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Review

Beyond Diet Reconstruction: Stable Isotope Applications to Human Physiology, Health, and Nutrition

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ABSTRACT: Analysis of stable carbon and nitrogen isotopes from soft or mineralized tissues is a direct and widely-used technique for modeling diets. In addition to its continued role in paleodiet analysis, stable isotope analysis is now contributing to studies of physiology, disease, and nutrition in archaeological and living human populations. In humans and other animals, dietary uptake and distribution of carbon and nitrogen among mineralized and soft tissue is carried out with varying efficiency due to factors of internal biology. Human pathophysiologies may lead to pathology-influenced isotopic fractionation that can be exploited to understand not just skeletal health and diet, but physiological health and nutrition. This study reviews examples from human biology, non-human animal ecology, biomedicine, and bioarchaeology demonstrating how stable isotope analyses are usefully applied to the study of physiological adaptation and adaptability. Suggestions are made for future directions in applying stable isotope analysis to the study of nutritional stress, disease, and growth and development in living and past human populations. Am. J. Hum. Biol. 25:445–456, 2013. © 2013 Wiley Periodicals, Inc.

INTRODUCTION

Analysis of stable isotope ratios from soft or mineralized tissues is a direct and widely-used technique for reconstructing diets. The utility of stable carbon, nitrogen, and sulfur isotope analysis derives from the fact that "you are what you eat": stable isotope signatures of foods are reflected by consumer tissues (for a recent review on principles of stable isotope analysis in anthropology, see Schoeninger (2011)). Since its initial applications in anthropology in the 1970s (van der Merwe and Vogel, 1978; Vogel and van der Merwe 1977), stable isotope analysis is now widely used to study past human diet through time and across space, and is important in the tool kit of bioarchaeologists, who are concerned with past human health. Along the way, assumptions have been made regarding the link between stable isotope data and nutrition. Diet and nutrition, though related, are not coterminous, and an understanding of diet is not necessarily an understanding of nutrition or of health. Stable isotope ratios do not reproduce exact menus, but rather distinguish between broad food categories: for example, meat versus plants, terrestrial versus aquatic protein sources, or C_4 grasses/cereals vs. C_3 fruits/vegetables (Honch et al., 2012; Nehlich et al., 2011; Schoeninger and DeNiro, 1984). Accordingly, stable isotope ratios do not reveal "good" and "bad" diets, which is a distinction difficult to make even among living humans. Although intra- and inter-population differences in diet may have implications for health disparities, the human body is extremely plastic and can synthesize many of its nutrients from a wide variety of foods, meaning human health outcomes are well-buffered against the types of changes in diet which stable isotope ratios are traditionally used to assess.

Diet reconstruction through traditional stable isotope analysis remains an indispensable tool in physical anthropology. However, stable isotope analysis is moving into other areas involving the study of past *health*—a term impossible to define, but used henceforth in general reference to pathophysiologies and nutritional stresses that reduce functionality. Beginning with the discovery that starvation in birds leads to ^{15}N enrichment of body tissues (Hobson et al., 1993), much work has been conducted in recent years to open the possibility of a stable isotope biochemistry for understanding past and present health. Human biology influences the dietary uptake, distribution and excretion of stable isotopes among mineralized and soft tissues (Schoeller, 1999). Associated pathology-dependent isotopic variation can be assessed to understand not just skeletal health, but physiological health, and not just diet, but nutrition. By taking into account not just foods and their characteristic stable isotope signatures, but also the physiological factors influencing how stable isotope ratios are fixed by the body, biological anthropologists stand to make significant inroads into our understanding of health and nutrition over a broad range of contexts and time periods.

This review is of areas in which innovations in the analysis of natural abundances of stable isotopes have moved beyond diet reconstruction into topics more closely allied with health, including physiology and disease processes among living humans, and paleopathology in the past. A particular goal of the review is to highlight the physiological mechanisms underlying stable isotope variation in humans, drawing frequently from human biology and animal ecology. In many cases only preliminary evidence is available, and this paper underscores future potential as much as it reviews efforts-to-date. This review focuses on stable carbon and nitrogen isotopes, but other elements are introduced as relevant to monitoring physiology and health. For more background on carbon, nitrogen, and oxygen isotope analysis in anthropology, see Katzenberg (2008) and White et al. (2004). Stable sulfur isotope ratios, the utility of which is increasingly apparent in studying migration patterns, marine food consumption, and weaning patterns, are dealt with in greater detail by Richards

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et al. (2001) and Nehlich et al. (2011, 2012). For more information on hydrogen see O'Grady et al. (2012), and for calcium, Heuser and Eisenhauer (2010) and Morgan et al. (2012). For more information on stable isotope analyses using individual amino acids, see the work by Petzke et al. (2005) and Honch et al. (2012). Stable isotope ratios are reported as delta (δ) values, which express the ratio of the heavier isotope to the lighter isotope relative to laboratory standards in parts per thousand, or "per mil" (‰).

PHYSIOLOGY

It is well-known that stable isotopes of foods are reflected in the tissues of consumers. However, the relationship is not direct, but is mediated by consumer physiology through a process known as fractionation (for a useful review of isotopic fractionation, see the work by Schoeller (1999)). Stress responses and compromised health affect the physiological processes underlying stable isotope fractionation and distribution in the body. Such pathophysiologies may create inter-individual isotopic variations that permit, in theory, identification of biologically meaningful health, stress, and/or nutrition shifts. These perspectives offer a new window into the physiological status of living humans. When interpreted alongside data from the archaeological record and paleopathology, the relationships between stable isotope signatures and health can become a useful tool to be tapped in bioarchaeology. In this section, physiological mechanisms which affect stable isotope signatures are reviewed, including negative nitrogen balance, positive nitrogen balance, and cases of associations between stable isotope ratios and specific disorders.

Catabolism in protein insufficiency

Both stable nitrogen (δ^{15}N) and stable carbon (δ^{13}C) ratios, but especially the former, have been used to study starvation and fasting in a variety of animals (e.g., Deschner et al., 2012; Doucett et al., 1999; Hobson et al., 1993; Polischuk et al., 2001). When protein is utilized for tissue building by the body, isotope fractionation occurs during transamination (synthesizing non-essential amino acids *in vivo*) and deamination (breaking down any excess protein not used for tissue building). After fractionation, body tissues are enriched in ^{15}N and body wastes (urea) are enriched in ^{14}N relative to the δ^{15}N values of the diet (Steele and Daniel, 1978). The overall effect of these fractionating processes on δ^{15}N ratios in body tissues is measurable and usually referred to as the diet-tissue space or a trophic effect. Between diet and bone collagen, the effect is on the order of approximately +3‰ (Minawaga and Wada, 1984; Schoeninger and DeNiro, 1984), and is the primary reason why δ^{15}N values are used in paleodiet reconstructions. Previously reported isotopic differences among vegans, ovo-lacto-vegetarians, and omnivorous humans due to this trophic effect are shown in Figure 1 (Petzke et al., 2005).

When an individual does not ingest sufficient protein ("sufficient" protein intake varies among species), the body can resort to catabolizing its own tissues. Recycled body tissues repeat the fractionating processes of transamination and deamination, leaving body tissues of a protein-stressed individual even more enriched in ^{15}N. This effect was first reported among birds (Hobson et al., 1993; Hobson and Clark, 1992). Captive quails fed a maintenance diet were shown to exhibit higher δ^{15}N ratios than quails on a growth diet, and wild Ross' geese exhibited significantly higher δ^{15}N ratios after 19–25 days of fasting associated with incubating new clutches (Hobson et al., 1993). Significant δ^{15}N differences in the liver, muscle, blood, and bone of the nutritionally stressed animals on the order of +0.5–2.0‰ were detected (Fig. 2). This study has been supported by numerous others with a variety of animals (Barboza and Parker, 2006; Barboza and Parker, 2008; Boag et al., 2006; Cherel et al., 2005; Gustine et al., 2011; Oelbermann and Scheu, 2002; Parker et al., 2005; Polischuk et al., 2001), although there are exceptions (Ambrose, 2000; Kempster et al., 2007; Williams et al., in press). Hatch (2012) reviews a number of

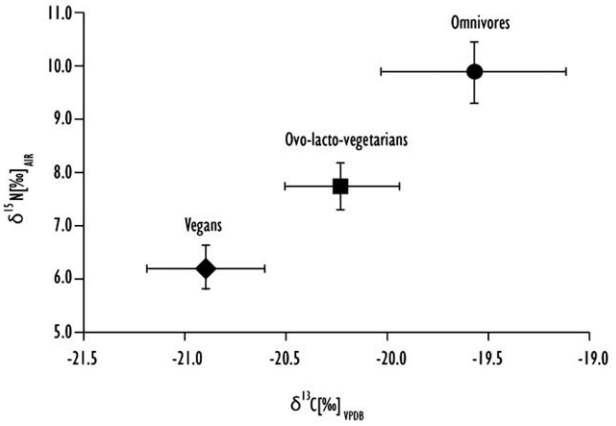

Fig. 1. Isotopic differences due to trophic-level enrichment, shown by comparing the hair stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope values of modern human vegans, ovo-lacto vegetarians (consumers of eggs and milk, but not animal flesh) and omnivores (data from Petzke et al. (2005b)).

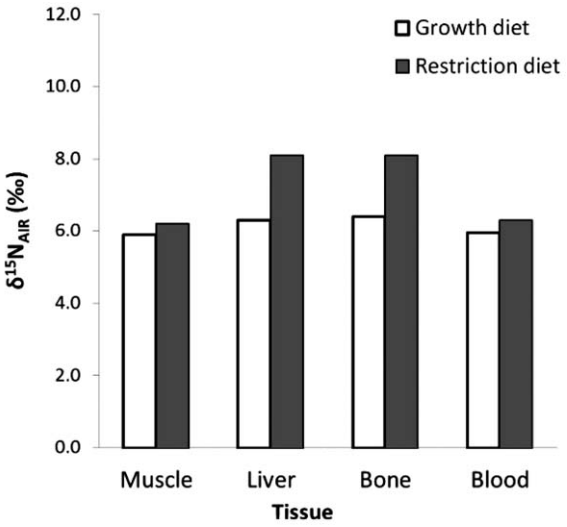

Fig. 2. Differences in mean stable nitrogen isotope (δ^{15}N) values of captive quails fed ad libitum (controls) and quails fed restriction diets after hatching which limited weight gain. The elevated δ^{15}N values of restricted diet quails are caused by fractionation due to catabolism. All differences are statistically significant ($P < 0.05$, Wilcoxon rank-sum test) (modified from Hobson et al. (1993).

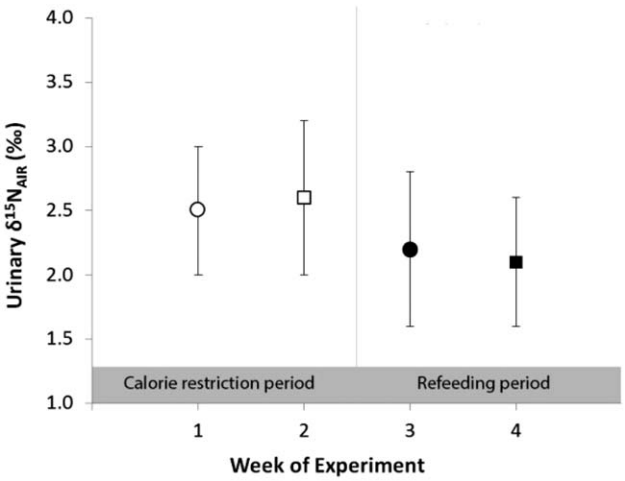

Fig. 3. Changes in the urinary stable nitrogen isotope (δ^{15}N) values of seven bonobos during the course of a diet restriction experiment. In Weeks 1 and 2, calorie-rich foods were gradually removed from the diet, and in Weeks 3 and 4, gradually replaced. δ^{15}N values were inversely related to body weight, reflecting the intensification, and eventual subsiding, of tissue catabolism during calorie restriction (data from Deschner et al. (2012)).

the exceptions and suggests there may be a threshold, below which δ^{15}N values are unaffected even in nutritional stress. That is, if an organism is not severely stressed, no effect may be detected. Ambrose (2000) proposes that while weight loss after maturation may occasion an isotopic effect, growth restriction during childhood growth may not (but see Kempster et al., 2007; Williams et al., 2012).

Other exceptions to the expected ^{15}N enrichment with food restriction likely have to do with the competing influence of positive nitrogen balance during growth, a comprehensive review of which is provided by Waters-Rist and Katzenberg (2010). Actively growing organisms route dietary protein directly to tissues, bypassing transamination and deamination. As an example, Williams et al. (2012) found δ^{15}N values of nutritionally restricted puffin chicks to be lower than chicks fed ad libitum, and suggest rapid growth (positive nitrogen balance) may reduce tissue δ^{15}N values as more protein is used for tissue building, and less nitrogen excreted with urea. In a study of humans, Fuller et al. (2004) observed that during pregnancy, while expectant mothers gained weight, the δ^{15}N values of their hair decreased, indicating a state of positive nitrogen balance during growth.

Protein stress has been demonstrated isotopically in non-human primate studies. Vogel et al. (2012), in their study of the effects of seasonal resource scarcity on nitrogen flux in wild Bornean orangutans, found that orangutan urine was slightly enriched in ^{15}N during times of low protein intake, reflecting tissue catabolism for cellular maintenance. Similar results were reported by Deschner et al. (2012), who found higher δ^{15}N values of urine in nutritionally stressed captive bonobos in a controlled feeding experiment. In the experiment, captive bonobos were fed restriction diets with low digestible energy, which occasioned weight loss, and were switched after 2 weeks to a calorie-rich diet that eventually led to an overall weight increase. Glucocorticoids, δ^{15}N, ($P = 0.002$) and δ^{13}C ratios were measured from urine alongside the weight changes. Consistent with previous findings on positive and negative nitrogen balance, during the restriction period δ^{15}N and δ^{13}C increased (Fig. 3). A novel contribution of this study was the incorporation of urinary glucocorticoid levels, which correlate with both δ^{15}N ($P = 0.002$) and δ^{13}C ($P = 0.005$), providing dual measures of the effects of nutritional challenges.

The findings of both Vogel et al. (2012) and Deschner et al. (2012) are particularly important because the animals were not starved. Foods were always available, but digestible energy content varied and the isotopic effects were still observed. The studies demonstrate the utility of stable isotope ratios to detect changes in nutrition without starvation, which is highly relevant among humans who are culturally and physiologically flexible when it comes to diet. Among humans, severe nutritional stress and/or starvation are not nearly as common as moderate nutritional stress.

An important window into relationships between δ^{15}N values and negative nitrogen balance among living humans is from investigations of individuals with eating disorders. In two separate studies, Hatch et al. (2006) and Mekota et al. (2006) sampled hair sequentially from individuals with anorexia and/or bulimics after their admittance to a recovery facility, obtaining a longitudinal record of isotopic shifts throughout the recovery period. The ends of hair represented the "sickest" period while the roots represented the "healthiest" period. Changes in δ^{13}C and δ^{15}N values were measured along the hair through the treatment phase (Hatch et al., 2006; Mekota et al., 2006) and also in comparison to controls (Hatch et al., 2006). During the "sickest" periods, δ^{15}N values from hair of affected individuals were approximately 0.5–2.0‰ higher than values after treatment. Mekota et al. (2006) showed these decreasing δ^{15}N values to be inversely associated with increasing BMI. Hatch et al. (2006) observed elevated δ^{15}N values among anorexic, but not bulimic, individuals. Enrichment in hair ^{15}N during the "sickest" periods was likely the result of catabolism of endogenous tissues necessitated by a state of gluconeogenesis among these malnourished individuals (Mekota et al., 2006).

Important is the absence of ^{15}N enrichment among bulimics, which suggests how, even in spite of a serious eating disorder that presumably occasioned weight loss, enough protein was ingested to avoid negative nitrogen balance and a corresponding isotopic effect (Hatch et al., 2006). This differs from the finding of an isotopic effect with moderate protein restriction among non-human primates (Deschner et al., 2012), and may have something to do with differences in the tissues analyzed. Hair sampled by Hatch et al. (2006) reflects a longer time-averaged period than does the urine sampled by Deschner et al. (2012), and it might be expected that the substrate with the rapid synthesis rate should be more sensitive to moderate changes in diet while the relatively inert tissue does not. Different tissue substrates reflecting different "chronological snapshots" are presented in Table 1. Such a difference between the isotope signals of hair and urine underscores the potential difficulty of observing these isotope effects in bones, which turn over on the order of years. However, for human biologists, the application of stable isotope analysis to soft tissues of living humans offers a highly productive method for understanding

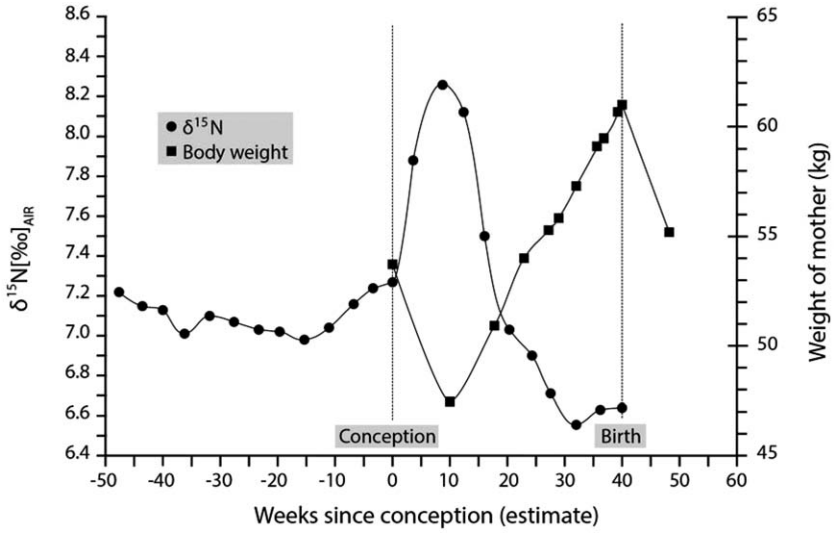

Fig. 4. Stable nitrogen isotope (δ^{15}N) and body weight changes during the pregnancy of a mother who experienced morning sickness (modified from Fuller et al. (2005)). After conception, morning sickness occasioned weight loss. This body weight change is accompanied by elevated δ^{15}N values, evidence of tissue catabolism. As the mother begins to regain weight, δ^{15}N values decline. The δ^{15}N values continue to decline as the mother gains weight in her pregnancy, reflecting a state of positive nitrogen balance in which much nitrogen is routed to tissue growth, and not fractionated and excreted with urea.

TABLE 1. Mineralized tissues representing different periods of an individual's life

Less recently formed		More recently formed	Example(s)
Diaphysis	vs.	Epiphysis/metaphysis	(Richards et al., 1998; Waters-Rist et al., 2010)
Teeth (enamel; dentine)	vs.	Bone (collagen; bone mineral)	(Reitsema and Vercellotti, 2012)
Early-mineralizing permanent teeth (e.g., incisors)	vs.	Late-mineralizing permanent teeth (e.g., second molar)	(Dupras and Tocheri, 2007; Sealy et al., 1995)
Deciduous teeth	vs.	Permanent teeth	(Dupras and Tocheri, 2007)
Tooth crown	vs.	Tooth root	(Fuller et al., 2003)
Cortical bone	vs.	Trabecular bone	(Sealy et al., 1995)
Insoluble bone fraction[a]	vs.	Soluble bone fraction[a]	(Bell et al., 2001)
Slowly remodeling bone	vs.	Rapidly remodeling bone	(Jørkov et al., 2009; Sealy et al., 1995)

[a]See Bell et al. (2001) for a description of the soluble and insoluble bone fractions.

nutritional stress, changes in BMI, and growth trajectories.

Pregnancy

The innovative work of Fuller et al. (2004, 2005) demonstrated that among pregnant mothers experiencing morning sickness (nutritional stress), hair δ^{15}N values are elevated compared to (1) other mothers, and (2) periods without morning sickness from the same individuals (Fig. 3). The sickest mothers exhibited the greatest ^{15}N enrichment, and δ^{15}N values returned to "normal" as weigh recovery progressed during pregnancy, indicating their more severe nutritional stress and a state of negative nitrogen balance (Fuller et al., 2005).

There are some indications from non-human animal ecology that lactation may engender stable isotope shifts in mothers (Habran et al., 2010; Jenkins et al., 2001; Kurle, 2002; Polischuk et al., 2001; Reitsema, 2012). Breast milk is a major source of low-^{14}N nitrogen excretion in the form of urea during lactation (Schoeller, 1999), which could leave maternal tissues comparatively enriched in ^{15}N. Indeed, the δ^{15}N values of milk are generally lower than those of body tissues (Fuller, 2003, p 77–85). Information on the stable isotope signatures of human breast milk is very limited (Fogel et al., 1989; Fuller, 2003), and more work is needed to identify the nature and extent of isotopic shifts among mothers associated with lactation.

Although virtually unexplored among living humans, stable isotope analysis has clear applications to studying breastfeeding and weaning. Infants who breast feed are essentially a trophic position higher than their mothers, which manifests in elevated δ^{15}N ratios of infants. The potential to identify weaning behavior has been widely tapped in bioarchaeology (reviewed by Katzenberg et al. (1996)) and to a lesser extent in animal ecology (see Reitsema (2012)) but has received very little attention among living humans (c.f., Fogel et al., 1989; Fuller et al., 2006). Stable isotope analysis is a highly useful way to assess an infant's nutritional dependence on its mother. Incrementally growing tissues, such as hair, offer weekly to monthly snapshots of weaning status that are recorded in the absence of researchers, and are not subject to informant recall.

Weaning has the potential to adversely affect health and fitness of both mother and infant (Hobcraft et al., 1983; Jelliffe and Maddocks, 1964; Mozumder et al., 2000;

Tracer, 1991; Valeggia and Ellison, 2009). Foods used to wean infants may be nutritionally inferior to breast milk. Furthermore, breast milk contains resistance factors, such as immunoglobins, that buffer a breastfeeding infant from outside pathogens. Alongside these challenges to an infant's physiological state is the additional problem of outside foods introducing a new vector for infection and illness. Early weaning and/or exclusive bottle-feeding have been shown to increase risk of infant mortality in both historical and modern human populations (reviewed by Katzenberg et al. (1996) and Valeggia and Ellison (2009)). In addition to monitoring weaning, stable isotope analysis has the potential to offer insights on infants' nutritional transitions during and after weaning by assessing nitrogen balance. How does infant physiology help navigate this transition? If body reserves are being mobilized to buffer a difficult weaning process, δ^{15}N signatures should theoretically be affected, showing an increase during the nutritionally stressful periods. Alternatively, it has also been shown that in less extreme cases of protein stress where tissue building and growth is still possible, urea nitrogen may be increasingly used for protein synthesis. This may cause lower δ^{15}N values among growing infants in a moderately low-protein environment (Fuller, 2003, p 90–91; Rikimaru et al., 1985). These cases are largely speculative, and more work is needed to identify the potential utility of stable isotope analysis in monitoring growth and development.

High-protein diets

Somewhat paradoxically considering the influence of low-protein diets and catabolism on raising tissue δ^{15}N, controlled feeding experiments with animals have indicated that unusually high δ^{15}N values also are associated with *high*-protein diets (Sponheimer et al., 2003a,b). This is because when there is a surfeit of protein in the diet, deamination occurs to rid the body of the excess. A fractionating process, deamination causes ^{15}N enrichment in the body. High protein in the diet or any other physiological condition leading to increased bioavailability of protein could instigate this metabolic response. Because diets including omnivore, suckling animal or fish protein will also lead to ^{15}N enrichment in consumer tissues, this physiological effect of high protein on δ^{15}N values may be obscured. Analysis of stable sulfur isotope ratios (Nehlich et al., 2012) and individual amino acids (Choy et al., 2010; Corr et al., 2005) can help distinguish among these other sources of high δ^{15}N values.

Whereas essential amino acids are useful to provide a more direct link between tissue and food stable isotope ratios, nonessential amino acids also have interesting advanced applications, as the various nonessential amino acids are synthesized by way of different mechanisms (Choy et al., 2010; Styring et al., 2010). In cases of high protein diets, non-essential amino acids may be increasingly synthesized from excess protein. For example, Choy et al. (2012) studied paleodiet using ancient human remains from the site of Tongsamdong, Korea by assaying δ^{13}C and δ^{15}N signatures of 16 individual amino acids from bone collagen. Two of the non-essential amino acids, serine and glycine, exhibited δ^{13}C values that were lower than what was anticipated for the expected marine-based diets of the population. The authors suggest that serine and glycine may have been synthesized from the essential

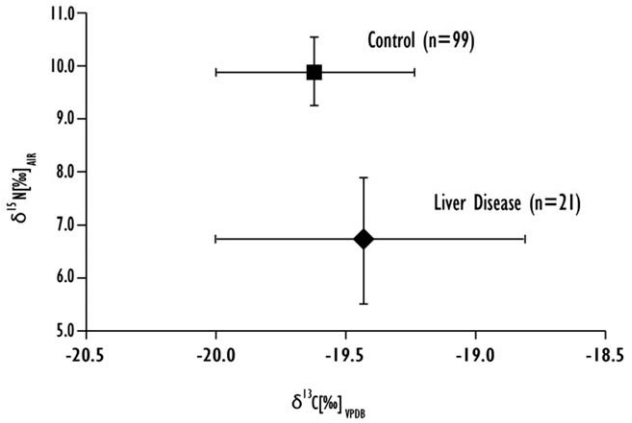

Fig. 5. Means and standard deviations of the hair stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope values of patients with cirrhosis ($n = 21$) and healthy controls ($n = 99$) reported by Petkze et al. (2006). Significant differences were detected in δ^{15}N values but not in δ^{13}C values.

amino acid threonine, which in marine fish is lower in ^{13}C than serine and glycine (Choy et al., 2010, Fig. 5; p 6108). This threonine dehydrogenase pathway is a relatively uncommon manner of serine and glycine biosynthesis, but is more often utilized in high-protein diets (Choy et al., 2010; Darling et al., 2000). Thus, both the utilization of this pathway as well as the mobilization of excess protein from fish for the synthesis of nonessential amino acids point to a diet high in protein. Careful consideration of the biosynthesis of nonessential amino acids may shed light on the significance of isotopic variation brought on through biosynthesis of other nonessential amino acids (c.f., Petzke et al., 2006; discussed next).

Specific pathologies

It is appreciated in biomedicine that some specific diseases and conditions may be directly associated with stable isotope signatures, independent of diet. Biomedical applications of stable isotopes typically involve isotopic labeling for tracer studies rather than assessments of natural abundances. Here, some examples of the effects of specific disorders on natural abundances of stable isotopes in the body are discussed, including liver disease, diabetes, and osteoporosis.

Liver disease. After protein is broken down in the stomach, constituent amino acids are transported to the liver where nitrogen is metabolized into serum proteins and urea in two separate fractionating processes (Sick et al., 1997). Fractionation during urea production leaves urea higher in ^{14}N than the original nitrogen pool. If there is a shift toward utilizing more nitrogen for protein synthesis rather than urea production, more ^{14}N is available in the liver for protein synthesis. Additionally, biosynthesis of different amino acids through transamination, which occurs primarily in the liver, is associated with different fractionation factors (Macko et al., 1986; Sick et al., 1997). In recognition of the fact that disorders of the liver are closely involved in amino nitrogen metabolism in the body, Petzke et al. (2006) compared the δ^{15}N and δ^{13}C values of hair from patients with liver disease and those of

healthy controls. Bulk protein δ^{15}N was 3.2‰ lower among the cirrhotics compared to healthy controls, without significant differences in δ^{13}C (Fig. 5). Additionally, nearly every essential and nonessential amino acid differed in δ^{15}N between the cirrhotic and healthy individuals: most, but not all, amino acid δ^{15}N values were lower among the cirrhotics. The lower δ^{15}N values of cirrhotics may have to do with "decreased nitrogen disposal"—that is, retention of relatively more nitrogen that in healthy individuals is fractionated and excreted. A complete explanation requires further investigation, but Petzke et al. (2006) suggest "disturbances in the liver amino nitrogen metabolism" are responsible for at least some of the isotopic differences (Petzke et al., 2006, p 2977). Disturbances in amino acid biosynthesis in other pathophysiological conditions that may affect amino acid metabolism warrant exploration.

Diabetes. Stable hydrogen (δ^{2}H) and oxygen (δ^{18}O) isotope ratios in body water are positively correlated with those of drinking water (Longinelli, 1984; Luz et al., 1984). This lends δ^{18}O analysis its utility in studies of migration and mobility (c.f., Mitchell and Millard, 2009). However, isotopic fractionation associated with the production of metabolic water from foods contributes to body water being slightly, but systematically, higher than drinking water and free water in foods (Luz et al., 1984; O'Grady et al., 2010). The δ^{2}H and δ^{18}O signatures of body water thus vary depending on the relative contributions of drinking/free water and metabolic water to the overall body water pool. Diabetes mellitus is a condition that causes dehydration, leading to increased fluid intake, and consequently, a larger proportion of the body pool oxygen deriving from fluids, and less from foods. O'Grady et al. (2010) demonstrated that among mice with streptozotocin-induced diabetes, δ^{2}H and δ^{18}O values of body water (urine) were more similar to those of drinking water than the δ^{2}H and δ^{18}O values of control mice, reflecting the relatively greater contribution of drinking water to the overall body pool among diabetics. Diabetic mice drank five to six times more water than control mice, and produced more urine daily. Isotopic differences between the mouse groups were significant, with diabetic mice exhibiting significantly lower values (δ^{2}H: -88.6 ± 2.3‰ vs. -101.9 ± 5.4‰; δ^{18}O: -7.9 ± 0.5‰ vs. -10.6 ± 1.0‰; $P < 0.0001$).

Kuo et al. (2012) also investigated δ^{2}H and δ^{18}O variation in the context of renal function using blood plasma from human subjects. The authors did not find significant differences among diabetics and healthy controls, but did identify significantly lower δ^{2}H and δ^{18}O values in the group of individuals with end stage renal failure. In addition to the effects of increased water flux, the authors note that accumulation of isotopically "light" metabolites in the kidneys may contribute to this difference. Interestingly, there is evidence that "heavy" water (e.g., water consisting of the heavier atoms ^{2}H and ^{18}O) is toxic to the kidney (Katz et al., 1957; Thomson and Klipfel, 1958). Together, these studies suggest a potentially useful role to urinary stable isotope analysis for understanding kidney disease, including early detection.

Osteoporosis. It has been shown that stable calcium isotope ratios ($\delta^{44/40}$Ca) are a biomarker for bone loss (Heuser and Eisenhauer, 2010; Morgan et al., 2012). Among humans, isotopic fractionation in the body causes bone mineral to consist of more of the light isotope ^{40}C, and soft tissues and urine to contain more of the heavy isotope ^{44}C (Heuser and Eisenhauer, 2010). Under conditions of chronic bone resorption, the light isotopes formerly fixed in bone are preferentially excreted in urine. Urine $\delta^{44/40}$Ca ratios are subsequently lower among individuals experiencing bone loss. This was demonstrated among patients undergoing prolonged bed rest, whose urinary $\delta^{44/40}$Ca ratios declined markedly over time (Fig. 6) (Morgan et al., 2012). Isotopic analysis of urine thereby offers a noninvasive method for investigating metabolic bone diseases, including early detection and assessment of treatment efficacy. Additionally, $\delta^{44/40}$Ca values may be used to monitor changes in calcium homeostasis relating to pregnancy and lactation, periods known to affect calcium excretion and resorption (Ritchie et al., 1998).

Summary of physiology

There is now ample evidence that pathophysiological responses to stress engender systematic isotope effects in tissues. Nitrogen balance is one source of isotopic variation, and has been explored in the contexts of eating disorders, dietary restriction, and pregnancy among humans. Some specific conditions, such as diabetes, liver disease, and osteoporosis, are other examples of pathology-specific fractionation used in biomedicine. These are all cases in which pathology and physiology—and not just diet—underlie isotopic variation. This potential offers enticing possibilities in human biology. Future directions include examining weaning and nitrogen balance in the case of normal growth and development from a life-history perspective, and investigating other pathologies known to affect metabolism.

PALEOPATHOLOGY

The examples reviewed in the previous section of pathology-specific isotopic variation are case studies of living subjects. Can stable isotope data inform on past physiology from archaeological remains? Skeletal stress indicators are used in bioarchaeology to inform on past paleopathologies such as scurvy, osteoporosis, and vitamin D deficiency (rickets and osteomalacia), endocrine disorders (e.g., acromegaly), hematological disorders (e.g., anemia), and infectious diseases (e.g., tuberculosis, treponemal diseases, and leprosy as indicated by periostitis and osteomyelitis) (White et al. 2011). Diet is a risk factor in many of these pathological conditions, potentially linking diet and skeletal health. Of interest is whether individuals with these disorders exhibit systematically different stable isotope signatures when compared to the "healthy" population at large (for a recent review of such case studies, see Richards and Montgomery (2012)). However, with few exceptions, skeletal stress indicators are non-specific, meaning a variety of environmental conditions may bring them about. For non-specific stress indicators, links with pathology and stable isotope ratios are expected because of diet, an independent variable that likely influences variation in both (e.g., maize consumption leading both to dental caries and high tissue δ^{13}C values). Links between non-specific pathology and stable isotope signatures are thus indirect. A more general problem with using paleopathological data to discern past

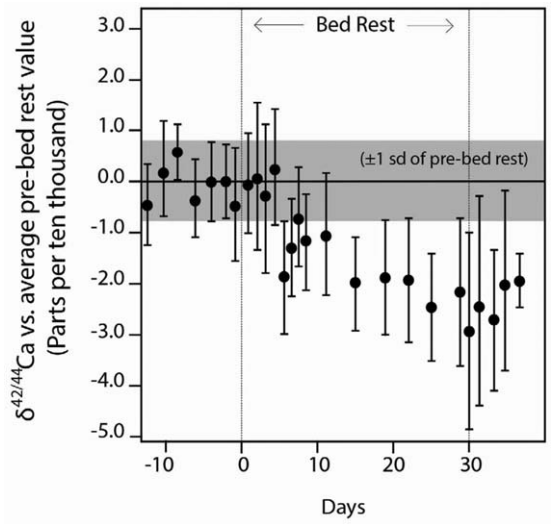

Fig. 6. Changes in urinary stable calcium isotope ratios ($\delta^{44/40}$Ca) over the course of prolonged bed rest. Bone resorption during relative immobility liberates the lighter isotope ^{40}C from bone mineral, leaving urine relatively ^{44}C-depleted (modified from Morgan et al. (2012)).

health is that skeletal stress and health are only imperfectly related (c.f., Flinn and England, 1997; Tanner et al., 2009). Certain skeletal stressors may not engender a decline in overall health, and vice versa. Even when working with modern human populations, health is an abstract concept with a continuum of expressions and with no single individual or population representing perfect health. Just as health and stress are not coterminous, neither are diet and nutrition.

In the following sections, examples of stable isotope applications to pathology that break away from the limitations of simple associations with diet are reviewed, including cases of nitrogen balance associated with AIDS, macronutrient availability, and sickle cell disease. In these cases, isotopic variation is directly influenced by physiology, and is not merely the by-product of an association with particular foods and diets. The utility of studying diet change during the life span from a life history perspective is also reviewed, as it pertains to a better understanding of past health.

Nitrogen balance and bone

The relationship between nitrogen balance and δ^{15}N variation has been explored primarily using soft tissues, although some efforts have been made using bone (Hobson et al., 1993; Waters-Rist and Katzenberg, 2010). Katzenberg and Lovell (1999) explored potential relationships between stable isotope ratios and skeletal responses in periostitis, fracture, atrophy, and osteomyelitis using seven cadavers with known medical histories, including three controls and four with skeletal pathologies. Excepting the atrophied bone, both pathological and non-pathological segments of the same bone were sampled. The osteomyelitic bone alone showed a δ^{15}N effect: the affected region of bone was nearly 2.0‰ higher than the two unaffected regions sampled. Although the δ^{15}N difference could possibly be due to a change in diet while pathological bone deposition was occurring, the fact that this patient suffered and died from AIDS suggests that the elevated δ^{15}N signature was due to negative nitrogen balance during illness and chronic wasting (Katzenberg and Lovell, 1999). This finding warrants future examination, and underscores the utility of using skeletal materials from individuals with known medical histories in model-building for bioarchaeology.

It is uncertain whether the isotopic effects of episodic events such as pregnancy and growth spurts can be detected in skeletal remains. Elevated tissue δ^{15}N during morning sickness is an intriguing finding among living humans (Fuller et al., 2005) which has not yet been identified with certainty in human skeletal populations (Nitsch et al., 2010). Waters-Rist and Katzenberg (2010) studied the effects of rapid growth and positive nitrogen balance on δ^{15}N values in bones by comparing rapidly growing and slowly and/or non-growing regions of long bones (epiphyses, diaphyses, and metaphyses). The researchers did not find the hypothesized ^{15}N depletion with growth, and advance the relative inertness of bone as one possible explanation for this. However, Fuller et al. (2006) have suggested the significantly lower δ^{15}N values found from female skeletal remains from Roman Britain could be due to growth and positive nitrogen balance with pregnancy. Diet differences between the sexes is often advanced as a possible explanation for lower δ^{15}N values among women, but the role of a pregnancy effect should not be discounted. Interestingly, bioarchaeologists often encounter a higher prevalence of dental caries among females compared to males, a difference usually attributed to diet (c.f. Larsen (1997) and references therein). However, it also has been proposed that pregnancy impedes salivation, thus increasing the risk of dental caries, independent of diet (Lukacs and Largaespada, 2006). Testing the hypothesis that dental caries prevalence is linked to parity is difficult in the archaeological record. A relationship could be sought between caries prevalence, parity, and δ^{15}N signatures of hair or nails among living subjects toward assessing the feasibility of δ^{15}N as an indicator of parity in explaining dental caries in the archaeological record.

Macronutrient scrambling versus routing in protein insufficiency

In the 1990s, two controlled feeding experiments demonstrated that stable isotope signatures in collagen from bone primarily reflect protein in the diet, whereas bone mineral more equitably reflects all macronutrients (Ambrose and Norr, 1993; Tieszen and Fagre, 1993). This knowledge has become ingrained in bone stable isotope approaches to diet reconstruction. However, when dietary protein is insufficient for tissue building, collagen amino acids might come "scrambled" from other macronutrients, such as carbohydrates (Keenleyside et al., 2006; Prowse et al., 2004). Unlike the case of catabolism in extreme protein insufficiency, this scrambling does not involve preexisting body reserves, but rather how new ingested foods are incorporated initially. Protein insufficiency may be revealed when an expected protein source is not reflected in collagen, suggesting collagen was synthesized from carbohydrates.

Possible examples of this are reported in regions of the Mediterranean, where high human δ^{15}N values suggest

marine foods were consumed, yet low $\delta^{13}C$ values suggest a terrestrial diet (Craig et al., 2009; Keenleyside et al., 2006; Prowse et al., 2004; Prowse et al., 2005) (Fig. 7). An explanation for these inconsistencies is that while nitrogen was adequate for amino acid synthesis and continued to be routed from marine foods to collagen, marine carbon was insufficient to manufacture all the necessary amino acids in collagen, causing $\delta^{13}C$ to be scrambled from other, low-^{13}C terrestrial macronutrients (lipids and carbohydrates). In the case of macronutrient scrambling, isotopic values reflect a homeostatic response to physiological stress brought on by a low-protein diet. Importantly, this physiological response reflects not just amounts of animal protein in the diet, but all protein in the diet, including protein from plants. Although macronutrient scrambling may account for cases of high and varied $\delta^{15}N$ values and low and less-varied $\delta^{13}C$ values, other possible explanations include consumption of omnivores, suckling animals, or animals fed on human refuse (Fuller et al., 2010; Müldner and Richards, 2007), and land management strategies that raise the source $\delta^{15}N$ of soils (Bogaard et al., 2007; Grogan et al., 2000), an issue which analysis of sulfur stable isotope ratios has helped resolve (Nehlich et al., 2011, 2012).

Analysis of individual amino acids could shed further light on the issue of unexplained $\delta^{15}N$ variation, including macronutrient scrambling. In their study of past human populations from Korea that differed in their marine fish intake, Choy et al. (2010) examined $\delta^{13}C$ differences in essential and nonessential amino acid. Among the "high-marine" population from Tongsamdong, $\delta^{13}C$ values of two amino acids—serine and glycine—were lower than what could be expected based on the serine and glycine values of marine fish. Although biosynthesis from threonine through the threonine dehydrogenase pathway is one possible explanation for this, discussed previously, another possible explanation is that serine and glycine might be more sensitive to synthesis from terrestrial lipids and carbohydrates, despite a bulk $\delta^{13}C$ signature consistent with a marine-based diet. By considering individual amino acids apart from bulk tissues, information about minor protein sources can be obtained to improve on the interpretations of the major or principal protein sources available through analysis of collagen or keratin.

If macronutrient scrambling underlies isotopic profiles, relevant questions remain, such as, what is "deficient" when it comes to dietary protein, and were these people "stressed" or "unhealthy" if they were apparently able to compensate for a stressful protein environment? Rather than illustrating a clear case of nutritional stress, examples of possible macronutrient scrambling are perhaps more significant as examples of human plasticity when it comes to subsistence. Although facing a stressful environment of scarce protein, the human body could compensate by extracting nutrients from other foods.

Sickle cell disease

Some preliminary evidence indicates $\delta^{18}O$ values may vary with anemia. In an experimental study with humans, it was noted that $\delta^{18}O$ from respired CO_2 of anemics differs from that of healthy individuals (Epstein and Zeiri, 1988). The different rates of oxygenation and deoxygenation of sickle-cell and normal hemoglobin are among several potential sources of this isotopic variation, as reaction rate is known to influence stable isotope fractionation (Hoefs, 2004). For example, increased ventilation rates of human subjects during exercise is associated with reduced fractionation (Widory, 2004; Zanconato et al., 1992), whereas diffusion of CO_2 across calloused lung tissue in smokers is associated with increased fractionation (Epstein and Zeiri, 1988).

Building on the work of Epstein and Zeiri (1988), Reitsema and Crews (2011) measured $\delta^{18}O$ values in bones of transgenic mice expressing human hemoglobin-S genes and control mice with healthy mouse hemoglobin. The sickle-cell mice exhibited significantly lower bone $\delta^{18}O$ values than the healthy mice ($-5.6 \pm 0.6‰$ vs. $-4.5 \pm 0.7‰$; $P = 0.002$) (Fig. 8). Whether this is due to oxygenation rates, the amount of hemoglobin trapped in lung tissue, water flux, or the activity levels of the mice is the subject of future research. However, the relationship between sickle cell disease and stable isotope ratios is intriguing, and represents another case of the pathophysiology of a disease underlying stable isotope variation in body tissues.

Strategic sampling in paleopathology: Circumventing the osteological paradox?

The osteological paradox has relevance in this discussion, because one of its salient points is the problem of making inferences about the health of a living population from the physiological state of individuals at the times of their deaths (Wood et al., 1992). Stable isotope signatures from different mineralized tissues capture life stages other than that which immediately precedes death (Table 1). Therefore, it is possible to obtain different snapshots of diet (e.g., in infancy, childhood, early and late adulthood) through strategic sampling. Adults in a skeletal sample may be thought of as children who lived, and these individuals possess isotopic records of both their adult and childhood diets in the form of bones and teeth, respectively. Comparing the teeth of adults and the bones or teeth of subadults (children who died) permits a better understanding of aspects of diet and "health" (using mortality as proxy for health) during the lifespan. A life history approach is now frequently applied in stable isotope studies, with researchers investigating diet at different periods in the lifespan (Bell et al., 2001; Berger et al., 2010; Chenery et al., 2010; Dupras and Tocheri, 2007; Fuller et al., 2003; Herrscher, 2003; Katzenberg, 2008; Kosiba et al., 2007; Lambert et al., 2012; Reitsema and Vercellotti, 2012; Sealy et al., 1995).

Reitsema and Vercellotti (2012) took a life-history approach to the influence of diet on skeletal health, comparing dentine and rib collagen from individuals buried in a medieval rural cemetery in Northern Italy. Previous bioarchaeological examinations from the population showed skeletal health disparities between low-status males and the remainder of the population, including high-status males, and both low- and high-status females (Vercellotti et al., 2011). It was expected that childhood diets low in animal protein may predispose the individuals to poor health, as estimated from skeletal stress indicators, later in life. Stable isotope analysis of rib collagen, reflecting adult diet, supported the hypothesis of marked- social and sex-based disparities, with low-status males consuming less animal protein and more millet (possibly as a gruel)

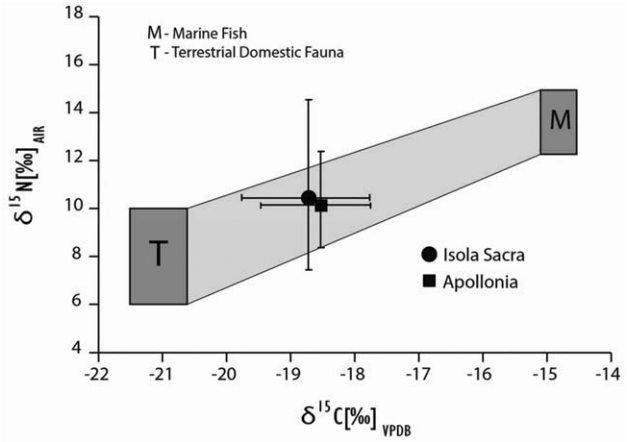

Fig. 7. Isotopic ranges of two hypothetical protein sources (T = terrestrial; M = marine) are shown. The shaded region between these depicts a range of expected values for human diets comprising more or less marine and terrestrial protein. When $\delta^{13}C$ values fall within the expected range but $\delta^{15}N$ ranges that surpass the expected range, "macronutrient scrambling" may be occurring. Data from Keenleyside et al. (2006) and Prowse et al. (2004).

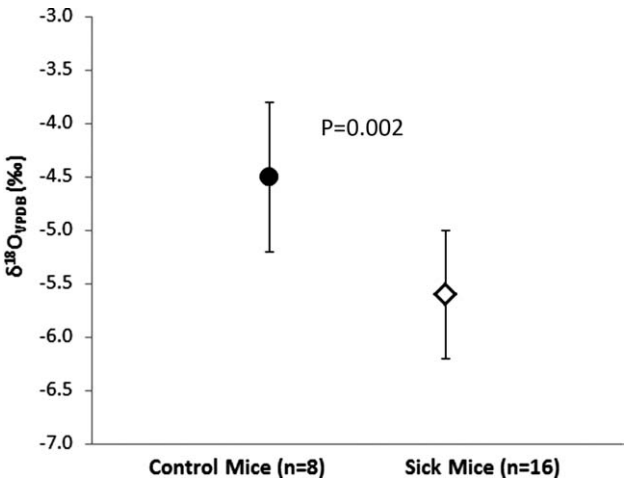

Fig. 8. Significant ($P = 0.002$) differences in the bone mineral $\delta^{18}O$ values of control mice and mice expressing human transgenes for sickle cell disease.

than other groups. However, stable isotope analyses of dentine from second molars indicated that childhood diets of the entire population were isotopically similar. Evidently, the conditions leading to skeletal stress and a low-protein diet among low-status males developed later in life, and did not stem back to childhood "malnutrition," at least as far as isotopic data could reveal. Although this may reflect culturally prescribed activity patterns in the population, it may also reflect a degree of cultural "buffering" for women in this society—including both the high- and the low-status women—possibly in recognition of their reproductive roles. The combination of a low-protein diet and skeletal stress points to poor health for low-status males.

Summary of paleopathology

To date, the most common use of stable isotope signatures to elucidate past "health" has involved comparing stable carbon and nitrogen isotope data to skeletal stress indicators (reviewed by Richards and Montgomery (2012)). A problem with this approach is that most of the association between stable isotope signatures and paleopathology has to do with diet's role in nutrition, which stable isotope analyses only indirectly assess, thus increasing uncertainty when linking stable isotopes to skeletal pathologies. Furthermore, nutrition is only one of many factors involved in these paleopathologies. Other factors include infection, parasites, genetics, and disease. Some applications of stable isotope analysis to pathological conditions break away from these limitations, because the association between isotopic signatures and pathology is caused by pathology-dependent fractionation, and is not simply intermediated by diet.

It is uncertain how useful $\delta^{15}N$ effects of nutritional stress will be useful when only skeletal materials are studied. With soft tissues such as muscle and hair, short-term stressors can be readily detected. Bone, with its much slower turnover rate and its time-averaged stable isotope signatures, may not reveal episodic nutritional deprivation. The chances of associating stable isotope ratios with nutritional stress in the future may depend on simultaneous advancements in sampling strategies—for example, bone density fraction sampling (Bell et al., 2001) or dental serial sectioning (Eerkens et al., 2011; Fuller et al., 2003). In addition to the problem of slow bone turnover rates, human plasticity may inhibit the isotopic effects and detection of even chronic nutritional deprivation. More studies of living humans experiencing physiological stress are needed to develop this research area.

CONCLUSIONS

Because physiology mediates the fixation of carbon, nitrogen, and other elements in consumer tissues, stable isotope analysis in anthropology is moving beyond diet reconstructions to providing insights about health and physiology. As relatively non-invasive natural tracers, stable isotope ratios from hair, nails, urine, blood, and feces stand to contribute important insights on nutrition and disease processes among living humans. To date, studies of natural abundances of stable isotopes have proven useful in understanding physiology and metabolism during pregnancy, nutritional stress including eating disorders, and in cases of other specific disorders, including osteoporosis, diabetes, and liver disease.

Human plasticity, an adaptive mechanism, can be an obstacle in actually understanding and studying human health, as it buffers against physiological manifestations of stress and "poor" health or nutrition. Stable isotope analysis offers a window into internal nutrient resource allocation that can yield otherwise invisible information not evident from other health assessments (including early detection of health disorders).

Several key problems need to be addressed to develop isotopic applications to health in past human populations. Possibly the most important of these is the metabolic inertness of bone which prevents episodic events from being "recorded" isotopically. Improved sampling strategies that target constituents of bone with higher turnover rates, or selective sampling of individual amino acids, will help provide more refined information from the skeletal record. There is already evidence that osteoporosis, sickle cell disease, and conditions affecting nitrogen

balance are associated with stable isotope variation in bone.

This review documents clear examples of stable isotope signatures reflecting physiological conditions independent of diet among living humans, non-human primates, and other animals, and there is a great need for advancing these research areas. To assist in breaking through the ceiling of stable isotope applications in anthropology, future researchers should take into consideration the sensitivity of stable isotope signatures to physiology, adopting creative and innovative sampling methods.

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