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

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Carbon (^{13}C/^{12}C) and nitrogen (^{15}N/^{14}N) stable isotope ratios were longitudinally measured in human hair that reflected the period from pre-conception to delivery in 10 pregnant women. There was no significant change in the δ^{13}C results, but all subjects showed a decrease in δ^{15}N values (−0.3 to −1.1‰) during gestation. The mechanisms causing this decrease in hair δ^{15}N have not been fully elucidated. However, since the δ^{15}N values of dietary nitrogen and urea nitrogen are significantly lower compared to maternal tissues, it is hypothesized that the increased utilization of dietary and urea nitrogen for tissue synthesis during pregnancy resulted in a reduction of the steady state diet to a body trophic level effect by approximately 0.5–1‰. An inverse correlation ($R^2 = 0.67$) between hair δ^{15}N and weight gain was also found, suggesting that positive nitrogen balance results in a reduction of δ^{15}N values independent of diet. These results indicate that δ^{15}N measurements have the ability to monitor not only dietary inputs, but also the nitrogen balance of an organism. A potential application of this technique is the detection of fertility patterns in modern and ancient species that have tissues that linearly record stable isotope ratios through time. Copyright © 2004 John Wiley & Sons, Ltd.

The naturally occurring stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) have become powerful tools for assessing the diets of humans and animals past and present.[1–6] Here, stable isotope ratios 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 (Ambient Inhalable Reservoir, AIR). For a detailed review, see Katzenberg.[7]

Fundamental to the correct interpretation of these results is the belief that the isotopic signatures of body tissues faithfully record dietary inputs, such that "you are what you eat"[8] plus a small positive fractionation factor (1–2‰ for δ^{13}C; 3–4‰ for δ^{15}N) known as a trophic level effect.[2,3,7] Studies of animals fed isotopically constant diets have demonstrated that this principle is valid for a steady metabolic state.[9–13] However, there is evidence that factors other than dietary inputs may influence nitrogen isotope ratios in acute situations.[14,15] In particular, conditions such as nutritional stress[16–19] and disease[20,21] have been shown to increase δ^{15}N values when an organism is catabolic and in negative nitrogen balance.

This study investigates the converse situation of pregnancy. In pregnancy, the body becomes anabolic and enters positive nitrogen balance, decreasing nitrogen excretion and increasing protein synthesis. In order to conduct a longitudinal study throughout human pregnancy, we analyzed hair samples taken at birth from recent mothers in the USA. The protein in sequential sections of the hair provided a record of the isotope ratios existing in the body when that section of hair was synthesized.[22] The findings of this research have significant implications regarding the interpretation of δ^{15}N values in archaeological and ecological samples and may lead to a method for investigating pregnancy and fertility patterns in past populations. In addition, this method has the potential to provide a simple and non-invasive means of monitoring nitrogen balance during pregnancy in contemporary women.

EXPERIMENTAL

Subjects

Ten women (subjects A–J) participated in this study during the period September 2001 to May 2003. All subjects gave birth to healthy singleton infants between 36 and 42 weeks of gestation except for subject D who delivered twins. Written informed consent was obtained from each subject after full explanation of the research, and the protocol was approved by the Sutter Health Institutional Review Committee (Sacramento, California, USA).

Dietary surveys

Stable isotope ratios are primarily influenced by dietary habits,[3,4] and it would be ideal to investigate non-dietary

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isotopic variations in humans consuming an isotopically homogeneous diet. However, the ethical and financial constraints of human research prohibit such studies. Thus, in order to assess dietary intakes, all subjects completed dietary surveys at least once a trimester (most subjects every 6–8 weeks) during the course of pregnancy. These forms were general in nature and asked subjects to list the frequency of dietary intakes (servings per week) for a number of different food groups: meats, dairy products, fish, vegetables, fruits, oils and fats, and sugar.

While there is debate about the accuracy of dietary questionnaires,[23] the forms used in this research were similar to those that have been accepted for use in other large-scale medical studies and provided a general semiquantitative measure of dietary intakes.[24,25] In addition, we are aware of problems such as the under-reporting of dietary intakes on dietary forms, but since we are primarily interested in dietary changes over the course of gestation we felt that these questionnaires would be valid for the scope of this research project.

Hair analysis

As hair is simple and painless to sample, it has been used in a number of isotopic studies for dietary reconstruction.[26–37] Hair is a good medium to study the isotopic effects of dietary and physiological changes since it is metabolically inert, resistant to degradation, isotopically representative of the body protein pool, and has a fast synthesis rate.[9,10,13,22,36] While there is some variation, 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 skin.[38] Thus, the isotopic signature of the body is linearly recorded along the length of a hair, and changes through time can be easily monitored.

Hair was sampled and analyzed according to standard laboratory procedures.[22] Between 1–3 days after birth, hair was cut from the crown of the subject's head and as close to the scalp as possible. All hair samples were greater than 12 cm in length so that pre-pregnancy isotopic values were obtained from each individual. Initially, the proximal end of the hair sample was wrapped in medical tape (which served as an anchor), and then the hair was stored in plastic bags until analysis. The samples were cleaned twice by soaking in a 2:1 mixture of methanol and chloroform for 30 min to remove any lipid or shampoo residue, then rinsed in deionized or distilled water for 15 min. Hair samples (30–60 strands) were placed lengthways onto a strip of aluminum foil, approximately 15 mm wide; this strip of foil was then folded over lengthways twice to enclose the hair sample. The foil-wrapped hair was then cut with a scalpel into 1 or 1.5 cm sections (corresponding to 4 or 6 weeks of growth, respectively), dried overnight under vacuum to remove any remaining water, and rolled into balls for isotopic analysis. The hair samples were then 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, Cheshire, UK). All samples were run in triplicate; precision was typically less than ±0.2‰ for the $\delta^{13}C$ and $\delta^{15}N$ results.

RESULTS

Diet

The dietary surveys revealed no systematic changes in the diets of the individuals during gestation. Some subjects increased their frequency of animal protein (meat and dairy products) consumption, while others had no change or a decrease. Since the subjects did not frequently consume fish during pregnancy (1–2 servings per week), the hair $\delta^{13}C$ values (−16.5‰ to −18.5‰) are consistent with a mixed diet containing both C_4 plants such as maize (expected consumer's $\delta^{13}C = -12‰$) and C_3 plants (expected consumer's $\delta^{13}C = -21‰$).[3] These values are in agreement with results from a previous North American food web study.[27] The pre-pregnancy $\delta^{15}N$ values are between 8.5‰ and 9.5‰, and this is consistent with the omnivorous diet of these subjects.[22,27]

Changes in isotopic ratios during pregnancy

A summary of the $\delta^{13}C$ and $\delta^{15}N$ data for the 10 subjects is shown in Table 1 and Figs. 1(a) and 1(b). There is no difference in the $\delta^{13}C$ values (mean ± SD) for all subjects between conception (−17.4 ± 0.5‰) and birth (−17.4 ± 0.5‰) (Fig. 1(a)). In contrast, all subjects show a dramatic reduction in their $\delta^{15}N$ values (mean ± SD) during pregnancy which, for the group as a whole, is highly significant (conception = 9.0 ± 0.3‰; birth = 8.4 ± 0.5‰; $p < 0.001$ using a Wilcoxon signed-rank test) (Fig. 1(b)).

Two typical graphs of the results from this study are shown for subjects A and C in Figs. 2(a) and 2(b). For subject A the $\delta^{13}C$ values are relatively constant during the course of the hair measurement period (40 weeks before conception to 40 weeks after conception). The $\delta^{15}N$ values, while increasing by 0.4‰ from −40 to −20 weeks before conception, are constant from −20 weeks to conception. In the hair estimated to have grown after conception, the $\delta^{15}N$ values show a slight increase followed by a marked downward trend, falling from 9.2‰ at 16 weeks of gestation to 7.8‰ at birth or 40 weeks of gestation (Fig. 2(a)). Subject C displays a similar graph to the results of subject A with little change in the $\delta^{13}C$ values (Fig. 2(b)). In the 38 weeks before conception the hair $\delta^{15}N$ values range between 8.5‰ and 8.9‰. After conception, the hair $\delta^{15}N$ values remain steady at 8.8‰ during the first trimester. At 26 weeks of gestation the hair $\delta^{15}N$ values start to decline from 8.7‰ to 7.9‰ at birth or 42 weeks (Fig. 2(b)). Subject D, who delivered twins, shows an early (after the 8th week) and steady decrease in $\delta^{15}N$ (Fig. 2(c)). As the combined birth weight of the twins (4.9 kg) was significantly greater than the average weight at birth of a single infant (3.0–3.5 kg), this sudden and rapid decrease in $\delta^{15}N$ may be linked to the increased demand for maternal nitrogen by the two fetuses. The results from subject J are particularly interesting, since the hair of the individual was sufficiently long to record two successive pregnancies (Fig. 2(d)). During both pregnancies the $\delta^{15}N$ values show a decreasing trend with no change in the $\delta^{13}C$ results. In addition, after the first birth, the $\delta^{15}N$ values tend to recover upwards, towards the 'steady state' pre-conception values.

Relationship between $\delta^{15}N$ and weight gain

Since weight gain during pregnancy should reflect a positive nitrogen balance, we decided to test the hair $\delta^{15}N$ data against

Table 1. Hair $\delta^{13}C$ and $\delta^{15}N$ values at conception and birth for subjects A–J. Also included are the infant birth weights

Subject	$\delta^{13}C$ at conception (‰)	$\delta^{13}C$ at birth (‰)	$\delta^{15}N$ at conception (‰)	$\delta^{15}N$ at birth (‰)	Birth weight (kg)
A	−17.3	−17.7	8.9	7.8	3.78
B	−16.9	−17.1	9.1	8.7	2.85
C	−16.8	−17.0	8.9	7.9	3.95
D	−17.8	−17.9	8.9	8.0	4.90†
E	−17.6	−17.2	8.7	7.9	3.50
F	−16.9	−16.7	9.3	8.5	3.82
G	−18.0	−17.5	8.9	8.3	3.86
H	−18.1	−17.7	8.6	7.9	3.38
I	−18.0	−18.2	9.3	9.0	3.63
J 1st*	−17.1	−17.2	9.4	8.9	3.05
J 2nd*	−17.1	−16.9	9.2	8.9	2.92

* Subject J's hair $\delta^{15}N$ values recorded two successive pregnancies.
† Twins

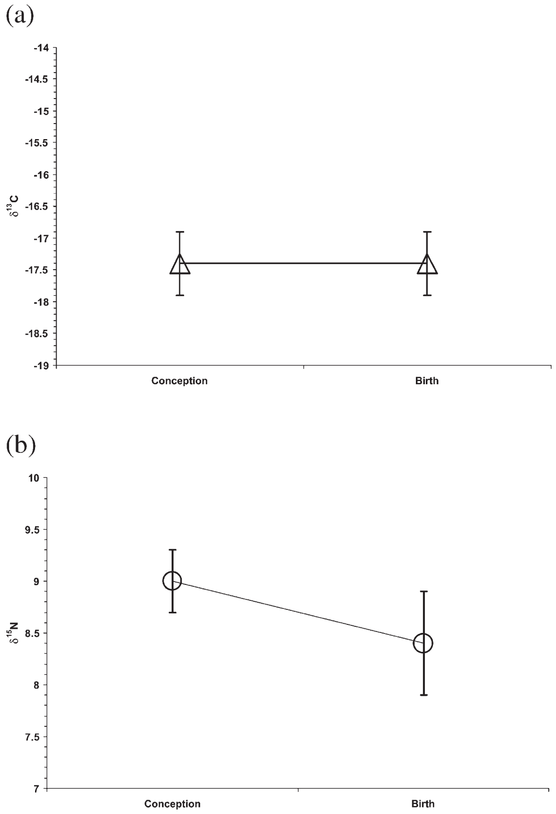


Figure 1. Graphs illustrating the change between conception and birth (mean ± SD) for the hair $\delta^{13}C$ (a) and $\delta^{15}N$ (b) results from 10 pregnant women. There is no consistent variation in the $\delta^{13}C$ values (conception = −17.4 ± 0.5‰; birth = −17.4 ± 0.5‰), but all subjects show a significant decrease in $\delta^{15}N$ between conception (9.0 ± 0.3‰) and birth (8.4 ± 0.5‰).

the weight variations recorded during gestation. Time was assigned to each of the hair segments (1 cm = 1 month) from the back estimation of the samples taken at birth, and a summary of the data for all subjects is shown in Fig. 3. While there is significant scatter, there is clearly an inverse correlation between $\delta^{15}N$ and weight gain such that increased maternal weight during pregnancy results in a drop in hair $\delta^{15}N$ values. This trend is seen in all subjects, and the data can be fitted to a linear function ($y = -0.0379x + 0.0174$) with $R^2 = 0.67$. The high degree of scatter observed in Fig. 3 was likely influenced by the lack of an isotopically homogenous diet, and by the fact that the monitored weight gain in this experiment included weight components from fat and water. As the protein gain represents only 8% of the total weight gain in pregnancy,[39] it is speculated that this correlation would improve significantly if only the protein portion of the weight gain had been measured. In addition, the hair was only sampled at birth and not at discreet intervals of time, and this potentially introduced error into the time measurements by the differences in individual hair growth rates, which in turn could have accounted for some of the scatter in Fig. 3.

The total decrease in maternal hair $\delta^{15}N$ from the pre-conception baseline to birth is plotted against the birth weight of each of the infants (Fig. 4). While a general trend is observed, the correlation is weak ($R^2 = 0.41$), and this suggests that total maternal weight gain (comprising the increases in maternal tissues, blood, placenta, amniotic fluid, and the fetus) is a better indicator of the magnitude of $\delta^{15}N$ decrease during pregnancy. This is not necessarily surprising since the fetus represents only approximately 40% of the total increase in protein gain during pregnancy.[40] Still, given the small number of subjects studied and our incomplete knowledge of the biochemical mechanisms involved in this decrease in $\delta^{15}N$, more research is required to better elucidate the relationships between $\delta^{15}N$, maternal weight gain, and infant birth weight.

DISCUSSION

The results of this study indicate that the isotopic values of hair appear to be altered by the metabolic and physiological changes of pregnancy, with $\delta^{15}N$ values decreasing significantly at birth compared to pre-pregnancy values. The discovery that all subjects display a decrease in $\delta^{15}N$ values (−0.3 to −1.1‰), even though they had access to a wide variety of foods with different isotopic signatures of nitrogen, strongly suggests that this depletion in ^{15}N is a metabolic and physiological phenomena of gestation and not the result of changing dietary habits. However, the lack of an isotopically uniform maternal diet possibly contributed to the large range of ^{15}N-depletions observed for the subjects. Similar hair ^{15}N-depletion patterns have also been observed in a cross-sectional study of pregnant and lactating women from The Gambia[41] and in another cross-sectional study of lactating horses from the USA.[42] Thus, the results of previous research combined with the findings of this study demonstrate that $\delta^{15}N$ values are influenced not only by diet, but also by perturbations in nitrogen homeostasis during pregnancy.

Nitrogen retention during pregnancy

As pregnancy is an anabolic state characterized by maternal and fetal protein synthesis, women are in positive nitrogen balance when given adequate nutrition.[43] A recent longitudinal human study of nitrogen balance where maternal energy and protein content were held constant revealed that nitrogen retention was +0.2 g/day before pregnancy and −0.4, +0.5, and +1.2 g/day at 12, 23, and 34 weeks gestation, respectively.[44] This progressive increase in nitrogen retention is attributed to a decrease in maternal urea synthesis and excretion and increased urea salvage.[45–49] Kalhan *et al.*[47] found that the rate of ureogenesis during pregnancy fell by 30% in the first trimester and by 45% in the third trimester, compared to non-pregnant controls, and Mojtahedi *et al.*[44] observed a statistically significant reduction in nitrogen excretion in urine during late pregnancy (11.0 ± 1.4 g/day) compared to early pregnancy (12.6 ± 1.3 g/day). Salvage of urea by microflora hydrolysis in the colon also increases significantly during gestation, suggesting that a failsafe mechanism exists whereby urea nitrogen can be returned to the maternal metabolic pool in times of nitrogen stress such as pregnancy.[45,46]

The physiological and biochemical mechanisms that cause this decrease in urea synthesis and excretion during pregnancy are not well defined. The response is likely driven by a rise in circulating hormone levels after conception; increases in progesterone and estrogen have been linked to suppression of the enzymes of the urea cycle.[50] Research by Kalhan *et al.*[47] suggests that increased insulin resistance could

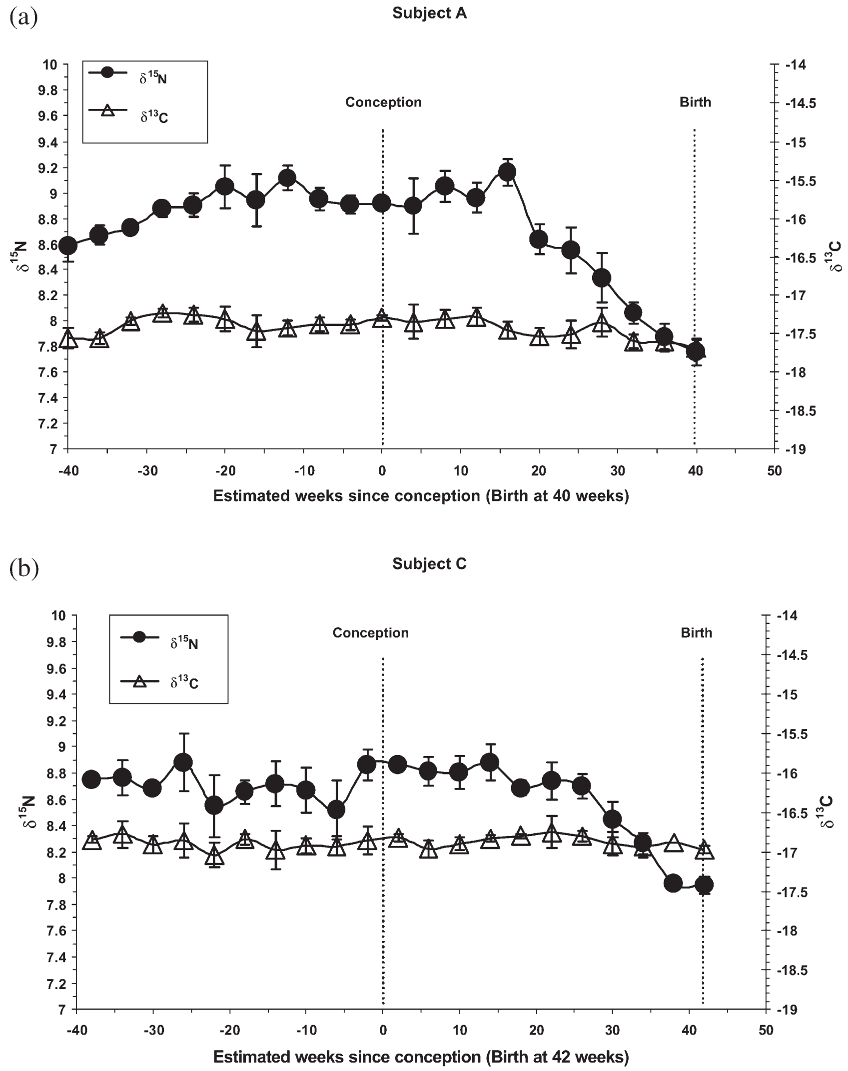
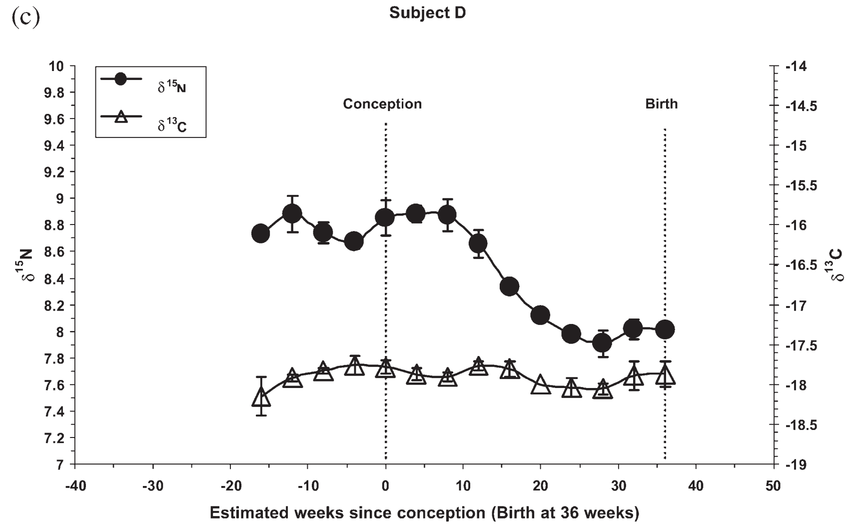
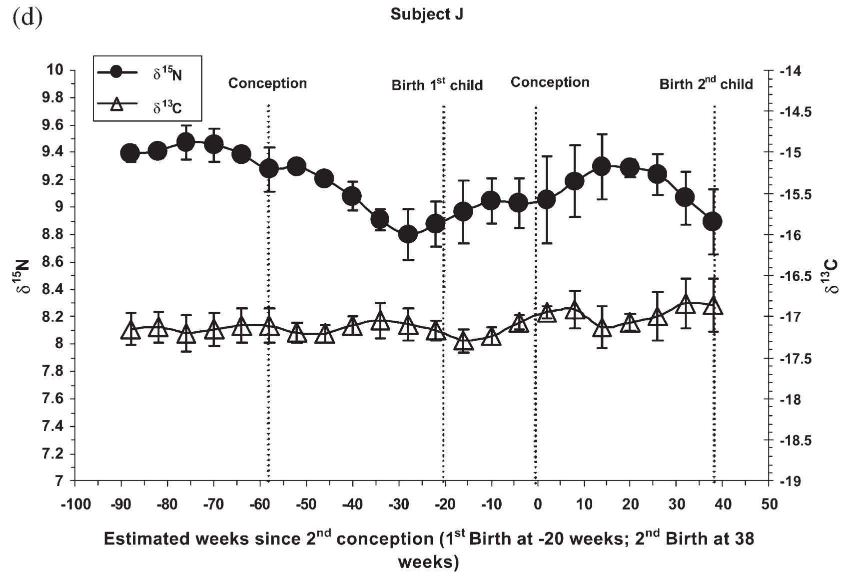


Figure 2. Representative graphs of typical $\delta^{13}C$ and $\delta^{15}N$ variations before and during pregnancy in human hair sampled at birth (a, b). Hair samples were analyzed in 1 or 1.5 cm sections corresponding to 4 or 6 week intervals of growth, respectively,[38] and thus the x-axis is time derived from measurement along the hair starting from the scalp. All samples were measured in triplicate with the error bars shown. In (c), subject D gave birth to twins, and in (d) the hair of subject J was sufficiently long to record two successive pregnancies.





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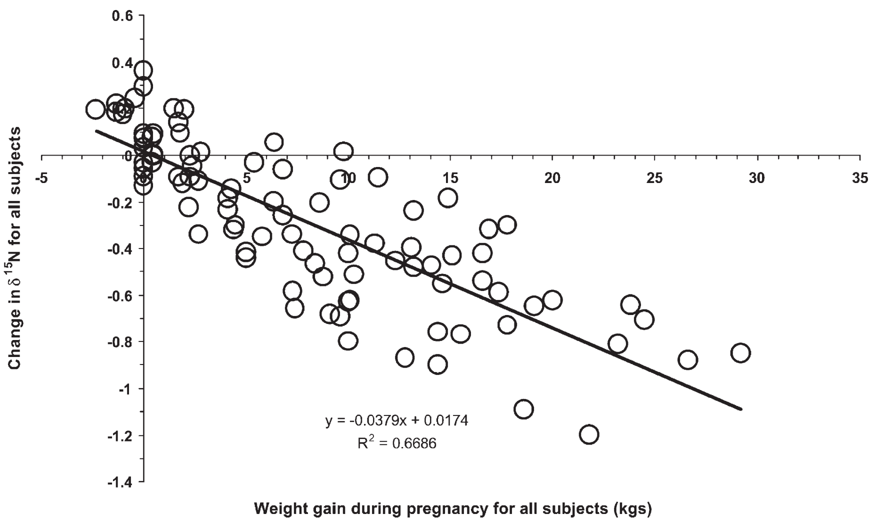

Figure 3. Changes in hair $\delta^{15}N$ plotted against maternal weight gain during pregnancy for all 10 subjects. An inverse correlation is observed such that decreasing hair $\delta^{15}N$ values correspond to increases in weight and thus positive nitrogen balance.

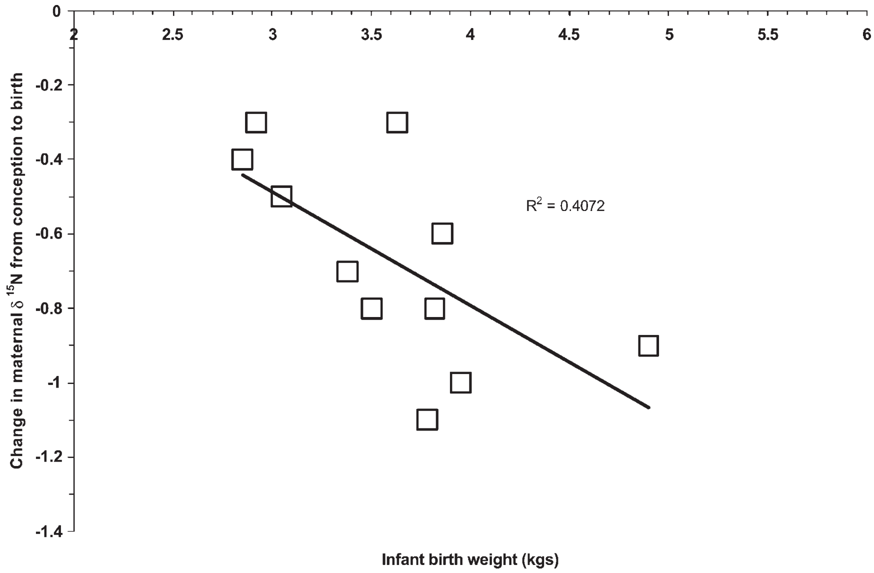

Figure 4. Infant birth weight plotted against total change in maternal hair δ^{15}N from conception to birth.

be a trigger for positive nitrogen balance during pregnancy since a decrease in the rate of urea synthesis was found to correlate with a decrease in the transamination of leucine. Pregnancy-induced resistance to insulin can result in higher levels of circulating glucose that is potentially available to the fetus for energy.[51] Since there is a close interrelationship between energy and nitrogen metabolism, this increased concentration of maternal blood glucose means that fewer amino acids need to be deaminated/transaminated and converted into glucose, resulting in a metabolic shift in the overall partitioning of amino acids in the mother from oxidation to deposition.[51] In a review of the literature, Duggleby and Jackson[51] estimate that protein synthesis during pregnancy is relatively unchanged for the first trimester, increases by 15% in the second trimester, and by an additional 25% in the third trimester. While the idea that insulin resistance is the main cause of nitrogen retention during pregnancy is speculative, it is interesting to note that other conditions of positive nitrogen balance (neonate growth, puberty) are also characterized by states of insulin resistance[47] and are predicted to have smaller diet to tissue trophic level effects for δ^{15}N.[13,41,52]

Possible mechanisms for the decrease in δ^{15}N during gestation

The reason for this decrease in hair δ^{15}N during pregnancy is likely complex, and the mechanism has not been fully elucidated. However, it is probable that the increased nitrogen retention during gestation plays a significant role. A recent study of llamas and goats fed identical alfalfa diets lends support to the importance of a ''urinary nitrogen excretion'' mechanism which influences the magnitude of tissue δ^{15}N values.[52] In this study the goats lost less urinary nitrogen (70%) and had lower diet to hair nitrogen fractionations (5.0‰) compared to the llamas that had urinary nitrogen losses of 77% and diet to hair fractionations of 6.3‰. It is speculated that increased nitrogen retention can lead to lower δ^{15}N values by two different pathways. First, in times of increased nutritional demands such as pregnancy, the body can preferentially reroute more dietary amino acids from oxidation and excretion toward deposition at sites of tissue synthesis.[51] Since δ^{15}N values increase in a relative step-wise fashion as one moves up the food chain (the trophic level effect), dietary δ^{15}N values are lower than consumer tissue δ^{15}N values by approximately 3–4‰.[2–4] Thus, the more direct assimilation of a greater percentage of dietary nitrogen with lower δ^{15}N values results in a reduction in the 'normal' or steady state diet to body trophic level fraction by approximately 0.5–1‰. Second, this decrease in maternal hair δ^{15}N values could also be influenced by increased urea salvage by microflora in the colon.[45,46] Since human urine is ^{15}N-depleted compared to body tissues, with δ^{15}N values ranging from 3–5‰,[53,54] the return of this isotopically light nitrogen to the metabolic pool for protein synthesis could potentially result in a decrease in maternal δ^{15}N values during gestation.

In addition, an alternate explanation for the drop in the hair nitrogen isotopic ratio during pregnancy is that, while the maternal tissue is decreasing in δ^{15}N, the fetus is becoming progressively enriched in ^{15}N such that the offset between the mother/fetus pair is essentially balanced. Thus, the fetus would be feeding off the maternal tissue and would be a trophic level higher than the mother. However, experimental evidence seems to contradict this since infant hair and fingernails formed *in utero* are only slightly enriched compared to maternal samples.[54–56] In addition, instead of increasing during gestation, the infant fingernail and hair δ^{15}N values tend to decrease in parallel with the falling maternal values.[54–56] This suggests that there is a small and relatively constant fractionation factor between the mother and fetus that is likely mediated by placental uptake and excretion. However, the intricacies and exact mechanism of this decrease in maternal δ^{15}N are not fully understood, and it is clear that more detailed research is needed to unravel the biochemical complexities of this issue.

Potential applications

The potential applications of these findings and this technique are far ranging. For palaeodietary and ecological studies, knowledge that $\delta^{15}N$ can be influenced by pregnancy and weight changes is crucial to correct dietary interpretation. The current assumption is that differences in $\delta^{15}N$ values between males and females primarily reflect changes in the amount of animal versus plant protein consumption.[3,4] In terms of archaeological research, it is unknown if this isotopic depletion observed in hair will be seen in bone collagen since it continuously remodels, but research suggests that there is increased bone formation and bone resorption during the third trimester of pregnancy and lactation.[57] Measurements of bone mineral density in the axial skeleton have found average reductions of 3–5% with some women having reductions as high as 10% during pregnancy and lactation.[58,59] The resorption of the bone matrix and the subsequent remineralization after weaning during a lifetime of pregnancies and periods of lactation could potentially result in changes in the $\delta^{15}N$ values of female skeletons, but there is yet to be research in this area.

While it is unknown if the nitrogen isotopic effects of gestation will be registered in bone collagen, the results of this study suggest the possibility of detecting pregnancy in other metabolically inert tissues (teeth, feathers, horns, tusks, otoliths, etc.) that record isotopic signatures through time. Previous research measuring the isotopic composition of annual growth lines in mammoth tusks,[60] whale baleen plates,[61] and marine mammal teeth,[62,63] has shown that this dentine preserves the dietary, environmental, and physiological conditions of the animals. Thus, analysis of $\delta^{13}C$ and $\delta^{15}N$ values in dentine along the length of a tusk from an extinct animal such as a mammoth could potentially detect fertility patterns in past species.[42] In addition, sampling and analyzing $\delta^{15}N$ values in hair, blood, and urine from modern animals provides ecologists and physiologists with a possible method of assessing pregnancy and gross nitrogen metabolism from different animal species.

This technique is not limited to archaeological and ecological research and would likely benefit modern medical studies of protein stress and nitrogen balance under a variety of conditions such as pregnancy, anorexia, bulimia, exercise, disease, weight loss, or burns. Currently, one of the most effective means of estimating nitrogen balance in pregnancy is by administering ^{15}N-labeled compounds such as glycine or urea.[45,64,65] While this method is effective, it does suffer from limitations that could be alleviated by $\delta^{15}N$ measurements in human hair. Due to the unpredictable nature and expense of recruiting women before they become pregnant, these enriched tracer experiments do not usually obtain data from pre-pregnancy, and thus comparison of any nitrogen variations relative to the non-pregnant state is difficult.[51] In addition, these ^{15}N-labels are only administered over a limited period of time (days) and cannot capture the nitrogen fluctuations during the course of the entire pregnancy. Analysis of $\delta^{15}N$ values in hair which was formed before and during gestation is a simple, inexpensive, and non-invasive technique, and it could be used in conjunction with the ^{15}N-labeled tracer method to gain more insight into the protein requirements and nitrogen metabolism of women during pregnancy.

CONCLUSIONS

We have conducted a longitudinal study of hair $\delta^{13}C$ and $\delta^{15}N$ values in women before conception and during gestation. While no consistent change was observed in the $\delta^{13}C$ results (Fig. 1(a)), all subjects showed a decrease in their $\delta^{15}N$ values (−0.3 to −1.1‰) during pregnancy (Table 1). An inverse correlation ($R^2 = 0.67$) between $\delta^{15}N$ and weight gain during pregnancy was also observed (Fig. 3), but there was little correlation ($R^2 = 0.41$) between infant birth weight and the drop in maternal hair $\delta^{15}N$ values. The biochemical mechanisms causing this decline in hair $\delta^{15}N$ values are not fully understood. It is speculated that during anabolic states (such as pregnancy) the redirection of dietary amino acids from oxidation/excretion to tissue synthesis, and an increase in urea salvage, result in falling $\delta^{15}N$ values during gestation since dietary nitrogen and urea nitrogen both have ^{15}N-depleted values compared to maternal protein. Thus, the increased utilization of this lighter nitrogen by the body results in a reduction in the steady state diet to body trophic level fractionation factor by approximately 0.5–1‰ during pregnancy.

The results of this study demonstrate that $\delta^{15}N$ values are influenced not only by diet, but also by fluctuations in nitrogen homeostasis during human pregnancy. Protein synthesis or positive nitrogen balance causes $\delta^{15}N$ values to decrease, while lean muscle catabolism or negative nitrogen balance results in increasing $\delta^{15}N$ values.[16–19] The finding that $\delta^{15}N$ results can change independently of diet does not invalidate their use in dietary reconstruction since the magnitude of the depletion in ^{15}N (0.5‰ to 1.0‰) is small compared to the trophic level effect. However, this fact needs to be considered when dietary interpretations are being formulated for palaeodietary studies and ecological research with special attention given to individuals or organisms that might be out of nitrogen balance. In addition, patterns of static $\delta^{13}C$ and decreasing $\delta^{15}N$ values can be examined in tissues that linearly record isotopic values to possibly deduce the number of pregnancies in modern and ancient organisms. Finally, while this research has demonstrated the potential of using $\delta^{15}N$ values from human hair to monitor perturbations in nitrogen balance in pregnancy, it is hypothesized that this technique can also be used to study other conditions in which humans and animals are not in a steady metabolic state.

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