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Identification of energy consumption and nutritional stress by isotopic and elemental analysis of urine in bonobos (*Pan paniscus*)

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A mounting body of evidence suggests that changes in energetic conditions like prolonged starvation can be monitored using stable isotope ratios of tissues such as bone, muscle, hair, and blood. However, it is unclear if urinary stable isotope ratios reflect a variation in energetic condition, especially if these changes in energetic condition are accompanied by shifts in dietary composition. In a feeding experiment conducted on captive bonobos (*Pan paniscus*), we monitored urinary $\delta^{13}C$, $\delta^{15}N$, total C (carbon), total N (nitrogen), and C/N ratios and compared these results with glucocorticoid levels under gradually changing energy availability and dietary composition. Measurements of daily collected urine samples over a period of 31 days showed that while shifts in urinary isotope signatures of $\delta^{13}C$ and $\delta^{15}N$ as well as total C were best explained by changes in energy consumption, urinary total N excretion as well as the C/N ratios matched the variation in dietary composition. Furthermore, when correcting for fluctuations in dietary composition, the isotope signatures of $\delta^{13}C$ and $\delta^{15}N$ as well as total C correlated with urinary glucocorticoid levels; however, the urinary total N and the C/N ratio did not. These results indicate for the first time that it is possible to non-invasively explore specific longitudinal records on animal energetic conditions and dietary compositions with urinary stable isotope ratios and elemental compositions, and this research provides a strong foundation for investigating how ecological factors and social dynamics affect feeding habits in wild animal populations such as primates.

Over the past few decades, stable isotope ratio analysis has become an important tool for examining ecologically based questions such as an organism's position in a food web, migration patterns, the effect of pollution, and physiological status.[1–3] The majority of these studies in ecological research have focused on the application of carbon and nitrogen ratios to distinguish dietary patterns: such as between marine and terrestrial diets, or between C_3 and C_4 plant-based diets and to determine trophic level feeding habits within a particular food chain.[1–5] However, evidence from feeding experiments involving various avian, reptile, and mammalian species indicates that stable isotope ratios in general, and nitrogen isotope ratios in particular, can be used to monitor fluctuations in nitrogen balance caused by situations such as nutritional stress.[6–13] During episodes of negative nitrogen balance and in response to mobilization of endogenous protein, elevated $\delta^{15}N$ values in body tissues have been observed.[8–10,13] This pattern of 'enrichment in loss'[14] has also been reported from humans during morning sickness,[15] in individuals diagnosed with eating disorders such as anorexia nervosa and bulimia,[16–18] and in athletes during physical activity.[19] This ^{15}N-enrichment is due to the fact that under periods of substantial nutritional stress, organisms catabolize endogenous amino acids to meet the demands of protein synthesis, and thus an organism literally 'lives on its own meat'.[20]

While the majority of studies that have explored the link between nutritional stress and ^{15}N-enrichment have used tissue samples such as hair, nails, feathers, muscle, and blood, little isotopic research has focused on urine.[6,12,14,21,22] It has been suggested that ^{15}N-enriched values in tissue can only be observed under conditions of extreme food restriction or among fasting animals, making isotopic ratios unreliable for monitoring moderate nutritional stress in non-fasting animals.[7,11] Given the fast synthesis and excretion time of urea,[23,24] the isotopic analysis of urine or uric acid samples should provide a means to determine subtle variations in the nitrogen balance of an organism. Previous research in

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reptiles found that changes in isotopic ratios due to short-term starvation were only detectable in excreta and not in body tissue.[6,12] Since the isotopic signatures of $\delta^{15}N$ and $\delta^{13}C$ in urine are not only influenced by nutritional stress but also by the ingested food, experiments need to control both the energy intake and the isotopic composition of the food itself. If stable isotope ratio analyses of urine samples are shown to detect moderate changes in energy balance, the technique will provide a useful tool for exploring food abundance and nutritional stress in animal populations in the wild.

In the present study we investigate how a change in food composition with decreasing digestible energy content influences the $\delta^{13}C$ and $\delta^{15}N$ values and the total carbon (%C) and nitrogen (%N) composition of urine. To this aim, a feeding experiment was conducted on a group of captive pygmy chimpanzees or bonobos (*Pan paniscus*) in which the animals were exposed to a controlled modification in energy availability and food composition over a period of 31 days. Data on digestible energy intake, body weight, and isotopic and elemental parameters of the food consumed were related to variations in urinary $\delta^{13}C$ and $\delta^{15}N$ signatures and to the carbon and nitrogen elemental analysis. Furthermore, these urinary isotopic and elemental results were compared with urinary glucocorticoid levels to investigate how well an activation of the Hypothalamic–pituitary–adrenal axis caused by nutritional stress correlates with isotope and elemental parameters. Specifically, this experiment was designed to address the following questions: How are urinary isotope and elemental measurements influenced by food characteristics and by changes in digestible energy intake associated with nutritional stress? How do these changes relate to other indicators of nutritional stress such as glucocorticoids?

EXPERIMENTAL

Study design and study animals

This study was conducted on a group of seven (males n = 1, females n = 6) adult bonobos from Frankfurt Zoo in Frankfurt, Germany (for details on the animals, see Supporting Information). The feeding experiment lasted 4 weeks and consisted of two parts. For the first 2 weeks, the amount of food items with high energy content was gradually decreased whereas the amount of low-caloric food was increased (period of energy restriction). Following the period of energy restriction the low caloric food items were gradually replaced by calorie-rich food items (refeeding). At the end of the second 2-week period the amount of high caloric items exceeded that which the bonobos normally received to cause a weight gain.[25] The experimental protocol was approved and conducted in accordance with the authority of animal welfare (Veterinaerdezernat, Regierungspraesidium Darmstadt, Germany).

Data collection

Before the start of the experiment, subjects were trained to deliver urine into containers held by zoo personnel. Urination was enhanced by providing nettle tea 20 min prior to the collection time. Samples were collected directly into plastic cups or taken off the ground with disposable plastic pipettes. Urine samples were collected each day, once in the morning (~08:00) and once in the afternoon (~14:00) and frozen immediately at −20 °C and transported on dry ice to the laboratory. Animals were weighed on a daily basis on a balance (EZI WEIGHT 2, Tru-Test Ltd., Auckland, New Zealand: range: 0–500 kg, resolution: 0.5 kg) except for one female that could not be weighed throughout the experiment (see Deschner *et al.*[25] for further details).

Elemental and isotope analysis

An aliquot of 1 mL of each urine sample was lyophilized in a freeze dryer for 48 h. The residue was homogenized and ~1 mg taken for isotopic analysis. Sample combustion and elemental analysis (%C, %N, C/N ratio) were performed in an elemental analyzer (Flash EA 2112, ThermoFinnigan, Bremen, Germany) coupled to a ThermoFinnigan Delta XP isotope ratio mass spectrometer at the Max Planck-Institute for Evolutionary Anthropology in Leipzig (Germany) for the measurement of isotopic ratios ($\delta^{15}N$, $\delta^{13}C$). In addition, ~1 mg of freeze dried matter of each food item was weighed into tin capsules for analysis as described above. Using the formula: $X(‰) = (R_{sample}/R_{standard} - 1) \times 1000$ (‰), R_{sample} was calculated for each food item and taking the total amount of N and C in the dry weight into consideration, the amount of each heavy and light isotope for each food item was calculated and then summed up over all food consumed per day. We then calculated average $\delta^{15}N$ and $\delta^{13}C$ values for the food consumed on each day. The total N (%) and total C (%) values were calculated accordingly.

Measurement of digestible energy intake

To calculate the daily digestible energy intake we summarized the energy content of each food item (fresh weight) considering the total amount that each food contributed to the meal. Food that was not consumed was weighed and the corresponding energy content was subtracted from the original figure. In addition, the feces of the entire group were collected each day, weighed, thoroughly mixed, and a sample was taken for measurement of the energy content.

The gross energy of each food item and feces sample was determined via bomb calorimetry (C5003 bomb calorimeter; IKA, Staufen, Germany) conducted in the nutritional physiology laboratory at IZW in Berlin, Germany.[26] 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.[26] Crude protein was accessed using Dumas-Combustion. A subsample of each food item was burned in an elemental analyzer (Elementar Rapid N III; Elementar Analysensysteme GmbH, Hanau, Germany) and the total nitrogen (N) of the sample provided an estimate of crude protein (protein level = N × 6.25).

Steroid hormone measures

Urinary glucocorticoid levels were measured using high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS). LC measurements were carried out using a Waters Alliance 2695 separation module equipped with a quaternary pump and a column oven (Waters, Milford, MA, USA) and separation was performed on a reversed-phase C-18

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column (Gemini, 150 × 2 mm, 3 µm; Phenomenex, Torrance, CA, USA) with gradient elution (acetonitrile/water and 0.1% formic acid).[27] MS analyses were carried out on a Quattro Premier XE tandem mass spectrometer (Micromass, Manchester, UK) using electrospray ionization (ESI) in positive mode.[27]

The quantitative analysis of urinary glucocorticoid metabolites by LC/MS was carried out in the range of 0.3 to 1000 ng/µL.[23,25] We excluded samples that had an internal standard recovery of more than ±50% from the expected values from our analysis. We examined LC/MS data with MassLynx (version 4.1, QuanLynx-Software; Waters/Micromass, Milford, MA, USA). The preparation of urine samples followed the protocol of Hauser *et al.*[27] By adding deuterated internal standards to samples before the extraction of steroids we were able to control for variations in extraction efficiency. Since bonobo urine contains an unidentified metabolite that co-elutes with the deuterated internal standard d_4-cortisol, d_3-testosterone was used as the reference component for all glucocorticoids. When the recovery of the internal standard deviated by more than ±50% from the expected value, samples were excluded from the analysis.

In chimpanzees and bonobos, only a minor part of the cortisol in the blood is found as native cortisol in urine[28] while other glucocorticoid metabolites are found in much higher quantities.[27] Therefore, in addition to urinary cortisol, four main metabolites of this glucocorticoid in primates, tetrahydrocortisol, tetrahydrocortisone, 11-hydroxyetiocholanolon, and 11-oxoetiocholanolon, were quantified[1] and the sum of cortisol and these metabolites was used as a measure of adrenal glucocorticoid production.[29,30] To control for differences in the water content of the urine, the creatinine level of each sample was determined via microtiterplate assay.[31] Since weight loss accompanying digestion of body protein could lead to changes in urinary creatinine levels and thereby render this correction method invalid, we tested for changes in urinary creatinine levels across the period of the feeding experiment. There were no significant temporal changes in urinary creatinine levels (GLMM: estimate = −0.089, SE = 0.055, p_{MCMC} = 0.110 for a quadratic relationship; estimate = −0.026, SE = 0.050 p_{MCMC} = 0.610 for a linear relationship, quadratic term removed). Overall 176 morning samples and 192 afternoon samples were used for our analysis. Individual sample sizes ranged from 23 to 26 for morning, and from 20 to 31 for afternoon samples.

Statistics

To explore how the isotope measurements were influenced by the nutrition and physical status of the subjects, five separate models were used: one with each of the two isotope ratios ($\delta^{13}C$, $\delta^{15}N$) and three measures of elemental composition (total C (%), total N (%), and urinary C/N ratio) as response variables. As predictor variables (with fixed effects) we used the digestible energy intake during the day prior to urine sampling, the value of the response variable measured in food consumed on the previous day (e.g. if urinary $\delta^{15}N$ was the response variable, the total $\delta^{15}N$ in the food consumed on the previous day was included as the predictor variable) and relative body weight (calculated as the actual body weight divided by the maximum body weight during the experiment). General Linear Mixed Models (GLMM[32]) into which we included subjects' identity as a random effect were used. For details about these and other analyses conducted, see the Supporting Information.

RESULTS

As expected, the digestible energy intake declined significantly during the restriction period (Spearman rank correlation, N_{days} = 15, r_s = −0.65, p = 0.011) and increased during the refeeding period (Spearman rank correlation, N_{days} = 16, r_S = 0.61, p = 0.014, Table 1). Other food parameters such as crude protein availability (gram protein/day), dry matter and the total amount N were affected differentially by the changes in food composition across the entire feeding experiment (Table 1). Energy restriction induced a decrease in body weight which was restored during the refeeding period (Table 2). Furthermore, temporal changes in digestible energy intake were associated with changes in isotope parameters and elemental levels in the consumed food (Table 2, Fig. 1). Elemental compositions and stable isotope ratios were influenced differently by both the digestible energy intake of the previous day and the elemental compositions and stable isotope ratios of the food (Table 3, Figs. 2, 3, and 4). Changes in urinary $\delta^{15}N$ and $\delta^{13}C$ levels and total C excretion were strongly related to the digestible energy intake of the previous day while the urinary C/N ratio and total N excretion were associated with corresponding levels in food. In addition, we investigated if urinary isotope signatures were correlated with urinary glucocorticoid measurements while controlling for isotope ratios and elemental compositions in the food consumed. The urinary glucocorticoid levels increased during the restriction period and the $\delta^{15}N$, $\delta^{13}C$ levels and total C excretion showed a significant correlation with urinary glucocorticoid levels; however, no significant correlation was observed for the C/N ratio and the total N excretion (Table 4).

Table 1. Correlations between food parameters and time during the energy restriction and refeeding period of the feeding experiment (N = number of days)

	Energy restriction phase			Refeeding phase		
	r	N	*p*	r	N	*p*
Digested energy per day (kJ/group)	**−0.65**	15	**0.011**	**0.61**	16	**0.014**
Protein	0.30	15	0.277	−0.09	16	0.745
C/N ratio	**−0.67**	15	**0.007**	**0.66**	16	**0.006**
$\delta^{13}C$	−0.48	15	0.074	**0.51**	16	**0.043**
$\delta^{15}N$	**−0.55**	15	**0.034**	−0.08	16	0.770
Total C (%)	−0.08	15	0.771	−0.07	16	0.812
Total N (%)	**0.64**	15	**0.010**	**−0.67**	16	**0.005**
Dry matter	**−0.63**	15	**0.012**	0.39	16	0.141

Table 2. Average levels of investigated parameters in food and urine during the 2 weeks of energy restriction and the 2 weeks of refeeding. Values are averages (and SD) across days and animals. The sample size was N = 6 for body weight and N = 7 for all other parameters (see Experimental section)

Parameters		Energy restriction 1.week		Energy restriction 2.week		Refeeding 1.week		Refeeding 2.week	
body weight (kg)		33.2	(7.9)	32.1	(7.5)	32.2	(7.2)	33.1	(6.2)
urine parameters	C/N ratio	2.3	(1.3)	1.5	(0.4)	2.1	(0.7)	4.2	(1.2)
	$\delta^{13}C$	−23.1	(1.3)	−22.4	(1.5)	−23.7	(1.9)	−23.8	(1.8)
	$\delta^{15}N$	2.5	(0.5)	2.6	(0.6)	2.2	(0.6)	2.1	(0.5)
	Total C (%)	19.6	(4.2)	17.1	(3.3)	19	(3.6)	22.3	(5.5)
	Total N (%)	11.6	(3.6)	13.8	(4.0)	11.4	(3.3)	6.9	(2.3)
food parameters	digested energy per day (kJ/group)	42536	(11552)	33516	(7654)	50051	(24832)	79142	(22613)
	C/N ratio	19.8	(5.1)	14.4	(1.0)	21.7	(6.0)	31.7	(7.3)
	$\delta^{13}C$	−17.1	(1.9)	−18.3	(1.7)	−17.8	(1.5)	−16.1	(0.5)
	$\delta^{15}N$	6.4	(0.6)	6.1	(0.4)	6.0	(1.4)	5.8	(1.0)
	Total C (%)	40.4	(1.0)	40.7	(1.4)	41.4	(1.9)	41.4	(1.3)
	Total N (%)	2.2	(0.5)	2.9	(0.3)	2.0	(0.5)	1.4	(0.5)
	total food mass consumed (kg/group/day)	33.2	(6.2)	35	(5.0)	29.5	(5.2)	37.3	(12.1)
	"% dry matter"	8.8	(3.0)	6.6	(1.8)	12.6	(4.7)	13.3	(1.6)
	"% protein of dry mass"	15.9	(3.4)	21.3	(1.5)	14.2	(2.8)	10	(4.8)

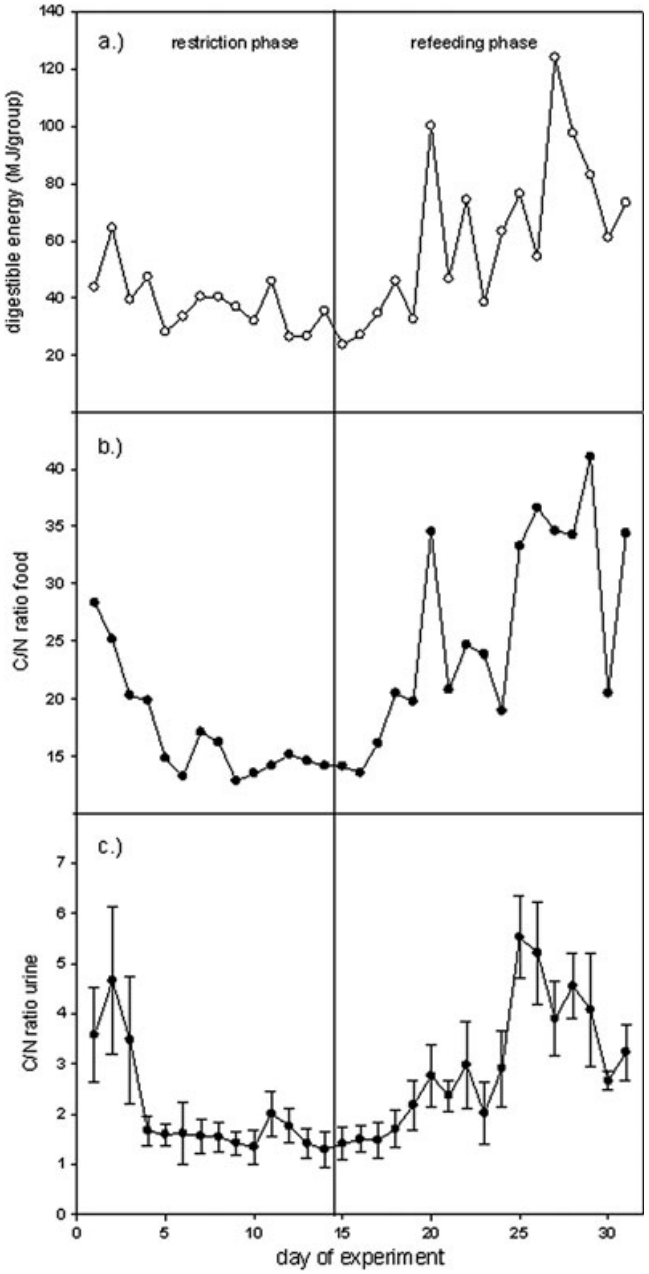

Figure 1. Temporal profile of digestible energy intake (a), C/N ratio in food (b), and C/N ratio in urine (c) throughout the energy restriction and refeeding period. The C/N ratio in urine is given as mean and SD across all adult animals.

DISCUSSION

The results of this controlled feeding experiment are critical to the understanding and application of stable isotope ratios in ecological studies as we could show that energy restriction in the diet induced an increase in urinary $\delta^{13}C$ and $\delta^{15}N$ values and a decrease in total C levels in the urine samples of mammals. These changes were found to be independent of the isotopic composition and total C levels in the consumed diet. On the other hand, urinary total N, and as a consequence the urinary C/N ratio, were primarily influenced by changes in the total N levels and the C/N ratio of the

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Table 3. Dependency of urinary isotope signatures and elemental parameters on changes in digestible energy intake, isotope signatures, elemental parameters of the food, and relative body weight. Note that for each response variable a different GLMM was built (see Experimental section). In all cases, the full model was highly significant compared with the null model (likelihood ratio tests: all χ^2 >16, all df = 3, all p <0.001). All predictors were z-transformed

Response	Predictor	Estimate	HPD L	HPD U	p
C/N ratio	Intercept	0.755	0.679	0.831	0.0001
	digested energy per day	0.080	−0.004	0.157	0.1372
	C/N ratio food prev. day	0.275	0.190	0.360	**0.0001**
	relative body weight	0.067	0.009	0.128	**0.0270**
$\delta^{13}C$	Intercept	−23.098	−23.769	−22.406	0.0001
	digested energy per day	−0.642	−0.989	−0.274	**0.0014**
	$\delta^{13}C$ food prev. day	0.111	−0.193	0.440	0.6612
	relative body weight	0.118	−0.276	0.472	0.5992
$\delta^{15}N$	Intercept	2.395	2.121	2.656	0.0001
	digested energy per day	−0.192	−0.272	−0.104	**0.0001**
	$\delta^{15}N$ food prev. day	0.001	−0.070	0.067	0.9780
	relative body weight	−0.015	−0.128	0.081	0.5394
	autocorrelation term	0.978	0.449	1.487	**0.0008**
Total C (%)	Intercept	4.375	4.300	4.462	0.0001
	digested energy per day	0.223	0.136	0.309	**0.0001**
	Total C (%) food prev. day	−0.002	−0.079	0.071	0.8498
	relative body weight	−0.023	−0.108	0.058	0.6904
	autocorrelation term	0.591	0.052	1.062	**0.0330**
Total N (%)	Intercept	3.279	3.187	3.380	0.0001
	digested energy per day	−0.090	−0.199	0.032	0.2396
	Total N (%) food prev. day	0.262	0.146	0.382	**0.0001**
	relative body weight	−0.157	−0.248	−0.071	**0.0006**
	autocorrelation term	0.737	0.160	1.170	**0.0120**

consumed diet. No significant correlation was found between digestible energy intake and total N and the C/N ratio in the urine (Table 3). In addition, we showed for the first time that urinary $\delta^{13}C$ and $\delta^{15}N$ values as well as total C levels (controlled for the content in the diet) correlated with urinary glucocorticoid levels, but no correlation was observed for the urinary total N levels and the C/N ratio.

Patterns of elemental measures and isotope signatures

The patterns observed in the isotope signatures of the urine are in agreement with the notion of the body 'living on its own meat'[20] and thus the isotopic phenomenon of 'enrichment in loss'.[14] While the $\delta^{13}C$ and $\delta^{15}N$ values in the urine both gradually increased during energy restriction, the levels of both isotopes dropped during the refeeding period (Fig. 4). The urinary total C levels decreased during the restriction period and increased again during refeeding. This pattern is consistent with a general decrease in the intake of digestible carbon, and an increase in carbon-containing synthesis products coming from the breakdown of tissues and body energy reserves. It is important to keep in mind that carbon excretion does not occur solely through urine and a vast amount of carbon is excreted via breath as CO_2.[33,34] Therefore, it is remarkable that a significant shift in carbon excretion was still observed in the urine. In addition to the abovementioned changes in the carbon, there was an increased excretion of nitrogen with ongoing energy restriction and a decrease during the refeeding period. These results are surprising since one would expect that when exposed to nutritional stress an organism would try to conserve nitrogen

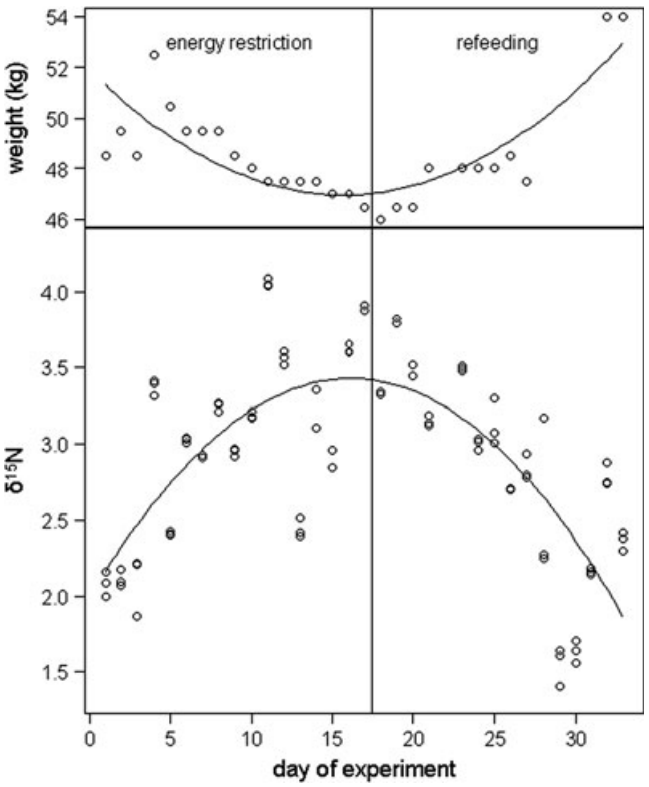

Figure 2. Changes in urinary $\delta^{15}N$ and body weight throughout the energy restriction and refeeding period of the food restriction experiment for the adult male Ludwig. Samples were measured in triplicate. The regression curves show the relation between day of the experiment and body weight or urinary $\delta^{15}N$, respectively, as determined using a univariate linear model.

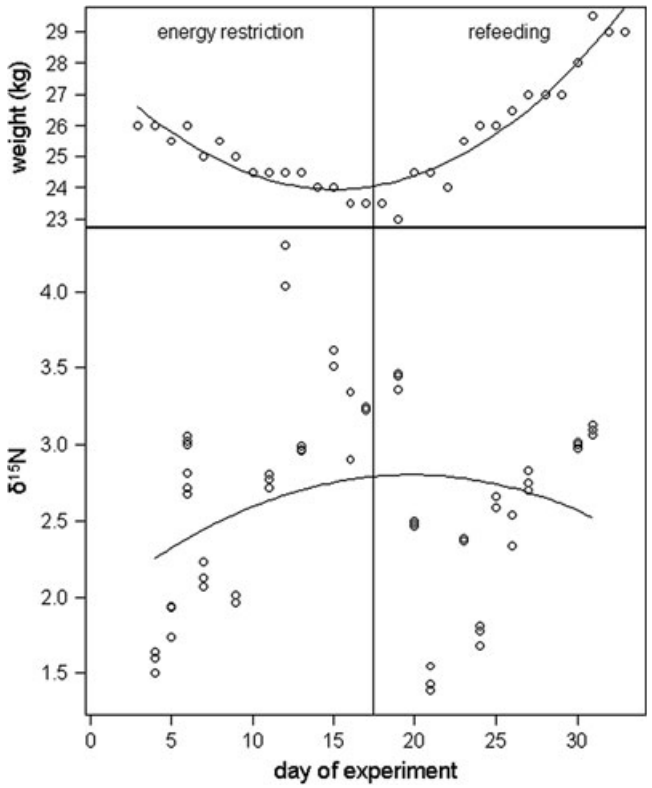

Figure 3. Changes in urinary $\delta^{15}N$ and body weight throughout the energy restriction and refeeding period of the experiment for the adult female Zomi. Samples were measured in triplicate. The regression curves show the relation between day of the experiment and body weight or urinary $\delta^{15}N$, as determined using a univariate linear model.

reserves.[35] Evidence for this comes from studies on humans and animals that have shown that energy restriction reduces nitrogen excretion via urine.[36,37] However, a shift in nitrogen excretion can also be caused by changes in the amount of nitrogen consumed.[38] While there was no overall temporal pattern in total protein consumption, the total N levels in the dry matter of the food items consumed increased during the energy restriction period and decreased during the refeeding period. This pattern arose because the increase in total N in the dry matter of the food was compensated for by a decrease in the total dry matter of the food during energy restriction (and a later increase during the refeeding period). Therefore, it seems that the increase in urinary nitrogen excretion was mainly caused by the shift in diet where energy-rich food items were replaced by energy-poor ones, with some of the main energy-poor food items (such as lettuce) having high total N levels and a very low C/N ratio. Thus, of all the parameters investigated, changes in digestible energy intake (loss or gain), were detected best by urinary $\delta^{13}C$ and $\delta^{15}N$ values as well as total C levels. The urinary total N levels and the C/N ratios were better indicators for the composition of food, namely the total N content in the dry matter (Table 3).

The results of this detailed feeding experiment are significant as a number of studies that investigated the influence of nutritional stress on isotope signatures in a variety of

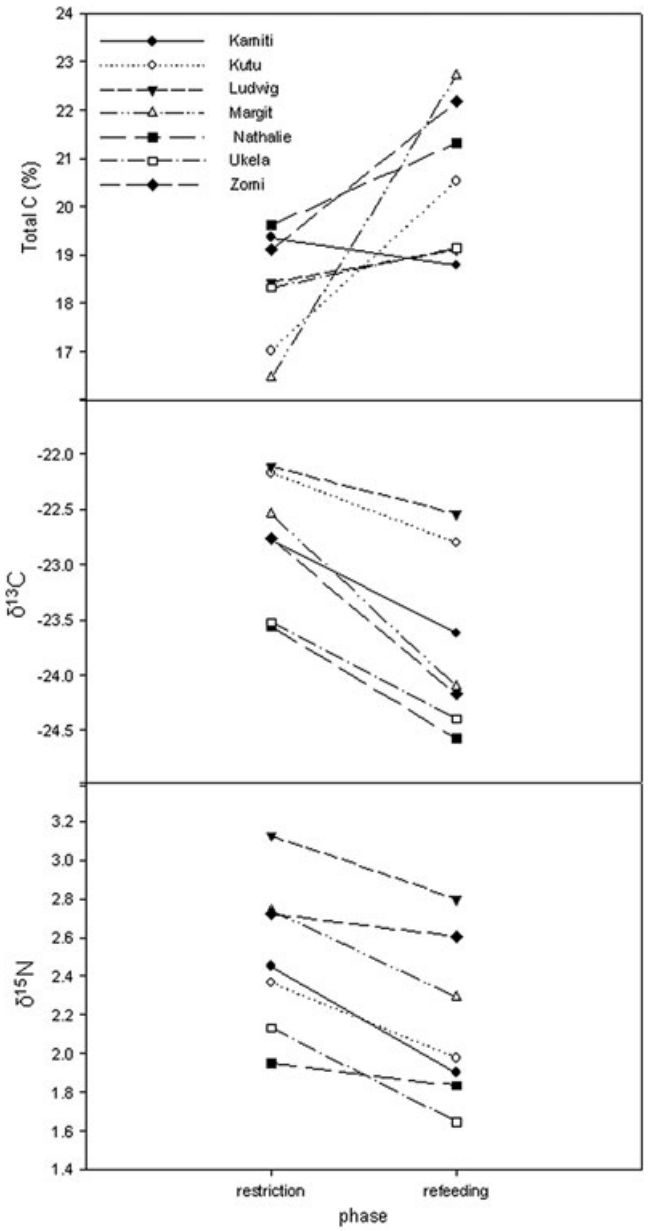

Figure 4. Changes in average urinary total C (%), $\delta^{13}C$ and $\delta^{15}N$ from the energy restriction to the refeeding period for all adult individuals.

Table 4. Partial correlations between isotope signatures and urinary glucocorticoid levels across the experimental period

Isotope parameters	Average rho	t	df	*p*
C/N ratio	−0.10	−1.12	6	0.305
$\delta^{13}C$	0.29	4.34	6	**0.005**
$\delta^{15}N$	0.36	5.43	6	**0.002**
Total C (%)	−0.36	−5.76	6	**0.001**
Total N (%)	0.04	0.36	6	0.732

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tissues from different animal species found inconsistent results.[7,11] Thus, the following question needs to be addressed: Why was the pattern of changes in isotope signatures and total C so pronounced in this study? Even in cases of severe starvation leading to a breakdown of energy reserves, only a fraction of tissue is metabolized at any given point in time, leading to a very slow change in isotope signatures that is difficult to quantify. In addition, the elemental compositions of tissues such as bone, hair, and muscle are relatively constant, so the elemental analysis of these tissues through time and under different dietary conditions or periods of nutritional stress would be insufficient for the detection of episodes of nutritional stress. However, in an excretion product like urine, a more pronounced shift in isotope signatures and elemental compositions can be expected since a reduction of digestible energy intake leads to a rapid change in the proportion of compounds stemming from the different sources (diet vs. energy reserves). During normal feeding or when consumers gain weight, most C and N excreted via urine originates from ingested food. However, during periods of nutritional stress or starvation an increasing amount of these urinary compounds and the main nitrogen source of urea originate from metabolized internal tissues (muscle) that are ^{15}N-enriched compared with the diet and this in turn causes an increase in δ^{15}N values of the urine.[9,10,15,39] Thus, the isotopic and elemental variations appear to be much more pronounced in urine compared with the slow and gradual changes that take place in tissues such as bone, muscle, hair, and blood.

Correlates of elemental measures and isotope signatures with glucocorticoid levels

A reduction in energy availability leads to the mobilization of stored energy reserves.[40] The breakdown of existing energy stores is induced by an increase in glucocorticoid excretion.[41] The measures of urinary isotope ratios and total C levels in this study did correlate well with changes in urinary glucocorticoid excretion (Table 4). When the digestible energy intake was reduced the glucocorticoid levels rose, leading to the mobilization of energy reserves. The excreted breakdown products of these stored energy reserves led to the observed changes in the isotope signatures and thereby to a correlation with glucocorticoid excretion patterns.

In this study, by simultaneously manipulating the C/N ratio and the energy content of the diet, it was possible to investigate how the urinary isotope and elemental parameters are affected by these changes. This situation of simultaneous shifts in dietary composition and energy content mimics conditions of tropical environments where consumers do not experience severe food scarcity but respond to seasonal changes in food availability by shifting from high-quality food such as fruit to other abundant plant foods that are of lower quality such as leaves.[42] Since mature leaves and other herbaceous vegetation are relatively rich in protein, the shift in diet is likely to be accompanied by a change in protein intake.[43] Bonobos are large fruit-eating hominoid primates, living in tropical lowland forests of the Congo basin. Changes in size and composition of foraging groups[44] have been related to changes in resource abundance.[45] Given the favorable environmental conditions and the flexible grouping pattern, bonobos are thought to avoid resource competition and maintain sufficient energy intakes throughout the year.[46] From this perspective it seems reasonable to assume that the selection pressure for physiological coping mechanisms of seasonal changes in food supply has been relatively low. Indeed, the results obtained in this feeding experiment indicate that bonobos are highly responsive to changes in digestible energy intake and it is tempting to speculate that other species that experience more pronounced seasonality in resource abundance may differ in their physiological response to changes in energy intake.

The results show that these two simultaneously occurring effects differentially influence urinary isotope and elemental parameters. While changes in urinary δ^{13}C and δ^{15}N values as well as total C levels indicate shifts in digestible energy intake, the urinary total N levels and the C/N ratios indicate shifts in the food composition, namely the total N content in dry matter. Furthermore, the induced changes were created by moderately shifting the diet. Although the bonobos lost weight during the energy restriction period and gained weight during the refeeding period there was always enough food matter available. This contrasts with other studies that created nutritional stress by exposing subjects to severe starvation. While the moderate changes in energy content used in this study would perhaps not have led to detectable shifts in isotope signatures in other tissues, it was sufficient to induce significant effects in urine. It remains to be investigated if during shifts to a different dietary trophic level, e.g. a shift from herbivorous to omnivorous or carnivorous diet, one could still distinguish between changes in urinary isotope signatures caused by nutritional stress and those caused by dietary changes. Ongoing feeding experiments including herbivorous and omnivorous diets will allow us to determine the differences between nutritional stress and dietary shift signals in urinary elemental compositions and isotope ratios.

The analysis of urinary stable isotope ratios and elemental compositions has the potential to become a valuable tool to assess how seasonal changes in food abundance and energy balance impact on the foraging decisions of wild animal species. This is of particular importance in species where fluctuations in food availability are moderate. For example, many nonhuman primates live in tropical forest habitats and the shortage of preferred food items such as ripe fruit does not lead to starvation but is compensated for by the consumption of low-quality food items such as leaves.[47,48] While nutrient intake may be relatively stable over time social constrains may cause significant inter-individual differences in energy balance. Moreover, in conjunction with corresponding information from other species, the results obtained in this study will be useful for testing existing socio-ecological theories.[49] Finally, the detection of moderate fluctuations in energy as used in this study on bonobos will be highly relevant and applicable to medical research in the form of diagnosing and monitoring conditions marked by changes in nitrogen balance such as eating disorders in humans.[16,17,50]

CONCLUSIONS

This experimental study on bonobos found that urinary carbon and nitrogen isotope ratios and measurements of total carbon facilitate the detection of moderate short-term fluctuations in energy intake and body weight while the urinary total nitrogen levels and C/N ratios are strongly related to the total nitrogen content of the food consumed. A comparison with urinary glucocorticoid levels indicates that the observed patterns are in line with the notion that fluctuations in energy intake lead to glucocorticoid-induced mobilization of body energy reserves. This study demonstrates the potential to longitudinally and non-invasively track dietary habits and changes in energy status under conditions of moderate nutritional stress, a condition that is typical for species living in tropical forest habitats. These findings are of great importance to ecologists working with terrestrial mammalian populations such as primates to determine seasonal variations in access to nutritional sources and the impact of social parameters in the context of resource competition. Finally, this technique can be applied to nutritional and medical studies of modern humans: isotopic measures of urine samples may be a useful tool for exploring nitrogen flux and balance under various conditions such as pregnancy, dieting, eating disorders, and physical activity associated with endurance training. The advantage of urine over previous research that has measured isotopic values in tissues, such as hair, is that the fast formation and excretion of urine permit a more rapid and direct measurement of the nitrogen condition and balance in humans, and this is an area of future research.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

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