Energetics at the urban edge: Environmental and individual predictors of urinary C-peptide levels in wild chacma baboons (*Papio ursinus*)

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Abstract

As human-modified landscapes encroach into natural habitats, wildlife face a reduction in natural food sources but also gain access to calorie-rich, human-derived foods. However, research into the energetics of wildlife living within and adjacent to urban and rural landscapes is lacking. C-peptide - a proxy for insulin production and a diagnostic tool for assessing pancreatic function in humans and domestic animals - can be quantified non-invasively from urine (uCP) and may provide a way to investigate the energetic correlates of living in human-altered landscapes. UCP is increasingly used in studies of primate energetics, and here we examine predictors of variation in uCP levels in n=17 wild chacma baboons (*Papio ursinus*) living at the urban edge on the Cape Peninsula, South Africa. We find that uCP was positively associated with provisioning and negatively with night fasting. UCP levels were comparable between winter and summer but significantly lower during spring, possibly driven by consumption of energy-rich seeds during summer and more human-derived foods during winter. UCP was elevated in pregnant females and similar for lactating and cycling females. We find no effect of dominance rank on uCP. Samples collected with synthetic Salivettes had significantly lower uCP levels than directly pipetted samples. Overall, our results indicate that uCP is a reliable, non-invasive measure of energy balance and intake in baboons, and suggest potential energetic benefits of living at the urban edge. More broadly, studies of uCP may offer unique insight into the environmental control of hormone-behaviour relationships in species crossing natural and urban environments.

Keywords: Baboons; C-peptide; Energy balance; Human-wildlife contact; Pregnancy; Primates; Provisioning; Urine; Season; Salivettes

Introduction

The loss of natural habitat to agricultural, industrial, and urban land uses is a global phenomenon that has been linked to increasing negative interactions between humans and wildlife (Woodroffe & Ginsberg 1998, Soulsbury & White 2016). Animals living in close proximity to human-modified landscapes may face a reduction in natural food sources but may also gain access to human-derived foods (for reviews see e.g. Fehlmann et al., in revision, Hill, 2017). Urban and crop foraging have been documented for numerous species, including birds (e.g. *Larus spp.;* Belant 1997), and mammals (e.g. *Loxodonta africana*, Naughton Treves 1998; *Ursus americanus,* Lewis et al. 2015; *Macaca fascicularis*, Yeo & Neo 2010; *Papio ursinus*, Lodge et al., 2013; Fehlmann et al. 2017). Human-derived foods are often rich in calories (e.g. Fehlmann et al. 2017; Lodge et al., 2013) and individuals may fundamentally change their behaviour in order to access and navigate in urban space (e.g. Fehlmann et al. 2017, Shepard et al., 2016) and/or alter their activity budgets (e.g. reduced foraging, more resting) as a result of feeding on human-derived foods (Strum 2010; Hoffman and O'Riain 2012a). Thus, living and foraging in a human-altered environment affects activity and energy budgets.

Whilst behavioural responses of wildlife to urban and rural environments are increasingly studied (reviewed by Fehlmann et al., in revision; Lowry et al., 2013); research into the energetic correlates is largely lacking. Recent works on glucocorticoids, the group of steroid hormones mainly involved in the regulation of glucose metabolism and energy mobilization, in crop-eating elephants (*Elephas maximus*), baboons (*Papio anubis*), and chimpanzees (*Pan troglodytes*) suggest that access to high-quality foods can offset energetic stress (McLennan et al., 2019; Pokharel et al., 2019; Lodge et al., 2013), but living in human-changed environments also presents psychological stressors potentially leading to increased glucocorticoids (see e.g. Bonier 2012). To understand energetic correlates of living within and adjacent to urban and rural landscapes therefore requires a physiological measure of energetic condition that is unaffected by psychological stress (see e.g. Dias et al., 2017).

Monitoring energetic condition, without relying on broad measures of energy consumption and expenditure (for a review see e.g. Emery Thompson, 2017) in wild animals is a key challenge for field biologists. Recent advances in non-invasive assessment of physiological markers include the measurement of urinary connecting peptide or C-peptide of insulin (Emery Thompson, 2016, Emery Thompson, 2017). Insulin is essential for glucose absorption, utilization, and storage and acts as hormonal regulator of energy balance in the brain (Schwartz et al., 1992, Havel, 2001, Norris and Carr, 2013). Insulin production is thus a major component of energy metabolism and modulator of an animal´s energetic condition. During insulin synthesis in the pancreas, C-peptide is cleaved from proinsulin and secreted on an equimolar (i.e. 1:1) basis to insulin and, in addition to serving as a diagnostic tool for assessing pancreatic function in humans and domestic animals (for reviews see e.g. Leighton et al., 2017; Rosenfield et al., 2017), can be used as a proxy for nutritional status or energy balance (Emery Thompson, 2016). C-peptide is excreted at a consistent rate into urine and, in contrast to insulin, can be assessed from urine samples, enabling the repeated and non-invasive assessment of energetic condition (Emery Thompson, 2016), even in wild study populations.

Positive associations between urinary C-peptide (uCP) and energy balance (or proxies thereof) have been demonstrated in various non-human primate species, including effects of body mass and experimentally induced weight change (e.g. fasting, provisioning) (e.g. Wolden-Hanson et al., 1993, Deschner et al., 2008, Girard-Buttoz et al., 2011), food and fruit availability and intake (e.g. Emery Thompson and Knott, 2008, Emery Thompson et al., 2009, Harris et al., 2009, Grueter et al., 2014), as well as energetic aspects of the social environment, e.g. dominance rank (Sherry and Ellison, 2007, Emery Thompson et al., 2009, Higham et al., 2011a, Lodge, 2012). Furthermore, age and sex (Thompson et al., 2020) as well as female reproductive state have been shown to affect uCP (e.g. Emery Thompson et al., 2012, McCabe et al., 2013, Nurmi et al., 2018; but see Lodge, 2012, Grueter et al., 2014). Pregnancy, for instance, has been associated with increased uCP in bonobos

(*Pan paniscus*: Nurmi et al., 2018) and Assamese macaques (*Macaca assamensis*: Touitou, pers. comm.), likely reflecting gestational changes in maternal carbohydrate metabolism (Butte, 2000). Furthermore, uCP has been linked to changes in energetic condition associated with conception (*Cercocebus sanjei*: McCabe et al., 2013; *Pan troglodytes:* Emery Thompson et al., 2012). A number of studies have also investigated effects of energy expenditure on uCP, including travel time, activity budgets, activity specific energy constants, or periods of high energetic demands such as lactation or illness (e.g. Emery Thompson et al., 2009, Higham et al., 2011a, Emery Thompson et al., 2012, Lodge, 2012, Bergstrom, 2015).

Given the vast evidence linking uCP and energetic condition, studies of uCP in species crossing natural and urban environments may offer unique insight into the environmental control of hormone-behaviour relationships and reveal/rule out energetic benefits of living in human-altered landscapes. However, to date, research on uCP has focussed on captive animals (see above) or wild animals in their natural habitats, despite non-human primate use of anthropogenic foods leading to frequent contact and conflict and presenting a major conservation challenge across Africa, Asia, and Neotropics (reviewed by Hill, 2017, Estrada et al., 2012). Therefore, devising novel methods to better understand the drivers for wild animals to live and forage within and adjacent to urban and rural landscapes is crucial to developing management plans that ultimately seek to improve the welfare of people and wildlife and the conservation value of the latter (Fehlmann et al., in revision).

Here, we investigate variation in uCP levels in wild, male and female, chacma baboons (*Papio ursinus*) living at the urban edge on the Cape Peninsula, South Africa where they forage in both natural and urban spaces (e.g. Fehlmann et al., 2017; van Doorn et al., 2010). First, we biologically validate our uCP assay (and assess how uCP responds to intake of high-calorie human foods), and test whether manipulation of food availability affects uCP, and predict baboons will show higher uCP during a period of provisioning compared to the periods before and after (see e.g. Wolden-Hanson et al., 1993, Deschner et al., 2008, Girard-Buttoz et al., 2011). We also investigate the effect of urine collection method (pipette versus synthetic swab) on uCP since both are becoming common but are not systematically compared (but see Danish et al., 2015). Second, we test for seasonal and circadian effects (night fasting) on levels of uCP. In the case of season, food availability and baboon activity budgets (e.g. Davidge, 1978, Weingrill et al., 2004, van Doorn, 2009, van Doorn et al., 2010, Lewis and O'Riain, 2017, Mucina and Rutherford, Reprint 2011) vary in accordance with a Mediterranean climate on the Cape Peninsula (hot, dry summers and cold, wet winters; Cowling et al., 1996) and we therefore expect seasonal variation in uCP. Specifically, we predict that an increased availability of energy-rich seeds (Fehlmann et al., 2017) during summer (van Doorn, 2009, van Doorn et al., 2010) should be reflected in higher uCP in summer compared to winter months. However, this difference could be offset by increased consumption of human foods during winter compared to summer (van Doorn, 2009, van Doorn et al., 2010). On a shorter temporal scale, we predict a circadian effect on uCP, i.e. lower uCP following night fasting (e.g. Girard-Buttoz et al., 2011, Georgiev, 2012; but see Deschner et al., 2008, Emery Thompson et al., 2009, Lodge, 2012 for studies that find no circadian effect). We also investigate how individual traits, i.e. dominance rank and female reproductive state, influence uCP. Predicting dominance rank effects is not straightforward because such effects appear to become prominent mainly during periods of high competition and/or increased energy expenditure (Sherry and Ellison, 2007, Emery Thompson et al., 2009, Higham et al., 2011a). However, because high-ranking baboons may have better access to food resources and/or higher energy intake (e.g. Post, 1980, Barton, 1993, Barton and Whiten, 1993, Kaplan et al., 2011), we expect higher uCP in high-ranked individuals. We expect (hormonally confirmed) female reproductive state (cyclic, pregnant, lactating) to influence uCP and predict higher uCP in pregnant (e.g. Nurmi et al., 2018), and lower uCP in lactating compared to cyclic females (e.g. Emery Thompson et al., 2012).

Methods

Study site and subjects

Data were collected on a troop of wild chacma baboons consisting of ~50 individuals (including n=21 adults) in Da Gama Park (34.1617° S, 18.4054° E), on the Cape Peninsula, South Africa. Urine samples were collected from n=17 individually identified adult individuals (n=2 males, n=15 females; Table 1) between 4th July and 24th November 2018. The troop was provisioned with fruit, vegetables, and corn for a period of ten days (24/07/2018-02/08/2018) at a location within their core home range (they located and consumed the food every day). This provisioning was to facilitate cage-trapping of the baboons so they could be fitted with tracking collars as part of a larger project and served to biologically validate our uCP assay given that an increase in food availability and intake should lead to elevations in uCP excretion (e.g. Emery Thompson and Knott, 2008, Emery Thompson et al., 2009, Harris et al., 2009, Grueter et al., 2014). The work was approved by local authorities and Swansea University's Ethics Committee (IP-1314-5).

Urine sample collection and storage

N=315 urine samples (mean±SD=18.5±3.5 per individual; Table 1) were collected opportunistically throughout the day and immediately after urination using Salivettes (Sarstedt Salivette Cortisol code blue, order number 51.1534.500; n=129 samples; Danish et al., 2015) or directly pipetted into 2.0 ml Eppendorf Safe-Lock microcentrifuge tubes using disposable pipettes (n=186 samples; also see Supplementary Material Figure S1). Only samples not obviously contaminated with faeces, which is known to substantially alter uCP levels (Higham et al., 2011b), were collected. Samples were kept on ice packs during the day to avoid uCP degradation (Higham et al. 2011b). In the evening, salivettes were centrifuged at 3000 RPM for 5 min and the urine was transferred into 2.0 ml Eppendorf Safe-Lock microcentrifuge tubes. All urine samples were stored at -20°C within a maximum of 12 hours of collection and were shipped to Swansea University on dry ice at the end of the data collection period. Samples were finally stored frozen at -20°C until analysis.

C-peptide analysis

Samples were analysed using a commercially available C-peptide ELISA Kit (IBL International GmbH, Hamburg, Germany; Art. No. RE 53011) which has been successfully used in other Cercopithecines (macaques: Girard-Buttoz et al., 2011, Higham et al., 2011 a,b, Girard-Buttoz et al., 2014, Müller et al., 2017; baboons: Lodge, 2012). A mixed-sex pool of serially diluted urine ran parallel to the uCP standard curve (Supplementary Material, Figure S2). The majority of samples were diluted 1:6 (range: 1:1 – 1:12) with IBL sample diluent (Art. No. RE 53017) to bring the uCP concentrations into the working range of the assay and samples were then assayed according to the manufacturer's instructions. Assay sensitivity was 0.064 ng/ml. Inter-assay coefficients of variation calculated from replicate determinations of low and high value quality controls (made from pooled baboon urine) included on each assay plate (n=10) were 7.4% (low) and 9.3% (high). To adjust for differences in urine concentration, C-peptide values were corrected for specific gravity (SG), i.e. the density of urine relative to the density of distilled water, measured with a manual handheld refractometer, using the following correction formula: $uCP_{SG} = uCP_{ng/ml}*(SG_{pop}-1)/(SG-1)$, where SG_{Pop} is the mean SG value for the population (1.019) (see e.g. Miller et al., 2004). All uCP values reported are the SG corrected uCP concentrations.

Faecal hormone analysis and assessment of reproductive state

N=3 females (F13, F16, F19; Table 1) transitioned from pregnancy to lactation and n=1 female was lactating (F1; Table 1) throughout the study period. Records of swelling patterns and births (see e.g. Weingrill et al., 2004) left uncertainties about reproductive state, hence, for the remaining n=11 females, reproductive state was assessed through analysis of faecal progestogen metabolites (e.g. Ziegler et al., 2000, Fürtbauer et al., 2010). From these females, n=336 faecal samples were collected (mean±sd=30.5±10.6 samples per female) directly after defaecation and were stored on ice packs during and frozen at the end of the day at -20°C (within a maximum of 12 hours). Samples were freeze-dried at the University of Cape Town and dried samples were shipped to Swansea University where they were processed and steroid hormones extracted following standard procedures (e.g. Fürtbauer et al., 2010). In brief, samples were pulverised with a mortar and pestle and sieved to remove undigested material. Approximately 0.1 g of each sample were weighed and extracted with 3ml 80% methanol. Faecal suspensions were vortexed at 1500 rpm for ten minutes and subsequently centrifuged at 3000 rpm for ten minutes. Extracts were split and decanted into two 2.0 ml Eppendorf Safe-Lock microcentrifuge tubes. 250µl were transferred to screw top microcentrifuge tubes and evaporated at 38°C under a stream of nitrogen. Dried extracts were sent to the endocrinology laboratory of the German Primate Centre, Göttingen, Germany, where samples were resuspended in 250µl 80% methanol by sonicating them in a water bath for 5 min, followed by 30 sec of vortexing (Shutt et al., 2012). Progestogen metabolites were then assayed by enzyme immunoassay (EIA) using the monoclonal antibody (Quidel clone no. 425) and HRP-labelled conjugate produced by late Coralie Munro (Clinical Endocrinology Laboratory, UC Davis, USA) against 4-pregnen-11-ol-3, 20-dione hemisuccinate:bovine serum albumin (BSA). The antibody cross-reacts with numerous progesterone metabolites and has been used successfully in various mammal species, including baboons, to assess female reproductive status (Wasser et al., 1988, Graham et al., 2001).

Prior to EIA, fecal extracts were diluted 1:30 - 1:300 with assay buffer to bring the hormone concentrations into the working range of the assay and duplicate 50µl aliquots of diluted samples and 5α-pregnane-3ß-ol-20-one standard (4.8 - 625 pg/50µl) were combined with labelled conjugate (50 µl) and antiserum (50 µl) and incubated overnight at 4°C. After incubation, the plates were washed four times after which TMB substrate solution was added and the plates incubated at room temperature in the dark for another 60 min. The enzyme reaction was finally stopped by adding 50 µl of 2 M H_2SO_4 to each well and absorbance measured at 450 nm (reference 630 nm). Serial dilutions of samples showed displacement curves that run parallel to the respective standard curve. Sensitivity of the assay was 9.8 pg. Inter-assay coefficients of variation (CV), calculated from replicate determinations of low and high value quality controls included on each assay plate (n=10), were 7.9% (low) and 2.3% (high).

The faecal progestogen data revealed that n=5 females were cyclic (F2, F4, F17, F18; Table 1), n=4 females (with brown infants of unknown age) showed ovarian acyclicity (amenorrhea) (F5, F6, F10, F14; Table 1), n=1 female was pregnant (F9; Table 1), and n=2 females conceived during the study period (F7, F15; Table 1).

Dominance rank

We calculated female dominance ranks based on the outcomes of directly observed dyadic interactions (displacements, chases and aggressive displays; n=634) for all females in the troop, using the packages *AniDom* (Farine and Alfredo Sanchez-Tojar, 2019) and *Compete* (Curley, 2016) in R (R Development Core Team, 2010). Due to a high sampling effort (ratio of interactions to individuals: 33.4, recommended: 10-20), and low sparseness of the data (observed proportion of known dyads: 0.82, which is above that estimated under a Poisson process: mean=0.57, 2.5% and 97.5% quantiles: 0.40, 0.74), we determined our hierarchy to be very steep (using the randomized Elo-rating method), repeatable (using the *estimate_uncertainty_by_repeatability* function in *AniDom*: score of 0.98) and highly linear (using triangle transitivity to assess ‘orderliness’ (McDonald and Shizuka, 2012). For these reasons we determined the hierarchy using the I&SI method (which minimizes the number of inconsistencies (I) in a dominance matrix and subsequently minimizes the strength of those inconsistencies (SI)) in the package *Compete* (see Schmid and de Vries, 2013, Sánchez-Tójar et al., 2017), which finds the “best order” of rank that deviates least from linearity, and is most appropriate for very steep hierarchies. We standardized ranks (across males and females to test for an overall effect of rank) between 0 and 1 (with 1 being the highest and 0 the lowest ranking individuals) using the function *range01* in the Package *funModeling* (Casas, 2019).

Table 1: Details of study animals, including sex, rank, reproductive state (females), median and range of SG corrected uCP concentrations (see text for details), and number of urine samples collected.

			Median uCP$_{SG}$ (range; n)			
ID	**Sex**	**Rank**	**male**	**cyclic**	**pregnant**	**lactating**
M1	Male	Alpha	11.6 (49.2; 20)			
M2	Male	Beta	24.0 (56.7; 21)			
F1	Female	1				16.6 (87.8; 18)[b]
F2	Female	2		8.8 (65.8; 20)		
F4	Female	4		13.19 (51.2; 17)		
F5	Female	5				11.9 (55.1; 16)[a]
F6	Female	6				18.0 (37.7; 16)[a]
F7	Female	7		14.1 (20.5; 9)	24.7 (54.9; 7)	
F9	Female	9			43.8 (228.4; 19)	
F10	Female	10				4.1 (19.02; 25)[a]
F13	Female	13			58.2 (63.9; 6)	12.5 (58.4; 13)[b]
F14	Female	14				10.4 (50.8; 17)[a]
F15	Female	15		18.6 (35.0; 3)	7.5 (81.9; 10)	
F16	Female	16			38.1 (149.1; 24)	10.0 (6.3; 2)[b]
F17	Female	17		7.0 (67.1; 15)		
F18	Female	18		9.9 (44.9; 22)		
F19	Female	19			14.5 (NA; 1)	14.5(38.0; 14)[b]
Total n=315			**41**	**86**	**67**	**121**

[a] brown infant (late lactation), [b] black infant (early lactation)

Data analysis

Data were analysed in RStudio version 1.2.1335 (RStudio Team, 2018) using the package *lmerTest* (Kuznetsova et al., 2017). First, to biologically validate our uCP assay and to test how uCP responds to intake of high-calorie human foods, we investigated the effect of manipulation of food availability on uCP levels, and ran a Linear Mixed Model (LMM; Baayen, 2008) including uCP as response, context (pre-provisioning = the ten days prior to provisioning, provisioning = the ten days during which the troop was provisioned, post- provisioning = the ten days following provisioning) as categorical fixed effect and ID and Date as random effects (n=46 samples, n=17 individuals; LMM1). Reproductive state (pregnant vs. non-pregnant to allow including males) and collection method (salivette vs. pipette) were controlled for as fixed effects (see LMM2).

Second, a LMM was used to investigate the effects of various predictors on uCP levels (n=315 samples, n=17 individuals; LMM2). Collection method (Salivette/pipette), standardized dominance rank (continuous, see above), sex (male/female; to control for potential sex differences), time of day (before/after 9AM; to account for a potential effect of night fasting; see e.g. Girard-Buttoz et al., 2011, Georgiev, 2012), and season (winter/spring/summer) were included as categorical fixed effects. Winter included the months July and August, spring included the months September and October, and summer included November. Due to winter rainfall, annually flowering grasses are mainly available in winter whereas fruits and flowers are most abundant in spring and summer (Johnson, 1993, Milton, 2004). An interaction between season and rank (which would be expected if competition for food varies with season) was tested but dropped due to non-significance. To control for individual differences in uCP, baboon ID was included as random effect. Date of collection was included as random effect to account for uneven sampling across time.

Third, to test for effects of female reproductive state on uCP, we ran a LMM on female data only (n=274 samples, n=15 females; LMM3), including uCP as response and female reproductive state (cyclic, lactating, pregnant) as categorical fixed effect. Season, collection method and time of day were controlled for based on results from our main model (LMM2, above). Baboon ID and date of collection were included as random effects. Additional analyses investigating influence of stage of pregnancy (early versus late, based on 190 days gestation; Weingrill et al., 2004) and lactation (early versus late, based on the presence of a black or brown infant), respectively, revealed no significant effects (see Supplementary Material).

UCP was log-transformed in all models to meet model assumptions of normally distributed residuals. The significance of the full models as compared to the null models (only including random effects and control predictors) was established using likelihood ratio tests (R function *anova*). To rule out potential collinearity issues between predictor variables, we obtained Variance Inflation Factors (VIFs; Field, 2005) for standard linear models (excluding random effects) using the package *car* (Fox & Weisberg, 2011). No issues with collinearity were found (maximum VIF=1.3). Model diagnostics were performed using graphical procedures (Q-Q plot and standardized residuals vs. fitted values). The significance level was set at $p<0.05$.

Results

Biological validation of uCP - effect of provisioning

The provisioning model was significantly different from the null model (LMM1: χ2 = 9.58, df = 2, p = 0.008). UCP levels were significantly lower before (median: 5.7 ng/ml, range: 22.2 ng/ml; LMM1: estimate±se=-1.32±0.47, t=-2.78, p=0.012; Figure 1) and after (median: 14.4 ng/ml, range: 69.3 ng/ml; estimate±se=-1.01±0.35, t=-2.93, p=0.011) than during provisioning (median: 27.1 ng/ml, range: 102.1 ng/ml; Figure 1).

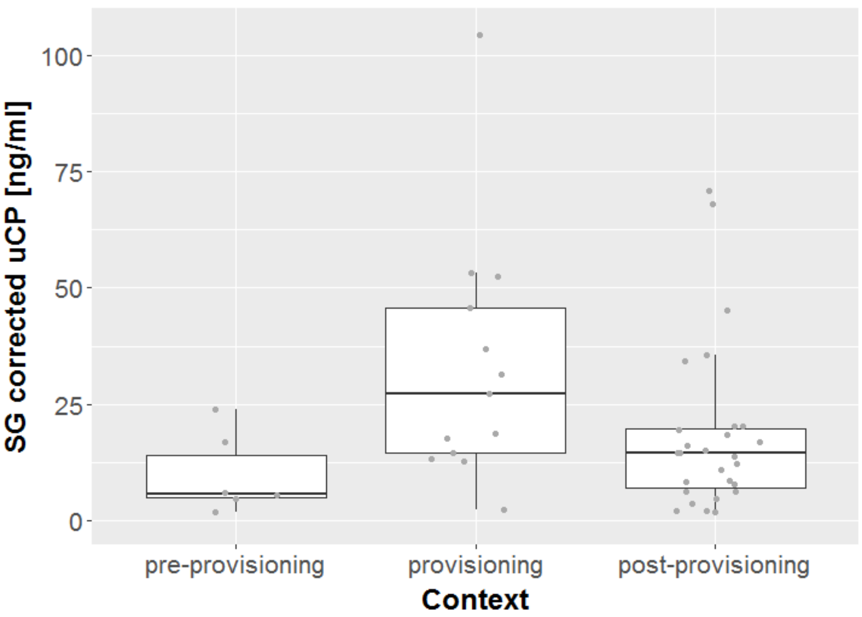

Figure 1: Effect of manipulation of food availability on UCP levels in wild chacma baboons. Baboons were provisioned with fruit, vegetables and corn during a 10-day period ("provisioning") and uCP levels compared to the ten days before and after (pre- and post-provisioning). Grey dots represent individual data points.

Effects of season, night fasting, dominance rank, and urine collection method on uCP

SG corrected uCP concentrations ranged from 0.28 ng/ml – 228.85 ng/ml. Our main model was significantly different from the null model (LMM2: $\chi2 = 64.36$, df = 5, $p < 0.001$). Samples collected before 9AM (n=46; median: 7.7 ng/ml, range: 62.5 ng/ml) had significantly lower uCP compared to samples collected after 9AM (n=269; median: 14.4 ng/ml, range: 228.6 ng/ml; LMM2: $p<0.001$; Table 2). Season affected uCP, with concentrations being significantly higher in summer (median: 29.1 ng/ml, range: 87.9 ng/ml) and winter (median: 14.3 ng.ml, range: 228.5 ng/ml) than during spring (median: 11.7 ng/ml, range: 123.8 ng/ml) (LMM2: summer: p=0.003; winter:, p=0.011; Table 2; Figure 2) and no significant difference was found between winter and summer (LMM2: p=0.139). Urine collection method significantly affected uCP levels, with concentrations being significantly lower for samples collected with Salivettes (median: 10.6 ng/ml, range: 75.3 ng/ml) compared to samples pipetted directly into microcentrifuge tubes (median: 16.9 ng/ml, range: 227.6 ng/ml; LMM2: $p<0.001$; Table 2; Figure 3). Sex and dominance rank, had no significant effect on uCP (LMM2; Table 2).

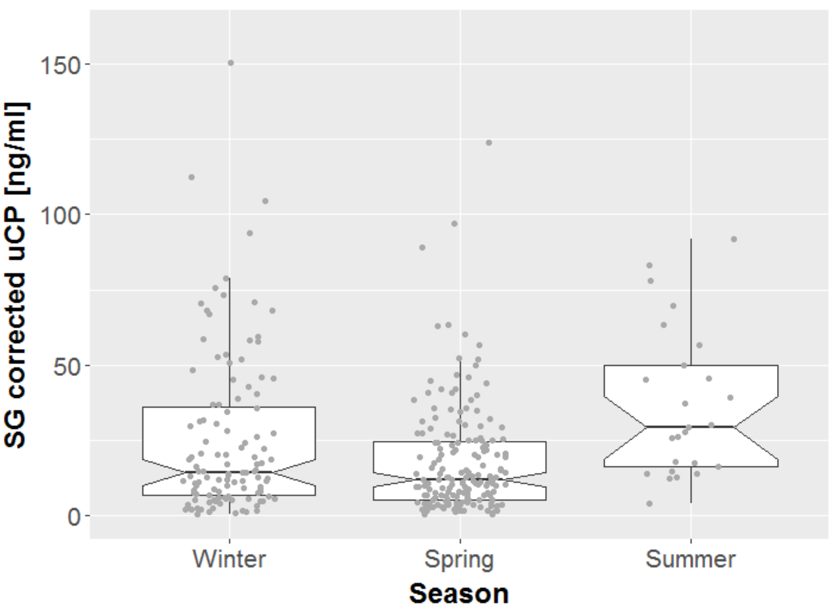

Figure 2: Effect of season on uCP levels in wild chacma baboons. UCP was significantly higher during winter (July and August) and summer (November) than during spring (September and October). Notches display 95% confidence intervals around the median (black line). Grey dots represent individual data points (n=1 data point for 'Winter' not shown; uCP=228.9ng/ml).

Table 2: Effects of sex, dominance rank, season, night fasting, and urine sample collection method, on SG corrected urinary C-peptide levels (ng/mg) in male (n=2) and female (n=15) chacma baboons (LMM2). Significant effects are highlighted in bold (n=315 samples). See text for further details.

Predictor variable	**Estimate**	**Std. Error**	**T-value**	**P-value**
Sex (male)	0.28	0.50	0.56	0.583
Dominance rank	0.10	0.47	0.22	0.828
Night fasting (before 9AM)	-0.52	0.15	-3.38	**<0.001**
Season[a] (summer)	0.70	0.23	3.06	**0.003**
Season[a] (winter)	0.35	0.13	2.63	**0.011**
Collection method (Salivette)	-0.60	0.11	-5.33	**<0.001**

[a] Reference category = spring

Figure 3: Effect of urine collection method on uCP levels in wild chacma baboons. Notches display 95% confidence intervals around the median (black line). Outliers are not shown.

Effect of female reproductive state on uCP

The female model was significantly different from the null model (LMM3: χ2 = 16.99, df = 2, p < 0.001). UCP levels were significantly lower in cyclic (median: 10.7 ng/ml, range: 67.9 ng/ml, n=86 samples, LMM3: estimate±se=-0.90±0.24, t=-3.82, p=0.001; Figure 4) and lactating (median: 11.6 ng/ml, range: 88.0 ng/ml, n=121 samples; LMM3: estimate±se=-0.81±0.22, t=-3.67, p<0.001; Figure 4) females compared to pregnant females (median: 34.8 ng/ml, range: 228.4 ng/ml, n=67 samples; Figure 4). No statistically significant differences in uCP were found between early versus late pregnancy and between early versus late lactation (Supplementary Material).

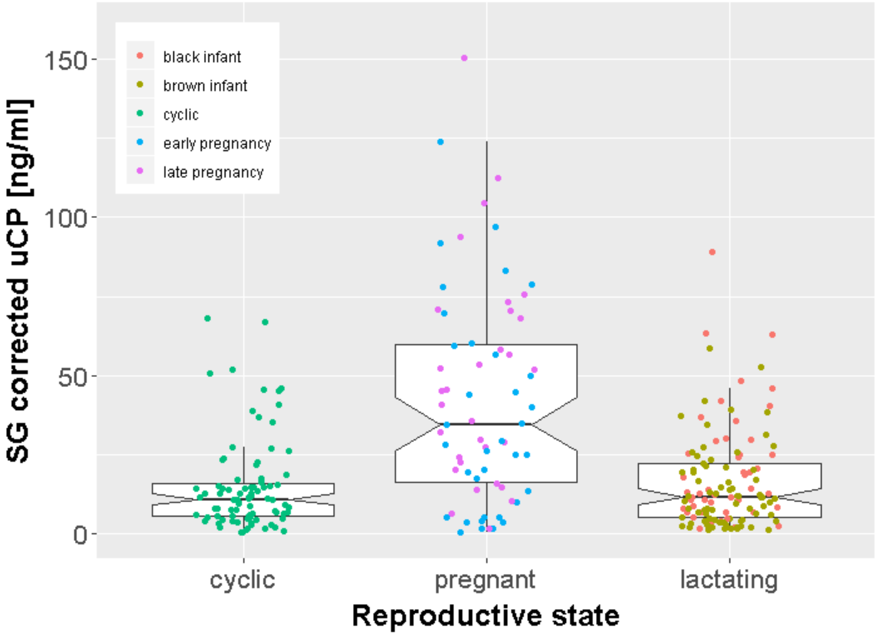

Figure 4: Effect of female reproductive state on uCP levels in wild chacma baboons. Early and late pregnancy represent the first and second half of pregnancy based on a gestation length of 190 days (Weingrill et al., 2004). Notches display 95% confidence intervals around the median (black line). Coloured dots represent individual data points (n=1 data point for 'pregnant' not shown; uCP=228.9ng/ml).

Discussion

Where there is human activity, there is likely to be predictable access to food, which is attractive to wildlife (Baruch-Mordo et al., 2013). Studies have demonstrated that animals can reap benefits from access to human foods, including higher reproductive success through decreased birth intervals and increased infant survival (Altmann & Alberts, 2003; Strum, 2010). Interactions between humans and wildlife, however, also carry negative consequences, such as economic losses, disease transmission, and injury and death (Soulsbury & White, 2016; Strum, 2010). While numerous behavioural studies have set out to better understand the causes and consequences of living within and adjacent to urban and rural landscapes (for reviews see e.g. Fehlmann et al., in revision; Lowry et al., 2013), research into the energetic correlates are largely lacking. In this study we investigated predictors of variation in uCP, a proxy for energy balance (Emery Thompson, 2016), in chacma baboons living at the urban edge on the Cape Peninsula. We (1) discuss the implications of our findings and (2) provide future directions and considerations for using uCP as a tool for understanding and mitigating human-wildlife conflict.

uCP as a marker of nutritional status in chacma baboons

The provisioning of the study troop as part of our larger research programme provided us with a semi-controlled experiment to biologically validate our uCP assay, i.e. to investigate how uCP changes with increased food availability, quality, and intake. As expected, uCP levels were significantly higher during the 10-day period when baboons were provisioned with human foods (fruit, vegetables, and corn). The results from this natural experiment are in line with i) results from controlled fasting/provisioning experiments (*Macaca spp*.: Wolden-Hanson et al., 1993, Girard-Buttoz et al., 2011; and *Pan paniscus:* Deschner et al., 2008) and ii) studies showing positive correlations between uCP and food availability and intake (e.g. Wolden-Hanson et al., 1993, Deschner et al., 2008, Emery Thompson and Knott, 2008, Emery Thompson et al., 2009, Harris et al., 2009, Girard-Buttoz et al., 2011, Grueter et al., 2014). Our findings therefore suggest that uCP responds rapidly to changes in energy intake (and consumption of human foods), making it a useful marker to track short term changes in nutritional status. Further support for this comes from our finding that uCP concentrations were significantly lower after night fasting (i.e. in samples collected before 9AM) which has been shown also in macaques (Girard-Buttoz et al., 2011) and chimpanzees (Georgiev, 2012; but see Deschner et al., 2008, Emery Thompson et al., 2009, Lodge, 2012 for studies that find no effect).

Effects of female reproductive state on uCP

Consistent with other studies that report higher uCP in pregnant females (*Pan paniscus*: Nurmi et al., 2018; *Macaca assamensis*: Touitou, pers. comm.; but see Grueter et al., 2014 for *Gorilla beringei beringei* and Lodge, 2012 for *Papio hamadryas anubis*) we found higher uCP in samples collected from pregnant females compared to lactating (acyclic), and cyclic females. Higher uCP levels in pregnancy are likely due to gestational changes in maternal carbohydrate metabolism (Butte, 2000), and perhaps also an energy conservation strategy by pregnant females (Dufour and Sauther, 2002, Murray et al., 2009), afforded by the high-quality resources available in the Cape Peninsula (natural and anthropogenic food: Fehlmann et al., 2017). Contrary to our prediction, however, uCP levels of lactating females were comparable to but not lower than those of cyclic females. It is possible that changes in energy intake may offset effects of lactation on energetic condition (see e.g. Emery Thompson et al., 2012). Energy intake in yellow baboons, for instance, is higher in pregnant and lactating females compared to cyclic females (Muruthi et al., 1991). At the same time or alternatively, energy expenditure may be reduced during lactation. Lactating chacma baboons have been shown to rest more than non-lactating females, whilst feeding times are comparable across lactating and non-lactating females (Barret et al., 2006). It is possible that the energetic constraints of lactation are dampened by the consumption of high-energy human foods. Female olive baboons feeding on crops in Nigeria, for instance, experience a quicker return to menstrual cycling and increased reproductive output (Higham et al., 2009).

Longer-term studies are now required to fully consider effects of reproductive state on uCP, so that researchers can study within-individual variation across states. For example, during our study, just two females conceived, preventing us from linking uCP to changes in energetic condition associated with conception as shown in Sanje mangabeys (*Cercocebus sanjei*) and chimpanzees (Emery Thompson et al., 2012, McCabe et al., 2013). Furthermore, the effects of reproductive state on uCP may vary across seasons; however, investigating such interactions requires a larger data set where the same females are sampled in different reproductive states and across seasons.

Effects of season and rank on uCP

We found a strong seasonal effect on uCP. Specifically, uCP concentrations were lower during spring compared to summer and winter, which is likely related to differences in food availability/quality and seasonal changes in activity budgets. uCP was particularly low in September (see Supplementary Material Figure S3) which was the month with the highest rainfall. While no data on baboon diet is available for the transient seasons for the Da Gama troop, high levels of rainfall have been linked to reduced feeding (see e.g. Bronikowski and Altmann, 1996, Thompson et al., 2020), which may explain the overall reduced uCP concentrations during spring, compared to summer and winter (see Figure 1).

Previous work on the Da Gama troop revealed no pronounced difference in diet between winter and summer, apart from an increase in the consumption of human-derived foods during the winter-period (van Doorn, 2009). However, caution should be exercised when extrapolating these feeding patterns to our current findings, as these data were collected ten years ago when management was less effective at preventing baboons from entering urban areas (van Doorn & O'Riain, 2020). Annual variations in weather patterns may translate into different food availability across years (e.g. Norton et al., 1987) and baboons are known for their "eclectic" feeding pattern, optimising their diet depending on what food is available (Whiten et al., 1991, van Doorn et al., 2010). Seeds are high-energy Cape baboon foods (see e.g. supplementary information in Fehlmann et al., 2017; Lewis and O'Riain 2017), and alien seeds are more prevalent in the summer than in winter. While we did not systematically record and quantify food availability and intake rates, the baboons' summer diet did include more energy-rich seeds (pers. obs. in the field and during processing of faecal samples for hormone analysis; see van Doorn, 2009), which may explain the overall increase in uCP from spring to summer (see Figure 1) and our findings related to food-provisioning and uCP (see above) support this interpretation.

The evenly distributed and abundant food resources and little or no natural predation risk experienced by baboons in our study population (Skead, 1980, Hoffman and O'Riain 2012b) likely reduces skew in access to food resources. Hence, rank differences in energy intake and expenditure may be minimal which is supported by our finding that we see no overall effect of rank and no interaction between rank and season on uCP (which would be expected if competition for food varies with season). To fully understand the potential for rank effects on uCP in chacma baboons, future studies should test for effects within and between sexes (the small sample size of n=2 adult males in the troop prevented us from investigating sex differences) across seasons. In addition, more detailed data on uCP and food availability and intake is needed to confirm that seasonal variation in uCP is linked to differences in activity and food availability, quality, and intake in both natural and urban space.

Seasonal fluctuation in the use of human-altered landscapes by animals strongly suggests that food scarcity in the natural space in conjunction with the ripening of palatable crops in agricultural areas are key drivers in attracting animals to human-modified landscapes (primates: Nowak et al. 2017, Naughton-Treves et al., 1998; Tweheyo et al., 2005; black bears: Lewis et al., 2015; African elephants: Osborn, 2004). Similarly, predation of livestock by carnivores increases when natural prey density is low (Khorozyan et al., 2015, Patterson et al., 2004) and the young of domestic animals are abundant (Nattrass et al. 2020). Previous studies have assessed food availability through comparing both the quality and quantity of food sources in natural and human-modified environments, suggesting that reduced nutritional state causes the movement of wildlife towards human-modified landscapes in search for food to meet energetic demands (Osborn, 2004, Lewis et al., 2015, Naughton-Treves et al., 1998, Van Doorn et al., 2010). However, some studies find no link between natural forage availability and the propensity of wildlife to exploit human foods (Ekanayaka et al., 2011) and highlight the importance of specific attractants (e.g. maturing crops) instead (Chiyo et al., 2005). In these cases, consuming human foods may be based on opportunity rather than energetics, with raiding being opportunistic, habitual and/or hedonistic without necessarily displaying a seasonal pattern (Chiyo et al., 2011; McLennan & Hockings, 2014; Wilkie & Douglas-Hamilton, 2018). Physiological markers such as uCP present a valuable tool to confirm the role of energetic state in the propensity to forage on human foods.

Feasibility of uCP as a tool for understanding and mitigating human-wildlife conflict

Assessment of uCP presents a clear-cut way to assess the role that energy status plays in drawing wildlife to urban and rural spaces and hence could help inform mitigation strategies to minimise human-wildlife conflict. Expanding the use of uCP as a biomarker of energetics beyond human and non-human primates would allow for its application in various conflict species. Insulin production is a key process in stimulating energy uptake in all mammals and is associated with a parallel release of C-peptides (Jin Chan & Steiner, 2000). Work comparing the amino acid sequences of C-peptides obtained from the pancreas of various mammal taxonomic families found that while the sequences vary, several prominent features of the C-peptide structures are preserved (Peterson et al., 1972; see also Landreh et al., 2014, Wahren 2004). Moreover, C-peptide has been successfully measured in the blood of domestic animals (dog, cat, horse; reviewed by Rosenfield et al., 2017). Together, these findings provide a promising basis for uCP measurements in non-primate mammals and future studies should validate existing immunoassays.

More practical considerations relate to the feasibility of urine collection which poses a methodological challenge due to its rapid absorption on most natural substrates (Schwartz et al., 2008). In the wild, urine samples have been successfully collected in several species that experience conflict with humans, including primates (Thompson & Knott, 2008, Wittig et al. 2015, Andelman et al., 1985, Girard-Buttoz et al., 2011), elephants (Rasmussen & Wittemyer, 2002), coyotes (Patterson et al., 2000) and lions (Gilfillan et al., 2017). In the present study, urine samples were primarily recovered from hard substrates both in the natural and urban environment. As non-permeable substrates are likely a key factor in successfully obtaining urine samples, uCP studies in the urban space (tarmac roads, buildings) might benefit from this. In contrast to direct pipetting, the use of new tools such as Salivettes open the possibility of collecting urine samples on various substrates; however, this requires methodological considerations as Salivettes may alter uCP concentrations (see below).

Effects of urine collection method on uCP

Our study also highlights methodological aspects of uCP as a non-invasive marker of energy status. In contrast to a recent study that found no significant difference in uCP concentrations in human and macaque urine transferred onto synthetic Salivettes and matched controls (Danish et al., 2015; note the small sample size of n=8 may have prevented an effect from being observed), uCP levels in the present study, on average, were significantly lower in samples collected with synthetic Salivettes than samples collected with pipettes. Given that we can rule out a potential storage effect/degradation (see e.g. Deschner et al., 2008, Higham et al., 2011b) as all urine samples, regardless of the collection method used, were placed in the freezer simultaneously at the end of each day (urine was recovered from Salivettes at the end of each day; see methods), this result suggests absorption of uCP by the Salivettes. There is some evidence for absorption of steroid hormones by synthetic Salivettes, for example, studies determining salivary androgen concentrations (e.g. testosterone and androstendione) using this method have reported lower recoveries (Turpeinen et al., 2012, Büttler et al., 2018). Similarly, studies comparing uCP in samples preserved on filter paper and matched original (frozen) samples suggest possible absorption of uCP but results are mixed (e.g. humans: Sherry and Ellison, 2007; macaques: Higham et al., 2011b; orang-utans: Emery Thompson and Knott, 2008). It is unlikely that synthetic Salivettes release substances that would increase the specific gravity (SG) measure (hence, lead to lower uCP measures) and SG values in our samples did not differ between the two collection methods (data not shown). Moreover, SG values in human urine samples measured following incubation of samples on

Salivettes for a couple of hours revealed no difference to SG values assessed from untreated control samples (Heistermann, unpubl.). Thus, absorption of uCP by the swab remains the most likely cause for reduced uCP concentrations found in samples collected with Salivettes.

Given that synthetic Salivettes are being increasingly used in the assessment of urinary markers (e.g. macaques: Danish et al., 2015, Müller et al., 2017, Müller-Klein et al., 2018, Müller-Klein et al., 2019), evaluation and knowledge of potential effects of using Salivettes on analyte recovery (and potentially SG measures) is crucial. In particular, studies comparing uCP within a species but using different urine collection methods should be aware of (and statistically control for) the potential effects that the applied collection methods may have on uCP measures.

Conclusion

Overall, our study indicates that uCP can be used as a reliable, non-invasive marker of energetic condition in baboons, providing a solid foundation for investigating the energetic causes and consequences of their behavioural, ecological and social flexibility in natural and human-modified landscapes across Africa (for reviews see Hill, 2017, Fischer et al., 2019). Our results suggest potential energetic benefits of living at the urban edge by offsetting constraints associated with season and reproduction. Thus, uCP measurements will allow testing theories of the physiological drivers of exploiting resource-rich human environments in primates (Hill, 2017) and potentially also in other mammals (Fehlmann et al., in revision, Lowry et al., 2013), which may ultimately help to inform mitigation strategies to minimise human-wildlife conflict. Future work in this area will require important methodological considerations regarding urine sampling and analysis techniques for uCP in non-primate mammals.

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Author contributions

IF conceived the study, analysed the urine samples, analysed the data, and wrote the manuscript with input from all authors. CC and AB conducted the field work and collected behavioural observation data, urine and faecal samples. CC processed and extracted faecal samples. MH analysed faecal samples for progestogen metabolites. AB calculated the dominance hierarchy. AJK and JO'R provided technical and logistical support.

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