Fecal thyroid hormones allow for the noninvasive monitoring of energy intake in capuchin monkeys

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Abstract

Measuring energetic condition of wild animals is of major importance in ecological research, as it is profoundly linked to fitness. However, noninvasive monitoring of energetic condition in wild-living animals is methodologically challenging. Measuring urinary C-peptide levels is a suitable method to noninvasively assess energy balance in wild-living animals. As collecting urine is not always feasible in the wild, it is essential to establish alternative biomarkers for other sample types to assess energy balance. Thyroid hormones (TH) are potential candidates as they are involved in the regulation of metabolic processes. During periods of low energy intake, serum TH levels are reduced, leading to a decrease in metabolic activity. To investigate whether fecal TH can serve as a biomarker for energy balance, we validated a total T_3 ELISA to measure immunoreactive T_3 (iT_3) in fecal samples of yellow-breasted capuchins. We restricted caloric intake of seven males, assessed daily group caloric intake and determined daily individual fecal iT_3 levels. Analytical validation of the assay showed that fecal iT_3 levels can be reliably measured; however, proper storage conditions must be implemented and possible degradation to be accounted for. IT_3 levels were significantly higher on days with high group caloric intake. However, individual iT_3 levels varied substantially, resulting in an overlap across individuals between conditions. Our results indicate that fecal iT_3 levels can serve as a useful biomarker to detect changes in energy intake of yellow-breasted capuchins. Overall, measuring fecal iT_3 levels may present a suitable method for monitoring energy balance when urine collection is impossible.

Key Words

- T_3
- primates
- feces
- caloric restriction
- energetic condition

Journal of Endocrinology (2016) **231**, 1–10

Journal of Endocrinology

Introduction

The situation-dependent allocation of energy to phenotypic traits throughout an animal's life affects the physical appearance of the animal and has an influence on traits such as body size, age of maturation and number of offspring, thereby ultimately determining lifetime reproductive success (Stearns 1992, Roff 2002). The energy allocation model assumes that energy can either be allocated to reproduction, somatic growth and maintenance, or storage (Perrin & Sibly 1993, Roff & Fairbairn 2007, French *et al.* 2009). Monitoring an individual's energy balance (defined as energy intake minus energy expenditure) is, therefore, essential to understanding how individuals allocate their energy between competing demands in relation to

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0152

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their environment. The investigation of energy balance in primates is particularly interesting, as they have exceptionally slow rates of growth, reproduction, aging and a low metabolic rate (Pontzer *et al.* 2014).

Assessing energetic condition of wild-living animals is a valuable tool in behavioral ecology when studying the effects of changing environmental conditions on the energy budget, behavior and life history of an individual. When food resources are limited, individuals have to allocate energetic resources to processes essential for survival to protect future reproductive possibilities (Stearns & Hoekstra 2005). Such a trade-off can be important in energetically costly contexts as, for example, competition over mates, mate guarding and the quest for high dominance rank (Bercovitch & Nurnberg 1996, Janson & Vogel 2006, Higham *et al.* 2011, Surbeck *et al.* 2015). The assessment of individual energetic condition allows for the ability to trace patterns of such trade-offs.

Noninvasive quantification of energy balance in wild-living animals remains a major methodological challenge. Up until the last decade, energy intake, energy expenditure and energy balance were assessed by collecting a variety of data, such as the amount of consumed food, time spent on processing food, analyzing nutritional values of food items, travel distance and time spent on finding food resources (e.g., Coelho 1974, Eley *et al.* 1989, Chivers 1998, Knott 1998). Such detailed measures require intensive observation times and data collection.

An alternative is the quantification of biomarkers of energy balance in noninvasively collected samples. Recent studies have validated the use of urinary C-peptide (UCP) as a physiological marker to assess energy balance in primates (Sherry & Ellison 2007, Deschner *et al.* 2008, Emery Thompson & Knott 2008, Girard-Buttoz *et al.* 2011). This method allows for the monitoring of energy balance in wild-living animal populations and thereby for the investigation of trade-offs in relation to competition for rank and reproduction, and food abundance. Dominant chimpanzee males have consistently lower C-peptide levels than low-ranking males (Emery Thompson *et al.* 2009), supporting previous findings that maintaining high dominance rank is associated with metabolic costs (Muller & Wrangham 2004). In wild bonobos and long-tailed macaques, mate guarding or mate competition does not affect male energy balance (Girard-Buttoz *et al.* 2014, Surbeck *et al.* 2015). Male rhesus macaques engage in competitive endurance rivalry leading to high-ranking males, who heavily invested into mating activities during the mating season, being in worse energetic condition than low-ranking males (Higham *et al.* 2011). Periods of low food availability or lower food quality led to a negative energy balance as measured with UCP levels in female orangutans (Emery Thompson & Knott 2008), mountain gorillas (Grueter *et al.* 2014) and black-and-white colobus (Harris *et al.* 2010). Furthermore, female chimpanzee mothers inhabiting lower quality foraging areas were found to be in worse energetic condition than mothers in food-rich areas, resulting in longer periods before resuming ovarian cyclic activity after lactational amenorrhea (Emery Thompson *et al.* 2012). Therefore, such noninvasive methods for the assessment of energetic condition open the door to investigate costs and benefits of different life history strategies.

However, in many wild-living animal species, such as capuchin monkeys, collecting urine samples is not regularly feasible. Fecal samples might present an alternative matrix for establishing a biomarker for energy balance as they have been successfully used to measure a variety of steroid hormones in animal species (Palme *et al.* 2005, Heistermann 2010, Goymann 2012). As C-peptide levels cannot be measured in feces, there is a compelling need to establish a biomarker to reliably assess the changes in energy balance in noninvasively collected fecal samples. Researchers have recently measured thyroid hormones (THs) in fecal samples in a validation study across several mammalian species (Wasser *et al.* 2010), in response to reduced food availability (Ingbar & Galton 1975, Abdullah & Falconer 1977, Ayres *et al.* 2012, Gobush *et al.* 2014, Joly *et al.* 2015) and in relation to ecological parameters (Cristóbal-Azkarate *et al.* 2016).

THs are crucially involved in the regulation of the metabolic activity of organisms (Kaack *et al.* 1979) and, therefore, represent suitable candidates for monitoring energy balance. In humans, during periods of acute or chronic starvation and hence restricted caloric intake, serum TH levels decrease and as a consequence, basal metabolism is reduced in order to conserve energy (Merimee & Fineberg 1976, Eales 1988, Blake *et al.* 1991, Flier *et al.* 2000, Fontana *et al.* 2006, Holloszy & Fontana 2007); in contrast, during high caloric intake, serum TH levels are elevated (Danforth *et al.* 1979, Galofré *et al.* 2010). Additionally, in several mammal species, changes in energy balance are associated with changes in serum TH levels, as for example, TH levels decreased during fasting in badgers (Harlow & Seal 1981), sheep (Blum *et al.* 1980) and rabbits (Menchetti *et al.* 2015). TH levels increase as well with increasing energy expenditure, for example, as a reaction to low temperatures (Silva 1995, Cristóbal-Azkarate *et al.* 2016) and in relation to the thermic effect

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DOI: 10.1530/JOE-16-0152

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of physical activity (Kim 2008). Consequently, TH are well-suited candidates for serving as a physiological marker for energy balance.

The two main TH are thyroxine (3,3′,5,5′-tetraiodothyronine, T_4) and triiodothyronine (3′,3,5-triiodothyronine, T_3) (Köhrle 1999). Although more than 99% of T_3 and T_4 are bound to serum proteins, it is mainly the free form of T_3 that is biologically active (Danforth & Burger 1989). The sum of free and bound T_3 is named total T_3 (TT_3) (Köhrle 1999). Radiometabolism studies on rats have revealed that while a majority of TH is excreted into the urine (Shakespear & Burke 1976), a considerable amount of TH can be recovered in feces (DiStefano & Sapin 1987). Additionally, in domestic dogs, the majority of thyroid metabolites in urine are excreted as T_3 and only a small fraction as T_4 (Wasser *et al.* 2010).

Recently, TH were measured in fecal samples of a variety of animal species, including nonhuman primates (Wasser *et al.* 2010). In howler monkeys, reduced food intake led to decreased fecal T_3 levels in females, but not in males (Wasser *et al.* 2010). Unfortunately, as no data on caloric intake were collected, an interpretation of a causal relationship between reduced energy intake and fecal T_3 levels was difficult to establish. However, while these results indicate that fecal T_3 is correlated with energy balance, increased precision in the tracking of energy intake is still needed to validate this hypothesis.

Thus, the objective of this study is to validate a method for the noninvasive assessment of energy intake in fecal samples of yellow-breasted capuchins. Our approach was (1) to investigate whether T_3 can be reliably measured in fecal samples of this species with a commercial TT_3 ELISA (analytical validation) and (2) to test whether fecal T_3 levels are affected by caloric restriction (biological validation). Therefore, we assessed daily caloric intake of two captive yellow-breasted capuchin monkey groups over a 3-week period during which the caloric intake was manipulated to simulate a period of restricted energy availability.

Methods

Subjects

We conducted a feeding experiment during a 3-week period between March and April 2014 on male yellow-breasted capuchins (*Sapajus xanthosternos*) from two different social groups kept in Zoo Frankfurt, Germany. Age ranged from 1 to 28 years. One group consisted of five males and one female (group A), and the other group consisted of three males (group B). We excluded one male from group A from further analysis, as sample size for the male was too low. We also excluded the only female because statistical analysis of one individual would not be possible and would not be representative of the female sex. All individuals were in good health at the time of data collection. The capuchins were fed with a mixture of fruits and vegetables and water was available *ad libitum* during the whole study period.

Fecal sample collection

All fecal samples were collected throughout the day (09:00 h–17:00 h) from the ground when explicitly assignable to the defecator and when not contaminated with urine. Fresh fecal samples were collected in plastic tubes and immediately stored at −20°C. After the termination of the experiment, samples were transported frozen to the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany (MPI-EVA).

Sample preparation and TT_3 assay

In the lab at MPI-EVA, we freeze-dried all samples for 24 h at −40°C, ground and then stored them in whirl packs at −20°C. Seventy-two samples from seven males underwent an ethanol–water extraction (70%) following Wasser *et al.* (2010) with the following modifications: For each sample, 7.5 mL ethanol–water mix (70%) were added to 0.05 g freeze-dried fecal powder, vortexed for 30 min and then centrifuged for 20 min at 1040 ***g***. The supernatant was decanted into a fresh vial, the procedure was repeated and the resulting supernatant was combined with the first.

We took 2400 µL of ethanol extract, evaporated the extract under nitrogen at 45°C and reconstituted the extract in 120 µL TT_3 null standard (a buffer provided by IBL International GmbH, Hamburg, Germany, consisting of human serum and stabilizers). Immunoreactive T_3 (iT_3) was measured with a commercial, competitive TT_3 ELISA (Ref. RE55251, IBL International GmbH) for human serum. Samples, standards and quality controls (50 µL per well) were run in duplicates, following the instructions from the supplier. Results were calculated as ng per g dried feces.

Out of 72 samples, 15 were above the linear range of the assay and, therefore, needed re-measurement at a concentration of 10:1 with 1200 µL EtOH extract reconstituted in 120 µL TT_3 null standard. Values for two of the samples were discarded from the data set because one was unrealistically high (more than three standard

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DOI: 10.1530/JOE-16-0152

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deviations above the mean), indicating a potential unobserved contamination with urine, and the other because we could not achieve consistent values despite repeated measurements.

Analytical validation

Extraction efficiency, parallelism, assay accuracy, inter- and intra-assay coefficients of variance, stability For the analytical validation of the TT_3 assay, we conducted the following steps:

(a) To assess extraction efficiency, four samples were spiked with two different concentrations of standard (high: 0.25 ng/mL, low: 0.1 ng/mL) before the evaporation step and subsequently measured with the assay. It is not feasible to reliably spike dry fecal powder in a homogeneous manner. Therefore, and to at least assess the extraction efficiency for part of the extraction process, we spiked the ethanol extract and referred to this as extraction efficiency throughout the article. Both the assay recovery and the extraction efficiency were calculated following Behringer *et al.* (2012).

(b) To test for parallelism, a pool sample (210 µL with a concentration of 2.67 ng/mL extract) consisting of two male samples was spiked with 40 µL of 30 ng/mL standard (3,3′,5-triiodo-L-thyronine (T_3) purchased from Sigma-Aldrich, CAS number 6893-02-3, batch number T2877) and diluted serially. As the concentration of T_3 in fecal samples of this species is relatively low, spiking the pool sample with a standard enabled us to obtain a reasonable dilution curve, which allowed us to estimate relevant matrix effects around the sample concentrations at which the samples are routinely measured.

(c) To assess the accuracy of the assay, a recovery experiment was conducted. After samples underwent extraction, four extracts with known concentration were spiked with high: 2.5 ng/mL and low: 1 ng/mL standard concentrations, respectively.

(d) Inter-assay coefficients of variance (CV) of high and low concentrations were 3.41% and 6.26%, respectively ($n=5$). Intra-assay variance was determined by running one high- and one low concentrated pool sample (concentration high pool sample: 61.6 ng/g dried feces; concentration low pool sample: 28.9 ng/g dried feces), each consisting of six samples with known concentration, equally distributed over one assay plate. Intra-assay CVs were 4.86% for high and 4.08% low concentrations.

(e) To assess whether TH concentrations degrade over time, we tested a subset of 15 fecal samples 17 months after the initial measurements were conducted. We tested two potential storage methods, fecal powder and ethanol extract, which are two successive steps in the sample preparation (as explained in detail above). Both the fecal powder and the ethanol extract were stored at −20°C during the 17-month period.

Biological validation: feeding experiment

During days of regular feeding, the two capuchin groups were fed with the usual amount of food (normal caloric intake); during days of reduced caloric intake, the groups received half of the usual amount of calories (reduced caloric intake). To keep overall food mass constant, during the period of reduced caloric intake, we replaced the amount of calorie-rich food items with low-calorie food items. Therefore, during both feeding regimes, the same food items were fed, however, in relatively different quantities. For each group, all fruits and vegetables

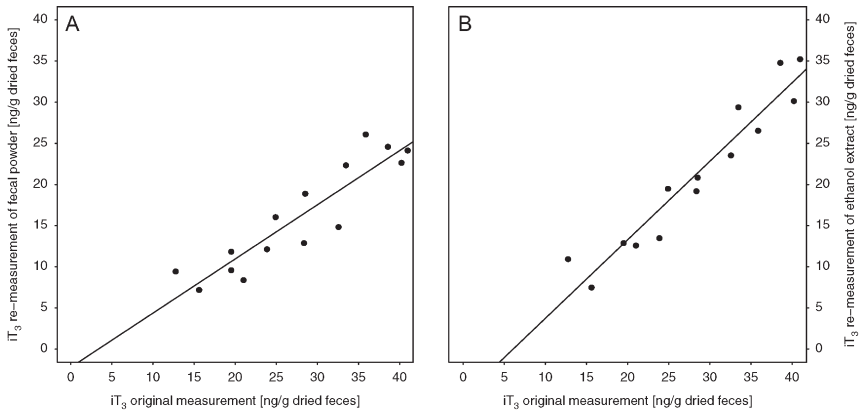

Figure 1
Loss of immunoreactive T_3 in (A) fecal powder and (B) ethanol extract after a storage period of 17 months at −20°C. Depicted are regression lines for each condition.

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DOI: 10.1530/JOE-16-0152

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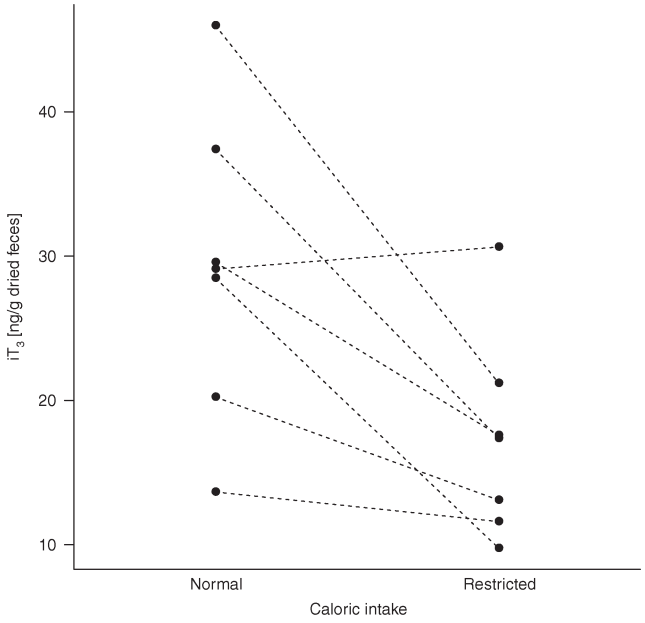

Figure 2
Change in fecal immunoreactive T_3 levels in male yellow-breasted capuchins during high (normal) and low (restricted) caloric intake periods. Each point represents average values of one individual. The dashed lines connect points referring to the same male ($n=7$ males with an average of ten samples per male).

were weighed before being fed. On the following day, the remaining food was collected and weighed again. We calculated the daily energy intake per group by subtracting the energy content of the food recovered from the energy content of the food provided for each day. The mean caloric intake in group A was 2562.3 kcal (S.D. ± 425; range: 2080.3–3257 kcal) and 1637.5 kcal (S.D. ± 244; range: 1471.8–2229.5 kcal) in group B during the periods of normal caloric intake. This corresponded to an estimated mean caloric intake per individual of 427.05 kcal in group A and 545.8 kcal in group B. In contrast, during the periods of reduced feeding, mean caloric intake was 1603.7 kcal (S.D. ± 343; range: 1234.9–1979.5 kcal) in group A and 928.8 kcal (S.D. ± 84.5; range: 838.1–1031.7 kcal) in group B, which corresponded to an estimated mean individual caloric intake of 267.3 kcal in group A and 309.6 kcal in group B.

Statistical analysis

To assess the impact of changes in caloric intake on fecal iT_3 levels, we ran a general linear mixed model (Baayen 2008), with iT_3 levels being the response variable and daily caloric intake being the test predictor with fixed effect. The model was fitted in R (R Core Team 2014) using the function lmer of the R-package lme4 (Bates *et al.* 2014). We included age of the individual, sampling time and group identity as control predictors with fixed effects. Individual ID was included as a random effect, and we included random slopes of caloric intake and time of sampling within individual ID to keep type I error rate at the nominal level of 5% (Schielzeth & Forstmeier 2009, Barr *et al.* 2013). We determined model stability by excluding individuals one at a time (see Results section for details) and checked for problems with collinearity by determining variance inflation factors (VIF, Field 2009) based on a standard linear model excluding the random effects, both of which were revealed not to be an issue (maximum VIF: 6.31). To meet the criterion of normal distribution, we subtracted the minimum iT_3 value from all iT_3 values and then square root transformed them. All quantitative fixed effects were z-transformed to a mean of zero and a standard deviation of 1 (Schielzeth 2010). Control predictors and random effects were kept in the reduced model. To establish the significance of

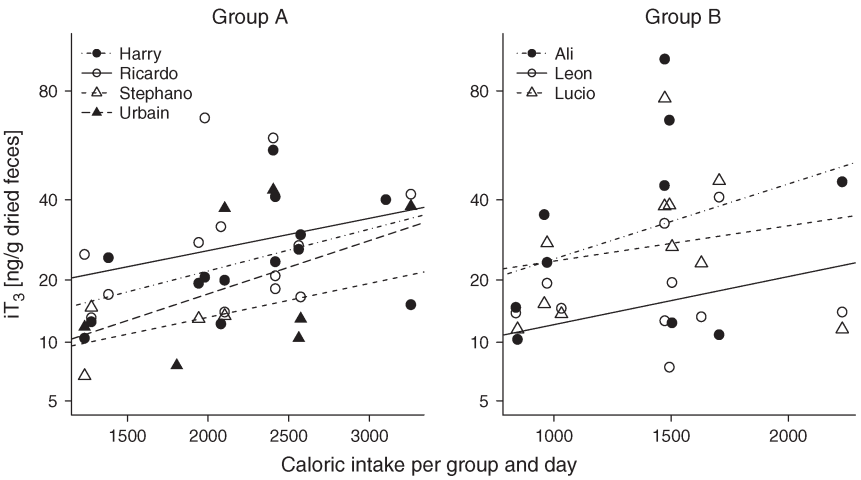

Figure 3
Individual fecal immunoreactive T_3 levels in male yellow-breasted capuchins changing with caloric intake per group and day. Depicted are regression lines for each individual.

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DOI: 10.1530/JOE-16-0152

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Table 1 Statistical summary of the impact of caloric intake on fecal iT_3 levels of male yellow-breasted capuchins; significant effects are indicated in bold.

Term	Estimate	S.E.M.	χ^2	Df	*P*	Min[b]	Max[b]
Intercept	3.467	0.513	[a]	[a]	[a]	2.951	3.659
Intake calories per day	**0.736**	**0.245**	**8.336**	**1**	**0.004**	**0.680**	**0.796**
Age at sampling	−0.130	0.225	0.317	1	0.573	−0.426	−0.049
Sampling time	−0.504	0.486	1.053	1	0.305	−0.831	−0.353
Group	1.927	1.006	3.511	1	0.061	1.275	2.791

[a]Not indicated because of having no meaningful interpretation; [b]Minimum and maximum of the model stability.

the individual predictors, we used likelihood ratio tests comparing the full with a respective reduced model lacking the particular predictor (Barr *et al.* 2013).

To test for parallelism, we ran a linear model with the interaction between the respective sample type (standard curve vs pool sample) and the concentration of the standard, with the percent binding as the response. The model was fitted in R (R Core Team 2014) using the function lm. We checked for the assumptions of normality and homogeneity of the residuals by inspecting a qq-plot of the residuals and residuals plotted against fitted values (Field 2009), which did not indicate any problems. We further assessed model stability by means of DFBeta (Field 2009), which indicated the model to be moderately stable. To test for the stability of TH across two potential storage methods, we used an exact Wilcoxon signed-rank test using the R-package exactRankTests (Hothorn & Hornik 2015) to assess whether TH concentrations significantly degrade during storage. In addition, to assess whether potential degradation processes affect relative TH levels, we used a Spearman's correlation.

Results

Analytical validation

Extraction efficiency, parallelism, assay accuracy, inter- and intra-assay coefficients of variance, stability The mean extraction efficiency was 102.94% (S.D. = 11.17, $n = 4$) for the high-spiked extracts and 75.49% (S.D. = 14.16, $n = 4$) for the low-spiked extracts. The serially diluted pool sample was parallel to the standard curve ($t(6) = -0.795$, $P = 0.4568$; confirmed by visual inspection, Supplementary Fig. 1, see section on supplementary data given at the end of this article). The assay accuracy was on average of 85.3% (S.D. = 9.08, $n = 4$) for the high-spiked samples and 93.14% (S.D. = 3.3, $n = 4$) for the low-spiked samples. IT_3 levels were significantly lower compared with initial measurements when repeated after 17 months (original measurement compared with re-measurement of fecal powder: Wilcoxon signed-rank test: T+ = 120, $n = 15$, $P < 0.001$; original measurement compared with re-measurement of ethanol extract: T+ = 120, $n = 15$, $P < 0.001$). The average proportion of iT_3 loss when re-extracting samples from fecal powder after 17 months was 42.8% (S.D. = 10.8; range: 26.19–60.43%) and 27.66% (S.D. = 12.26; range: 9.77–52.43%) from ethanol extracts. However, the measurements at both time points were strongly and significantly correlated for both conditions (Fig. 1; original measurement and re-measurement of fecal powder: rs = 0.92, $n = 15$, $P < 0.001$; original measurement and re-measurement of ethanol extract: rs = 0.975, $n = 15$, $P < 0.001$).

Biological validation: feeding experiment

The mean fecal iT_3 level across individuals during days of normal caloric intake was 31.03 ng/g dried feces ($n = 43$; S.D. ± 19.36) and 18.59 ng/g dried feces ($n = 27$; S.D. ± 12.39) during the periods of restricted caloric intake (Fig. 2). The full model was highly significant as compared with the reduced model ($\chi^2 = 8.34$, df = 1, $P = 0.004$), indicating that caloric intake per day was a significant predictor of iT_3 levels (Figs 2, 3 and Table 1). Levels of iT_3 were significantly lower on days when caloric intake was restricted, as indicated by the positive estimate derived by the model.

Additionally, we found a considerable overlap of iT_3 levels across individuals between days of low and high caloric intake (Fig. 2). Several individuals had lower iT_3 levels during days of normal caloric intake than the highest average level achieved by an individual during days of restricted caloric intake.

Discussion

With this study, we were able to successfully validate the measurement of iT_3 in fecal samples of

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male yellow-breasted capuchin monkeys. By conducting experiments to test for extraction efficiency, parallelism and assay accuracy, as well as providing information about the reproducibility of measurements within and between plates, we successfully conducted an analytical validation of the measurement of iT_3 levels in fecal samples of this species. Furthermore, by conducting a feeding restriction experiment and monitoring changes in individual iT_3 levels of seven males while at the same time linking them to group levels of daily caloric intake, we showed that individual fecal iT_3 levels were significantly correlated with the caloric intake of the entire group. Due to the noninvasive design of this study, we were restricted to linking iT_3 levels solely to energy intake. However, previous studies found that TH concentrations reflect energy balance (Blum *et al.* 1980, Harlow & Seal 1981, Menchetti *et al.* 2015). Therefore, although in this study we could only show strong correlations between energy intake and fecal iT_3 levels, measuring iT_3 levels may have the potential to be a powerful alternative method for assessing energy balance in animals in which serum or urine collection is impossible.

Detailed studies on degradation patterns of TH in fecal samples during storage are rare; however, degradation of TH was reported for fecal samples of African elephants (Wasser *et al.* 2010). Our results indicate a significant degree of degradation of TH for both storage methods. Comparing the degree of degradation of the two storage methods suggests that storing fecal samples in the form of ethanol extracts is highly preferable to storing them as dried fecal powder. Taken together, this suggests that within one project, the time between sampling and measuring should be kept short in order to avoid hormone degradation. Alternatively, if that is not possible, the delay should be kept as constant as possible so that measurements are comparable. In cases where this is not possible, a detailed analysis of the degree of degradation is essential, and if this reveals to be important, the time from sampling to measurement should be included as a control variable in the statistical analysis.

In our study, changes in caloric intake were reflected in fecal iT_3 levels on the same day. In carnivores, serum T_3 levels also reflect reduced caloric intake when measured within the same day (Harlow & Seal 1981, Delgiudice *et al.* 1987). Due to the time delay associated with excretion via the gut, it would be expected that changes in fecal iT_3 levels are detected later than those in serum. However, smaller bodied primates, to which capuchins and most of the other new world primates belong to, have a very fast gut passage time (e.g., 8–24 h in common marmosets: Bahr *et al.* 2000 and 2.1–3.0 h in tufted capuchins: Wheeler *et al.* 2013 as estimated by ACTH challenge). This fast response of hormone excretion into feces would not be expected for larger bodied primates because they are characterized by a rather long gut passage time (e.g., 2–3 days in western lowland gorillas, Shutt *et al.* (2012), and around 29 h in chimpanzees, Murray *et al.* (2013), as estimated by ACTH challenge). Therefore, when adapting this methodology to other animals, species-specific information on gut passage times should be taken into account to properly detect the endocrinological signal of iT_3 excretion in feces in relation to fluctuations in caloric intake.

Our results revealed considerable intra- and inter-individual differences in iT_3 levels under both feeding conditions and an overlap in levels between the conditions across individuals, for example, some animals had higher iT_3 levels during the days of caloric restriction than others had during the days of normal feeding regime. In this study, we were limited to measures of daily caloric intake on a group rather than on an individual level. Therefore, the variability in individual levels within a feeding regime could be due to the differences in individual energy intake and expenditure. To explain additional variance in individual iT_3 levels within and between individuals, in future studies, efforts should be made to include more control variables, such as individual caloric intake, energy expenditure and body mass.

TH are involved in developmental processes (for review, see e.g., Crockford 2006), with levels remaining high after birth and during development and then characteristically decreasing after reaching adulthood as shown in humans (Oliner *et al.* 1957, Beckers *et al.* 1966, Ryness 1972, Hesch *et al.* 1977), bonobos and chimpanzees (Behringer *et al.* 2014). In our study, one subadult individual of approximately 3 years of age did not show the expected decline in iT_3 levels when caloric intake was restricted. It is possible that individuals during growth phases are less sensitive to environmental stimuli and, therefore, keep metabolic rates constant, even if caloric intake is restricted. This suggests that individuals in different life history stages (e.g., development or pregnancy) can show different energy allocation patterns according to their respective needs. However, as we did not collect individual intake rates, another possibility that would explain this lack of reactivity in iT_3 levels is that this individual had a higher energy intake rate than the rest of the group and hence its iT_3 levels did not change across the conditions.

This novel method to assess energetic condition by measuring iT_3 in noninvasively collected fecal samples provides the possibility to investigate research

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DOI: 10.1530/JOE-16-0152

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questions about trade-offs in relation to somatic growth, maintenance and reproduction. For example, mate guarding, a behavior many male primates conduct to monopolize the fertilization of females, potentially comes along with metabolic costs to the males caused by higher rates of agonistic and sexual behaviors and less time spent on feeding (e.g., Alberts *et al.* 1996, Matsubara 2003, Ancona *et al.* 2010, Girard-Buttoz *et al.* 2014). This suggests that energetic condition might be negatively affected by mate-guarding events. Future studies using iT_3 levels to monitor energetic condition can now focus on energy allocation during energetically constraining phases, for example, mating season or pregnancy.

To conclude with this study, we have shown that fecal iT_3 levels of a nonhuman primate species can be used as a reliable physiological marker of energy intake in a noninvasively collected medium. In the future, fecal iT_3 measurements might be used to explore to what extent social, developmental and physiological factors impact individual decisions on energy allocation into somatic growth, maintenance or storage and reproduction. However, detailed validations as exemplified in this study are necessary when this method is applied to a different species or a different matrix such as urine. Importantly, it has to be kept in mind that iT_3 levels varied considerably within and between individuals, which means that defining a general threshold of high vs low iT_3 levels to assign an individual to a category of energetic condition is not feasible. Rather, this inter-individual variability in iT_3 levels necessitates the implementation of within-subject comparisons in which each individual serves as its own control. In addition, to account for within-individual variation, several samples per individual and nutritional state should be analyzed. Finally, future studies should aim at including detailed data on individual intake rates of calories as well as individual rates of energy expenditure to enable more precise insights into patterns of energy allocation in the study species.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/JOE-16-0152.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research was supported by the Max Planck Society.

Acknowledgements

The support from the curator (Dr Wilms), the veterinaries (Drs Geiger and Schauerte) and the keepers (Harald Thomas, Annie Fuchs and Richard Weigl) of Frankfurt Zoo is gratefully acknowledged. The authors are grateful to Roisin Murtagh for laboratory assistance and advice. The authors are also grateful to Roger Mundry for help in statistical analysis and to Mimi Arandjelovic for editing the manuscript. The authors also thank two anonymous reviewers for their helpful and constructive comments, which significantly improved the quality of this article. The protocol for the restriction study, including the collection of fecal samples, was approved by the regional council of the city of Darmstadt, number: V54 – 19c 20/15, and the authorities of Frankfurt Zoo.

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Received in final form 13 July 2016
Accepted 26 July 2016
Accepted Preprint published online 26 July 2016

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-16-0152

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