Physiology & Behavior 127 (2014) 13–19

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Socioecological correlates of energy balance using urinary C-peptide measurements in wild female mountain gorillas

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HIGHLIGHTS

- Quantifying energy balance in wild animals is fraught with difficulties.
- Urinary C-peptides are a valuable biomarker of energy balance in primates.
- We measured C-peptide levels in female mountain gorillas in Rwanda.
- C-peptide levels were significantly influenced by habitat, season and day time.
- Ecological variability can affect energy balance even in herbivorous species.

ARTICLE INFO

Article history:
Received 29 May 2013
Received in revised form 22 October 2013
Accepted 14 January 2014

Keywords:
Gorilla beringei beringei
Energy balance
Urinary C-peptide
Seasonality

ABSTRACT

Maintaining a balanced energy budget is important for survival and reproduction, but measuring energy balance in wild animals has been fraught with difficulties. Female mountain gorillas are interesting subjects to examine environmental correlates of energy balance because their diet is primarily herbaceous vegetation, their food supply shows little seasonal variation and is abundant, yet they live in cooler, high-altitude habitats that may bring about energetic challenges. Social and reproductive parameters may also influence energy balance. Urinary C-peptide (UCP) has emerged as a valuable non-invasive biomarker of energy balance in primates. Here we use this method to investigate factors influencing energy balance in mountain gorillas of the Virunga Volcanoes, Rwanda. We examined a range of socioecological variables on energy balance in adult females in three groups monitored by the Karisoke Research Center over nine months. Three variables had significant effects on UCP levels: habitat (highest levels in the bamboo zone), season (highest levels in November during peak of the bamboo shoot availability) and day time (gradually increasing from early morning to early afternoon). There was no significant effect of reproductive state and dominance rank. Our study indicates that even in species that inhabit an area with a seemingly steady food supply, ecological variability can have pronounced effects on female energy balance.

1. Introduction

Maintaining a positive energy balance by juggling uptake and expenditure is key to survival and reproduction [1–4]. Because animals rarely live under stable, unchanging conditions, a variety of factors may cause energy balance to vary in the short and/or long term. Variability in food availability may be caused by seasonal variation or small scale patterns of habitat structure, which may in turn lead to fluctuations in energy intake and/or energy expenditure [5,6]. Energy balance also can vary according to reproductive condition [7,8] or level of infection [9]. For animals living in social groups, especially primates, energy balance and its fitness consequences are predicted to be influenced by socioecological parameters such as dominance rank and group size [10–13]. Furthermore, the question remains as to whether animals living in less seasonal, more stable environmental conditions, yet still experience variation in reproductive status and social conditions, are able to maintain a stable energy balance even with minor environmental fluctuations.

The goal of this study is to investigate ecological, reproductive, and social parameters that may influence variability in energy balance in wild mountain gorillas. Mountain gorillas (*Gorilla beringei beringei*) of the Virunga Massif are an interesting species in which to investigate environmental and social correlates of energy balance because their food supply shows little seasonal variation and is abundant, easily attainable, and easily digestible [14]. However, they also live at one of the highest altitudes, up to 3800 m [15, Grueter, unpubl.], and hence cooler environments of any primate species. While the diet of the Virunga mountain

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http://dx.doi.org/10.1016/j.physbeh.2014.01.009

gorillas largely consists of abundant herbs available year-round, one important dietary item, highly nutritious bamboo shoots, is available only during some months of the year and in a distinct habitat type [16–18]. Additionally, there are distinct wet and dry seasons, with corresponding variation in average daily temperature, which could affect thermoregulatory and energy balance patterns on a daily and seasonal level [19,20].

Mountain gorillas live in stable social groups, which is predicted to result in feeding competition and variability in individual fitness, depending on the distribution of resources and group size [21,22]. Because of the relatively even distribution and constant abundance of the dietary items mountain gorillas consume, it has been hypothesized that they experience little feeding competition with dominance rank expected to have little impact on female reproductive success. However, dominance rankings of adult females do remain fairly robust and stable for many years [23–25] and a positive, but weak correlation between dominance ranks and reproductive success has been observed [22].

Few studies have measured energy balance in wild primates [but see 8,26–30] and other mammals [31,32] because obtaining accurate measures is notoriously difficult, requiring time-intensive observations of intake rates, activity budgets, distance traveled or some other proxy for energy expenditure, and the energy content and estimations of digestibility of food items [e.g. 5,29,33]. To assess energy balance in female mountain gorillas we measured the levels of urinary C-peptide (UCP). C-peptide is a small polypeptide produced during insulin synthesis in the pancreas and secreted in equimolar amounts as insulin into the bloodstream [34–36]. In controlled tests, UCP levels correlated positively with changes in body mass [37,38] and in wild living animals with estimates of food intake [39]. An increasing number of studies in feeding ecology in wild living primates have used UCP as a marker of energy balance, examining correlations with environmental, physiological or social conditions. For example, UCP levels were found to decrease with decreasing food availability [9,40] and to decrease during periods of elevated endurance rivalry in rhesus macaques [41].

Combining UCP measurements with behavioral and ecological data, we tested several predictions. First, we examined if urinary energy balance changed in relation to season and time spent in different habitat types, including bamboo. Furthermore, because many hormones exhibit diurnal effects [42] and the gorillas have daily cyclic patterns of feeding and resting periods [43], we also considered if time of day correlated with UCP levels. Because gestation and (early) lactation are phases of high energy needs for female primates/mammals [44–48], reproductive state was also predicted to cause changes in energy balance. In humans and chimpanzees UCP levels were particularly low during early lactation as a result of low insulin production (brought about by the energetic requirements of milk production, [49]), then gradually increased during lactational amenorrhea and then slightly dropped just before menstrual resumption [50,44]. Therefore, we expected UCP levels to be higher in pregnant than in lactating or cycling females because of the physiological changes that the body undergoes during this period, most notably a progressive decline in insulin sensitivity [51,52]. Lastly, the relationship between dominance rank and C-peptide levels in female primates has not yet been evaluated, but studies have shown that rank can positively influence energy gain [29,53; Wridght et al., unpublished]. We thus predicted that dominant females would have higher levels of urinary C-peptide as a result of access to higher quality food and/or higher quantities of food.

2. Methods

2.1. Behavioral observations

Behavioral observations were made between October 2009 and December 2010 in Volcanoes National Park, Rwanda, an Afromontane forest (ca 2500–4500 m) that is characterized by a mosaic of vegetation communities [15,54]. The study area consisted of three distinct habitat types: *bamboo* vegetation between 2500 and 2800 m, *Hagenia–Hypericum* woodland interspersed with open canopy herbaceous slopes between 2800 and 3300 m, and the subalpine and *alpine* zones between 3300 and 3800 m [for details on habitat composition, see 15,54]. There are two distinct rainy seasons, from March to May and from September to December, with dry seasons in between. Annual rainfall in Bisate (altitude 2400 m), ca. 2 km from the park boundary was 1823 mm and 2076 mm in 2009 and 2010, respectively.

Three habituated gorilla groups monitored by the Karisoke Research Center were observed for a maximum of 4 h per group per day between 0800 and 1500 h. Longer observation periods were not allowed, as per regulations of the Rwanda Development Board to minimize anthropogenic disturbance. Group size averaged 45 in Pablo (with five silverbacks and 11 females), eleven in Ntambara (with two silverbacks and four females), and eleven in Bwenge (with one silverback and five females). All adult females ($n = 20$) aged eight years or older were included in the study; one was nulliparous and all the others were parous. Females were classified into the reproductive state of pregnant, lactating or having cyclic ovarian activity. Pregnancy was determined based on the date of birth and back-counting a gestation length of 8.5 months [55]. We assumed that females were exhibiting estrous cycles for the 3–4 months prior to pregnancy, and were lactating for the remainder of the time [55]. Four females were pregnant during most of the study, 12 lactating and four cycling. Three of them transitioned from pregnancy to lactation during the study period and three from cycling to pregnancy.

We conducted focal animal sampling (either 30 min or 50 min periods) on all the adult females on a rotating basis during which incidents of aggression, spatial displacements and avoidances were recorded. Each female was observed for an average of 68.4 h (range: 60.7–97.8 h) of focal animal sampling. Concurrently, instantaneous scan samples were conducted at 10-min intervals during which the activity (feed, rest, travel, groom, etc.) of the focal individual, the habitat type and, in case of feeding, the food item being consumed were recorded.

The proportion of time each group spent in the three habitat zones and the percentage of bamboo shoots in the diet were calculated on a monthly basis from instantaneous scans. The total number of scans were 6170 in Pablo (2250 feeding scans), 2254 in Ntambara (1188 feeding scans) and 2287 in Bwenge (882 feeding scans). Scan data were evenly distributed across months throughout the sampling period, but there was some variation among months (mean per month for all three groups: 1190, standard deviation = 368.7, range = 691–1778).

Dominance ranks of the females were based on the outcomes of dyadic approach–retreat events (displacements and avoidances) observed during focal animal sampling and all occurrence observations. Dominance matrices were calculated using the I&SI method [56] using MATMAN with an interpolation procedure [57]. We then standardized the rankings for each group. All females with a standardized rank larger than 0.5 were assigned high ranks whereas those smaller than 0.5 were considered low-ranking.

2.2. Sample collection

Urine samples were collected on an ad libitum basis between 08:00 h and 14:30 h from April to December 2010 by field assistants and researchers. A total of 170 samples were collected from 20 adult female gorillas (mean per individual: 8.2, SD = 4.4; range = 2–19). We used disposable plastic pipettes to collect urine (at least 0.3 mL per sample) from vegetation and from leaves on the ground and then subsequently pipetted the urine into 2 mL Cryovials. Samples contaminated with feces or soil were discarded. Tubes were labeled with identity of individual, date, time and habitat type where sample was collected (this is typically also the habitat type where the gorillas spent the hours prior to sample collection) stored in an insulated freezer bag until upon arrival in camp they could be transferred into a freezer (temperature: −20 °C). Storing samples after collection on ice in a freezer bag for a limited time does not have a significant effect on UCP levels [38]. Samples were kept on dry ice while being transported from Rwanda to the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany. This research adhered to all animal welfare, legal and ethical requirements of Rwanda.

2.3. Sample preparation and C-peptide measurement

In the lab, frozen urine samples were thawed at room temperature. After 10 s of shaking (VX-2500 Multi-Tube Vortexer) the samples were centrifuged for 10 min at 2000 *g* (Multifuge Heraeus). Then 100 μl urine was added in duplicates into the wells of a commercially available solid phase enzyme amplified sensitivity immunoassay kit from DIAsource (C-Pep-EASIA KAP0401), designed to measure C-peptide in human serum. Serial dilutions of urine samples gave displacement curves parallel to those obtained with standards from the assay kit. To assess the accuracy of the C-peptide measurement in gorilla urine, recovery experiments were performed with a pool sample consisting of urine of eight different females. The pool sample was divided into 3 aliquots and spiked with the standard provided by the assay kit in five different concentrations. For the two lower standard concentrations (around which most of the samples values were situated) recoveries were 119% (N = 3; SD = 10.49%) and 116% (N = 3; SD = 12.10%), respectively. The pool samples spiked with higher standard concentrations resulted in 95% (N = 3; SD = 14.27%), 83% (N = 3, SD = 9.13%) and 82% (N = 3; SD = 17.24%) recoveries. The intra-assay coefficients of variation determined from five replicates of female gorilla urine samples of low (0.81 ng/mL) and high (2.06 ng/mL) concentrations were 5.64%, and 9.28%, respectively. The inter-assay variability was determined from four separated runs. Inter-assay variations calculated for the mean of average duplicated for high (2.88 ng/mL), and low (0.98 ng/mL) measurements were 10.16%, and 10.94%, respectively.

To adjust for the variation in the volume and concentration of the urine, we measured creatinine (crea) concentrations in each sample [58] and expressed all UCP values in ng/mg creatinine. All urine samples with creatinine levels <0.1 mg/mL were excluded (N = 7 of 170 assayed samples) to avoid overestimation of C-peptide concentrations by a correction with very low creatinine values.

2.4. Statistics

We used general linear mixed effects or hierarchical models [59,60] to investigate the effects of ecological, reproductive status and social variables, with UCP levels as a proxy for energy balance as the dependent variable. The following fixed effects, covariates, and interactions were included in the model: habitat, monthly rainfall, season, reproductive state, dominance rank (low vs. high), time of day, autocorrelation term (see below), and the interaction between rank and reproductive state. The autocorrelation term was computed using a code written by Roger Mundry [61]. The term 'season' was composed of the sine and cosine of the date transformed into a circular variable (2*pi*day/365). The variable 'rainfall' was z-transformed to a mean of zero and a standard deviation of one to achieve easier interpretable coefficients [62], and the response variable 'C-peptide concentration' was square root transformed due to a deviation from a normal distribution.

We controlled for autocorrelation in the models because the response variable was likely to show some degree of temporal autocorrelation which could have led to non-independent residuals, with possibility that such autocorrelation could seriously diminish the validity of the model. We did this by first running the full model as described above and obtaining the residuals from it. For each data point, we then averaged the residuals of all other data points (considering only residuals derived from the same individual). The contribution of the residuals to the average was weighted according to their lags in time to the specific data point, with the weight function following a Gaussian distribution. Its mean was set to zero (i.e. maximum weight at small time lags) and its standard deviation was determined such that the likelihood of the full model with the derived variable ('autocorrelation term') included was maximized. Time lags between data points were measured in days and fractions of days (according to the precise timing of the observations). The resulting 'autocorrelation term' was included as an additional fixed effect in the final model.

We controlled for repeated sampling of the same individual and group by including individual and group as random factors in the model. The model was fitted with Gaussian error structure and identity link function. We checked the following diagnostics of model validity: distribution of residuals and quantile–quantile plots to verify normality and residuals plotted against predicted values to assess homogeneity [63].

Significance testing was done by first comparing the full model with a null model containing only autocorrelation term and random effects using a likelihood ratio test [64]. When this produced a significant result we considered the significance of the individual parameters and dropped non-significant interactions to get interpretable estimates and p-values for the lower terms the interactions comprised. p-Values for the individual effects were obtained using Markov chain Monte Carlo (MCMC) analysis to establish significance [59].

All analyses were performed in R [65]. Generalized linear mixed models (GLMMs) were conducted using the function 'lmer' of the R package lme4 [66]. P values of individual effects in Gaussian models were generated with MCMC [59] and derived using the functions aovlmer.fnc and pvals.fnc of the R package languageR [67]. Likelihood ratio tests were ran using the R function 'anova' with the argument 'test' set to 'Chisq'.

3. Results

The whole model with UCP as response was found to be statistically significantly different from the null model containing only the autocorrelation term and the random effect (chi sq = 44.509, df = 11, $p < 0.001$). The interaction of rank and reproductive state was not significant and was therefore dropped. Three variables had a significant effect on UCP concentrations: habitat type, season, and day time. Reproductive state, rank, and rainfall did not have a significant influence on UCP levels (Table 1).

UCP levels were highest in urine samples collected when the animals were in the bamboo zone (mean = 16.5 ng/mg Crea, SD = 11.3), followed by the alpine zone (mean = 15.6, SD = 10.1) and lowest in the Hagenia zone (mean 11.4, SD = 8.8) (Fig. 1). UCP levels were highest in samples collected in November (mean = 19.1, SD = 10.0)

Table 1

Results of the generalized linear mixed models examining the impact of ecological, reproductive status and social predictor variables on urinary C-peptide level (ng/mg Crea) in female mountain gorillas. Estimates, standard errors, MCMC (Markov chain Monte Carlo)-based p-values and overall p values for individual fixed effects and covariates are shown. Significant effects are highlighted in bold.

	Estimate	Std. error	P_{MCMC}	Overall p value[a]
Intercept	1.639	1.006	[b]	
Habitat (Bamboo)[c]	−0.728	0.364	0.026	**0.008**
Habitat (Hagenia)[c]	−0.861	0.284	0.002	
Rainfall	0.225	0.137	0.210	NA
Rank (low)	−0.109	0.207	0.979	NA
Reproductive state (lactating)[c]	0.516	0.257	0.039	0.110
Reproductive state (pregnant)[c]	0.354	0.327	0.137	
Day time	0.169	0.076	**0.034**	NA
sin (season)	−0.360	0.206	0.193	**0.005**
cos (season)	0.462	0.200	0.010	
Autocorrelation term	0.525	0.092	<0.001	NA

[a] Overall p-value for these factors obtained from the function aovlmer.fnc of the R package languageR [67].

[b] Not shown because it has no meaningful interpretation.

[c] Categorical predictors with three levels were dummy-coded; R does this by taking the alphanumerically first group as the reference level. The estimated slopes and p-values for the dummy-coded variables represent their difference to the uncoded level (i.e. the reference level).

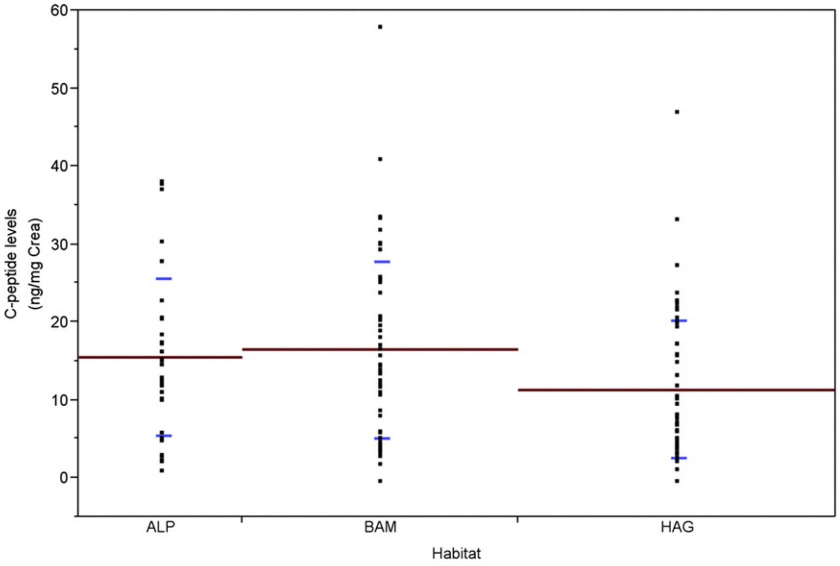

Fig. 1. Urinary C-peptide levels in samples collected in different habitat types of mountain gorillas (ALP = alpine, BAM = bamboo, HAG = Hagenia zone). Long horizontal lines indicate means and short horizontal lines standard deviations. The size of the columns of the x-axis is proportional to the number of samples obtained from the three habitat types (ALP: n = 34; BAM: n = 57; HAG: n = 66). Habitat type was not recorded for six samples.

(Fig. 2), which coincides with heaviest use of the bamboo zone (ranging from 75 to 89% of time per group calculated from the instantaneous scan samples) and the highest reliance on bamboo shoots as a food source (ranging from 73 to 95% of all feeding records in that month per group) (Figs. 3, 4). The decline in UCP levels from November to December corresponds to the end of the bamboo season. UCP levels increased significantly as the day progressed (Fig. 5).

4. Discussion

Results of this study reveal that UCP levels in female mountain gorillas in the Virunga Volcanoes are correlated with habitat type in which the animals foraged, seasonality, and time of day, but not with reproductive status or dominance rank. UCP concentrations were highest when the gorillas were foraging in the bamboo during the 2–3 month season when the shoots were available. Bamboo shoots are the only highly seasonal food resource consumed by the gorillas in the Virunga Volcanoes [17,18]. They are rich in energy and protein compared to their other food resources (Grueter et al., unpubl.), and they likely provided the gorillas with a high amount of energy that manifested itself in higher C-peptide concentrations. Seasonal variation in the availability and consumption of high-quality/preferred food (fruit) and correlation with higher baseline C-peptide levels has also been demonstrated for chimpanzees and orangutans in the wild [9,68]. Based on a comparative study, it also has been suggested that energetic condition is compromised in habitats of lower productivity [9,44,69]. Therefore it would be interesting to compare the patterns observed in UCP in the Virunga mountain gorillas with those in Bwindi Impenetrable National Park, Uganda because those gorillas live in an environment with more seasonally available fruit and lower abundance of herbaceous vegetation [70]. Furthermore, the Bwindi mountain gorillas have a longer interbirth interval than those in the Virungas, which could likely be due to energetic constraints [71].

Contrary to our prediction, there was no correlation between reproductive state and energy balance. In contrast, female orangutans showed the highest levels when in cycling state and lowest while lactating [72]. Similarly, during times of food scarcity, C-peptide levels have been shown to decline in lactating colobus monkeys [40]. In humans, C-

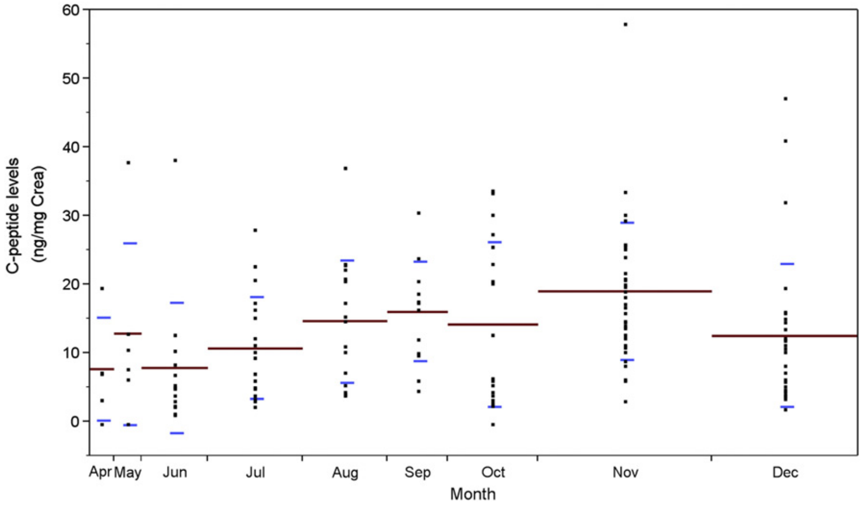

Fig. 2. Monthly variation in urinary C-peptide concentrations in mountain gorillas. The size of the columns of the x-axis is proportional to the number of samples collected per month. Long horizontal lines indicate means and short horizontal lines standard deviation.

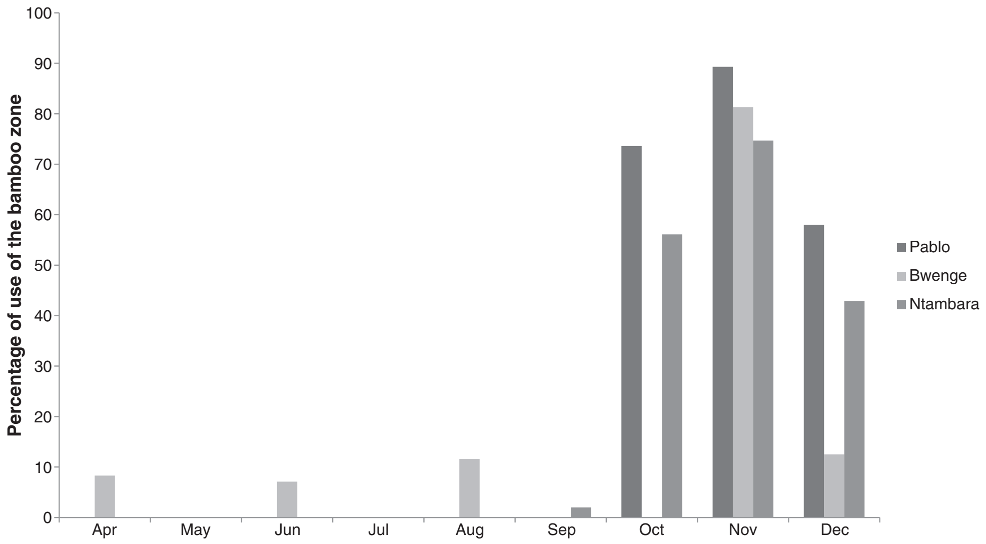

Fig. 3. Monthly variation in the use of the bamboo zone for the three gorilla groups.

peptide levels increased in the last trimester of pregnancy [73], and in female chimpanzees C-peptide levels were depressed for several months postpartum [44]. The lack of significant differences in energy balance for females in different reproductive states suggests that female mountain gorillas may cope with the demands of these reproductive phases by altering their behavior such as increasing feeding effort and/or enhancing consumption of high-quality food [7,74–77] or curtailing physical activity [8,78–81] or both [82]. Indeed, Watts [43] found that pregnant and lactating mountain gorilla females spent significantly more time feeding than non-pregnant, non-lactating ones. Given how energetic costs of producing offspring has implications for lifetime reproductive success, further study of the relationship between reproductive state and energy balance levels in female apes would be beneficial. In particular, since milk production is not static, an examination of changes in energy balance levels across different phases of the lactation period would be desirable. Gradual increases in C-peptide concentrations during lactational amenorrhea have been documented in humans and chimpanzees [50,44].

We predicted that higher-ranking females would have higher UCP levels as a result of better access to food, but did not observe such an effect. The lack of an effect may be due to few samples per individual and/or using a crude high/low estimate of dominance rank. The absence of a rank-related benefit in energetic condition could also reflect costs associated with dominance such as by elevated aggression [9]. Rate of aggression given is correlated with rank in these female mountain gorillas (Grueter et al., unpublished). No other studies that we are aware of have examined rank-related variation in C-peptide levels in female primates, but conflicting results have been observed for male chimpanzees: Emery Thompson et al. [9] documented higher levels in low-ranking males whereas Sherry and Ellison [68] found that top ranking chimpanzee males had higher levels than low ranking males, but only during times of fruit abundance (note that the sample size in the latter study was limited). These seemingly contradictive results could however be explained if high ranking males invest considerable energy budgets during periods of mate competition as recently shown for rhesus macaques [41].

Variation in C-peptide levels can reflect two processes, the response to acute food intake [73] and general stored energy [38]. Our results show that UCP concentrations increase as the day progresses, which is a pattern expected if UCP indeed reflects acute food intake. However,

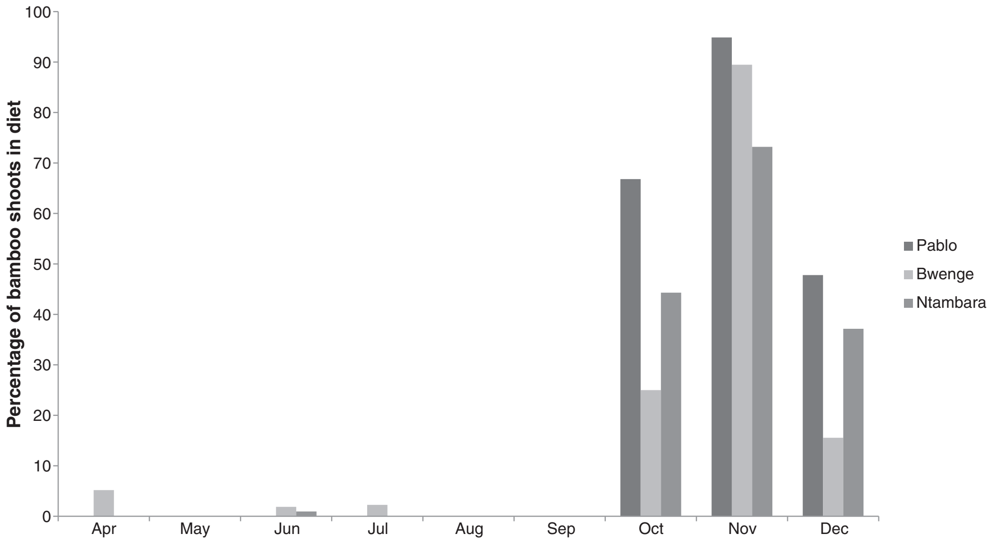

Fig. 4. Monthly variation in the percentage of bamboo shoots in the diet for the three gorilla groups.

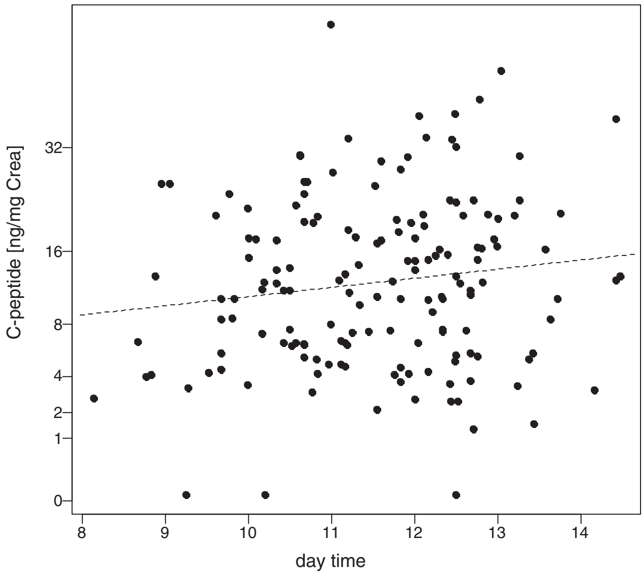

Fig. 5. Variation in urinary C-peptide levels across time of day (dashed line from standard linear regression).

it should be noted that we did not have any samples from the earliest and latest hours of the day. Gorilla feeding activity is most intense between 8 and 9 am and then drops precipitously toward midday when they typically rest, and then increases again [43]. No samples were collected later than 14:30 h in this study; for a full understanding of how energy balance fluctuates during the day we would need samples across the whole day. That C-peptide can be a sensitive marker of energetic balance on a short-term scale has recently been shown for chimpanzees: Georgiev [83] reported that hourly changes in ripe fruit intake in chimpanzees were related to diurnal variation in UCP. A study on humans has shown that C-peptide levels fluctuate with time of day, i.e. lower levels were found at night and in the early morning and increments were noted following meals [73]. How well the patterns of UCP excretion in this study reflect stored energy in from of fat deposits cannot be estimated.

That habitat and seasonality were significant predictors of energy balance suggests that there are energetic effects pertinent to spatio-temporal variability in resource availability and foraging behavior, even in a species that shows relatively little variation in food availability. These results contribute to our current understanding of energy balance in gorillas based on previous studies of foraging behavior and nutritional analysis of foods. Western gorillas, that experience a much greater fluctuation in food availability due to a distinct fruiting season, were shown to have an overall steady energy balance, as the increase in energy intake during times of high fruit availability was counterbalanced with higher travel costs and energy expenditure [84]. Additionally, dominance rank was found to correlate with energy balance in Bwindi mountain gorillas (Wright et al., unpublished). Future studies should involve more inter-population and inter-species comparisons using both UCP and foraging behavior to best understand the long term fitness consequences of how patterns of energy balance respond to variation in ecological and social parameters.

Acknowledgments

We thank Tony Mudakikwa, Rosette Chantal Rugamba and Prosper Uwingeli at Rwanda Development Board for permission to conduct research at Volcanoes National Park, Tara Stoinski and Christophe Boesch for endorsing this collaborative project, Felix Ndagijimana and Veronica Vecellio for general organizational support during the implementation of this research, Didier Abavandimwe, Sosthene Habumuremyi and the Karisoke trackers for assistance in collecting samples, and Roger Mundry for help with statistical analyses. We thank James Higham and one anonymous reviewer for their helpful comments on our manuscript. Funding for this study was provided by the Max Planck Society, the Dian Fossey Gorilla Fund International, and the Swiss National Science Foundation/Schweizerischer Nationalfonds (PBZHP3-128152). The research conducted adhered to the legal requirements of Rwanda.

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