General and Comparative Endocrinology 285 (2020) 113290

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Urinary total T3 levels as a method to monitor metabolic changes in relation to variation in caloric intake in captive bonobos (*Pan paniscus*)

Tobias Deschner[a,*], Gottfried Hohmann[a], Sylvia Ortmann[b], Franka S. Schaebs[a], Verena Behringer[a]

[a] Max Planck Institute for Evolutionary Anthropology, Department of Primatology, Deutscher Platz 6, 04103 Leipzig, Germany
[b] Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany

ARTICLE INFO

Keywords:
Urine
Total T3
Pan paniscus
Apes
Nutritional stress
Metabolism

ABSTRACT

Monitoring metabolic activity in wild living animals has become of particular interest in the field of ecological research. Methods for the repeated non-invasive sampling of individuals are needed. Thyroid hormones (TH) are involved in the regulation of metabolic activity, and their measurement can be used as a proxy to monitor metabolic changes. During periods of low energy intake, serum TH levels are reduced, leading to a decrease in metabolic activity. Using urine samples collected during a food restriction experiment in captive bonobos we validated a total triiodthyronin (TT3) enzyme immunoassay (EIA) for the monitoring of metabolic changes. We found that the majority of immune reactivity of the assay in the urine samples could be explained through immunoreactivity to T3. Furthermore, urinary T3 was stable through repeated freeze-thaw cycles but prolonged exposure to room temperature lead to degradation. Most importantly, we found that for all animals urinary total T3 levels were higher when more digestible energy was consumed. We concluded that urinary total T3 measurements are a suitable method for monitoring metabolic changes in bonobos and potentially in a wide range of animal species.

1. Introduction

Natural populations of animals and many human societies are exposed to temporal changes in energy availability (Ebling and Barrett, 2008; Fu et al., 2017; Ngidi and Hendriks, 2014). Declining availability in preferred food resources can, to a certain degree, be compensated through the consumption of fallback foods, changes in diet, and adaptations in the digestive physiology (Lambert and Rothman, 2015; Marlowe and Berbesque, 2009; Marshall et al., 2009). However, a decline in energy availability can be severe enough to challenge physical condition, reproductive performance, and survival (Hobbs, 1989; Schneider, 2004; Wingfield and Kitaysky, 2002). Accordingly, species and/or individuals differ in terms of optimal energy allocation strategies causing individual and species-specific life history patterns (Perrin and Sibly, 1993; Roff and Fairbairn, 2007; Stearns and Koella, 1986). During periods of food scarcity, energy allocation shifts from growth and reproduction to maintenance in order to assure survival (Perrin and Sibly, 1993). Given the fitness consequences of changes in energy intake, the assessment of temporal metabolic changes across individuals is of crucial importance in behavioral ecology. One way to estimate the consequences of varying food availability is to monitor the ratio of energy intake and energy expenditure in order to calculate energy balance. This method necessitates detailed data on food intake, the energy content of different food items, the time needed to process food, and the time and energy that is needed to move between food patches (Emery Thompson and Knott, 2008). Others have used changes in body mass and/or body weight (Drewnowski et al., 2004; Groscolas, 1986; Heldmaier, 1989) to estimate shifts in energy balance, which can be challenging in wild animals. Another approach is the measurement of biomarkers such as ketones (Knott, 1998) or urinary c-peptide levels (Sherry and Ellison, 2007). Such biomarkers can be assessed non-invasively by collecting urine samples (Hoogwerf and Goetz, 1983; Knott, 1998; Sherry and Ellison, 2007). Particularly, urinary c-peptide measures have been successfully applied to monitor energy balance in relation to food availability in wild chimpanzees (Emery-Thompson et al., 2009), black and white colobus monkeys (Harris et al., 2009), and mountain gorillas (Grueter et al., 2014).

Thyroid hormones (TH) can be used to monitor changes in metabolic activity, because they regulate basal metabolic rate and thereby control growth and development (Kaack et al., 1979; López et al., 2013). The two major TH in the circulation are thyroxine (3,3′,5,5′-tetraiodothyronine, T4) and triiodothyronine (3′,3,5-triiodothyronine,

* Corresponding author.
E-mail address: deschner@eva.mpg.de (T. Deschner).

https://doi.org/10.1016/j.ygcen.2019.113290
Received 2 July 2019; Received in revised form 23 September 2019; Accepted 25 September 2019
Available online 26 September 2019

T3). Both hormones, T3 and T4, are secreted by the thyroid gland. There is peripheral conversion of T4 to T3. T4 is a prohormone that acts as the reservoir for the production of T3, the biologically active hormone (Soukhova et al., 2004, Burke and Eastman, 1974; Fisher and Polk, 1989; Tomasi, 1991). The majority of circulating TH are reversibly bound to serum proteins with < 1% remaining free. The sum of bound and free TH is referred to as total TH, e.g., total T3 (Köhrle, 1999; Refetoff and Nicoloff, 1995).

The response of TH on metabolic changes is well established with data from humans and mammals (Behringer et al., 2018): When caloric intake is restricted, the concentration of TH declines thereby reducing metabolic rate and facilitating the organism to conserve energy (Eales, 1988; Flier et al., 2000). While human plasma and serum T4 levels did not dramatically decrease during food restriction (Danforth and Burger, 1989; Merimee and Fineberg, 1976), free as well as total serum T3 levels decreased in response to reduced energy intake to as low as 50% of the normal levels in human serum samples (Danforth and Burger, 1989; Fontana et al., 2006). A similar decline in total and free blood T3 levels was found during experimental food restriction in rats (*Rattus norvegicus domestica*) (e.g., Reichlin, 1957; Vijayan and McCann, 1977), domestic animals like goats (*Capra aegagrus hircus*) and fowl (*Gallus gallus domesticus*) (e.g., Abdullah and Falconer, 1977; Klandorf and Harvey, 1985), and carnivores like badgers (*Taxidea taxus*) and bears (*Ursus americanus*) (e.g., Harlow and Seal, 1981; Tomasi et al., 1998). Monitoring total blood T3 levels allows for the monitoring of metabolic changes during periods of changing energy supply.

The majority of previous studies investigating the effect of metabolic changes in total T3 levels in humans and other animals relied on serum samples. The collection of blood samples is invasive and alternative methods are in demand to be able to assess metabolic changes via repeated measurements, especially in wild large-bodied species for which repeated capture is not an option. Alternative matrices like urine and fecal samples can be collected repeatedly and non-invasively in zoos and wild animals without disturbing or impairing the individual and can be used to quantify total T3 levels. Methods for measuring total T3 levels in fecal samples have been developed and validated for a variety of bird and mammal species, including captive and wild howler monkeys (*Alouatta palliate*) (Dias et al., 2017; Wasser et al., 2010) and yellow-breasted capuchin monkeys (*Sapajus xanthosternos*) (Schaebs et al., 2016). Fecal total T3 levels increased during the mating season and with high levels of food availability in wild Barbary macaques (*Macaca sylvanus*) (Cristóbal-Azkarate et al., 2016). In dogs, a radiometabolism study revealed that THs are mostly excreted as T3 into the urine and only to a small amount as T4 (Wasser et al., 2010). The measurement of urinary total T3 levels as a marker for growth and maturation was validated successfully in bonobos and chimpanzees. In both species total T3 levels declined with age as shown in studies on humans (Behringer et al., 2014). However, to our knowledge a controlled study that relates urinary total T3 levels to metabolic changes in relation to variation in energy intake in nonhuman animals has yet to be carried out. Furthermore, information on assay specificity and sample treatment related degradation patterns of urinary T3 levels have not been investigated yet.

This study investigated urinary total T3 levels as a marker of metabolic changes in captive bonobos during periods of controlled restriction of caloric intake. We predicted urinary total T3 levels to decline during a period of low caloric intake and to increase when dietary energy content was restored and to positively correlate with c-peptide levels already measured in the same samples (Deschner et al., 2008). To validate the method of measuring urinary total T3, we established an HPLC/ELISA immunogram, to confirm that the immunoreactivity measured in urine samples with the enzyme-linked immunosorbent assay (ELISA) was representative of total T3 reactivity. Additionally, we tested the stability of urinary total T3 to freeze-thaw cycles and when samples were exposed to room temperature for different time intervals.

2. Methods

2.1. Study design and study animals

The study was conducted on a bonobo group for four weeks in January and February 2007 in Frankfurt Zoo, Germany. Urine samples were collected from seven adult bonobos (males: N = 1, females: N = 6) as described in detail in Deschner et al. (2008). The feeding experiment consisted of two periods. During the first period (two weeks of energy restriction), caloric content of food was gradually reduced by decreasing the amount of energy rich food items and replacing them with low caloric food items. In the second period (two weeks of refeeding), the low calorie food items were gradually replaced by calorie-rich food items to increase the total energy content of the food. By the end of the energy restriction phase food contained 21.2% less digestible energy than during the first week of the of the energy restriction phase, while average digestible energy of the food provided in the second week of the refeeding phase, exceeded the one of the food provided in the second week of the energy restriction phase by more than a double (Deschner et al., 2012). The protocol of the feeding experiment was approved by the authorities of Frankfurt Zoo and the authority of animal welfare (Veterinaerdezernat, Regierungspraesidium Darmstadt, Germany).

2.2. Sampling protocol

Before the onset of the experiment, bonobos were trained to urinate on command close to the enclosure's fence to facilitate urine collection. Urination was enhanced by providing nettle tea 20 min prior to the collection time. Urine samples were collected directly into plastic cups or were pipetted off the ground. Samples were collected twice a day, once in the morning (~08:00) and once in the afternoon (~14:00). Immediately after collection, urine samples were frozen and stored in a freezer at − 20 °C. After completion of the experiment, samples were transported on dry ice to the Endocrinology laboratory at the Max-Planck Institute for Evolutionary Anthropology (MPI-EVA), Leipzig, Germany.

2.3. Sample preparation and total T3 assay

We measured total T3 in 194 urine samples of seven adult bonobos with a total T3 ELISA (Ref. RE55251, IBL International GmbH, Hamburg, Germany). In a previous study, we showed that the ontogenetic changes in urinary total T3 levels observed in bonobos and chimpanzees corresponded to the pattern found in humans (Behringer et al., 2014). Here we added a number of analytical validations steps, such as testing stability and specificity of the antibody, as well as whether urinary total T3 correlates with energy consumption. 87 urine samples (average 12 samples ± 1 per individual) were collected during the energy restriction period, and 104 samples (average 15 samples ± 1 per individual) during the refeeding period.

Intra-and interassay coefficients of variance of low and high concentrations (calculated from replicate measurements of samples and quality controls) were 4.1% and 3.0%; and 5.5% and 4.7%, respectively.

To compensate for variation in volume and concentration of the urine, specific gravity (SG) was assessed using a digital handheld refractometer (TEC, Ober-Ramstadt, Germany) and total T3 corrected for SG was calculated as described in Miller et al. (2004).

Three out of the 194 urine samples were excluded preceding analysis, because in these three samples SG measurement equaled zero; indicating that the urine was heavily diluted and making a proper concentration correction impossible, leaving a total sample set of 191 urine samples.

2.4. C-peptide assay

We measured urinary c-peptide with AutoDELFIA® C-peptide, a commercially available time-resolved fluoroimmunoassay kit from PerkinElmer, designed to measure C-peptide in human serum and plasma. The assay was validated for the use in bonobo urine and proved to provide information on changes in body mass in bonobos (Deschner et al., 2008).

2.5. Analytical validation

2.5.1. HPLC/ELISA- immunogram

For the assessment of immunoreactivity of the total T3 ELISA (Ref. RE55251, IBL International GmbH, Hamburg, Germany), a solution with only T3 standard (T3: Sigma Aldrich, product number T2877) and a pool sample of three different bonobo urine was fractioned using a high-performance liquid chromatograph (HPLC).

100 µl of the pooled urine sample and T3 standard was injected in a Waters Alliance 2695 HPLC equipped with a Gemini C18 column (Phenomenex, Torrance, CA, USA) with a flow rate of 0.2 ml/min using a gradient of eluent A (5% acetonitrile with 0.1% formic acid) and eluent B (95% acetonitrile with 0.1% formic acid). 18 fractions were collected with a Waters Fraction Collector 3 (Waters, Milford, MA, USA). Fractions were lyophilized (Heto PowerDry LL300C) overnight and kept frozen at − 20 °C until they were reconstituted in 150 µl of assay buffer. Every fraction was run on the total T3 ELISA for the assessment of immunoreactivity in each fraction.

2.5.2. Stability experiment

To test the degree of degradation of total T3 in urine samples of bonobos, two experiments were carried out with four samples each. In the first experiment we left aliquots of the samples at room temperature for 0, 5, 19, 33, and 61 h, respectively. In the second experiment we exposed aliquots of four samples to four freeze and thaw cycles.

2.6. Physiological validation

2.6.1. Measurement of digestible energy intake

For the calculation of daily digestible energy intake we summarized the energy content of each food item considering the total amount that each food contributed to the meal (Deschner et al., 2012) for details on caloric measures of food items). Food that was not consumed was weighed and the corresponding energy content was subtracted from the original amount. In addition, the feces from the entire group were collected daily, weighed, thoroughly mixed, and a sample was taken for measurement of the energy content.

The gross energy of each food item and fecal sample was determined via bomb calorimetry (C5003 bomb calorimeter; IKA, Staufen, Germany) conducted in the nutritional physiology laboratory at Leibniz-Institute for Zoo and Wildlife Research (IZW) in Berlin, Germany (Ortmann et al., 2006). The determined values for gross energy of provisioned food items in kJ per gram dry matter and of the energy excreted via feces the next day allowed us to calculate the daily consumed amount of digestible energy in kJ for the entire group (Ortmann et al., 2006). For further information, see Deschner et al. (2012).

2.7. Statistics

2.7.1. Urinary total T3 level changes in relation to energy consumption

To explore changes in urinary total T3 levels with available calories, we ran a linear mixed model (LMM, (Baayen, 2008)) with urinary total T3 levels corrected for SG (log transformed) as the response variable and digestible energy consumption of the previous day as the predictor variable. We included individual ID as a random effect, digestible energy consumption of the previous day was included as random slopes within individual ID. We included the random slope of digestible energy on the previous day to account for the possibility that the effect of this predictor varied between individuals. Not accounting for such a potential effect can inflate type I error rates (Barr et al., 2013; Schielzeth and Forstmeier, 2009). Indeed, the presence of such variation of the effect of digestible energy among individuals seems quite likely in this study, for instance, due to differential impact of genetic factors, social skills, or rank. The model was fitted in R, version 3.3.2, (R Core Team, 2018) using the function lmer of the R package lme4 (Bates et al., 2015).

Model stability was determined by excluding individuals one at a time and comparing estimates for the fixed effects with those of the full model. Model stability results did not indicate any influential levels of random effects to exist.

Values for digestible energy consumption were square-root transformed, the minimum was subtracted and the resulting value was z-transformed to a mean of zero and a standard deviation of one to improve the interpretability of regression coefficients (Schielzeth, 2010).

Likelihood ratio tests were used to determine the significance of the full model as compared to the null model, (Dobson, 2002) and to determine significance of individual effects (Barr et al., 2013).

As one female was in her last trimester of pregnancy during the experimental period and TH, especially T3 levels, are known to be elevated during pregnancy (Soldin et al., 2004), TH levels during this period may not reflect acute metabolic changes. Therefore, we ran all models with and without this individual.

2.7.2. Urinary total T3 levels in relation to urinary c-peptide levels

We investigated the association between urinary total T3 levels and c-peptide levels by calculating the Spearman correlation for each individual and then testing with a one-sample *t*-test if this association across subjects was significantly different from 0.

3. Results

3.1. Analytical validation

3.1.1. HPLC/ELISA- immunogram

For the T3 standard, immunoreactivity was found in fractions 5–7, while for the pooled urine immunoreactivity was found in fractions 4–8 (Fig. 1). Total immunoreactivity of urine fractions (100%) added up to 4.67 (ng/ml). 3.58 (ng/ml) of the reactivity was found in fractions 5–7, the same fractions in which immunoreactivity was found for the

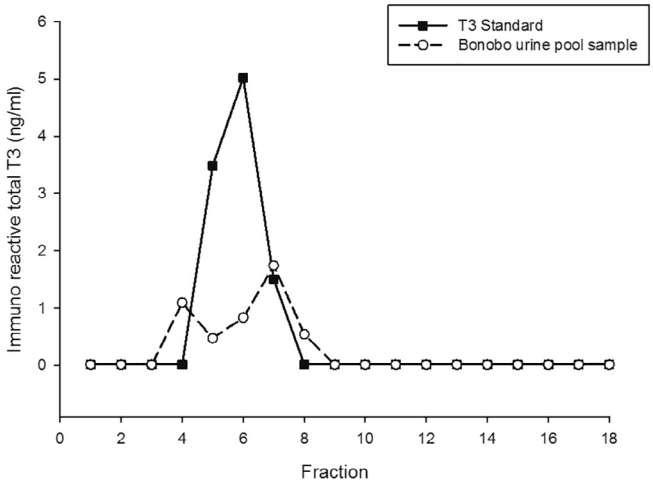

Fig. 1. High-performance liquid chromatographic separations representing total T3 (ng/ml per fraction) reactivity of an ELISA to pooled bonobo urine sample (open circles) in comparison to a T3 standard (filled squares) after separation in 18 fractions.

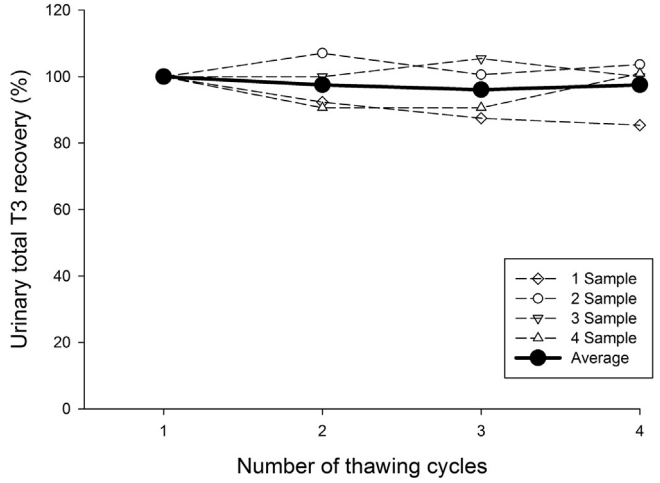

Fig. 2. Mean urinary total T3 recovery in four bonobo samples during four thawing cycles (dashed lines represent the four samples, the solid line represents the mean across all samples).

standard. Therefore, 77% of immunoreactivity in the pool urine sample could be explained through immunoreactivity to T3, while the immunoreactivity in fractions 4 and 8 represented 23% of the total immunoreactivity.

3.1.2. Stability experiment

i) Freeze-and thaw cycles

Urinary total T3 levels remained stable for up to four thawing cycles, with only a slight decline of on average < 5% and no drastic variation in decline across samples (Fig. 2).

ii) Exposure to room temperature

Exposing urine samples to room temperature led to a gradual decrease in urinary total T3 levels with increasing exposure duration (Fig. 3). However, the magnitude of degradation differed widely across samples. While urinary total T3 levels remained relatively stable in one sample across the entire duration of exposure to room temperature, the other three showed a considerable decline. After five hours, samples still contained on average 88% of the original urinary total T3 concentration. After 19 h of exposure, samples contained on average of 75% of the original samples and after 33 h of exposure, urinary total T3 concentration was 65% of the original value. An additional measurement after 61 h of exposure to room temperature did not result in further degradation.

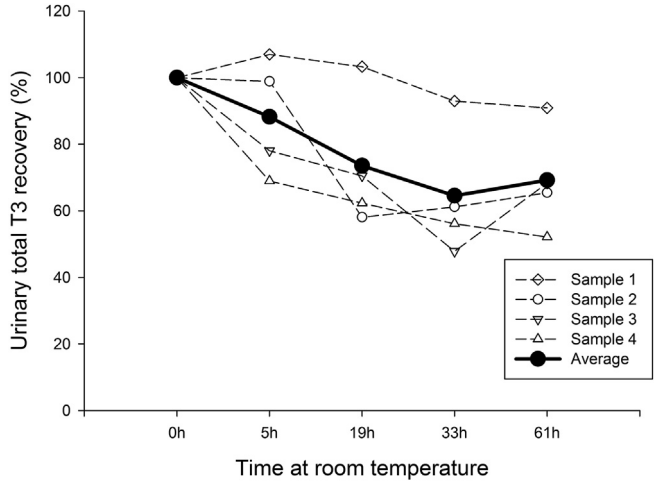

Fig. 3. Mean urinary total T3 recovery in four bonobo samples left at room temperature for up to 61 h (dashed lines represent the four samples, the solid line represents the mean across all samples).

3.2. Physiological validation

3.2.1. Urinary total T3 level changes in relation to energy consumption

The average of urinary total T3 concentration across all individuals was 1.3 (ng/ml) SD ± 0.9 during the calorie restriction phase and 4.1 (ng/ml) SD ± 3.3 during the refeeding period.

The progression in energy restriction was associated with a gradual decrease in average daily urinary total T3 levels (Fig. 4). Total T3 levels immediately increased at the first day of refeeding, and after the fifth day into the refeeding period, total T3 levels were even higher compared to the beginning of the experiment (Fig. 4).

Comparing the full model, including the test predictor digestible energy, to the null model, lacking this test predictor, revealed significance (whole group: $\chi^2 = 21.5$, df = 1, $P < 0.001$; without the pregnant female: $\chi^2 = 18.4$, df = 1, $P < 0.001$). Urinary total T3 levels were significantly higher when more digestible energy was consumed (whole group: Est. = 0.51, SE = 0.05, $P < 0.001$; without the pregnant female: Est. = 0.53, SE = 0.06, $P < 0.001$). All individuals showed the same pattern, with urinary total T3 levels increasing with increasing digestible energy consumption (Fig. 5).

Average urinary total T3 levels during the restriction phase were lower than during the refeeding phase of the feeding experiment for every animal (Fig. 6). The bonobo with the highest urinary total T3 levels during the restriction period had on average 1.8 (ng/ml) SD ± 1.0 and the lowest individual 0.8 (ng/ml) SD ± 0.3. During the refeeding period, the highest average individual urinary total T3 level was 9.9 (ng/ml) SD ± 4.6, and the lowest level was on average 2.8 (ng/ml) SD ± 2.8. The adult male showed the strongest change in urinary total T3 levels, and the pregnant female showed the weakest change.

3.2.2. Urinary total T3 levels in relation to urinary c-peptide levels

Urinary total T3 levels were significantly correlated to c-peptide during the energy restriction phase (average r = 0.30, t = 2.67, df = 5, p = 0.044), as well as during the refeeding phase of the experiment (average r = 0.394, t = 3.18, df = 5, p = 0.024).

4. Discussion

In this study we have shown that the total T3 assay measures mainly T3 in urine samples. Urinary total T3 is stable during freeze-thaw cycles, but should not be stored for hours at room temperature. The urinary total T3 levels are also positively related to caloric intake.

HPLC/ELISA immunograms are one procedure to determine the immunoreactivity of an assay (Rettenbacher et al., 2013; Stöwe et al., 2013). Most commercial assays are designed to measure a certain native hormone in human blood samples. Assays may cross-react with substances not present in blood but present in matrixes like urine or feces, which therefore were not tested for their cross-reactivity with the antibody of the assay by the supplier (Rettenbacher et al., 2013). With the performance of a HPLC/ELISA immunogram we were able to show that the majority of immunoreactivity of the total T3 ELISA in urine was indeed due to cross-reactivity to T3. Based on our findings, we conclude that urinary total T3 measurements with the assay used are sufficiently specific for detecting changes in T3 levels, because free T3 is the biological active TH, and only unbound TH are filtrated by the kidney into the urine (Shakespear and Burke, 1976).

Total T3 in urine was stable during freeze-thaw cycles. This allows for the use of urine samples, which had already been thawed for other purposes. However, total T3 levels declined with exposure time to room

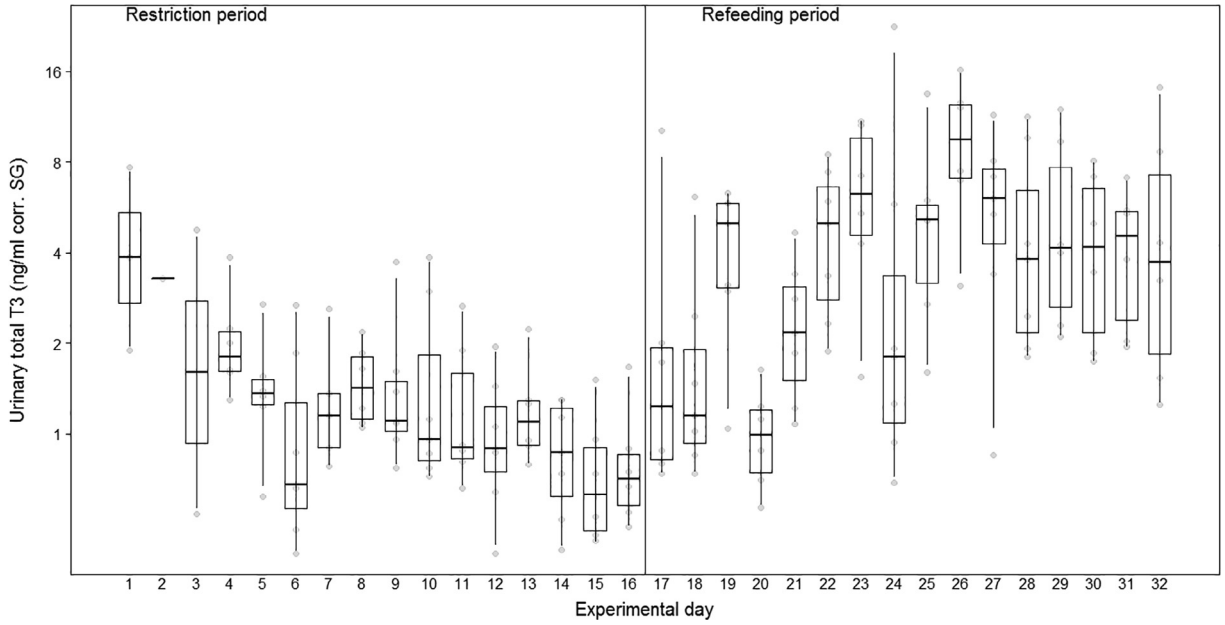

Fig. 4. Temporal profile of average urinary total T3 levels (ng/ml corrected for specific gravity) of seven adult bonobos (N = 191 samples) throughout the energy restriction (N = 87 samples) and refeeding period (N = 104 samples). Urinary total T3 levels are given as group means (± SD).

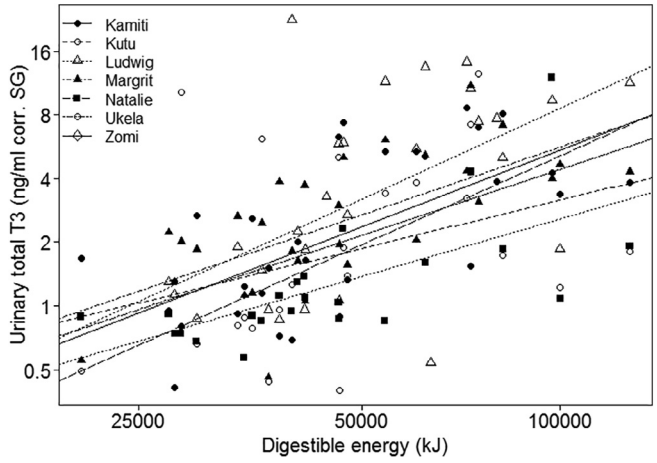

Fig. 5. Daily urinary total T3 levels (ng/ml corrected for specific gravity (corr. SG)) in relation to amount of digestible energy for seven adult bonobos (N = 191 samples).

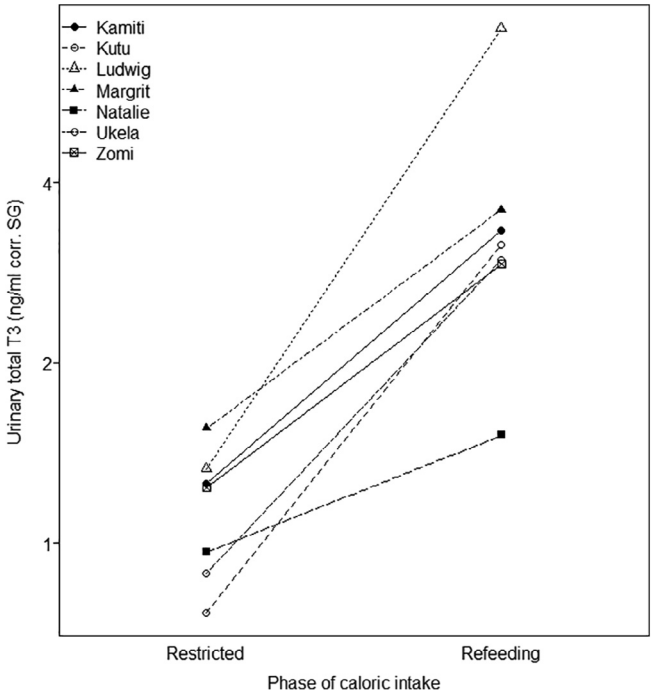

Fig. 6. Changes in average urinary total T3 levels (ng/ml corrected for specific gravity) from the energy restriction to the refeeding period for seven adult individuals. Y-axis is log transformed.

temperature. As a result, the handling of thawed urine samples should be as short as possible or samples should be kept cold during the handling process after thawing, e.g. by storing them on ice.

The results from the feeding experiment indicate that bonobos adapted their physiology to a reduced caloric intake. The decline of urinary total T3 levels at times of food restriction is consistent to previous findings of minimized energy expenditure at times of low energy intake (Delgiudice et al., 1987; Harlow and Seal, 1981; Palmblad et al., 1977; Rosenbaum et al., 2000). Caloric restriction tends to reduce thyroid function and thereby allows the body to conserve energy by reducing the metabolic rate (e.g., Blake et al., 1991; Eales, 1988; Flier et al., 2000; Silva, 1995). The fast response of changes in TH levels in our study corresponds to results of studies exposing their subjects to more severe energy restrictions such as starvation studies in carnivores (Delgiudice et al., 1987; Harlow and Seal, 1981). The overall increase of urinary total T3 levels during refeeding and even higher values during this period compared to onset levels are in line with other studies (Danforth et al., 1979; Galofré et al., 2010).

Urinary total T3 levels in this study correlated positively with urinary c-peptide levels an established physiological marker for the assessment of energetic condition. Urinary c-peptide has been validated for the monitoring of variation in body mass, nutritional status, and energetic condition in urine of nonhuman primates (Deschner et al., 2008; Emery Thompson and Knott, 2008; Girard-Buttoz et al., 2011; Sherry and Ellison, 2007). In humans, c-peptide levels correlated positively with body mass index across individuals (French et al., 1992; Polonsky et al., 1988), and increased with weight gain within individuals (Yoshida et al., 2006).

Because metabolic rate is sensitive to energetic condition the

observed correlation between urinary total T3 levels and urinary c-peptide levels was expected, particularly because energy intake was the only factor manipulated in this study. However, T3 regulates a variety of metabolic activities throughout lifetime and factors other than energy intake contribute independently to variation in T3 excretion, such as, cold ambient temperatures which force the body to increase basal metabolic rate to produce heat (van der Lans et al., 2013). TH levels then increase independent of energy intake and energetic status (Eastman et al., 1974). When the body increases its temperature, for example during fever, free TH levels increase even when the individuals eats less due to the sickness (Rastogi et al., 1976; Shafer et al., 1980). Increases in body temperature (adaptive thermogenesis) are associated with an increase in metabolic rate and as a result change the circulating TH levels (Bianco et al., 2005). C-peptide on the other hand is a by-product of insulin production. Insulin is involved in the carbohydrate metabolism and is secreted as a response to low blood sugar levels (Blix et al., 1982). Given that c-peptide levels are related to the amount of glucose present in blood, changes in T3 and c-peptide levels can happen independently of each other. For example, during exercise urinary c-peptide levels decline in response to a declining blood sugar level, while T3 levels increase in response to an increase in metabolic demands (Blix et al., 1982; Frisch et al., 1984). T3 levels increase in response to increased energy intake independent of whether the energy source mainly consists of protein or carbohydrates, whereas c-peptide levels decline with a protein biased diet which resembles excretion patterns observed during periods of starvation (Landau et al., 1981). A meat-based increase in energy intake would lead to an increase in T3 levels but not to an increase in c-peptide levels. C-peptide relates more to carbohydrate intake. TH are related to energy intake, but they also show parallel changes to energy expenditure (Al-Adsani et al., 1997; Bianco et al., 2005; Lowell and Spiegelman, 2000).

Measuring T3 levels cannot serve as a mere substitute for measuring c-peptide levels to estimate variation in nutritional status. Given the multitude of parameters influencing T3 excretion and thereby metabolic rate and the scarcity of studies on non-invasive measurements of T3 levels in wild animals, explorative studies are needed to estimate the impact of environmental and developmental factors on T3 excretion. With the ability to control for irrelevant factors, the parallel measurement of T3 and c-peptide levels might allow for the monitoring of important life history decisions. One example could be periods of high male-male competition for access to fertile females. One could expect that the necessity of mate guarding might lead to a reduced investment in feeding and thereby lower c-peptide levels. Males, dependent on their rank, age and overall physical condition, might need to keep metabolic rates high to be able to successfully compete for mating opportunities, and therefore a divergence in changes of c-peptide and T3 levels might be expected.

5. Conclusion

We validated urinary total T3 measurements for the monitoring of metabolic changes in relation to variation in energy intake in bonobos with an ELISA. We have shown that freeze-thaw cycles do not affect urinary total T3 levels; however, storing the samples at room temperature should be avoided. With this study we have shown that urinary total T3 measurements are a suitable method for monitoring metabolic changes in bonobos and potentially in a wide range of animal species.

Acknowledgements

We thank Frankfurt Zoo for the permission to conduct this research. Jan Bauer collected the samples and the keepers of the Great Ape facility provided invaluable assistance. The help of Róisín Murtagh and Vera Schmeling with lab work is gratefully acknowledged. The authors thank Lillian Fornof for editing the manuscript. This research was supported by the Max-Planck-Society.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.113290.

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