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Compound-specific amino acid isotopic proxies for detecting freshwater resource consumption

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ABSTRACT

Of central importance to palaeodietary reconstruction is a clear understanding of relative contributions of different terrestrial (i.e., C_3 vs. C_4 plants) and aquatic (i.e., freshwater vs. marine) resources to human diet. There are, however, significant limitations associated with the ability to reconstruct palaeodiet using bulk collagen stable isotope compositions in regions where diverse dietary resources are available. Recent research has determined that carbon-isotope analysis of individual amino acids has considerable potential to elucidate dietary protein source where bulk isotopic compositions cannot. Using $\delta^{13}C_{AA}$ values for human and faunal remains from Zvejnieki, Latvia (8th – 3rd millennia BCE), we test several isotopic proxies focused on distinguishing freshwater protein consumption from both plant-derived and marine protein consumption. We determined that the $\Delta^{13}C_{Gly\text{-}Phe}$ and $\Delta^{13}C_{Val\text{-}Phe}$ proxies can effectively discriminate between terrestrial and aquatic resource consumption, and the relationship between essential $\delta^{13}C_{AA}$ values and the $\Delta^{13}C_{Gly\text{-}Phe}$ and $\Delta^{13}C_{Val\text{-}Phe}$ proxies can differentiate among the four protein consumption groups tested here. Compound-specific amino acid carbon-isotope dietary proxies thus enable an enhanced understanding of diet and resource exploitation in the past, and can elucidate complex dietary behaviour.

1. Introduction

Stable carbon and nitrogen isotopic analyses of archaeological human and faunal remains have been used for over three decades to reconstruct palaeodiet and to investigate palaeoecology, and these studies make valuable contributions to our understanding of subsistence practices in diverse environments, and of changing patterns of resource use temporally and spatially (Katzenberg and Pfeiffer, 2000). In recent years, however, it has become increasingly evident that there are significant limitations in the assessment of palaeodiet using bulk protein stable isotope compositions in ecologically-complex regions where diverse dietary resources are available (e.g., Hedges, 2004; Lidén et al., 2004; Milner et al., 2004). Differential resource access and exploitation by individuals or societies can have important implications for socioeconomic behaviour, or reveal the social and ecological impact of environmental perturbations over time. Thus, of central importance in many archaeological contexts is the ability to determine the relative contributions of different protein sources to diet, such as terrestrial C_3 plant-derived protein, freshwater protein, terrestrial C_4 plant-derived protein, or marine protein resources. Bulk carbon-isotope compositions, however, may mask dietary variability if the contributions from specific resources (e.g., marine protein) are small (Hedges, 2004), or if the natural variability in carbon-isotope compositions between two classes of resources is low, as is often the case when comparing the isotopic compositions of terrestrial plants and freshwater fauna from adjacent lakes and rivers.

Nitrogen-isotope compositions reflect the trophic position of consumed protein and are known to increase several per mil per trophic level between producers and consumers, and often help in disentangling complex diets (DeNiro and Epstein, 1981; Schoeninger, 1985). There is considerable variability in the degree of ^{15}N-enrichment between diet and consumer tissues; among humans from European archaeological sites, for example, the tissue – diet nitrogen-isotope offsets ranged from ~+2 to +5‰ (reviewed

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in Hedges and Reynard, 2007), and a recent controlled dietary study estimated trophic offsets as high as +6‰ (O'Connell et al., 2012). Trophic relationships inferred from nitrogen-isotope compositions can, however, be obscured or altered, for example, if manure is applied to terrestrial crops (Bogaard et al., 2007; Fraser et al., 2011), in particularly arid environments (Ambrose and DeNiro, 1986; Craine et al., 2009; Grocke et al., 1997; Heaton et al., 1986), or if nitrogen metabolism is affected by physiological stress (Reitsema, 2013; Williams et al., 2011). The possible influence of these factors on $\delta^{15}N$ values can, however, be difficult to assess. Focusing on compound-specific carbon-isotope compositions, particularly of essential amino acids, eliminates the potentially confounding effect of tissue-diet fractionation and routing vs. biosynthesis of non-essential amino acids on bulk carbon-isotope compositions, and of the environmental and physiological effects discussed above on nitrogen-isotope compositions.

Controlled feeding studies and the relatively few published papers that have analyzed archaeological material have demonstrated that carbon-isotope analysis of individual amino acids has considerable potential to elucidate palaeodiet where traditional bulk isotopic data fail (Choy et al., 2010; Corr et al., 2005; Fogel and Tuross, 2003; Hare et al., 1991; Honch et al., 2012; Howland et al., 2003; Jim et al., 2006). For example, intra-individual offsets between the amino acids phenylalanine and glycine ($\Delta^{13}C_{Gly\text{-}Phe}$; Corr et al., 2005) reliably distinguish among individuals consuming a high marine protein diet and those consuming a terrestrial C_4 plant diet, and the feasibility of using the relationships between other paired amino acids to identify dietary protein source has also been investigated (e.g., $\Delta^{13}C_{Val\text{-}Phe}$ for assessing freshwater resource consumption; Honch et al., 2012). Nonetheless, identifying instances of freshwater protein consumption isotopically remains challenging. In temperate environments, high freshwater protein consumption is typically inferred based on high nitrogen-isotope compositions, C_3 plant-like carbon-isotope compositions, and the presence of fish bones or artefacts used to exploit aquatic resources in the archaeological record (Lillie and Richards, 2000; Minagawa and Wada, 1984; Richards et al., 2001). In the absence of corroborative archaeological evidence, however, it is very difficult to assess the significance of freshwater resource contribution to human diet. The development of an array of isotopic proxies which are sensitive to changes in dietary protein source and are highly discriminatory among different protein resources is thus essential to increasingly refined palaeodiet reconstruction.

Here, the objective is to test new and previously proposed isotopic proxies and relationships focused on distinguishing freshwater resource consumption from both plant-derived (C_3 and C_4) and marine protein consumption using compound-specific carbon-isotope compositions of collagen. We present new amino acid carbon-isotope data for archaeological humans and fauna from Zvejnieki, Latvia (c. 8th – 3rd millennia BCE). Archaeological and zooarchaeological data, as well as previously published bulk carbon- and nitrogen-isotope compositions, strongly indicate that the inhabitants consumed considerable quantities of freshwater resources. Hence, these hunter-gatherer-fisher settlements offer an ideal opportunity to evaluate and refine compound-specific isotopic proxies for disentangling palaeodiet, which can then be used in other, less well-understood archaeological contexts where multiple resources from marine, terrestrial and freshwater environments were available for consumption.

2. Theoretical considerations for stable carbon-isotope analysis

The reconstruction of palaeodiet using stable isotope analysis is based on the well-tested assumption that tissue isotopic composition reflects the isotopic composition of consumed food (Ambrose, 1993). Systematic differences exist in isotopic composition between tissue and diet (Ambrose, 1993; DeNiro and Epstein, 1978), which are a result of isotopic discrimination, the differential partitioning of isotopes between phases in a reaction (e.g., ingested food → consumer tissue) caused by the slight mass differences among isotopes of the same element (e.g., ^{13}C, ^{12}C). When diet is protein-sufficient, the $\delta^{13}C$ values of proteinaceous consumer tissues (e.g., collagen and dentin) largely reflect the carbon-isotope composition of dietary protein as it is derived from the base of the food web (Ambrose, 1993; Kellner and Schoeninger, 2007). Tissue carbon-isotope composition is a weighted average of the isotopic compositions of all component essential and non-essential amino acids ($\delta^{13}C_{AA}$). Essential amino acids (e.g., threonine, valine, methionine, isoleucine, leucine, histidine, lysine, and phenylalanine) cannot be generated by the body and therefore must be ingested in sufficient quantities. In contrast, non-essential amino acids (e.g., asparagine/aspartic acid, hydroxyproline, glutamic acid/glutamate, serine, glycine, alanine, proline and arginine) can be assimilated with minimal modification from a dietary source, or may be synthesised *de novo* using components drawn from the body's biochemical pools; the latter will result in isotopic fractionation (Ambrose and Norr, 1993; Newsome et al., 2014). Tissue essential amino acid $\delta^{13}C$ values are thus expected to closely approximate dietary essential amino acid $\delta^{13}C$ values due to direct routing, i.e., $\Delta^{13}C_{\text{tissue AA}-\text{diet AA}} \approx 0‰$. Non-essential amino acid $\delta^{13}C$ values, however, may show evidence of both direct routing and biosynthesis, depending on the quality, digestibility and amino acid composition of consumed food.

Differences in the way that C_3 and C_4 plants incorporate $^{13}C/^{12}C$ during photosynthesis allow assessment of relative dietary contributions of these different classes of foods, consumed as both plants and plant-consuming fauna. C_3 plants (e.g., grains, rice, tubers, fruits and vegetables) have lower (i.e., more negative) $\delta^{13}C$ values (global average ≈ −26.5‰), whereas C_4 plants (e.g., tropical grasses, maize, millet and sorghum) have higher $\delta^{13}C$ values, with an average of around −12.5‰ (Ambrose, 1993). Terrestrial plants obtain carbon from atmospheric CO_2 (average −8‰; Marino and McElroy, 1991). In contrast, marine plants obtain carbon from several inorganic sources (e.g., dissolved bicarbonate, carbonate-containing minerals), which are generally ^{13}C-enriched relative to the terrestrial carbon source. Dissolved inorganic carbon enters the marine food web via photosynthetic uptake by algae and phytoplankton (Corbisier et al., 2006; Mook and Tan, 1991). The carbon-isotope compositions of marine organisms are therefore dependent on several factors, including the $\delta^{13}C$ values of the dissolved inorganic carbon, isotopic fractionation associated with photosynthetic uptake, and the rate of diffusion of CO_2 across photosynthetic cells in plants, which is slow for marine plants compared to terrestrial plants (Mook and Tan, 1991; Rau et al., 1996). As a result, C_3 marine plant carbon-isotope compositions are often indistinguishable from the isotopic compositions of terrestrial C_4 plants. This comparatively small difference in carbon-isotope compositions makes disentangling consumption of marine protein (−5–−17‰) from C_4 plant-derived protein (≈−12.5‰) challenging. The isotopic composition of freshwater carbon is contingent on a similarly complex array of environmental sources, including, for example, CO_2 and dissolved organic carbon evolved from bacterial decay of terrestrial organic matter, dissolution of inorganic carbonates from rocks and soils in the catchment area, photosynthesis of aquatic flora, and carbonate precipitation (Mook and Tan, 1991). In the predominately C_3 plant-based ecosystems of northern Europe, freshwater resource $\delta^{13}C$ values are ~≈−27‰ (Dufour et al., 1999; Katzenberg and Weber, 1999).