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Interpreting Stable Carbon and Nitrogen Isotope Ratios in Archaeological Remains: An Overview of the Processes Influencing the δ13C and δ15N Values of Type I Collagen

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Abstract

The application of isotopic ratio mass spectrometry to archaeological science has produced many important contributions to the study and understanding of ancient human and animal populations. Paleodietary reconstruction through the analysis of stable isotope ratios in skeletal, dental, and soft tissue remains presents another avenue for interpreting the past. The methodology employed to obtain isotopic data from archaeological remains directly influences the types of questions that can be addressed and the interpretation of the data. Furthermore, there are fundamental idiosyncrasies of archaeological specimens and their ante- and post-mortem environments that may influence the results of an isotopic study. This paper explores the ways in which the stable isotopic signatures of carbon and nitrogen in type I collagen in archaeological bones and teeth are formed, modified, or destroyed throughout life and in the post-depositional environment. For a comprehensive review of the methodological and interpretive implications of paleodietary reconstruction using stable isotopic analysis, see Ambrose (1993).

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Introduction

The paleodietary reconstruction method of stable isotope analysis is an invaluable tool for archaeologists. It has been used for the reconstruction of microenvironments, human mobility, nutrition and disease, status differentiation, and the role of seasonality in human mortuary practices (Ambrose 1993, 1998; Katzenberg and Lovell 1999; Williams 2008). Stable isotope signatures can also be used to answer questions about the migration, ecology, ritual involvement, and domestication of animals (Burleigh and Brothwell 1978; Wassenaar 2008; White et al. 2001). Morris, White, Hodgetts, Longstaffe, and Booth (unpublished data) use the diets of domesticated dogs (*Canis familaris*) as a proxy for studying the diets of their human keepers. The analysis of stable carbon and nitrogen isotope ratios from bone and dentinal collagen is useful for reconstructing the relative dependency on types of dietary foods. For instance, stable isotopic analysis of type I bone collagen has provided biochemical evidence for understanding the rise and spread of maize agriculture across the Americas (Bender et al. 1981; Katzenberg et al. 1995; Schwarcz et al. 1985; Vogel and van der Merwe 1977). Molecular, physiological, and environmental factors can influence the carbon and nitrogen isotope compositions of archaeological collagen. It is crucial for archaeologists to understand the multifactorial processes that may influence the isotopic signatures of ancient tissues and their implications for paleodietary reconstruction using bone or dentinal collagen in order to produce and interpret isotopic data in a meaningful way. This paper reviews many of the molecular, ecological, and environmental factors that can dramatically impact the relationship between the isotopic compositions of food sources and their consumers, specific to humans and archaeological fauna.

Theoretical Background of Stable Isotopes in Archaeological Science

The basis for stable isotope analysis stems from the observation that atoms may differ in their atomic weight due to a varying number of neutrons within the nucleus (Nier and Gulbransen 1939). Atoms of a single element with varying atomic mass are referred to as isotopes, which have measurable quantitative differences in reaction rate and bond strength (Sulzman 2007). The molecular composition of the tissues within an organism is in part a product of its dietary sources of various elements metabolised during its lifetime. Because they do not decay over time, stable isotopes can be used to infer dietary behaviour based on the molecular composition of ancient tissue (Ambrose 1993). The relative abundances of stable carbon and nitrogen isotopes are the foundation of paleodietary reconstruction using bone and dentinal collagen.

There are three mutually exclusive photosynthetic pathways used by plants: the Calvin (C3) cycle, the Hatch-Slack (C4) pathway, and Crassulacean acid metabolism (CAM) (O'Leary 1981; Smith and Epstein 1971). Each of these pathways metabolise atmospheric CO_2 during photosynthesis in radically different ways, producing different fractionations of carbon isotopes. Fractionation is the non-linear transfer of isotopes from the source to the product; fractionations are the variable results of this process (Sulzman 2007). Due to quantitative

differences in velocity and bond strength, molecules will typically discriminate against the heavier, slower isotopes in producing compounds, leading to different fractionations of carbon isotopes relative to their source of carbon (Ambrose 1993; Marshall et al. 2007). The amount of ^{13}C relative to ^{12}C, or the $\delta^{13}C$ value, is a measure of relative dietary dependence on C3- and C4-based foods (Marshall et al. 2007). Both C3 and C4 plants are ^{13}C-depleted relative to their source of inorganic carbon (atmospheric CO_2), but C3 plants are much more depleted than C4 plants (Bocherens et al. 2000). O'Leary (1988) shows that the distribution of the $\delta^{13}C$ values of C3 and C4 plants is bimodal, with average values of -27.1 ± 2.0‰ and -13.1 ± 1.2‰, respectively (Bocherens et al. 2000). As a result, the carbon isotope compositions in ecosystems primarily reflect the types of plants and environmental parameters forming the basis of the food webs (Bocherens et al. 2000). DeNiro and Epstein (1981) note that the relative dependency of C3 versus C4 plants should therefore be represented in the $\delta^{13}C$ values of the tissues of their consumers. Maize is historically the dominant C4 plant in North America (van der Merwe and Vogel 1978). Maize originated in hot and dry areas and spread north in antiquity through cultural interactions and the adoption of agriculture (Tykot 2006). The changes in the relative dependency on C4 foods represented in human skeletal remains have allowed archaeologists to track the spread and intensification of maize agriculture throughout North America (Katzenberg et al. 1995; Schwarcz et al. 1985; Vogel and van der Merwe 1977).

The proportion of ^{15}N relative to ^{14}N isotopes, or the $\delta^{15}N$ value, in the tissues of plants and animals can be used to assess trophic level distinctions, nursing and weaning behaviours, consumption of specific plant types, and various kinds of prolonged nutritional stresses. Both herbivores and carnivores are enriched in ^{15}N relative to their dietary source of nitrogen by approximately 3‰ due to fractionation during metabolism and tissue synthesis (Katzenberg 2000). DeNiro and Epstein (1981) demonstrate that animals appear to incorporate dietary ^{15}N preferentially over dietary ^{14}N. The enrichment of ^{15}N in tissues relative to their dietary source progresses across successive trophic levels. As a result, the $\delta^{15}N$ value indicates the trophic level of an animal in a specific ecosystem. This trophic level effect also applies to infants consuming breast milk.

When breast milk is the primary source of nourishment for an infant, there will be an enrichment of ^{15}N due to a trophic level effect because the infant is consuming its mother's tissues in the form of breast milk (Jenkins et al. 2001; Metcalfe et al. 2010). There is no enrichment of ^{13}C in the offspring relative to its mother because breast milk is mainly composed of lipids that are ^{13}C-depleted relative to the blood plasma of the mother (Jenkins et al. 2001). Due to fractionation during the metabolism of dietary carbon, there is approximately 5‰ enrichment of ^{13}C in the tissues of their consumers (van der Merwe and Vogel 1978). This negates any enriching effect on the $\delta^{13}C$ values of tissues in the infant; however, this model does not account for the routing of certain dietary proteins and amino acids to collagen biosynthesis, so small differences may persist (Metcalfe et al. 2010; Schwarcz 2000).

The $\delta^{15}N$ values of bodily tissues can be used to indicate the timing and duration of nursing and weaning in infants. Moreover, in some animals there are certain tissue structures that synthesise during a restricted time period that coincides with

nursing such as enamel and dentine (Linde and Goldberg 1993). This means that the $\delta^{13}C$ and $\delta^{15}N$ values of dentinal collagen will likely reflect the diet of the individual's mother throughout the nursing stage while the carbon and nitrogen isotope compositions of bone collagen reflect cumulative dietary history, excluding the effects of fractionation. The $\delta^{15}N$ value of collagen is also related to the consumption of certain plant types. Legumes have a symbiotic relationship with bacteria of the genus *Rhizobium*. These bacteria live in the roots of the plants and fix nitrogen from surrounding soils, making the nitrogen available to the plant (Katzenberg 2000). As a result, leguminous plants have $\delta^{15}N$ values closer to atmospheric nitrogen whereas other plants are much more enriched in ^{15}N. This allows the relative abundance of leguminous plant consumption to be studied isotopically.

Type I Collagen Molecular Structure and Biosynthesis

Type I collagen (which will hereafter be referred to as collagen) is a structural protein found in bones and dentine. In bones, collagen is continually synthesised and resorbed throughout the lifetime of an individual. Hypothetically, the enzymes secreted by osteoclasts during bone resorption may preferentially liberate molecules composed of lighter isotopes, which can alter the $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen. Rates of cellular turnover in bones vary spatially between skeletal elements and within single bones. This variance may result in fractionations of the stable isotope composition specific to skeletal features. Dentine is normally not remodelled following mineralisation, although there is some addition of secondary dentine after dentinogenesis. Tertiary dentine may also form in response to varying external stimuli such as irritants or trauma (Linde and Goldberg 1993). Some estimates state that collagen accounts for approximately 30% of the mass of bones and only about 20% of mass in dentine (Ambrose 1990; Davis 1987; Linde and Goldberg 1993). Approximately 65% of the carbon content in collagen is derived from intact amino acids in dietary protein (Ambrose 1993; Schwarcz 2000). The $\delta^{13}C$ and $\delta^{15}N$ values in collagen are a function of its amino acid composition (Ambrose 1993; Harbeck and Grupe 2009). Glycine (NH_2CH_2COOH) is the primary constituent of collagen and accounts for 33.4% of its mass, but contributes only 17.5% of its carbon and 28.2% of its nitrogen (Harrison and Katzenberg 2003). Glycine is a non-essential amino acid for most organisms meaning it is synthesized within the body (Tuross et al. 1988).

The source material of carbon for biosynthesis influences what portions of the diet are represented in the stable carbon and nitrogen isotope compositions of specific tissues. Structural carbonate in hydroxylapatite is derived from bicarbonate in the serum, specifically blood plasma CO_2, which is largely derived from the metabolism of carbohydrates (Ambrose and Norr 1993; Harrison and Katzenberg 2003). Collagen derives its carbon from ingested and synthesised amino acids (Harrison and Katzenberg 2003; Schwarcz 2000). As a consequence of its biosynthesis process, collagen is more representative of the protein portion of the diet rather than the bulk metabolic carbon pool (Ambrose and Norr 1993). Carbohydrates, lipids, and proteins not used in protein synthesis are less represented in collagen than in structural carbonates due to the routing of certain amino acids described by Schwarcz (2000).

Harrison and Katzenberg (2003) maintain that animal protein is likely to make a greater contribution to collagen

synthesis due to its amino acid composition, whereas only some of the amino acids from plant tissues will contribute. Harrison and Katzenberg (2003) observed that the $\delta^{13}C$ values of collagen samples falling within a range of -20 to -17‰ had corresponding carbonate $\delta^{13}C$ values in the range of -14 to -9‰. The relative abundance of carbohydrates in the diet is less strongly represented in collagen than in the mineral phase of bones and dentine. Furthermore, the $\delta^{13}C$ and $\delta^{15}N$ values of collagen may over-represent the dietary importance of animal proteins enriched in ^{13}C or ^{15}N relative to low protein plant foods that are enriched in ^{13}C such as maize. Such considerations are essential to the interpretation of the carbon and nitrogen isotope compositions of collagen, especially when attempting to reconstruct the dietary importance of low protein foods. When possible, stable carbon and nitrogen isotopic analyses of collagen should be interpreted alongside complementary carbonate data. However, the carbon isotope composition of structural carbonate in bones overestimates the proportion of ^{13}C in the diet by approximately 5‰ (van der Merwe and Vogel 1978). Typically, the $\delta^{13}C$ value of collagen provides a lower limit of the relative dependency on C3 and C4 foods, whereas the $\delta^{13}C$ value of carbonate provides an upper limit, with the real value likely falling within that range.

Physiology and Dietary Ecology

The relationship between the isotopic signatures of dietary elements and their consumers varies consistently across species. The different biochemical fractions that occur in an organism's body also vary in carbon and nitrogen isotope composition due to fractionation during metabolism (Bocherens et al. 2000). Among other factors, Froehle et al. (2010) note that habitat, body size, and digestive physiology may confound the relationship between diet and tissues; however, they maintain that when the protein source is controlled, body size does not contribute to this relationship. When diet is unknown, but potentially non-monoisotopic, the $\delta^{13}C$ value of collagen does not give a strong indication of the $\delta^{13}C$ value of diet (Froehle et al. 2010). Ambrose and Norr (1993) show that as diet varies, so should the spacing between the $\delta^{13}C$ values of collagen and diet as well as collagen and carbonate. This spacing is a consequence of the metabolic pathways used in the biosynthesis of collagen and carbonates as described previously (Harrison and Katzenberg 2003; Schwarcz 2000). When there is a higher proportion of protein in the diet, there is greater congruence between the isotopic signatures of collagen and diet (Froehle et al. 2010). Substantial variations may also occur in the $\delta^{13}C$ and $\delta^{15}N$ values resulting from nursing and weaning, both between and within species (Jenkins et al. 2001). Ultimately, the dietary ecology of an organism is restricted by the environmental parameters in which it grows, develops, and lives. Microenvironments can be the source of substantial isotopic variation and contribute to a variety of nutritional stresses that alter the isotopic signatures of plants and animals.

Environmental Contributions to Stable Isotope Ratios

The environmental parameters during the life of an organism can impact the stable isotope composition of its tissues. Bocherens et al. (2000) outline some of the local environmental factors that may influence the carbon isotope signatures of plants and their consumers. In closed forest environments, the inorganic source of carbon (atmospheric CO_2) for under-storey plants is ^{13}C-depleted relative to the general atmosphere. The depletion is a result of respiration by animals and the

decomposition of organic matter. Moreover, in environments with low light intensity, the $\delta^{13}C$ values of plants may be as low as or lower than -30‰. Conversely, water and saline stress environments lead to less isotopic fractionation in C3 plants resulting in $\delta^{13}C$ values as high as -20‰.

Katzenberg (2000) notes that there is more variation in the $\delta^{13}C$ values of freshwater plants and fish than terrestrial flora and fauna. Unlike terrestrial plants, freshwater plants have varying sources of carbon that include atmospheric CO_2, CO_2 in the water, bicarbonate and carbonate from rocks and soils, and organic carbon from waste and decomposing plant and animal remains (Katzenberg 2000). Additionally, carnivorous species of freshwater fish are enriched in both ^{13}C and ^{15}N due to the consumption of higher trophic level proteins. In diets consisting of both fish and plants, the routing of carbon atoms during collagen biosynthesis results in the over-representation of the proteins of fish enriched in ^{13}C in the carbon isotope composition of collagen relative to the proteins in C4 plants (Schwarcz 2000). Marine and coastal environments can further confound interpretations of isotopic data because of the variable isotopic signatures of ocean flora forming the basis of the food webs. Marine fauna have an average $\delta^{13}C$ value of about -15‰, with values becoming more negative with decreasing ocean temperatures (van der Merwe and Vogel 1978). In coastal environments, the fertilization of crops using seabird guano may greatly influence the nitrogen isotope composition of harvested plants by introducing large quantities of exogenous nitrogen into the soils. Szpak et al. (2012) show that the fertilization of maize with seabird guano can dramatically affect its $\delta^{15}N$ value and by extension, the tissue of its consumers. Environmental parameters are an essential consideration in the interpretation of any isotopic dataset due to their profound and multiple effects on the isotopic signatures of plants, animals, and their human consumers.

Post-Mortem Alteration

Taphonomic processes during the inhumation period of skeletal remains can affect bones and teeth, both structurally and chemically. Collins et al. (2002) and Hedges (2002) demonstrate that taphonomic degradation of remains can lead to structural changes, both macro- and micro-scopically, or even a complete loss of molecules. Chemical changes to archaeological collagen frequently occur due to microbial activity, hydrolysis, or soil pH. Gillespie et al. (1984) note that archaeological bones often contain exogenous nitrogen from sources such as humic acids, soil amino acids, or fertilizers. Structural changes may influence the rate and degree of post-mortem chemical alterations as well. For instance, increases in micro-porosity, or distortion of the crystalline structure of hydroxylapatite in bones, correlates negatively with the preservation of collagen. Nielsen-Marsh et al. (2000) argue that microbial degradation of collagen heavily relies on the initial breakdown of the inorganic matrix to permit access for bacteria and fungi.

Hedges (2002) states that the majority of collagen loss in archaeological remains is due to microbial activity. Severely affected bone can lose over 80% of its collagen. Bacteria and fungi in surrounding soils produce collagenases, which break down bone collagen and liberate organic carbon and nitrogen to the microorganisms (Pate 1994). Mayer (1994) notes that the organization of proteins such as collagen within hard tissues inherently protects them from microbial enzymolysis until the mineral structure has been altered. Changes in the diameter of micro-pores permit access to large molecules such as

collagenases. The integrity of the micro-porosity of bones physically restricts the enzymatic digestion of collagen. Degradation aside, soil bacteria and fungi are also a major source of post-mortem alteration of $\delta^{13}C$ and $\delta^{15}N$ values. Heterotrophic bacteria preferentially metabolise amino acids with a higher number of carbon atoms leading to changes in the molecular composition of collagen (Grupe 2001). Balzer et al. (1997) examine the effects of soil bacteria on the carbon and nitrogen isotope compositions of bone collagen. The $\delta^{13}C$ values shifted more negatively whereas the $\delta^{15}N$ values shifted more positively. Balzer et al. (1997) posit that changes to the carbon isotope composition arise from modification of the amino acid composition by soil bacteria. Conversely, changes in the nitrogen isotope composition are a consequence of the cleavage of peptide bonds in which heavier ^{15}N atoms preferentially remain in the substrate.

In environments protected from microbial activity, the primary cause of collagen deterioration is the hydrolysis of peptide bonds leading to the formation of protein fragments that will eventually be leached from the bone (Collins et al. 1995). Smith et al. (2002) observed that rapid collagen loss due to hydrolysis may occur in histologically unaltered bone. Hydrolytic chemical changes can be a product of the post-depositional environment or of the organism itself. Upon death, the process of autolysis causes the chemical digestion of cells by hydrolytic enzymes (Pate 1994). Grupe (2001) notes that collagen molecules are extremely difficult to hydrolyse due to strong intra- and inter-molecular bonds. Hypothetically, due to the variation in bond strength of carbon and nitrogen isotopes, lighter isotopes may be preferentially hydrolysed resulting in changes to the isotopic composition of the collagen.

Mediating the impact of microbial activity and hydrolysis is the post-depositional environment. Temperature and aridity can either promote or inhibit microbiological activity. Temperature influences the rates of chemical reactions and microbial growth patterns (Child et al. 1993; Ortner et al. 1972). Bacterial activity is greatly reduced at low temperatures (Grupe 2001). The effects of microbial activity also vary depending on whether the bones were cooked or buried with or without soft tissues (Hedges 2002). Natural or cultural removal of soft tissues inhibits the process of putrefaction and the associated microbial activity (Pate 1994). Hedges (2002) maintains that concentrations of solutes and soil pH affect the deterioration of collagen through hydrolysis. Grupe (2001) argues that overall, the decomposition of dead organic matter is a function of its burial environment rather than time elapsed since burial.

Conclusions

It is clear that aside from the methodological constraints of producing and interpreting isotopic data, there are many other factors that must be considered in the interpretation of the data. The molecular composition of carbon isotopes and their frequency within dietary amino acids directly affects the accuracy of isotopic analyses of the dependency on certain food sources in human and animal skeletal remains. The isotopic signatures of carbon and nitrogen from type I collagen are also altered by the physiology of the organism itself. The microenvironments in which an organism lives, dies, and is deposited can have substantial effects on the stable isotope compositions of carbon and nitrogen in bone or dentinal collagen.

Contemporary research in stable isotope anthropology is focused on further understanding the idiosyncrasies of isotopic

variation in plants and animals, while applying this knowledge to an ecological and biocultural understanding of the past. Currently, the range and causes of carbon stable isotope variation are well understood relative to nitrogen. Future research on the role of nutrition, disease, stress, seasonality, and environment are critical for a more holistic understanding of nitrogen stable isotope variation in plant and animal tissues. Another limitation facing current research is the quantification of rates of cellular turnover. The timing of cellular metabolism of the four primary constituents of bone – calcium phosphates, collagen, lipids, and non-collagenous proteins – is a relatively poorly understood concept. Contemporary analyses of the mobility of human and animal populations are unable to precisely measure the chronology of isotopic changes based on known rates of cellular turnover in tissues.

The future of stable isotope analysis in archaeology is likely to incorporate further advancements from the fields of cell biology, physiology, biogeochemistry, and molecular genetics as its application to understanding the past continues to diversify. As the technology and skilled technicians required to conduct molecular analyses become more accessible, the interpretation of stable isotopic signatures in human and animal remains will likely become an analytical staple in both cultural resource management (CRM) and research contexts in archaeology, alongside radiocarbon dating. Archaeologists rely on multiple lines of evidence to accurately interpret the past and stable isotope analysis provides an opportunity for a multitude of biochemical evidence on a variety of complex topics. Stable isotope ratios in archaeological remains are increasingly being used to address questions about human socio-economic and political systems, status and occupational hierarchies, and the movement of people and objects across the physical landscape. As the complexity and breadth of the discipline increases, so will its ability to illuminate the past.

References Cited

Ambrose, S.H. 1998. Chronology of the Later Stone Age and Food Production in East Africa. *Journal of Archaeological Science* 25:377–392.

---. 1993. Isotopic Analysis of Palaeodiets: Methodical and Interpretive Considerations. In *Investigation of Ancient Human Tissue: Chemical Analysis in Anthropology*. M.K. Sandford, ed. Pp. 59–130. New York: Gordon & Breach.

---. 1990. Preparation and Characterization of Bone and Tooth Collagen for Isotopic Analysis. *Journal of Archaeological Science* 17:431–451.

Ambrose, S.H., and L. Norr. 1993. Experimental Evidence for the Relationship of the Carbon Isotope Ratios of Whole Diet and Dietary Protein to Those of Bone Collagen and Carbonate. In *Prehistoric Human Bone: Archaeology at the Molecular Level*. J.B. Lambert and G. Grupe, eds. Pp. 1–38. Berlin: Springer-Verlag.

Balzer, A., G. Gleixner, G. Grupe, H.L. Schmidt, S. Schramm, and S. Turban-Just. 1997. In Vitro Decomposition of Bone Collagen by Soil Bacteria: the Implication for Stable Isotope Analysis in Archaeometry. *Archaeometry* 39:415–429.

Bender, M.M., D.A. Baerreis, and R.L. Steventon. 1981. Further Light on Carbon Isotopes and Hopewell

Agriculture. *American Antiquity* 46:346–353.

Bocherens, H., D. Billiou, V. Charpentier, and M. Mashkour. 2000. Palaeoenvironmental and Archaeological Implications of Bone and Tooth Isotopic Biogeochemistry (13C, 15N) in Southwestern Asia. In *Archaeozoology of the Near East IV*, vol. B. H. Buitenhuis, M. Mashkour, and F. Poplin, eds. Pp. 104–115. Groningen: ARC 32.

Burleigh, R., and D. Brothwell. 1978. Studies on American Dogs, 1. Carbon Isotopes in Relation to Maize in the Diet of Domestic Dogs from Early Peru and Ecuador. *Journal of Archaeological Science* 5:355–362.

Child, A.M., R.D. Gillard, and A.M. Pollard. 1993. Microbially-Induced Promotion of Amino Acid Racemization in Bone: Isolation of the Organisms and Detection of Their Enzymes. *Journal of Archaeological Science* 20:159–168.

Collins, M.J., M.S. Riley, A.M. Child, and G. Turner-Walker. 1995. A Basic Mathematical Simulation of the Chemical Degradation of Ancient Collagen. *Journal of Archaeological Science* 22:175–183.

Collins M.J., C.M. Nielsen-Marsh, J. Hiller, C.I. Smith, and J.P. Roberts. 2002. The Survival of Organic Matter in Bone: A Review. *Archaeometry* 44(3):383–394.

Davis, S.J.M. 1987. What Are Bones and Teeth? In *The Archaeology of Animals*. Pp. 47–60. London: Routledge.

DeNiro, M.J., and S. Epstein. 1981. Influence of Diet on the Distribution of Nitrogen Isotopes in Animals. *Geochimica et Cosmochimica Acta* 45:341–351.

Froehle, A.W., C.M. Kellner, and M.J. Schoeninger. 2010. Effect of Diet and Protein Source on Carbon Stable Isotope Ratios in Collagen: Follow Up to Warinner and Tuross (2009). *Journal of Archaeological Science* 37:2662–2670.

Gillespie, R., R.E.M. Hedges, and J.O. Wand. 1984. Radiocarbon Dating of Bone by Accelerator Mass Spectrometry. *Journal of Archaeological Science* 11:165–170.

Grupe, G. 2001. Archaeological Microbiology. In *Handbook of Archaeological Sciences*. D.R. Brothwell, and A.M. Pollard, eds. Pp. 351–363. Chichester: John Wiley & Sons, Ltd.

Harbeck, M., and G. Grupe. 2009. Experimental Chemical Degradation Compared to Natural Diagenetic Alteration of Collagen: Implications for Collagen Quality Indicators for Stable Isotope Analysis. *Archaeological and Anthropological Sciences* 1:43–57.

Harrison, R.G., and M.A. Katzenberg. 2003. Paleodiet Studies Using Stable Carbon Isotopes from Bone Apatite and Collagen: Examples from Southern Ontario and San Nicolas Island, California. *Journal of Anthropological Archaeology* 22:227–244.

Hedges, R.E.M. 2002. Bone Diagenesis: An Overview of Processes. *Archaeometry* 44(3):318–328.

Jenkins, S.G., S.T. Partridge, T.R. Stephenson, S.D. Farley, and C.T. Robbins. 2001. Nitrogen and Carbon Isotope Fractionation Between Mothers, Neonates, and Nursing Offspring. *Oecologia* 129:336–341.

Katzenberg, M.A., H.P. Schwarcz, M. Knyf, and F.J. Melbye. 1995. Stable Isotope Evidence for Maize Horticulture and Paleodiet in Southern Ontario, Canada. *American Antiquity* 60(2):335–350.

Katzenberg, M.A., and N.C. Lovell. 1999. Stable Isotope Variation in Pathological Bone. *International Journal of Osteoarchaeology* 9:316–324.

Katzenberg, M.A. 2000. Stable Isotope Analysis: A Tool for Studying Past Diet, Demography, and Life History. In *Biological Anthropology of the Human Skeleton*. M.A. Katzenberg, and S.R. Saunders, eds. Pp. 413–442. Hoboken: Wiley-Liss.

Linde, A., and M. Goldberg. 1993. Dentinogenesis. *Critical Reviews in Oral Biology and Medicine* 4(5):679–728.

Marshall, J.D., J.R. Brooks, and K. Lajtha. 2007. Sources of Variation in the Stable Isotope Composition of Plants. In *Stable Isotopes in Ecology and Environmental Science: A Primer*. R. Michener and K. Lajtha, eds. Pp. 22–60. Malden: Blackwell Publishing.

Mayer, L.M. 1994. Surface Area Control of Organic Carbon Accumulation in Continental Shelf Sediments. *Geochimica et Cosmochimica Acta* 58:1271–1284.

Metcalfe, J.Z., F.J. Longstaffe, and G.D. Zazula. 2010. Nursing, Weaning, and Tooth Development in Woolly Mammoths from Old Crow, Yukon, Canada: Implications for Pleistocene Extinctions. *Palaeogeography, Palaeoclimatology, Palaeoecology* 298(3):257–270.

Nielsen-Marsh C.M., A. Gernaey, G. Turner-Walker, R. Hedges, A. Pike, and M.J. Collins. 2000. The Chemical Degradation of Bone. In *Human Osteology in Archaeology and Forensic Science*. M. Cox and S. Mays, eds. Pp. 439–454. London: Greenwich Medical Media.

Nier, A.O., and E.A. Gulbransen. 1939. Variations in the Relative Abundance of the Carbon Isotopes. *Journal of the American Chemical Society* 61:697–698.

O'Leary, M.H. 1981. Carbon Isotope Fractionation in Plants. *Phytochemistry* 20(4):553–567.

---. 1988. Carbon Isotopes in Photosynthesis. *Bioscience* 38:328–336.

Ortner, D.J., D.W. von Endt, and M.S. Robinson. 1972. The Effect of Temperature on Protein Decay in Bone: Its Significance in Nitrogen Dating of Archaeological Specimens. *American Antiquity* 37:514–520.

Pate, D.F. 1994. Bone Chemistry and Paleodiet. *Journal of Archaeological Method and Theory* 1(2):161–209.

Schwarcz, H.P., J. Melbye, M.A. Katzenberg, and M. Knyf. 1985. Stable Isotopes in Human Skeletons of Southern Ontario: Reconstructing Paleodiet. *Journal of Archaeological Science* 12:187–206.

Schwarcz, H.P. 2000. Some Biochemical Aspects of Carbon Isotopic Paleodiet Studies. In *Biogeochemical Approaches to Paleodietary Analysis*. S.H. Ambrose and M.A. Katzenberg, eds. Pp. 189–209. New York: Kluwer Academic/Plenum.

Smith, B.N., and S. Epstein. 1971. Two Categories of 13C/12C Ratios for Higher Plants. *Plant Physiology* 47:380–384.

Smith, C. I., C.M. Nielsen-Marsh, M. Jans, P. Arthur, A.G. Nord, and M.J. Collins. 2002. The Strange Case of Apigliano: Early 'Fossilization' of Medieval Bone in Southern Italy. *Archaeometry* 44(3):405–16.

Sulzman, E.W. 2007. Stable Isotope Chemistry and Measurement: A Primer. In *Stable Isotopes in Ecology and Environmental Science*. R. Michener and K. Lajtha, eds. Pp. 1–21. Malden: Blackwell Publishing.

Szpak, P., F.J. Longstaffe, J-F Millaire, and C.D. White. 2012. Stable Isotope Biogeochemistry of Seabird Guano Fertilization: Results from Growth Chamber Studies with Maize (*Zea Mays*). PLoS One 7(3). http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0033741.

Tuross, N., M.L. Fogel, and P.E. Hare. 1988. Variability in the Preservation of the Isotopic Composition of Collagen from Fossil Bone. *Geochimica et Cosmochimica Acta* 52:929–935.

Tykot, R.H. 2006. Isotope Analyses and the Histories of Maize. In *Histories of Maize: Multidisciplinary Approaches to the Prehistory, Linguistics, Biogeography, Domestication, and Evolution of Maize*. J.E. Staller, R.H. Tykot, and B.F. Benz, eds. Boston: Elsevier Academic Press.

van der Merwe, N.J., and J.C. Vogel. 1978. 13C Content of Human Collagen as a Measure of Prehistoric Diet in Woodland North America. *Nature* 276:815–816.

Vogel, J.C., and N.J. van der Merwe. 1977. Isotopic Evidence for Early Maize Cultivation in New York State. *American Antiquity* 42(2):238–242.

Wassenaar, L.I. 2008. An Introduction to Light Stable Isotopes for Use in Terrestrial Animal Migration Studies. In *Tracking Animal Migration with Stable Isotopes*. K.A. Hobson and L.I. Wassenaar, eds. Boston: Academic Press.

White, C.D., M.E. Pohl, H.P. Schwarcz, and F.J. Longstaffe. 2001. Isotopic Evidence for Maya Patterns of Deer and Dog Use at Preclassic Colha. *Journal of Archaeological Science* 28:89–107.

Williams, L.J. 2008. Investigating Seasonality of Death at Kellis 2 Cemetery Using Solar Alignment and Isotopic Analysis of Mummified Tissues. PhD Dissertation, University of Western Ontario, London.