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The Diet-Body Offset in Human Nitrogen Isotopic Values: A Controlled Dietary Study

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ABSTRACT The “trophic level enrichment” between diet and body results in an overall increase in nitrogen isotopic values as the food chain is ascended. Quantifying the diet–body $\Delta^{15}\text{N}$ spacing has proved difficult, particularly for humans. The value is usually assumed to be +3–5‰ in the archaeological literature. We report here the first (to our knowledge) data from humans on isotopically known diets, comparing dietary intake and a body tissue sample, that of red blood cells. Samples were taken from 11 subjects on controlled diets for a 30-day period, where the controlled diets were designed to match each individual's habitual diet, thus reducing problems with short-term changes in diet causing iso-

topic changes in the body pool. The $\Delta^{15}\text{N}_{\text{diet-RBC}}$ was measured as +3.5‰. Using measured offsets from other studies, we estimate the human $\Delta^{15}\text{N}_{\text{diet-keratin}}$ as +5.0–5.3‰, which is in good agreement with values derived from the two other studies using individual diet records. We also estimate a value for $\Delta^{15}\text{N}_{\text{diet-collagen}}$ of $\approx 6\%$, again in combination with measured offsets from other studies. This value is larger than usually assumed in palaeodietary studies, which suggests that the proportion of animal protein in prehistoric human diet may have often been overestimated in isotopic studies of palaeodiet. *Am J Phys Anthropol* 149:426–434, 2012. © 2012 Wiley Periodicals, Inc.

Light element isotopic analyses of human and animal body tissues are increasingly used to elucidate dietary patterns in past and living populations, with applications in archaeology, ecology, and nutritional epidemiology. However, the full potential of those analyses remains constrained by our limited understanding of the mechanisms involved in the transfer of the isotopic signature to the body during the absorption and incorporation of food. This is particularly the case with nitrogen isotopes, where there is an observed enrichment between diet and body (the “trophic level effect” or $\Delta^{15}\text{N}_{\text{diet-body}}$), resulting in an increase in $\delta^{15}\text{N}$ as the food chain is ascended (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984). Despite its clear empirical success as a dietary indicator, we do not yet know metabolically how and where the ^{15}N enrichment between diet and body occurs. Ecological studies suggest that mammals, fish, birds, reptiles, and insects all have similar enrichments (Caut et al., 2009), so it seems to be independent of the mode of nitrogen excretion, but there has been little exploration of the cause. Quantifying the enrichment has proved difficult: large-scale ecological studies suggest that the enrichment associated with each trophic level is $\approx +3\text{--}4\%$, while small-scale animal feeding experiments show values anywhere between +1.5 and +6‰ (see review in Caut et al., 2009). In addition to being poorly quantified and understood, the trophic level effect also seems capable of quite large variation under a range of environmental conditions (temperature, altitude, aridity), as well as being potentially affected by physiological factors such as water stress, starvation and growth, digestive physiology and diet composition (for a review see McCue and Pollock, 2008).

For isotopic studies of human diet, the resolution of our interpretations is limited because we do not know what value to use for the ^{15}N enrichment in humans (see Hedges and Reynard, 2007). While broad-scale changes in diet are easily observed in human isotopic values (Vogel and van der Merwe, 1977; Tauber, 1981; Buikstra and Milner, 1991; Lubell et al., 1994; Bonsall et al., 1997; Richards et al., 2003), our lack of knowledge of the $\Delta^{15}\text{N}_{\text{diet-body}}$ value, and of influencing factors on this parameter, means that we cannot with confidence identify isotopic shifts resulting from small-scale dietary changes. For this, we need to quantify better the $\Delta^{15}\text{N}_{\text{diet-body}}$ in humans.

QUANTIFYING THE ENRICHMENT

It has been generally assumed that the nitrogen isotopic enrichment in mammals, including humans, is broadly similar, with a $\Delta^{15}\text{N}_{\text{diet-body}}$ value initially taken

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to be about 3‰ (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984; Hare et al., 1991), but more recently values of up to 5‰ have been postulated (Ambrose, 2000; Jenkins and Partridge, 2001; Bocherens and Drucker, 2003; Sponheimer et al., 2003; Robbins et al., 2005; Caut et al., 2009). General reviews of the ecological literature for animals ranging from invertebrates to large mammals and aquatic and terrestrial species give overall mean $\Delta^{15}\text{N}_{\text{diet-body}}$ values of 2.5–3.5‰, with a high degree of variability, based on analyses of a range of body tissues (Post, 2002; McCutchan et al., 2003; Vanderklift and Ponsard, 2003). A value of around 3‰ fits with numerous predator–prey relationships in terrestrial ecological situations (see a summary in Bocherens and Drucker, 2003).

A large number of controlled animal feeding studies have been carried out, to attempt to quantify the offset (see summary in Caut et al., 2009). But for humans, the situation is more complicated, as there are significant difficulties in obtaining reliable data on which to base an estimate of human $\Delta^{15}\text{N}_{\text{diet-body}}$. A number of human studies have looked at isotopic variation within populations depending on self-reported diet type (O'Connell and Hedges, 1999a; Bol and Pflieger, 2002; Petzke et al., 2005b), or compared human isotopic variation to estimated diets, either at a population level (Minagawa et al., 1986; Schoeller et al., 1986; Minagawa, 1992; Thompson et al., 2011; Valenzuela et al., 2011) or on household basis (Yoshinaga et al., 1996). A few studies have compared individuals' isotopic values to self-reported dietary records (Petzke et al., 2005a; Hedges et al., 2009; Huelsemann et al., 2009; O'Brien et al., 2009; Nash et al., 2012). Most studies of humans have used hair keratin, and some have used blood proteins (RBC, plasma, serum). Some short term feeding studies have measured other samples (such as urine and feces: Kuhnle et al., in press).

A significant problem with controlled diet isotopic studies is that of tissue turnover rates. When measuring the $\Delta^{15}\text{N}_{\text{diet-body}}$, the tissues usually of interest (e.g., bone collagen, hair keratin, blood proteins) isotopically reflect medium or long-term diet (months or years), so that a short-term dietary intervention study is not possible, due to issues with tissue turnover and isotopic equilibration (Jones et al., 1981; Tieszen et al., 1983; O'Connell and Hedges, 1999a; Ayliffe et al., 2004; Huelsemann et al., 2009; Petzke and Lemke, 2009). This has long been recognized, and all robust published controlled animal feeding studies are of animals raised on a single diet over a long time period of several years, if not a lifetime. Such a study is not ethically or practically possible in humans.

Here we report isotopic analyses from humans on known and controlled diets for a short period, where the controlled diets were designed to match each individual's habitual diet, thus reducing problems with short-term changes in diet causing isotopic changes in the body pool. We measured dietary intake and a body tissue sample, red blood cells (RBCs).

MATERIALS AND METHODS

Samples were collected from healthy subjects taking part in a 30-day dietary intervention study to develop dietary biomarkers during the period of October 2002 to June 2003. Participants were provided with their habitual diet under controlled conditions for 30 days; blood

samples and duplicate diets were collected. Details of the study protocol can be found in Tasevska et al., (2005, 2006). The study was approved by the Cambridgeshire Local Research Ethics Committee (LREC No 02/232) and all participants gave their full informed written consent. Samples were archived in a controlled storage facility (Fisher Bioservice, Bishop's Stortford, UK) at -80°C for RBC and -20°C for all other specimens, and analyzed for this study in 2009–2010.

Subjects

A total of 13 healthy subjects from Cambridgeshire, UK, were recruited with advertisements. All participants were medically examined before the beginning of the study, including an assessment of the individual's past and family medical history, details of recent and current medications, vitamin supplements, and tobacco/alcohol intake, and a cardiovascular examination. Blood analysis of fasting plasma glucose and glycated hemoglobin (HbA_{1c}) was undertaken and all subjects were within the normal range (fasting plasma glucose <6.1 mmol/l, $\text{HbA}_{1c} < 6\%$). For this study, only samples from 11 participants (five males and six females, aged 23–66 y (39.7 ± 14.7 y), with a mean BMI of 25.8 ± 4.6 kg/m²; Table 1) were suitable, as the 30-day study period for the remaining two was not continuous (a brief break for Christmas).

Study design

For the duration of the study, participants lived in the volunteer suite of the MRC Dunn Human Nutrition Unit (Cambridge, UK), where all food provided was prepared by trained technicians, and all specimens collected and processed. Participants followed their normal daily routine but were only allowed to consume foods prepared by the diet technicians. Subjects weighed themselves daily on an electric balance without shoes and in light clothing and recorded their body weight in the study diary. Physical activity was assessed using a questionnaire validated by the EPIC study (Wareham et al., 2003). Physical activity was recorded in the study diary on a daily basis as time (minutes) engaged in different type of exercise. A four-level score (inactive, moderately inactive, moderately active, and active) was assigned by combining occupational physical activity together with time participating in higher-intensity physical activities such as cycling, aerobics, swimming, jogging, exercising at a gym on a regular basis, etc.

Diets

Prior to the study, participants were asked to keep 7-day food diaries for 4 weeks while living at home. Weekly interviews with one of the investigators provided additional information, such as brand names. These data were used to replicate the habitual diet of each participant for the duration of the study. From approximately two-and-a-half times the amount of food expected to be eaten by the participant, one-half was prepared and one-half was kept for the preparation of a duplicate meal. The prepared half was weighed to the nearest gram, labeled with the name and the day, and left in a separate refrigerator for each individual. During the day, participants helped themselves and returned the uneaten food to the containers in the refrigerator. The next day, the

TABLE 1. Subject details, and blood and diet isotopic results

Subject	Sex	BMI	Age (y)	Mean energy intake (MJ/d)	Energy intake %CV	Mean protein intake (g/d)	Protein intake %CV	Mean N intake (g/d)	N intake %CV	Arith mean diet $\delta^{15}\text{N}$ (‰)	Weighted mean diet $\delta^{15}\text{N}$ (‰)	Std dev diet $\delta^{15}\text{N}$ (‰)	Median diet $\delta^{15}\text{N}$ (‰)	IQR diet $\delta^{15}\text{N}$ (‰)	Blood1 $\delta^{15}\text{N}$ (‰)	Blood2 $\delta^{15}\text{N}$ (‰)	Mean blood $\delta^{15}\text{N}$ (‰)
V1	M	27.9	52	10.9	15.2	99.1	15.4	15.4	18.2	5.5	5.5	1.2	5.2	4.8–5.8	8.9	8.8	8.9
V2	M	27.3	46	11.2	19.5	118.2	23.1	18.8	27.3	4.5	4.6	1.0	4.8	3.8–5.2	7.6	7.4	7.5
V5	F	27.5	23	10.3	16.1	82.4	31.9	12.8	21.3	4.9	5.0	0.8	4.7	4.4–5.2	8.5	8.3	8.4
V6	F	19.3	24	10.2	17.5	78.8	30	15.2	34.9	5.1	5.3	1.1	4.7	4.5–5.4	7.8	7.9	7.8
V7	M	23.1	66	15.6	19	110	29.9	18.4	33.1	4.4	4.4	1.0	4.5	3.6–5.2	8.1	8.0	8.0
V8	F	22.3	29	11.7	17.3	99	23.7	15.2	20.8	5.0	5.0	1.4	4.4	4.0–5.8	8.2	8.1	8.2
V9	M	24.5	26	11.6	25.2	110.6	42.2	20.2	37.7	4.4	4.5	1.1	4.3	3.5–5.4	7.8	7.8	7.8
V10	F	34.5	48	12.7	17.4	103.4	23	15.6	24.8	4.4	4.5	1.0	4.4	3.8–4.8	8.9	8.8	8.9
V11	M	32.2	38	14.1	21.7	120.5	24.4	18.1	25.3	4.9	4.9	1.2	4.7	4.1–5.1	8.6	8.5	8.6
V12	F	23.2	56	11.6	19.2	107.3	23.7	15.7	22.4	4.7	4.7	0.7	4.7	4.3–5.1	8.1	8.1	8.1
V13	F	22.2	29	9.4	15.9	84.8	23	12.8	21.4	5.4	5.4	1.3	5.2	4.5–5.8	8.1	8.1	8.1
Mean		25.8	39.7	11.8	18.5	101.3	26.4	16.2	26.1	4.8	4.9	1.1			8.3	8.2	8.2
Std dev		4.6	14.7	1.8	2.9	14.2	6.9	2.4	6.4	0.4	0.4	0.2			0.5	0.4	0.4
Median		24.5	38.0	11.6	17.5	103.4	23.7	15.6	24.8	4.9	4.9	1.1	4.7		8.2	8.1	8.1
IQR		22.7–27.7	27.5–50.0	10.6–12.2	16.7–19.4	91.9–110.3	23.1–30.0	15.2–18.3	21.3–30.2	4.5–5.1	4.5–5.1		4.4–4.8		7.9–8.6	7.9–8.4	7.9–8.5

uneaten food was weighed out and the amount of food eaten was calculated.

Dietary intake was calculated from the UK food-composition tables using DINER (Data Into Nutrients for Epidemiological Research) (Welch et al., 2001). Tea and coffee were consumed freely during the course of the study, but participants were asked to keep their intake consistent and estimated intake was included in the data analysis. Five of the participants occasionally consumed alcohol; as this was not permitted in the volunteer suite, participants consumed alcohol outside the premises and recorded amount and type. The calculated dietary intake for alcoholic drinks was also added into the consumption data obtained in the study.

Duplicate diets were prepared daily for each participant. All food and drink items (excluding coffee, tea, alcoholic drinks, water, added salt, and pepper) were weighed to the nearest 1 g, chopped up and crushed, mixed with a weighed amount of boiling deionized water, and homogenized with a Magimix 5100 automatic food processor, usually for 10–15 min, until a smooth emulsion was obtained. Aliquots of each duplicate were stored at -20°C for analysis.

Blood collection, handling, and storage

Blood was sampled twice from each subject, at the start and in the last week of the study, by a trained phlebotomist. For one subject (V12), only blood collected at the end of the study was available for analysis. Fasting venous blood was collected into 10 ml lithium heparin monovettes. Within 1 h, the monovettes were centrifuged, the red blood cells removed from below the LiHep beads, washed thrice in chilled physiological solution, and then stored at -80°C prior to analysis.

Isotopic analyses

Duplicate diet samples were analyzed as liquid homogenates representative of 24 h food intake for each individual's diet. Eight to twelve days' diets were analyzed per subject, from the last half of the study. Samples were lyophilized and weighed into tin capsules (0.8 mg per aliquot). Red blood cell samples (0.2 ml) were lyophilized and then weighed into tin capsules (0.8 mg per aliquot). Diet samples were isotopically analyzed in duplicate, while blood samples were run in triplicate.

Isotopic analyses were performed using a Costech (Valencia, CA) automated elemental analyzer coupled in continuous-flow mode to a Thermo Finnigan MAT253 (Bremen, Germany) mass spectrometer at the Godwin Laboratory, Department of Earth Sciences, University of Cambridge. Stable isotope concentrations are measured as the ratio of the heavier isotope to the lighter isotope relative to an internationally defined standard, AIR (Hoefs, 1997). Isotopic results are reported as $\delta^{15}\text{N}$ values in parts per 1000 or "permil" (‰) values, where $\delta^{15}\text{N} = [^{15}\text{N}/^{14}\text{N}_{\text{sample}}/^{15}\text{N}/^{14}\text{N}_{\text{standard}} - 1] \times 1,000$. Based on replicate analyses of international and laboratory standards, measurement errors are less than $\pm 0.2\text{‰}$ for $\delta^{15}\text{N}$.

Statistical analysis

Because of the sample size and distribution of the data, nonparametric tests were conducted to investigate differences. The main objective of this study was to investigate differences in $\delta^{15}\text{N}$ between diet and blood;

TABLE 2. The $\Delta^{15}\text{N}_{\text{diet-RBC}}$ of the population calculated in different ways, using the mean and median measures of the subjects' nitrogen isotopic values

Subject	Arith mean $\delta^{15}\text{N}_{\text{diet}}$ (‰)	Median $\delta^{15}\text{N}_{\text{diet}}$ (‰)	Blood 2 $\delta^{15}\text{N}_{\text{RBC}}$ (‰)	Mean $\delta^{15}\text{N}_{\text{RBC}}$ (‰)	$\Delta^{15}\text{N}_{\text{diet-RBC}}$ (mean blood - mean diet) (‰)	$\Delta^{15}\text{N}_{\text{diet-RBC}}$ (blood 2 - mean diet) (‰)	$\Delta^{15}\text{N}_{\text{diet-RBC}}$ (mean blood - median diet) (‰)	$\Delta^{15}\text{N}_{\text{diet-RBC}}$ (blood 2 - median diet) (‰)
V1	5.5	5.2	8.8	8.9	3.3	3.3	3.7	3.6
V2	4.5	4.8	7.4	7.5	3.0	2.9	2.7	2.7
V5	4.9	4.7	8.3	8.4	3.4	3.3	3.7	3.6
V6	5.1	4.7	7.9	7.8	2.7	2.7	3.1	3.1
V7	4.4	4.5	8.0	8.0	3.7	3.6	3.6	3.5
V8	5.0	4.4	8.1	8.2	3.2	3.1	3.7	3.7
V9	4.4	4.3	7.8	7.8	3.5	3.5	3.5	3.5
V10	4.4	4.4	8.8	8.9	4.4	4.4	4.4	4.4
V11	4.9	4.7	8.5	8.6	3.7	3.7	3.9	3.8
V12	4.7	4.7	8.1	8.1	3.4	3.4	3.4	3.4
V13	5.4	5.2	8.1	8.1	2.8	2.8	2.9	2.9
Mean	4.8		8.2	8.2	3.4	3.3	3.5	3.5
Stdev	0.4		0.4	0.4	0.5	0.5	0.5	0.4
Median	4.9	4.7	8.1	8.1	3.4	3.3	3.6	3.5
IQR	4.5–5.1	4.4–4.8	7.9–8.4	7.9–8.5	3.1–3.6	3.0–3.5	3.2–3.7	3.3–3.7
Max	5.5	5.2	8.8	8.9	4.4	4.4	4.4	4.4
Min	4.4	4.3	7.4	7.5	2.7	2.7	2.7	2.7

assuming a standard deviation of 10% (higher than observed in this study) and a sample size of 11, changes of 15% can be detected with a power ($1-\beta$) of 0.9 at a significance level of $\alpha = 0.05$. Power calculations were performed with G*Power 3.1.2 (Faul et al., 2009). Data analyses were conducted using Stata 11.2 (Statacorp, College Station, TX). The bivariate boxplot (bagplot: Rousseeuw et al., 1999) was prepared in R 2.12.1 (Team, 2009). Unless indicated otherwise, data are given as mean \pm standard deviation.

RESULTS

Results are shown in Table 1. Overall, the body weight remained constant throughout the study (75.6 ± 15.7 kg at start vs. 75.8 ± 15.6 kg at end; Wilcoxon signed rank test, $P = 0.56$) which suggests that the intake achieved in the study was a valid reflection of the usual dietary habits in these volunteers. Weight changed by less than 2% in 10 participants; in one participant, the weight increased from 63.1 kg to 64.8 kg. However, this can be explained by normal fluctuations in the body weight, and changes in activity patterns during the study. Thus we take this population as being in a good approximation to steady state. True steady-state conditions are rarely achieved in free-living individuals, because abrupt changes in nitrogen balance occur from day to day, related to changes in dietary intake. Net accumulations and loss in nitrogen can be as much as $\pm 2\text{SE}$ for free-living individuals, largely due to day-to-day variations in dietary nitrogen intake which can take several days to be reflected in excreted nitrogen (Bingham and Cummings, 1985). Of the 11 subjects, three of the subjects were physically inactive, three moderately inactive, four moderately active, and one active. They mostly practiced cycling, swimming, exercising at the gym, and jogging.

The median diet nitrogen isotopic value for all subjects was 4.7‰ (range in subject medians of 4.3–5.2‰). The mean diet nitrogen isotopic value for all subjects was 4.8 ± 0.4 ‰ (range in subject means of 4.4–5.5‰). We investigated whether daily variation in dietary nitrogen content would affect the average dietary nitrogen isotopic value for each subject, since individuals did not consume

the same amount of protein on each of the 30 days of the study. For nine of the subjects, the difference between the arithmetical mean $\delta^{15}\text{N}$ and the mean $\delta^{15}\text{N}$ of each subject's diets weighted by the nitrogen contribution from each day's diet was less than 0.1‰, and for two individuals, the difference was less than 0.2‰; overall there was no statistically significant difference (Wilcoxon signed rank test, $P = 0.37$) between the two means (Table 1), so we consider that varying nitrogen intake had little if any quantifiable effect. Total protein intake and total nitrogen intake were inversely correlated with diet $\delta^{15}\text{N}$, although this correlation was only marginally significant (Spearman rank correlation: $\rho = -0.59$, $P = 0.05$, and $\rho = 0.57$, $P = 0.07$, respectively).

The range of RBC nitrogen isotopic values for all subjects was 7.6–8.9‰ at the start of the study and 7.4–8.8‰ at the end of the study. The median $\delta^{15}\text{N}_{\text{RBC}}$ for all subjects was 8.2‰ (IQR = 7.9–8.6‰) at the start of the study, and 8.1‰ (IQR = 8.0–8.4‰) at the end of the study; the mean $\delta^{15}\text{N}_{\text{RBC}}$ for all subjects was 8.3 ± 0.5 ‰ at the start of the study, 8.2 ± 0.4 ‰ at the end of the study, and 8.2 ± 0.4 ‰ for the two values averaged. Comparison of the $\delta^{15}\text{N}_{\text{RBC}}$ of blood taken at the start and end of the study shows a small decrease (comparison possible for 10 of the 11 subjects: median difference = -0.1 ‰, Wilcoxon test, $P = 0.02$; Table 1).

The overall difference between blood RBC and diet $\delta^{15}\text{N}$ ($\Delta^{15}\text{N}_{\text{diet-RBC}}$) in the population can be calculated in several ways, depending on whether the mean or median for the population is used (Table 2). The range of individual $\Delta^{15}\text{N}_{\text{diet-RBC}}$ is between 2.7 and 4.4‰, whichever way is used, and the average $\Delta^{15}\text{N}_{\text{diet-RBC}}$ for the group is between +3.3 and +3.6‰, with the statistically most parsimonious value (using the final blood sample $\delta^{15}\text{N}_{\text{RBC}}$ and the median diet $\delta^{15}\text{N}$) of +3.5‰ (Fig. 1). We did not observe any statistically significant difference between men and women, and no significant correlation with age or physical activity. The study was carried out over a period of months, but the sample size was too small to investigate the possible effects of seasonal changes in metabolic activity. However, $\Delta^{15}\text{N}_{\text{diet-RBC}}$ and $\delta^{15}\text{N}_{\text{RBC}}$ —but not $\delta^{15}\text{N}_{\text{diet}}$ —correlated significantly with BMI (Spearman rank correlations, respectively: $\rho =$

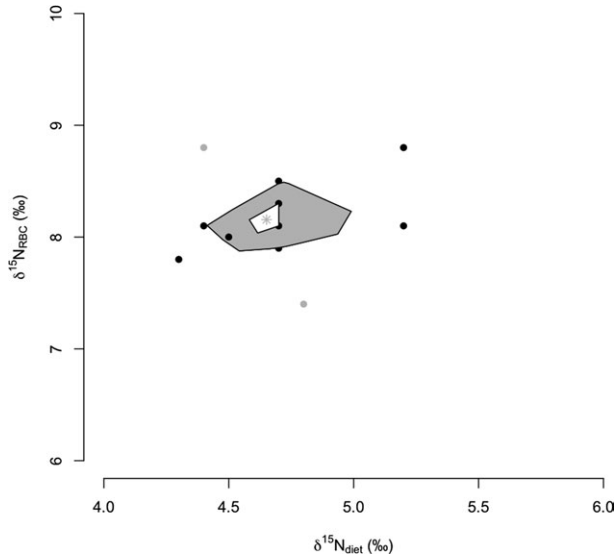


Fig. 1. Nitrogen isotopic values of red blood cells and study diets for each subject. Data for the final blood sample and the median diet for each individual are shown as a bagplot (Rousseeuw et al., 1999): 50% of samples are within the gray area, the median is marked as a star, the central white region is a 95% confidence region for the depth median of the group, and the two identified outliers are shown in gray.

0.73, $P = 0.02$; $\rho = 0.62$, $P = 0.04$; $\rho = -0.29$, $P = 0.38$). If we exclude those who are obese ($BMI > 30$), all three correlations are non-significant, thus it is possible that the two obese subjects skew the data. For the nine subjects with $BMI < 30$ (nonobese) the mean $\Delta^{15}N_{\text{diet-RBC}}$ is $+3.2\%$, as compared with $+3.4\%$ for all 11 subjects, using the mean $\delta^{15}N$ values of diet and RBC.

DISCUSSION

The assumption underlying the premise of this study is that the controlled diet consumed by subjects over the 30-day study was isotopically similar to their habitual diets. The study for which these samples were collected was not designed as an isotopic study, so no consideration was made of isotopic variability in foods. However, the diets were carefully designed so as to match the composition of habitual diets, including the matching of brands consumed. A small but significant average decrease of 0.1% in $\delta^{15}N_{\text{RBC}}$ suggests that the study diets were not isotopically identical to habitual diets (bearing in mind that each subject's study diet was specific to them, so some may have been different and others not). Red blood cells have a mean in vivo life span of 120 days (Landaw, 1991), so a median change of -0.1% in $\delta^{15}N_{\text{RBC}}$ over the duration of the 30-day study suggests that there could be a median difference of -0.4% over 120 days. Thus the measured $\delta^{15}N_{\text{RBC}}$ of bloods taken at the end of the study may be an overestimate by $+0.3\%$ compared with that which would be measured if the subjects continued on the controlled diets for several months. Therefore we suggest that the range of $\Delta^{15}N_{\text{diet-RBC}}$ values that we derive, of $+3.3$ to $+3.6\%$ (Table 2), should be expanded to be $+3.0$ – 3.6% , but that $\Delta^{15}N_{\text{diet-RBC}}$ is highly likely to be larger than $+3\%$. For the further discussion in this paper, we use the value of $+3.5\%$, based on the most parsimonious

value of $\Delta^{15}N_{\text{diet-RBC}}$, with the recognition that it may be a slight overestimate.

Studies have shown that isotopic differences between diet and animal tissues can vary under different conditions (e.g., Ambrose and DeNiro, 1986; Heaton et al., 1986; Sealy et al., 1987; Hobson and Clark, 1992; Hobson et al., 1993; Gröcke et al., 1997), and that human nitrogen isotopic values vary under different conditions, including pregnancy, growth, illness and pathology (e.g., Katzenberg and Lovell, 1999; Fuller et al., 2004; Fuller et al., 2005; Mekota et al., 2006; Waters-Rist and Katzenberg, 2010). Thus it is likely that the offset measured here will not be universally constant for all humans on all diets. However, this is the first quantified isotopic study of the diet to body enrichment in humans on controlled diets, and therefore gives an indication of the magnitude of the offset that we can expect. We found no effect of sex or age on $\Delta^{15}N_{\text{diet-body}}$ offset in these subjects. The observed positive correlation with BMI, driven by the two obese subjects, is intriguing and requires further investigation: the possibility of an effect of differential bioavailability of nutrients and differential uptake between individuals may be a factor here, and one that should be considered further.

Offsets from diet to keratin and collagen

To be able to use this measured diet-body offset for humans in palaeodietary studies, we must estimate what it equates to in terms of tissues analyzed in other studies, such as keratin or collagen. We can combine our data with that of three other studies, all on North American residents, to derive a value for $\Delta^{15}N_{\text{diet-keratin}}$ (Table 3). Nash et al. (2009) showed a mean increase of $+1.5 \pm 0.6\%$ from RBCs to hair keratin. Kraft et al. (2008) showed that blood plasma has a higher $\delta^{15}N$ than red blood cells by 1.5% on average. Schoeller et al. (1986) showed a mean increase of $+0.3 \pm 0.7\%$ from plasma protein to hair keratin. Combining the plasma/RBC/keratin results from these two latter studies, we get an estimated offset of $+1.8\%$ from RBCs to hair keratin, in fairly good agreement with the value of $+1.5\%$ observed by Nash et al. Our measured $\Delta^{15}N_{\text{diet-RBC}}$ value of $+3.5\%$ equates to a $\Delta^{15}N_{\text{diet-keratin}}$ of $\approx +5.0\%$ using the Nash offset, and to $\approx +5.3\%$ using the Kraft-Schoeller combined offset (no errors propagated).

Our derived $\Delta^{15}N_{\text{diet-keratin}}$ value can be compared to estimates from two studies specifically examining the offset from diet to hair keratin, based on estimates of dietary intake combined with food and hair isotopic analysis (Table 3). Yoshinaga et al. (1996) analyzed 49 males in Papua New Guinea, in the period 1980–1982. Through food consumption surveys, food isotopic analysis, and hair isotopic analysis, they derived an estimated value of $+5.0$ – 6.9% for $\Delta^{15}N_{\text{diet-keratin}}$ based on a calculated diet for each individual. Hedges et al. (2009) analyzed 20 females in Fiji sampled in 1999. Through diet diaries, food isotopic analysis, and hair isotopic analysis, they derived an estimated value of $+4.1 \pm 0.7\%$ for $\Delta^{15}N_{\text{diet-keratin}}$ based on a calculated diet for each individual. Our measured data with a combination of the Nash-Jahren-Schoeller offsets gives an estimate of $\Delta^{15}N_{\text{diet-keratin}}$ of $+5.0$ – 5.3% , which falls between the estimated values from Yoshinaga and Hedges. Studies estimating dietary intake at the population level have estimated a $\Delta^{15}N_{\text{diet-keratin}}$ of ca. $+4.3\%$ (Minagawa et al., 1986; Schoeller et al., 1986).

TABLE 4. Nitrogen isotopic offsets between diet, blood and other tissues in published controlled mammal feeding studies

Reference	Species common name	$\Delta^{15}\text{N}_{\text{diet-tissue}}$ (‰)					
		Liver	Muscle	Hair	Whole Blood	RBC	Plasma
Nakagawa et al., 1985	Rats	2.6		1.6	1.5		
Arneson et al., 2005	Mice	4.3	3.1		3.2		
Arneson et al., 2005	Mice	4.7	3.1		3.2		
Arneson et al., 2005	Mice	3.8	2.0		2.9		
Hobson et al., 1996	Harp, Harbor, Ringed seals	3.1	2.4	3.0		1.7	
Kurle, 2002 ^a	Northern fur seals					3.9	5.2
Lesage et al., 2002	Gray, Harbor, Harp seals					1.7	3.1 ^b
Lesage et al., 2002	Harp seals					2.0	3.6
Roth and Hobson, 2000	Red fox	3.6	3.6	3.4		2.6	4.2
Yoneyama et al., 1983	Rats	3.4	3.1			2.0	3.9

^a Data from 5 seals, excluding that from pregnant/lactating Baabs.

^b Data measured on serum, not plasma.

will lead to an overestimation of the dietary importance of foods with higher nitrogen isotopic values, usually higher trophic level foods such as meat, milk and fish. As Hedges and Reynard (2007) note, using a $\Delta^{15}\text{N}_{\text{diet-collagen}}$ value of 3–4‰ produces an estimate of dietary animal protein percentage (as a proportion of total protein intake) of 60% and sometimes up to 80% for prehistoric farmers in Europe, which is greater than animal protein dietary fraction of modern “developed” countries and twice that of modern “developing” countries (Sluijs et al.; Frassetto et al., 2000; FAOSTAT, 2012), as well as being in excess of that consumed by most ethnographically documented hunter-gatherer populations (Cordain et al., 2000). If a value of +6‰ were used as $\Delta^{15}\text{N}_{\text{diet-collagen}}$ offset, this would typically reduce the dietary animal protein intake estimate by about a third to a half, bringing such estimates for prehistoric farmers in line with dietary animal/plant protein ratios in living horticultural/agricultural populations (Yoshinaga et al., 1996; Frassetto et al., 2000; MacIntyre et al., 2002; Muhammad-Lawal and Balogun, 2007; Hedges et al., 2009; Iyagbe and Orewa, 2009; Baroudi et al., 2010).

CONCLUSIONS

In 11 subjects consuming their habitual diets under controlled conditions, we have measured the $\Delta^{15}\text{N}_{\text{diet-RBC}}$ as +3.5‰. This is the first study to measure the $\Delta^{15}\text{N}_{\text{diet-body}}$ offset in humans on controlled diets of known isotopic composition. Using measured offsets from other studies, we estimate the human $\Delta^{15}\text{N}_{\text{diet-keratin}}$ as +5.0–5.3‰, which is in good agreement with estimates derived from the two other studies using individual diet records (Yoshinaga et al., 1996; Hedges et al., 2009). We also derive a value for $\Delta^{15}\text{N}_{\text{diet-collagen}}$ of \approx 6‰, larger than usually assumed in palaeodietary literature. This larger value goes some way to resolving the conundrum of interpretations of very high animal protein intake in isotopic studies of prehistoric farmers—we suggest that this has often been overestimated. We advocate that dietary interpretations of previously published archaeological human isotopic data are reconsidered in the light of our work.

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LITERATURE CITED

- Ambrose SH. 2000. Controlled diet and climate experiments on nitrogen isotope ratios of rats. In: Ambrose SH, Katzenberg MA, editors. Biogeochemical approaches to paleodietary analysis. New York: Kluwer Academic/Plenum. p 243–259.
- Ambrose SH, DeNiro MJ. 1986. The isotopic ecology of East African mammals. *Oecologia* 69:395–406.
- Arneson LS, MacAvoy SE. 2005. Carbon, nitrogen, and sulfur diet-tissue discrimination in mouse tissues. *Can J Zool* 83:989–995.
- Ayliffe LK, Cerling TE, Robinson T, West AG, Sponheimer M, Passey BH, Hammer J, Roeder B, Dearing MD, Ehleringer JR. 2004. Turnover of carbon isotopes in tail hair and breath CO₂ of horses fed an isotopically varied diet. *Oecologia* 139:11–22.
- Baroudi T, Ben Maiz H, Abid HK, Benammar-Elgaied A, Alouane LT. 2010. Dietary intakes of essential nutrients among Arab and Berber ethnic groups on rural Tunisian island. *Nutrition* 26:75–81.
- Bingham SA, Cummings JH. 1985. Urine nitrogen as an independent validity measure of dietary-intake: a study of nitrogen-balance in individuals consuming their normal diet. *Am J Clin Nutr* 42:1276–1289.
- Bocherens H, Drucker D. 2003. Trophic level isotopic enrichment of carbon and nitrogen in bone collagen: case studies from recent and ancient terrestrial ecosystems. *Int J Osteoarchaeol* 13:46–53.
- Bol R, Pflieger C. 2002. Stable isotope (C-13, N-15 and S-34) analysis of the hair of modern humans and their domestic animals. *Rapid Commun Mass Spectrom* 16:2195–2200.
- Bonsall JC, Lennon RJ, McSweeney K, Stewart C, Harkness DD, Boroneant V, Bartosiewicz L, Payton R, Chapman J. 1997. Mesolithic and Early Neolithic in the Iron Gates: a palaeodietary perspective. *J Eur Archaeol* 5:50–92.
- Buikstra JE, Milner GR. 1991. Isotopic and archaeological interpretations of diet in the Central Mississippi Valley. *J Archaeol Sci* 18:319–329.
- Caut S, Angulo E, Courchamp F. 2008. Discrimination factors (Delta N-15 and Delta C-13) in an omnivorous consumer: effect of diet isotopic ratio. *Funct Ecol* 22:255–263.
- Caut S, Angulo E, Courchamp F. 2009. Variation in discrimination factors (Delta(15)N and Delta(13)C): the effect of diet isotopic values and applications for diet reconstruction. *J Appl Ecol* 46:443–453.
- Cordain L, Miller JB, Eaton SB, Mann N, Holt SHA, Speth JD. 2000. Plant-animal subsistence ratios and macronutrient energy estimations in worldwide hunter-gatherer diets. *Am J Clin Nutr* 71:682–692.

- DeNiro MJ, Epstein S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta* 45:341–351.
- FAOSTAT. 2012. Food Balance Sheets. FAO.
- Faul F, Erdfelder E, Buchner A, Lang A-G. 2009. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav Res Methods* 41:1149–1160.
- Felicetti LA, Schwartz CC, Rye RO, Haroldson MA, Gunther KA, Phillips DL, Robbins CT. 2003. Use of sulfur and nitrogen stable isotopes to determine the importance of whitebark pine nuts to Yellowstone grizzly bears. *Can J Zool* 81:763–770.
- Frassetto LA, Todd KM, Morris RC, Sebastian A. 2000. Worldwide incidence of hip fracture in elderly women: relation to consumption of animal and vegetable foods. *J Gerontol A Biol Sci Med Sci* 55:M585–M592.
- Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, Hedges REM. 2004. Nitrogen balance and delta N-15: why you're not what you eat during pregnancy. *Rapid Commun Mass Spectrom* 18:2889–2896.
- Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, Hedges REM. 2005. Nitrogen balance and ¹⁵N: why you're not what you eat during nutritional stress. *Rapid Commun Mass Spectrom* 19:2497–2506.
- Grücke DR, Bocherens H, Mariotti A. 1997. Annual rainfall and nitrogen-isotope correlation in macropod collagen: application as a palaeoprecipitation indicator. *Earth Planet Sci Lett* 153:279–285.
- Hare PE, Fogel ML, Stafford TW Jr, Mitchell AD, Hoering TC. 1991. The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. *J Archaeol Sci* 18:277–292.
- Heaton THE, Vogel JC, von la Chevallerie G, Collett G. 1986. Climatic influence on the isotopic composition of bone collagen. *Nature* 322:822–823.
- Hedges R, Rush E, Aalbersberg W. 2009. Correspondence between human diet, body composition and stable isotopic composition of hair and breath in Fijian villagers. *Isotopes Environ Health Stud* 45:1–17.
- Hedges REM, Clement JG, Thomas CDL, O'Connell TC. 2007. Collagen turnover in the adult femoral mid-shaft: modeled from anthropogenic radiocarbon tracer measurements. *Am J Phys Anthropol* 133:808–816.
- Hedges REM, Reynard LM. 2007. Nitrogen isotopes and the trophic level of humans in archaeology. *J Archaeol Sci* 34:1240–1251.
- Hilderbrand GV, Farley SD, Robbins CT, Hanley TA, Titus K, Servheen C. 1996. Use of stable isotopes to determine diets of living and extinct bears. *Can J Zool* 74:2080–2088.
- Hobson KA, Alisauskas RT, Clark RG. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. *Condor* 95:388–394.
- Hobson KA, Clark RG. 1992. Assessing avian diets using stable isotopes. II. Factors influencing diet-tissue fractionation. *The Condor* 94:189–197.
- Hobson KA, Schell DM, Renouf D, Noseworthy E. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Can J Aquatic Sci* 53:528–533.
- Hoefs J. 1997. *Stable isotope geochemistry*. Berlin: Springer.
- Huelsemann F, Flenker U, Koehler K, Schaezner W. 2009. Effect of a controlled dietary change on carbon and nitrogen stable isotope ratios of human hair. *Rapid Commun Mass Spectrom* 23:2448–2454.
- Iyangbe CO, Orewa SI. 2009. Determinants of daily protein intake among rural and low-income urban households in Nigeria. *Am-Eurasian J Sci Res* 4:290–301.
- Jenkins S, Partridge S. 2001. Nitrogen and carbon isotope fractionation between mothers, neonates, and nursing offspring. *Oecologia* 129:336–341.
- Jones RJ, Ludlow MM, Troughton JH, Blunt CG. 1981. Changes in natural carbon isotope ratio of the hair from steers fed diets of C₄, C₃ and C₄ species in sequence. *Search* 12:85–87.
- Katzenberg MA, Lovell NC. 1999. Stable isotope variation in pathological bone. *Int J Osteoarchaeol* 9:316–324.
- Kuhnle GGC, Joosen AMCP, Kneale CJ, O'Connell TC. In press. Carbon and nitrogen isotopic ratios of urine and faeces as novel nutritional biomarkers of meat and fish intake. *Eur J Nutr*. PMID: 22406837
- Kurle CM. 2002. Stable-isotope ratios of blood components from captive northern fur seals (*Callorhinus ursinus*) and their diet: applications for studying the foraging ecology of wild otariids. *Can J Zool* 80:902–909.
- Landaw SA. 1991. Homeostasis, survival, and red cell kinetics: measurement and imaging of red cell production. In: Hoffman R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, editors. *Hematology: basic principles and practice*. New York: Churchill Livingstone. p 274–290.
- Lubell D, Jackes M, Schwarcz H, Knyf M, Meiklejohn C. 1994. The Mesolithic-Neolithic transition in Portugal: isotopic and dental evidence of diet. *J Archaeol Sci* 21:201–216.
- MacIntyre UE, Kruger HS, Venter CS, Vorster HH. 2002. Dietary intakes of an African population in different stages of transition in the North West Province, South Africa: the THUSA study. *Nutr Res* 22:239–256.
- McCue MD, Pollock ED. 2008. Stable isotopes may provide evidence for starvation in reptiles. *Rapid Commun Mass Spectrom* 22:2307–2314.
- McCutchan JH Jr, Lewis WM Jr, Kendall C, McGrath CC. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102:378–390.
- Mekota AM, Grupe G, Ufer S, Cuntz U. 2006. Serial analysis of stable nitrogen and carbon isotopes in hair: monitoring starvation and recovery phases of patients suffering from anorexia nervosa. *Rapid Commun Mass Spectrom* 20:1604–1610.
- Minagawa M. 1992. Reconstruction of human diet from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in contemporary Japanese hair: a stochastic method for estimating multi-source contribution by double isotopic tracers. *Appl Geochem* 7:145–158.
- Minagawa M, Karasawa K, Kabaya Y. 1986. Carbon and nitrogen abundances in human feeding ecosystem. *Chikyū-kagaku (Geochemistry)* 20:79–88.
- Minagawa M, Wada E. 1984. Stepwise enrichment of ¹⁵N along food chains: further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochim Cosmochim Acta* 48:1135–1140.
- Miron MLL, Herrera MLG, Ramirez PN, Hobson KA. 2006. Effect of diet quality on carbon and nitrogen turnover and isotopic discrimination in blood of a New World nectarivorous bat. *J Exp Biol* 209:541–548.
- Muhammad-Lawal A, Balogun G. 2007. Animal protein consumption among rural households in Kwara State, Nigeria. *Afr J Gen Agr* 3:21–27.
- Lesage V, Hammill MO, Kovacs KM. 2002. Diet-tissue fractionation of stable carbon and nitrogen isotopes in phocid seals. *Marine Mamm Sci* 18:182–193.
- Nakagawa A, Kitagawa A, Asami M, Nakamura K, Schoeller DA, Slater R, Minagawa M, Kaplan IR. 1985. Evaluation of isotope ratio (IR) mass spectrometry for the study of drug metabolism. *Biomed Mass Spectrom* 12:502–506.
- Nash SH, Bersamin A, Kristal AR, Hopkins SE, Church RS, Pasker RL, Luick BR, Mohatt GV, Boyer BB, O'Brien DM. 2012. Stable nitrogen and carbon isotope ratios indicate traditional and market food intake in an indigenous circumpolar population. *J Nutr* 142:84–90.
- O'Brien DM, Kristal AR, Jeannet MA, Wilkinson MJ, Bersamin A, Luick B. 2009. Red blood cell delta N-15: a novel biomarker of dietary eicosapentaenoic acid and docosahexaenoic acid intake. *Am J Clin Nutr* 89:913–919.
- O'Connell TC, Healey MA, Hedges REM, Simpson AHW. 2001. Isotopic comparison of hair, bone and nail: modern analyses. *J Archaeol Sci* 28:1247–1255.
- O'Connell TC, Hedges REM. 1999a. Investigations into the effect of diet on modern human hair isotopic values. *Am J Phys Anthropol* 108:409–425.
- O'Connell TC, Hedges REM. 1999b. Isotopic comparison of hair and bone: archaeological analyses. *J Archaeol Sci* 26:661–665.
- Petzke KJ, Boeing H, Klaus S, Metges CC. 2005a. Carbon and nitrogen stable isotopic composition of hair protein and amino

- acids can be used as biomarkers for animal-derived dietary protein intake in humans. *J Nutr* 135:1515–1520.
- Petzke KJ, Boeing H, Metges CC. 2005b. Choice of dietary protein of vegetarians and omnivores is reflected in their hair protein C-13 and N-15 abundance. *Rapid Commun Mass Spectrom* 19:1392–1400.
- Petzke KJ, Lemke S. 2009. Hair protein and amino acid C-13 and N-15 abundances take more than 4 weeks to clearly prove influences of animal protein intake in young women with a habitual daily protein consumption of more than 1 g per kg body weight. *Rapid Commun Mass Spectrom* 23:2411–2420.
- Post DM. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83:703–718.
- Richards MP. 2006. Palaeodietary reconstruction. In: Brickley M, Buteux S, Adams J, Cherrington R, editors. *St Martin's uncovered: investigations in the churchyard of St Martin's-in-the-Bull Ring, Birmingham, 2001*. Oxford: Oxbow Books. p 147–151.
- Richards MP, Schulting RJ, Hedges REM. 2003. Sharp shift in diet at onset of Neolithic. *Nature* 425:366–366.
- Robbins CT, Felicetti LA, Sponheimer M. 2005. The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia* 144:534–540.
- Roth JD, Hobson KA. 2000. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction. *Can J Zool* 78:848–852.
- Rousseeuw PJ, Ruts I, Tukey JW. 1999. The bagplot: a bivariate boxplot. *Am Stat* 53:382–387.
- Schoeller DA, Minagawa M, Slater R, Kaplan IR. 1986. Stable isotopes of carbon, nitrogen and hydrogen in the contemporary North American human food web. *Ecol Food Nutr* 18:159–170.
- Schoeninger MJ, DeNiro MJ. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochim Cosmochim Acta* 48:625–639.
- Sealy JC, van der Merwe NJ, Lee-Thorp JA, Lanham JL. 1987. Nitrogen isotopic ecology in Southern Africa: implications for environmental and dietary tracing. *Geochim Cosmochim Acta* 51:2707–2717.
- Sluijs I, Beulens JWW, van der A DL, Spijkerman AMW, Grobbee DE, van der Schouw YT. Dietary intake of total, animal, and vegetable protein and risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-NL Study. *Diabetes Care* 33:43–48.
- Sponheimer M, Robinson T, Ayliffe L, Roeder B, Hammer J, Passey B, West A, Cerling T, Dearing D, Ehleringer J. 2003. Nitrogen isotopes in mammalian herbivores: hair $\delta^{15}\text{N}$ values from a controlled feeding study. *Int J Osteoarchaeol* 13:80–87.
- Tasevska N, Runswick SA, Bingham SA. 2006. Urinary potassium is as reliable as urinary nitrogen for use as a recovery biomarker in dietary studies of free living individuals. *J Nutr* 136:1334–1340.
- Tasevska N, Runswick SA, McTaggart A, Bingham SA. 2005. Urinary sucrose and fructose as biomarkers for sugar consumption. *Cancer Epidemiol Biomarkers Prev* 14:1287–1294.
- Tauber H. 1981. ^{13}C evidence for dietary habits of prehistoric man in Denmark. *Nature* 292:332–333.
- Team RDC. 2009. R: A language and environment for statistical computing. Vienna, Austria.
- Thompson AH, Chesson LA, Podlesak DW, Bowen GJ, Cerling TE, Ehleringer JR. 2011. Stable isotope analysis of modern human hair collected from Asia (China, India, Mongolia, and Pakistan). *Am J Phys Anthropol* 141:440–451.
- Tieszen LL, Boutton TW, Tesdahl KG, Slade NA. 1983. Fractionation and turnover of stable isotopes in animal tissues: implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia* 57:32–37.
- Valenzuela LO, Chesson LA, O'Grady SP, Cerling TE, Ehleringer JR. 2011. Spatial distributions of carbon, nitrogen and sulfur isotope ratios in human hair across the central United States. *Rapid Commun Mass Spectrom* 25:861–868.
- Vanderklift MA, Ponsard S. 2003. Sources of variation in consumer-diet delta N-15 enrichment: a meta-analysis. *Oecologia* 136:169–182.
- Vogel JC, van der Merwe NJ. 1977. Isotopic evidence for early maize cultivation in New York State. *Am Antiq* 42:238–242.
- Wareham NJ, Jakes RW, Rennie KL, Schuit J, Mitchell J, Hennings S, Day NE. 2003. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutr* 6:407–413.
- Waters-Rist AL, Katzenberg MA. 2010. The effect of growth on stable nitrogen isotope ratios in subadult bone collagen. *Int J Osteoarchaeol* 20:172–191.
- Welch AA, McTaggart A, Mulligan AA, Luben R, Walker N, Khaw KT, Day NE, Bingham SA. 2001. DINER (Data Into Nutrients for Epidemiological Research): a new data-entry program for nutritional analysis in the EPIC-Norfolk cohort and the 7-day diary method. *Public Health Nutr* 4:1253–1265.
- Williams LJ, White CD, Longstaffe FJ. 2011. Improving stable isotopic interpretations made from human hair through reduction of growth cycle error. *Am J Phys Anthropol* 145:125–136.
- Yoneyama T, Ohta Y, Ohtani T. 1983. Variations of natural ^{13}C and ^{15}N abundances in the rat tissues and their correlation. *Radioisotopes* 32:330–332.
- Yoshinaga J, Minagawa M, Suzuki T, Ohtsuka R, Kawabe T, Inaoka T, Akimichi T. 1996. Stable carbon and nitrogen isotopic composition of diet and hair of Gidran-speaking Papuans. *Am J Phys Anthropol* 100:23–34.