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Combined influence of meteoric water and protein intake on hydrogen isotope values in archaeological human bone collagen

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ABSTRACT

Hydrogen isotopes in archaeological human bone collagen are poorly understood, but present an opportunity to add new depth to our understanding of ancient populations. The competing influences of meteoric water versus protein intake on human bone collagen hydrogen isotope values were examined through comparison with the well-understood proxies of hydroxyapatite oxygen and collagen nitrogen isotopes, respectively. Consideration of the data set as individual points compared to averaged pools of individuals in each of 11 archaeological sites suggested the latter partially eliminates inherent variability due to food choice or regional movement. Collagen hydrogen isotopes were moderately correlated with hydroxyapatite oxygen isotopes (R = 0.695, site averages) and collagen nitrogen isotopes (R = 0.562, site averages). Correlation improved with a multiple linear regression including both oxygen and nitrogen (R = 0.745, site averages). Correlation between meteoric water hydrogen and oxygen isotope values converted from hydroxyapatite and collagen values, respectively, yielded a slope well below the expected value of ~8 observed directly in meteoric water (i.e. the "meteoric water line"). Correlation between converted meteoric water hydrogen and the measured collagen non-exchangeable hydrogen isotope values showed a slope well below the expected value of 1.0. Theoretical meteoric water hydrogen isotope values and theoretical herbivorous collagen hydrogen isotope values were calculated based on previously established equations in order to construct a hypothetical framework free of trophic level influences. Deviations between actual values and these theoretical values correlated weakly with collagen nitrogen isotope values, suggesting that direct trophic level enrichment/depletion is not controlling the disparity between expected and measured values. The deviations are hypothetically caused by non-local food sources, and a decoupling of expected oxygen and hydrogen relationships as individuals consumed more meat and decreased in vivo non-essential amino acid production. This work presents a new model that facilitates understanding of the complex relationship between meteoric water and protein intake controls on hydrogen isotopes in omnivorous human populations that can potentially inform about past meteoric water values and amounts of animal protein consumption.

1. Introduction

Stable isotope analysis of bones is relatively common in archaeology and paleontology to determine dietary components, provenance, migrations, climate proxies, metabolic functioning, and social demographics. Several decades of research have established a solid understanding of stable carbon, nitrogen, and oxygen isotope dynamics in archaeological bone collagen and hydroxyapatite. Hydrogen isotopes in bone have been addressed only recently. The routing of hydrogen into bone collagen in particular is less well-understood, but presents new options for understanding archaeological remains.

Hydrogen isotopes have been examined more thoroughly in tissues which are similar to the collagen protein and can serve as basic comparisons. Keratin (i.e. feathers, claws, nails) has been studied most heavily, although blood, muscle, lipids, and other organ tissues have been examined as well (Chesson et al. 2009, 2011; Hobson et al., 1999; Tuross et al., 2008; Wolf et al., 2011). Hydrogen is routed to keratin from both dietary food and drinking water, where the former pathway provides trophic information and the latter indicates latitudinal provenance (Bowen et al., 2005, 2009; Ehleringer et al., 2008; O'Brien and Wooller, 2007; Sellick et al., 2009). Where some studies suggest keratin hydrogen isotopes largely reflect drinking water isotope values (Hobson et al., 1999, Wolf et al., 2011), others suggest secondary dietary hydrogen input as well (Bowen et al., 2009; Ehleringer et al., 2008;

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Kirsanow and Tuross, 2011; Pietsch et al., 2011). Bulk blood, muscle, lipid, and organ hydrogen isotope values reflect largely drinking water sources (Chesson et al., 2011; Hobson et al., 1999; Wolf et al., 2011), although dietary input may have some influence (Commerford et al., 1983). While this previous research provides background for understanding hydrogen isotopes in bone collagen, keratin and other tissues have a more rapid turnover, a fundamentally different structure, and potentially different hydrogen sources rendering them inadequate proxies for collagen.

Collagen is the primary protein in animal bones and includes hydrogen atoms bound to carbon, or bound within carboxyl, amide, and minimal sulfhydryl side-groups. These side-group hydrogen atoms are labile and exchange with hydrogen from other water sources. The more stable carbon-bound hydrogen atoms comprise a calculated fraction of 0.742–0.829 (majority 0.77–0.81) of all hydrogen atoms in collagen (Cormie et al. 1994b, 1994c; Leyden et al., 2006; Sauer et al., 2009; Topalov et al., 2013) and are generally non-exchangeable with external water sources. The total (i.e. TOT) hydrogen isotope composition of bone collagen (i.e. COLL) can be represented as

$$\delta^2H_{COLL\text{-}TOT} = (1\text{-}f) * \delta^2H_{COLL\text{-}NEX} + f * \delta^2H_{COLL\text{-}EX}$$

where f represents fraction of exchangeable hydrogen (i.e. ~0.19-0.23), $\delta^2H_{COLL\text{-}NEX}$ represents the isotope value of non-exchangeable hydrogen atoms, and $\delta^2H_{COLL\text{-}EX}$ represents the isotope value of exchangeable hydrogen atoms. Isotope values are in standard delta notation:

$$\delta X = [(R_{sample} - R_{standard})/R_{standard}]$$

where R is the ratio (i.e. $^2H/^1H$), values are in parts per thousand (‰), and the standard is V-SMOW.

The $\delta^2H_{COLL\text{-}NEX}$ represents the isotope signal incorporated via water or dietary food and can be considered with a general conceptual framework:

$$\delta^2H_{COLL\text{-}NEX} = (\delta^2H_{ingested\ water} + \varepsilon_a) + (\delta^2H_{dietary\ amino\ acids} + \varepsilon_b).$$

The $\delta^2H_{ingested\ water}$ represents δ^2H of water taken into the body via food water or direct drinking and incorporated into amino acids synthesized in vivo during collagen construction (i.e. “non-essential” amino acids). The ε_a represents hydrogen isotope fractionation during this process. The $\delta^2H_{dietary\ amino\ acids}$ represents δ^2H of amino acids synthesized ex vivo and are incorporated directly from consumed dietary proteins (i.e. “essential” amino acids). The ε_b represents subsequent fractionation as these amino acids are incorporated into collagen, although this value is suspected to be minimal and constant within a given species. Cormie et al. (1994a), Cormie et al. (1994c) and Chesson et al. (2011) present a thorough review of factors contributing to bone collagen $\delta^2H_{ingested\ water}$, ε_a, $\delta^2H_{dietary\ amino\ acids}$, and ε_b; additional insight is gained from detailed discussions of keratin hydrogen incorporation (Ehleringer et al., 2008; Bowen et al., 2009).

A relatively strong linear correlation between $\delta^2H_{ingested\ water}$ and $\delta^2H_{COLL\text{-}NEX}$ exists in strict herbivores obtaining all dietary fractions (i.e. amino acids, carbohydrates, water) from plants. Since leaf and stem δ^2H values reflect local precipitation δ^2H values, the herbivore $\delta^2H_{COLL\text{-}NEX}$ correlates with these local precipitation δ^2H values (Cormie et al. 1994a, 1994c; Pietsch et al., 2011; Reynard and Hedges, 2008). Hydrogen isotope values in herbivore bone collagen can be considered with the simpler representation of $\delta^2H_{COLL\text{-}NEX} = \delta^2H_{ingested\ water} + \varepsilon_a$. Carnivores tend to show an apparent trophic level effect where $\delta^2H_{COLL\text{-}NEX}$ deviates from the expected correlation with $\delta^2H_{ingested\ water}$ (Birchall et al., 2005; Pietsch et al., 2011; Reynard and Hedges, 2008; Topalov et al., 2013; Tuross et al., 2008). This is due likely to the additional $\delta^2H_{dietary\ amino\ acids}$ variable which can show considerable range depending on the type and amount of animal protein consumed.

Humans present a complex case of omnivory. Limited research examining human collagen δ^2H values suggests a combination of ingested water and dietary input (Reynard and Hedges, 2008), which agrees with limited data from other omnivorous mammals (Reynard and Hedges, 2008; Tuross et al., 2008). As archaeological human remains are of high interest, examining human collagen δ^2H could provide another dimension by which to examine dietary input and ingestion of environmental water in a uniquely coupled pathway. It has the potential to contribute additional information to the study of geographic origin, migrations, and dietary choices or available foods.

This study uses the well-known relationships of bone nitrogen and oxygen with trophic structure and meteoric water, respectively, to explore these mechanisms’ effects on $\delta^2H_{COLL\text{-}NEX}$. Nitrogen in collagen (i.e. $\delta^{15}N_{COLL}$) is represented in standard delta notation as indicated previously where R is $^{15}N/^{14}N$ and the standard is atmospheric air. The $\delta^{15}N_{COLL}$ increases approximately 3–4‰ with trophic level (Bocherens and Drucker, 2003; DeNiro and Epstein, 1981; Schoeninger and DeNiro, 1984) providing a proxy for amount and type of dietary protein intake. Oxygen is found in the hydroxyapatite mineral fraction of bone in both the phosphate (i.e. PHOS) and carbonate (i.e. CARB) sites. Phosphate and carbonate oxygen isotopes (i.e. $\delta^{18}O_{PHOS}$ and $\delta^{18}O_{CARB}$) are represented in standard delta notation where R is $^{18}O/^{16}O$ and the standard is V-SMOW. Both $\delta^{18}O_{PHOS}$ and $\delta^{18}O_{CARB}$ correlate with drinking water isotopes (Bryant and Froelich, 1995, Daux et al., 2008, Kohn, 1996, Longinelli, 1984, Luz and Kolodny, 1985, Luz et al., 1984) providing a proxy for geographic locality. The $\delta^{18}O$ and δ^2H values of meteoric water (i.e. MW) are strongly correlated according to the known meteoric water line: $\delta^2H_{MW} = 8 * \delta^{18}O_{MW} + 10$ (Craig, 1961; Kendall and Coplen, 2001). In the absence of dietary influence, the $\delta^2H_{COLL\text{-}NEX}$ is expected to correlate to $\delta^{18}O_{PHOS}$ and $\delta^{18}O_{CARB}$ with a similar slope to that of the meteoric water line. Deviations from this end member were compared to associated $\delta^{15}N_{COLL}$ values, and multiple linear regression models constructed to determine the combined relative influence of ingested water and dietary proteins on the $\delta^2H_{COLL\text{-}NEX}$ values. Combinations of δ^2H and $\delta^{18}O$ values in bone collagen have been used to examine herbivores, but this study adds to the sparser comparisons with $\delta^{15}N$, omnivores, and carnivores (Cormie et al., 1994a; Kirsanow and Tuross, 2011; Kirsanow et al., 2008; Pietsch et al., 2011; Topalov et al., 2013; Tuross et al., 2008).

2. Materials and methods

2.1. Sample collection and preparation

Human remains were sampled from 11 North American archaeological sites primarily on the east coast with one southern site including individuals from Texas (Fig. 1, Table 1, Supplementary Table S1). These sites were selected based on availability of samples, range of geographic localities, and range of potential protein consumption. The sites are primarily temperate regions with similar humidity and temperature conditions. The exception is Glorieta Pass wherein the individuals hailed from the warmer dryer regions of Texas (Alberts, 1984). Carbon, nitrogen, and oxygen isotope data for some samples were published previously in France et al. (2014) and France and Owsley (2015).

Mechanical and chemical preparation methods followed France et al. (2014). Briefly, ~500 mg of solid bone cross section (majority cortical with traces of trabecular) was removed for collagen analysis using pliers or a rotary tool. This cross section yields a homogenized average isotope value across the final ~10–20 years of life. Approximately 50 mg of powdered bone for phosphate and carbonate analysis was obtained by crushing with an agate mortar and pestle or using a rotary tool. Phosphates were extracted via dissolving mineral phases in hydrofluoric acid (2 M), buffering in ammonium hydroxide (20%), and precipitating silver phosphate using a silver nitrate solution (2 M). Carbonates were isolated by eliminating organics with sodium hypochlorite (2–3%) and eliminating secondary carbonates using acetic acid