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Investigation of amino acid $\delta^{13}C$ signatures in bone collagen to reconstruct human palaeodiets using liquid chromatography–isotope ratio mass spectrometry

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Abstract

This research presents the individual amino acid $\delta^{13}C$ values in bone collagen of humans ($n = 9$) and animals ($n = 27$) from two prehistoric shell midden sites in Korea. We obtained complete baseline separation of 16 of the 18 amino acids found in bone collagen by using liquid chromatography–isotope ratio mass spectrometry (LC–IRMS). The isotopic results reveal that the humans and animals in the two sites had similar patterns in essential amino acids (EAAs) and non-essential amino acids (NEAAs). The EAA and NEAA $\delta^{13}C$ values in humans are intermediate between those in marine and terrestrial animals. However, the threonine $\delta^{13}C$ values in humans and animals measured in this study are more highly enriched than those of other amino acids. At both sites, all amino acids in marine animals are ^{13}C-enriched relative to those of the terrestrial animals. The isotopic evidence suggests that the Tongsamdong human had EAAs and NEAAs from marine food resources, while the Nukdo humans mainly had EAAs from terrestrial food resources but obtained NEAAs from both terrestrial and marine resources. The $\delta^{13}C$ isotopic differences in amino acids between marine and terrestrial animals were the largest for glycine (NEAA) and histidine (EAA) and the smallest for tyrosine (NEAA) and phenylalanine (EAA). In addition, threonine among the EAAs also had a large difference (~8‰) in $\delta^{13}C$ values between marine and terrestrial animals, and has the potential to be used as an isotopic marker in palaeodietary studies. Threonine $\delta^{13}C$ values were used in conjunction with the established $\Delta^{13}C_{Glycine–phenylalanine}$ values and produced three distinct dietary groups (terrestrial, omnivorous, and marine). In addition, threonine $\delta^{13}C$ values and $\Delta^{13}C_{Serine–phenylalanine}$ values were discovered to separate between two dietary groups (terrestrial vs. marine), and these $\delta^{13}C$ values may provide a potential new indicator for investigating the distinction between marine and terrestrial protein sources in human diets.

1. INTRODUCTION

Stable isotope ratios of bulk carbon and nitrogen in bone collagen can reveal information about the dietary history of humans and animals (van der Merwe and Vogel, 1978; Schoeninger et al., 1983; DeNiro, 1985; Schwarcz and Schoeninger, 1991; Schoeninger and Moore, 1992; Katzenberg, 2000) such as C_3 vs. C_4 terrestrial diets (van der Merwe and Vogel, 1978; Vogel and van der Merwe, 1978; Barton et al., 2009) or terrestrial vs. marine diets (Chisholm et al., 1982; Schoeninger et al., 1983; Schoeninger and Moore, 1992; Richards et al., 2001; Lee-Thorp, 2008). While bulk stable isotope ratio analysis is a well-established technique, there is increasing interest in isotope ratio

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measurements of single amino acid fractions of bone collagen from archaeological samples (Tuross et al., 1988; Hare et al., 1991; Fogel and Tuross, 2003; Howland et al., 2003; Jim et al., 2006; McCullagh et al., 2006). Compound-specific stable isotope ratio analysis permits more accurate measurements through the removal of contamination, as well as providing additional knowledge about isotopic fractionations of the constituent amino acids of bone collagen. In particular, the application of compound-specific stable isotope ratio analysis to archaeological samples can be used to overcome limitations of bulk stable isotope ratio analysis, for example, to differentiate C_4 terrestrial and marine protein diets where $\delta^{13}C$ and $\delta^{15}N$ bulk values overlap (Corr et al., 2005).

Over the past decades, the technique of gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IRMS) has been used for the separation and measurement of amino acid carbon isotope compositions in bone collagen (Hare et al., 1991; Docherty et al., 2001; Howland et al., 2003; Corr et al., 2005, 2009; Jim et al., 2006). While this has been an effective technique, a major drawback of GC–C–IRMS is that it requires derivatization (which involves the addition of carbon atoms) to make the amino acids volatile for analysis (Corr et al., 2007a,b). The recent development of coupling liquid chromatography (LC) to IRMS enables online carbon isotope ratio measurement of compounds such as amino acids (Godin et al., 2005, 2008a; McCullagh et al., 2006, 2008), alcohols (Cabanero et al., 2008; Tagami and Uchida, 2008), peptides (e.g. glutathione) (Schierbeek et al., 2007), carbohydrates (Cabanero et al., 2006; Boschker et al., 2008), amino sugars (Bodé et al., 2009) and fatty acids (Heuer et al., 2006; Godin et al., 2008b). The main advantage of LC–IRMS over GC–C–IRMS is that no derivatization is needed resulting in easier sample preparation and improved accuracy and reproducibility (Smith et al., 2009). McCullagh et al. (2006) were the first to use LC–IRMS to study amino acid $\delta^{13}C$ values in archaeological bone collagen. The authors tested the LC–IRMS technique on six archaeological and modern samples, each having a different dietary isotopic signature to demonstrate its potential for palaeodietary studies. This research suggested that the LC–IRMS technique could be useful in palaeodietary reconstruction since it found a difference in amino acid $\delta^{13}C$ values between terrestrial and marine-based diets. Unfortunately, this study was unable to obtain complete baseline separation of some amino acids (notably threonine, serine, glutamate, and glycine) in bone collagen in a single analytical run. Recently, a robust three-phase chromatographic method was developed for the analysis of $\delta^{13}C$ values in amino acids, able to obtain, in a single analytical run, complete baseline separation of 16 of the 18 amino acids found in bone collagen and a variety of other protein hydrolysates using LC–IRMS (Smith et al., 2009).

Stable isotope ratio analysis of carbon in amino acids of bone collagen permits the investigation of amino acid metabolic pathways as well as specific dietary information about human diets. Amino acids can be classified into two groups based on their metabolic activities (Nelson and Cox, 2000). Essential amino acids (EAAs) are those that an organism cannot synthesize and therefore must come from dietary intake. Non-essential amino acids (NEAAs), however, are those that can be synthesized by an organism from other carbon sources (i.e. carbohydrate, lipid or other amino acids). The EAA $\delta^{13}C$ values in a tissue thus reflect the EAA $\delta^{13}C$ values in foods consumed, and this can be used to reconstruct dietary sources in humans and animals in more detail (DeNiro and Epstein, 1978; O'Brien et al., 2002; Petzke et al., 2005). At the base of the food chain, all amino acids are synthesized by metabolic pathways that occur in plants and microorganisms, and the $\delta^{13}C$ values of these individual amino acids reflect the biosynthetic processes involved in the formation of individual amino acids (Abelson and Hoering, 1961; Macko et al., 1987; Fogel and Tuross, 2003; Larsen et al., 2009). As higher organisms consume these amino acids, they can be metabolically converted to other amino acids or intermediates (and potentially isotopically) fractionated and then incorporated into a tissue (Reeds, 2000).

Archaeological human ($n = 9$) and animal ($n = 27$) bone samples examined in this study were obtained from the Tongsamdong collections in the Busan City Museum and the Nukdo collections in the Busan National University, South Korea. The two sites are located in temperate coastal regions of southern parts of the Korean peninsula, which is dominated by C_3 terrestrial vegetation. Although the two sites were both shell middens, archaeological evidence has revealed different food consumption patterns between the two sites (Crawford and Lee, 2003; Suzuki et al., 2008). Recent studies on bulk $\delta^{13}C$ and $\delta^{15}N$ values from bone collagen indicate that human diet at the Nukdo shell midden was based on terrestrial protein resources (Choy and Richards, 2009), but that humans in the Tongsamdong shell midden mainly consumed marine protein resources (Choy and Richards, 2010). In this study, LC–IRMS was used to measure amino acid $\delta^{13}C$ values in bone collagen hydrolysates of human and animal remains excavated from the Tongsamdong ($n = 12$) and Nukdo ($n = 24$) shell midden sites in South Korea. The goal of this study was to develop alternative isotopic markers of specific marine food consumption in human palaeodiets. To this purpose, the EAA and NEAA $\delta^{13}C$ patterns were examined in the animals from marine and terrestrial ecosystems, and compared to the human $\delta^{13}C$ values. In addition, the marine and terrestrial amino acid $\delta^{13}C$ values were also examined in relation to the amino acid biosynthetic pathways in bone collagen from humans and animals. This project represents the most detailed application of LC–IRMS technology to archaeological human and faunal samples to date, and this research presents the first reported $\delta^{13}C$ values from the 18 most abundant amino acids in bone collagen from humans and different animal species.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

All mobile phases and oxidation reagents were made with Rotisolv® HPLC Gradient Grade water (Carl Roth, Karlsruhe, Germany). Mobile phases were made with