RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2005; **19**: 2497–2506
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.2090

RCM

Nitrogen balance and δ^{15}N: why you're not what you eat during nutritional stress

Benjamin T. Fuller[1,2*,†], James L. Fuller[3], Nancy E. Sage[3], David A. Harris[2], Tamsin C. O'Connell[4] and Robert E. M. Hedges[1]

[1]Research Laboratory for Archaeology and the History of Art, University of Oxford, 6 Keble Road, Oxford OX1 3QJ, UK
[2]Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK
[3]Sutter Auburn Faith Hospital, 11815 Education Street, Auburn, CA 95602, USA
[4]Department of Archaeology, University of Cambridge, Downing Street, Cambridge CB2 3DZ, UK

Received 20 June 2005; Revised 13 July 2005; Accepted 13 July 2005

While past experiments on animals, birds, fish, and insects have shown changes in stable isotope ratios due to nutritional stress, there has been little research on this topic in humans. To address this issue, a small pilot study was conducted. Hair samples from eight pregnant women who experienced nutritional stress associated with the nausea and vomiting of morning sickness (hyperemesis gravidarum) were measured for carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope ratios. The δ^{13}C results showed no change during morning sickness or pregnancy when compared with pre-pregnancy values. In contrast, the δ^{15}N values generally increased during periods of weight loss and/or restricted weight gain associated with morning sickness. With weight gain and recovery from nutritional stress, the hair δ^{15}N values displayed a decreasing trend over the course of gestation towards birth. This study illustrates how δ^{15}N values are not only affected by diet, but also by the nitrogen balance of an individual. Potential applications of this research include the development of diagnostic techniques for tracking eating disorders, disease states, and nitrogen balance in archaeological, medical, and forensic cases.

The stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) are routinely used to elucidate the diets of humans and animals in modern and ancient ecosystems. This research is based on the principle that the isotopic values of body tissues reflect the isotopic signatures of the foods and liquids consumed plus a small predictable fractionation factor that is unique to each isotopic ratio, known as a trophic level shift (the difference between diet and tissue isotopic values). While animals raised on isotopically homogeneous diets have demonstrated the merits of this research,[1–8] evidence is emerging that additional factors affect isotopic results such that trophic level shifts are variable under certain conditions.[9–15] This uncertainty in the magnitude of the fractionation factors for stable isotope ratios is problematic and can lead to the erroneous reconstruction of feeding habits.

In particular, δ^{15}N values appear to be influenced by the nitrogen balance of an organism such that anabolic states can cause a decrease in consumer δ^{15}N values[16,17] and catabolic states and high protein diets can increase consumer δ^{15}N values.[12,18–25] While previous research has shown that human pregnancy results in a decrease in hair δ^{15}N values probably as a result of increased nitrogen retention,[16] there has been little experimental evidence examining how human δ^{15}N values are affected by nutritional stress. In one of the few studies to address this topic, Katzenberg and Lovell[26] studied stable isotope ratios in pathological bone and found elevated δ^{15}N values that were probably the result of tissue catabolism.

This research records isotopic variations in hair from pregnant women who experienced varying degrees of nutritional stress as manifested by weight loss or restricted weight gain that was primarily the result of morning sickness or nausea and vomiting during pregnancy (hyperemesis gravidarum). The results of this study are important for the correct interpretation of isotopic values in archaeological, ecological, medical, and forensic research, and suggest that, in addition to diet, δ^{15}N values can be affected by nitrogen balance and nutritional stress in humans.

EXPERIMENTAL

Subjects

As part of an ongoing study of isotopic changes in modern humans, hair samples were collected from eight pregnant women (subjects A–H) residing in Auburn, California, USA. These women were identified from a wider study investigating the isotopic changes during pregnancy, and all the subjects studied here are different from the individuals reported in Fuller *et al*.[16] All subjects gave birth to healthy singleton infants between 38 and 40 weeks of gestation. Maternal weight changes, the duration and intensity of morning sickness, and other medical/special conditions were recorded for each subject during pregnancy. Informed written consent

*Correspondence to: B. T. Fuller, P.O. Box 707, Auburn, CA 95604, USA.
E-mail: benjamin.fuller@sthughs-oxford.com
†Current address: P.O. Box 707, Auburn, CA 95604, USA.

was obtained from each subject after full explanation of the research and the protocol was approved by the Sutter Health Institutional Review Committee (Sacramento, CA, USA).

Dietary surveys

One of the limitations of this experiment was the lack of an isotopically controlled diet for the subjects. However, providing an isotopically homogeneous diet to pregnant women is prohibitive in terms of cost and ethics. Thus, dietary intakes were monitored during gestation by the completion of dietary surveys at least once each trimester. These questionnaires were general in nature and asked each subject to list the frequency (servings per weeks) and type of dietary intake for a number of different food groups: meats, dairy products, fish, vegetables, fruits, oils and fats, and sugar.

The validity and accuracy of dietary surveys for assessing dietary habits have been questioned, and the under-reporting of dietary intakes can confound results.[27] While these limitations need to be considered, the questionnaires used in this study were similar to those that have been successfully employed in other research projects.[28,29] Since the primary aim of these dietary surveys was to detect changes in dietary patterns (mainly variations in the consumption of animal protein), the forms were sufficiently valid for the questions being addressed in this study. Thus, the dietary questionnaires were only a semi-quantitative gauge of the dietary intakes for these subjects.

Hair analysis

The use of hair for stable isotope ratio analysis is increasing in frequency due to the ease of sampling hair and its advantageous characteristics such as being metabolically inert, resistant to degradation, isotopically representative of the body protein pool, and having a fast rate of synthesis.[16,30–37] Although individual variations exist, human hair generally grows at a rate of 1 cm per month or 0.35 mm/day, and it takes approximately 6 days for hair to emerge from the scalp.[38] Thus, the stable isotope ratios measured along the length of a hair shaft will provide a record of the isotopic composition of the body amino acid pool through time.[16,30,34]

Maternal hair samples were collected between 1 and 5 days after birth, and the hair was cut from the crown of the subject's head and as close to the scalp as possible. The samples were then prepared as outlined in O'Connell and Hedges[30] and Fuller *et al.*[16] The hair was cleaned twice by being soaked in a 2:1 mixture of methanol/chloroform for 30 min to remove lipid and shampoo residue, and rinsed in deionized or distilled water for 15 min. Hair samples (30–60 strands) were then wrapped in aluminum foil, cut into 1 or 1.5 cm sections (corresponding to approximately 4 or 6 weeks of growth, respectively), and dried overnight under vacuum to remove moisture.

Stable isotope ratio analysis

Stable isotope results are measured as the ratio of the heavier isotope to the lighter isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) and reported as δ values in parts per 1000 or 'per mil' (‰) relative to internationally defined standards for carbon (Vienna Pee Dee Belemnite, vPDB) and nitrogen (atmospheric N_2).[39] For example, the stable isotope ratios of nitrogen are expressed as:

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R_{sample} and $R_{standard}$ represent the ratios of $^{15}N/^{14}N$ for the sample being analyzed and the standard (atmospheric N_2), respectively. For detailed reviews, see Schwarcz and Schoeninger,[39] Schoeninger,[40] Kelly,[41] and McCutchan *et al.*[42] The hair samples (30–60 strands) were combusted to CO_2 and N_2 in an automated carbon and nitrogen analyzer (Carlo Erba, Milan, Italy) coupled to a continuous-flow isotope ratio monitoring mass spectrometer (PDZ Europa Geo 20/20, Crewe, UK). When possible, all samples were analyzed in triplicate (subject G measured in duplicate);

Table 1. Duration and symptoms of morning sickness, medical and special dietary information, pre-conception body mass index (BMI), pre-conception maternal weight, and infant birth weights for subjects A–H

Subject	Duration and symptoms of morning sickness	Medical and special dietary information	BMI (kg/m^2)	Maternal weight (kg)	Birth weight (kg)
A	Nausea (week 7)	Smoking during pregnancy	30.5	78.2	2.52
B	Nausea, hunger, and fatigue (weeks 6–12); fatigue, dizziness, and fainting spells which led to falls (weeks 20–30)	The fainting spells and falls were unusual during pregnancy and the subject had reduced dietary intake during this period	24.3	73.6	3.99
C	Nausea and vomiting (first trimester)	Smoking during pregnancy	27.7	73.2	3.03
D	Nausea and vomiting (weeks 4–13; 22–24)	Subject reported increased appetite and dietary intake during morning sickness	28.6	78.2	3.49
E	Nausea, vomiting, and taste-bud changes (weeks 10–16)	Mild hypertension	30.7	89.1	3.61
F	Nausea and vomiting (first trimester)	Smoking during pregnancy	36.4	113.6	3.47
G	Nausea, vomiting, and fatigue (weeks 7–10)	Subject was on a vegetarian diet but during the last 6 weeks of pregnancy started to consume chicken and turkey	20.4	51.4	3.07
H	Severe nausea and vomiting (first trimester); very difficult for the subject to eat and keep food down during this period, resulting in pronounced weight loss (6.4 kg)	Subject has been a strict vegan for 5 years. No animal protein was consumed during the pregnancy except for a single egg; mild anemia during pregnancy	20.3	53.6	3.17

precision was typically less than ±0.2‰ for $\delta^{13}C$ and $\delta^{15}N$ results.

RESULTS

Dietary intake and isotopic results

In general, the subjects reported a reduced dietary intake and weight loss during episodes of morning sickness. No systematic variations in animal protein consumption (increases or decreases) were reported during gestation. Once the morning sickness had subsided, the subjects returned to their normal dietary routines. The omnivorous subjects (A–F) have remarkably consistent $\delta^{13}C$ (−16.5‰ to −18.5‰) and $\delta^{15}N$ values (8.8‰ to 9.5‰) considering the wide variety of foods available, and these results are similar to previous research from North America.[16,37,43] Subject G is a vegetarian and had $\delta^{13}C$ values between −18.5‰ and −19.0‰ and $\delta^{15}N$ values between 7.8‰ and 8.1‰ before conception. Subject H is a vegan and her $\delta^{13}C$ (≈−20.0‰) and $\delta^{15}N$ (≈7.0‰) values were the least enriched of all the subjects. The $\delta^{15}N$ results of subject H are comparable with those from vegans from Oxford and Okehampton, UK.[30,31]

Subjects

Table 1 lists the duration and symptoms of morning sickness, medical conditions, special dietary information, pre-conception BMI (body mass index), pre-conception maternal weight, and the infant birth weights for subjects A–H. The pregnancy weight data zeroed to the pre-pregnancy weight and the $\delta^{15}N$ values for the eight subjects are plotted in Figs. 1(a)–1(h). The

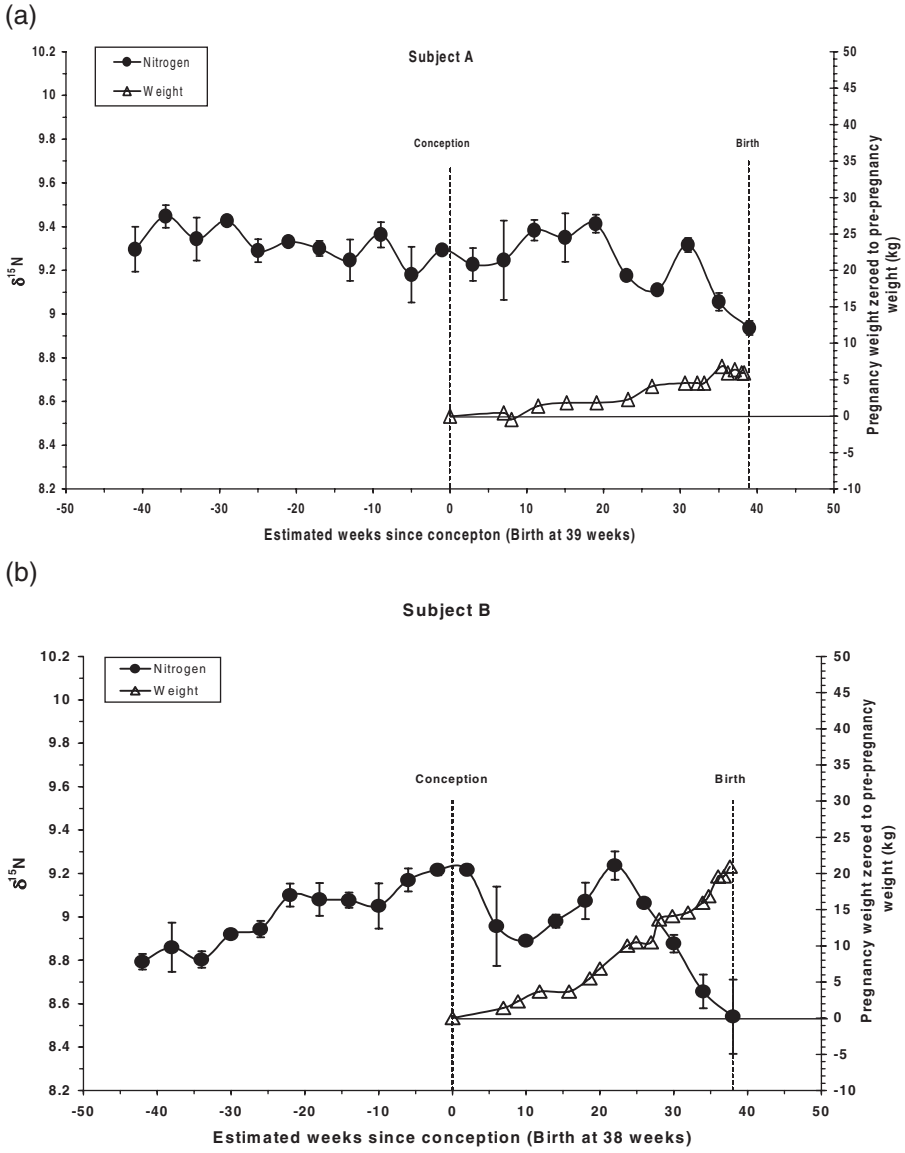

Figure 1. (a–h) Individual hair $\delta^{15}N$ variations and weight fluctuations during pregnancy for subjects A–H. Hair samples were isotopically analyzed in 1 or 1.5 cm sections corresponding to 4 or 6 week intervals of growth, respectively.[38] During periods of either restricted weight gain or weight loss, there was a general increase in hair $\delta^{15}N$ values. Weight changes during pregnancy were zeroed to the pre-pregnancy weights of the subjects.

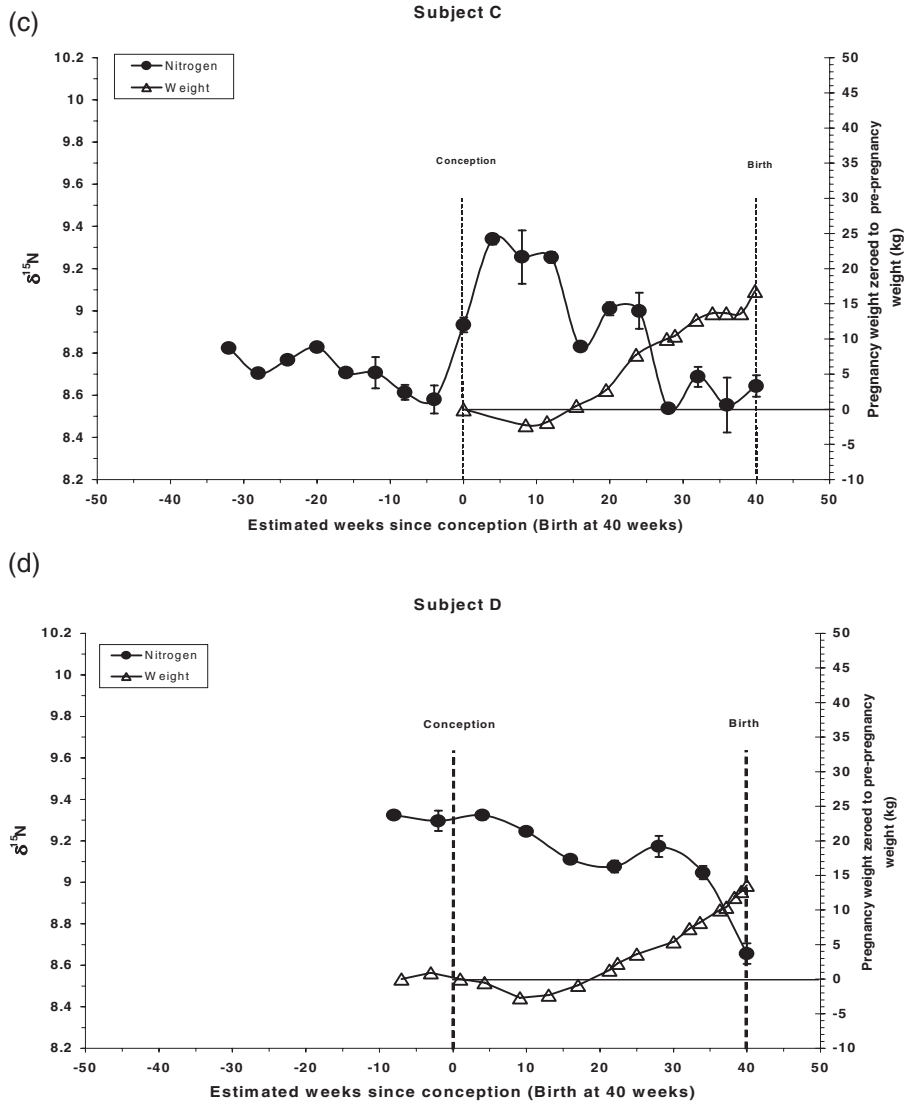

Figure 1. (*Continued*)

δ^{13}C values showed little uniform change during pre-pregnancy, nutritional stress, or birth, and the results at these periods are listed in Table 2.

Subject A

Subject A complained of nausea during the 7th week of gestation and displays relatively stable hair δ^{15}N values during the first 19 weeks of pregnancy (Fig. 1(a)). The δ^{15}N values decrease (0.3‰) between 19 and 27 weeks, display an upward trend between 27 and 31 weeks, and then show a decrease (0.4‰) between 31 and 39 weeks. The reason for the small δ^{15}N spike at 31 weeks is unknown, but it could correspond to a period when subject A was not gaining weight between 30 and 33 weeks.

Subject B

Subject B exhibits a unique hair δ^{15}N pattern where there is a decrease soon after conception, an increase (0.3‰) between 10 and 22 weeks, and then a sharp and steady decrease (0.7‰) until birth at 40 weeks (Fig. 1(b)). This isotopic pattern seems to be associated with the fact that subject B experienced nausea, hunger, and fatigue between 6 and 12 weeks, as well as fatigue, dizziness, and fainting spells between 20 and 30 weeks gestation. However, there does seem to be some offset between the weight plateau at 23–27 weeks and the elevated δ^{15}N value at 22 weeks and this is probably the result of a small difference in the estimated hair growth rate. The symptoms between 6 and 12 weeks are consistent with morning sickness, but the cause of the fatigue, dizziness, and fainting spells between 20 and 30 weeks is unknown. Subject B also reported that these symptoms affected her eating habits, since she recorded a decrease in appetite during this time.

In contrast to subjects A and B who only complained of nausea, subjects C–H experienced varying degrees of nausea and vomiting accompanied by weight loss (Figs. 1(c)–1(h)).

Subject C

During the first trimester, subject C experienced nausea, vomiting, and weight loss, and displayed a sudden rise ($\approx$0.7‰) in her hair δ^{15}N values (Fig. 1(c)). After approximately 10 weeks, the hair δ^{15}N values decreased and subject C began to recover from her weight loss of 2.3 kg. Between 12 and 16 weeks there is a drop of 0.4‰, an upward trend at 20–24 weeks, and then a similar 0.5‰ drop between

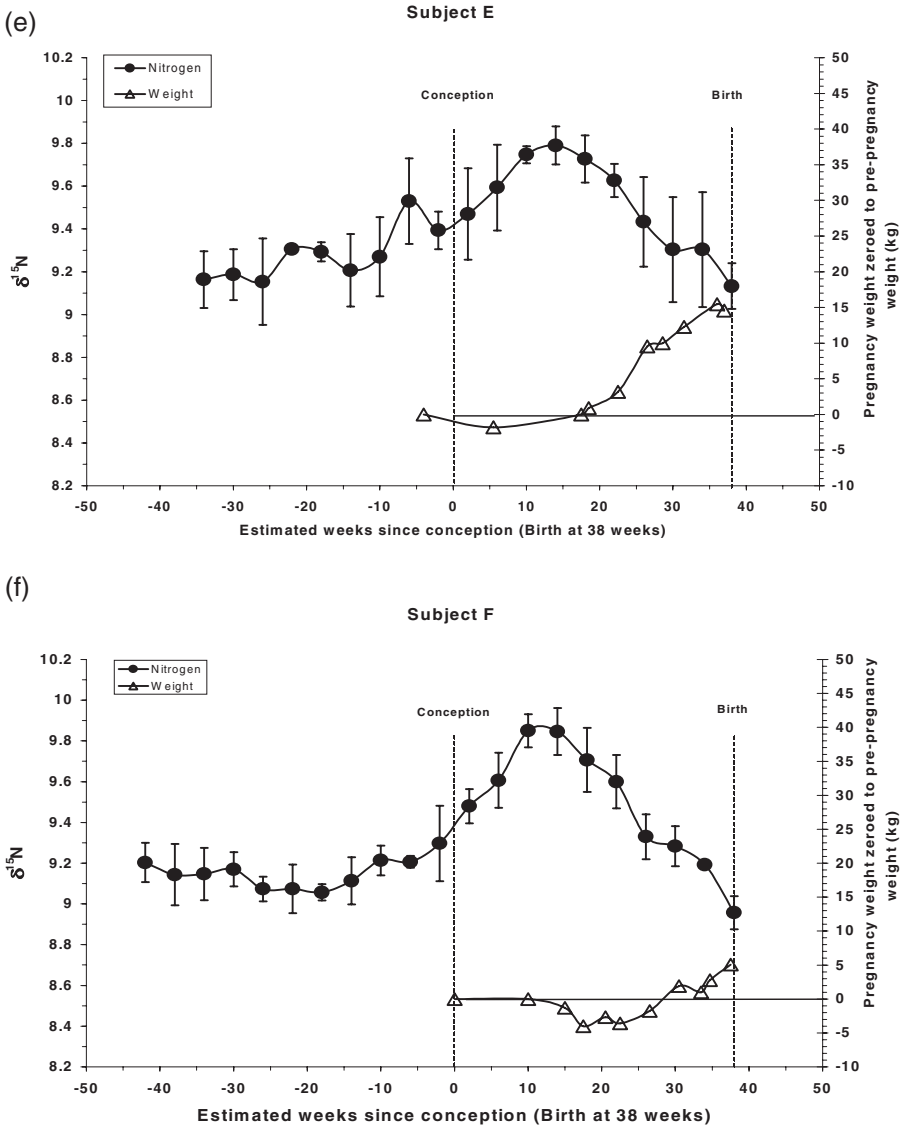

Figure 1. (*Continued*)

24 and 28 weeks. Little change in the hair δ^{15}N values was displayed during the last 8 weeks of pregnancy, and this corresponded to a near static weight between 32 and 38 weeks. In addition, it is clear that there is an offset between the δ^{15}N values and the estimated weeks since conception, and we attribute this to a discrepancy between the subject's actual rate of hair growth and that estimated from literature values.[38]

Subject D

Subject D complained of nausea and vomiting between weeks 4 and 13, and 22 and 24, and showed little change in her hair δ^{15}N values, despite a loss of 2.7 kg during the first trimester (Fig. 1(d)). It is unknown why subject D did not show an increase in hair δ^{15}N values with weight loss, but we speculate that it could be the result of her reported increase in appetite and dietary intake during the periods of morning sickness. There is a very small upward trend in hair δ^{15}N values between 22 and 28 weeks, and this is possibly the result of the nausea and vomiting between 22 and 24 weeks. Between 28 and 40 weeks, the hair δ^{15}N values of subject D display a decrease ($\approx$0.5‰) that corresponds to weight gain.

Subject E

Subject E experienced nausea, vomiting, and taste-bud changes between 10 and 16 weeks and displays a weight loss of 1.8 kg during the first trimester (Fig. 1(e)). The hair δ^{15}N values increase (0.4‰) from 0 to 14 weeks and then progressively decrease (0.7‰) towards birth at 38 weeks.

Subject F

During the first trimester, subject F complained of nausea, vomiting, and reduced dietary intake, and her hair δ^{15}N values increased ($\approx$0.5‰) from conception to 14 weeks (Fig. 1(f)). In addition, subject F experienced a weight loss of 4 kg at 18 weeks and this roughly corresponds to the increased hair δ^{15}N values in the first trimester. Again, there seems to be some offset due to a difference between the subject's actual rate of hair growth and that estimated from literature values.[38] After 14 weeks, there is a decrease (0.9‰) in hair δ^{15}N values towards birth at 38 weeks.

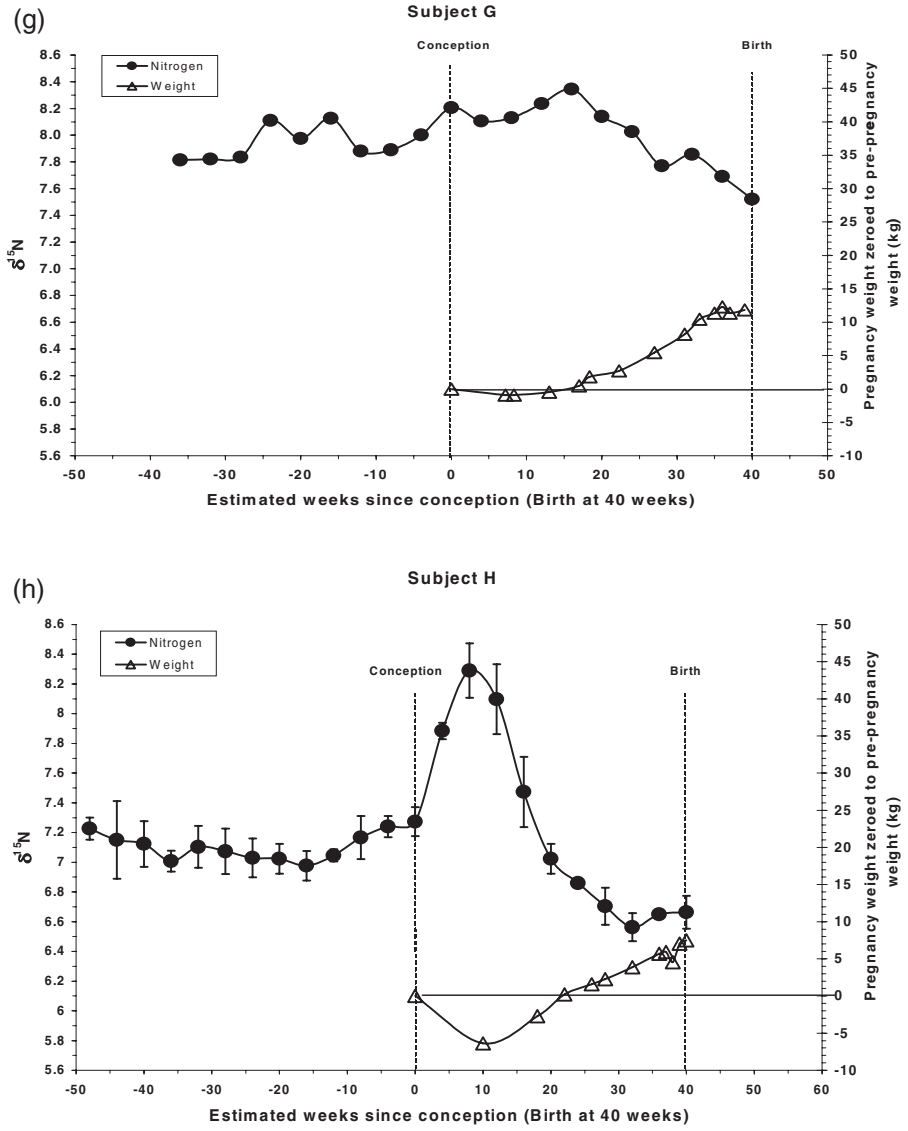

Figure 1. (*Continued*)

Subject G

Due to sampling constraints, hair from subject G could only be measured in duplicate (Fig. 1(g)). Subject G was unique in that she is a vegetarian and her low pre-pregnancy δ^{15}N values (7.8‰ to 8.1‰) confirm this fact. Subject G had a small weight loss ($\approx$1 kg) during the first trimester, and, while she experienced nausea and some vomiting, her complaints were fewer than those of the other subjects. This milder experience of morning sickness probably contributed to the small upward trend in hair δ^{15}N values between 4 and 16 weeks. Between 16 and 40 weeks, the hair δ^{15}N values decrease by 0.8‰ and there is an increase in weight of 12 kg. The subject remained a vegetarian until the last 6 weeks of pregnancy at which point she started supplementing her diet with chicken and turkey.

Table 2. δ^{13}C results for subjects A–H at conception, during nutritional stress, and at birth

Subject	δ^{13}C at conception (‰)	δ^{13}C during nutritional stress (‰)	δ^{13}C at birth (‰)
A	−17.1	−16.9	−17.6
B	−17.0	−17.0	−16.8
C	−17.5	−17.4	−17.2
D	−18.0	−17.8	−17.6
E	−17.3	−17.0	−17.3
F	−17.5	−17.4	−17.5
G	−18.7	−18.8	−18.8
H	−20.2	−20.2	−20.2

Subject H

Subject H exhibits the most striking evidence for the effects of nutritional stress on δ^{15}N results (Fig. 1(h)). This individual was a strict vegan for approximately 5 years before pregnancy, and the low δ^{15}N values ($\approx$7.0‰) confirm this dietary lifestyle. Shortly after becoming pregnant and throughout the first trimester, subject H experienced persistent nausea and vomiting that greatly limited her ability to eat and keep down foods. This period of nutritional stress resulted in a loss of 6.4 kg during the first trimester and a simultaneous increase in δ^{15}N of $\approx$1‰. After the 10th week, the morning sickness subsided, resulting in a rapid increase in weight gain (13.9 kg) accompanied by a sharp decrease in δ^{15}N of 1.7‰.

A summary of the data in Figs. 1(a)–1(h) is plotted in Fig. 2, where the maternal weight fluctuations zeroed to pre-pregnancy weights are plotted against the corresponding change in hair δ^{15}N values zeroed to pre-conception δ^{15}N values. A slight inverse trend between weight gain and δ^{15}N is observed, but the correlation is weak ($R^2 = 0.40$).

DISCUSSION

The hair δ^{13}C results did not display uniform patterns during periods of nutritional stress or pregnancy, and these results corroborate previous research on nutritional stress in birds[12,18] and pregnancy in humans.[16] However, the lack of a change in δ^{13}C values was in contrast to results from food restricted chicks,[44] starving spiders,[22] and fish fed slightly below maintenance levels,[15] where the δ^{13}C values were found to increase. It is unknown why some animals and insects exhibit changes in δ^{13}C during nutritional stress and others do not, and more research is necessary to fully understand these different observations.

The findings of this study confirm that δ^{15}N values are influenced not only by dietary intakes, but also by deviations in nitrogen homeostasis such that a catabolic state results in an increase in the nitrogen isotope ratio and an anabolic state causes a decrease in the nitrogen isotope ratio of the body protein pool. While previous research has demonstrated a decrease in hair δ^{15}N that is probably the result of maternal nitrogen conservation during pregnancy,[16] this experiment shows evidence of the effects of transient nutritional stress on human δ^{15}N values during gestation. When these results are compared with past research on animals, birds, fish, and insects, it is evident that many organisms have a biochemical response to nitrogen stress that causes an increase in δ^{15}N tissue values,[9–12,18–22,25] and this fact is not entirely surprising given the biochemical similarities of nitrogen conservation and excretion among organisms.[45]

Nutritional stress and increasing δ^{15}N values

Pregnancy is an anabolic state requiring enhanced nutritional intake to support the growth of the mother and the fetus and has been described as a period of 'accelerated starvation'.[46] Pregnant women are advised to consume an additional 6–10 g of protein per day, compared with non-pregnant women, with the greatest demand for protein arising in the third trimester.[47] When dietary consumption falls short of this increased demand for nitrogen, women can utilize their own body reserves in the form of tissue stores. Since muscle mass is the largest reservoir of nitrogen in the body, the endogenous amino acids represent a major source of nitrogen for protein metabolism.[48]

All subjects experienced some form of restricted weight gain and/or weight loss during pregnancy as a result of morning sickness. Thus, the women were mobilizing their tissue reserves to fuel the increased nutritional demands of pregnancy. Previous research on rats offers some evidence for the biochemical mechanisms involved. The livers of fasted pregnant rats were able to convert more amino acid carbon (alanine) into glucose than were fasted non-pregnant rats as a result of the increased uptake of gluconeogenic precursors by hepatocytes.[49,50] In addition, more of the metabolized nitrogen from the glucose synthesis was found in a metabolically usable form (ammonia) in the fasted pregnant rats than in the fasted non-pregnant rats where the nitrogen had been converted into urea. This reduced urea formation during pregnancy has been verified in humans and, while the exact mechanism has not been elucidated, it is hypothesized that it could be the result of a decrease in the delivery of ureogenic substrates to the liver and/or a reduction in enzymatic activity in the urea cycle.[51–53]

Results from this study show that this 'anabolic catabolism'[49] of maternal tissues during starvation, or the shift from the assimilation of dietary nitrogen to nitrogen derived from consumer tissue, caused an increase in hair δ^{15}N values. The

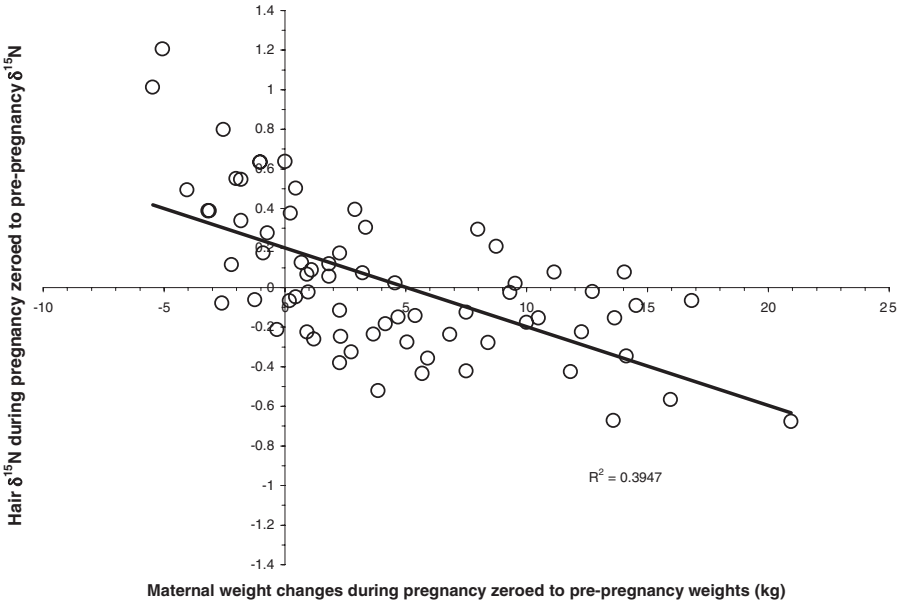

Figure 2. A summary of the hair δ^{15}N changes and weight fluctuations during pregnancy for all subjects A–H. A subtle inverse trend between weight gain and hair δ^{15}N is observed, but the correlation is very weak ($R^2 = 0.40$).

biochemical mechanisms associated with this δ^{15}N increase are believed to be the same as those causing the trophic level fractionation between diet and consumer tissue; in this instance, however, the diet or nitrogen source is the consumer's tissue.[12,18,54] As the breakdown rate of skeletal muscle protein increases, the excreted lighter nitrogen (^{14}N) is not replaced by dietary protein, and the remaining tissues become progressively enriched in heavier nitrogen (^{15}N) as the duration of nutritional stress and weight loss progresses.[12,18,54]

In this study, a slight inverse trend between weight gain and δ^{15}N is observed, but the correlation is weak ($R^2 = 0.40$). Fuller *et al*.[16] found a stronger correlation ($R^2 = 0.67$) between increasing weight gain and decreasing δ^{15}N values in pregnant women who were not significantly nutritionally stressed. This discrepancy suggests the possibility that during periods of inadequate nutritional intake such as morning sickness, the mixing of dietary amino acids having a lower δ^{15}N value (trophic level effect) with nitrogen derived from muscle catabolism having a higher δ^{15}N value (starvation effect) results in the deterioration of the inverse relationship between weight gain and δ^{15}N during pregnancy. This could be because weight loss by the mobilization of muscle mass to fuel the increased demands of maternal and fetal protein synthesis is being masked by the changes of water weight, adipose tissue, and fetal mass during pregnancy. Thus, monitoring weight gain during gestation only provides an estimate of nitrogen balance during pregnancy since the protein weight gain represents only 8% of the total weight gain in pregnancy.[55] In addition, some of the scatter in Fig. 2 is probably the result of individual differences in hair growth rates, and this is clearly seen in subjects B, C, and F (Figs. 1b, 1c and 1f)).

The δ^{15}N results of subject H (vegan pregnancy) best demonstrate a switch from dietary to tissue nitrogen, and the possible mixing of these two nitrogen sources during the third trimester. During the first trimester, the severe hyperemesis gravidarum and inability to consume her vegan diet probably resulted in tissue catabolism acting as the major source of nitrogen for protein synthesis. This is illustrated in Fig. 3, where there is a correlation between weight loss and increasing hair δ^{15}N values. After the morning sickness subsided, subject H was able to resume her diet, resulting in a shift in the primary source of nitrogen from muscle tissue to dietary intake. This is also illustrated in Fig. 3, where there is an inverse relationship between weight gain and decreasing hair δ^{15}N values until the weight gain reaches $\approx$4 kg. After this weight, the final two points in Fig. 3 show a small increase and a plateau in δ^{15}N despite a general increase in weight gain. Since the demand for nitrogen continues to increase towards full term,[47,53] and since these last two points were obtained at 36 and 40 weeks of gestation, it is speculated that the increasing fetal demands for nitrogen may have outstripped the supply of nitrogen available in the vegan diet. This could have led to an input of ^{15}N-enriched muscle derived nitrogen into the metabolic pool which might have contributed to the decline in weight gain ($\approx$1.4 kg) between 37 and 38 weeks of gestation.

In addition, it is interesting to note that the δ^{15}N results in the third trimester from subject H are nearly identical to the δ^{15}N results in the third trimester from subject D discussed in Fuller *et al*.[16] Since this subject D gave birth to twins with a

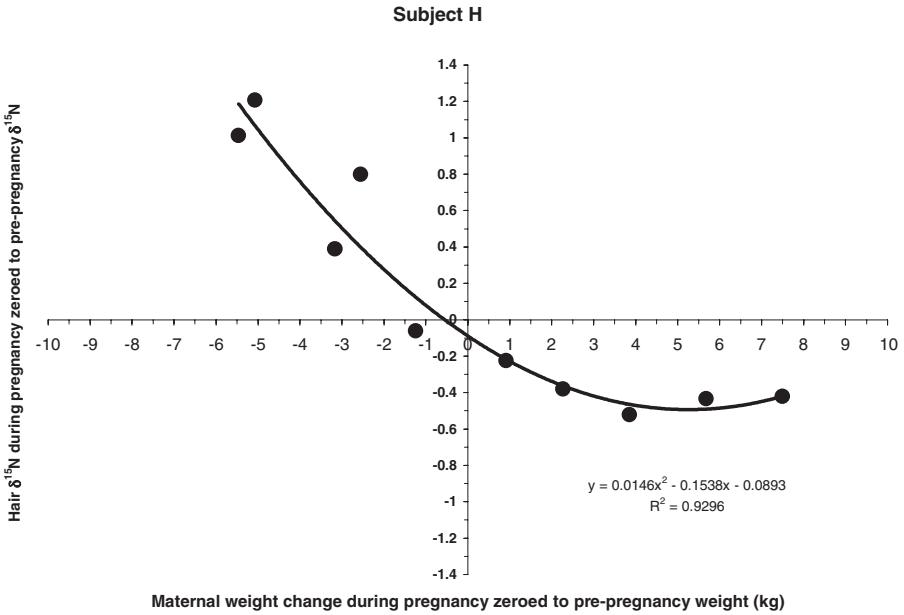

Figure 3. Hair δ^{15}N variations plotted against corresponding weight changes for subject H during pregnancy. Subject H is a vegan and appears to display a metabolic shift in the source of nitrogen for protein synthesis from tissue catabolism to dietary protein and the possible mixing of the two sources during the 3rd trimester. This produced a relationship between weight changes and hair δ^{15}N values. After a weight gain of 4 kg, a smoothing of the relationship is observed which could be due to an input of ^{15}N-enriched nitrogen into the metabolic pool from the breakdown of muscle tissue to support the growing demand for nitrogen by the mother and fetus.

substantially greater combined birth weight (4.9 kg) than the average birth weight of a single infant (3.0–3.5 kg), the increased demand for nitrogen during the third trimester might have been achieved by 'anabolic catabolism'[49] which could have contributed to a curtailed weight gain (1.1 kg) between 31 to 35 weeks and the rise in hair $\delta^{15}N$ around the same time. Indeed, studies of urinary 3-methylhistidine, a marker for the catabolism of muscle tissue, have found an increased concentration toward term, suggesting an increased rate of protein degradation, probably fueled by the breakdown of maternal protein stores.[56,57] While it is speculative at this point to attribute the late-term plateaus and increases in hair $\delta^{15}N$ to ^{15}N-enriched nitrogen from muscle catabolism, it is clear from this research, and from the results for subject H in particular, that the exogenous (dietary protein) and endogenous (tissue catabolism) reservoirs of nitrogen have distinct $\delta^{15}N$ signatures that can be used as natural labels to monitor nitrogen balance in humans.

Potential applications and future directions

The confirmation that human $\delta^{15}N$ values increase in synthesized protein in times of nutritional stress opens up many avenues for future research. In addition to monitoring nitrogen balance during pregnancy, the analysis of hair $\delta^{15}N$ values could potentially be developed as a diagnostic tool for eating disorders, disease states, and nutritional stress in archaeological, medical, and forensic cases. While this technique shows promise, there are areas for improvement. A limiting aspect of this current research was the fact that we had to estimate the time since conception by assigning each 1 cm section of hair to 4 weeks of growth. While this estimated hair growth rate did produce sound results, it is clear that some individuals (subjects B, C, and F) had more variable hair growth rates such that there was an offset between the weight fluctuations and the recorded time of the $\delta^{15}N$ variations. Thus, it would be beneficial to obtain multiple hair samples at different times during gestation so that hair growth rates can be individually monitored. In addition, the isotopic analysis of smaller hair segments (1–5 mm) would permit a more detailed identification of nitrogen fluctuations due to dietary or metabolic patterns of the order of weeks or days.

As was discussed by Koch[58] and Fuller *et al*.,[16] it should be theoretically possible to monitor pregnancy and nitrogen balance in other tissues that do not remodel after formation such as tusks, teeth, horns, and feathers. However, the realization that $\delta^{15}N$ results can also increase during pregnancy as a result of nutritional stress may hamper the detection of pregnancy and fertility patterns in isotopic studies, and this fact needs to be kept in mind for future research. Indeed, limited isotopic studies of polar bears, seals, and caribou have shown that $\delta^{15}N$ values can become elevated during pregnancy and lactation.[59–62] Thus, particular attention needs to be focused on organisms that fast or reduce feeding during gestation, and more results are needed from animals with established fertility patterns in order to determine if it is possible to obtain pregnancy and birth spacing information about extinct species.

When the results of this research are viewed in context with the mounting evidence from past studies,[2,9–22,24,25,42,63] it becomes clear that it is no longer possible to assume that there is a constant $\approx 3‰$ diet-tissue fractionation factor for $\delta^{15}N$. In addition to the isotopic composition of the diet, the protein content of the diet and the nitrogen balance of an organism must be considered so that correct inferences about unknown dietary habits can be made. This lack of a uniform trophic level effect for $\delta^{15}N$ should not invalidate the use of $\delta^{15}N$ values in food web studies, but it suggests that care is needed in how isotopic results are interpreted. In particular, we strongly agree with Gannes *et al*.[54] that more controlled laboratory experiments are needed, especially those of the caliber of Ambrose,[7] Sponheimer *et al*.,[14,24] Gaye-Siessegger *et al*.,[15,17,23] Voigt and Matt,[25] and Parker *et al*.,[62] so that it will be possible to unravel how and why there is variability in the $\delta^{15}N$ fractionation factor.

In addition, advances in measuring nitrogen isotopic ratios in single amino acids will, it is hoped, provide a more detailed investigation of the $\delta^{15}N$ trophic level effect at the molecular level.[4,36,64–67] As is already the case with plankton, it would be ideal to use nitrogen single amino acid measurements to develop an 'internal index to trophic position'[65,66] for humans so that isotopic differences at the protein level could be attributed to a specific cause such as diet, pregnancy, nutritional stress, disease, etc. This emerging field of molecular research should help refine our knowledge of the inner workings of the trophic level effect and of amino acid metabolism. Thus, a clearer understanding of the biochemical mechanisms that cause nitrogen fractionation *in vivo* will lead to greater confidence in the assumptions and conclusions drawn from $\delta^{15}N$ results in a variety of academic disciplines.

CONCLUSIONS

We measured hair $\delta^{13}C$ and $\delta^{15}N$ values in eight pregnant women who experienced varying degrees of nutritional stress as a result of the nausea and vomiting from morning sickness (hyperemesis gravidarum). No uniform change in $\delta^{13}C$ results was observed in the subjects during nutritional stress or pregnancy. In contrast, the $\delta^{15}N$ results displayed a general increase during nutritional stress and then a general decrease as gestation progressed towards full term. The findings of this small study indicate that, in addition to diet, human $\delta^{15}N$ values are influenced by the nitrogen balance of an individual where a catabolic state such as morning sickness results in tissue protein becoming ^{15}N-enriched. This evidence that human $\delta^{15}N$ values increase as a result of nutritional stress needs to be considered when stable isotope ratio results are being used to reconstruct the feeding habits of an individual. In conclusion, while the effects of nutritional stress associated with morning sickness during pregnancy show clear isotopic and metabolic parallels to starvation studies in animals, one is not necessarily a model for the other and more research involving nutritional stress (dieting, sickness, eating disorders, etc.) in humans is needed to enhance our understanding of the $\delta^{15}N$ trophic level effect.

Acknowledgements

We are greatly indebted to the subjects who participated in this study. Without their help, interest, and enthusiasm this

research would never have been possible. Thanks are due to Peter Ditchfield, Martin Humm, Angela Bowles, and Christine Tompkins of the RLAHA, at the University of Oxford, for technical assistance and advice during isotopic analysis. This manuscript was greatly improved by the comments and suggestions of Mandy Jay and two anonymous reviewers.

REFERENCES

1. DeNiro MJ, Epstein S. *Geochim. Cosmochim. Acta* 1978; **42**: 495.
2. DeNiro MJ, Epstein S. *Geochim. Cosmochim. Acta* 1981; **45**: 341.
3. Tieszen LL, Boutton TW, Tesdahl KG, Slade NA. *Oecologia* 1983; **57**: 32.
4. Hare PE, Fogel ML, Stafford TW, Mitchell AD, Hoering TC. *J. Archaeol. Sci.* 1991; **18**: 277.
5. Tieszen LL, Fagre T. In *Prehistoric Human Bone: Archaeology at the Molecular Level*, Lambert JB, Grupe G (eds). Springer-Verlag: Berlin, 1993; 121–155.
6. Ambrose SH, Norr L. In *Prehistoric Human Bone: Archaeology at the Molecular Level*, Lambert JB, Grupe G (eds). Springer-Verlag: Berlin, 1993; 1–37.
7. Ambrose SH. In *Close to the Bone: Biogeochemical Approaches to Paleodietary Analysis in Archaeology*, Ambrose SH, Katzenberg MA (eds). Plenum Press: New York, 2000; 243–259.
8. Howland MR, Corr LT, Young SMM, Jones V, Jim S, van der Merwe NJ, Mitchell AD, Evershed RP. *Int. J. Osteoarch.* 2003; **13**: 54.
9. Ambrose SH, DeNiro MJ. *Oecologia* 1986; **69**: 395.
10. Sealy JC, van der Merwe NJ, Lee-Throp JA, Lanham JL. *Geochim. Cosmochim. Acta* 1987; **51**: 2707.
11. Ambrose SH. *J. Archaeol. Sci.* 1991; **18**: 293.
12. Hobson KA, Alisauskas RT, Clark RG. *Condor* 1993; **95**: 388.
13. Focken U. *Isotopes Environ. Health Stud.* 2001; **37**: 199.
14. Sponheimer M, Robinson TF, Roeder BL, Passey BH, Ayliffe LK, Cerling TE, Dearing MD, Ehleringer JR. *J. Archaeol. Sci.* 2003; **30**: 1649.
15. Gaye-Siessegger J, Focken U, Muetzel S, Abel H, Becker K. *Oecologia* 2004; **138**: 175.
16. Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, Hedges REM. *Rapid Commun. Mass Spectrom.* 2004; **18**: 2889.
17. Gaye-Siessegger J, Focken U, Abel H, Blecker K. *Naturwissenschaften* 2004; **91**: 90.
18. Hobson KA, Clark RG. *Condor* 1992; **94**: 189.
19. Scrimgeour CM, Gordon SC, Handley LL, Woodford JAT. *Isotopes Environ. Health Stud.* 1995; **31**: 107.
20. Webb SC, Hedges REM, Simpson SJ. *J. Exp. Biol.* 1998; **201**: 2903.
21. Adams TS, Sterner RW. *Limnol. Oceanogr.* 2000; **45**: 601.
22. Oelbermann K, Scheu S. *Oecologia* 2002; **130**: 337.
23. Gaye-Siessegger J, Focken U, Abel HJ, Becker K. *Isotopes Environ. Health Stud.* 2003; **39**: 125.
24. Sponheimer M, Robinson T, Ayliffe L, Roeder B, Hammer J, Passey B, West A, Cerling T, Dearing D, Ehleringer J. *Int. J. Osteoarchaeol.* 2003; **13**: 80.
25. Voigt CC, Matt F. *J. Exp. Biol.* 2004; **207**: 1741.
26. Katzenberg MA, Lovell NC. *Int. J. Osteoarchaeol.* 1999; **9**: 316.
27. Becker W, Welton D. *Public Health Nutr.* 2001; **4**: 683.
28. Kroke A, Klipstein-Grobusch K, Voss S, Moseneder J, Thielecke F, Noack R, Boeing H. *Am J. Clin. Nutr.* 1999; **70**: 439.
29. Williams JH, O'Connell TC. *J. Gerontol. A Biol. Sci. Med. Sci.* 2002; **57**: M797.
30. O'Connell TC, Hedges REM. *Am. J. Phys. Anthropol.* 1999; **108**: 409.
31. Bol R, Pflieger C. *Rapid Commun. Mass Spectrom.* 2002; **16**: 2195.
32. Schwertl M, Auerswald K, Schnyder H. *Rapid Commun. Mass Spectrom.* 2003; **17**: 1312.
33. Sharp ZD, Atudorei V, Panarello HO, Fernandez J, Douthitt C. *J. Arch. Sci.* 2003; **30**: 1709.
34. Ayliffe LK, Cerling TE, Robinson T, West AG, Sponheimer M, Passey BH, Hammer J, Roeder B, Dearing MD, Ehleringer JR. *Oecologia* 2004; **139**: 11.
35. West AG, Ayliffe LK, Cerling TE, Robinson TF, Karren B, Dearing MD, Ehleringer JR. *Funct. Ecol.* 2004; **18**: 616.
36. Petzke KJ, Boeing H, Metges CC. *Rapid Commun. Mass Spectrom.* 2005; **19**: 1392.
37. Roy DM, Hall R, Mix AC, Bonnichsen R. *Am. J. Phys. Anthropol.* 2005; in press.
38. Saitoh M, Uzuka M, Sakamoto M, Kobori T. Rate of hair growth. In *Hair Growth*, Montagna W, Dobson RL (eds). Pergamon Press: Oxford, 1969; 183–201.
39. Schwarcz HP, Schoeninger MJ. *Yearb. Phys. Anthropol.* 1991; **34**: 283.
40. Schoeninger MJ. *Evol. Anthropol.* 1995; **4**: 83.
41. Kelly JF. *Can. J. Zool.* 2000; **78**: 1.
42. McCutchan JH Jr, Lewis WM, Kendall C, McGrath CC. *Oikos* 2003; **102**: 378.
43. Schoeller DA, Minagawa M, Slater R, Kaplan IR. *Ecol. Food Nutr.* 1986; **18**: 159.
44. Hatch KA, Sacksteder KA, Treichel IW, Cook ME, Porter WP. *Biochem. Biophys. Res. Commun.* 1995; **212**: 719.
45. Singer MA. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 2003; **134**: 543.
46. Freinkel N, Metzger BE, Nitzan M, Hare JW, Shambaugh GE III, Marshall RT, Surmaczynska BZ, Nagel TC. *Isr. J. Med. Sci.* 1972; **8**: 426.
47. Duggleby SL, Jackson AA. *Curr. Opin. Clin. Nutr. Metab. Care* 2002; **5**: 503.
48. Castaneda C. *J. Anim. Sci.* 2002; **80**: E98.
49. Metzger BE, Agnoli FS, Freinkel N. *J. Clin. Invest.* 1970; **49**: 65A.
50. Metzger BE, Agnoli FS, Freinkel N. *Horm. Metab. Res.* 1970; **2**: 367.
51. Kalhan SC, Tserng KY, Gilfillan C, Dierker LJ. *Metabolism* 1982; **31**: 824.
52. Kalhan SC, Rossi KQ, Gruca LL, Super DM, Savin SM. *Am. J. Physiol.* 1998; **275**: E423.
53. Mojtahedi M, de Groot LCPGM, Boekholt HA, van Raaij JMA. *Am. J. Clin. Nutr.* 2002; **75**: 1078.
54. Gannes LZ, O'Brien DM, del Rio CM. *Ecology* 1997; **78**: 1271.
55. Hytten FE. In *Clinical Physiology in Obstetrics*, Hytten FE, Chamberlain G (eds). Blackwell Scientific Publications: Oxford, 1991; 150–172.
56. Fitch WL, King JC. *Hum. Nutr. Clin. Nutr.* 1987; **41**: 327.
57. Naismith DJ, Emery PW. *Eur. J. Clin. Nutr.* 1998; **42**: 483.
58. Koch PL. Abstract presented at the 5th Advanced Seminar on Palaeodiet, Valbonne, France, 1997.
59. Kurle CM. *Can. J. Zool.* 2002; **80**: 902.
60. Kurle CM, Worthy GAJ. *Oecologia* 2001; **126**: 254.
61. Polischuk SC, Hobson KA, Ramsay MA. *Can. J. Zool.* 2001; **79**: 499.
62. Parker KL, Barboza PS, Stephenson TR. *J. Mammal.* 2005; **86**: 610.
63. Minagawa M, Wada E. *Geochim. Cosmochim. Acta* 1984; **48**: 1135.
64. Fogel ML, Tuross N, Johnson BJ, Miller GH. *Org. Geochem.* 1997; **27**: 275.
65. McClelland JW, Montoya JP. *Ecology* 2002; **83**: 2173.
66. Schmidt K, McClelland JW, Mente E, Montoya JP, Atkinson A, Voss M. *Mar. Ecol. Prog. Ser.* 2004; **266**: 43.
67. Petzke KJ, Boeing H, Klaus S, Metges CC. *J. Nutr.* 2005; **135**: 1515.