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

T.C. O’Connell,1,2* C.J. Kneale,2 N. Tasevska,3 and G.G.C. Kuhnle4,5

1Department of Archaeology and Anthropology, University of Cambridge, UK
2McDonald Institute for Archaeological Research, University of Cambridge, UK
3MRC Dunn Human Nutrition Unit, Wellcome Trust/MRC, Building, Cambridge, UK
4Department of Food and Nutritional Sciences, University of Reading, UK
5Department of Public Health and Primary Care, MRC Centre for Nutritional Epidemiology in Cancer Prevention and Survival, University of Cambridge, UK

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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 $\Delta^{15}$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 isotopic changes in the body pool. The $\Delta^{15}$N$_{\text{diet-RBC}}$ was measured as $+3.5\%$. Using measured offsets from other studies, we estimate the human $\Delta^{15}$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 $\Delta^{15}$N$_{\text{diet-collagen}}$ of $\approx 6\%$, again in combination with measured offsets from other studies. This value is larger than usually assumed in palaeodiets, 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 $\Delta^{15}$N$_{\text{diet-body}}$), resulting in an increase in $\delta^{15}$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 $\approx +3$–$4\%$, 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 $\Delta^{15}$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 $\Delta^{15}$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 $\Delta^{15}$N$_{\text{diet-body}}$ value initially taken

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*Correspondence to: T.C. O’Connell, McDonald Institute for Archaeological Research, Downing St, Cambridge, CB4 3DZ, UK.
E-mail: tco21@cam.ac.uk

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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 $\Delta^{15}N_{\text{diet-body}}$ 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 $\Delta^{15}N_{\text{diet-body}}$. 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 Pfieger, 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; Hulsemann 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 $\Delta^{15}N_{\text{diet-body}}$ the tissues usually of interest (e.g., bone collagen, hair keratin, blood proteins) isotope 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; Hulsemann 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 Tasevská 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 (HbA1c) was undertaken and all subjects were within the normal range (fasting plasma glucose <6.1 mmol/l, HbA1c <8%). 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) | Weighted Std dev | Median | Mean | IQR (Mean) | Mean | Median | IQR (Mean) | Weighted Std dev | Median | Mean | IQR (Mean) | Mean | Median | IQR (Mean) |
|---------|-----|-----|---------|------------------|--------|------|-----------|------|--------|-----------|------------------|--------|------|-----------|------|--------|-----------|
| V1      | M   | 27.9| 52      | 10.9             | 23.9   | 15.5 | 27.6      | 15.4 | 18.2   | 26.5      | 15.4             | 27.5   | 15.4 | 26.5      | 15.4 | 18.2   | 23.9      |
| V2      | F   | 27.5| 46      | 10.2             | 23.3   | 15.5 | 27.6      | 15.4 | 18.2   | 23.3      | 15.4             | 27.3   | 15.4 | 21.3      | 15.4 | 18.2   | 18.2      |
| V3      | M   | 27.5| 64      | 10.2             | 23.3   | 15.5 | 27.6      | 15.4 | 18.2   | 23.3      | 15.4             | 27.3   | 15.4 | 21.3      | 15.4 | 18.2   | 18.2      |
| V4      | M   | 27.5| 21.7   | 10.2             | 23.3   | 15.5 | 27.6      | 15.4 | 18.2   | 23.3      | 15.4             | 27.3   | 15.4 | 21.3      | 15.4 | 18.2   | 18.2      |
| V5      | F   | 27.5| 63      | 10.2             | 23.3   | 15.5 | 27.6      | 15.4 | 18.2   | 23.3      | 15.4             | 27.3   | 15.4 | 21.3      | 15.4 | 18.2   | 18.2      |
| V6      | M   | 27.5| 31.9   | 10.2             | 23.3   | 15.5 | 27.6      | 15.4 | 18.2   | 23.3      | 15.4             | 27.3   | 15.4 | 21.3      | 15.4 | 18.2   | 18.2      |
| V7      | M   | 27.5| 31.9   | 10.2             | 23.3   | 15.5 | 27.6      | 15.4 | 18.2   | 23.3      | 15.4             | 27.3   | 15.4 | 21.3      | 15.4 | 18.2   | 18.2      |
| V8      | F   | 27.5| 31.9   | 10.2             | 23.3   | 15.5 | 27.6      | 15.4 | 18.2   | 23.3      | 15.4             | 27.3   | 15.4 | 21.3      | 15.4 | 18.2   | 18.2      |
| V9      | M   | 27.5| 31.9   | 10.2             | 23.3   | 15.5 | 27.6      | 15.4 | 18.2   | 23.3      | 15.4             | 27.3   | 15.4 | 21.3      | 15.4 | 18.2   | 18.2      |
| V10     | F   | 27.5| 31.9   | 10.2             | 23.3   | 15.5 | 27.6      | 15.4 | 18.2   | 23.3      | 15.4             | 27.3   | 15.4 | 21.3      | 15.4 | 18.2   | 18.2      |

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 a 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 δ15N values in parts per 1000 or “permil” (‰) values, where δ15N = ([15N/14N sample/15N/14N standard] − 1) × 1000. Based on replicate analyses of international and laboratory standards, measurement errors are less than ±0.2‰ for δ15N.

**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 δ15N between diet and blood;
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-β) of 0.9 at a significance level of α = 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 ± 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 ±25E 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%o (range in subject medians of 4.3–5.2%). The mean diet nitrogen isotopic value for all subjects was 4.8 ± 0.4%o (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 δ15N and the mean δ15N of each subject’s diets weighted by the nitrogen contribution from each day’s diet was less than 0.1%o, and for two individuals, the difference was less than 0.2%o; 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 δ15N, although this correlation was only marginally significant (Spearman rank correlation: ρ = −0.59, P = 0.05, and ρ = 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%o at the end of the study. The median δ15N_RBC for all subjects was 8.2%o (IQR = 7.9–8.6%)o at the start of the study, and 8.1%o (IQR = 8.0–8.4%)o at the end of the study; the mean δ15N_RBC for all subjects was 8.3 ± 0.5%o at the start of the study, 8.2 ± 0.4%o at the end of the study, and 8.2 ± 0.4%o for the two values averaged. Comparison of the δ15N_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 δ15N (δ15N_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 Δ15N_diet-RBC is between 2.7 and 4.4%o, whichever way is used, and the average Δ15N_diet-RBC for the group is between +3.3 and +3.6%, with the statistically most parsimonious value (using the final blood sample δ15N_RBC and the median diet δ15N) of +3.5%o (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, Δ15N_diet-RBC and δ15N_RBC—but not δ15N_diet—correlated significantly with BMI (Spearman rank correlations, respectively: ρ =

| TABLE 2. The Δ15N_diet-RBC of the population calculated in different ways, using the mean and median measures of the subjects' nitrogen isotopic values |
|---|---|---|---|---|---|---|---|
| Subject | Arith mean Δ15N_diet (%o) | Median Δ15N_diet (%o) | Blood 2 Δ15N_RBC (%o) | Mean Δ15N_RBC (%o) | Δ15N_diet-RBC (mean blood - mean diet) (%o) | Δ15N_diet-RBC (blood 2 - mean diet) (%o) | Δ15N_diet-RBC (mean blood - median diet) (%o) |
| V1 | 5.5 | 5.2 | 8.8 | 8.9 | 3.3 | 3.3 | 3.7 |
| V2 | 4.5 | 4.8 | 7.4 | 7.5 | 3.0 | 2.9 | 2.7 |
| V5 | 4.9 | 4.7 | 8.3 | 8.4 | 3.4 | 3.3 | 3.7 |
| V6 | 5.1 | 4.7 | 7.9 | 7.8 | 2.7 | 2.7 | 3.1 |
| V7 | 4.4 | 4.5 | 8.0 | 8.0 | 3.7 | 3.6 | 3.5 |
| V8 | 5.0 | 4.4 | 8.1 | 8.2 | 3.2 | 3.1 | 3.7 |
| V9 | 4.4 | 4.3 | 7.8 | 7.8 | 3.5 | 3.5 | 3.5 |
| V10 | 4.4 | 4.4 | 8.8 | 8.9 | 4.4 | 4.4 | 4.4 |
| V11 | 4.9 | 4.7 | 8.5 | 8.6 | 3.7 | 3.7 | 3.9 |
| V12 | 4.7 | 4.7 | 8.1 | 8.1 | 3.4 | 3.4 | 3.4 |
| V13 | 5.4 | 5.2 | 8.1 | 8.1 | 2.8 | 2.8 | 2.9 |
| Mean | 4.8 | 4.8 | 8.2 | 8.2 | 3.4 | 3.3 | 3.5 |
| Stdev | 0.4 | 0.4 | 0.4 | 0.4 | 0.5 | 0.5 | 0.5 |
| Median | 4.9 | 4.7 | 8.1 | 8.1 | 3.4 | 3.3 | 3.6 |
| 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 |
| Max | 5.5 | 5.2 | 8.8 | 8.9 | 4.4 | 4.4 | 4.4 |
| Min | 4.4 | 4.3 | 7.4 | 7.5 | 2.7 | 2.7 | 2.7 |
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 δ¹⁵N_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 δ¹⁵N_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 δ¹⁵N_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 Δ¹⁵N_diet-RBC values that we derive, of +3.3 to +3.6% (Table 2), should be expanded to +3.0–3.6‰, but that Δ¹⁵N_diet 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 Δ¹⁵N_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 Δ¹⁵N_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 Δ¹⁵N_diet-keratin (Table 3). Nash et al. (2009) showed a mean increase of +1.5 ± 0.6% from RBCs to hair keratin. Kraft et al. (2008) showed that blood plasma has a higher Δ¹⁵N than red blood cells by 1.5‰ on average. Schoeller et al. (1986) showed a mean increase of +0.3 ± 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 Δ¹⁵N_diet-RBC value of +3.5‰ equates to a Δ¹⁵N_diet-keratin of +5.0‰ using the Nash offset, and to +5.3‰ using the Kraft-Schoeller combined offset (no errors propagated).

Our derived Δ¹⁵N_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 Δ¹⁵N_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 ± 0.7‰ for Δ¹⁵N_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 Δ¹⁵N_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 Δ¹⁵N_diet-keratin of ca. +4.3‰ (Minagawa et al., 1986; Schoeller et al., 1986).
To consider how our data would translate to a $\Delta^{15}N_{\text{diet-collagen}}$ offset, we must then consider the offset between human hair keratin and bone collagen. Three published studies have measured this in humans, one in a modern population ($+0.9 \pm 0.2\%$: O’Connell et al., 2001) and two in archaeological individuals ($+1.0 \pm 1.1\%$: O’Connell and Hedges, 1999b; $+1.0 \pm 1.4\%$: Richards, 2006) (Table 3). There are problems in using such data (such as the small sample sizes and the large standard deviations in the two archaeological studies) but it is noteworthy that all studies have similar mean offsets for the $\Delta^{15}N_{\text{keratin-collagen}}$ offset. Adding these corrections to the estimated $\Delta^{15}N_{\text{diet-keratin}}$ of $+5.0-5.3\%$ derived from our data and the offsets measured by Nash/Kraft/Schoeller et al., we derive a range of $+5.9-6.3\%$ for the $\Delta^{15}N_{\text{diet-collagen}}$ offset (again no errors propagated).

As we discuss earlier, the measured $\delta^{15}N_{\text{RBC}}$ may be an overestimate, and thus the derived values of $\Delta^{15}N_{\text{diet-keratin}}$ and $\Delta^{15}N_{\text{diet-collagen}}$ may also be overestimated. Possible problems with studies comparing keratin to diet include issues with growth cycle errors (Williams et al., 2011). Problems with studies comparing collagen and keratin include differential time periods represented in the two tissues (O’Connell et al., 2001; Hedges et al., 2007). However, even with a very conservative approach, assuming a $\Delta^{15}N_{\text{diet-RBC}}$ value of $+3\%$, and using minimum offset values to keratin (Nash study, $+0.9\%$, i.e., $1\sigma$ less than the mean), and to collagen (O’Connell 2001 modern study, $+0.7\%$, i.e., $1\sigma$ less than the mean), our results suggest a $\Delta^{15}N_{\text{diet-collagen}}$ offset of $+4.6\%$, which is at the upper end of the currently accepted range. These data suggest therefore a larger offset than commonly assumed.

We can place the limited human data in the context of that from other animal studies. All controlled feeding studies on animals so far have observed isotopic inhomogeneity in different tissues, and such isotopic differences can be substantial (Caut et al., 2009). Other mammalian studies have shown a similar pattern to that summarized above for humans: whole blood and red blood cells generally have low nitrogen isotopic values relative to other tissues, or at the low end of the range, and in comparisons of plasma and red blood cells, plasma always has a higher nitrogen isotopic value, often by more than 1\% (Table 4). As regards the magnitude of the offsets, similar values to our estimates are found for a range of species in the literature. A number of animal studies have found $\Delta^{15}N_{\text{diet-body}}$ differences of greater than 4\% for a variety of tissues (DeNiro and Epstein, 1981; Hilderbrand et al., 1996; Roth and Hobson, 2000; Sponheimer et al., 2003; Arneson and MacAvoy, 2005; Miron et al., 2006; Caut et al., 2008), and studies of goat, alpaca, seal and bear have shown differences larger than 5\%, up to 6.4\% (Kurle, 2002; Felicetti et al., 2003; Sponheimer et al., 2003).

**Implications of this study for palaeodietary work**

Overall, our data suggest that the $\Delta^{15}N_{\text{diet-collagen}}$ offset in this group is ca. $+6\%$, larger than that usually assumed in the archaeological literature, typically around $+3-5\%$ (Bocherens and Drucker, 2003). Using a very conservative approach to the data, the estimate is still ca. $+4.6\%$, at the upper end of the currently accepted range. Such an observation has implications for the interpretation of human palaeodiet from isotopic data: an underestimation of the $\Delta^{15}N_{\text{diet-collagen}}$ offset

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**Table 3.** Nitrogen isotopic values of tissues, diet and calculated diet-tissue offsets in published human studies (all given in units of \(\%\)).

| Population | Sex | Sex | Hair $\delta^{15}N$ | RBC $\delta^{15}N$ | Plasma $\delta^{15}N$ | Diet $\delta^{15}N$ | $\Delta^{15}N_{\text{diet-keratin}}$ | $\Delta^{15}N_{\text{diet-collagen}}$ |
|------------|-----|-----|-------------------|-----------------|-------------------|-----------------|-----------------|-----------------|
|             |     |     |                  |                 |                   |                 |                 |                 |
| Human       |     |     |                  |                 |                   |                 |                 |                 |
| Modern      | M   |     | 9.1 ± 0.5        | 8.9 ± 0.7       | 9.0 ± 0.6         | 9.4 ± 0.6       | 9.3 ± 0.7       | 9.3 ± 0.7       |
| Modern      | F   |     | 9.1 ± 0.5        | 8.9 ± 0.7       | 9.0 ± 0.6         | 9.4 ± 0.6       | 9.3 ± 0.7       | 9.3 ± 0.7       |
| Ancient     | M   |     |                  |                 |                   |                 |                 |                 |
| Ancient     | F   |     |                  |                 |                   |                 |                 |                 |
| Animal      |     |     |                  |                 |                   |                 |                 |                 |
|             |     |     |                  |                 |                   |                 |                 |                 |

**Notes:**
- Values calculated from the individual subject data, rather than the reported averages in Table 4 of the paper.
- Values taken from Table 3b of the paper, where the mean but no standard deviations are given.

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**American Journal of Physical Anthropology**
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 $\Delta^{15}N_{\text{diet-collagen}}$ 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.; Fras- setto 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 $\Delta^{15}N_{\text{diet-collagen}}$ 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; Fras- setto et al., 2000; Maclntyre et al., 2002; Muhammad- Lawal and Balogun, 2007; Hedges et al., 2009; Iyan- ghe and Orewa, 2009; Baroudi et al., 2010).

**CONCLUSIONS**

In 11 subjects consuming their habitual diets under controlled conditions, we have measured the $\Delta^{15}N_{\text{diet-RBC}}$ as +3.5%. This is the first study to measure the $\Delta^{15}N_{\text{diet-body}}$ offset in humans on controlled diets of known isotopic composition. Using measured offsets from other studies, we estimate the human $\Delta^{15}N_{\text{diet-keratin}}$ 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 $\Delta^{15}N_{\text{diet-collagen}}$ 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, MacAvey 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-Elgaaied 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 validatory 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, McSweeny K, Stewart C, Harkness DD, Boroneant V, Bartosiewicz L, Payton R, Chapman J. 1997. Mesolithic and Early Neolithic in the Iron Gates: a palaeodi- etary perspective. J Eur Archaeol 5:50–92.

Buikstra JE, Milner GR. 1991. Isotopic and archaeological interpre- tations of diet in the Central Mississippi Valley. J Archaeol Sci 18:319–329.

Caut S, Angulo E, Orellana R, Curchamp 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, Curchamp F. 2009. Variation in discrimination factors (Delta15N and Delta13C): the effect of diet iso- topic 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.

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

| Reference               | Species common name | Liver   | Muscle | Hair   | Whole Blood | RBC   | Plasma |
|-------------------------|---------------------|---------|--------|--------|-------------|-------|--------|
| Nakagawa et al., 1985   | Rats                | 2.6     | 1.6    | 3.2    |             |       |        |
| 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             | Northern fur seals  | 3.9     | 5.2    |        |             |       |        |
| Lesage et al., 2002     | Gray, Harbor, Harp seals | 3.0  | 1.7    | 3.1    | 1.7         |       |        |
| 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         |       |        |

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

*b Data measured on serum, not plasma.
DeNiro MJ, Epstein S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Act 45:341–351.

FAOSTAT. 2012. Food Balance Sheets. FAO.

Faul F, Erdferder 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.

Feliciotti 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.

Frascione LA, Trehub 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: what 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 19:2507–2506.

Groeke DR, Bocherens H, Mariotti A. 1997. Annual rainfall and nitrogen-isotope correlation in macropod collagen: application as a paleo precipitation indicator. Earth Planet Sci Lett 153:279–285.

Hare PE, Vogel 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, Bush E, Aalbersberg W. 2009. Correspondence between human diet, body composition and stable isotope 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.

Helderbrand 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.

Huebsemann F, Fenker U, Koehler K, Schaenzer 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.

Iyange 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 GCC, 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: 22046837.

Kurile 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 species. 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 Ed, Shattil SJ, Furie B, Cohen HJ, editors. Hematology: basic principles and practice. New York: Churchill Livingstone, p 274–290.

Lubell D, Jackes M, Schwarz H, Knuf 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 intake 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.

McLetchan 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 13C and 15N in contemporary Japanese hair: a stochastic method for estimating multi-source contribution by double isotopic tracers. Appl Geochim 7:145–158.

Minagawa M, Karasawa K, Kabaya Y. 1986. Carbon and nitrogen abundances in human feeding ecosystem. Chikyu-kagaku (Geochemistry) 20:79–88.

Minagawa M, Wada E. 1984. Stepwise enrichment of 15N along food chains: further evidence and the relation between 15N and animal age. Geochim Cosmochim Act 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 15N: a novel biomarker for estimating multi-source contribution by double isotopic tracers. J Nutr 140:861–867.

O’Connell TC, Healy 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.

Pezzino KJ, Boening HO, Klaus S, Metges CC. 2005a. Carbon and nitrogen stable isotope composition of hair protein and amino acids in natural and cultured eicosapentaenoic acid and docosahexaenoic acid intake. Am J Clin Nutr 89:913–919.

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.

R, Benz EJ, Shattil SJ, Furie B, Cohen HJ, editors. Hematology: basic principles and practice. New York: Churchill Livingstone, p 274–290.
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.

Schoeninger MJ, Busey 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 δ15N values from a controlled feeding study. Int J Osteoarchaeol 13:80–87.

Tasevska N, Runswick SA, Bingham SA. 2006. Urinary potassium 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.

Taubner H. 1981. 13C 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, Boutron TW, Tesdahl KG, Slade NA. 1983. Fractionation and turnover of stable isotopes in animal tissues: implications for δ13C 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.

Vanderkilt 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, Henning 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.

Watson-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 J, Minagawa M, Suzuki T, Ohtsuka R, Kawabe T, Iinoaki T, Akahama T, Ohtani T. 1983. Variations of natural 13C and 15N abundances in the rat tissues and their correlation. Radioisotopes 32:330–332.

Yoshinaga J, Minagawa M, Suzuki T, Ohtsuka R, Kawabe T, Iinoaki T, Akahama T, Ohtani T. 1983. Variations of natural 13C and 15N abundances in the rat tissues and their correlation. Radioisotopes 32:330–332.

Yoshinaga J, Minagawa M, Suzuki T, Ohtsuka R, Kawabe T, Iinoaki T, Akahama T, Ohtani T. 1983. Variations of natural 13C and 15N abundances in the rat tissues and their correlation. Radioisotopes 32:330–332.