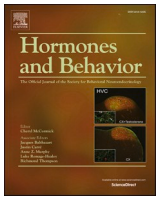
Hormones and Behavior 152 (2023) 105355

Contents lists available at ScienceDirect

Hormones and Behavior

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



Effects of accelerometry-derived physical activity energy expenditure on urinary C-peptide levels in a wild primate (*Papio ursinus*)

Angharad Morgan [a], Charlotte Christensen [a,b], Anna M. Bracken [a,c], M. Justin O'Riain [d], Andrew J. King [a], Ines Fürtbauer [a,*]

[a] Biosciences, School of Biosciences, Geography and Physics, Faculty of Science and Engineering, Swansea University, SA2 8PP Swansea, United Kingdom
[b] Department of Evolutionary Biology and Environmental Studies, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland
[c] School of Biodiversity, One Health and Veterinary Medicine, Graham Kerr Building, Glasgow G12 8QQ, Scotland, United Kingdom
[d] Institute for Communities and Wildlife in Africa, Department of Biological Sciences, University of Cape Town, Rondebosch 7701, South Africa

ARTICLE INFO

Keywords:
Baboons
C-peptide
Dynamic body acceleration
Energy balance
Energy expenditure
Tri-axial accelerometry
VeDBA

ABSTRACT

Animals have finite energy reserves for growth, survival, and reproduction and must maintain a stable energy balance. Measuring energy balance in the wild, however, is beset with methodological challenges. Quantification of urinary C-peptide (uCP), a proxy for insulin secretion, has enabled researchers to non-invasively estimate energy balance, and positive relationships between uCP levels and energy intake have been documented in numerous non-human primates. Comparatively few studies show that, consistent with insulin physiology, energy expenditure also alters levels of uCP. The timescale and extent of this relationship, however, remains unclear given the reliance on crude measures of activity and inferred energy expenditure. Here, for the first time, we test for effects of accelerometer-derived Vectorial Dynamic Body Acceleration (VeDBA) - a continuous measure of physical activity energy expenditure - on urinary C-peptide (uCP) levels in n = 12 wild chacma baboons (*Papio ursinus*). Applying a model selection approach, we show that VeDBA summed over short timescales (30 min to 1 h) prior to urine collection was negatively associated with uCP levels. Using the acceleration-based time individuals spent 'non-stationary' (i.e. locomoting) prior to urine collection as a predictor - instead of summed VeDBA - revealed similar but less clear results. Overall, the negative relationship between VeDBA and uCP levels highlights the importance of quantifying physical activity energy expenditure when using uCP measures to estimate energy balance and has potential implications for the field of energetics accelerometry.

1. Introduction

All animals take in and use energy, and the ratio of intake to expenditure, i.e. energy balance, has important physiological, behavioural, and ecological consequences (reviewed by Ellison, 2008). Energy intake, or calorie consumption, is influenced by the quality, availability, and distribution of food in an animal's environment, as well as individual preferences, and the level of competition (reviewed by Emery Thompson, 2017). Energy is expended in support of internal metabolism, specific dynamic action and thermoregulation, as well as in physical activity (Bergstrom, 2015; Bonneaud et al., 2003; reviewed by Green, 2011). Consequently, variation in physical activity leads to variation in energy expenditure which could lead to changes in overall energy balance unless compensated by energy intake. Insight into energy balance is crucial to understanding how animals allocate finite energy reserves to growth, maintenance, and reproduction, with the ultimate goal of maximising fitness (Brown et al., 2004; reviewed by Tomlinson et al., 2014). Positive energy balance can lead to energy storage over time, and a greater surplus of energy to allocate towards reproduction (reviewed by Emery Thompson, 2017). Negative energy balance, on the other hand, can increase morbidity, mortality risk, and negatively affect reproductive success (Archie et al., 2014; reviewed by Ellison, 2008).

The peptide hormone insulin plays a major role in energy and glucose metabolism. In response to high blood glucose levels, insulin is secreted to stimulate the uptake of glucose into cells and convert excess glucose to glycogen (Goodman, 2009). This maintains glucose homeostasis and supplies tissues and organs with energy. Insulin secretion is lower in subjects with a lower body mass index, suggesting that poorer energetic condition may lead to lower insulin secretion, and lower C-

* Corresponding author.
E-mail address: i.fuertbauer@swansea.ac.uk (I. Fürtbauer).

https://doi.org/10.1016/j.yhbeh.2023.105355
Received 7 September 2022; Received in revised form 13 March 2023; Accepted 27 March 2023
Available online 7 April 2023

peptide levels (Polonsky et al., 1988; Wolden-Hanson et al., 1993; Yoshida et al., 2006; Deschner et al., 2008; Girard-Buttoz et al., 2011). C-peptide, a polypeptide segment cleaved during the conversion of proinsulin to insulin, is secreted on an equimolar (1:1) basis with insulin (Sherry and Ellison, 2007). Unlike insulin, C-peptide is excreted at a constant rate into urine, bypassing liver metabolism, and allowing its use as a non-invasive proxy for changes in levels of secreted insulin, and therefore changes in individual energy balance (reviewed by Emery Thompson, 2017; Kruszynska et al., 1987).

Positive relationships between urinary C-peptide (uCP) levels and energy intake have been documented in several non-human primates (e.g. *Pongo pygmaeus*, Emery Thompson and Knott, 2008; Sherry and Ellison, 2007; *Pan paniscus*, Georgiev et al., 2011; *Papio ursinus*, Fürtbauer et al., 2020; *Papio hamadryas anubis*, Lodge, 2012; *Cebus imitator*, Bergstrom, 2015; Bergstrom et al., 2020; *Macaca fascicularis*, Girard-Buttoz et al., 2011; *Colobus guereza*, Harris et al., 2010; *Pan troglodytes*, Emery Thompson et al., 2009; Sherry and Ellison, 2007). Other studies find no effects of food availability/intake on uCP levels (e.g. *Pan paniscus*, Surbeck et al., 2015; *Alouatta palliata*: Rangel Negrín et al., 2021) or show that reductions in energy intake can be compensated by lowered energy expenditure, and thus balancing energetic condition (*Macaca fascicularis*; Girard-Buttoz et al., 2014).

Physical activity can result in reduced blood glucose levels and improved glycemic control (e.g. Solomon et al., 2018). A recent systematic review into postprandial responses in human adults suggests that engaging in any physical activity, regardless of intensity, is effective in lowering postprandial glycemia (Aqeel et al., 2020). This, along with the increased insulin sensitivity associated with physical activity (Huus et al., 2016), results in reduced secretion of insulin, and thus also reduced secretion of C-peptide during physical activity (Krotkiewski and Górski, 1986). In contrast to research into effects of energy intake on uCP levels (see above), very few studies have focused on the relationship between uCP levels and energy expenditure, and usually rely on inferred energy expenditure, e.g. mating activity, mate guarding and competition, or lactation (e.g., Higham et al., 2011a; Emery Thompson et al., 2012, Surbeck et al., 2015), or calculations based on known energetic constants (e.g. Lodge, 2012). More recently, declines in uCP levels following physical exercise have been shown in captive tufted capuchin monkeys (*Sapajus apella*, Sacco et al., 2021). In children, physical activity (daily step counts) is negatively correlated with fasting C-peptide (Huus et al., 2016). Although these studies suggest that energy expenditure, in addition to intake, alters levels of uCP, the extent and timescale of this relationship is unclear as previous findings are based on small sample sizes (Sacco et al., 2021), crude measures of activity (Higham et al., 2011a), or without time-matching of the two variables (Huus et al., 2016).

Accelerometry-derived proxies can measure energy expenditure during activity (Wilson et al., 2006) and have been used in various species across taxa (see e.g. references in Supplementary Table S1). Triaxial acceleration measures from surge, heave, and sway dimensions can be combined to provide measures of Dynamic Body Acceleration (DBA), i.e. Overall DBA Acceleration (ODBA) or Vectorial DBA (VeDBA) of an animal at any one point in time (Gleiss et al., 2011; Qasem et al., 2012). The use of these metrics depends on the relationship between the rate of mechanical work and the acceleration produced by muscle contraction (Gleiss et al., 2011). Although comparative studies have shown that ODBA and VeDBA (which are strongly correlated) can both be used as reliable proxies of physical activity energy expenditure (Qasem et al., 2012), VeDBA minimises error associated with device attachment and orientation, and thus, appears to be more appropriate (Gleiss et al., 2011).

Here, for the first time, we test for effects of VeDBA on uCP levels in wild chacma baboons (*Papio ursinus*). Predicting a negative relationship between physical activity energy expenditure and uCP levels, we first determine the timescale over which VeDBA and uCP levels are associated through summing VeDBA across various time frames prior to urine excretion. Second, to inform future works and, in particular, studies without high-resolution acceleration data, we tested whether replacing summed VeDBA with (acceleration-based) 'time spent non-stationary' would reveal comparable results.

2. Methods

2.1. Study animals and site

Data were collected as part of a larger study on a troop of wild chacma baboons, *Papio ursinus*, living at the urban edge in Da Gama Park, on the Cape Peninsula, South Africa (34.1617°S, 18.4054°E) between July and November 2018. The 'Da Gama' troop consisted of approximately 50 individuals (n = 21 adults). 17 adults were fitted with tracking collars including tri-axial accelerometers ('daily diaries'; Wildbyte Technologies, Swansea University; for details see Christensen et al., 2023; McCann et al., 2021; Bracken et al., 2021; Bracken et al., 2022). All work was approved by local authorities and Swansea University's ethics committee (IP-1314-5). Accelerometry and uCP data were available for n = 12 individuals (2 males, 10 females).

2.2. Acceleration data

Tri-axial acceleration was recorded at 40 Hz, allowing for the study of locomotory behaviour and general physical activity (Fehlmann et al., 2017). Data from the three axes – X (surge), Y (sway) and Z (heave) – were combined to calculate VeDBA:

$$VeDBA = \sqrt{\left(A_x^2 + A_y^2 + A_z^2\right)}$$

where A_x, A_y, and A_z are the dynamic body accelerations at any point in time from the three orthogonal axes represented by the accelerometer (Qasem et al., 2012). Smoothed VeDBA, which uses the running mean of values over three seconds to reduce the impact of short high-amplitude bursts of activity (Fehlmann et al., 2017), was used in all further analysis.

2.3. Urine collection and uCP analysis

N = 123 urine samples (10.3 ± 3.5 per individual, n = 12) had associated acceleration data. Full details on urine sample collection and uCP analysis are described in Fürtbauer et al. (2020). In brief, urine samples were collected opportunistically throughout the day, immediately after urination, using Salivettes or pipettes. To avoid uCP degradation, samples were kept on ice packs throughout the day (Higham et al., 2011b) and in the evening, Salivettes were centrifuged at 3000 rpm for five minutes, and urine was transferred to 2.0 ml Eppendorf Safe-Lock microcentrifuge tubes. All samples were stored at −20 °C within 12 h of collection and shipped to Swansea University on dry ice at the end of the data collection period (CITES export permit No: 208683; APHA import authorisation No: ITIMP18.1181) and stored frozen at −20 °C until analysis. uCP levels were quantified using a commercially available ELISA kit (IBL International GmbH, Hamburg, Germany; Art No. RE 53011), used successfully in other Cercopithecines (*Macaca fascicularis & M. mulatta*: Girard-Buttoz et al., 2011; Higham et al., 2011b; Sadoughi et al., 2021; *P. hamadryas anubis*: Lodge, 2012) and validated for chacma baboons (Fürtbauer et al., 2020). Assay sensitivity was 0.064 ng/ml and intra-assay variability <6.5 % according to the manufacturer. Samples with inter-assay coefficients of variation >10 % between duplicates were re-measured. 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). uCP values were corrected for specific gravity (SG) to adjust for differences in urine concentration (Miller et al., 2004; Fürtbauer et al., 2020; population

mean SG = 1.019).

2.4. Statistical analysis

Statistical analysis was carried out in RStudio version 1.1.463 (RStudio Team, 2018) using base R (version 4.2.2; R Core Team, 2022) and the package *lme4* (Bates et al., 2011) to run linear mixed models (LMMs). We used second-order Akaike Information Criterion (AICc) based model selection (Burnham and Anderson, 2002) to determine the timescale over which VeDBA and uCP levels are associated, and present results (and null model comparison) of the model with the best fit (lowest AICc; for full model comparison see Supplementary Material). Models with a difference in AICc (ΔAICc) >2 were considered to have less support (Burnham and Anderson, 2002).

First, to investigate the effect of physical activity on uCP levels, we summed VeDBA over various time intervals (15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 8 h, 12 h, 16 h and 24 h) prior to urine excretion and included these as fixed effects in linear mixed models. Season, collection method (Salivette or pipette), night fasting (before versus after 9 AM), and reproductive state (pregnant versus non-pregnant, the latter including the two males as no effect of sex on uCP levels was detected in previous work; Fürtbauer et al., 2020) have a significant influence on uCP levels in the study troop and were therefore included as additional fixed effects (for details see Fürtbauer et al., 2020) in all models, and only the summed VeDBA time interval (see above) was replaced. Winter included July and August, and spring included September and October. We used Fisher's z-transformation on VeDBA in all LMMs to allow model comparison and compare fixed effect sizes. Baboon ID and date of urine sampling were fitted as a random intercept in all LMMs to account for individual differences in uCP levels and uneven sampling across time, respectively. Random slopes were included for VeDBA. We considered random slopes for other predictors that could vary within individual ID; however, this resulted in poor model fit and did not qualitatively affect the results. We therefore chose not to include additional random slopes. uCP levels were log-transformed in all models to meet assumptions of normality. Model assumptions were checked using the package *performance* (Lüdecke et al., 2021).

Second, using the same approach, we tested whether acceleration-based 'time spent non-stationary' could also be used to predict uCP levels (instead of summed VeDBA), which could inform future studies without high-resolution acceleration data. To do this, we included minutes of non-stationary activity over the various time intervals (see above) as a fixed effect. Time spent non-stationary included seconds (converted to minutes) with VeDBA values equal or above 0.1 g, as values above this threshold likely represent locomotory activities such as running, walking and foraging (Christensen et al., 2023; Fehlmann et al., 2017).

3. Results

VeDBA summed across different time windows prior to urine collection was negatively correlated with uCP levels at short timescales (Fig. 1a; Fig. 2). The 45-min model had the lowest AICc (381.59) and was different from the null model (ΔAICc = 2.14; for model output see Table 1). The models with VeDBA summed over 30 min and 1 h received

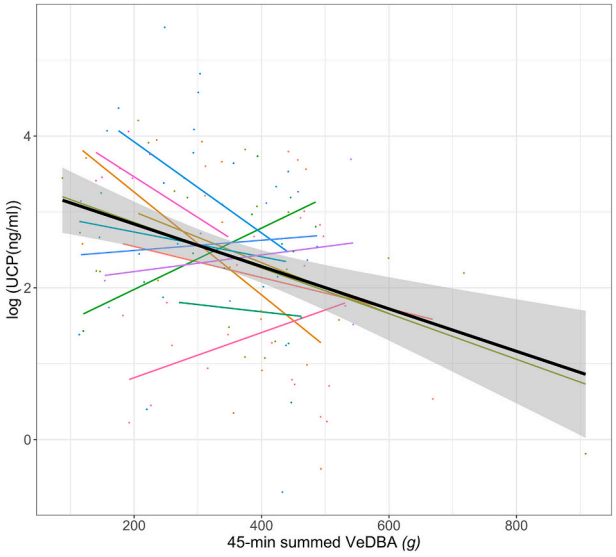


Fig. 2. Effect of Vectorial Dynamic Body Acceleration (VeDBA; *g*) summed over 45 min prior to urine collection on specific gravity corrected urinary C-peptide levels (ng/ml), showing the overall effect (thick black line, with 95 % confidence intervals), individual slopes (thin coloured lines), and individual data points (coloured according to Baboon ID; n = 12).

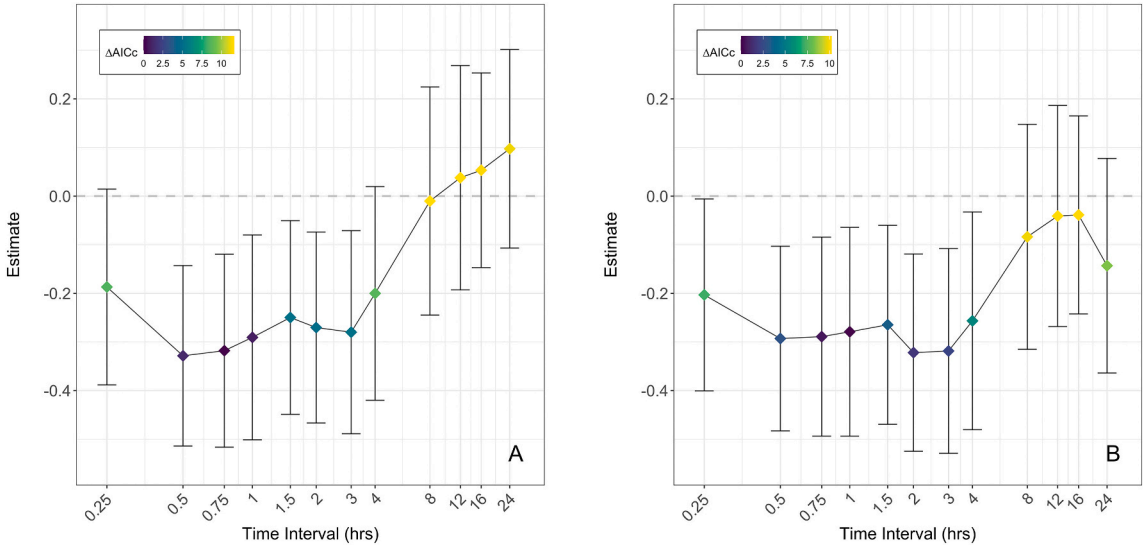


Fig. 1. Model estimates and 95 % confidence intervals for the LMMs testing the effect of a) summed Vectorial Dynamic Body Acceleration (VeDBA) and b) time spent non-stationary across different time windows prior to urine collection on specific gravity corrected urinary C-peptide levels. Dot colours represent ΔAICc values. Note that x-axes are on a log-scale.

Table 1
Effects of A) summed Vectorial Dynamic Body Acceleration (VeDBA) and B) time spent non-stationary prior to urine collection on specific gravity corrected urinary C-peptide levels (ng/ml) in n = 12 chacma baboons (n = 123 samples). Season, night fasting, urine collection method, and reproductive state were controlled for based on results from Fürtbauer et al. (2020). Baboon ID and date of urine collection were included as random effects and random slopes were included for VeDBA/time spent non-stationary.

Predictor variables	Estimate ± S.E.	t-value
A) Summed VeDBA model (AICc = 381.6)		
45 min summed VeDBA	−0.32 ± 0.1	−3.14
Season (winter)[a]	0.54 ± 0.19	2.83
Collection method (Salivette)[b]	−0.65 ± 0.19	−3.53
Night fasting (before 9 AM)[c]	−0.44 ± 0.24	−1.82
Reproductive state (pregnant)[d]	0.44 ± 0.34	1.31
B) Time spent non-stationary model (AIC = 382.8)		
Time non-stationary in 1 h	−0.28 ± 0.11	−2.55
Season (winter)[a]	0.52 ± 0.19	2.72
Collection method (Salivette)[b]	−0.69 ± 0.19	−3.69
Night fasting (before 9 AM)[c]	−0.55 ± 0.25	−2.22
Reproductive state (pregnant)[d]	0.47 ± 0.34	1.38

[a] Reference category = spring.
[b] Reference category = pipette.
[c] Reference category = after 9 AM.
[d] Reference category = non-pregnant.

similar support (ΔAICc <2; Fig. 1a; Supplementary Table S2) whereas all other models received less support (ΔAICc >2; Fig. 1a; Supplementary Table S2). For time spent non-stationary, the 1-h model had the lowest AICc (382.81) and was slightly better than the null model (ΔAICc = 0.93; for model output see Table 1). Models for time intervals of 45 min and 2 h received similar support (ΔAICc <2; Fig. 1b; Supplementary Table S3) whereas all other models received less support (ΔAICc >2; Fig. 1b; Supplementary Table S3).

4. Discussion

Quantification of uCP allows for the non-invasive assessment of energy balance in primates and potentially other mammals (see discussion in Fürtbauer et al., 2020), and positive relationships between uCP levels and measures of energy intake have been documented across species (e.g. Emery Thompson et al., 2009; Fürtbauer et al., 2020; Georgiev et al., 2011; Girard-Buttoz et al., 2011; Grueter et al., 2014; Harris et al., 2010; Sherry and Ellison, 2007). Comparatively few studies have tested or controlled for the impact of energy expenditure on uCP levels (Emery Thompson et al., 2009; Higham et al., 2011a; Sacco et al., 2021; Lodge, 2012; Rangel Negrín et al., 2021; Bergstrom et al., 2020; Surbeck et al., 2015), despite known effects of acute and regular physical exercise on insulin secretion (see below). Here, through investigating the relationship between accelerometer-derived VeDBA - a continuous measure of physical activity energy expenditure - and uCP levels, we show that physical activity energy expenditure affects uCP levels at short timescales.

4.1. Relationships between physical activity energy expenditure and uCP levels

During physical activity, insulin sensitivity and effectiveness increase (e.g. Huus et al., 2016), resulting in a larger physiological response from a submaximal concentration of insulin (Heath et al., 1983). Acute exercise also has an 'insulin-like' effect, increasing glucose uptake for glycolysis and subsequent glycogen synthesis in an insulin-independent manner via the recruitment and translocation of GLUT-4 glucose transporters (reviewed by Borghouts and Keizer, 2000; Christ-Roberts et al., 2004; reviewed by Hayashi et al., 1997; Houmard et al., 1996). Consequently, less insulin is secreted into the bloodstream during and immediately after exercise, due to enhanced insulin sensitivity. As C-peptide is secreted on an equimolar basis to insulin, C-peptide levels follow a similar pattern during physical activity. During recovery after exercise, insulin and C-peptide levels promptly increase to pre-exercise levels (Krotkiewski and Górski, 1986; Minuk et al., 1981). Consistent with this short-term physiological response described for humans, recent work demonstrated that captive capuchin monkeys show reduced uCP levels shortly after a 1-h treadmill activity, with a greater difference between pre- and post-exercise uCP at higher speed (Sacco et al., 2021).

Here, we show that summed VeDBA is negatively associated with uCP levels in chacma baboons at short time scales (30 min - 1 h; Fig. 1a). This indicates that higher physical activity shortly prior to urine collection is associated with lower uCP levels and should therefore be quantified and controlled for to allow for more comprehensive interpretation of variation in uCP measurements. Time intervals of under 30 min are likely too short to capture any potential relationship, due to the time lag between insulin and C-peptide secretion and incorporation of C-peptide into urine (Hodges and Heistermann, 2011). The decreasing support of models beyond 1 h (Fig. 1a) can be explained by insulin and C-peptide levels returning to pre-exercise levels during recovery. Models with VeDBA summed over periods between 8 and 24 h received very little support (Fig. 1a, Supplementary Table S2). Similarly, in olive baboons, no association between uCP levels and daily energy expenditure (based on time-activity budgets and activity-specific energy constants) was found (Lodge, 2012). Together, this indicates that the time window over which relationships between uCP levels and activity exist is relatively short, whereas uCP-energy intake relationships are often found over longer timeframes (e.g. Emery Thompson and Knott, 2008; Sherry and Ellison, 2007; Emery Thompson et al., 2009), highlighting a possible mismatch between timescales of energy intake/expenditure and uCP levels.

For (acceleration-based) time spent non-stationary, the 1 h model received the strongest support, with similar support for models at timeframes of 45 min and 2 h (Fig. 1b; Supplementary Table S3). The small difference in timeframes for the two measures of physical activity may be due to the level of information they capture - one is a measure of time spent active, while the other is a more detailed measure of activity, taking into account the intensity of activity during a given timeframe. Together, our results indicate that both direct (summed VeDBA) and indirect (minutes of non-stationary activity; reflecting walking, running and foraging; Christensen et al., 2023) measures of physical activity are valid proxies of physical activity energy expenditure in chacma baboons. This further suggests that even if high-resolution acceleration data are not available, physical activity energy expenditure could be quantified through measures of time spent moving or non-stationary from continuous behavioural observations prior to urine sampling.

One limitation of the present study is that energy intake could not be restricted or quantified. Previous studies demonstrating effects of physical activity on C-peptide levels used fasting C-peptide, thereby ruling out any effects of energy intake (humans: Huus et al., 2016; capuchins: Sacco et al., 2021). Collecting urine samples from wild baboons is challenging and restricting sample collection to fasting samples (i.e. before 9 AM, see Fürtbauer et al., 2020) would result in a considerably smaller sample size. Note, however, that random non-fasting C-peptide levels correlate with fasting C-peptide levels in humans (Berger et al., 2000). In our study population, uCP levels are highly repeatable within individuals, and – apart from lower uCP levels before 9 AM (Fürtbauer et al., 2020), we find no diurnal effects on uCP levels. These findings are likely due to the fact that baboons spend a large portion of the day feeding/foraging (Christensen et al., 2023; Weingrill et al., 2004) and their natural diet mainly includes low-calorie plant-derived foods (Johnson et al., 2013), presumably resulting in less fluctuations in glucose, insulin, and thus C-peptide levels (e.g. Fabbrini et al., 2013). Taken together and given the short timeframe over which physical activity and uCP levels were associated, we believe that any potential effects due to absence/presence of energy intake on uCP levels in our study is minimal. Future studies, however, should consider both energy

expenditure and intake.

4.2. uCP: an endocrine tool in energetics accelerometry?

Accelerometry studies of animal energetics usually test VeDBA (and/or ODBA) against physiological measures of energy expenditure, i.e. doubly labelled Water (DLW) or heart rate (fH) in the wild, or the estimation of metabolic rate in the laboratory through measurement of the rate of oxygen consumption (VO_2) (see Supplementary Table S1). Both methods can be invasive, and often involve drawing blood samples (DLW), or surgical implantation of data loggers (fH) (Butler et al., 2004; although see Green, 2011 for discussion on external attachment of heart rate loggers). DLW can only be used in the short-term, ruling out the potential for long-term studies in natural settings (Speakman, 1997). While heart rate loggers provide data at high temporal resolutions, heart rate can be influenced by other factors such as stress and physical fitness, altering its relationship with energy expenditure and oxygen consumption (Green, 2011).

In addition to the shortcomings of these methods, Halsey (2017) proposed that many reported acceleration-energy expenditure relationships have been falsely interpreted, and that accelerometers do not measure the amount of energy expended from an activity, but instead the amount of time in an activity. Known as the 'time trap' hypothesis, this suggests that relationships between summed measures of energy expenditure, such as total oxygen consumption and summed tri-axial acceleration values, should be treated with caution because time is included on both sides of the equation. In diving seals and sea lions, for instance, acceleration-energy expenditure relationships fail to persist when total measures are converted to rates or mean values (Ladds et al., 2017), suggesting that accelerometers do not accurately measure energy expenditure. Since the problem arises due to the regression of two variables that include time, uCP could offer a means of validating VeDBA without falling into the time trap. This is assuming that due to C-peptide being filtered at a consistent rate by the kidney, the resulting concentrations in the bladder do not change over time. Future work could investigate the presence or absence of a relationship between uCP levels and time since previous miction.

Overall, uCP offers a non-invasive, relatively inexpensive, and repeated measure of energetic condition both in captivity and in the wild. Whether relationships between VeDBA and uCP levels could be studied in other species will depend on the practicality of using accelerometers and the feasibility of both urine collection and uCP detection. Urine samples can be retrieved using pipettes, filter paper, or synthetic Salivettes, however collection can be complicated by surfaces which quickly absorb fluids, and the need for sufficient amounts of urine for analysis (Danish et al., 2015). The fluid recovery rate of collection devices is thus important, with synthetic Salivettes proving superior compared to alternatives (Danish et al., 2015), however potential absorption of uCP by Salivettes should be considered (see Fürtbauer et al., 2020). Finally, although the majority of uCP studies to date are on non-human primates (e.g., Girard-Buttoz et al., 2011; Sherry and Ellison, 2007), uCP has also been quantified in other mammals (e.g., Rosenfield et al., 2017; see discussion in Fürtbauer et al., 2020), forming a promising basis for using uCP measurements as a new tool in energetics accelerometry also in non-primate mammals.

5. Conclusions

The negative uCP-physical activity relationship has important implications for the interpretation of past studies and the design of future studies using uCP as a proxy of energy balance. Considering both energy intake and expenditure, and the time frames over which these two factors (and their proxies of various temporal nature) influence uCP levels will be crucial. Future research on species where C-peptide can be detected in urine (see above) can now use the present study (and time frames) as a basis to explore relationships between VeDBA - or non-stationary activity if accelerometry is infeasible - and uCP measurements, opening new avenues into understanding animal energy balance.

CRediT authorship contribution statement

IF conceived the study and analysed the urine samples. CC and AB conducted the field work and collected urine samples. AM analysed the data and wrote the first draft of the manuscript under supervision of IF. All authors commented on the manuscript. AJK and JO'R provided technical and logistical support.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

Acknowledgements

We thank the Baboon Technical Team, Cape Nature, and SANParks for authorisation to conduct our research. We also thank Human Wildlife Solutions, field assistants Lucy Robertson, Charlotte Solman, and Francesca Marshall-Stochmal, vets Dorothy Breed and Gary Buhrman, Esme Beamish, and Layla King for their help and support and Stuart Semple, Emily Shepard, and two anonymous reviewers for providing comments on earlier drafts. We are grateful to the Association for the Study of Animal Behaviour for supporting this work with a research grant awarded to IF.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2023.105355.

References

Aqeel, M., Forster, A., Richards, E.A., Hennessy, E., McGowan, B., Bhadra, A., Guo, J., Gelfand, S., Delp, E., Eicher-Miller, H.A., 2020. The effect of timing of exercise and eating on postprandial response in adults: a systematic review. Nutrients 12 (1), 221.

Archie, E.A., Altmann, J., Alberts, S.C., 2014. Costs of reproduction in a long-lived female primate: injury risk and wound healing. Behav. Ecol. Sociobiol. 68 (7), 1183–1193. https://doi.org/10.1007/s00265-014-1729-4.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Grothendieck, G., 2011. Package "lme4." *Linear-Mixed Effects Models Using S4 Classes.* R Package Version, 1. Retrieved from. http://dk.archive.ubuntu.com/pub/pub/cran/web/packages/lme4/lme4.pdf. Retrieved from.

Berger, B., Stenström, G., Sundkvist, G., 2000. Random C-peptide in the classification of diabetes. Scand. J. Clin. Lab. Invest. 60, 687–693. https://doi.org/10.1080/00365510050216411.

Bergstrom, M.L., 2015. Seasonal Effects on Nutrition and Energetic Condition of Female White-Faced Capuchin Monkeys (University of Calgary). University of Calgary. https://doi.org/10.11575/PRISM/27720.

Bergstrom, M.L., Kalbitzer, U., Campos, F.A., Melin, A.D., Emery Thompson, M., Fedigan, L.M., 2020. Non-invasive estimation of the costs of feeding competition in a neotropical primate. Horm. Behav. 118, 104632 https://doi.org/10.1016/j.yhbeh.2019.104632.

Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B., Sorci, G., 2003. Assessing the cost of mounting an immune response. Am. Nat. 161 (3), 367–379. https://doi.org/10.1086/346134.

Borghouts, L.B., Keizer, H.A., 2000. Exercise and insulin sensitivity: a review. Int. J. Sports Med. 21, 1–12. Georg Thieme Verlag Stuttgart ·New York. https://doi.org/10.1055/s-2000-8847. Georg Thieme Verlag Stuttgart ·New York.

Bracken, A.M., Christensen, C., O'Riain, M.J., Fehlmann, G., Holton, M.D., Hopkins, P. W., Fürtbauer, I., King, A.J., 2021. Socioecology explains individual variation in urban space-use in response to management in Cape chacma baboons (*Papio ursinus*). Int. J. Primatol. 43, 1159–1176. https://doi.org/10.1007/s10764-021-00247-x.

Bracken, A.M., Christensen, C., O'Riain, M.J., Fürtbauer, I., King, A.J., 2022. Flexible group cohesion and coordination, but robust leader-follower roles, in a wild social primate using urban space. Proc. R. Soc. B Biol. Sci. 289, 20212141 https://doi.org/10.1098/rspb.2021.2141.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. Ecology 85 (7), 1771–1789. John Wiley & Sons, Ltd. https://doi.org/10.1890/03-9000. John Wiley & Sons, Ltd.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer Science & Business Media, Berlin, Germany.

Butler, P.J., Green, J.A., Boyd, I.L., Speakman, J.R., 2004. Measuring metabolic rate in the field: the pros and cons of the doubly labelled water and heart rate methods. Funct. Ecol. 18, 168–183. https://doi.org/10.1111/j.0269-8463.2004.00821.x.

Christensen, C., Bracken, A.M, O'Riain, M.J., Fehlmann, G., Holton, M., Hopkins, P., King, A.J., Fürtbauer, I., 2023. Quantifying allo-grooming in chacma baboons (*Papio ursinus*) using tri-axial acceleration data and machine learning. R. Soc. Open Sci. 10, 221103 https://doi.org/10.1098/rsos.221103.

Christ-Roberts, C.Y., Pratipanawatr, T., Pratipanawatr, W., Berria, R., Belfort, R., Kashyap, S., Mandarino, L.J., 2004. Exercise training increases glycogen synthase activity and GLUT4 expression but not insulin signaling in overweight nondiabetic and type 2 diabetic subjects. Metab. Clin. Exp. 53 (9), 1233–1242. https://doi.org/10.1016/j.metabol.2004.03.022.

Danish, L.M., Heistermann, M., Agil, M., Engelhardt, A., 2015. Validation of a novel collection device for non-invasive urine sampling from free-ranging animals. PLoS One 10 (11), e0142051. https://doi.org/10.1371/journal.pone.0142051.

Deschner, T., Kratzsch, J., Hohmann, G., 2008. Urinary C-peptide as a method for monitoring body mass changes in captive bonobos (*Pan paniscus*). Horm. Behav. 54 (5), 620–626.

Ellison, P., 2008. Energetics, reproductive ecology, and human evolution. PaleoAnthropology 2008, 172–200.

Emery Thompson, M., 2017. Energetics of feeding, social behavior, and life history in non-human primates. Horm. Behav. 91, 84–96. https://doi.org/10.1016/j.yhbeh.2016.08.009.

Emery Thompson, M., Knott, C.D., 2008. Urinary C-peptide of insulin as a non-invasive marker of energy balance in wild orangutans. Horm. Behav. 53 (4), 526–535. https://doi.org/10.1016/j.yhbeh.2007.12.005.

Emery Thompson, M., Muller, M.N., Wrangham, R.W., Lwanga, J.S., Potts, K.B., 2009. Urinary C-peptide tracks seasonal and individual variation in energy balance in wild chimpanzees. Horm. Behav. 55 (2), 299–305. https://doi.org/10.1016/j.yhbeh.2008.11.005.

Emery Thompson, M., Muller, M.N., Wrangham, R.W., 2012. The energetics of lactation and the return to fecundity in wild chimpanzees. Behav. Ecol. 23 (6), 1234–1241. https://doi.org/10.1093/beheco/ars107.

Fabbrini, E., Higgins, P.B., Magkos, F., Bastarrachea, R.A., Voruganti, V.S., Comuzzie, A.G., Shade, R.E., Gastaldelli, A., Horton, J.D., Omodei, D., Patterson, B.W., Klein, S., 2013. Metabolic response to high-carbohydrate and low-carbohydrate meals in a nonhuman primate model. Am. J. Phys. Endocrinol. Metab. 304, E444–E451.

Fehlmann, G., O'Riain, M.J., Hopkins, P.W., O'Sullivan, J., Holton, M.D., Shepard, E.L.C., King, A.J., 2017. Identification of behaviours from accelerometer data in a wild social primate. Anim. Biotelemetry 5 (1), 1–11. https://doi.org/10.1186/s40317-017-0121-3.

Fürtbauer, I., Christensen, C., Bracken, A., O'Riain, M.J., Heistermann, M., King, A.J., 2020. Energetics at the urban edge: environmental and individual predictors of urinary C-peptide levels in wild chacma baboons (*Papio ursinus*). Horm. Behav. 126, 104846 https://doi.org/10.1016/j.yhbeh.2020.104846.

Georgiev, A.V., Thompson, M.E., Lokasola, A.L., Wrangham, R.W., 2011. Seed predation by bonobos (*Pan paniscus*) at Kokolopori, Democratic Republic of the Congo. Primates 52 (4), 309–314. https://doi.org/10.1007/s10329-011-0256-4.

Girard-Buttoz, C., Higham, J.P., Heistermann, M., Wedegärtner, S., Maestripieri, D., Engelhardt, A., 2011. Urinary C-peptide measurement as a marker of nutritional status in macaques. PLoS One 6 (3), e18042. https://doi.org/10.1371/journal.pone.0018042.

Girard-Buttoz, C., Heistermann, M., Rahmi, E., Marzec, A., Agil, M., Fauzan, P.A., Engelhardt, A., 2014. Mate-guarding constrains feeding activity but not energetic status of wild male long-tailed macaques (*Macaca fascicularis*). Behav. Ecol. Sociobiol. 68, 583–595.

Gleiss, A.C., Wilson, R.P., Shepard, E.L.C., 2011. Making overall dynamic body acceleration work: on the theory of acceleration as a proxy for energy expenditure. Methods Ecol. Evol. 2 (1), 23–33. https://doi.org/10.1111/j.2041-210X.2010.00057.x.

Goodman, H.M., 2009. Basic Medical Endocrinology, Third edition. Academic Press, San Diego. https://doi.org/10.1016/B978-0-12-373975-9.X0001-8.

Green, J.A., 2011. The heart rate method for estimating metabolic rate: review and recommendations. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 158 (3), 287–304. https://doi.org/10.1016/j.cbpa.2010.09.011.

Grueter, C.C., Deschner, T., Behringer, V., Fawcett, K., Robbins, M.M., 2014. Socioecological correlates of energy balance using urinary C-peptide measurements in wild female mountain gorillas. Physiol. Behav. 127, 13–19. https://doi.org/10.1016/j.physbeh.2014.01.009.

Halsey, L.G., 2017. Relationships grow with time: a note of caution about energy expenditure-proxy correlations, focussing on accelerometry as an example. Funct. Ecol. 31, 1176–1183. Blackwell Publishing Ltd. https://doi.org/10.1111/1365-2435.12822. Blackwell Publishing Ltd.

Harris, T.R., Chapman, C.A., Monfort, S.L., 2010. Small folivorous primate groups exhibit behavioral and physiological effects of food scarcity. Behav. Ecol. 21 (1), 46–56. https://doi.org/10.1093/beheco/arp150.

Hayashi, T., Wojtaszewski, J.F.P., Goodyear, L.J., 1997. Exercise regulation of glucose transport in skeletal muscle. Am. J. Physiol. Endocrinol. Metab. 273, 1039–1051. https://doi.org/10.1152/ajpendo.1997.273.6.e1039.

Heath, G.W., Gavin, J.R., Hinderliter, J.M., Hagberg, J.M., Bloomfield, S.A., Holloszy, J.O., 1983. Effects of exercise and lack of exercise on glucose tolerance and insulin sensitivity. J. Appl. Physiol. Respir. Environ. Exerc. Physiol. 55 (2), 512–517. https://doi.org/10.1152/jappl.1983.55.2.512.

Higham, J.P., Heistermann, M., Maestripieri, D., 2011a. The energetics of male-male endurance rivalry in free-ranging rhesus macaques, *Macaca mulatta*. Anim. Behav. 81 (5), 1001–1007. https://doi.org/10.1016/j.anbehav.2011.02.001.

Higham, J.P., Girard-Buttoz, C., Engelhardt, A., Heistermann, M., 2011b. Urinary C-peptide of insulin as a non-invasive marker of nutritional status: some practicalities. PLoS One 6 (7), e22398. https://doi.org/10.1371/journal.pone.0022398.

Hodges, J.K., Heistermann, M., 2011. Field endocrinology: monitoring hormonal changes in free–ranging primates. In: Field and Laboratory Methods in Primatology: A Practical Guide, Second edition, pp. 353–370. https://doi.org/10.1017/CBO9780511921643.022.

Houmard, J.A., Tyndall, G.L., Midyette, J.B., Hickey, M.S., Dolan, P.L., Gavigan, K.E., Dohm, G.L., 1996. Effect of reduced training and training cessation on insulin action and muscle GLUT-4. J. Appl. Physiol. 81 (3), 1162–1168. https://doi.org/10.1152/jappl.1996.81.3.1162.

Huus, K., Åkerman, L., Raustorp, A., Ludvigsson, J., 2016. Physical activity, blood glucose and C-peptide in healthy school-children, a longitudinal study. PLoS One 11 (6), e0156401. https://doi.org/10.1371/journal.pone.0156401.

Johnson, C.A., Raubenheimer, D., Rothman, J.M., Clarke, D., Swedell, L., 2013. 30 days in the life: daily nutrient balancing in a wild Chacma baboon. PLoS One 8 (7), e70383. https://doi.org/10.1371/journal.pone.0070383.

Krotkiewski, M., Górski, J., 1986. Effect of muscular exercise on plasma C-peptide and insulin in obese non-diabetics and diabetics, Type II. Clin. Physiol. 6 (6), 499–506. https://doi.org/10.1111/j.1475-097X.1986.tb00783.x.

Kruszynska, Y.T., Home, P.D., Hanning, I., Alberti, K.G.M.M., 1987. Basal and 24-h C-peptide and insulin secretion rate in normal man. Diabetologia 30 (1), 16–21. https://doi.org/10.1007/BF01788901.

Ladds, M.A., Rosen, D.A.S., Slip, D.J., Harcourt, R.G., 2017. Proxies of energy expenditure for marine mammals: an experimental test of "the time trap". Sci. Rep. 7 (1), 1–10. https://doi.org/10.1038/s41598-017-11576-4.

Lodge, E., 2012. Energetics and Life-history of Olive Baboons (Papio hamadryas anubis) in the Gashaka Gumti National Park. University of Roehampton.

Lüdecke, D., Ben-Shachar, M., Patil, I., Waggoner, P., Makowski, D., 2021. Performance: an R package for assessment, comparison and testing of statistical models. J. Open Source Softw. 6 (60), 3139. https://doi.org/10.21105/joss.03139.

McCann, R., Bracken, A.M., Christensen, C., Fürtbauer, I., King, A.J., 2021. The relationship between GPS sampling interval and estimated daily travel distances in chacma baboons (*Papio ursinus*). Int. J. Primatol. 42 (4), 589–599. https://doi.org/10.1007/s10764-021-00220-8.

Miller, R.C., Brindle, E., Holman, D.J., Shofer, J., Klein, N.A., Soules, M.R., O'Connor, K.A., 2004. Comparison of specific gravity and creatinine for normalizing urinary reproductive hormone concentrations. Clin. Chem. 50, 924–932.

Minuk, H.L., Vranic, M., Marliss, E.B., 1981. Glucoregulatory and metabolic response to exercise in obese noninsulin-dependent diabetes. Am. J. Physiol. Endocrinol. Metab. 3 (5) https://doi.org/10.1152/ajpendo.1981.240.5.e458.

Polonsky, K.S., Given, B.D., Hirsch, L., Shapiro, E.T., Tillil, H., Beebe, C., Van Cauter, E., 1988. Quantitative study of insulin secretion and clearance in normal and obese subjects. J. Clin. Investig. 81 (2), 435–441. https://doi.org/10.1172/JCI113338.

Qasem, L., Cardew, A., Wilson, A., Griffiths, I., Halsey, L.G., Shepard, E.L.C., Wilson, R., 2012. Tri-axial dynamic acceleration as a proxy for animal energy expenditure; should we be summing values or calculating the vector? PLoS One 7 (2), e31187. https://doi.org/10.1371/JOURNAL.PONE.0031187.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rangel Negrín, A., Coyohua Fuentes, A., de la Torre Herrera, A., Cano Huertes, B., Reynoso Cruz, E., Ceccarelli, E., Dias, P.A.D., 2021. Female reproductive energetics in mantled howler monkeys (*Alouatta palliata*): a follow-up study. Am. J. Phys. Anthropol. 174 (3), 396–406. https://doi.org/10.1002/ajpa.24222.

Rosenfield, D.A., Nichi, M., Pizzutto, C.S., 2017. C-peptides for diagnostics and therapy: a veterinary medicine point of view. Pesqui. Vet. Bras. 37 (1), 36–40. https://doi.org/10.1590/S0100-736X2017000100006.

RStudio Team. (2018). R Studio Version 1.1.463.

Sacco, A.J., Granatosky, M.C., Laird, M.F., Milich, K.M., 2021. Validation of a method for quantifying urinary C-peptide in platyrrhine monkeys. Gen. Comp. Endocrinol. 300, 113644 https://doi.org/10.1016/j.ygcen.2020.113644.

Sadoughi, B., Girard-Buttoz, C., Engelhardt, A., Heistermann, M., Ostner, J., 2021. Non-invasive assessment of metabolic responses to food restriction using urinary triiodothyronine and cortisol measurement in macaques. Gen. Comp. Endocrinol. 306, 113736 https://doi.org/10.1016/j.ygcen.2021.113736.

Sherry, D.S., Ellison, P.T., 2007. Potential applications of urinary C-peptide of insulin for comparative energetics research. Am. J. Phys. Anthropol. 133 (1), 771–778. https://doi.org/10.1002/ajpa.20562.

Solomon, T.P., Eves, F.F., Laye, M.J., 2018. Targeting postprandial hyperglycemia with physical activity may reduce cardiovascular disease risk. But what should we do, and when is the right time to move? Front. Cardiovasc. Med. 5, 99.

Speakman, J.R., 1997. Doubly Labelled Water: Theory and Practice. Chapman & Hall, London.

Surbeck, M., Deschner, T., Behringer, V., Hohmann, G., 2015. Urinary C-peptide levels in male bonobos (*Pan paniscus*) are related to party size and rank but not to mate competition. Horm. Behav. 71, 22–30.

Tomlinson, S., Arnall, S.G., Munn, A., Bradshaw, S.D., Maloney, S.K., Dixon, K.W., Didham, R.K., 2014. Applications and implications of ecological energetics. Trends

Ecol. Evol. 29, 280–290. Elsevier Current Trends. https://doi.org/10.1016/j.tree.2014.03.003. Elsevier Current Trends.

Weingrill, T., Gray, D., Barrett, L., Henzi, S., 2004. Fecal cortisol levels in free-ranging female chacma baboons: relationship to dominance, reproductive state and environmental factors. Horm. Behav. 45, 259–269.

Wilson, R.P., White, C.R., Quintana, F., Halsey, L.G., Liebsch, N., Martin, G.R., Butler, P. J., 2006. Moving towards acceleration for estimates of activity-specific metabolic rate in free-living animals: the case of the cormorant. J. Anim. Ecol. 75 (5), 1081–1090. https://doi.org/10.1111/j.1365-2656.2006.01127.x.

Wolden-Hanson, T., Davis, G.A., Baum, S.T., Kemnitz, J.W., 1993. Insulin levels, physical activity, and urinary catecholamine excretion of obese and non-obese rhesus monkeys. Obes. Res. 1 (1), 5–17.

Yoshida, N.M., Yoshiuchi, K., Kumano, H., Sasaki, T., Kuboki, T., 2006. Changes in heart rate with refeeding in anorexia nervosa: a pilot study. J. Psychosom. Res. 61 (4), 571–575.