number of pregnancies) rather than a categorical predictor (e.g., one vs. more than one) and compare continuous parity to age.

With regards to our ecological predictors, previous studies in this gelada population found that elevated iGCMs were associated with low temperatures, with little to no effect of rainfall (Beehner and McCann, 2008; Tinsley Johnson et al., 2018). We therefore hypothesized that geladas experience cold stress. However, in this analysis of pregnant females, our pooled model indicated that neither minimum temperature not cumulative rainfall were informative predictors for iGCMs (Table 2). Because higher rainfall indicates greater green grass availability (Jarvey et al., 2018), we predicted that during times of heavy rain pregnant females would exhibit signs of reduced metabolic costs reflected by lower iGCM levels. However, we found no effect of rainfall on iGCM levels. There is evidence from several sources (Hunter, 2001; Fashing et al., 2014; Jarvey, 2016) that geladas have ample fallback foods during low rainfall when green grass is less available, potentially explaining why rainfall's correlation with green grass availability does not affect iGCMs. In sum, we emphasize that the social and individual factors of rank and parity are better predictors of iGCMs across gestation than are the ecological factors of rainfall and temperature.

With our validation of a group-specific 11ß-hydroxyetiocholanolone EIA for assessing adrenocortical activity in female geladas, we have established a normative profile of fecal GC output across gelada gestation and identified that low maternal dominance rank and perhaps also primiparity are associated with higher iGCM concentrations, which could have downstream effects on the developing fetus. Although the placenta prevents the majority of maternal cortisol from reaching the fetus via enzymatic deactivation processes through 11β-hydroxysteroid dehydrogenase 2 (11B-HSD2) (Welberg et al., 2000), there is evidence that high levels of maternal cortisol decrease the effectiveness of 11B-HSD2 (Peña et al., 2012; Belkacemi et al., 2011; Mairesse et al., 2007). Thus, low rank and primiparity likely indicate conditions under which fetuses are exposed to higher concentrations of cortisol. This knowledge can help us identify the evolutionary pathways via which GCs affect offspring. In short-lived mammals, GCs seem to serve as a cue about the external environment, informing offspring about the expected environment in adulthood. However, the social environment may be more meaningful for long-lived species such as primates, as it may exhibit greater correlation between early-life and adulthood (Frankenhuis et al., 2019). Thus, maternal GCs may be better indicators of a mother's social environment, as dominance rank is often inherited. Alternatively, maternal GCs may be more directly related to current maternal condition or investment strategies, which may or may not be a direct correlate with the external environment (Berghänel et al., 2016; Lu et al., 2019; Wells, 2007, 2003). Future work will examine the relationships between maternal GCs and offspring development in geladas and consider how rank and parity may have direct effects on offspring.

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CRediT authorship contribution statement

Sofia C. Carrera: Conceptualization, Formal analysis, Investigation, Writing - original draft, Visualization. **Sharmi Sen:** Investigation. **Michael Heistermann:** Methodology, Validation, Writing - review & editing. **Amy Lu:** Writing - review & editing. **Jacinta C. Beehner:** Conceptualization, Writing - review & editing, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2020.113494.

References

Albers, P.C.H., de Vries, H., 2001. Elo-rating as a tool in the sequential estimation of dominance strengths. Anim. Behav. 61, 489–495. https://doi.org/10.1006/anbe.2000.1571.

Altmann, J., Alberts, S.C., 2005. Growth rates in a wild primate population: ecological influences and maternal effects. Behav. Ecol. Sociobiol. 57, 490–501. https://doi.org/10.1007/s00265-004-0870-x.

Altmann, J., Lynch, J.W., Nguyen, N., Alberts, S.C., Gesquiere, L.R., 2004. Life-history

correlates of steroid concentrations in wild peripartum baboons. Am. J. Primatol. 64, 95–106. https://doi.org/10.1002/ajp.20064.
Anderson, D.R., Burnham, K.P., 2002. Avoiding Pitfalls when using information-theoretic methods. J. Wildl. Manage. 66, 912–918.
Arnold, T.W., 2010. Uninformative parameters and model selection using Akaike's information criterion. J. Wildl. Manage. 74, 1175–1178. https://doi.org/10.2193/2009-367.
Bales, K.L., French, J.A., Hostetler, C.M., Dietz, J.M., 2005. Social and reproductive factors affecting cortisol levels in wild female golden lion tamarins (Leontopithecus rosalia). Am. J. Primatol. 67, 25–35. https://doi.org/10.1002/ajp.20167.
Barton, K., 2019. MuMIn: Multi-Model Inference. R package version 1.43.15. https://CRAN.R-project.org/package=MuMIn.
Bashaw, M.J., Sicks, F., Palme, R., Schwarzenberger, F., Tordiffe, A.S.W., Ganswindt, A., 2016. Non-invasive assessment of adrenocortical activity as a measure of stress in giraffe (Giraffa camelopardalis). BMC Vet. Res. 12, 1–13. https://doi.org/10.1186/s12917-016-0864-8.
Beehner, J.C., Bergman, T.J., 2017. The next step for stress research in primates: to identify relationships between glucocorticoid secretion and fitness. Horm. Behav. 91, 68–83. https://doi.org/10.1016/j.yhbeh.2017.03.003.
Beehner, J.C., Bergman, T.J., 2008. Infant mortality following male takeovers in wild geladas. Am. J. Primatol. 70, 1152–1159. https://doi.org/10.1002/ajp.20614.
Beehner, J.C., McCann, C., 2008. Seasonal and altitudinal effects on glucocorticoid metabolites in a wild primate (Theropithecus gelada). Physiol. Behav. 95, 508–514. https://doi.org/10.1016/j.physbeh.2008.07.022.
Beehner, J.C., Nguyen, N., Wango, E.O., Alberts, S.C., Altmann, J., 2006. The endocrinology of pregnancy and fetal loss in wild baboons. Horm. Behav. 49, 688–699. https://doi.org/10.1016/j.yhbeh.2005.12.016.
Behringer, V., Deschner, T., 2017. Non-invasive monitoring of physiological markers in primates. Horm. Behav. 91, 3–18. https://doi.org/10.1016/j.yhbeh.2017.02.001.
Belkacemi, L., Jelks, A., Chen, C.H., Ross, M.G., Desai, M., 2011. Altered placental development in undernourished rats: role of maternal glucocorticoids. Reprod. Biol. Endocrinol. 9, 1–11. https://doi.org/10.1186/1477-7827-9-105.
Berghänel, A., Heistermann, M., Schülke, O., Ostner, J., 2016. Prenatal stress effects in a wild, long-lived primate: predictive adaptive responses in an unpredictable environment. Proc. R. Soc. B Biol. Sci. 283. https://doi.org/10.1098/rspb.2016.1304.
Bolker, B., R Development Core Team, 2019. bbmle: Tools for general maximum likelihood estimation. R package version 1.0.22. https://CRAN.R-project.org/package=bbmle.
Braga Goncalves, I., Heistermann, M., Santema, P., Dantzer, B., Mausbach, J., Ganswindt, A., Manser, M.B., 2016. Validation of a fecal glucocorticoid assay to assess adrenocortical activity in meerkats using physiological and biological stimuli. PLoS ONE 11, 1–22. https://doi.org/10.1371/journal.pone.0153161.
Brown, G.R., 2001. Sex-biased investment in nonhuman primates: Can Trivers and Willard's theory be tested? Anim. Behav. 61, 683–694. https://doi.org/10.1006/anbe.2000.1659.
Cavigelli, S.A., 1999. Behvioral patterns associated with fecal cortisol levels in free-ranging ring-tailed lemurs, Lemur catta. Anim. Behav. 57, 935–944.
Cavigelli, S.A., Caruso, M.J., 2015. Sex, social status and physiological stress in primates: the importance of social and glucocorticoid dynamics. Philos. Trans. R. Soc. B Biol. Sci. 370. https://doi.org/10.1098/rstb.2014.0103.
Cavigelli, S.A., Dubovick, T., Levash, W., Jolly, A., Pitts, A., 2003. Female dominance status and fecal corticoids in a cooperative breeder with low reproductive skew: Ring-tailed lemurs (Lemur catta). Horm. Behav. 43, 166–179. https://doi.org/10.1016/S0018-506X(02)00031-4.
Chapman, C.A., Chapman, L.J., 2000. Constraints on group size in red colobus and red-tailed guenons: examining the generality of the ecological constraints model. Int. J. Primatol. 21, 565–585. https://doi.org/10.1023/A:1005557002854.
Chapman, C.A., Pavelka, M.S.M., 2005. Group size in folivorous primates: ecological constraints and the possible influence of social factors. Primates 46, 1–9. https://doi.org/10.1007/s10329-004-0093-9.
Cockrem, J.F., 2013. Individual variation in glucocorticoid stress responses in animals. Gen. Comp. Endocrinol. 181, 45–58. https://doi.org/10.1016/j.ygcen.2012.11.025.
Crespi, B., Semeniuk, C., 2004. Parent-offspring conflict in the evolution of vertebrate reproductive mode. Am. Nat. 163, 635–653. https://doi.org/10.1086/382734.
Dantzer, B., Newman, A.E.M., Boonstra, R., Palme, R., Boutin, S., Humphries, M.M., McAdam, A.G., 2013. Growth in a wild mammal. Science (80-) 340, 1215–1218. https://doi.org/10.1126/science.1235765.
Dantzer, B., Westrick, S.E., Van Kesteren, F., 2016. Relationships between Endocrine traits and life histories in wild animals: insights, problems, and potential pitfalls. Integr. Comp. Biol. 56, 185–197. https://doi.org/10.1093/icb/icw051.
Dettmer, A.M., Novak, M.A., Meyer, J.S., Suomi, S.J., 2014. Population density-dependent hair cortisol concentrations in rhesus monkeys (Macaca mulatta). Psychoneuroendocrinology 42, 59–67. https://doi.org/10.1016/j.psyneuen.2014.01.002.
Dettmer, A.M., Rosenberg, K.L., Suomi, S.J., Meyer, J.S., Novak, M.A., Chavatte-Palmer, P., 2015. Associations between parity, hair hormone profiles during pregnancy and lactation, and infant development in rhesus monkeys (Macaca mulatta). PLoS ONE 10, 1–13. https://doi.org/10.1371/journal.pone.0131692.
Edwards, P.D., Boonstra, R., 2018. Glucocorticoids and CBG during pregnancy in mammals: diversity, pattern, and function. Gen. Comp. Endocrinol. 259, 122–130. https://doi.org/10.1016/j.ygcen.2017.11.012.
Emery Thompson, M., Muller, M.N., Kahlenberg, S.M., Wrangham, R.W., 2010. Dynamics of social and energetic stress in wild female chimpanzees. Horm. Behav. 58, 440–449. https://doi.org/10.1016/j.yhbeh.2010.05.009.
Engh, A.L., Beehner, J.C., Bergman, T.J., Whitten, P.L., Hoffmeier, R.R., Seyfarth, R.M., Cheney, D.L., 2006. Female hierarchy instability, male immigration and infanticide increase glucocorticoid levels in female chacma baboons. Anim. Behav. 71, 1227–1237. https://doi.org/10.1016/j.anbehav.2005.11.009.
Fanson, K.V., Keeley, T., Fanson, B.G., 2014. Cyclic changes in cortisol across the estrous cycle in parous and nulliparous Asian elephants. Endocrine Connect. 3, 57–66. https://doi.org/10.1530/EC-14-0025.
Fashing, P.J., Nguyen, N., Venkataraman, V.V., Kerby, J.T., 2014. Gelada feeding ecology in an intact ecosystem at Guassa, Ethiopia: variability over time and implications for theropith and hominin dietary evolution. Am. J. Phys. Anthropol. 155, 1–16. https://doi.org/10.1002/ajpa.22559.
Fichtel, C., Kraus, C., Ganswindt, A., Heistermann, M., 2007. Influence of reproductive season and rank on fecal glucocorticoid levels in free-ranging male Verreaux's sifakas (Propithecus verreauxi). Horm. Behav. 51, 640–648. https://doi.org/10.1016/j.yhbeh.2007.03.005.
Foerster, S., Cords, M., Monfort, S.L., 2011. Social behavior, foraging strategies, and fecal glucocorticoids in female blue monkeys (Cercopithecus mitis): potential fitness benefits of high rank in a forest guenon. Am. J. Primatol. 73, 870–882. https://doi.org/10.1002/ajp.20955.
Frankenhuis, W.E., Nettle, D., Dall, S.R.X., 2019. A case for environmental statistics of early-life effects. Philos. Trans. R. Soc. B Biol. Sci. 374. https://doi.org/10.1098/rstb.2018.0110.
Frigerio, D., Dittami, J., Möstl, E., Kotrschal, K., 2004. Excreted corticosterone metabolites co-vary with ambient temperature and air pressure in male Greylag geese (Anser anser). Gen. Comp. Endocrinol. 137, 29–36. https://doi.org/10.1016/j.ygcen.2004.02.013.
Ganswindt, A., Palme, R., Heistermann, M., Borragan, S., Hodges, J.K., 2003. Non-invasive assessment of adrenocortical function in the male African elephant (Loxodonta africana) and its relation to musth. Gen. Comp. Endocrinol. 134, 156–166. https://doi.org/10.1016/S0016-6480(03)00251-X.
Gesquiere, L.R., Altmann, J., Archie, E.A., Alberts, S.C., 2018. Interbirth intervals in wild baboons: environmental predictors and hormonal correlates. Am. J. Phys. Anthropol. 166, 107–126. https://doi.org/10.1002/ajpa.23407.
Gesquiere, L.R., Onyango, P.O., Alberts, S.C., Altmann, J., 2011. Endocrinology of year-round reproduction in a highly seasonal habitat: Environmental variability in testosterone and glucocorticoids in baboon males. Am. J. Phys. Anthropol. 144, 169–176. https://doi.org/10.1002/ajpa.21374.
Gluckman, P.D., Hanson, M.A., Spencer, H.G., 2005. Predictive adaptive responses and human evolution. Trends Ecol. Evol. 20, 527–533. https://doi.org/10.1016/j.tree.2005.08.001.
Gómez-Espinosa, E., Rangel-Negrín, A., Chavira, R., Canales-Espinosa, D., Dias, P.A.D., 2014. The effect of energetic and psychosocial stressors on glucocorticoids in mantled howler monkeys (Alouatta palliata). Am. J. Primatol. 76, 362–373. https://doi.org/10.1002/ajp.22240.
Grant, K.S., Worlein, J.M., Meyer, J.S., Novak, M.A., Kroeker, R., Rosenberg, K., Kenney, C., Burbacher, T.M., 2017. A longitudinal study of hair cortisol concentrations in Macaca nemestrina mothers and infants. Am. J. Primatol. 79, 1–9. https://doi.org/10.1002/ajp.22591.
Hämäläinen, A., Heistermann, M., Fenosoa, Z.S.E., Kraus, C., 2014. Evaluating capture stress in wild gray mouse lemurs via repeated fecal sampling: Method validation and the influence of prior experience and handling protocols on stress responses. Gen. Comp. Endocrinol. 195, 68–79. https://doi.org/10.1016/j.ygcen.2013.10.017.
Heistermann, M., Palme, R., Ganswindt, A., 2006. Comparison of different enzymeimmunoassays for assessment of adrenocortical activity in primates based on fecal analysis. Am. J. Primatol. 68, 257–273.
Hinde, K., Skibiel, A.L., Foster, A.B., Del Rosso, L., Mendoza, S.P., Capitanio, J.P., 2015. Cortisol in mother's milk across lactation reflects maternal life history and predicts infant temperament. Behav. Ecol. 26, 269–281. https://doi.org/10.1093/beheco/aru186.
Hunter, C.P., 2001. Ecological Determinants of GELADA Ranging PATTERNS (*Theropithecus gelada*). Doctoral dissertation. University of Liverpool.
Janson, C.H., Goldsmith, M.L., 1995. Predicting group size in primates: Foraging costs and predation risks. Behav. Ecol. 6, 326–336. https://doi.org/10.1093/beheco/6.3.326.
Jarvey, J.C., 2016. The importance of underground foods in female felada (Theropithecus Gelada) socioecology. Master's Thesis, University of Michigan.
Jarvey, J.C., Low, B.S., Bergman, T.J., Beehner, J.C., 2016. The roots of all evil: Aggression and below-ground feeding in female geladas. 85th Annual Meeting of the American Association of Physical Anthropologists. Atlanta, GA.
Jarvey, J.C., Low, B.S., Pappano, D.J., Bergman, T.J., Beehner, J.C., 2018. Graminivory and fallback foods: annual diet profile of geladas (Theropithecus gelada) living in the Simien Mountains National Park, Ethiopia. Int. J. Primatol. 39, 105–126. https://doi.org/10.1007/s10764-018-0018-x.
Jensen, E., Wood, C.E., Keller-Wood, M., 2004. Chronic alterations in ovine maternal corticosteroid levels influence uterine blood flow and placental and fetal growth. Am. J. Physiol. Integr. Comp. Physiol. 288, R54–R61. https://doi.org/10.1152/ajpregu.00149.2004.
Keay, J.M., Singh, J., Gaunt, M.C., Kaur, T., 2006. Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: a literature review. J. Zoo Wildl. Med. 37, 234–244. https://doi.org/10.1638/05-050.1.
Korgun, E.T., Ozmen, A., Unek, G., Mendilcioglu, I., 2012. The effects of glucocorticoids on fetal and placental development. In Glucocorticoids – New Recognition Our Familiar Friend. https://doi.org/10.5772/50103.
Kuijper, B., Johnstone, R.A., 2018. Maternal effects and parent–offspring conflict. Evolution 72, 220–233. https://doi.org/10.1111/evo.13403.
le Roux, A., Beehner, J.C., Bergman, T.J., 2011. Female philopatry and dominance patterns in wild geladas. Am. J. Primatol. 73, 422–430. https://doi.org/10.1002/ajp.20916.

Lu, A., Bergman, T.J., McCann, C., Stinespring-Harris, A., Beehner, J.C., 2016. Growth trajectories in wild geladas (Theropithecus gelada). Am. J. Primatol. 78, 707–719. https://doi.org/10.1002/ajp.22535.

Lu, A., Petrullo, L., Carrera, S., Feder, J., Snyder-mackler, N., 2019. Developmental responses to early-life adversity: evolutionary and mechanistic perspectives. Evol. Anthro. 1–18. https://doi.org/10.1002/evan.21791.

MacDougall-Shackleton, S.A., Bonier, F., Romero, L.M., Moore, I.T., 2019. Glucocorticoids and "stress" are not synonymous. Integr. Org. Biol. https://doi.org/10.1093/iob/obz017.

Maestripieri, D., Georgiev, A.V., 2016. What cortisol can tell us about the costs of sociality and reproduction among free-ranging rhesus macaque females on Cayo Santiago. Am. J. Primatol. 78, 92–105. https://doi.org/10.1002/ajp.22368.

Mairesse, J., Lesage, J., Breton, C., Bréant, B., Hahn, T., Darnaudéry, M., Dickson, S.L., Seckl, J., Blondeau, B., Vieau, D., Maccari, S., Viltart, O., 2007. Maternal stress alters endocrine function of the feto-placental unit in rats. Am. J. Physiol. Metab. 292, E1526–E1533. https://doi.org/10.1152/ajpendo.00574.2006.

Mas-Rivera, A., Bercovitch, F.B., 2008. Postpartum recuperation in primiparous rhesus macaques and development of their infants. Am. J. Primatol. 70, 1047–1054. https://doi.org/10.1002/ajp.20596.

Möstl, E., Palme, R., 2002. Hormones as indicators of stress. Domest. Anim. Endocrinol. 23, 67–74.

Möstl, E., Rettenbacher, S., Palme, R., 2005. Measurement of corticosterone metabolites in birds' droppings: an analytical approach. N.Y. Acad. Sci. 1046, 17–34. https://doi.org/10.1196/annals.1343.004.

Murray, C.M., Stanton, M.A., Wellens, K.R., Santymire, R.M., Heintz, M.R., Lonsdorf, E.V., 2018. Maternal effects on offspring stress physiology in wild chimpanzees. Am. J. Primatol. 80, 1–12. https://doi.org/10.1002/ajp.22525.

Nakagawa, S., Freckleton, R.P., 2011. Model averaging, missing data and multiple imputation: a case study for behavioural ecology. Behav. Ecol. Sociobio. 65, 103–116. https://doi.org/10.1007/s00265-010-1044-7.

Nettle, D., Bateson, M., 2015. Adaptive developmental plasticity: What is it, how can we recognize it and when can it evolve? Proc. R. Soc. B Biol. Sci. 282, 1–9. https://doi.org/10.1098/rspb.2015.1005.

Neumann, C., Duboscq, J., Dubuc, C., Ginting, A., Irwan, A.M., Agil, M., Widdig, A., Engelhardt, A., 2011. Assessing dominance hierarchies: validation and advantages of progressive evaluation with Elo-rating. Anim. Behav. 82, 911–921. https://doi.org/10.1016/j.anbehav.2011.07.016.

Nguyen, N., Gesquiere, L.R., Wango, E.O., Alberts, S.C., Altmann, J., 2008. Late pregnancy glucocorticoid levels predict responsiveness in wild baboon mothers (Papio cynocephalus). Anim. Behav. 75, 1747–1756. https://doi.org/10.1016/j.anbehav.2007.09.035.

Palme, R., 2019. Non-invasive measurement of glucocorticoids: Advances and problems. Physiol. Behav. 199, 229–243. https://doi.org/10.1016/j.physbeh.2018.11.021.

Palme, R, Möstl, E, 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Int. J. Mammalian Biol. 62 (2), 192–197.

Peña, C.J., Monk, C., Champagne, F.A., 2012. Epigenetic effects of prenatal stress on 11β-hydroxysteroid dehydrogenase-2 in the placenta and fetal brain. PLoS ONE 7, 1–9. https://doi.org/10.1371/journal.pone.0039791.

Petrullo, L., Lu, A., 2019. Natural variation in fetal cortisol exposure is associated with neonatal body mass in captive vervet monkeys (Chlorocebus aethiops). Am. J. Primatol. 81. https://doi.org/10.1002/ajp.22943.

Piersma, T., Reneerkens, J., Ramenofsky, M., 2000. Baseline corticosterone peaks in shorebirds with maximal energy stores for migration: a general preparatory mechanism for rapid behavioral and metabolic transitions? Gen. Comp. Endocrinol. 120, 118–126. https://doi.org/10.1006/gcen.2000.7543.

R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria https://www.R-project.org/.

Rimbach, R., Heymann, E.W., Link, A., Heistermann, M., 2013. Validation of an enzyme immunoassay for assessing adrenocortical activity and evaluation of factors that affect levels of fecal glucocorticoid metabolites in two New World primates. Gen. Comp. Endocrinol. 191, 13–23. https://doi.org/10.1016/j.ygcen.2013.05.010.

Roberts, E.K., Lu, A., Bergman, T.J., Beehner, J.C., 2017. Female reproductive parameters in wild geladas (Theropithecus gelada). Int. J. Primatol. 38, 1–20. https://doi.org/10.1007/s10764-016-9939-4.

Roberts, E.K., Lu, A., Bergman, T.J., Beehner, J.C., 2012. A Bruce effect in wild geladas. Science (80-.). 335, 1222–1225. https://doi.org/10.1126/science.1213600.

Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The reactive scope model – a new model integrating homeostasis, allostasis, and stress. Horm. Behav. 55, 375–389. https://doi.org/10.1016/j.yhbeh.2008.12.009.

Rubin, D.B., 1987. Multiple Imputation for Nonresponse in Surveys. Wiley, New York.

Rudolph, K., Fichtel, C., Schneider, D., Heistermann, M., Koch, F., Daniel, R., Kappeler, P.M., 2019. One size fits all? Relationships among group size, health, and ecology indicate a lack of an optimal group size in a wild lemur population. Behav. Ecol. Sociobiol. 73. https://doi.org/10.1007/s00265-019-2746-0.

Saltzman, W., Maestripieri, D., 2011. The neuroendocrinology of primate maternal behavior. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 1192–1204. https://doi.org/10.1016/j.pnpbp.2010.09.017.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Preparative actions. Endocr. Rev. 21, 55–89. https://doi.org/10.1210/edrv.21.1.0389.

Schafer, J.L., Olsen, M.K., 1998. Multiple imputation for multivariate missing-data problems: a data analyst's perspective. Multivariate Behav. Res. 33, 545–571.

Setchell, J.M., Smith, T., Wickings, E.J., Knapp, L.A., 2008. Factors affecting fecal glucocorticoid levels in semi-free-ranging female mandrills (Mandrillus sphinx). Am. J. Primatol. 70, 1023–1032. https://doi.org/10.1002/ajp.20594.

Sheriff, M.J., Bell, A., Boonstra, R., Dantzer, B., Lavergne, S.G., McGhee, K.E., MacLeod, K.J., Winandy, L., Zimmer, C., Love, O.P., 2017. Integrating ecological and evolutionary context in the study of maternal stress. Integr. Comp. Biol. 57, 437–449. https://doi.org/10.1093/icb/icx105.

Sheriff, M.J., Krebs, C.J., Boonstra, R., 2010. Assessing stress in animal populations: Do fecal and plasma glucocorticoids tell the same story? Gen. Comp. Endocrinol. 166, 614–619. https://doi.org/10.1016/j.ygcen.2009.12.017.

Shutt, K., Setchell, J.M., Heistermann, M., 2012. Non-invasive monitoring of physiological stress in the western lowland gorilla (Gorilla gorilla gorilla): validation of a fecal glucocorticoid assay and methods for practical application in the field. Gen. Comp. Endocrinol. 179, 167–177. https://doi.org/10.1016/j.ygcen.2012.08.008.

Smith, R.J., Leigh, S.R., 1998. Sexual dimorphism in primate neonatal body mass. J. Hum. Evol. 34, 173–201. https://doi.org/10.1002/ajpa.10011.abs.

Snaith, T.V., Chapman, C.A., Rothman, J.M., Wasserman, M.D., 2008. Bigger groups have fewer parasites and similar cortisol levels: a multi-group analysis in red colobus monkeys. Am. J. Primatol. 70, 1072–1080. https://doi.org/10.1002/ajp.20601.

Snyder-Mackler, N., Beehner, J.C., Bergman, T.J., 2012. Defining higher levels in the multilevel societies of geladas (Theropithecus gelada). Int. J. Primatol. 33, 1054–1068. https://doi.org/10.1007/s10764-012-9584-5.

Symonds, M.R.E., Moussalli, A., 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behav. Ecol. Sociobiol. 65, 13–21. https://doi.org/10.1007/s00265-010-1037-6.

Tazumi, T., Hori, E., Uwano, T., Umeno, K., Tanebe, K., Tabuchi, E., Ono, T., Nishijo, H., 2005. Effects of prenatal maternal stress by repeated cold environment on behavioral and emotional development in the rat offspring. Behav. Brain Res. 162, 153–160. https://doi.org/10.1016/j.bbr.2005.03.006.

Tetsuka, M., 2007. Actions of glucocorticoid and their regulatory mechanisms in the ovary. Animal Sci. J. 78, 112–120. https://doi.org/10.1111/j.1740-0929.2007.00414.x.

Thayer, Z.M., Feranil, A.B., Kuzawa, C.W., 2012. Maternal cortisol disproportionately impacts fetal growth in male offspring: evidence from the Philippines. Am. J. Hum. Biol. 24, 1–4. https://doi.org/10.1002/ajhb.21226.

Thayer, Z.M., Kuzawa, C.W., 2014. Early origins of health disparities: material deprivation predicts maternal evening cortisol in pregnancy and offspring cortisol reactivity in the first few weeks of life. Am. J. Hum. Biol. 26, 723–730. https://doi.org/10.1002/ajhb.22532.

Thayer, Z.M., Kuzawa, C.W., 2015. Ethnic discrimination predicts poor self-rated health and cortisol in pregnancy: Insights from New Zealand. Soc. Sci. Med. 128, 36–42. https://doi.org/10.1016/j.socscimed.2015.01.003.

Tinsley Johnson, E., Snyder-Mackler, N., Beehner, J.C., Bergman, T.J., 2014. Kinship and dominance rank influence the strength of social bonds in female geladas (Theropithecus gelada). Int. J. Primatol. 35, 288–304. https://doi.org/10.1007/s10764-013-9733-5.

Tinsley Johnson, E., Snyder-Mackler, N., Lu, A., Bergman, T.J., Beehner, J.C., 2018. Social and ecological drivers of reproductive seasonality in geladas. Behav. Ecol. 29, 574–588. https://doi.org/10.1093/beheco/ary008.

Touma, C., Palme, R., 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: The importance of validation. N.Y. Acad. of Sci. 74, 54–74. https://doi.org/10.1196/annals.1343.006.

van Buuren, S., Groothuis-Oudshoorn, K., 2011. mice: Multivariate imputation by chained equations in R. J. Statistical Software, 45(3), 1–67. https://www.jstatsoft.org/v45/i03/.

Wasser, S.K., Hunt, K.E., Brown, J.L., Cooper, K., Crockett, C.M., Bechert, U., Millspaugh, J.J., Larson, S., Monfort, S.L., 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. Gen. Comp. Endocrinol. 120, 260–275. https://doi.org/10.1006/gcen.2000.7557.

Weingrill, T., Willems, E.P., Zimmermann, N., Steinmetz, H., Heistermann, M., 2011. Species-specific patterns in fecal glucocorticoid and androgen levels in zoo-living orangutans (Pongo spp.). Gen. Comp. Endocrinol. 172, 446–457. https://doi.org/10.1016/j.ygcen.2011.04.008.

Welberg, L.A.M., Seckl, J.R., Holmes, M.C., 2000. Inhibition of 11β-hydroxysteroid dehydrogenase, the foetoplacental barrier to maternal glucocorticoids, permanently programs amygdala GR mRNA expression and anxiety-like behaviour in the offspring. Eur. J. Neurosci. 12, 1047–1054. https://doi.org/10.1046/j.1460-9568.2000.00958.x.

Wells, J.C.K., 2007. The thrifty phenotype as an adaptive maternal effect. Biol. Rev. 82, 143–172. https://doi.org/10.1111/j.1469-185X.2006.00007.x.

Wells, J.C.K., 2003. The thrifty phenotype hypothesis: thrifty offspring or thrifty mother? J. Theor. Biol. 221, 143–161. https://doi.org/10.1006/jtbi.2003.3183.

Wheeler, B.C., Tiddi, B., Kalbitzer, U., Visalberghi, E., Heistermann, M., 2013. Methodological considerations in the analysis of fecal glucocorticoid metabolites in tufted capuchins (Cebus apella). Int. J. Primatol. 34, 879–898. https://doi.org/10.1007/s10764-013-9703-y.

Wingfield, J.C., 2013. Ecological processes and the ecology of stress: the impacts of abiotic environmental factors. Funct. Ecol. 27, 37–44. https://doi.org/10.1111/1365-2435.12039.

Wood, S.N., 2004. Stable and efficient multiple smoothing parameter estimation for generalized additive models. J. Am. Stat. Assoc. 99, 673–686.