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Energy balance but not competitive environment corresponds with allostatic load during development in an Old World monkey

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A B S T R A C T

Primates develop slowly relative to their body size, a pattern posited to result from ecological risk aversion. Little is known, however, about how energy balance contributes to allostatic load in juveniles. Using data collected over 8 consecutive months, we examined variation in energy balance (as measured by urinary C-peptide) and how energy balance, life history status, and social competition related to allostatic load (as measured by deviation from baseline fecal glucocorticoid metabolites, dfGCs) in 41 wild juvenile blue monkeys from 3 social groups. Juvenile energy balance was higher among females, older juveniles, when ripe fruit was more available, and when rainfall was lower. Energy balance, but not life history or competitive environments, predicted dfGC concentrations, such that juveniles generally had lower mean dfGCs when they had higher energy balance. An additional exploratory analysis of how dfGCs relate to social strategies revealed that subjects had lower dfGCs when they groomed less, and played more. Time spent grooming interacted with energy balance in predicting dfGC concentrations, so that individuals that groomed more actually had higher dfGCs when they had higher energy balance. Together these results reveal that energetic deficiencies are a true ecological risk factor in blue monkeys, and suggest that navigating the social environment via overt affiliative behavior is potentially both a stress-relieving and stress-inducing endeavor during development.

1. Introduction

Extended juvenile periods in primates are thought to have evolved to help individuals avoid food shortages as they grow physically and remain vulnerable both to social competition and predation (Janson et al., 2003; O'Mara, 2015; Stone, 2007). Little is known, however, about how energetic challenges relate to allostatic load in juveniles, or the cost of maintaining homeostasis through predictable and unpredictable environmental change (Romero et al., 2009). Although internal and external environmental challenges to homeostasis can vary by life stage, most studies of allostasis, its energetic and environmental correlates, and its behavioral mediators in wild populations have focused on adults, with effects of age explored primarily during infancy or senescence (Reeder and Kramer, 2005). In group-living species, adults can use affiliative social ties to ameliorate environmental challenges and navigate competitive social environments (Cords and Thompson, 2017); nevertheless, how affiliative ties relate to allostatic load during the juvenile period has been largely overlooked.

Juveniles, which are neither dependent on parental care nor yet reproductively active, are particularly vulnerable to the negative effects of food shortage because of the energetic demands of physical growth and brain development (Douhard et al., 2014; Janson et al., 2003; Kuzawa et al., 2014). In addition, they are vulnerable to social competition from older and larger individuals because of their small size and relative inexperience (Pereira and Fairbanks, 2003; Stanton et al., 2011). Meeting growth requirements, particularly in the face of resource instability and competitive exclusion, can increase individuals' exposure to predators during foraging, disrupt cell function, and advance the deterioration of telomeres (Metcalfe and Monaghan, 2001). In baboons, for example, females have shorter lifespans if they experience a combination of poor nutritional and social conditions during development, such as early maternal loss, competing siblings, and drought during the first year of life (Altmann, 1991; Tung et al., 2016). Understanding how energy balance contributes to juveniles' short-term allostatic costs therefore helps clarify both how long-term damage may accumulate and how life histories can evolve to trade off fast growth for adult health and competence.

Circulating concentrations of glucocorticoids (GCs) are one critical mediator of allostasis (Goymann and Wingfield, 2004; Romero et al., 2009), as they mobilize energy to cope with challenges to homeostasis

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and inhibit bodily maintenance that is not immediately essential for survival (Sapolsky et al., 2000). Because of their metabolic function, elevated GC concentrations indicate that individuals experience stressors in the environment and must expend energy to navigate them. If individuals sustain GC concentrations higher than those within their reactive scope, their physiological systems can succumb to allostatic load or wear and tear (Romero et al., 2009), with negative effects on cardiovascular health, fertility, and memory (Sapolsky et al., 2000). Whether such chronic elevations of GCs *directly* reduce fitness, or even regularly occur in wild-living animals, is not clear (Beehner and Bergman, 2017; Boonstra, 2013), however elevated GCs, particularly in tandem with food shortages, may signal that individuals are vulnerable to disease or death (Bonier et al., 2009; Pride, 2005; Wilkening and Ray, 2016).

Food shortages can lead individuals into negative energy balance and, in several vertebrates, cause increased glucocorticoid secretion to mobilize energy for daily activities and bodily maintenance (Bonier et al., 2009). A food shortage associated with an El Niño event caused iguanas in the Galapagos to lose body mass, which led to large spikes in GCs (Romero and Wikelski, 2001). The iguanas with the highest GCs were most likely to die during the year. Food shortage during periods of great energetic demand, such as reproduction and growth, can lead to pronounced energetic deficiencies. For example, low-ranking adult female blue monkeys had higher GCs than high-ranking females when undergoing the energetically costly process of lactating (Foerster et al., 2011).

Energy balance is defined as the rate of energy intake minus the rate of energy expenditure (Hall et al., 2012) and is difficult to calculate precisely for wild animals. Specifically, measuring expenditure requires administering doubly labeled water (Schoeller, 1988) and measuring intake requires records of all items consumed and their caloric content. C-peptide of insulin (hereafter, CP), however, is one biomarker that corresponds with energy balance and can be analyzed relatively easily from field collected urine samples (Emery Thompson and Knott, 2008; Emery Thompson et al., 2010; Emery Thompson et al., 2009; Higham et al., 2011b). Insulin secretion often corresponds with changes in body mass (Deschner et al., 2008; Girard-Buttoz et al., 2011) and signals energy balance to the brain (Emery Thompson, 2016). CP is produced on an equimolar basis to insulin after the cleavage of pro-insulin and is excreted in urine (Norman and Litwack, 1997). CP is therefore a useful biomarker to assess whether allostatic load derives from lowered energy balance.

Both energy balance and allostatic load may relate to living in social groups. Group living can increase competition for resources, either through indirect scramble competition and/or direct contest competition (Isbell, 1991), but in addition, affiliative social ties with groupmates may help individuals avoid or cope with competition. Ties may be particularly important in helping juveniles, in light of their high energetic demands for growth and vulnerability to aggressive competition (Cords et al., 2010). In adults, affiliative relationships are often seen to increase tolerance, helping individuals to avoid harassment (e.g., feral horses and Assamese macaques, Cameron et al., 2009; Haunhorst et al., 2017), peaceably co-feed (baboons, King et al., 2011), and maintain body contact for homeothermy (Barbary macaques, Lehmann et al., 2016; McFarland et al., 2015). Close affiliates may also buffer the experience of stressors via socio-positive contact (Crockford et al., 2013; Heinrichs et al., 2003; Hostinar et al., 2014; Kikusui et al., 2006; Young et al., 2014). In some studies of captive animals, GC concentrations are lower among individuals with even a single familiar social partner during a stressful event (e.g. experimental transfer to a new social group, rhesus macaques, Gust et al., 1996) and among those that engage more frequently in social play (marmosets, Mustoe et al., 2014). Nevertheless, as juveniles are navigating a relatively novel social environment, social interaction may present more challenges than support. For example, post-fledgling ravens and juvenile chimpanzees that spent more time in friendly and cooperative interactions with partners had higher GC concentrations (Anestis, 2005; Stoewe et al., 2008). Together, these studies suggest that juveniles may actually bear some physiological cost of affiliative interaction.

We examined the relationship of internal and external environmental challenges with allostatic load among juveniles in wild blue monkeys. These monkeys are group-living and inhabit an environment with seasonal fluctuations in both fruit availability and rainfall (Mitchell, 2009; Pazol and Cords, 2005). Juvenile blue monkeys face challenges similar to those of other juvenile animals: they receive more agonism compared to adult females (Cords et al., 2010) and have a wider range of annual mortalities (Cords and Chowdhury, 2010), which are possibly related to variation in food availability and predation. They grow particularly slowly, with developmental periods that are long even for primates (ca. 7 yrs., Cords, 2012), making it likely that they employ an ecologically risk-averse growth strategy.

Here, we tested the hypothesis that energetic demands and vulnerability to competition are major challenges during the juvenile period, contributing to individual allostatic load. Our study comprised 3 aims. **Aim 1** was to assess variation in energy balance according to juveniles' life history (i.e., age, sex), their experience of indirect and direct competition (i.e., group size and maternal dominance rank), and their physical environment (i.e., local fruit availability and rainfall). Under Aim 1, we predicted that the availability of ripe fruit would be the strongest measured predictor of energy balance among juveniles because fruit is an energy-rich and preferred food (P1; Foerster et al., 2011). As male growth rates increase relative to females' at the very end of juvenility and before puberty in blue monkeys (Leigh, 1995), we predicted that the challenges of matching intake with expenditure would cause lower energy balance among males vs. females to emerge with age (P2). We also predicted that juvenile energy balance would decrease with group size (via indirect scramble competition, P3) and decrease with lower maternal dominance rank (via losses in direct contest competition, P4). We predicted the latter also because energetic stress is higher among low vs. high ranking adult females during lactation (Foerster et al., 2011), an energetic demand perhaps similar to juveniles' physical growth.

Aim 2 was to then assess how allostatic load varied according to life history, competitive environments, and energy balance. We predicted that individuals with higher energy balance would have lower GC concentrations (P5) and that the threat of aggression associated with lower maternal dominance rank would cause low ranking juveniles to have higher GCs (P6).

Lastly, we conducted an exploratory analysis of the relationships between broadly characterized social strategies and allostatic load in juveniles (**Aim 3**). As blue monkeys interact infrequently relative to other cercopithecines, such as baboons and macaques, (Cords, 2000), we undertook this exploration to determine if any suggestive relationships between sociality and allostatic load exist, which could be tested in a future, more focused behavioral study. We first characterized juveniles' multi-dimensional social strategies in 2-mo periods, including affiliative and agonistic behavior, using a principal components analysis (Aim 3a). We then assessed how average GC levels in each period related to social strategies, energy balance, life history variables, and social status from the corresponding time period (Aim 3b). We predicted that social strategies involving positive social contact, such as social play or grooming, would also correspond with lower GC concentrations (P7), and that strategies involving a high receipt of aggression would correspond with higher GCs (P8). We predicted that affiliative behavior would buffer the relationship between stressors and GCs as seen under Aim 2 (P9).

2. Methods

2.1. Study site and population

The wild study population inhabits the Isecheno area of Kakamega

Forest in western Kenya (0°19′ N, 34°52′ E; elevation 1580 m, mean annual rainfall 1997–2011 1942 mm; (Mitchell, 2009). We collected data on 41 pre-pubertal juveniles (21 males, 20 females, mean age 4.5 ± 1.7 yrs) for 8 months (August 2015–March 2016). Subjects lived in 3 social groups that neighbored one another (mean group size: 37–65 individuals; mean juveniles per group: 16–31), and male juveniles of neighboring groups often interacted with one another. Subjects were individually identifiable by their natural physical variation. Subjects' ages were known from precise, long-term demographic records of the study population (Cords, 2012). A team of 4 observers, including author NAT, collected all biomarker and behavioral samples after a 2-month training period to ensure inter-observer agreement in behavioral coding. Training ended when observers reached 90% agreement in behavioral coding and NAT checked observers at 1-month intervals to ensure continued agreement.

2.2. Fruit availability and rainfall

We calculated a fruit availability index (FAI) using data from monthly plant phenology surveys of 36 major food species (consistently constituting > 0.05% of annual adult feeding time) and their basal areas in 44 group-specific transects (N = 13, 6 and 9 10 m × 100 m uniformly distributed transects for groups 1, 2 and 3 respectively), which represented approximately 10% of each group's home range area (similar to Foerster and Monfort, 2010). One field assistant collected data on ca. 10 focal trees of each food species, recording each as fruiting or not, and for fruiting trees, counting number of fruits (twice, to check for accuracy) on an exponential-based scale (e.g., 70–99, 100–399, 400–699, 700–1000, 1000–3999) and estimating percentage of ripe fruit to the nearest 25% (Leighton, 1993). Not all focal trees designated as "fruiting" received a fruit count, therefore we averaged number of ripe fruits among trees that received counts and assigned that average to all fruiting trees. We then calculated the average number of ripe fruits across all focal trees of a given species (i.e., fruiting or not). To calculate group-specific FAIs, we summed across all tree species the product of the average mid-point estimate of number of ripe fruits and average basal area in group-specific transects (Ganzhorn et al., 2003). We used daily rainfall data collected locally by Kenya Forest Service staff.

2.3. Fecal and urine sample collection and fecal glucocorticoid (fGC) and urinary c-peptide (uCP) analysis

Fecal and urine collection occurred between 07:30 and 17:00, whenever observers witnessed excretion from identified subjects. Subjects were targeted to ensure that fecal and urine samples were collected approximately once every 2 weeks. For fecal samples, we homogenized the whole sample with a stick and placed ca. 1 g of feces, uncontaminated with dirt, urine or other feces in 1.5–15 ml plastic tubes for storage. We pipetted urine from leaves or other substrates that were uncontaminated with dirt, feces, or urine from other animals, and stored samples in 1.5 ml polypropylene tubes. Urine and fecal samples were immediately placed in thermoses with ice packs until they were placed in a −20 °C freezer within 4 h. Samples remained frozen and in the dark until they were shipped to the USA on ice and transferred to a −20 °C freezer at New York University, where they remained frozen until further processing. In total, we collected 627 fecal and 612 urine samples, averaging 15.3 ± 2.1 fecal and 15.0 ± 2.4 urine samples per subject.

Author NAT extracted glucocorticoid metabolites from feces following the protocol of Heistermann et al. (1995) and Palme et al. (2013) at the Anthropology Department of New York University (NYU). After lyophilizing and pulverizing samples, NAT extracted an aliquot of ca. 0.05–0.1 g (exact weights recorded) of fecal powder into 3 ml of 80% methanol in water by vortexing for 15 min. Following centrifugation (2000g, 20 min) of the fecal suspension, NAT removed 1 ml of the supernatant and stored it at −20 °C until hormone analysis.

NAT assayed fecal extracts for concentrations of cortisol metabolites at the German Primate Center using an enzyme immunoassay (EIA) for immunoreactive 11β-hydroxyetiocholanolone, a group-specific assay for the measurement of 5-reduced 3α,11β-dihydroxylated cortisol metabolites. These metabolites represent a major and quantitatively abundant portion of cortisol metabolites in the feces of mammals: they have been validated and shown to track changes in glucocorticoid output reliably in several species (Braga Goncalves et al., 2016; Ganswindt et al., 2003; Palme and Möstl, 1997) including primates in all major taxa, i.e., lemurs, South American monkeys, Cercopithecoid monkeys and great apes (Hämäläinen et al., 2014; Heistermann et al., 2006; Rimbach et al., 2013; Shutt et al., 2012; Weingrill et al., 2011; Wheeler et al., 2013).

The assay was carried out as described in detail by Heistermann et al. (2004). Samples were diluted at 1: 80 or 1:800 (depending on concentration) in assay buffer (0.04 M PBS, pH 7.2) to bring hormone concentrations into the working range of the assay. Sensitivity of the assay at 90% binding was 0.6 pg. Serial dilutions of fecal extracts from samples of different animals gave displacement curves that were parallel to the 11β-hydroxyetiocholanolone standard curve. Inter-assay coefficients of variation (CV), assessed by replicate determinations of high- and low-value quality controls run in each assay, were 8.9% (high, N = 42 wells) and 11.9% (low, N = 42) and intra-assay CVs were 3.9% (high; N = 20 plates) and 6.0% (low, N = 20). All hormone concentrations are expressed as mass hormone per fecal dry mass.

NAT assayed urinary C-peptide of insulin (uCP) by radioimmunoassay (RIA) using a Merck Millipore™ RIA kit for human C-Peptide in the Anthropology Department at Rutgers University. The C-peptide molecule is extremely well conserved among mammals (Peterson et al., 1972) and C-peptide in blue monkey samples dilutes in parallel when using Merck Millipore kits™ (Michelle Brown, personal communication). Prior to assay, samples were diluted at 1:2 or 1:20, depending on concentration. Inter-assay coefficients of variation of high- and low-value quality controls were 5.1 (high) and 7.3 (low, N = 9 batches), and average intra-assay coefficient of variation was 4.4% (N = 666 CVs including samples, standards, and controls). We standardized uCP concentrations by samples' specific gravity, measured by an Atago™ handheld refractometer, following Miller et al. (2004), at NYU. To control for variable water content of urine samples, the uCP level in a given sample was multiplied by the average specific gravity of all samples divided by the specific gravity of the given sample (Miller et al., 2004).

Concentrations of fGCs did not vary across samples by hour of collection during the day, however uCP concentrations did decrease with time of day (linear mixed model, or LMM, of log uCP concentrations, subject ID as random effect, N = 612, β = −0.1, p = .007; Fig. 1). We therefore expressed uCP concentrations as residuals of log uCP concentrations vs. time of day (Emery Thompson et al., 2010). To focus on variation in fGCs relevant to a subject's own reactive scope (e.g., Romero et al., 2009), we expressed each sample fGC concentration in terms of its deviation (dfGC) from the subject's 8-month baseline (mean) and then averaged individuals' deviations from baseline for each 2-month observation period (see Behavioral data collection and analysis). The dataset included 3 pairs of fecal and 2 pairs of urine samples that were collected from the same subject on the same day, so we averaged the fGCs and uCP residuals for each pair to get a daily value, yielding N = 623 dfGC and N = 610 uCP daily values, and N = 160 subject-period average dfGCs and N = 156 subject-period average uCP residuals.

2.4. Data binning, life history and competitive environmental variables

We divided the study into four consecutive 2-month periods, for which we calculated all average residual biomarker concentrations, life history status, and competitive environmental variables. We chose 2-

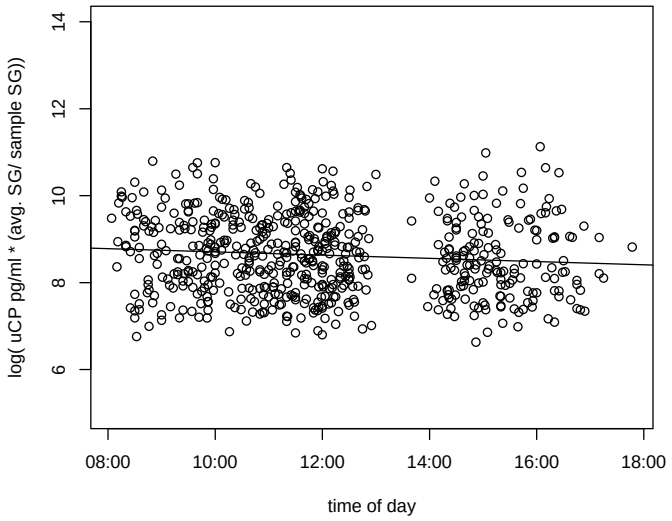

Fig. 1. Log urinary c-peptide (uCP) concentration vs. time at collection. Trend line is fitted from simple linear regression, unlike linear mixed model used for statistical analysis. N = 612 urine samples.

month periods to capture distinct variation in fruit availability and rainfall over time (Fig. 2). We matched variables calculated over these periods to their average biomarker concentrations (mean 3.9 ± 0.9 fecal and 4.0 ± 1.0 urine samples/subject/period).

We calculated subjects' ages at the mid-date of each 2-month period, and group size as the number of non-infant individuals present in a subject's social group for > 25% of the period. We used mothers' dominance rank to represent juvenile dominance relations because offspring appear to acquire rank maternally in blue monkeys (Klass and Cords, 2015). Maternal rank was calculated based on decided winner-loser interactions either from data collated over the study period if mothers were still alive, or over the mother's last year of life, using the I &SI method in DomiCalc (Schmid and de Vries, 2013). Dominance rank ranged from 0 to 1 representing the proportion of co-resident adult females that a mother outranked. We counted subjects' maternal kin present in the social group based on known pedigrees, where maternal aunts and nieces (r = 0.125) were the most distant relations included.

2.5. Behavioral data collection for exploratory analysis

Field observers conducted 20-min focal follows in which they recorded a focal subject's activity (e.g., resting, grooming, playing, feeding, moving) at 1 min intervals (i.e., point samples) and recorded the identities of social partners and of neighbors resting within 1 m of and in contact (but not grooming) with the subject and food item if the subject was feeding (e.g., fruit, leaves, insects). They recorded whether a subject self-scratched or self-groomed during a given minute as a 0–1 occurrence and kept a continuous record of all affiliative approaches (i.e., an individual arriving and remaining within 1 m) and agonistic behavior (i.e., aggressive threats, lunges, growls, contact, and approach-retreat interactions), and social partners in either scenario. Focal follows occurred between 07:30 and 17:00, and observers chose focal subjects throughout the day to maintain even numbers of follows each week per subject, and per day-period (i.e., morning, midday and afternoon). We collected a total of 1591 h of behavioral data, averaging 39.0 ± 3.1 h per subject.

Although we aimed to collect both behavioral and biomarker samples at uniform time intervals, social behavior and biomarker samples could not be consistently paired over a time scale relevant to fGC clearance in the study species (i.e. ca. 24 h, Foerster and Monfort, 2010), limiting our ability to draw strong conclusions about the relationship of behavior to biomarker levels. Specifically, we collected behavioral samples at 2.4 ± 2.7 day intervals per subject-period (mean ± sd, N = 3837 follows), fecal samples at 12.8 ± 10.1 day intervals per subject-period (mean ± sd, N = 623 samples), and urine samples at 11 ± 9.3 day intervals (N = 610 samples). Nevertheless, only 25 ± 11% (mean ± sd) of a subject's fecal samples for GC metabolites were collected on the day after a focal follow, and overt social interaction was absent in 61 ± 18% of these pre-sample follows. Thus, 308 of 623 fecal samples were paired with 20 min follows on the previous day, and only 115 of these contained overt social interaction. Similarly, only 294 of 610 urine samples paired with previous-day follows, and only 177 of these contained overt social interaction.

From behavioral point samples, we calculated the proportion of each subject's observation time per period that it spent in a given

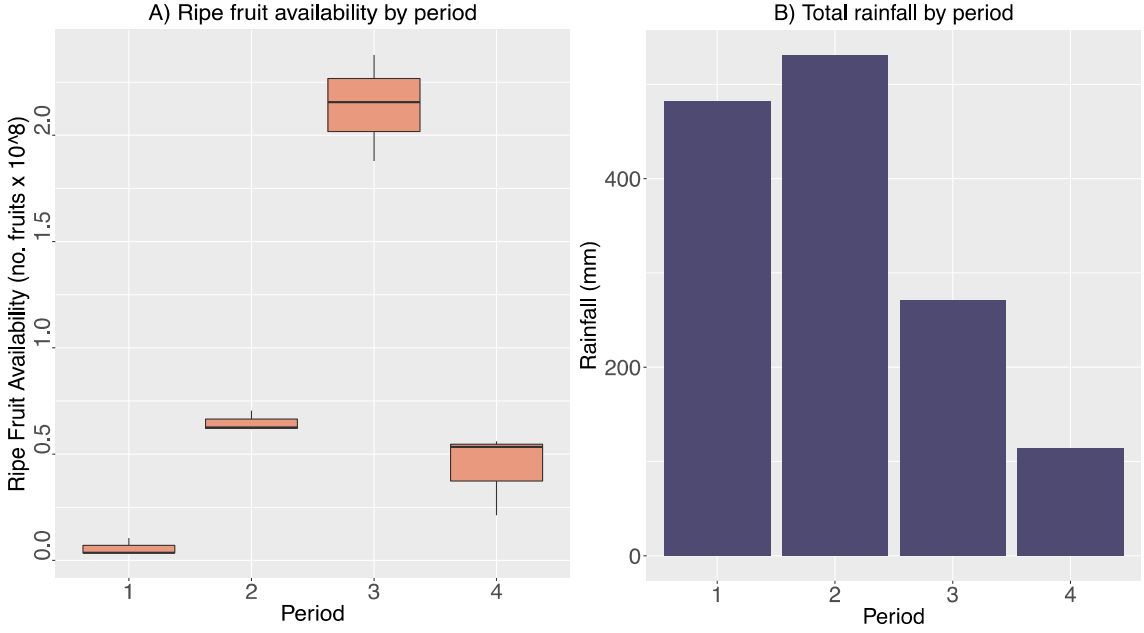

Fig. 2. Seasonal variation in A) group home-range-specific availability of ripe fruit (FAI; medians, IQRs, and ranges across 3 groups) and B) total rainfall by observation period.

activity (e.g., grooming, playing, resting). We pooled focal data to calculate each subject's number of affiliative partners (i.e., neighbors during resting, grooming and play partners) per period, and standardized them relative to the average amount of observation time it shared with other members of the study population.

We calculated subjects' tie or bond strength using a dyadic sociality index (DSI, Silk et al., 2013). The DSI included dyadic time spent grooming, sitting in contact without grooming, resting within 1 m without grooming, and hourly rate of approaches. We included these types of behavior because they were positively correlated among dyads within groups for each period (QAP matrix correlation with double Dekker semi-partialling technique, using function “netlm” in R package “sna”, Table S1; Dekker et al., 2007). The only affiliative behavior that did not consistently correlate positively with other types of affiliation was play (Table S1): therefore, we calculated a separate measure of the amount of play with closest partners. To calculate affiliative tie strength, we averaged DSIs over each subject's top 3 partners, excluding its mother, as ties with mothers are disproportionately strong relative to non-mothers among all juveniles (Cords et al., 2010). For play tie strength, we similarly averaged dyadic time spent playing among a subject's top 3 partners. We calculated subjects' rates of agonism given and received per hour of shared observation time from all-occurrence records during focal follows, pooled among subjects. Lastly, we calculated the proportion of observation minutes in which a subject self-scratched or self-groomed.

To characterize juveniles' social strategies, we entered behavioral variables in a principal components analysis (function “princomp” in R “base” package). We tested for sampling adequacy of all variables using a Kaiser-Meyer-Olkin test and for adequate correlations between variables using Bartlett's test (Budaev, 2010). We chose to retain 3 components based on Kaiser's rule, a scree test, and parallel analysis with 1000 iterations using the function “paran” in the R “paran” package (Dinno and Dinno, 2010; Zwick and Velicer, 1986). We chose not to rotate components after retaining them because rotation did not increase components' interpretability (Jolliffe, 2002).

2.6. Statistical analysis

For all analyses we used linear mixed models using the “lmer” function in R package “lme4” (Bates et al., 2014). We used standardized Z scores of continuous predictors for interpretability and standardized response variables to adjust for their scales (Schielzeth, 2010). We assessed collinearity of fixed effects via their variance inflation factors (“vif.mer” function in R (Frank, 2014) and confirmed normality of model residuals with Q-Q plots. We considered a predictor's influence on an outcome variable to be significant if the 95% confidence interval of its parameter estimate did not include zero (Nakagawa and Cuthill, 2007). Each model included subject ID as a random effect to account for repeated measures.

3. Results

3.1. Aim 1: Life history, competitive, and ecological predictors of energy balance, measured by uCP

To test P1–4 of variation in energy balance, we examined how life history status (age, sex), competitive environments (group size and maternal rank), and ecological variables (FAI measured over the 2-month periods, mean daily rainfall per period) predicted energy balance as measured by average time-residual uCP (N = 156 subject-periods). In agreement with P1, juvenile energy balance increased as the amount of ripe fruit increased in their home range (β = 0.15, 95% CI = 0.09–0.22, Fig. 3A,C, Table 1). Juvenile energy balance increased with higher average daily rainfall (β = −0.1, 95% CI = −0.17 to −0.03, Fig. 3A,D). Although ripe fruit availability corresponded roughly with lower rainfall across the four periods, these variables did not mirror one another (Fig. 2), and including them in a single model did not introduce problems of collinearity (maximum VIF in any model was < 2). Fruit availability and rainfall are therefore likely to have contributed independent effects on individual energy balance. Energy balance as measured by uCP concentrations was lower in males than females over all ages (LMM, β = − 0.15, 95% CI = −0.27 to −0.02) and showed a significant increase with age (β = 0.08, 95% CI = 0.01–0.15; Fig. 3A,B, Table 1). However, in contrast to P2, the difference between male and female energy balance did not increase with age (β sex (male) ∗ age = −0.01, 95% CI −0.15 −0.12). Sex differences in energy balance did not result from males and females' differential time feeding on fruit (LMM of % Time feeding on fruit, β sex (male) = 0.16, 95% CI −1.33 −1.64). Counter to P3–4, energy balance was not related to group size or maternal dominance rank. All VIFs in these models were < 2.5.

3.2. Aim 2: Life history, competitive, and energetic predictors of allostatic load, measured by mean dfGC per period

In agreement with P5, juveniles' average dfGC concentrations were lower when they maintained higher energy balance (β = −0.2, 95% CI −0.36 to −0.04; Fig. 4A Table 2). In contrast to P6, maternal dominance rank did not predict dfGCs (Fig. 4A, Table 2), nor did the relationship between energy balance and dfGCs vary according to rank (rank ∗ uCP β = 0.06, 95% CI −0.11–0.22). Juvenile age, sex, and group size also did not predict dfGC concentrations. All VIFs in these models were < 2.5.

3.3. Aim 3a: Social strategies characterized by principal components

All social variables had appropriate sampling adequacy for PCA (Kaiser-Meyer-Olkin test, min measure of sampling adequacy (MSA) = 0.57, overall MSA = 0.72; Dziuban & Shirky, 1974). Correlations between variables were also appropriately strong for PCA (Bartlett's test, $\chi^2(45) = 601.64$ $p < .0001$). Both Kaiser's rule and parallel analysis indicated that retaining the top 3 PCs was appropriate and these collectively explained 65% of variance in behavioral measures (Table S2).

We characterized each component according to the variables that loaded on it most strongly. Grooming and play measures both loaded strongly on PC1, however in opposite directions (% Time grooming and playing loadings = 0.43 and −0.41, respectively, Table S2). This led us to characterize PC1 as the groomer vs. player component. Number of play partners (0.38), number of neighbors while resting (0.56), and both agonism given (0.43) and received (0.44) loaded strongly on PC2. Variables loading weakly or in the opposite direction included time spent grooming (−0.04) and rates of self-directed behavior (−0.09), which either do not require a diversity of partners (time grooming) or are largely done solitarily (self-directed behavior). Indeed, 89% of self-directed behavior occurred when subjects were resting and not within 1 m or in contact with neighbors. Given this pattern of loadings, we characterized PC2 as social vs. solitary. Of the several variables that loaded strongly on PC3, only agonism given loaded positively (0.43). Variables that loaded negatively were socio-positive (e.g., play and affiliative bonds, −0.35 & −0.44) or solitary (e.g., self-directed behavior, −0.49), leading us to characterize PC3 as aggressive vs. peaceful.

As part of the PCA of juveniles' social strategies, we also assessed how life history (age, sex), demographic (number maternal kin, maternal rank), and ecological variables (FAI, rainfall) predicted scores on PCs 1–3 per period (N = 162 subject-periods). Scores on PC1 were lower in males than females (β = −2.79, 95% CI −3.17 to −2.4). Older individuals were more likely to have higher scores on PC1 (β = 0.47, 95% CI 0.28–0.68). Scores on PC1 decreased (less grooming, more play) when ripe fruit was more available (β = −0.26, 95% CI −0.41 to −0.10) and increased with greater rainfall (β = 0.30, 95% CI

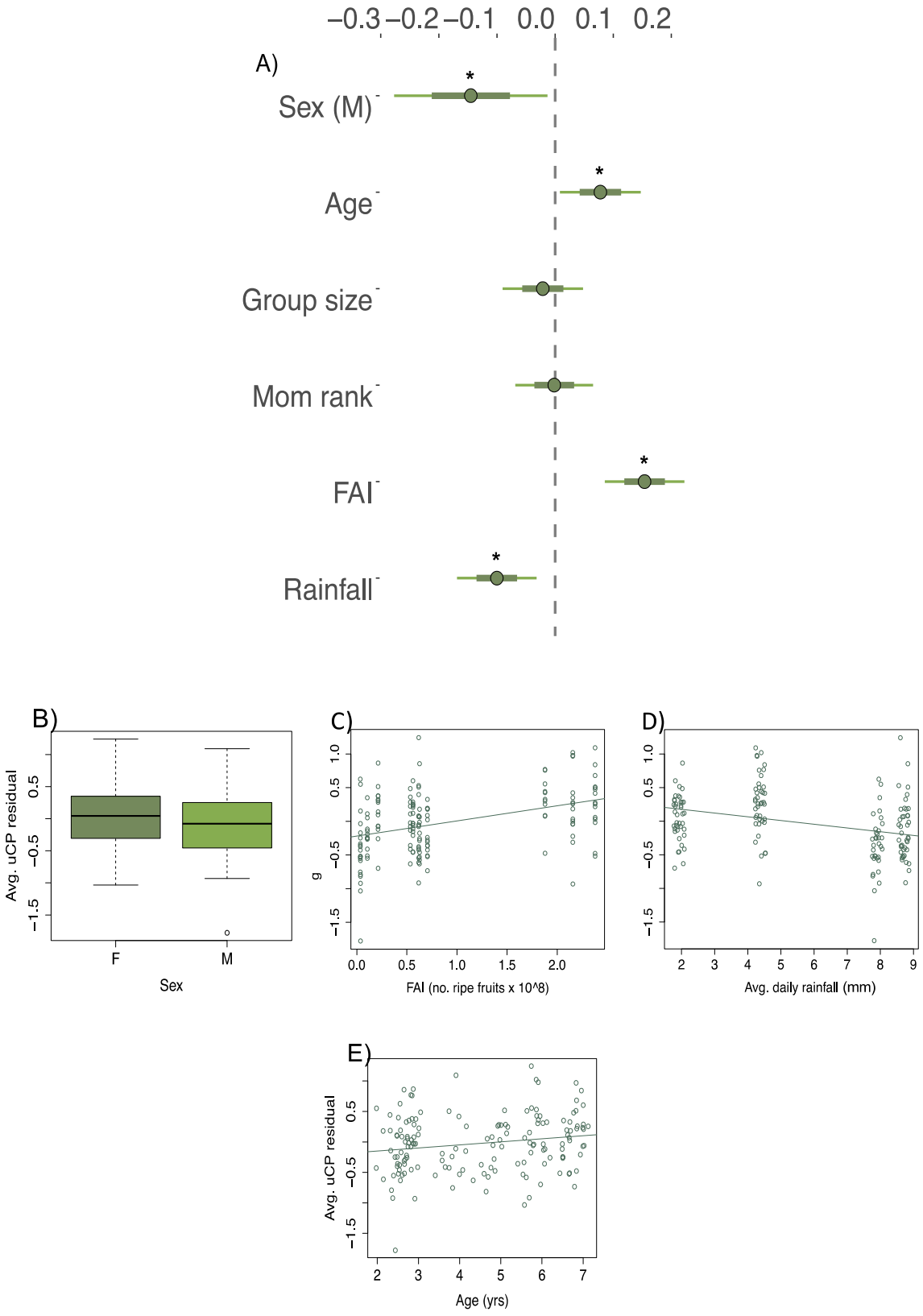

Fig. 3. Variation in juvenile average time-residual of log urinary c-peptide (uCP) concentrations per period as predicted by life history, competition, and physical environment. A) Relationship of standardized predictors with subject uCP concentrations in linear mixed model. Point is restricted maximum likelihood estimated coefficient. Thick and thin lines represent 50 and 95% confidence intervals, respectively. Dashed vertical line indicates a parameter estimate of zero. * 95% CI does not include zero. Variation in average uCP residuals by B) sex, C) fruit availability index, D) average daily rainfall, and E) age. Trend lines added with simple linear regression.

Table 1
Relationship of standardized predictors on energy balance as measured by average time-residual of log urinary c-peptide (uCP pg/ml urine) vs. time of day. Linear mixed effects regression, N = 156 subject-periods.

Predictor	Beta	SE	95% CI
Sex (M)	**−0.15**	**0.07**	**[−0.27, −0.02]** *
Age	**0.08**	**0.04**	**[0.01, 0.15]** *
Group size	−0.02	0.04	[−0.09, 0.05]
Maternal rank	0	0.03	[−0.07, 0.06]
FAI	**0.15**	**0.03**	**[0.09, 0.22]** *
Rainfall	**−0.10**	**0.03**	**[−0.17, −0.03]** *

Model results for significant relationships in bold.
* 95% CI does not cross zero.

Table 2
Individual life history, competitive environment, and energy balance's relationship with allostatic load, measured as average deviation from its baseline fGC level (dfGC ng/g feces). Linear mixed effects regression, *N* = 155 subject-periods.

Predictor	Beta	SE	95% CI
Sex (M)	−0.01	0.16	[−0.32, 0.31]
Age	−0.003	0.09	[−0.17, 0.16]
Group size	0.04	0.08	[−0.12, 0.20]
Maternal rank	0.01	0.08	[−0.14, 0.17]
Energy balance	**−0.2**	**0.08**	**[−0.36, −0.04]** *

Model results for significant relationships in bold.
* 95% CI does not cross zero.

Table 3
Exploratory results: individual social strategies' relationship with allostatic load, measured as average deviation from its baseline glucocorticoid concentration (dfGC ng/g feces). Linear mixed effects regression, *N* = 155 subject-periods.

Predictor	Beta	SE	95% CI
PC1: grooming vs. playing	**0.18**	**0.07**	**[0.05, 0.32]** *
PC2: social vs. solitary	−0.01	0.07	[−0.15, 0.13]
PC3: aggressive vs. peaceful	0.04	0.09	[−0.13, 0.20]
Sex (M)	**0.51**	**0.26**	**[0.01, 1.01]** *
Age	−0.08	0.09	[−0.25, 0.10]
Group size	−0.02	0.10	[−0.22, 0.18]
Maternal rank	0.02	0.08	[−0.14, 0.18]
Energy balance	**−0.16**	**0.08**	**[−0.32, −0.004]** *

Model results for significant relationships in bold.
* 95% CI does not cross zero.

0.14–0.46). Juveniles had higher scores on PC2 as they aged (β = 0.23, 95% CI 0.03–0.45), and lower scores (fewer social partners, less agonism) with higher fruit availability and rainfall (FAI β = −0.22, 95% CI -0.38 – −0.06; rainfall β = −0.42, 95% CI −0.58 to −0.25). Scores on PC3 (aggressive vs. peaceful) did not vary according to life history or ecological variables. All VIFs in these models were < 2.5.

3.4. Aim 3b: Relationship of social strategies with allostatic load

To examine whether affiliative and aggressive social behavior predicted allostatic load and appeared to buffer energetic stressors (P7–P9), we included three retained principal components, age, sex, maternal dominance rank, and energy balance as fixed effects in a linear mixed model predicting mean dfGC per 2-month period (*N* = 155 subject-periods). We also tested for an interaction effect between PC1 and sex. Juveniles' dfGCs varied according to social behavior as measured by PC1 (grooming vs. playing), such that dfGCs were higher among subjects that groomed more and played less (i.e., had a higher score on PC1, β = 0.18, 95% CI 0.05–0.32, N = 155; Fig. 4A, B, Table 3). Thus in partial agreement with P7 that affiliative behavior and dfGCs would be negatively related, more time spent playing corresponded with lower dfGCs, but contrary to P7, more time grooming corresponded with higher dfGCs. DfGCs did not relate to subjects' scores on social vs. solitary (PC2) and, counter to P8, aggressive vs. peaceful (PC3) components.

In these models, males had slightly higher average dfGCs than females (β sex = 0.51, 95% CI 0.01–1.01, Table 3), which likely emerged when controlling for social strategy because of males' frequent participation in social play. Nevertheless, the relationship of PC1 with dfGCs did not vary according to sex (β PC1 * sex = −0.04, 95% CI −0.36–0.29). General activity level also did not significantly predict dfGCs (LMM, % Time locomoting replacing all behavioral PCs in model, β locomoting = −0.11, 95% CI = −0.29–0.07). All VIFs were < 2.8, except in the interaction model of PC1 by sex where VIFs were < 7.15.

Again in agreement with P5, juveniles' energy balance was

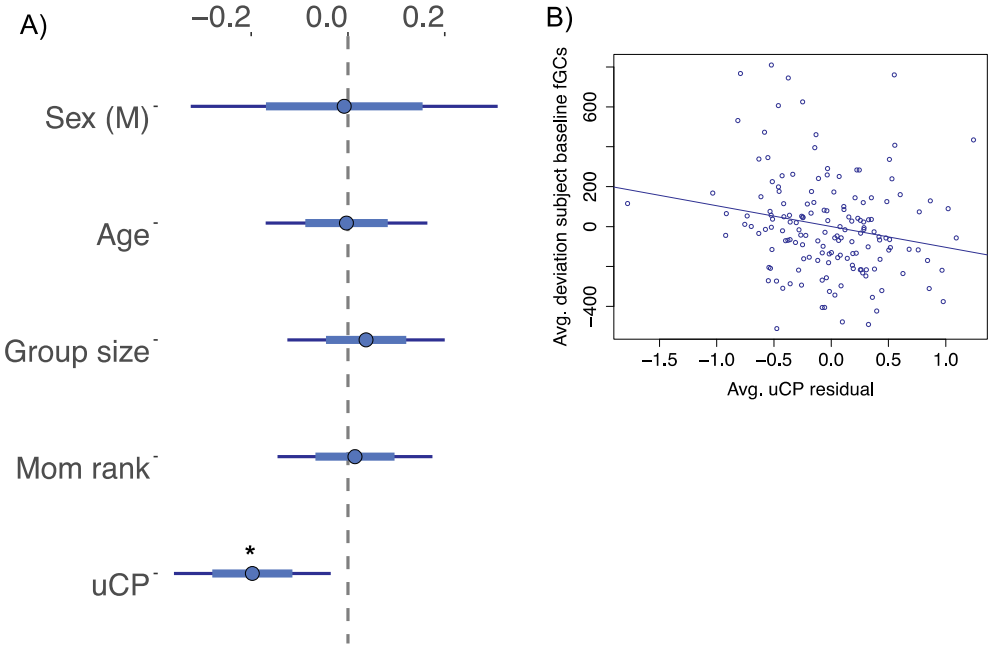

Fig. 4. Variation in juvenile average deviation in fecal glucocorticoid (dfGC) concentrations per period according to life history, competition, and energy balance as measured by average time-residual of log urinary c-peptide (uCP). A) Relationship of standardized predictors with subject dfGC concentrations. Point is restricted maximum likelihood estimated coefficient. Thick and thin lines represent 50 and 95% confidence intervals, respectively. Dashed vertical line indicates a parameter estimate of zero. *95% CI does not include zero. B) Variation in dfGCs by energy balance. Trend lines added with simple linear regression.

Table 4
Relationships with average deviation from baseline glucocorticoid concentration (dfGC ng/g feces) of % time grooming and % time playing, variables that loaded most strongly on PC1, and their interactions with energy balance in linear mixed effect models. Significant relationships in bold. N = 155 subject-periods.

Response variable	Predictor variables	β	95% CI
fGCs	**% Time grooming**	**0.26**	**[0.06, 0.46]**
	Sex (M)	0.32	[−0.08, 0.72]
	Age	−0.06	[−0.23, 0.11]
	Group size	0.07	[−0.09, 0.22]
	Maternal rank	0.03	[−0.13, 0.18]
	Energy balance	**−0.17**	**[−0.33, −0.01]**
fGCs	**% Time grooming**	**0.29**	**[0.09, 0.49]**
	Sex (M)	0.34	[−0.05, 0.73]
	Age	−0.08	[−0.24, 0.09]
	Group size	0.07	[−0.09, 0.23]
	Maternal rank	0.05	[−0.11, 0.2]
	Energy balance	**−0.18**	**[−0.34, −0.03]**
	% Time grooming: Energy balance	**0.19**	**[0.05, 0.33]**
fGCs	**% Time playing**	**−0.22**	**[−0.4, −0.04]**
	Sex (M)	0.24	[−0.13, 0.6]
	Age	−0.01	[−0.17, 0.15]
	Group size	0.01	[−0.15, 0.17]
	Maternal rank	0.02	[−0.13, 0.18]
	Energy balance	**−0.18**	**[−0.34, −0.02]**
fGCs	**% Time playing**	**−0.23**	**[−0.41, −0.05]**
	Sex (M)	0.25	[−0.12, 0.62]
	Age	−0.02	[−0.18, 0.15]
	Group size	0.02	[−0.14, 0.18]
	Maternal rank	0.03	[−0.13, 0.19]
	Energy balance	**−0.19**	**[−0.35, −0.03]**
	% Time playing: Energy balance	−0.06	[−0.23, 0.11]

negatively related to average dfGC concentrations in the social strategies model (β = −0.16, 95% CI −0.32 to −0.004; Fig. S1A Table 3). In this model, dfGCs again did not vary according to age, group size, or maternal rank.

Because PC1 represented time spent both playing and grooming, we created two post-hoc linear mixed models to test how percentage of time grooming and playing each corresponded to dfGC concentrations (Table 4). According to these models, the relationship of PC1 and dfGCs reflected both a positive association of percentage time grooming on dfGCs and a negative association of percentage time playing on dfGCs (Table 4).

To then test P9 that affiliation would buffer energetic stressors, we assessed whether time spent playing or grooming moderated the relationship between energy balance and dfGCs. Counter to P6, play behavior did not moderate the negative relationship of energy balance on dfGCs, but grooming behavior did. Surprisingly, energy balance had an increasingly *positive* relationship with dfGCs as time spent grooming increased (Table 4), again counter to P6. Increasing energy balance should not increase HPA activity directly (Sapolsky et al., 2000). Therefore one might best express this interaction as the higher a juvenile's energy balance, the more strongly its grooming positively corresponded with dfGCs (Fig. 5A). Indeed, it appeared that juveniles that spent the most time giving and receiving grooming were those that were most likely to have a positive relationship between energy balance and dfGCs (Fig. 5B).

Because percentage time grooming moderated the relationship between energy balance and dfGCs in an unexpected way, we further predicted that individuals spending the most time grooming could be grooming with less familiar social partners, such as non-kin. We found that individuals in the top grooming quartile spent significantly more time grooming non-kin vs. kin, but there were no differences in grooming non-kin vs. kin among subjects in other quartiles (LMM comparing % time grooming non-kin vs. kin, resetting reference level for each quartile, β quartile 4 non-kin vs. kin = 0.9, % 95 CI 0.41–1.39, Fig. 5C, Table S3).

4. Discussion

Consistent with the ecological risk aversion hypothesis, energy balance appears to contribute strongly to allostatic load in juvenile blue monkeys, as their dfGC concentrations decreased with higher energy balance. We did not find evidence that life history status (i.e., sex, age) or competitive environment (group size, maternal dominance rank) corresponded with dfGCs, nor did maternal rank modify the relationship between energy balance and dfGCs. Simultaneously, individual energy balance itself did not vary according to competitive environment, though energy balance was higher among females and older juveniles.

We cannot draw hard and fast conclusions from our exploratory analysis of juvenile social strategies because of the limited temporal concordance between social behavior and biomarker sampling (see Methods). Results suggest that juveniles' social strategies correspond with dfGC concentrations. Analyzing the proximate effects of affiliation on allostatic load among juveniles in a future, focused study is therefore warranted. Juveniles that groomed more and played less had higher dfGCs. Neither grooming nor playing appeared to buffer juveniles' experiences of energetic stressors. In fact, dfGCs surprisingly increased with energy balance among juveniles that groomed the most. Time playing had no such moderating effect on energy balance and dfGCs. It appeared that juveniles that groomed most were engaging less familiar partners, as only they groomed more with non-kin than kin.

4.1. Variation in energy balance during development (Aim 1)

Juvenile energy balance, as measured by urinary C-peptide, increased with the availability of ripe fruit in the habitat, as reported for wild chimpanzees and orangutans (Emery Thompson and Knott, 2008; Emery Thompson et al., 2009). Thus, as in other primate species (e.g. Deschner et al., 2008; Girard-Buttoz et al., 2011; Grueter et al., 2014; Harris et al., 2010; Higham et al., 2011a), uCP also appears to be a valid measure of energy balance in wild juvenile blue monkeys, confirming the general usefulness of this biomarker for investigating the association of environmental, social and life history variables with energetic condition of wild-living primates.

Females had higher energy balance than males, though they did not spend a larger percentage of their observation time feeding on fruit than males. Future studies of feeding and nutritional strategies might explore whether males and females select different qualities of food or feed at different rates during development. It is also possible that males had lower energy balance on average than females because males spent more time playing, which is energetically costly (Held and Špinka, 2011). Sex differences in energy balance did not appear to result from the growth spurt that males undergo prior to dispersal and puberty, as uCPs did not vary with age differently according to sex. As energy balance increased with age, challenges to meet energy expenditure with intake appear greater in early development.

Neither group size nor maternal rank predicted juvenile energy balance, even though juveniles lived in groups of various sizes and higher rank has corresponded with more time feeding on fruits among adult females (Foerster et al., 2011). It is possible that individuals in larger groups spread out to avoid competition and that access to food does not differ with dominance rank as strongly in juvenility as it does during adulthood. Finally, perhaps because heavy rainfall led individuals to cease all activity, including feeding (pers. obs.), individuals had lower energy balance during rainy periods.

4.2. Relationship of energy balance with juvenile allostatic load (Aim 2)

As predicted, juveniles with higher energy balance had lower dfGCs.

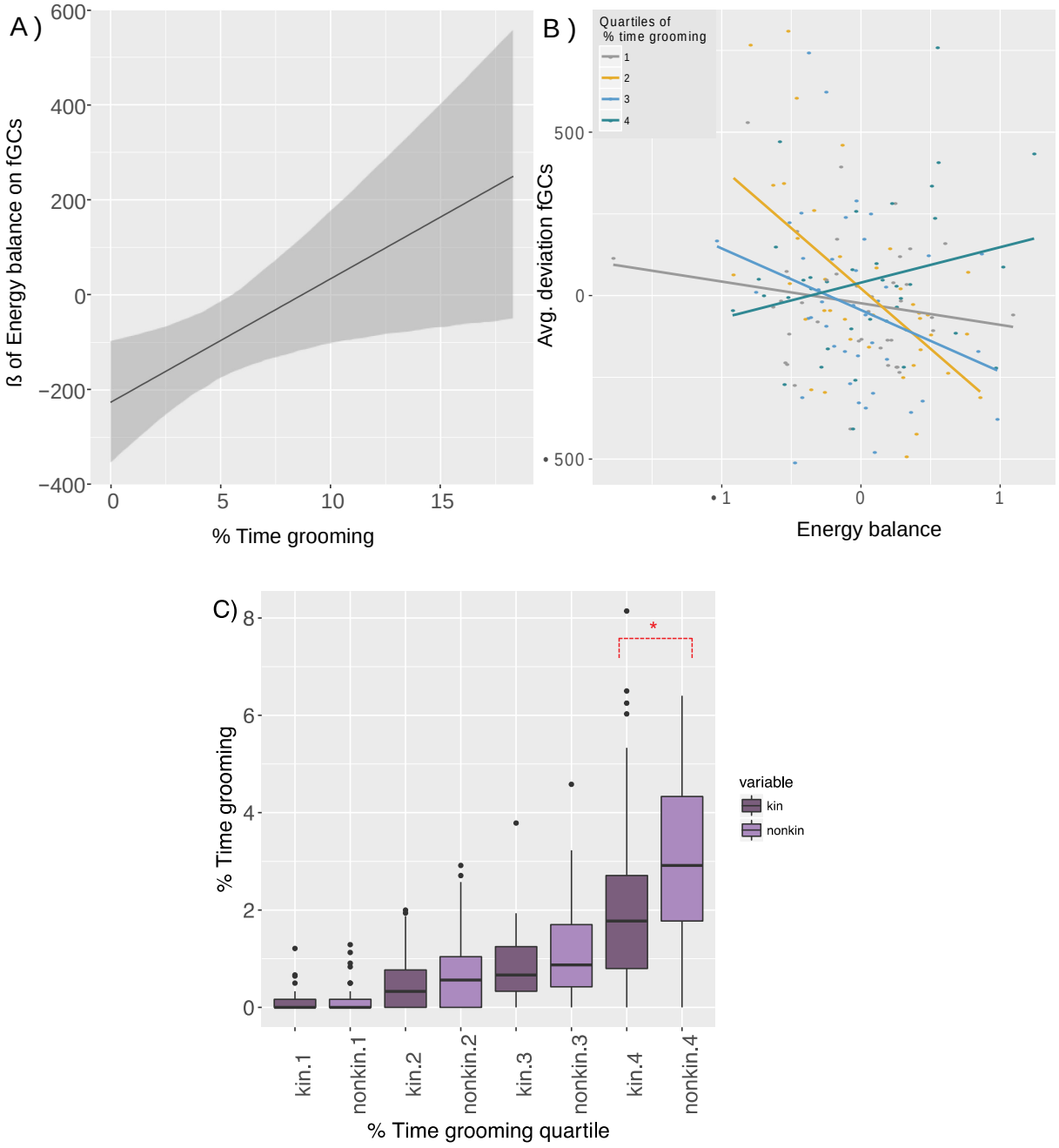

Fig. 5. Exploring the relationship between time grooming and deviation in fecal glucocorticoid (dfGC) concentrations. A) Conditional coefficient of average time-residual of log urinary c-peptide (uCP) concentrations on dfGCs as % time grooming varies. Predictors unstandardized. B) dfGC concentrations on uCP concentrations by quartiles of % time grooming. C) % Time grooming kin vs. non-kin among subjects in each grooming quartile. We reset the reference quartile-kinship class in an LMM to compare time grooming with kin vs. non-kin within each quartile and found a significant difference within the top quartile only.

This finding reveals that energy balance is indeed a driver of allostatic load among juveniles, consistent with the ecological risk aversion hypothesis that juveniles' grow slowly in part to avoid the risk of starvation. Importantly, *all* juveniles appeared prone to experience energetic stressors, not just low-ranking individuals as in adult female blue monkeys (Foerster et al., 2011, 2012). DfGCs did not vary with age, similar to patterns seen in juvenile baboons (Gesquiere et al., 2005), suggesting that individuals experience similar levels of internal and external stressors throughout development. Similarly, maternal dominance relations did not confer any advantage to juveniles in terms of avoiding stressors. Although counter to our prediction that the threat of aggressive competition among lower-ranking juveniles would correspond with higher dfGCs, the absence of a relationship between rank and dfGCs does concur with other studies that showed limited consequences of adult female dominance relations in this species (Cords, 2000; Foerster et al., 2011; Pazol and Cords, 2005; Roberts and Cords, 2013).

4.3. Social strategies during development and their ecological and life history correlates (Aim 3a)

PCA revealed that playing and grooming accounted for the greatest variation in juvenile behavioral patterns (PC1), and were driven by differences in age, sex, fruit availability, and rainfall. Sex differences in playing and grooming in this species are pronounced, and higher scores on PC1 among older individuals likely derive from females increasing

their time spent grooming with age (Cords et al., 2010; Thompson and Cords, in prep.). PC2, characterized as social vs. solitary, revealed that interacting with more affiliative partners frequently corresponded with giving and receiving agonism from them. Sexes did not differ in their scores on PC2 and, with age, both sexes appeared to expand their social networks and, concomitantly, their involvement in agonism. Scores on PC1 and 2 both decreased with higher fruit availability possibly because juveniles are more dispersed in the forest when foraging for widely available fruit, though males may overcome dispersion to play more often when fruit provides high energy stores (Thompson and Cords in prep.).

4.4. Preliminary findings of social strategies' role in juvenile allostatic load (Aim 3b)

Again, given that the majority of 20 min focal follows on days prior to fecal and urine sampling contained zero overt social interaction, we limit our confidence in the relationships identified between allostatic load and social behavior. Juveniles appeared to have higher dfGC concentrations when they spent more time grooming and were more bonded with grooming partners (opposite to what was reported in adult females over similar multi-month periods; Foerster et al., 2011), and if they spent less time playing and were less bonded to close play partners (i.e., had higher scores on PC1). These patterns appear to result from both a positive relationship between grooming and dfGCs, and a negative relationship between play and dfGCs. Although the latter relationship was expected, the former was not.

Neither play nor grooming appeared to act as a buffer of energy balance on dfGC concentrations. In fact, the relationship between energy balance and dfGCs was increasingly positive as time spent grooming increased, a reverse of the main effect of dfGC concentrations decreasing with energy balance. This suggests that grooming with partners could present a stressor to juveniles, particularly when fruit availability is high.

The relationship between GCs and sociality in juvenile blue monkeys, if true, may proceed in either causal direction. Energy mobilized by GCs could motivate affiliative behavior because an individual recently experienced a stressor (Stoewe et al., 2008) or because of an individual's general metabolic state leads them to be more active (Anestis, 2005). Nevertheless, one would expect grooming to correspond with lower GCs if it acts as a buffering strategy, and further, general activity levels did not predict higher dfGCs in blue monkey juveniles. It is perhaps more likely then that overt affiliation like playing and grooming could alter GC concentrations. Play may transform already potentially aggressive situations into friendly ones, as in adult sifakas (Antonacci et al., 2010), thereby lowering GCs, as in juvenile marmosets (Mustoe et al., 2014). Meanwhile, navigating social groups by grooming could be risky because maintaining proximity and contact introduces opportunities for agonism (Kalbitzer et al., 2017; Moser et al., 1991; Schino and Alessandrini, 2015). For example, in Japanese macaques, individuals that groom are more likely to subsequently receive agonism from groomees, although over time groomees reduce agonism and become more tolerant of groomers (Schino and Alessandrini, 2015). Although direct involvement in agonism (i.e., high scores on PC2) did not correspond with higher dfGCs in the juvenile study subjects. Grooming could also be stress-inducing because of the unfamiliarity of the grooming partner, e.g. non-kin vs. kin. Indeed, among savannah baboons, unrelated individuals are more likely to aggress juveniles (Pereira, 1988) and in chimpanzees, adults consistently have higher urinary cortisol after bouts of grooming non-bond partners than after bouts of grooming bond partners, with whom they are more familiar (Wittig et al., 2016). If blue monkey juveniles often groom with non-kin in feeding contexts, this might lead to the observed positive relationship between energy balance and dfGCs among individuals that groomed the most.

5. Conclusions and future directions

This study is the first to show in detail that energy balance is a major driver of allostatic load in a juvenile primate. Indeed, all juveniles regardless of sex, age, group size, or maternal dominance rank, were vulnerable to energetic stressors. As energy balance varied strongly according to seasonal fruit availability and rainfall, juveniles' risk of experiencing energetic stressors also appears seasonal. Future studies can employ C-peptide as a measure of energy balance in testing the function of extended juvenile periods in avoiding ecological risks. Comparative analyses to further test the ecological risk aversion hypothesis could also compare the relationship of CP and GCs among juveniles in species whose juvenile period varies in length (relative to total lifespan), with larger effects of CPs on GCs expected in species with longer juvenile periods.

Contrary to our initial hypothesis regarding social strategies and allostatic load, the results of our exploratory analysis suggest that affiliative behavior does not always help juveniles cope with social and ecological challenges, but rather appears to exert challenges of its own. Indeed, overt affiliation such as grooming could be challenging to individuals at this vulnerable life stage when grooming occurs with unfamiliar and potentially risky partners. In contrast, engaging in social play may decrease social uncertainty and corresponding allostatic load. Time-pairing social behavioral and biomarker samples by targeting juvenile subjects for glucocorticoid and C-peptide sampling immediately following social interaction could be a particularly useful study design for testing this hypothesis in the future (e.g., Wittig et al., 2016).

A further possibility is that, while overt affiliation is perhaps a short-term cost for juveniles, it could yield and be outweighed by a long-term benefit, especially for individuals of the philopatric sex. These individuals will spend the rest of their life potentially cooperating and competing with their groupmates, including non-relatives, so investing in partners during development is a way to develop familiarity and potentially life-long ties (e.g. Fairbanks, 2003). Research on adult female blue monkeys and baboons (Silk et al., 2010; Thompson and Cords, 2018) shows that if females are able to develop strong affiliative relationships with social partners that are consistent over years, this social investment pays off in greater survival. Data from long-term data sets will be particularly valuable to evaluate this hypothesis in future studies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2019.104664.

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