

Reconstructing the diets of southern African farmers: comparing stable isotopes across body tissues

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Abstract

Expanding on existing data, this thesis presents the largest (N=51) isotopic dataset for Iron Age (2000-100 years BP) agriculturalists in southern Africa. Four isotopic values are presented for each individual ($\delta^{13}\text{C}_{\text{bone apatite}}$, $\delta^{13}\text{C}_{\text{tooth enamel}}$, $\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{15}\text{N}_{\text{collagen}}$). Dietary reconstructions using both typical ‘fractionation factor’ methods and recent bi- and multi-variate models are presented and compared. For this population, which consumed primarily C_4 protein and energy sources (including protein-rich crops such as sorghum and millet), multiple tissue isotopes provide valuable insight into diet that cannot be achieved with single tissues, but current models are limited by the lack of isotopic diversity in the data on which they are based. Multi- and bi-variate models are unable to distinguish between C_4 plants and animals in some cases, and recreation of cluster analysis including the Iron Age data results in a reduction in the parsimony of the dietary clusters derived in Froehle et al. 2012. Isotopic reconstructions suggest that C_3 dietary components contributed limited protein or energy to Iron Age farmers, elaborating on archaeological evidence for their use.

Iron Age agriculturalists are found to have consumed highly variable and heterogeneous diets, especially after the 18th century (for all agriculturalists: $\delta^{13}\text{C}_{\text{bone apatite}} = -6.25 \pm 2.49\text{‰}$, $\delta^{13}\text{C}_{\text{tooth enamel}} = -2.88 \pm 2.48\text{‰}$, $\delta^{13}\text{C}_{\text{collagen}} = -8.65 \pm 2.16\text{‰}$, $\delta^{15}\text{N}_{\text{collagen}} = 10.05 \pm 1.9\text{‰}$). The expansion of settlement into higher-altitude grassland areas obscures the introduction of maize in the region in the 15th and 16th centuries, and there is no significant difference between the Early and Late Iron Age, with more diversity than expected overall. Environmental effects impact $\delta^{15}\text{N}_{\text{collagen}}$ significantly, but there is no distinct geographical patterning in ^{13}C between grassland and savanna biomes. Evidence of regional variation including along rivers and coastal regions is apparent in both the Early and Late Iron Age.

The pre-treatment of bone apatite for isotopic use was explored through a series of experiments that compared reaction time in acetic acid, sodium hypochlorite, and particle size of the bone powder to $\delta^{13}\text{C}_{\text{bone apatite}}$. Extremely small (<25 μm) particles were very sensitive to pre-treatment and significantly altered $\delta^{13}\text{C}_{\text{bone apatite}}$. Time in sodium hypochlorite was more impactful on the isotopic value of the sample than time in acid, but for short exposure times (<3.5 hours) even poorly preserved bone showed robust isotopic values. $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{tooth enamel}}$ were uncorrelated ($R^2=0.24$) and $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ ($4.77 \pm 1.42\text{‰}$) and $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ ($5.67 \pm 1.66\text{‰}$) are comparable to other published values indicating that tooth enamel and bone apatite are mineralogically and isotopically distinct. The effects of pre-treatment on isotope values in previous studies is examined and possible sources of difference in metabolic processes or fractionation for bone apatite and tooth enamel are explored.

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1. Introduction

Stable isotopes have been a valuable tool for understanding the diet of past people for decades. This technique has enabled broad reconstructions of the ecology and food production strategies of humans worldwide. Typical analysis involves a single body tissue, or at most two, and most reconstructions still rely on interpretive methods similar to those of early isotope work. Multivariate or combinatorial interpretive methods can offer additional insight into past diets. These techniques are based on characteristics of complex metabolic processes that are far from fully understood, but are worth pursuing.

This study analyses the isotopic evidence for diet among the Iron Age farmers of southern Africa. Combining previously published data on bone collagen with tooth enamel and bone apatite isotopic data, it establishes the largest (N=51) multi-tissue isotopic dataset for southern Africa and complements other datasets worldwide. The use of isotopes from bone for archaeological reconstruction has increased substantially in the last five years (Pestle et al., 2014), but bone apatite is still the least-utilised tissue for isotopic analysis. There is a lack of studies that compare the isotopes from this tissue to those from bone collagen and tooth enamel in the same individuals, and the possibilities of dietary reconstructions that are based on multiple tissues have been explored in only a limited and preliminary way.

Some work has been done to develop multivariate models, based on published data from controlled feeding experiments, wild animal experiments, and existing data on archaeological human remains (Kellner and Schoeninger, 2007; Froehle et al., 2012; Pestle et al., 2015). These models draw from the same human and animal datasets, which are geographically restricted and do not represent all of the major combinations of possible human diets. This is an artifact of the currently available data, and this study will contribute a valuable additional dataset from a different region and distinct dietary regime.

This project augments and expands on existing work to reconcile the isotopic distinctions between tissues, and contributes to efforts to develop dietary reconstructions that offer greater detail on the components of past diet. Iron Age farmers in the summer rainfall zone primarily grew crops such as millet and sorghum, which are both plants that utilize the C4 photosynthetic pathway (Sage, 1999). This population stands as an excellent comparison with the North American agriculturalists utilized in the models of Froehle et al., and would expand the data both numerically and regionally (2010). By analysing isotopes from bone

collagen, apatite, and tooth apatite in the same individuals, this study also seeks to provide greater insight into the offsets between the different tissues and explore the dietary conditions under which they can provide greater nuance to our understanding of past diets.

Chapter two provides an introduction to isotope chemistry and the way in which it is used to reconstruct and interpret human diet. It explores the two multivariate models that are analysed in the context of southern Africa, and the metabolism and structure of the body tissues of interest. Background to the ongoing debate around the preparation and analysis of these tissues, and how these methods effect their isotopic analysis, is also given. Context to the last 2000 years of southern African agricultural settlement and the archaeological evidence for it is given in chapter three. This chapter also provides background on the archaeological sites that feature in this study and the geographical and ecological context where they are found.

Chapter four outlines the methods employed for all analyses in this study, including two additional experiments that were conducted around the preparation and analysis of bone apatite. The full list of all sampled individuals and the institutions where they are housed can be found here. This chapter also discusses the sources of the previously analysed isotope data (both published and unpublished sources).

The results of all analysis are in chapter five. Isotopic data from all three tissues are explored and compared in terms of time, geography, subsistence strategy, and notable anomalies or outliers are indicated. Chapter five also explores the results of the experiments that were conducted to provide further support for the use of bone apatite as a tissue of archaeological interest.

The final chapter, six, examines the results of the analysis and explores possible reconstructions of Iron Age diet in light of the other lines of archaeological evidence. It analyses the multivariate models discussed in chapter two with the new Iron Age data, and presents a critique of previously employed reconstruction methods. This chapter also explores the implications of the results of the bone apatite experiments and makes recommendations for all isotopic analysis of this tissue going forward. This chapter concludes the study with a reflection on the various methods for dietary reconstruction that are possible with isotopes, and their relevance and appropriateness for Iron Age farmers in southern Africa and the rest of the world.

2. Isotopic Analysis of Human Diet

2.1. Introduction to Isotope Chemistry

2.1.1. Natural abundance, fractionation, and interpretation

Of all the hydrogen, carbon, nitrogen, oxygen, and sulfur atoms on Earth, 95% occur as the major isotope of those elements. The major isotope contains equal numbers of neutrons and protons in the nucleus: in the major isotope of carbon, ^{12}C , there are 6 protons and 6 neutrons. Other common isotopes of carbon are ^{13}C (7 neutrons) and ^{14}C (which is radioactive; ^{12}C and ^{13}C are stable). Each isotope of an element has the same chemical properties, but the different atomic masses cause different physical properties, particularly reaction rates. In most cases, the major isotope is the ‘light’ isotope—i.e. ^{12}C is physically lighter than ^{13}C because it lacks the extra neutron. For all the elements listed above, the major isotope is the lightest.

Isotopes occur naturally in varying abundances, and their different physical properties cause them to pattern across the bio- and lithosphere in predictable ways. For example, water molecules containing the heavy isotope of oxygen, ^{18}O , condense before those containing the lighter isotope (^{16}O), making rainfall at the initial point of a storm more enriched in ^{18}O . The reverse also occurs, when water molecules containing the lighter isotopes of oxygen (and hydrogen (^1H)) evaporate preferentially compared with those containing the heavy isotopes. The natural process of separating isotopes via their physical properties is fractionation. Fractionation occurs primarily through the differential reaction rates of heavier and lighter isotopes: heavier isotopes form stronger bonds, and so tend to accumulate on the side of an exchange reaction that is most energetically favorable. They also have slower reaction rates; in kinetic reactions lighter isotopes tend to undergo reaction disproportionately (Schwarcz and Schoeninger, 2012). Fractionation results in uneven distribution of isotopes of the same element, producing substances that have distinct, predictable isotopic ratios. The proportion of light:heavy isotopes of an element in a substance contains information about the formation processes it underwent, and the environment from which it drew constituent atoms. The isotope ratio of a sample is expressed as the following (in this example, carbon is used):

$$\text{Sample Ratio } (R_{\text{sample}}) = \left(\frac{^{12}\text{C}}{^{13}\text{C}} \right)_{\text{sample}}$$

The abundance of an isotope is expressed through δ (*delta*), which compares the abundance ratio of the sample to an internationally accepted standard value. The international standard is the ‘zero’ value for the heavy isotope on Earth, and so the heavy isotope content of the sample is the difference between the two ratios (expressed in parts per thousand (‰)). This notation focuses on the abundance of the heavy isotope in the sample relative to the abundance in the standard. If a sample has a more positive δ value than the standard, it contains more of the heavy isotope; i.e. the sample is enriched in the heavy isotope relative to the standard. The equation for δ is:

$$\delta[^{13}\text{C}] (\text{‰}) = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

In the case of carbon, the international standard is a marine belemnite from the PeeDee formation (PDB)¹. Relative to PDB, current atmospheric CO₂ has a $\delta^{13}\text{C}$ value of -8‰. Prior to the Industrial Revolution and associated increase in atmospheric carbon, $\delta^{13}\text{C}$ was -6.5‰ (Marino and McElroy, 1991). Delta values for other isotopes are calculated in the same way, using different internationally recognized standards.

2.1.2. Carbon

The most widespread and significant cause of the fractionation of carbon isotopes takes place during photosynthesis. Green plants combine CO₂ from the atmosphere with H₂O to form cellulose, and their tissues take on $\delta^{13}\text{C}$ values that reflect those of the CO₂, plus an offset. The offset reflects the chemical reactions the carbon atoms undergo—i.e. the fractionation of the carbon as it is transformed from CO₂ into cellulose.

The majority of terrestrial plants photosynthesize using the C₃ (Calvin-Benson) metabolic pathway: this is true of all trees and woody plants, temperate grasses, and most aquatic plants. C₃ plants have $\delta^{13}\text{C}$ values between -19‰ and -37‰, averaging globally around -26.7±2.3‰ (they are negative relative to the standard; i.e. more depleted in ¹³C than PDB) (Cerling et al., 1997b; Codron et al., 2005b). The wide range of $\delta^{13}\text{C}$ values observed in C₃ plants results from specific growing conditions which effect fractionation: enriched or depleted $\delta^{13}\text{C}$ values are found in plants growing under high water stress, or in closed, dark environments with limited CO₂ exchange (such as forest floors), respectively (Cerling et al., 1993, 1997a). $\delta^{13}\text{C}$ in C₃ plants is somewhat negatively correlated with rainfall ($r^2=0.20$,

¹ The original PDB was found in the south-eastern United States; that supply being exhausted, today VPDB (Vienna PDB) is used. The $\delta^{13}\text{C}$ values of VPDB and PDB are identical.

$p < 0.01$) for plants in southern Africa, which is a less strong relationship than is observed in comparable regions in Australia (Swap and Aranibar, 2004).

Tropical grasses that are adapted for higher levels of light, aridity, and temperatures during the growing season utilize the C₄ (Hatch-Slack) photosynthetic pathway, which captures CO₂ before following the C₃ pathway, and results in a 4-carbon end product (as opposed to the 3-carbon product of the Calvin-Benson cycle). C₄ photosynthesis reduces the loss of CO₂ through the leaf stomata, resulting in $\delta^{13}\text{C}$ values between -9‰ and -16‰, with an average of -12.5 ± 1.1 ‰ (Cerling et al., 1997b; Codron et al., 2005b). The distribution of C₄ plants correlates with seasonality of rainfall and temperature in the growing season: C₃ plants can only flourish in regions with low temperatures (below 25°C) during the wet/growing season (Edwards et al., 2010). No relationship is observed between C₄ $\delta^{13}\text{C}$ and rainfall amongst the plants of southern Africa, which is consistent with the correspondence between C₄ photosynthesis and limited water availability, and the effect on water use efficiency of the metabolic pathway (Swap and Aranibar, 2004). Some species of marine algae also utilize the C₄ pathway. Over 95% of grass species in inland South Africa are C₄, and surveys of hundreds of species from the region reported non-overlapping ranges for C₃ and C₄ plants and significantly different mean $\delta^{13}\text{C}$ (-26.5‰ and -12.5‰) (Vogel et al., 1978). An extensive survey of plants in the savanna region of north-eastern South Africa confirmed the distinction between C₃ and C₄ plants there, and found that natural variation based on species, plant body part, geology, etc does not contribute significantly enough to obscure the isotopic separation of the two groups (Codron et al., 2005b).

Because plants form the basis of the food chain, animals that consume plants of a particular photosynthetic pathway form body tissues with $\delta^{13}\text{C}$ values that reflect those of the plants, plus a fractionation offset. Secondary consumers that eat the flesh of primary consumers further reflect the isotopic composition of their prey, plus another offset—making them ultimately reflective of the plants in their environment. This process is discussed in sections 2.2 and 2.3. By measuring the $\delta^{13}\text{C}$ of body tissues from humans and animals at archaeological sites, the proportion of C₃ and C₄ plants (or animals consuming those plants) in the diet can be determined. This information is also often used to extrapolate climatic conditions at the time of occupation at the site, since the differences in photosynthetic pathway between the plants are based on adaptations to different environmental conditions.

2.1.3. Nitrogen

The international standard for nitrogen is AIR (ambient inhalable reservoir)/atmospheric N₂, defined as having a $\delta^{15}\text{N}$ of 0‰. Fractionation of nitrogen isotopes is driven by a complex cycle of nitrogen fixation and denitrification, primarily in soils and the ocean, which is much less well understood than the effects of fractionation on $\delta^{13}\text{C}$ (Handley and Raven, 1992; Santoro, 2016). There is a marked difference between the $\delta^{15}\text{N}$ values of organisms from marine and terrestrial environments, and $\delta^{15}\text{N}$ also generally increases with trophic level (i.e. becomes enriched in ¹⁵N with each subsequent consumer). The simple relationship between trophic level and $\delta^{15}\text{N}$ that was initially expressed when isotope studies began (e.g. Minagawa and Wada, 1984), however, is now known to be substantially more complex, varying with the ratio of ¹⁵N:¹⁴N in the diet, the class of the consumer, and the specific tissue under study (Caut et al., 2009).

Most inland plants in South Africa, both C₃ and C₄, have $\delta^{15}\text{N}$ values between +1‰ and +6‰, and there is no significant difference between the two photosynthetic pathways in terms of $\delta^{15}\text{N}$ (Heaton, 1987; Codron et al., 2005b). However, rainfall accounts for nearly 50% of all $\delta^{15}\text{N}$ values of C₃ plants in this region ($r^2=0.54$, $p<0.01$), while only 4% of C₄ plant $\delta^{15}\text{N}$ values are explained by rainfall (Swap and Aranibar, 2004). In savanna-specific plants in South Africa (largely C₄), climate, geology, and taxon-related factors can alter $\delta^{15}\text{N}$ by up to 4‰, but the overall range of $\delta^{15}\text{N}$ values is within the 1-6‰ interval (Codron et al., 2005b). In general, the abundance of N isotopes in southern Africa patterns inversely with rainfall, but this relationship is asymptotic around 1000mm of rainfall/year. This relationship holds on a largely subcontinental scale, and has been confirmed through studies with overlapping study areas of various sizes (Heaton, 1987; Sealy et al., 1987; Swap and Aranibar, 2004). Worldwide, leaf $\delta^{15}\text{N}$ values increase with increasing mean annual temperature for regions where MAT > -0.5°C (Craine et al., 2009).

Although there is a marked correlation between aridity and the $\delta^{15}\text{N}$ of animal bones, Heaton found a much smaller influence of rainfall on plant $\delta^{15}\text{N}$ in southern Africa (Heaton et al., 1986; Heaton, 1987). A large study of plants experiencing between 400-800mm/year of rainfall in the north-east of South Africa found no relationship between plant $\delta^{15}\text{N}$ and rainfall, suggesting that the relationship may be non-linear and only detectable in very arid conditions (Codron et al., 2013). Increased isotope fractionation in animal bones relative to plants is thought to be due to changes in the animal's water consumption and excretion in arid environments, rather than elevated $\delta^{15}\text{N}$ in the plants they eat (Heaton, 1987). Humans living

in arid environments reflect this pattern, with Holocene human remains from the coast and interior of South Africa demonstrating a correlation between higher $\delta^{15}\text{N}$ and decreasing rainfall (Heaton et al., 1986; Sealy et al., 1987). A study of terrestrial and marine animals, as well as Holocene humans, from the South-Western coast of South Africa supports the high impact of aridity on $\delta^{15}\text{N}$ values of animals and humans (Sealy et al., 1987).

In contrast, however, Murphy and Bowman found that dietary $\delta^{15}\text{N}$ was the primary cause of the relationship between kangaroo $\delta^{15}\text{N}_{\text{bone collagen}}$ and rainfall, suggesting that change in plant $\delta^{15}\text{N}$ was driving the change in $\delta^{15}\text{N}_{\text{bone collagen}}$. Metabolic factors appear to have little influence in the response of $\delta^{15}\text{N}_{\text{bone collagen}}$ to rainfall; why this case should be different from those of southern Africa is not yet clear (Murphy and Bowman, 2006).

2.2. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in Humans

2.2.1. Tissues sampled for archaeological isotope studies

Human tissues form from the plants and animals they consumed, incorporating carbon and nitrogen from dietary sources. The most commonly sampled tissues in archaeological studies are bone collagen (protein), the mineral phase of bone, and tooth enamel.

Collagen is the organic component of bone, enabling structural functions like bending and flexing. Collagen is also replaced as bones remodel, and the resulting isotope value represents the diet during the period when the collagen formed. Turnover rates are highest during adolescence (5-15% per annum during ages 10-15) and slow during adulthood (to 1.5-3% per annum). Collagen in most bones probably reflects the isotopic composition of diet over a period of at least 10 years, but femoral bone collagen even in older adults includes a substantial portion of collagen synthesized during adolescence (Hedges et al., 2007).

The inorganic phase of bone is often grouped with other minerals in the body, such as tooth enamel, but each is a unique biomineral, with specific properties and formation conditions related to their environment and function. Tooth enamel forms the extremely hard outer layer of teeth to protect them from the abrasive effects of mastication, and to resist dissolution in the acidic environment of the oral cavity (Hillson, 1996). Bone mineral provides the rigid framework and strength required for the load-bearing role of the skeleton in the body, and bone contains significantly more protein than enamel, such as collagen. Unlike tooth enamel, bone is remodeled throughout life by osteoclasts and osteoblasts, cells which

absorb and replace sections of mineral. Tooth enamel must resist dissolution to maintain function, and is not remodeled.

Both of these minerals are commonly referred to as ‘bioapatite’ or hydroxylapatite (sometimes written as ‘hydroxyapatite,’ which is not recognized by the International Mineralogical Association), terms which have caused some confusion and will be avoided in this study. Hydroxylapatite is a class of apatite containing hydroxyl groups (-OH) in the ‘channel site’ of the apatite structure. Both bone mineral and tooth enamel are apatites (general formula $\text{Ca}_5(\text{PO}_4)_3(\text{F},\text{OH},\text{Cl})$), but Raman spectroscopic comparison of tooth enamel and cortical bone reveals that while enamel shows the characteristic bands of -OH groups at the same positions as geologic and synthesized hydroxylapatite, bone shows no such bands (Wopenka and Pasteris, 2005; Pasteris et al., 2008). The term ‘bioapatite’ is also misleading as it does not distinguish between enamel and bone mineral (Sponheimer, 1999), so the terms tooth enamel and bone apatite will be used in this study. Functional-mineralogical differences have a significant impact on the preparation and use of these tissues for isotope analysis, which is discussed in section 1.6. For now, we turn to the pathways through which isotopes become incorporated in these tissues in the first place.

2.2.2. *Macronutrient Routing: from diet to tissue*

In early isotope studies, $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{collagen}}$ values of the same individuals did not agree as expected, casting doubt on the validity of using apatites for isotopic analysis (Schoeninger and Deniro, 1982). Today, $\Delta_{\text{collagen-apatite}}$ is recognized as a naturally occurring offset, and a valuable tool for dietary reconstruction (Krueger and Sullivan, 1984; Lee-Thorp et al., 1989; Krueger, 1991; Stott et al., 1997; Kellner and Schoeninger, 2007; Froehle et al., 2012). Collagen and the biominerals in bones and teeth undergo distinct metabolism and synthesis, drawing their constituent atoms from different parts of the diet. Each tissue reflects the isotopic signal of the dietary components that are metabolized to synthesize it (Schoeninger and Deniro, 1982; Krueger and Sullivan, 1984; Krueger, 1991).

2.2.2.1. *Routing: collagen*

As a protein, collagen is composed of essential and inessential amino acids. Essential amino acids cannot be synthesized in the body and are incorporated directly from dietary proteins, and in some cases inessential amino acids may also be directly incorporated from the diet. Between 17.3-21% of the amino acids in collagen are essential or have essential precursors, and are incorporated directly from the diet along with their constituent isotopes.

Even the isotopic values of non-essential amino acids have been shown to correlate with the isotopic value of body collagen, reflecting a combination of direct incorporation and *de novo* synthesis (Howland et al., 2003). Thus, the isotopic composition of dietary proteins is reflected in $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{15}\text{N}_{\text{collagen}}$ (Hare et al., 1991; Ambrose and Norr, 1993; Howland et al., 2003). Jim et al. (2004) calculated that for diets that are 20% protein in rats, 51.3% of carbon atoms in rat bone collagen are directly routed from dietary protein.

Bulk $\delta^{13}\text{C}_{\text{collagen}}$ correlates well with theoretical $\delta^{13}\text{C}_{\text{collagen}}$ values calculated from the $\delta^{13}\text{C}$ values of constituent amino acids from controlled feeding studies in rats ($R^2=0.95$) and pigs ($R^2=0.96$) (Howland et al., 2003; Jim et al., 2006). In general there is a strong correlation between $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{dietary protein}}$ (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004, 2006; Froehle et al., 2010). This correlation is surprising in view of the complex environment in which collagen forms. Non-essential amino acids can be incorporated intact or synthesized from dietary precursors that have their own isotopic signatures, and low protein diets cause more *de novo* amino acid synthesis from non-protein food sources. In high protein diets, dietary protein contributes a greater proportion of carbon atoms to collagen than in lower protein diets, and protein type also affects amino acid isotope values (feeding studies with mixed C_3/C_4 diets reveal the inclusion of non-protein dietary isotopes into non-essential amino acids varies widely) (Jim et al., 2006; Froehle et al., 2010).

2.2.2.2. Routing: tooth enamel and bone apatite

$\delta^{13}\text{C}$ of bone and enamel apatite reflects the $\delta^{13}\text{C}$ values of carbon atoms in the whole diet, proteins and non-protein components, carbohydrates and lipids. Bone apatite forms in equilibrium with blood carbonate, a product of energy metabolism, and initially $\delta^{13}\text{C}_{\text{bone apatite}}$ was theorized to reflect $\delta^{13}\text{C}_{\text{dietary energy}}$ (the ‘energy’ components of diet are generally carbohydrates and lipids, or the non-protein elements) (Krueger and Sullivan, 1984; Lee-Thorp et al., 1989).

Subsequent controlled feeding studies showed that $\delta^{13}\text{C}_{\text{bone apatite}}$ more closely tracks $\delta^{13}\text{C}_{\text{whole diet}}$, suggesting that carbon atoms from protein are incorporated into apatite as well (presumably those not directly routed into collagen) (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004). Based on pooled data from feeding studies, Kellner and Schoeninger (2007) report strong correlation between $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{whole diet}}$ ($R^2=0.97$) compared to that of $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{dietary energy}}$ ($R^2=0.59$). Dietary

protein content and body size of the animal (rats versus swine) had no effect on the offset between $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{whole diet}}$. Jim et al. (2006) estimate that diets with protein in excess of 12% contain more atoms than necessary for collagen synthesis, which likely includes all experimental diets and most human ones. This suggests that for most diets, $\delta^{13}\text{C}_{\text{whole diet}}$ is influenced by all dietary components including protein.

2.2.1. Fractionation Factors: from tissue to diet

Isotopic reconstruction of archaeological diet seeks to extrapolate the source of isotopes (the diet) from a distorted reflection (the isotopes recorded in body tissues). To do this, it has been necessary to calculate the specific effect of fractionation between diet and human tissues—to reverse-engineer the process of fractionation that results in tissue isotope values.

Initial estimates of the fractionation factor between diet and whole body $\delta^{13}\text{C}$ in primary consumers ($\Delta^{13}\text{C}_{\text{diet-body}}$) were between +1‰ and +3‰ (DeNiro and Epstein, 1978; Schoeninger and De Niro, 1984) and +5 or +6‰ was most commonly applied to human bone collagen (Vogel et al., 1977; Van Der Merwe and Vogel, 1978; Krueger and Sullivan, 1984). Experimental conditions have yielded published values for $\Delta^{13}\text{C}_{\text{diet-collagen}}$ between +3.7-6‰ (Bocherens and Drucker, 2003; Froehle et al., 2010). $\Delta^{15}\text{N}_{\text{diet-body}}$ was initially hypothesized to be $\sim +3-5\text{‰}$ per trophic level, although at the outset variation was observed both within and between diets (DeNiro and Epstein, 1981; Schoeninger and De Niro, 1984); between +1 and +6‰ is reported for a variety of wild and controlled animal feeding studies (Caut et al., 2009). In the first-ever semi-controlled-diet study of humans, O’Connell et al. (2012) found a significantly larger $\Delta^{15}\text{N}_{\text{diet-collagen}}$ (+4.6‰ at minimum), which suggests that previous interpretations may have overestimated the amount of ^{15}N enriched foods in the diet.

It is also becoming clear that fractionation in carbon isotopes associated with the formation of bone and tooth enamel apatite is different, and that the effect is greater in the case of enamel (resulting in a larger diet to tissue spacing) (Webb et al., 2014). Animal studies have yielded a range of fractionation offsets between apatite and diet: consolidating animal studies in rats and swine, Kellner and Schoeninger (2007) report a $\Delta^{13}\text{C}_{\text{apatite-whole diet}}$ of +9.7‰ (close to +9.4‰ and +9.5‰ in rats (Ambrose and Norr, 1993; Jim et al., 2004)), while swine not included in their study showed a $\Delta^{13}\text{C}_{\text{apatite-whole diet}}$ of 12‰ (Warinner and Tuross, 2009). A $\Delta^{13}\text{C}_{\text{enamel-whole diet}}$ of +14.1‰ was reported in pigs (Warinner and Tuross, 2009) and between +11.5±0.3‰ to +14.6±0.3‰ in animals varying in size from voles to cattle (Passey

et al., 2005). Several literature surveys report offsets between ~12-15‰ for $\Delta^{13}\text{C}_{\text{carbonate-diet}}$ in large herbivores, with carbonate deriving from a number of sources (primarily tooth enamel) (Kohn and Cerling, 2002; Hedges, 2003). Warinner and Tuross (2009) suggest that if tooth-bone difference in humans is similar to that of pigs, dietary reconstructions that have applied fractionation factors derived from bone apatite to $\delta^{13}\text{C}_{\text{tooth enamel}}$ values may have overestimated the presence of C_4 plants in the diet.

Sponheimer and Cerling (2014) suggest that the different offsets may be due to digestive physiology: methane produced by large herbivores is depleted in ^{13}C , raising the $\delta^{13}\text{C}$ value of dissolved inorganic carbonate from which $\delta^{13}\text{C}_{\text{apatite}}$ is derived. Digestive physiology is an important consideration when comparing isotope values between species; methanogenesis would affect both $\delta^{13}\text{C}_{\text{enamel}}$ and $\delta^{13}\text{C}_{\text{apatite}}$, but does not occur in small mammals such as rats or swine, or in primates and hominins.

After reviewing over 500 different fractionation factors from 66 publications, Caut et al. (2009) concluded that fractionation factors vary with taxon, type of tissue, and diet isotopic ratio—suggesting that specific, diet-dependent ratios should be calculated and used from now on for dietary reconstructions, and that cross-species comparison should be avoided if possible. Given that human diet and geographical distribution is the most diverse on Earth, and the difficulty of conducting human feeding experiments, it is unlikely that such precise factors could ever be calculated for reconstructing human diets. Other factors, such as activity level (affecting metabolic rates) and dietary fibre content have been shown to also affect diet to tissue spacing (Howland et al., 2003).

Given this, reconstructions should seek to use fractionation factors that are as population, diet, and region specific as possible, and ranges of factors more realistically account for natural complexity of isotopic analysis, especially when comparing across species (Bocherens and Drucker, 2003; Hedges and Reynard, 2007; Caut et al., 2009; O’Connell et al., 2012). Interpretation of archaeological diets should consider these uncertainties and be limited accordingly. Dietary reconstruction should move away from attempts to quantify the isotopic values of past diets, and new methods of dietary reconstruction that provide similar dietary information without the use of fractionation factors offer another, complementary approach (Kellner and Schoeninger, 2007; Froehle et al., 2012).

2.3. Beyond fractionation factors: multi-tissue dietary reconstruction

2.3.1. Dietary characteristics and tissue isotope values: protein content

Because they reflect different aspects of the diet, apatite and bone collagen isotope values are often studied in combination. These studies have revealed that the relationships between $\delta^{13}\text{C}_{\text{apatite}}$, $\delta^{13}\text{C}_{\text{collagen}}$, and diet differ according to the type of diet. As mentioned before, diets with above 12% protein provide sufficient amino acids to synthesize tissue proteins such as collagen, and the remaining carbon atoms are available for uptake in apatite. $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ is smaller in high protein diets, likely because in those cases $\delta^{13}\text{C}_{\text{whole diet}}$ resembles $\delta^{13}\text{C}_{\text{diet protein}}$ (Lee-Thorp et al., 1989). Conversely, although $\Delta^{13}\text{C}_{\text{whole diet-bone apatite}}$ was unaffected by changes in dietary protein content, $\delta^{13}\text{C}_{\text{bone apatite}}$ was predicted by $\delta^{13}\text{C}_{\text{dietary energy}}$ most accurately for low protein (5%) diets, with accuracy decreasing as protein concentration increased (Kellner and Schoeninger, 2007). The same is broadly true for $\delta^{13}\text{C}_{\text{tooth enamel}}$, but much less research has compared it to $\delta^{13}\text{C}_{\text{collagen}}$. Subsequent studies have further established that dietary protein levels significantly affect $\Delta^{13}\text{C}_{\text{collagen-apatite}}$ and $\Delta^{13}\text{C}_{\text{whole diet-collagen}}$, underscoring the importance of estimating protein content in archaeological diets through independent means in order to interpret isotopic values (Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004).

Harrison and Katzenberg (2003) report $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$ for two populations, one shifting from a C_3 plant and animal diet to one including maize (southern Ontario) and one with a consistent diet of marine protein and C_3 plant material (San Nicholas Island). The introduction of maize (a C_4 plant) is apparent in the wide range of $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ over time in southern Ontario (5.2‰ to 10.8‰), in contrast to the constant offset found on San Nicholas Island (2.5‰ to 2.8‰). The authors also note that the introduction of maize appears gradually in continuously enriched $\delta^{13}\text{C}_{\text{bone apatite}}$ values, while $\delta^{13}\text{C}_{\text{collagen}}$ does not shift to a more mixed (C_3/C_4) signal until a significant amount of maize has entered the diet (Harrison and Katzenberg, 2003). It is important to note that the maize agriculturalists in southern Ontario ate primarily C_3 resources aside from maize, including freshwater fish and animals feeding on C_3 plants. The isotopic distinction between that diet and the new resource (maize) contributes to how apparent it is in especially $\delta^{13}\text{C}_{\text{bone apatite}}$.

2.3.2. Diet characteristics and tissue isotope values: protein type

$\Delta^{13}\text{C}_{\text{collagen-apatite}}$ also varies with $\delta^{13}\text{C}_{\text{collagen}}$, and a wide range of human $\Delta^{13}\text{C}_{\text{collagen-apatite}}$ values have been reported even for individuals living in the same area (Sealy, 1997;

Loftus and Sealy, 2012). Ambrose and Norr (1993) demonstrated the large impact of the isotopic value of dietary protein on $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$: adding just 5% isotopically different protein to a monoisotopic diet was found to change $\delta^{13}\text{C}_{\text{collagen}}$ values in mice by up to 50%. Mice raised on a diet of C_3 protein ($\delta^{13}\text{C}_{\text{diet protein}} = -26.3\text{‰}$) and energy ($\delta^{13}\text{C}_{\text{energy}} = -25.1\text{‰}$) had $\delta^{13}\text{C}_{\text{collagen}}$ of -21.4‰ ; replacing the protein with a C_4 source ($\delta^{13}\text{C}_{\text{diet protein}} = -14.2\text{‰}$) but keeping energy the same shifted $\delta^{13}\text{C}_{\text{collagen}}$ to -14.7‰ (Ambrose and Norr, 1993). $\delta^{13}\text{C}_{\text{diet protein}}$ shifted $\delta^{13}\text{C}_{\text{collagen}}$ non-linearly as diets increased in protein content (a diet with 5% protein, which was isotopically distinct, shifted $\delta^{13}\text{C}_{\text{collagen}}$ by 41-52%, while a diet with 20% distinct protein caused a 66% shift in $\delta^{13}\text{C}_{\text{collagen}}$).

Froehle et al. (2010) also demonstrated that $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ is consistent within, but varies across, protein type: experimental data for rats and swine show highly diverse relationships between bone collagen and apatite, but when diets are separated by C_3 and C_4 protein content, the correlations are much higher ($R^2=0.814$ for C_4 /marine protein, and $R^2=0.921$ for C_3) even across taxa. This suggests that when dietary protein source is not known, collagen should be interpreted as reflecting primarily protein components of the diet, but that it can also provide useful whole-diet information if the dietary protein is monoisotopic and known. In the case of mixed protein sources, interpreting $\delta^{13}\text{C}_{\text{collagen}}$ can be challenging. In the case of high protein diets, $\delta^{13}\text{C}_{\text{bone apatite}}$, reflecting $\delta^{13}\text{C}_{\text{whole diet}}$, can resemble $\delta^{13}\text{C}_{\text{collagen}}$ as they both are tracking $\delta^{13}\text{C}_{\text{dietary protein}}$. Diets low in protein, with distinct (C_3 and C_4) protein and non-protein food sources, are easiest to interpret, as in these cases $\delta^{13}\text{C}_{\text{bone apatite}}$ is most distinct from $\delta^{13}\text{C}_{\text{collagen}}$.

The fact that the correlations are strong and consistent for data from both swine and rats is encouraging, as it suggests that despite differences in body size and digestive physiology, these animal models are reflecting biological processes that could be extrapolated to other animals. However, the very substantial impact of even a small amount of protein of a different isotopic signature, which may not be readily visible in the archaeological record, suggests that truly monoisotopic human diets should not be assumed without strong ecological or environmental constraints, such as island conditions.

The Harrison and Katzenberg (2003) study also provides two contrasting studies on the impact of isotopically distinct food sources in the diet. In the case of southern Ontario, isotopically distinct (C_4) maize was introduced into the diet, and even relatively small (small enough to not affect $\delta^{13}\text{C}_{\text{collagen}}$) quantities shifted $\delta^{13}\text{C}_{\text{bone apatite}}$. This resulted in a large $\Delta^{13}\text{C}$

collagen-bone apatite (7‰), which indicates that protein and non-protein dietary components are isotopically distinct even if the presence of maize had not been verified archaeologically. In the case of San Nicholas Island, marine protein resembles C₄ foods, in contrast to the terrestrial C₃ plants that were consumed; in this case, $\delta^{13}\text{C}_{\text{collagen}}$ was enriched in ¹³C in comparison to $\delta^{13}\text{C}_{\text{bone apatite}}$, resulting in a small (2.6‰) $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ (Harrison and Katzenberg, 2003). These two cases provide further evidence that multiple tissue isotopes can shed significant light on complex diets in cases where protein and non-protein components diverge isotopically. They also emphasize, however, that isotopic results must be interpreted in the context of local diets and other knowledge of their composition.

2.3.3. Reconstruction by model

It is now clear that different body tissues reflect dietary constituent elements to different extents, and can provide more detailed dietary information than is derived in most studies of a single tissue. In particular, separating the protein component from the rest of the diet, and differentiating between diets with monoisotopic components, or mixed ones, is the logical next step in isotopic dietary reconstructions. The wealth of existing data on experimental and wild animal studies, as well as archaeological humans, provides the opportunity to compare, combine, and seek larger patterns among terrestrial mammals or wider human populations.

Kellner and Schoeninger (2007) combined the results of four experimental animal studies (all those that analyzed multiple tissues and reported $\delta^{13}\text{C}_{\text{diet}}$ and $\delta^{13}\text{C}_{\text{diet protein}}$), studies of free-ranging fauna that reported $\delta^{13}\text{C}$ values for bone apatite and collagen, and ten archaeological human populations which consumed diets with monoisotopic protein sources, as confirmed archaeologically. They concluded that isotope values for single tissues are insufficient to derive dietary reconstructions of meaningful strength or depth. Such reconstructions correlate to a range of possible dietary values that often overlap, complicating potential interpretations. For instance, $\delta^{13}\text{C}_{\text{collagen}}$ can predict 100% C₄ ($\delta^{13}\text{C}_{\text{collagen}} \sim -8\text{‰}$) or C₃ ($\delta^{13}\text{C}_{\text{collagen}} \sim -22$ to -20‰) diets fairly accurately, but between -18‰ and -12‰ (i.e. mixed diets, as most real diets are), $\delta^{13}\text{C}_{\text{collagen}}$ can provide little information besides the fact that both sources are present. Confounding effects such as dietary protein content, animal body size, and digestive physiology are also a concern, and are discussed below.

By combining $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$ for all available controlled feeding studies, Kellner and Schoeninger derive a dietary reconstruction model based on two lines: one which describes diets with C_3 protein, and one for C_4 /marine protein. The lines are parallel with significantly different intercepts; a separate line for marine protein has a steeper slope and is near, and intersects, the C_4 protein line. Diets with mainly C_3 non-protein (“energy”) cluster at the lower end of both lines, and those with C_4 energy group at the top (Figure 2-1). Plotting wild faunal data and ten archaeological human populations with a variety of diets onto the lines indicates the primary isotopic content of both parts of the diet with a relatively high degree of accuracy (theorized diets were checked against prior conclusions based on archaeological evidence) (Kellner and Schoeninger, 2007). However, this simple model cannot resolve mixed diets, which fall either between the two lines (mixed protein sources) and/or in the middle of each line (mixed energy sources).

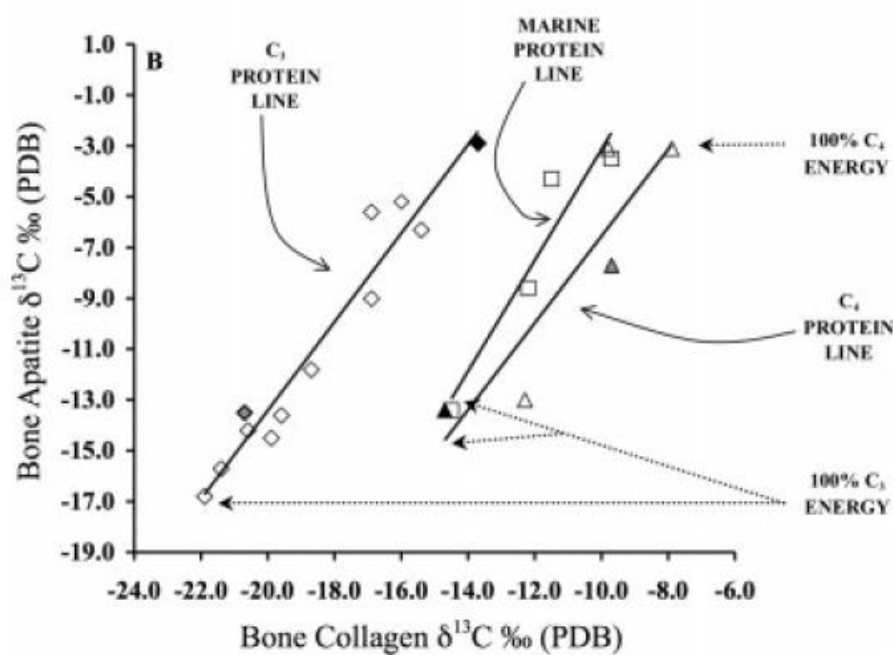


Figure 2-1 A simple model for dietary reconstruction based on protein source. From Kellner and Schoeninger 2007 (fig. 2b). Isotopic data from experimental animals divide into three statistically significant lines when separated by protein type; in each case, diets with primarily C_3 energy correspond to more negative collagen and apatite values

While Kellner and Schoeninger (2007) constructed a bivariate model from laboratory data and extrapolated it for wild animals and humans, Froehle et al. (2012) combined eight of the ten human populations studied in Kellner and Schoeninger to derive a multivariate diet reconstruction model using carbon and nitrogen. Attempting to resolve the overlap between C_4 and marine protein sources in the bivariate model, as well as provide more clarity on

mixed protein/energy diets, the multivariate model combines $\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{13}\text{C}_{\text{bone apatite}}$, and $\delta^{15}\text{N}_{\text{collagen}}$. Using cluster analysis to confirm that each diet in the human samples is isotopically distinct, Froehle et al. (2012) interpreted the diet represented by each cluster by comparing them to the protein-separated lines from Kellner and Schoeninger (also Froehle et al., 2010).

Discriminant function analysis then predicted which cluster a new human sample would fall into, based on a ‘training sample’ of known cluster membership (the Froehle et al. study sample) (Figure 2-2). Two discriminant functions were derived that accounted for 98.8% of the variance between the clusters—function one correlates to $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$, and function two to $\delta^{15}\text{N}$ (they are called ‘carbon’ and ‘nitrogen’). This allows new human isotope values to be converted to DFA scores and plotted against the five diet clusters, comparing them to the study sample and predicting their diets relative to the clusters. This was verified with a population from Saipan Island with an ambiguous diet, which were compared to hypothetical diet clusters for each of the candidate food sources and resolved (Froehle et al., 2012).

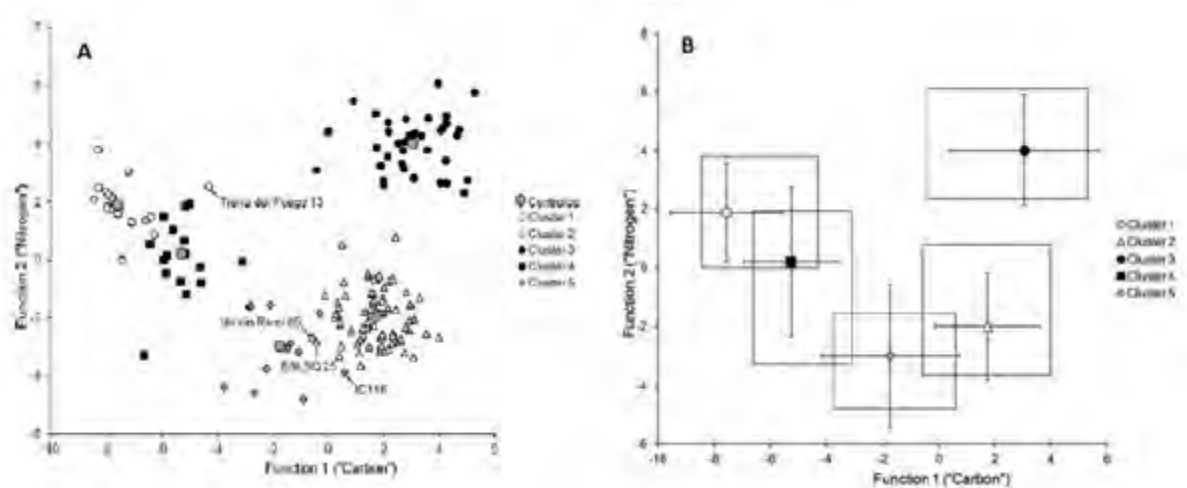


Figure 2-2 A multivariate model for diet reconstruction. Based on discriminant function scores and dietary clusters from Froehle et al 2012 (figure 4): (A) Discriminant function analysis converts three dimensions ($\delta^{15}\text{N}$, $\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{13}\text{C}_{\text{bone apatite}}$) into two scores that predict the group membership of a new sample based on known group memberships. In this case, the study sample of eight archaeological human populations was used as the known sample, the scores of which are plotted against pre-determined centroids representing different diets. (B) Mean and 2 standard deviation bars for each cluster. The limits of the boxes represent the minimum and maximum function score for each dietary cluster identified by the centroid. The dietary clusters are described as: percent C3:C4 diet and protein composition: Cluster 1=diet 100:0, protein 100% C3.

Cluster 2=diet 30:70, protein >50% C₄, Cluster 3=diet 50:50, protein 100% marine, Cluster 4=diet 70:30, protein >65% C₃, Cluster 5=diet 30:70, protein >65% C₃.

Both models attempt to combine the available isotopic measurements to provide a more holistic and informative image of past diets. They demonstrate that multiple-tissue isotope data can be used to great effect to provide more detailed insights into diet, including change over time, social differentiation, and resolve food sources that are archaeologically indistinct (Kellner and Schoeninger, 2007; Froehle et al., 2012; Somerville et al., 2013). It is clear that single isotopic measurements and reconstructions based off single fractionation factors can be improved upon as a source of dietary information. Comparing, combining, and contrasting the values of multiple tissues can help resolve issues of protein type, high and low protein diets, and mixed diets.

However, gathering isotopic data on additional tissues is only the start of this process. Fundamental understanding of key processes such as the impact of high and low protein content, aridity, and stress on macronutrient routing—which is crucial to interpreting the variance between tissues—is still lacking. It is also probable that unifying functions or lines derived from a wide range of human data obfuscate more than they reveal. Based on what (albeit little) we know about the effects on tissue isotope values of even small differences in dietary preference amongst feeders of the same type, the effects of aridity, and stress, it seems more likely than not that humans would present a wide range of geographically-correlated isotope variation. The diversity of human habitats presumably will have physiological and metabolic consequences that make grouping them all together under a unifying function counterproductive to the project of developing more detailed understandings of past diets.

The natural range of isotopic variation within a population—which is already a significant and unresolved issue, as individuals exhibit their own slight, but distinct isotopic variance—further complicates the problem of distinguishing between truly different diets, and simply different individual preferences, metabolisms, or localities. How much variation constitutes a true difference, both inter- and intra-population as well as intra-species or region, cannot be resolved simply by studying more tissues. Developing geographically informed dietary reconstructions based on multiple lines of evidence and restricting comparison only to the closest possible analogs (with both other regions and other species) will be crucial to moving dietary reconstruction away from simply “a C₃ diet” and into more detailed, but more complex, territory.

Although macronutrient routing provides the opportunity for more detailed dietary reconstruction, it also indicates that isotopic fractionation between diet and tissue, and interpretation thereof, is much more complex than researchers initially thought. Combining multiple tissue values is a necessary next step, but researchers should bear in mind that as isotope studies have expanded, the systems have proved more, not less, complex. Dietary interpretations may need to become more conservative before they can be more specific, as the usefulness of generalized theories, functions, and comparisons dwindles. The future of isotopic dietary reconstruction lies in more individualized, population-specific interpretations that are more detailed and less generalizable, in keeping with the complexity and variation that underlies human behavior, metabolism, and physiology.

2.4. Limitations and concerns when extrapolating from animal studies

Controlled feeding studies have been instrumental in revealing the effects of macronutrient routing and provide experimental support for the metabolic theories first advanced by Krueger and Sullivan (1984). However, there are some challenges to extrapolating the data from controlled feeding studies, and animal studies in general, to humans and the archaeological record.

Swine and rats differ substantially from humans, and each other, in terms of body size, metabolic rate, and digestive physiology. All three animals utilize simple monogastric bacterial digestion, but it is not yet clear how discrepancies in isotopic values between them should be interpreted (Schoeninger, 2014). Animals such as ruminants, or those utilizing other forms of fermentation for digestion, are probably not applicable analogs at all for humans, which rules out some studies of wild terrestrial herbivores (Lee-Thorp et al., 1989; Hedges, 2003), although not all wild herbivores are ruminants (Levin et al., 2008). Drawing from experimental data on a number of different species, Schoeninger (2014) proposed a $\delta^{13}\text{C}_{\text{tooth enamel}} - \delta^{13}\text{C}_{\text{diet}}$ offset of 14‰ for animals relying on microbial fermentation, and 10‰ for those with simple digestive tracts (such as primates, including humans), which is only one of many necessary taxa- or system-specific offsets and considerations (Sponheimer and Cerling, 2014). A $\delta^{13}\text{C}_{\text{bone apatite}} - \delta^{13}\text{C}_{\text{collagen}}$ offset of $5.6 \pm 1.2\text{‰}$ has been reported across the primate order, an important contribution of omnivorous human analogues, although the authors do note they only studied primates with monisotopic diets (Crowley et al., 2010).

Although some studies have shown similar isotopic patterning in swine and rats (e.g. the relationship between $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$ when separated by C_3/C_4 protein type) (Froehle et al., 2010), others suggest that the differences in body size (and species) could significantly affect fractionation factors even among animals with similar dietary preferences (e.g. discrepant $\Delta^{13}\text{C}_{\text{diet-collagen}}$ values between mice, rats, and gerbils) (Ambrose and Norr, 1993). The consistent offset between $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{whole diet}}$ regardless of body size and dietary type suggests that some tissues' isotopic values may be less affected by physiological and metabolic factors than others (Kellner and Schoeninger, 2007).

The resemblance between experimental diet and actual diets is also relevant. The populations that are of most interest, namely archaeological humans, inevitably consumed a wide range of food sources (the proverbial 'mixed diet'), and their diets would be expected to have varied in protein content and composition over time. Isotopes can only provide a homogenized reflection of a highly complex and conditional system, and animals fed on strictly calculated, unvarying diets—even those that are designed to simulate the 'mixed' diet of free-ranging omnivores—can provide general trends or patterns at best. Experimental animals also tend to be more specialist feeders, restricting the range of diets they can be fed (especially in the case of generational experiments, where low protein diets can affect fertility) (Tieszen and Fagre, 1993). Models derived from studies of such diets are also limited, as illustrated by the inability for the bivariate model to distinguish mixed diets of any kind.

Multivariate models to date have all been dependent on animal data of some kind, and will remain so as long as human data is scarce and difficult to source. Although Froehle et al. (2012) built their multivariate model off of human population data, they interpreted the dietary clusters (which are the source of meaningful dietary information in the model) based on the bivariate plot in Kellner and Schoeninger (2007). The limitations of animal study data are thus still present when interpreting human data from on the multivariate model. The majority of dietary reconstructions produced, however, did agree with previous conclusions reached through archaeological or ecological means (151 out of 158 individuals classified correctly) (Froehle et al., 2012). Anomalies between dietary reconstructions based on other lines of evidence, and those returned by the bi- or multivariate models may in fact be more illustrative of significant differences between the source datasets, rather than outright errors.

2.5. Bone and tooth enamel apatite: all apatites are not created equal

The tissues sampled for isotope studies (bone collagen and mineral, tooth enamel) are used out of necessity, as they are usually the only materials that are preserved in archaeological contexts. The very qualities that allow them to be preserved, however, also have implications for isotopic analysis, particularly for the laboratory methods used to analyze a piece of bone or tooth enamel for $\delta^{13}\text{C}_{\text{apatite}}$. Since both tissues are formed from blood plasma, it is unlikely that dietary components are routed to them differently, although there is minimal study to confirm this assumption. As will be discussed, however, bone and tooth apatite are mineralogically distinct due to their different functions within the body. These differences do impact the protocols and methods used to extract the isotopes in the first place.

2.5.1. Structural and functional variation in bone apatite and tooth enamel

There is increasing evidence that tooth enamel and bone mineral should be treated as mineralogically and chemically distinct (Sponheimer, 1999; Pasteris et al., 2004; Wopenka and Pasteris, 2005; Webb et al., 2014). Bone apatite crystals are much more poorly organized, and much smaller (one-tenth to one-hundredth the size) than those of tooth enamel. The smaller and more disordered the crystals, the less energetically favorable it is for the mineral to incorporate $-\text{OH}$ groups. This could explain the difference in hydroxylation between bone apatite and tooth enamel (Wopenka and Pasteris, 2005; Sponheimer and Cerling, 2014). In bone apatite, carbonate substitutes for phosphate in many cases; as the $\text{Ca}-\text{CO}_3^{2-}$ bond is weaker than the $\text{Ca}-\text{PO}_4^{3-}$ bond, bone apatite is more soluble in acid than an apatite with less carbonate (for instance, enamel) (Wopenka and Pasteris, 2005). The presence of carbonate, smaller crystal size, and lack of buffering capacity conferred by more $-\text{OH}$ groups renders bone significantly more soluble than enamel.

Acid dissolution is the mechanism by which osteoclasts resorb bone mineral, and the higher solubility of bone mineral facilitates this periodic remodeling. In contrast, tooth enamel must resist dissolution by oral fluids and by the acid attacks of bacteria that form on the surface of teeth. Enamel displays the same $-\text{OH}$ Raman spectroscopic bands as geologic or synthesized hydroxylapatite (Pasteris et al., 2004; Wopenka and Pasteris, 2005). Functional-structural differences between the two apatites do not necessarily lead to isotopic differences, but consistent offsets have been reported between them across several species,

probably due to their different formative processes and functional requirements (Webb et al., 2014).

2.5.2. Isotopic variation between bone apatite and tooth enamel

$\delta^{13}\text{C}$ values from bone and tooth apatite have been treated interchangeably as derived from 'structural carbonates' (e.g. France and Owsley, 2012), and studies that include isotope values for bone and tooth apatite from the same individuals are rare. Carbonates that are substituted into the mineral structure (bone apatite or tooth enamel) are formed in equilibrium with the bicarbonate mass balance within the body (Passey et al., 2005). Despite this fact, a range of variation has been reported in the isotopic values of these two biominerals. Webb et al. (2014) report an average $+4.3\pm 1.2\%$ offset between co-forming enamel and bone apatite in juvenile archaeological specimens. Santana-Sagredo et al. (2015) report an offset of $+1.8\pm 2.3\%$ for the same tissues in adults, and $+1.7\pm 2.5\%$ has also been documented (France and Owsley, 2012). Loftus and Sealy (2012) report poorly correlated bone and tooth apatite $\delta^{13}\text{C}$ values in Holocene adults ($R^2=0.37$), and enamel was found to be consistently enriched by $+2.3\%$ over bone apatite in swine (Warinner and Tuross, 2009). Warinner and Tuross (2009) caution that previously determined relationships between the isotopic value of diet and bone apatite may not hold true for tooth enamel (i.e. the same fractionation factor may not apply). Other swine studies reported a $\Delta^{13}\text{C}_{\text{bone apatite-diet}}$ of $+10.2\pm 1.3\%$ (Howland et al., 2003) and $\Delta^{13}\text{C}_{\text{enamel-diet}}$ of $+13.1\%$ (Passey et al., 2005). These relatively few studies nonetheless point to a significant difference between enamel and bone apatite, and suggest that they should no longer be compared directly without accounting for different diet to tissue fractionation.

Given that they form in equilibrium with the body, there are a limited number of explanations for the diversity of isotopic values found in biominerals. Maturation rates and formation times are distinct for tooth enamel (which forms early in life) and bone apatite (which is remodeled over the course of life), so dietary shifts over a lifetime may be revealed by differences between these two tissues (Sealy et al., 1995; Hillson, 1996; Lee-Thorp and Sealy, 2008). Third molars are typically sampled in an attempt to avoid the effects of weaning on the dietary signal, but even post weaning changes in diet related to age may be detected in tooth enamel. Although life history may well contribute to the offset between bone and tooth apatite, the process by which dietary isotopes are incorporated into the apatite structure must also be considered as a source of the inherent difference between the two sources of isotopic information. No studies have specifically compared the process of isotope

metabolism in these two apatites, or explored explicit routing through controlled feeding studies.

Aside from variation due to diet at the time of formation and possible differences in formation energies, the remaining explanation for these differences is that they are caused by the process of deriving and analyzing the isotopes themselves. If pre-treatment is the primary cause of the offset between tooth enamel and bone apatite, then experiments that attempt to control for pre-treatment effects should result in reduced offsets. The common pre-treatment protocols and their possible effect on the measured isotopic values are discussed in the next section.

2.6. Archaeological realities: diagenesis and pre-treatment methods

Diagenesis is the process that alters material as it transitions from the biosphere to the lithosphere. Almost any material preserved in the archaeological record has undergone diagenesis, which alters the chemical-physical properties to stabilize the material and preserve it, including changing the original isotopic value of the sample. This can occur broadly in two ways: through the incorporation or adsorption of foreign material (minerals, ions, etc) with different isotopic values to the sample, or isotopic changes to the original sample during processes of dissolution and re-precipitation (Zazzo et al., 2004). Archaeological tissues, and especially paleontological ones, are pre-treated before analysis to prevent diagenetic material from the depositional environment influencing the analysis of the original isotopic composition of the sample. Protocols for extracting collagen from bone samples and detecting some degree of diagenesis are established (Hedges, 2002; Lee-Thorp, 2002). Apatites (especially bone apatite), which are relatively newer to isotopic analysis, pose more challenges for mitigating the effects of diagenesis.

2.6.1. Bone and tooth enamel apatite: preservation

Tooth enamel is much more robust than bone apatite, resisting diagenesis over time, and material that is well over a million years old has been shown to preserve the original biogenic isotopes (e.g. Lee-Thorp and van der Merwe, 1991; Lee-Thorp and Sponheimer, 2006; Lee-Thorp, 2002). Initially, use of bone apatite for isotope studies was questioned due to the tendency for bone mineral to exchange atoms with its environment, especially in the form of 'adsorbed' carbonate that adheres to the exterior of the apatite mineral structure (Koch et al., 1997; Krueger, 1991; Lee-Thorp and van der Merwe, 1991; Schoeninger and

DeNiro, 1982). Attempts to derive radiocarbon dates from bone apatite were unsuccessful for this reason. Stable isotopes, however, are much more abundant than ^{14}C and so stable isotope analysis is much less sensitive to contamination than radiocarbon analysis.

The fidelity of isotopes in bone apatite from archaeological contexts was ultimately verified by comparing the $\delta^{13}\text{C}$ values of apatite and collagen from the same bones, and tracing the expected $\delta^{13}\text{C}$ offset between browsers (C_3 consumers) and grazers (C_4) through time. The validity of $\delta^{13}\text{C}_{\text{bone apatite}}$ has been confirmed subsequently, although the possibility of problematic diagenetic alteration of ions in bone mineral remains a concern (Sullivan and Krueger, 1981; Lee-Thorp and van der Merwe, 1991; Lee-Thorp, 2002; Lee-Thorp and Sponheimer, 2003; Shin and Hedges, 2012).

Crystallinity and other mineralogical characteristics are often assessed to verify the integrity of biominerals and show that they have not undergone substantial alteration. Experimental work has suggested that bone apatite, and to a lesser extent enamel, can undergo substantial changes to both carbonate and phosphate, even before mineralogical change is detected (Nelson et al., 1986; Wang and Cerling, 1994; Koch et al., 1997; Zazzo et al., 2004). Such changes are detected by shifts in crystallinity index through techniques such as FTIR and IRSF, but crystallinity fails to predict isotopic integrity for severely altered samples (Trueman et al., 2008). Although changes in crystallinity can demonstrate that minerals have not been structurally reorganized during diagenesis, the link between the crystallinity of a biomineral and its isotopic value—and why changes in crystallinity necessarily predicts diagenetic alteration of isotopes—is yet to be firmly established.

2.6.2. Bone and tooth enamel apatite: pre-treatment

Pre-treatment protocols to remove adsorbed, environmental carbonate from mineral prior to analysis were adopted very early on in isotope studies, and have varied little since (e.g. Sullivan and Krueger, 1981; Koch et al., 1997; Kohn and Cerling, 2002; Webb et al., 2014). Powdered samples are rinsed (in sodium hypochlorite or hydrogen peroxide) to remove organic contaminants (and in the case of bone, collagen) and in an acid (usually acetic acid) to remove soluble minerals (despite consistencies in the methodological steps, laboratories vary widely in the strength of reagents and length of treatment times, see Pestle et al., 2014). Exogenous contaminants are assumed to be largely on the surface of the sample, and thus should react first in the acid (Shin and Hedges, 2012). Substituted mineral is also more soluble due to increased imperfections in the crystal structure of the sample and should

be removed more easily (except in the case of material which has adsorbed a significant proportion of fluoride ions, which stabilize the apatite structure and will result in less soluble mineral) (Shin and Hedges, 2012). The remaining mineral should contain less substituted or contaminated material after leaching.

This process can reduce the presence of foreign material on the sample, but the dissolution and re-precipitation of bone in isotopically heterogeneous depositional environments during diagenesis remains a concern (Zazzo et al., 2004). The same process can occur during pre-treatment itself. From the outset there was controversy about the effects of pre-treatment on sample mineral recrystallization (and accompanying re-incorporation of dissolved mineral) and the subsequent isotopic value of the sample (i.e. Schoeninger and Deniro, 1982; Sullivan and Krueger, 1981). Systematic studies of the effect of acid concentration and treatment time on bone apatite and tooth enamel have revealed that samples may risk dissolution and recrystallization when concentrated acids or long reaction times are used (Koch et al., 1997; Sponheimer, 1999; Garvie-Lok et al., 2004). It is also possible that differently-soluble areas of mineral in a single sample, which have different isotopic values, are isolated by dissolution and when analyzed do not reflect the isotopic value of the whole (Koch et al., 1997; Shin and Hedges, 2012). FTIR spectroscopy indicates that even 15 minutes in dilute (0.1M) acetic acid can induce more substantial crystallographic changes in tooth enamel than 3 million years of fossilization (Sponheimer, 1999; Garvie-Lok et al., 2004).

After reacting bone and enamel powders in simulated diagenetic environments, Zazzo et al. (2004) determined that acetic acid pre-treatment was not sufficient to restore samples to their pre-reaction isotopic values or crystallinity indices, although the process was more effective for enamel than bone. However, those samples were treated with 1M acid for a minimum of 24 hours; in light of the documented effects of such concentrated acid and long treatment times, it is likely that the divergence between pre-diagenesis and post-treatment values was actually caused by recrystallization of the sample (Nielsen-Marsh and Hedges, 1997; Garvie-Lok et al., 2004). Changes in crystallinity and porosity after prolonged (16 hour) exposure to 0.1M acetic acid are observed in bone of varying degrees of preservation, but is especially acute for poorly preserved bones (Nielsen-Marsh and Hedges, 2000). That being said, a clear relationship between changes in crystallinity and ultimate isotopic

composition has not yet been established (Lee-Thorp and Sponheimer, 2003; Trueman et al., 2008).

The goal of pre-treatment of apatites remains the same—to remove exogenous organic and inorganic contaminants—but increasing use of apatite, especially enamel, for isotopic analysis, necessitated revising the accepted pre-treatment method. Initial treatment times were as long as two days in acid for large pieces (Schoeninger and Deniro, 1982), but it quickly became clear that prolonged reaction with acid risked recrystallization and possible alteration of the isotope values (Lee-Thorp and van der Merwe, 1991). With the development of small (~2.5 mg) sample preparation, recommended acid treatment times shortened to fifteen minutes, and concentrations shifted to 0.1M from 1.0M (Lee-Thorp et al., 1997; Sponheimer, 1999). Garvie-Lok et al. report a precipitous change in the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of modern and archaeological bone apatite after 4 hours, with recrystallization remaining a risk thereafter (2004). Even one hour in dilute (0.1M) acetic acid was found to be long enough to induce substantial changes in mineralogy and structural crystallinity, although no direct measurements were made to test the effects of increased crystallinity on the isotopic values of the sample (Nielsen-Marsh and Hedges, 1997).

The protocol for small, powdered enamel samples proposed by Lee-Thorp et al. (1997) also recommends more dilute (0.1M) acid, and notes the increased potential for reaction with the fine particles produced by common rotary drill collection methods (Sponheimer, 1999). Garvie-Lok et al. (2004) and Koch et al. (1997) both report isotopic variation for samples treated with 0.1M acid versus 1.0M in order to remove diagenetic carbonates. They concluded that 0.1M is preferred to prevent substantial changes to the crystallinity index of samples as well as changes to $\delta^{13}\text{C}$, however, Koch et al. (1997) also warn that even with 0.1M acid, some recrystallization can occur, especially if reaction time is lengthy. In contrast, Pestle et al. (2014) studied inter-laboratory variation in isotopic values and found that the use of stronger (1.0M) acid changed $\delta^{13}\text{C}_{\text{bone apatite}}$ of the same bone by only 0.03‰.

Mineralogical differences between enamel and bone apatite warrant further attention to the issues of recrystallization and reaction time. In some cases, the pre-treatment protocol for tooth enamel (30-45 minutes in sodium hypochlorite, and 15 minutes in 0.1M acetic acid) has been applied to bone apatite (Smith, 2005; Santana-Sagredo et al., 2015), although more lengthy and concentrated methods (such as 24-12 hours in sodium hypochlorite and 4 hours

in 1.0M acetic acid) remain in use (White et al., 2001; Reitsema et al., 2010; France and Owsley, 2012; Webb et al., 2014). Research into bone pre-treatment has largely tested long exposure to acid (~4 hour to 3 days) and varying concentrations (1.0M versus 0.1M) (Koch et al., 1997; Garvie-Lok et al., 2004). Neither study compared times in NaClO (samples were soaked in sodium hypochlorite for 2 and 3 days respectively).

Smith (2005) subjected bone apatite samples from modern and archaeological fauna to two different sodium hypochlorite times (3 and 4 hours) and three 0.1M acetic acid treatments (10, 15, and 30 minutes). Smith found $\delta^{13}\text{C}_{\text{bone apatite}}$ values consistently offset from those of bone collagen and tooth enamel from the same individuals, and very little variation due to pre-treatment overall. Based on this, and FTIR results for some samples, Smith concluded 3-4 hours in sodium hypochlorite and 10-15 minutes in acid yields the most consistent results for the archaeological bone in the study, which was also Iron Age bone from South Africa (reproducibility <1.5‰) (Smith, 2005). This experiment emphasizes the need for different pre-treatment methods for bone apatite and tooth enamel, and is also promising in that it demonstrated bone apatite from this region could, at times, be robust to some degree of pre-treatment.

In the first-ever study of the effect of NaClO to remove organics from archaeological bone and tooth enamel, Snoeck and Pellegrini (2015) reported that NaClO appeared to add CO_3 to the samples as revealed via FTIR. This is a troubling finding that should be explored further—although they also found that subsequent treatment by acetic acid to remove adsorbed carbonates was effective in removing the CO_3 contributed by the NaClO, this points to a chemical process that is not yet understood and could affect the sample's isotopic value (Snoeck and Pellegrini, 2015; Pellegrini and Snoeck, 2016).

There is consensus around this less aggressive pre-treatment for tooth enamel and bone apatite, but differences in particle size that result from standard collection methods have not been addressed. Tooth enamel powder collected with a diamond-tipped dental drill bit attached to a Dremel or other drill is extremely fine (15-22 μm) (Lee-Thorp et al., 1997; Sponheimer, 1999). As this practice is widespread and the highly-organized structure of enamel is likely to result in fine particles when abraded, it is reasonable to assume that such particle sizes are common. In contrast, bone carbonate is typically ground to a powder using a variety of instruments (SPEX mills, pestle and mortar, drills, etc) and can result in a wide variety of particle sizes. Harrison and Katzenberg (2003) sieved bone apatite particles to

0.375 mm, but did not compare the isotope results of those particles to any other particle size or tissue. That powder was reacted with 1.0M acetic acid for 24 hours, which is substantially longer than the pre-treatment for tooth enamel of a similar size (Harrison and Katzenberg, 2003). It is likely that all isotopic studies of bone apatite have tested varying and mixed particle sizes, which poses a problem for interpreting the results and comparing between studies.

Finer particle sizes produce more surface area for reaction, which can result in the loss of sample, or even the dissolution of the mineral of interest, potentially leaving behind exogenous, more insoluble components such as trace fluorine, to be analyzed (Sponheimer, 1999). More aggressive reaction with acid during pre-treatment may also cause fine-particle samples to recrystallize during treatment. Webb et al. (2014) found the largest offset between tissues came from tooth and bone apatite; since particle size was not considered and both tissues were treated following the same method, differences in isotopic values could be due to recrystallization of finer particles as much as inherent tissue differences. Shin and Hedges (2012) report a significant shift in $\delta^{13}\text{C}_{\text{bone apatite}}$ values for samples that are separated by bone density fractionation prior to pre-treatment, further suggesting that the mineral properties of bone are isotopically relevant and effected by pre-treatment methods.

In light of the concerns raised above, additional experiments were conducted to reduce the effects of particle size and pre-treatment protocol on the apatites analyzed in this study. The fourth chapter describes the methodology of these experiments as well as the preparation and analysis of tooth enamel, bone apatite, and bone collagen from the Iron Age individuals studied here. Context on the economy, diet, and ecology of these individuals, as well as the provenience of their remains, is discussed in the next chapter.

3. The Iron Age in southern Africa

3.1. Ecology of southern Africa

Studies of ancient humans seek to document and understand patterns of behavior in the context of their environment, including how humans actively alter and adapt to it. Environmental characterizations vary in scale and detail, and researchers should match the spatial scale of the characterization to that of their investigation. This study analyses a human population across a broad unit of space and time: those that practised agriculture in southern Africa prior to the arrival of European settlers, a period of over 2000 years and across almost one half of modern-day South Africa. For these purposes, broad environmental units are sufficient.

Biomes designate a geographic unit where a similar vegetative structure is exposed to the same macroclimatic forces and types of disruption, which correspond well to broad studies of large, mobile animals such as humans. (Mucina and Rutherford, 2006). Two biomes, savanna and grassland, dominate the northern half of South Africa and overlap in many areas. Both biomes experience similar rainfall patterns, with rainfall concentrated in January and February.

Distinct wet-summer/dry-winter periodicity is a key defining characteristic of the region, often called the ‘summer rainfall’ zone (Mucina and Rutherford, 2006). Vegetation is mainly C₄ grasses, with tree cover in the savanna ranging from 1-75%. Grassland is typically higher altitude (above 1500 meters above sea level) than savanna and is thus colder (high-altitude grasslands experience the most frost days of any biome in southern Africa). A small portion of samples for this study come from the Indian Ocean coast and just inland, which is extremely variable in vegetation cover, but has the highest annual rainfall and combines savanna-like areas with patches of sub-tropical forest.

Characterization of the southern African landscape is a legacy project with a great deal of diversity. Previous studies referred to in this thesis may refer to the ‘woodland savanna’ (Lee-Thorp et al., 1993) or the ‘bushveld,’ which describes patches of interlocking small trees and shrubs. These areas are all part of the savanna. Other common terms combine topography and vegetation, such as ‘Highveld’ (the largely grassland area of the Central

African Plateau), ‘bushveld’ (savanna north of the Highveld) and ‘Lowveld’ (region below 500 meters above sea level, including the Kruger Park). Highveld is maintained in this text for continuity to refer to the central grassland region occupied during the Late Iron Age (see below). The regions of interest in this study, divided by biome and modern mean annual rainfall, can be found in Figures 3-1, 3-2, and 3-3, which also include the sites included in this study. Table 3-1 lists the sites described below and their corresponding biome.

3.2. The Iron Age

The Iron Age refers to a new way of life based on agriculture and settled, village-based societies that arrived in southern Africa approximately 2000 years ago. Bantu-speaking people expanded southwards from East and West Africa (for review, see Mitchell, 2002), bringing this socio-cultural complex with them. These people were the first to smelt and work metals in southern Africa, leading to the term ‘Iron Age,’ but their plant husbandry was also new to the region, and some contemporary scholars prefer the terms ‘farmers’ or ‘agriculturalists.’ It is the diet and food procurement strategy of this group that is of interest in this study, so ‘farmers’ is apt. Contemporaneous individuals, sometimes in the same region, procured their food by hunting and gathering (e.g. Ribot et al., 2010) and will be referred to as ‘hunter gatherers.’ For the sake of clarity with previous work, however, the entire complex of new behaviors and economic practices associated with the arrival of the Bantu-speaking people (i.e. metallurgy, agriculture, and distinctive ceramics) will continue to be referred to as the ‘Iron Age’ (Phillipson, 1977; Huffman, 1982).

The farmers who arrived in southern Africa relied primarily on cultigens and domestic animals for food, but also hunted wild game and collected wild plant foods when available. Shellfish would have remained an important food source on the eastern coast, and exploitation of wild plants such as marula is evident (Maggs, 1994; Mitchell, 2002). There is also considerable evidence for overlap and interaction between agropastoral groups and those who practiced hunting and gathering (Maggs and Whitelaw, 1991; Ribot et al., 2010).

The Iron Age can be roughly divided into the Early Iron Age (EIA), which broadly corresponds to the first millennium AD, and the Late Iron Age (LIA) which corresponds to the second. On the Indian Ocean coast in modern-day KwaZulu-Natal, EIA sites are located in fertile soils adjacent to rivers, and LIA sites are located further from rivers. Building in stone is first documented in the second millennium AD, and ceramic sculpture recorded at a number of EIA sites (most notably the Lydenburg Heads) are not found at LIA sites. Scholars

disagree as to the degree of continuity or disjunct between these two periods, and whether they represent new waves of immigrants or *in situ* cultural developments. The term ‘Middle Iron Age’ is especially ill-defined and will not be used here (Badenhorst, 2009). For the purposes of this study, which does not closely examine timescale differences within the agricultural period, these broad distinctions are sufficient.

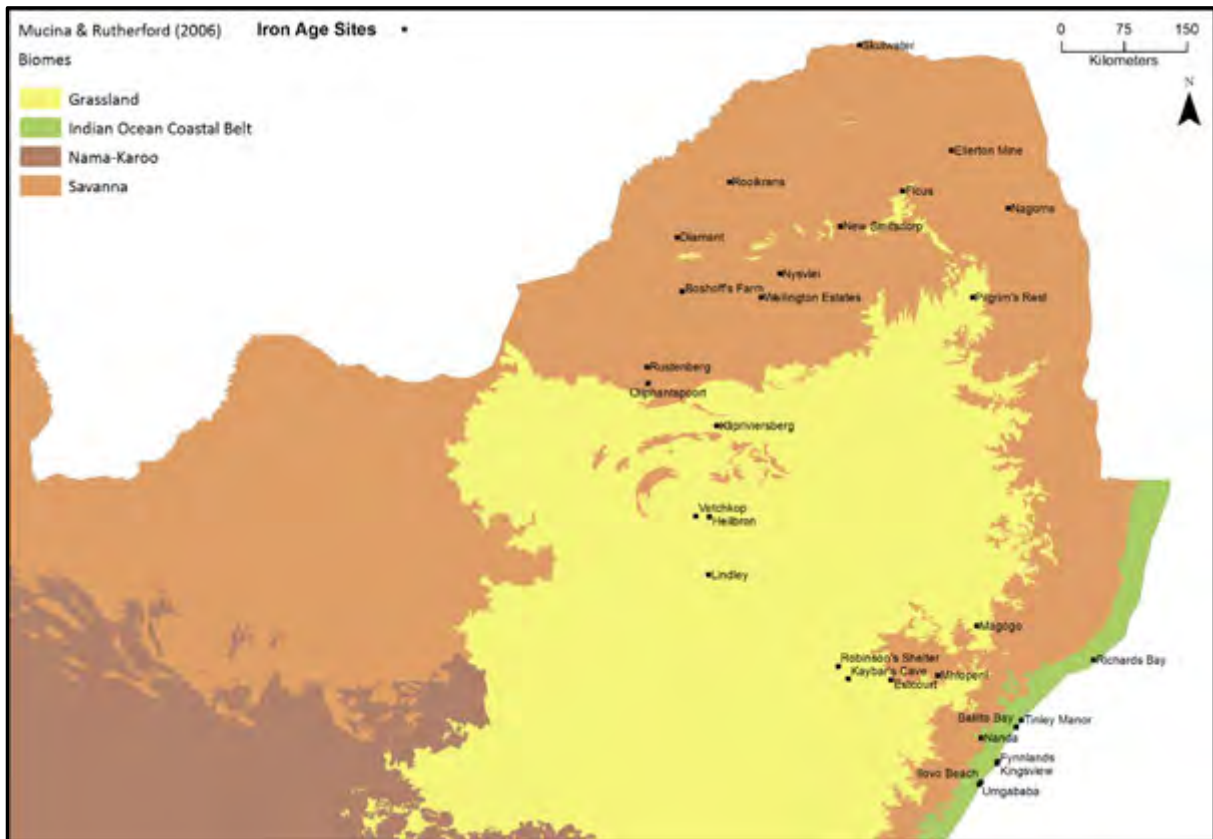


Figure 3-1 Map of Iron Age sites included in this study, by biome as designated in Mucina and Rutherford 2006

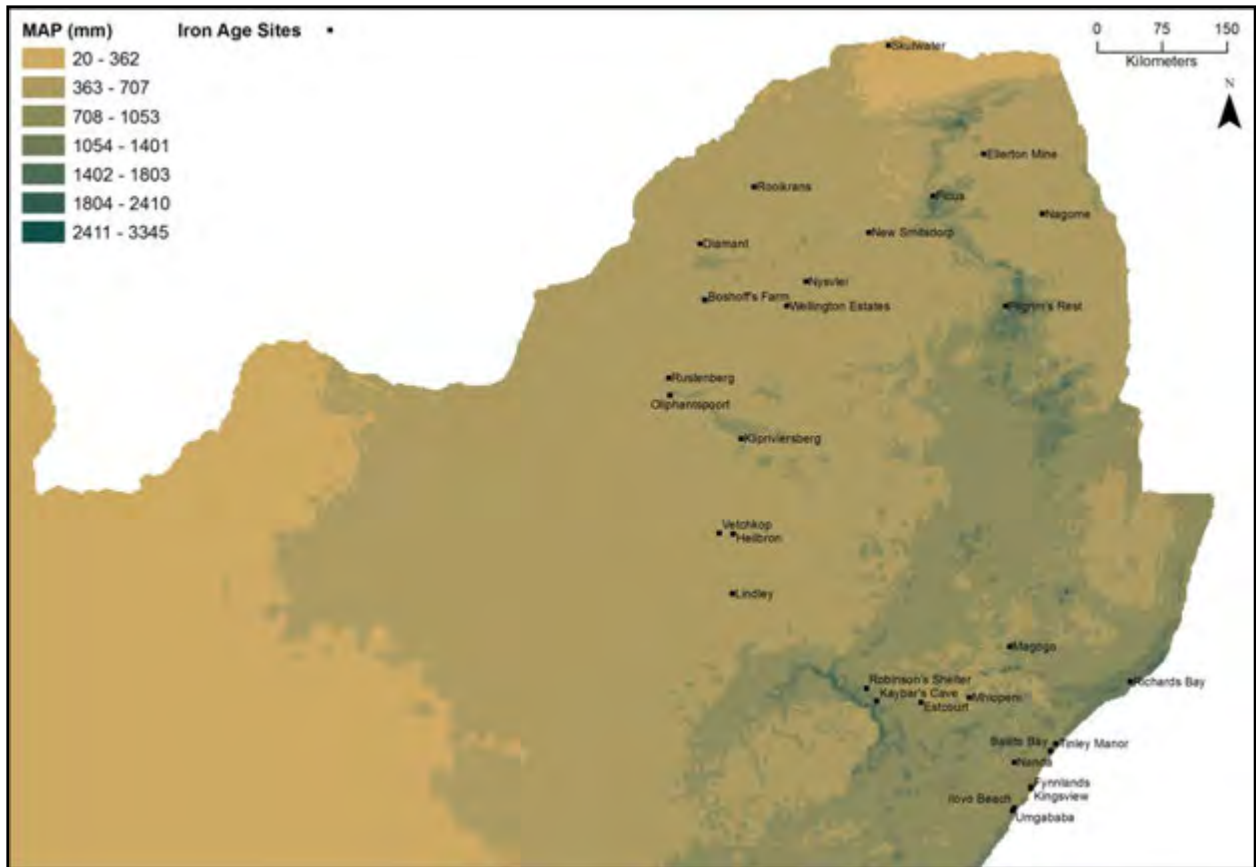


Figure 3-2 Map of modern-day mean annual precipitation in southern Africa, with Iron Age archaeological sites included in this study. Intervals (mm) are based on the peaks and troughs of the regional data to create meaningful distinctions in rainfall. Intervals are cut off at troughs in the original data to preserve real distinctions in rainfall intervals. Skutwater is the only site that receives less than 400mm of precipitation a year.

3.3. Subsistence and Food Production in the Iron Age

There has been a great deal of archaeological investigation into the subsistence practices, settlement patterns, symbolic systems and other aspects of Iron Age communities. In contrast, there has been very little work on skeletal bone chemistry: in South Africa, three studies have investigated carbon and nitrogen isotopes in bone collagen (Lee-Thorp et al., 1993; Gilbert, 1995; Ribot et al., 2010). Mosothwane (2010) did similar work in Botswana. All of these studies were limited to bone collagen, and many of the results remain unpublished. There is clearly an opportunity to use more up-to-date approaches to palaeodietary reconstruction, especially incorporating analysis of multiple tissues, in order to explore questions about Iron Age diet, and to make a contribution to current debates on multi-tissue analysis and multivariate reconstruction of palaeodiet.

Sorghum and millet, the main cultigens of Iron Age farmers, require warm, wet growing seasons (grown together, sorghum and millet need at least 350mm of rain during the

growing season) (Mitchell, 2002). The summer rainfall zone encompasses most of northern and eastern South Africa, stretching north-east of the Cape Fold Mountain belt (west of these mountains, seasonal expansion of the westerly winds and disruption of the easterlies produces wet winters and dry summers) and extending northward across the present South African border. Rainfall in southern Africa is highly variable, with the western Karoo receiving as little as 50 mm of rainfall a year, and the semi-tropical/tropical zones of the Indian Ocean coast receiving over 1000 mm a year (Figure 3-2).

Most of the archaeological sites discussed here are located in the north-east of modern-day South Africa, from the eastern coast to the Zimbabwe border, which receives less than 1000 mm of rainfall each year (Figure 3-2). With the exception of the coast, these areas receive rain almost exclusively from November to May, when they also reach their highest temperatures (Maggs, 1976; Lee-Thorp et al., 1993; Mucina and Rutherford, 2006). Iron Age settlement appears to have been restricted to regions with sufficient rainfall to grow crops (greater than 400 mm/year), suggesting that climate, controlling vegetation, impacted the geographic dispersion of agriculturalists (Maggs, 1976, 1994; Mitchell, 2002). EIA sites track the modern-day geographical range for land suitable for growing sorghum quite closely (Russell and Steele, 2009), and many sites cluster around more moist pockets of the savanna or grassland (see Figure 1-2, for example Rustenberg, Oliphantspoort, and Boshoff's Farm).

3.3.1. Diet in the EIA

The primary source of information on diet and economy among Iron Age populations is excavated food remains. Archaeological evidence such as grindstones and seed impressions on pottery indicate that cultigens were consumed during the Early Iron Age, with the earliest direct evidence impressions of pearl millet (*Pennisetum americanum*) seeds in clay at the 3rd century AD site of Silver Leaves (Huffman, 1982). Sorghum (*Sorghum bicolor*), and melon (*Citrullus*) were found at Magogo, in the 6th century AD (Maggs and Whitelaw, 1991). Domesticated animals, such as ovicaprines and to a lesser extent cattle, are evident from the 4th century at Happy Rest and Broederstroom (Mason, 1981; Voigt and Plug, 1984; Maggs and Whitelaw, 1991), with sheep and goats in clear evidence in coastal regions at Magogo (Maggs and Ward, 1984; Voigt, 1984). Despite the presence of domesticates at some sites, there are other contemporaneous sites where the faunal assemblages show a high reliance on hunting wild animals. Gathered plants would have been a key supplement to diet during period of drought and crop failure (Maggs, 1976; Plug and Voigt, 1985; Gilbert, 1995).

For EIA populations living in the coastal savanna in the east, previous isotopic results from bone collagen suggest consumption of C₄ plants or animals fed on C₄ plants, as well as marine protein sources for those individuals found near the coast (Ribot et al., 2010). Inland agricultural sites with evidence of marine resource exploitation suggest that farmers periodically travelled to the coast to collect shellfish, possibly on a seasonal basis, before returning inland (Maggs, 1994; Mitchell, 2002).

Increased production and consumption of crops, as reflected by a significant isotopic shift in human skeletons towards C₄ foods and away from marine resources, is reported by Ribot et al. after 400 AD (2010). At this time, there is also evidence for larger, more settled populations at higher altitude areas among EIA sites (Maggs and Whitelaw, 1991). Finger and pearl millet (*Eleusine corocana*, *Pennisetum typhoides*) were identified at Ndongondwane (8th century AD), along with the crops found at Magogo. Grindstones and iron agricultural implements such as hoes have also been recovered from a variety of sites (Maggs and Ward, 1984; Maggs and Whitelaw, 1991).

3.3.2. Diet in the LIA

The Late Iron Age begins with the resumption of most sequences around 1300-1500 AD, after many sequences reflect a hiatus from 1000-1500 AD. The hiatus has been interpreted to suggest environmental pressure or other stressors forced populations to relocate (Plug and Voigt, 1985). The LIA is a period for which much more is known about food production, diet, and life in general. Agriculturalists spread into new areas, including the grasslands of the interior modern-day KwaZulu-Natal and adjacent areas. By 1640, agriculture was found throughout the higher elevation portions of the savanna and grassland (modern-day Gauteng and Free State provinces and Lesotho), covering the entire climatic range for millet and sorghum (the modern day 550-600mm MAR isohyet) (Mitchell, 2002). The overall drier and cooler conditions of the Little Ice Age (1400-1800 AD) reduced riverine and forest cover, facilitating the expansion of savanna and grassland species, but there is historical and dendrochronological evidence for rainfall fluctuation and drought throughout this period (Tyson et al., 2002; Gillson and Ekblom, 2009; Ekblom et al., 2012; Woodborne et al., 2015). Agriculturalists would have been subject to intervals of famine and prosperity, and their reliance on crops would have fluctuated along with the climate (Huffman, 1996; Gillson and Ekblom, 2009).

As trade networks spread and resulted in the unequal distribution of wealth across societies (notably the site of Bambandyanalo (K2), from which interior African trade was

controlled), food production expanded and individual diets varied more with social status (Gilbert, 1995). Cattle took on a much more prominent role, although whether they were consumed in proportion to their presence in the archaeological record is debated (Huffman, 1989; Mitchell, 2002; Badenhorst, 2009). Communities living in the grassland appear to have consumed more livestock (and specifically, more cattle) than those living in the savanna. Domestic fauna, along with wild animals, may have sustained food supplies during intermittent crop failures in the higher-altitude and colder grassland (Plug and Voigt, 1985; Lee-Thorp et al., 1993; Mitchell, 2002). Diet continued to diversify as towns grew and social strata solidified: it is theorized that the inhabitants of Skutwater (a satellite town of Mapungubwe) may have consumed more wild plants and animals than those in the regional capital, although the effect may be caused by differential preservation and sample sizes at the two sites (Van Ewyk, 1987).

3.3.3. Maize

Maize produces higher yields than sorghum or millet and has a shorter growing season (Board on Science and Technology for International Development, 1996). Maize cultivation may have begun as early as 1635 on the southern Indian Ocean coast, where there is an accompanying increase in site density and expansion into marginal lands (Mitchell, 2002). Maize pollen has been identified in sediment cores from the Limpopo River Valley between 1600-1700 AD, and carbonized maize cobs in the savanna region of the eastern coast fall within the same period (Maggs, 1980; Ekblom et al., 2012). Cores from Lake Sibaya in northern KwaZulu-Natal province contain palynological evidence for cereal cultivation in the second millennium AD with pollen from *Zea mays* (maize) dated between 150-300 years BP (Neumann et al., 2008). The density of large Bokoni settlements on the Mpumalanga Escarpment suggests maize may have been cultivated as early as 1600 AD to support large populations, estimated to range as high as 19,000-57,000 people at maximum occupancy. Conditions on the Escarpment are too wet to support other cereal crops: although maize is more productive, it also requires 25% more rain than sorghum or millet (Maggs, 2008). Maize agriculturalists would have been more exposed to drought and crop failure, potentially forcing them to fall back on wild foods (Gilbert, 1995; Huffman, 1996; Mitchell, 2002). The exact date of maize introduction has been difficult to trace and will likely never be known, but historical evidence for Portuguese trade with communities in modern-day Zimbabwe combined with the archaeological evidence suggest that the crop would have

definitely been introduced to northern South Africa by the early to mid-16th century (Gilbert, 1995).

Differences in the economies and settlement patterns of EIA and LIA people have been thoroughly discussed and debated elsewhere (Huffman, 1982; Mitchell, 2002; Badenhorst, 2009). For the purposes of this study, only a few key differences will be highlighted: EIA settlements were closer to rivers or lower-lying areas, as opposed to higher altitude LIA settlements (Maggs and Ward, 1984), and coastal EIA people consumed a larger proportion of marine resources than those in the LIA (Maggs, 1994; Ribot et al., 2010). EIA sites are restricted to the savanna, while LIA settlements expanded to the grassland as the climate shifted throughout the second millennium (Maggs, 1994; Huffman, 1996; Tyson et al., 2002). Some researchers have suggested that the dominant domesticates in the EIA were ovicaprids, with increased emphasis on cattle beginning in the LIA (Plug and Voigt, 1985; Badenhorst, 2009), but others disagree (Huffman, 1982).

3.4. Isotopic predictions of Iron Age diet

The animal component of Iron Age diet can be inferred from the ecology of the region's vegetation. Wild and domesticated grazers consume primarily C₄ grass, while wild browsers reflect more mixed or C₃ diets, as most browse, such as the leaves of shrubs and trees, is C₃. As grazers, cattle strongly reflect C₄ grasses; ovicaprids tend to have more negative $\delta^{13}\text{C}$ values than cattle as they browse more, but this difference is not likely to be apparent in human tissues (Smith et al., 2007; Mosothwane, 2010). Wild game from this region can be either grazers or browsers (Lee-Thorp et al., 1993).

Savanna and grassland grasses are almost entirely C₄, as are the cereal crops grown in those regions. Gathered wild plant foods such as tubers and fruits are nearly all C₃ (Lee-Thorp et al., 1993), as are cultivated legumes and cucurbits. Increased reliance on C₄ cultigens and domesticated C₄ feeding animals probably came at the expense of wild food sources such as C₃ plants or wild browsers, and this should be apparent in the $\delta^{13}\text{C}$ values of the farmer's body tissues. There is no evidence for Iron Age settlement in the high altitude C₃ grassland of the Drakensberg mountains, where there are few woody plants to use as fuel and conditions are too harsh for grazing.

Limited ethnography supports the general archaeological image of Iron Age diet. The Pedi primarily consumed C₄ crops and C₃ legumes and cucurbits, ate domestic animal products or wild game, and supplemented their diets with wild herbs (Quin, 1964). The wide variety in this example underlines the heterogeneity of Iron Age subsistence strategies and the resources available to people at this time. In the EIA, archaeology has demonstrated strategies from a high reliance on domestic stock in the savanna areas (sites such as Happy Rest, Broederstroom, and others), while those on the coast show evidence of extensive use of marine resources such as mollusks (Voigt and Plug, 1984; Plug and Voigt, 1985; Lee-Thorp et al., 1993; Maggs, 1994). Lee-Thorp et al studied $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bone collagen from individuals from LIA sites in the savanna and grassland (all the area covered by the present study except the Indian Ocean coast), and found a considerable degree of variation across populations, even within similar biomes and rainfall zones. Individuals from the central grassland sites (Vrede, Lindley, Welgegund, Irene Cave) show the highest $\delta^{13}\text{C}$ values ($-7.43 \pm 1.70\text{‰}$) while those in the most arid savanna region (Skutwater) have the most depleted results ($-10.58 \pm 1.24\text{‰}$), suggesting the former consumed more C₄ based resources, probably cultivated grains (Lee-Thorp et al., 1993).

Ambrose (1986) reported relatively low nitrogen values (maximum $\delta^{15}\text{N} = 10.4\text{‰}$) for eight LIA farmers included in his study (two sites, Nysvlei and Ficus, are also included in this study although the individuals sampled are different). This was interpreted as an indication that they consumed very few domestic animal products, especially when compared the $\delta^{15}\text{N}$ values of pastoralists. Ambrose (1986) interpreted these values through the Pedi diet, which is low in animal products. However, the aridity and physiological water retention effects in animals and humans complicate simple trophic-level reconstructions with $\delta^{15}\text{N}$, and it is possible that environmental factors account for some of the low nitrogen values. Such confounding variables are another motivating factor in the development of other, multivariate reconstruction techniques.

The bulk of the specimens included in this study were first reported in Gilbert's 1995 study of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bone collagen. Table 5-4 lists the isotopic values for all individuals.

3.5. Background to sampled sites

The human skeletal record for the summer rainfall zone of southern Africa is comprised mainly of skeletons from individual burials, which limits their numbers. The combination of warm summer temperatures and moisture is highly destructive to bone and

preservation is often poor. The individuals sampled in this study represent a highly diverse population from sites across the north-eastern portion of South Africa and from both the EIA and LIA. Constraining the sample to more specific time and place intervals would have resulted in dramatically smaller sample size and limited the value of the analysis. Despite being dispersed in space and time, however, most of the individuals sampled have been established as being part of the Iron Age by previous scholars, mainly through archaeological context (especially associated ceramic traditions and settlement layout) (e.g. Gilbert, 1995; Maggs, 1980; Morris, 1992; Ribot et al., 2010; Van Ewyk, 1987).

The exceptions are a small number of individuals from several sites (Illovo Beach, Richard's Bay, Ballito Bay). Previous isotopic analysis of bone collagen suggests they consumed a higher proportion of C₃ foods, and they are dated to >2000 BP (Ribot et al., 2010). This suggests they practiced a subsistence pattern based more on hunting and gathering than on agriculture. These individuals are included in the analysis for comparative purposes.

The archaeological sites selected for this study are listed in Table 3-1, along with the biome in which they are situated and the rainfall as recorded by the nearest weather station (see also Figure 3-2). All individuals studied have been securely placed in the Iron Age, either through direct or indirect dating. The majority of sites occur in either the savanna or grassland biomes, and all are in areas that typically receive >200mm of mean annual precipitation per year.

Many of the individuals analyzed in this project were discovered accidentally in the course of agricultural activities, construction projects, or erosion. Some of them (individuals from Tinley Manor, Illovo Beach, Wellington Estates) were discovered in the early 1920s and 30s and sent to specialists for analysis, so very little contextual information is available (Wells and Dart, 1934; Galloway, 1936; Fichardt, 1960). Other sites analyzed here (Magogo Hills, Nanda, Mhlopheni, Rooikrans, Ficus, Nysvlei, Oliphantspoort, Rustenberg, Skutwater) were formally excavated and described (e.g. Maggs and Ward, 1984; Plug, 1984; Voigt, 1984). Many individuals—both those formally excavated and those that were discovered through erosion—were found in formal graves with grave goods, including ceramics, iron tools, and bead ornaments (e.g. some burials from Wellington Estates, Skutwater, Magogo Hills, and Mhlopheni).

All excavated sites that contained faunal material contained a mix of domesticated and wild animal remains. At Rooikrans, wild animals and hunting appeared to make up the majority of animal protein consumed; in contrast the bulk of animal remains at Skutwater were domesticates, and Van Ewyk suggests 51% of dietary protein was from cattle (Plug, 1984; Van Ewyk, 1987). Broederstroom, a 4th-7th century AD village, contained very few cattle remains and some ovicaprines, but hunting seemed to be the main source of animal protein (Maggs and Whitelaw, 1991). At Ficus and Happy Rest, herding is inferred through the presence of many ovicaprine remains (Voigt and Plug, 1984; Ambrose, 1986). The well-preserved fauna assemblage at Magogo also forms early evidence for herding (Voigt, 1984).

The scattered and varied nature of the Iron Age human remains that have been found to date means that in many cases, isotopic analysis is one of the few avenues of research into the diet and economy of the individual. By comparing the isotopic values of less-studied individuals with those of remains from sites with other, archaeological lines of evidence for consumption patterns at these sites (primarily faunal and plant remains), it is possible to create a more complete understanding of the economy and consumption of food-producing people during the Iron Age.

Table 3-1 Regional and archaeological context for individuals included in the study.

**These areas are all contemporary regions, rather than excavated sites. Individuals recovered there and included in this study have been dated to >2000 BP and they are included for comparison (see Table 5-3) for radiocarbon dates for each. ** Two individuals recovered at Ballito Bay are included in this study, one ascribed to the LIA and the other dated to >2000 BP (Ribot et al., 2010)*

Site Name	Biome²	Rainfall (mm/y)	Iron Age
Ballito Bay	Coastal Savanna	1054-1401	**
Boshoff's Farm	Savanna	708-1053	LIA
Diamant ^{3, 4}	Savanna	363-707	EIA
Ellerton Mine	Savanna	363-707	LIA
Estcourt ³	Grassland	708-1053	LIA
Ficus ⁵	Savanna	708-1053	LIA
Fynnlands	Coastal Savanna	708-1053	EIA
Heilbron ⁶	Grassland	363-707	EIA
Illovo Beach ⁷	Coastal Savanna	1054-1401	*

² (Mucina and Rutherford, 2006)

³ (Morris, 1992)

⁴ (Plug, 1985)

⁵ (Plug and Voigt, 1985)

⁶ (Lee-Thorp et al., 1993)

⁷ (Galloway, 1936)

Kaybars Cave ⁸	Grassland	1054-1401	LIA
Lindley ⁹	Grassland	363-707	LIA
Magogo ^{6, 10}	Grassland	708-1053	EIA
Mhlopeni	Savanna	708-1053	EIA
Nagome ¹¹	Savanna	363-707	LIA
Nanda ¹²	Coastal Savanna	708-1053	EIA
New Smitsdorp ¹³	Savanna	363-707	LIA
Nysvlei ¹³	Savanna	363-707	LIA
Olifantspoort ¹⁴	Savanna	708-1053	LIA
Pilgrim's Rest ⁶	Savanna/Grassland	1054-1401	LIA
Richards Bay	Coastal Savanna	1054-1401	*
Robinsons Shelter	Grassland	1054-1401	LIA
Rooikrans ^{6, 15, 16}	Savanna	363-707	LIA
Rustenburg ⁶	Savanna	708-1053	LIA
Skutwater ^{6, 17}	Savanna	20-362	LIA
Tinley Manor ^{18, 8}	IOCB	1054-1401	EIA
Vetchkop	Grassland	363-707	LIA
Wellington Estates ^{6, 18}	Savanna	363-707	LIA

3.6. Study aims and overview

This study builds on previous isotopic analysis of individuals from these sites, which consisted of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements of bone collagen (Lee-Thorp et al., 1993; Gilbert, 1995; Ribot et al., 2010). Supplementing these data with $\delta^{13}\text{C}$ from bone apatite and tooth enamel and adding all four isotopic values for additional individuals, this study (N=51) represents the largest known body of multivariate isotopic data on Iron Age agriculturalists.

This study has three main aims:

⁸ (Wells and Dart, 1934)

⁹ (Maggs, 1976)

¹⁰ (Maggs and Ward, 1984)

¹¹ (Rightmire and van der Merwe, 1976)

¹² (Whitelaw, 1993)

¹³ (Myers, 1958)

¹⁴ (Mason, 1986)

¹⁵ (Plug, 1984)

¹⁶ (Hall, 1984)

¹⁷ (Van Ewyk, 1987)

¹⁸ (Wells and Dart, 1934)

- 1) Measure the carbon isotope¹⁹ ratios of additional body tissues (bone and tooth enamel apatite) for previously published and new individuals to expand global multi-tissue isotopic data and compare with the results of previous studies of the region.
- 2) Utilizing the isotopic composition of different tissues to build a higher-resolution dietary reconstruction for farmers of the Iron Age.
- 3) Position the South African population sample in global models for macronutrient routing and dietary reconstruction.

The following chapter lays out the methodology employed, including two additional experiments in the pre-treatment of bone apatite. Based on the considerations given in section 2.2, these experiments were used to establish the usability of bone apatite of the studied individuals, and bone from Iron Age South Africa more generally. The methods used for all preparation and isotopic analysis of bone apatite and tooth enamel are then described, as well as the few samples for which collagen was not analysed in a previous study.

¹⁹ Analysis of carbonate also measures $\delta^{18}\text{O}$, which is reported in Appendix A. Oxygen results are not discussed in this study.

4. Methods for isotopic analysis

This chapter describes the pre-treatment and analysis of all three tissues (bone apatite, tooth enamel, bone collagen) studied. The pre-treatment methods described in this chapter are intended to isolate the component of interest in each tissue (bone apatite, tooth enamel, bone collagen) by removing contaminants and diagenetic materials. For apatites, there are a variety of protocols in the literature and so preliminary experiments were carried out to contribute to the ongoing debate around the efficacy and effects of varied pre-treatments. The preparation of bone collagen follows a generally well-accepted protocol that is outlined below.

4.1. Apatite pre-treatment experiments

All protocols for apatite pre-treatment involve the oxidation of organic contaminants on bone or enamel, followed by treatment with acid to remove carbonates that have formed on the sample during deposition or through diagenesis. Section 2.6.2 outlined the variety of pre-treatment protocols and concerns around particle size, recrystallization, and other effects of prolonged exposure to the pre-treatment reagents. The pre-treatment of tooth enamel is established and warranted no further investigation, but given the concerns around bone apatite, two experiments were conducted comparing the effects of pre-treatment to the final isotopic values of bone apatite samples. The first examined the effect of bone apatite particle size on the outcome of pre-treatment, and the second looked more closely at sodium hypochlorite exposure times and their effect on poorly preserved human bone.

4.1.1. Pre-Treatment Test 1: particle size

To compare the effect of pre-treatment protocols on bone powders of varying particle size, a well preserved bovid rib from the site of Olifantspoort was collected along with the

human material. $\delta^{13}\text{C}_{\text{collagen}}$ of the rib was -5.9‰ (C:N=3.19), determined via the method described in sections 4.2.3 and 4.3.2 below.

The rib was mechanically cleaned and cut into 2 cm long pieces which were ground in a SPEX mill, cooled with liquid nitrogen. As different particle sizes were desired, the rib pieces were milled using different impact frequencies and milling times and all powder was pooled together, mixed, and sieved through the following mesh sizes: 180 μm , 106 μm , 63 μm , and 25 μm . This resulted in the five particle size categories, defined as:

Size A: particles that did not pass through 180 μm mesh ($>180 \mu\text{m}$)²⁰

Size B: particles that passed through 180 μm but not 106 μm mesh (179-107 μm)

Size C: particles that passed through 106 μm but not 63 μm mesh (105-64 μm)

Size D: particles that passed through 63 μm but not 25 μm mesh (62-26 μm)

Size E: particles that passed through 25 μm mesh ($<24 \mu\text{m}$)

Mechanical sieving was sufficient to separate all particle sizes except those $<25 \mu\text{m}$. In that case, the bone powder was washed through the sieve with ethanol overnight.

Approximately 2mg of each size fraction was placed in 1.5 ml snap-top microcentrifuge tubes. Each of the five size fractions was subjected to six pre-treatments, adapted from Smith (2005), found in Table 4.1.

Table 4-1 Protocol for pre-treatment experiment 1. Reaction times in sodium hypochlorite and acetic acid were varied for different particle sizes. Sodium hypochlorite solution is diluted 1:1 with distilled water, resulting in ~1.75% NaClO v/v.

Particle Size	10 mins in 0.1M CH ₃ COOH			15 mins in 0.1M CH ₃ COOH			Hours in sodium hypochlorite
	2.5	3	3.5	2.5	3	3.5	
Size A	2.5	3	3.5	2.5	3	3.5	
Size B	2.5	3	3.5	2.5	3	3.5	
Size C	2.5	3	3.5	2.5	3	3.5	
Size D	2.5	3	3.5	2.5	3	3.5	
Size E	2.5	3	3.5	2.5	3	3.5	

Following the standard procedure for most pre-treatment methods, 1.0 ml of sodium hypochlorite was pipetted into each microcentrifuge tube and allowed to react. Tubes were shaken by hand every 15 minutes during the specified reaction time, and then centrifuged, the sodium hypochlorite pipetted off, and the powders rinsed three times in distilled water. 1.0 ml of acetic acid was added to each tube, which was shaken thoroughly to re-suspend the bone

²⁰These sizes will be referred to in the text by a range in μm . This is merely for ease of reference, and is not to suggest that these are the exact known sizes of all particles.

powder. Samples were centrifuged two minutes before the end of the treatment time and acid removed at exactly 10 or 15 minutes; then the powders were rinsed three times in distilled water and freeze dried. Visual inspection of sample powder showed minimal loss of sample due to treatment.

Mass spectrometry for all apatite samples is described in section 4.3.1.

4.1.2. Pre-treatment Test 2: poorly preserved bone

As pre-treatment is intended to mitigate the effects of post-depositional change to the isotopic composition of samples, especially for bone apatite, the preservation of individual samples is an important consideration. Authors analyzing bone apatite often emphasize the high levels of preservation in the contexts from which they draw their samples, and it is logical to conclude that pre-treatment methods may be more efficacious on well-preserved bone (Smith, 2005; France and Owsley, 2012; Santana-Sagredo et al., 2015).

In order to test the effects of pre-treatment on more poorly preserved bone, five human bone samples were selected from the study population. Poor preservation was assessed on the basis of discoloration, fragmentation or ‘crumbliness,’ and overall appearance of the bone. The samples were cleaned and ground as described for the faunal rib in pre-treatment test 1 in the SPEX mill, and sieved.

Particle size B (179-107 μm) was selected on the basis of the results of pre-treatment test 1 and treated for 2.5, 3, and 3.5 hours in sodium hypochlorite and 15 minutes in acid as described. The results of pre-treatment test 1 had suggested greater variation in the isotopic value of the sample due to NaClO treatment than time in acid, so NaClO was varied in this experiment while time in acid was not.

4.2. Archaeological human samples

Human remains were selected based on 1) association with Iron Age sites and farming practices, 2) inclusion in previous studies confirming their status as food producers, and 3) preservation. Well preserved bone was selected as much as possible and specimens were eliminated in cases where there were no well-preserved bones available. Individuals without second or third molars with sufficient enamel to sample were also eliminated. The enamel of first molars begins forming very early in life and can carry a pre-weaning isotopic signal that would not be comparable to the adult diet analyzed in bone apatite and collagen (Hillson, 1996).

Teeth or bones coated in glue or other preservatives were avoided. 3-8 cm long bone fragments were collected for each individual, preferably rib shaft fragments or phalanges to avoid damaging skeletal elements most often studied by biological anthropologists. Bone was visually inspected for the preservation of cortical bone, and pieces that were fragmented or ‘crumbly’ were discarded, as well as those that were heavily discolored by soil contaminants.

4.2.1. Tooth enamel preparation

The area selected for sampling, on the buccal or lingual surfaces of third (if available) or second permanent molars, was brushed clean and the surface lightly abraded before enamel powder was collected using a hand-held Dremel drill with a diamond-tipped dental drill bit. Approximately 5-10 mg of enamel was removed above the cementum-enamel junction and below the occlusal surface. Tooth enamel forms from the occlusal surface towards the cementum-enamel junction and can preserve time-based dietary signals, so teeth were sampled up the entire height of the crown to obtain a sample representing the entire period of crown formation. Care was taken to remove enamel evenly and avoid penetrating into the underlying dentin.

Approximately 2 mg of enamel powder was treated with sodium hypochlorite (~1.75% v/v) in 1.5 ml snap-top centrifuge tubes for 45 minutes, with tubes shaken every 15 minutes. These times follow the established method for tooth enamel pre-treatment (Lee-Thorp et al., 1997). After centrifugation the supernatant was pipetted off and the powder was rinsed three times in distilled water. Samples were reacted with 0.1M acetic acid for precisely 15 minutes to remove soluble mineral components (Lee-Thorp et al., 1997; Loftus and Sealy, 2012; Stowe and Sealy, 2015). Samples were centrifuged and rinsed in distilled water three more times before being freeze dried and 2 ± 0.2 mg weighed into 12 ml borosilicate tubes for mass spectrometry.

4.2.2. Bone apatite preparation

Bone fragments were ground in a liquid nitrogen-cooled SPEX mill and sieved through 180 and 106 μm mesh. Based on results from pre-treatment test 1 and 2, only powder size B (179-107 μm) was measured into 1.5 ml centrifuge tubes and treated with sodium hypochlorite for 3 hours, rinsed three times, and reacted with 0.1M acid for exactly 15 minutes. The same care was taken to ensure exact reaction times as for all other samples. After three rinses in distilled water, samples were freeze dried and weighed in the same manner as the tooth enamel.

4.2.3. Bone collagen preparation

The majority of $\delta^{13}\text{C}_{\text{collagen}}$ values presented in this study have been previously reported (Lee-Thorp et al., 1993; Gilbert, 1995). All analyses (previously published and those for this study) have been conducted at the Archaeometry Laboratory at the University of Cape Town. The protocol for collagen extraction in this laboratory (described below) has remained largely similar since the 1990s. When compared to a recently developed and more rigorous method, the collagen extracted by the two approaches from reasonably well-preserved bone showed no significant differences in isotopic composition (Sealy et al., 2014). The Cape Town laboratory therefore continues to use the older protocol for routine collagen extraction. Bone fragments for analysis were surface-cleaned with sandpaper to remove visible contaminants, weighed, and decalcified in 50 ml of 0.2M HCL. HCL was changed every 48 hours until a collagen pseudomorph remained; in some cases the pseudomorphs were fragmented or discolored (pseudomorphs of well-preserved collagen should be nearly transparent; in these cases pseudomorphs were yellowed). The pseudomorphs were rinsed three times in distilled water and treated with 0.1M NaOH for 24 hours to remove humic contaminants. Samples were then immersed in distilled water for 7 days, changed every 36-48 hours, or until the pH returned to neutral.

All samples were frozen in a -4°C freezer before being freeze-dried. Samples were weighed again and collagen yields calculated. Samples were weighed and analyzed as described in section 4.3.2.

The majority of collagen isotopic values used in this study are previously published results from as far back as the 1990s, and in many cases collagen yields were not reported. Collagen yield alone is not a particularly reliable indicator of collagen quality, and authors vary in the minimum yield considered sufficient (DeNiro, 1985; Ambrose, 1990; van Klinken, 1999; Sealy et al., 2014). For collagen extracted for this thesis, quality was assessed based on %C and %N, as well as C:N ratios.

As noted, archaeological sites in the summer rainfall zone are subject to wet/warm-cool/dry periods that are not conducive to a high degree of bone preservation. Of the eleven bone samples that were processed for collagen in this study to supplement published values, five had elemental C:N ratios above 3.6, exceeding the usually accepted upper limit of 3.4.

In almost all cases high C:N ratios were caused by low %C and/or %N. The accepted range of %C in this study was 26-47% and 11-17.3% for % N after Ambrose, 1990 and van

Klinken, 1999. These five samples had %C or %N that fell below these acceptable ranges. Low %C and %N indicate that a proportion of the collagen has been lost, probably the result of diagenetic processes in the environment. Humic contaminants can also result in low C:N. Although all samples were soaked in NaOH to remove humic material, some remained very discolored and disintegrated in the solution. Filtration was attempted to further remove contaminants and isolate organic material, but some samples were simply too degraded to include and those individuals were removed from the study population.

4.3. Mass Spectrometry

4.3.1. Apatites

All apatites were weighed in the same manner and analysed at the University of Cape Town stable isotope laboratory on the same equipment.

0.2 g of powder were weighed into 12 mL round-bottomed borosilicate glass tubes capped with screw-top extainer caps, which had been thoroughly cleaned with phosphoric acid as described in Loftus and Sealy (2012). All samples were analyzed at the University of Cape Town stable isotope laboratory using a ThermoFinnigan (Germany) model II gas bench as described by previous authors (Loftus and Sealy, 2012; Sealy et al., 2014; Stowe and Sealy, 2015) and ThermoFinnigan delta plus XP isotope ratio mass spectrometer. Three standards, Cavendish Marble ($\delta^{13}\text{C} = 0.34\text{‰}$, $\delta^{18}\text{O} = -8.95\text{‰}$), NBS 18 ($\delta^{13}\text{C} = -5.01\text{‰}$, $\delta^{18}\text{O} = -23.03\text{‰}$) and NBS 19 ($\delta^{13}\text{C} = 1.95\text{‰}$, $\delta^{18}\text{O} = -2.20\text{‰}$), were included in each run. $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ are calculated relative to the Vienna PeeDee Belemnite standard. Precision for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ was better than 0.2‰, based on repeated analyses of laboratory standards (Cavendish Marble) (N=30).

Apatite powders are reacted with 100% phosphoric acid and the resultant CO₂ gas is repeatedly sampled by the gas bench for each sample and the resultant values are averaged for a final result. Samples for which the precision of repeated readings was less than 0.2‰ were rejected and re-analyzed.

4.3.2. Collagen

Approximately 0.5mg of collagen was weighed into tin capsules and combusted to N₂ and CO₂ in an automated carbon and nitrogen analyzer (Carlo-Erba), coupled to a continuous-flow isotope ratio mass spectrometer (Delta V Plus (Thermo-Finnigan)). Samples were analyzed in duplicate and averaged if results were within 0.2‰, if not they were re-analyzed. The precision (1s) of repeated measurements of homogenous materials (in-house

laboratory standards) is less than 0.2‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Values of in-house standards have been determined by measurement relative to international standard materials NBS 21, IAEA N1 and N2, and standards exchanged with other laboratories (Sealy et al., 2014).

The final sample of 51 archaeological humans discussed in this study represent those individuals for whom reliable isotopic values were secured for bone apatite, bone collagen and enamel apatite. The isotopic results of analysis of these individuals is discussed in the following chapter, along with the results of the two pre-treatment experiments.

5. Results of Isotopic Analysis

5.1. Pre-treatment Test 1: effect of particle size and pre-treatment time on $\delta^{13}\text{C}_{\text{bone apatite}}$ of well-preserved bone

Pre-treatment test 1 investigated the effect of particle size and pre-treatment times on $\delta^{13}\text{C}_{\text{bone apatite}}$, using a well-preserved bovid rib from the site of Oliphantspoort. Preservation was assessed based on colour and the fact that the bone did not crumble at all when cut into sections. Cutting revealed thick cortical bone and demineralisation for $\delta^{13}\text{C}_{\text{collagen}}$ resulted in a high quality collagen pseudomorph that was not fragmented or discoloured. A sample of this rib was ground and sieved into five different particle size groups: Size A: >180 μm , Size B: 179-107 μm , Size C: 105-64 μm , Size D: 62-26 μm , Size E: <24 μm . Table 5.1 lists $\delta^{13}\text{C}_{\text{bone apatite}}$ for all experimental treatments and particle sizes. The four largest particle size groups had overlapping, similar mean $\delta^{13}\text{C}_{\text{bone apatite}}$ across all treatments (group A: $-0.35 \pm 0.30\%$, group B: $-0.40 \pm 0.16\%$, group C: $-0.26 \pm 0.12\%$, group D: $-0.25 \pm 0.22\%$). Group E, the smallest particles, produced a wider range of $\delta^{13}\text{C}_{\text{bone apatite}}$ across the treatments (range of 2.4‰) and on average was distinct from the other four (mean $\delta^{13}\text{C}_{\text{bone apatite}} -1.38 \pm 0.91\%$).

Table 5-1 Bovid rib $\delta^{13}\text{C}_{\text{bone apatite}}$ by particle size and treatment type (pre-treatment test 1).

All values are reported relative to VPDB. Each sample was analysed only once. The overall average $\delta^{13}\text{C}_{\text{bone apatite}}$ based on all 5 particle sizes was $-0.56 \pm 0.60\%$.

CH ₃ COOH (mins)	NaClO (hours)	Size A $\delta^{13}\text{C}$ (‰)	Size B $\delta^{13}\text{C}$ (‰)	Size C $\delta^{13}\text{C}$ (‰)	Size D $\delta^{13}\text{C}$ (‰)	Size E $\delta^{13}\text{C}$ (‰)
10 mins	2.5	-0.2	-0.4	-0.2	0.0	-0.4
	3	-0.4	-0.2	-0.2	0.0	-1.1
	3.5	-0.8	-0.4	-0.3	-0.3	-2.8
15 mins	2.5	-0.3	-0.6	-0.3	-0.3	-0.5
	3	-0.5	-0.4	-0.5	-0.6	-1.6
	3.5	0.1	-0.5	-0.1	-1.1	-1.8
$\bar{x} \pm \sigma$ (‰)		-0.35 ± 0.30	-0.40 ± 0.16	-0.26 ± 0.12	-0.25 ± 0.22	-1.38 ± 0.91
Range (‰)		0.9	0.4	0.3	0.5	2.4
$\delta^{13}\text{C}_{\text{collagen}}$	-5.9‰					
$\Delta^{13}\text{C}_{\text{collagen-apatite}}$		$-5.61 \pm 0.3\%$	$-5.54 \pm 0.14\%$	$-5.70 \pm 0.12\%$	$-5.56 \pm 0.41\%$	$-4.58 \pm 0.91\%$

Each group has few observations (<10) and so statistics should be treated descriptively. Tests for significance are reported for illustrative purposes, but differences should be interpreted with caution. In the case of the smallest particle size group, however, the magnitude of the difference in $\delta^{13}\text{C}_{\text{bone apatite}}$ supports the conclusion that particle size has a distinct impact on the ultimate isotopic value of the sample.

Initial results indicated that particle size has a significant effect on $\delta^{13}\text{C}_{\text{bone apatite}}$ values across all particle size groups and for all treatments (single factor ANOVA, $p=0.002$, $\alpha=0.05$). However, the overall range of variability across the groups (-0.5‰ to 0‰), all of which were from the same bovid rib, indicates that most particle sizes produce comparable isotopic results despite different pre-treatment methods. Mean $\delta^{13}\text{C}_{\text{bone apatite}}$ for the smallest particle size (Group E) is very different from the others ($-0.35\pm 0.26\text{‰}$ compared to -1.38‰)²¹, and is driving the significant result observed in the ANOVA. This particle size (which is comparable to that achieved by removing tooth enamel with a drill) is substantially more depleted in ^{13}C than any other size, with the most depleted values seen in material with the longest exposure to NaClO. Despite significant results in the case of group E, $\delta^{13}\text{C}$ does not clearly pattern with either NaClO or acid exposure time within or across the other particle sizes. The overall range in $\delta^{13}\text{C}_{\text{bone apatite}}$ between the largest and smallest particle sizes is illustrated in Figure 4.1, which also makes clear the relatively narrow range of values for sizes A-D. The range of values returned for size E is more than 4 times that of the other size groups; the mean $\delta^{13}\text{C}_{\text{bone apatite}}$ of group E does not fall within the range of sizes A-D at all (nor does the median value).

When all particle sizes were grouped together, no significant difference was observed between samples treated in 0.1M acetic acid for 10 minutes or 15 minutes (two-factor ANOVA with replication, $p=0.76$, $\alpha=0.05$) or for different lengths of time in NaClO (two-factor ANOVA with replication, $p=0.21$, $\alpha=0.05$). Size E showed the highest sensitivity to pre-treatment, but neither time in acid nor NaClO significantly contributed to variation in $\delta^{13}\text{C}_{\text{bone apatite}}$, although the effect of sodium hypochlorite was close to significant (single-factor ANOVA, $p=0.06$, $\alpha=0.05$). Given the small number of observations (6 for Group E), it is not possible to determine if this difference is ‘real’ or not. Differences of 30 minutes

²¹ As the data are not normally distributed (and there is no expectation that isotope values should be normally distributed), T-tests cannot be used to compare them. In this case, sample size is too small to effectively use non-parametric tests for difference between groups (the Mann-Whitney U Test or Wilcoxon signed-rank test), so statistics are used descriptively.

(each time interval in NaClO), however, are more likely to be detectable in the isotopic value of the sample than 5 minute differences in acid. This suggests that it is prolonged exposure to NaClO that has an effect on the isotopic value of the sample. A 5-minute difference in acid reaction does not appear to have any effect on $\delta^{13}\text{C}$.

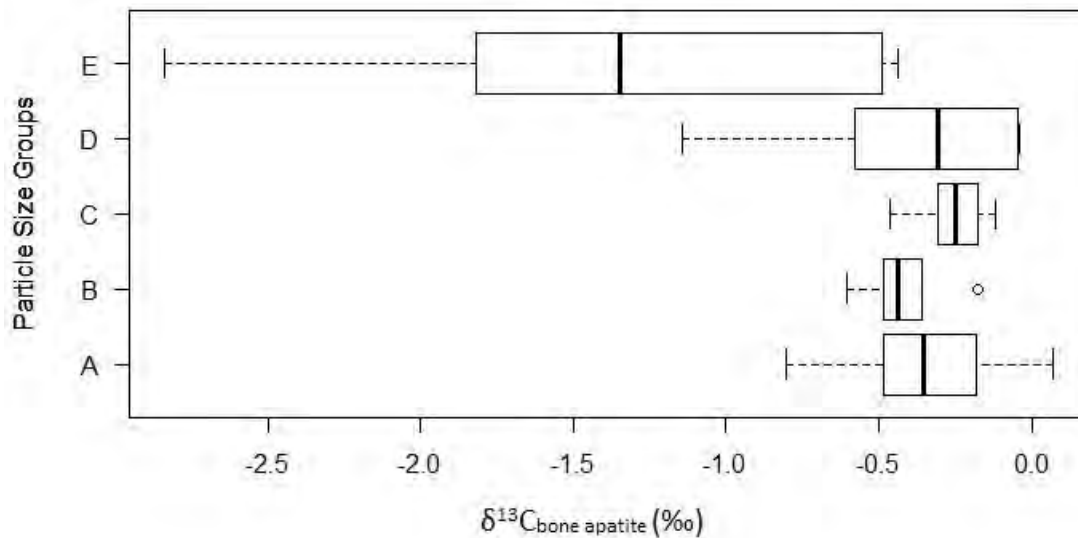


Figure 5-1 Pre-treatment test 1 results. $\delta^{13}\text{C}_{\text{bone apatite}}$ varies with particle size. Box plot illustrating the effect of particle size on $\delta^{13}\text{C}_{\text{bone apatite}}$ across all treatments. The boundaries of the box represent the first and third quartiles and center lines represent the median. Bars extend to 1.5 x interquartile range from each quartile, and circles (outliers) are defined as greater or less than 1.5 x IQR.

Groups C and B are the least sensitive to variations in pre-treatment. Variation in $\delta^{13}\text{C}$ for group B cannot be attributed to different times in acid (single-factor ANOVA, $p=0.31$, $\alpha=0.05$) or in NaClO (single-factor ANOVA, $p=0.156$, $\alpha=0.05$). This suggests that particles of this size are small enough for uniform reaction with pre-treatment reagents, but not so small that they undergo isotopic change (perhaps as a result of structural alteration such as recrystallisation).

$\delta^{13}\text{C}_{\text{collagen}}$ of the rib was -5.9‰ , and $\Delta^{13}\text{C}_{\text{collagen-apatite}}$ does not vary significantly with either time in acid or sodium hypochlorite for groups A-D (two-factor ANOVA with replication, acid time, $p=0.3$, NaClO time, $p=0.49$, $\alpha=0.05$). Particle size, however, does have a significant effect on $\Delta^{13}\text{C}_{\text{collagen-apatite}}$ (single-factor ANOVA, $p=0.002$, $\alpha=0.05$), which is driven by size E. The mean $\Delta^{13}\text{C}_{\text{collagen-apatite}}$ for size E ($-4.58\pm 0.91\text{‰}$) is notably smaller than the overall mean $\Delta^{13}\text{C}_{\text{collagen-apatite}}$ for all sizes ($-5.40\pm 0.46\text{‰}$).

The mean $\delta^{13}\text{C}$ value of particle size B ($-0.40\pm 0.16\text{‰}$) was closest to the overall mean of the bone ($-0.56\pm 0.60\text{‰}$) and the mean value for groups A-D ($-0.35\pm 0.26\text{‰}$). Group B is also the largest well-constrained particle size that yielded consistent results. Based on this

result, all samples of human bone used for $\delta^{13}\text{C}_{\text{bone apatite}}$ measurements were ground and sieved and particle size B (179-107 μm) was selected for pre-treatment and isotope measurements.

5.2. Pre-treatment Test 2: effect of pre-treatment time on $\delta^{13}\text{C}_{\text{bone apatite}}$ of poorly preserved bone

Pre-treatment test 2 was intended to examine the effect of varying durations of treatment in NaClO on poorly preserved bone. Bone from five individuals included in the study were selected on the basis of poor preservation, as described in section 4.1.2 of the Methods chapter. The bone was cleaned, ground, and sieved as described, and size B (179-107 μm) powder was treated in NaClO for varying lengths of time, followed by 15 minutes in 0.1M acetic acid, based on the results of pre-treatment test 1. The results of pre-treatment test 2 indicate that between 2.5 and 3.5 hours, NaClO reaction does not result in significantly different $\delta^{13}\text{C}_{\text{bone apatite}}$, even for poorly preserved bone. Four out of five samples have a standard deviation of $<0.2\text{‰}$ across the three treatments, and the standard deviation of the fifth (UCT 4148) is 0.23‰ (Table 5.2).

Table 5-2 Pre-treatment test 2 $\delta^{13}\text{C}_{\text{bone apatite}}$ results for pre-treatment test 2. All values are reported relative to VPDB.

Time in NaClO (hours)	UCT 3685 $\delta^{13}\text{C}$ (‰)	UCT 4148 $\delta^{13}\text{C}$ (‰)	UCT 4915 $\delta^{13}\text{C}$ (‰)	UCT 4922 $\delta^{13}\text{C}$ (‰)	UCT 16367 $\delta^{13}\text{C}$ (‰)
2.5	-5.7	-4.2	-6.5	-2.7	-5.0
3	-5.6	-3.9	-6.4	-2.9	-5.2
3.5	-5.9	-4.4	-6.5	-3.1	-4.9
$\bar{x} \pm \sigma$	$-5.75 \pm 0.18\text{‰}$	$-4.19 \pm 0.23\text{‰}$	$-6.46 \pm 0.05\text{‰}$	$-2.90 \pm 0.19\text{‰}$	$-5.05 \pm 0.14\text{‰}$

Figure 5.2 further illustrates that varying durations of treatment with NaClO did not significantly change $\delta^{13}\text{C}_{\text{bone apatite}}$ for the poorly preserved bone samples. The flatness of each line indicates little change across the treatment times, and even in cases where the lines curve slightly there is no systematic pattern of enrichment or depletion due to longer times in NaClO. The precision of repeated measurements on the mass spectrometer was better than 0.2‰ , suggesting the differences across treatment times are simply random analytical variations. No significant difference was observed across treatment times for each sample (2-factor ANOVA without replication, $p=0.24$, $\alpha=0.05$).

These data indicate that $\delta^{13}\text{C}_{\text{bone apatite}}$ of particles between 179-107 μm is not significantly altered by a one-hour range of reaction times in NaClO, even for poorly-preserved bones. This particle size group returns robust $\delta^{13}\text{C}$ values even when the bone is in

poor condition. Based on these results, pre-treatment for 3 hours in NaClO and 15 minutes in acetic acid was used for all human bone samples for which $\delta^{13}\text{C}_{\text{bone apatite}}$ is reported below.

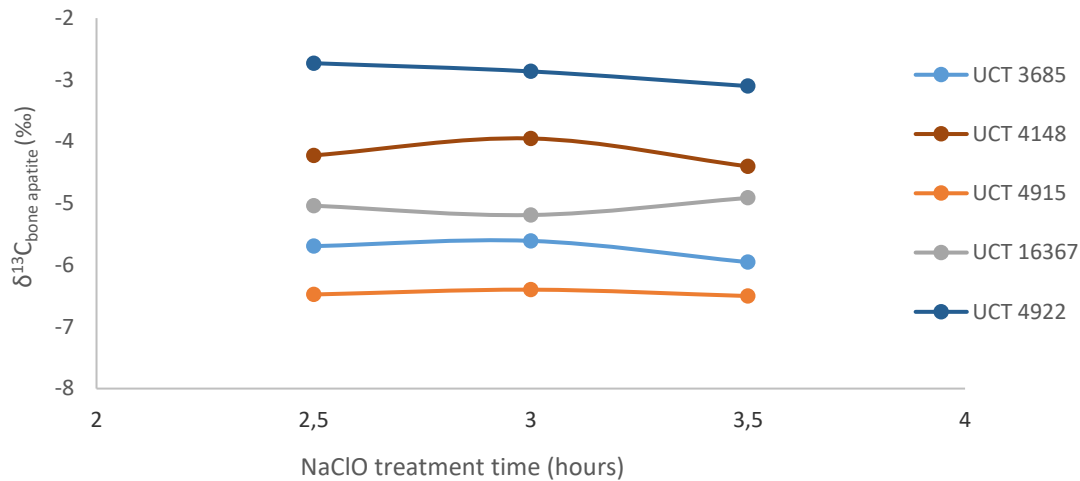


Figure 5-2 Pre-Treatment Test 2: $\delta^{13}\text{C}_{\text{bone apatite}}$ over time for poorly preserved human bone apatite. $\delta^{13}\text{C}_{\text{bone apatite}}$ for group B (179-107 μm) size bone across NaClO treatment times. All samples were reacted with 0.1M acetic acid for 15 minutes after reaction with NaClO as per the method described.

5.3. Human Isotopic Results

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from bone collagen and $\delta^{13}\text{C}$ from bone apatite and tooth enamel for all individuals included in the study are listed in Table 5.3. As discussed in section 4.2.3 of the methods chapter, collagen was assessed based on %C and %N because many previously published collagen values did not report C:N ratios. Collagen quality indicators for all collagen samples analysed in this study fell within the ranges for well-preserved collagen (%C: 26-47% and %N: 11-17.3% after Ambrose, 1990 and van Klinken, 1999). Collagen C:N ratios for those samples that reported them, and $\delta^{18}\text{O}$ for tooth enamel and bone apatite can be found in Appendix A.

Three individuals included in the study were hunter-gatherers, because they pre-date the beginning of the Iron Age in the coastal region where they were buried: UCT 5398, 5431, and 10853 (Ribot et al., 2010). All four isotopic values for these individuals cluster and are substantially different from those of the Iron Age agriculturalists (Table 5.4) (for $\delta^{13}\text{C}_{\text{bone apatite}}$ $-3.82 \pm 2.49\text{‰}$ compared to $-10.06 \pm 0.29\text{‰}$; $\delta^{13}\text{C}_{\text{tooth enamel}}$ $-2.88 \pm 2.48\text{‰}$ compared to -

9.32±1.45‰; $\delta^{13}\text{C}_{\text{collagen}}$ -8.65±2.16‰ compared to -13.83±0.25‰; $\delta^{15}\text{N}_{\text{collagen}}$ 10.05±1.9‰ compared to 12.53±0.93‰)²².

A fourth individual, UCT 3686, has been attributed to the 10th century AD and was found at the site of Heilbron, in the Highveld grassland. There is no evidence of Iron Age farmer settlement in the Highveld before the second millennium (Maggs, 1976), so this individual was also a hunter gatherer, despite being classed as an agriculturalist by Lee-Thorp et al. (1993). This was likely due to the isotopic similarity between UCT 3686 and the Iron Age agriculturalists from that area, which can be seen in Figure 5-3. This similarity is due to the dominance of C₄ grass in the Highveld: a hunter gatherer consuming plants and animals from this area would be expected to have similar isotopic values to those of early agriculturalists there.

*Table 5-3 $\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{15}\text{N}_{\text{collagen}}$, $\delta^{13}\text{C}_{\text{bone apatite}}$, $\delta^{13}\text{C}_{\text{tooth enamel}}$ for sampled individuals from southern Africa. Tooth enamel and bone apatite were prepared and isotope values measured for this study. Most $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{15}\text{N}_{\text{collagen}}$ were first reported in: *Lee-Thorp et al., 1993; ** Gilbert 1995; ***Ribot et al., 2010. Collagen values for individuals not marked with asterisks were analysed by the author for this study unless otherwise indicated. Skeletons that have been directly dated are indicated. Otherwise, all chronological indicators are the same as in Table 3-1 and reproduced here for convenience. †The provenience of these skeletons beyond their general location (Pilgrim's Rest) has been lost, and it is probable they were salvaged or sent to Revil Mason in the early 20th century. It is likely that they are Late Iron Age individuals but no other details can be found. ‡ Located in the archaeological collection of the Ditsong Museum in Pretoria, South Africa. † Located in the Raymond Dart Collection at the School of Anatomical Sciences, University of the Witwatersrand, Johannesburg, South Africa. ‡ Located in the anatomical collection of the Department of Human Biology, University of Cape Town, Cape Town, South Africa. † Located in the archaeological collections of the KwaZulu-Natal Museum in Pietermaritzburg, South Africa. ‡ Located in the collection of the University of South Africa (UNISA).*

Museum Accession Code ²³	UCT Code	Site	Date (Century) ²⁴	Pre- or post-maize introduction	IA	Collagen		Enamel $\delta^{13}\text{C}$ (‰)	Tooth sampled	Bone $\delta^{13}\text{C}$ (‰)
						$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)			
NAS 30 [†]	3682	Skutwater*	12	Pre	LIA	-12.6	14.1	-4.2	M3	-3.2
NAS 28 [†]	3683	Skutwater*	12	Pre	LIA	-10.7	14.4	-5.4	M3	-7.2
NAS 29 [†]	3684	Skutwater*	12	Pre	LIA	-10.6	11.6	-6.2	M3	-4.7
NAS 27 [†]	3685	Skutwater*	12	Pre	LIA	-11.3	13.1	-3.8	M3	-5.6
A 233 [‡]	3686 ²⁵	Heilbron*	10	Pre	EIA	-11.0	10.6	-4.4	M2	-8.4
UCT 326 [†]	3690	Lindley (Makwareng) ²⁶	17 to 19	Post	LIA	-7.04	11.3	-0.3	M3	-3.9

²² For comparison of nonparametric data in sizes of unequal groups, the Mann-Whitney U Test would be appropriate. However, with only 3 data points in the hunter gatherer group, the test would be unable to detect differences at a meaningful level of significance and so they are compared descriptively here.

²³ Due to the closure and consolidation of collection, as well as the re-naming of museums, museum accession codes may be inconsistent within the same collection or institution. The current host institution is indicated for each individual.

²⁴ Dates are given at century intervals in Gilbert 1995, so individuals published elsewhere have been converted to maintain consistency. As precise chronologies are not required, broad ranges are given here to associate individuals with either 'pre maize' or 'post maize' agricultural practices.

²⁵ Pta-5218 (1000±60yrs BP), Morris 1992

²⁶ Collagen values taken from the UCT Archaeometry Lab Register, entered by Julia Lee-Thorp

UCT 327 [†]	3691	Lindley (Makwareng)*	17 to 19	Post	LIA	-7.2	10.4	-0.3	M3	-2.8
A 410 [‡]	3696 ²⁷	New Smitsdorp*	16	Post	LIA	-8.4	9.9	-1.5	M2	-3.3
A 1836 [‡]	3704	Wellington Estates*	16 to 19	Post	LIA	-6.2	10.5	-0.9	M3	-1.8
A 1837 [‡]	3705	Wellington Estates*	16 to 19	Post	LIA	-7.1	12.2	-1.4	M2	-1.5
A 2798 [‡]	3708	Rustenburg	18	Post	LIA	-7.4	9.07	0.5	M2	-3.4
A 2853/2854 ²⁸ ‡	3710	Oliphantspoort*	19	Post	LIA	-6.7	9.3	-2.9	M3	-2.7
A 2855 [‡]	3711	Oliphantspoort	18	Post	LIA	-6.7	7.9	-0.1	M3	-2.5
A 2856 [‡]	3712	Oliphantspoort	15	Post ²⁹	LIA	-7.7	8.9	-0.5	M2	-3.2
KCM 86/1 [†]	4148	Nanda***	6 to 7	Pre	EIA	-9.9	9.2	-4.9	M3	-3.9
A 4221/WA 25 [‡]	4152	Oliphantspoort	19	Post	LIA	-8.9	9.1	-0.7	M3	-4.4
A 220/WA 15 [‡]	4171	Nylsvlei*	16	Post	LIA	-7.9	10.3	-1.3	M2	-3.3
A 2230 [‡]	4309	Wellington Estates	16 to 19	Post	LIA	-7.8	11.2	-1.6	M2	-2.8
A 2231 [‡]	4310	Wellington Estates	16 to 19	Post	LIA	-6.6	11.5	-0.5	M3	-0.1
PMB 91/45 ¹¹	4497 ³⁰	Venus/Estcourt***	19	Post	LIA?	-7.0	11.0	-1.2	M3	-2.7
A 121 [‡]	4913	Vetchkop**	18	Post	LIA	-6.5	9.6	0.9	M3	-2.3
A 294 [‡]	4914	Kaybars Cave**	19	Post	LIA	-9.8	8.8	-3.0	M3	-5.5
A 295 [‡]	4915	Kaybars Cave**	19	Post	LIA	-11.0	8.0	-4.3	M3	-6.4
A 300 [‡]	4916	Robinsons Shelter**	19	Post	LIA	-11.6	7.5	-4.9	M3	-4.2
A 302 [‡]	4917	Robinsons Shelter**	19	Post	LIA	-9.7	6.6	-3.6	M3	-6.2
A 414 [‡]	4919	Ellerton Mine ³¹ **	14	Pre	LIA	-9.6	7.8	-5.1	M3	-5.3
A 416 [‡]	4920	Ellerton Mine**	14	Pre	LIA	-7.4	7.9	-3.6	M3	-3.2
A 1838 [‡]	4921	Wellington Estates**	16 to 19	Post	LIA	-6.6	8.4	-2.6	M2	-1.2
A 2122 [‡]	4922 ³²	Boshoff's Farm**	16	Post	LIA	-6.0	9.2	-1.5	M3	-2.9

²⁷ Pta-5224 (380±30yrs BP), Morris 1992

²⁸ In some cases, the same individual has acquired multiple accession numbers, in these cases all numbers are given for reconciliation and reference purposes.

²⁹ The stratigraphy of Oliphantspoort is highly complex and not well documented. The 15th century AD date for this individual is based on a dated ash heap which may not be securely associated with the burial; this represents one of the earliest dates for a burial at Oliphantspoort, all the rest of whom are considered by archaeologists to have consumed maize (Mason, 1986, Simon Hall pers. comm.). Considered with their isotopic similarity to the other individuals at the site, this individual was considered a 'post-maize' consumer in further analysis.

³⁰ Pta-5776 (140±40yrs BP), Ribot et al., 2010

³¹ Pta-4973 (600±50yrs BP), Morris 1992

³² Pta-4985 (400±45yrs BP), Morris 1992

A 2232 [‡]	4923	Wellington Estates**	16 to 19	Post	LIA	-6.1	11.7	-1.9	M2	0.5
A 2233 [‡]	4924 ³³	Wellington Estates**	16	Post	LIA	-6.9	9.5	-1.2	M3	0.5
A2234 [‡]	4925	Wellington Estates**	16 to 19	Post	LIA	-7.2	10.8	-1.0	M3	-0.7
A 2235 [‡]	4926 ³⁴	Wellington Estates**	19	Post	LIA	-7.5	10.7	-1.6	M3	-3.9
A 2237 [‡]	4928	Wellington Estates**	16 to 19	Post	LIA	-6.7	12.2	-3.7	M3	-1.4
WA 16 [‡]	4932	Ficus**	16	Post	LIA	-7.1	10.2	-1.7	M3	-2.4
A 7230/WA 131-78B2 [‡]	4948	Rooikrans**	17	Post	LIA	-9.5	8.2	-1.5	M3	-3.6
A 4227/WA131-78B3 [‡]	4949	Rooikrans**	17	Post	LIA	-9.5	8.9	-3.7	M2	-3.3
UNISA 1 [†]	5387	Diamant	6 to 7	Pre	EIA	-8.4	9.1	-0.9	M3	-3.0
KZ 86/1-B2 [†]	5392	Nanda***	6 to 7	Pre	EIA	-9.3	9.1	-2.3	M2	-7.2
A 609 [‡]	5395 ³⁵	Tinley Manor***	15	Pre	LIA	-7.4	6.0	-2.5	M3	-4.0
A 2120 [‡]	5396 ³⁶	Ballito Bay***	12	Pre	LIA	-9.9	8.2	-2.0	M3	-5.2
NM 2009/8 [†]	5398 ³⁷	Ballito Bay***	>2000 BP	Pre	HG	-13.6	13.3	-9.2	M3	-10.2
DBN 4381/NM 2009/6 [†]	5428 ³⁸	Fynnlans***	12	Pre	EIA	-12.9	9.9	-7.3	M3	-6.5
UCT 154 [†]	5431 ³⁹	Illovo Beach***	>2000 BP	Pre	HG	-13.8	11.5	-7.9	M3	-9.7
A 635 [‡]	10853 ⁴⁰	Richards Bay***	>2000 BP	Pre	HG	-14.1	12.8	-10.8	M3	-10.2
UCT 430 [†]	16364	Nagome	17	Post	LIA	-8.3	8.6	-6.1	M3	-4.0
PMB 2000/11 [†]	16365	Mhlopeni***	6	Pre	EIA	-6.6	10.0	-2.0	M3	-4.0
98/14 [†]	16367	Magogo	6 to 7	Pre	EIA	-7.4	8.0	-5.0	M2	-5.2
A 275 [‡]	16565	Wellington Estates	16 to 19	Post	LIA	-9.6	12.8	-2.7	M3	-2.7
A 1078 [‡]	17511	Pilgrim's Rest	[†] LIA	Post	LIA	-6.8	10.3	-6.2	M2	-2.0
A 1079 [‡]	17512	Pilgrim's Rest	[†] LIA	Post	LIA	-6.39	9.46	-1.4	M2	-1.6

³³ Pta-4981 (400±40yrs BP), Morris 1992

³⁴ Pta-4982 (160±50yrs BP), Morris 1992

³⁵ Pta-6126 (530 ±45yrs BP), Ribot et al., 2010

³⁶ Pta-6108 (940 ±20yrs BP), Ribot et al., 2010

³⁷ Pta-5803 (2940 ±50yrs BP), Ribot et al., 2010

³⁸ Pta-5789 (780±50yrs BP), Ribot et al., 2010

³⁹ Pta-6968 (1980±50yrs BP), Ribot et al., 2010

⁴⁰ OxA-V-2064-55 (2532±26yrs BP), Ribot et al., 2010

Table 5-4 Summary isotope values for all individuals. Average isotope values for all four isotopes across hunter-gatherer (HG)⁴¹, Early Iron Age (EIA) and Late Iron Age (LIA) groups.

	HG ($\bar{x} \pm \sigma$) (‰)	EIA ($\bar{x} \pm \sigma$) (‰)	LIA ($\bar{x} \pm \sigma$) (‰)	All ($\bar{x} \pm \sigma$) (‰)
	N=4	N=6	N=41	N=51
$\delta^{13}\text{C}_{\text{bone apatite}}$	-9.64 ± 0.87	-4.96 ± 1.63	-3.18 ± 1.76	-3.90 ± 2.45
$\delta^{13}\text{C}_{\text{tooth enamel}}$	-8.10 ± 2.72	-3.73 ± 2.38	-2.39 ± 1.86	-2.99 ± 2.50
$\delta^{13}\text{C}_{\text{collagen}}$	-13.13 ± 1.43	-9.10 ± 2.22	-8.17 ± 1.74	-8.67 ± 2.20
$\delta^{15}\text{N}_{\text{collagen}}$	12.05 ± 1.23	9.22 ± 0.70	9.97 ± 1.92	10.01 ± 1.87

The three hunter gatherers from the coast cluster tightly together, and are depleted in ^{13}C , due to the marine component in their diets, which is evidenced in Figure 5-3. Living in the Highveld grassland, far from marine resources and in a solidly C_4 region, the isotope values for the Heilbron hunter gatherer cluster more closely with Iron Age farmers. This accounts for the wide range in isotopic values across the hunter gatherers: $\delta^{13}\text{C}_{\text{bone apatite}}$ ranges from -10.2 to -8.4‰, $\delta^{13}\text{C}_{\text{tooth enamel}}$ from -10.8 to -4.4‰, $\delta^{13}\text{C}_{\text{collagen}}$ from -14.1 to -11‰, and $\delta^{15}\text{N}_{\text{collagen}}$ from 10.6 to 13.3‰.

There is a pattern of enrichment in ^{13}C across all tissues from the Early Iron Age to the Late Iron Age, although Early Iron Age farmers are spread evenly throughout the extent of values encompassed by those of the Late Iron Age. For EIA farmers, mean $\delta^{13}\text{C}_{\text{bone apatite}} = -4.96 \pm 1.63\%$, $\delta^{13}\text{C}_{\text{tooth enamel}} = -3.73 \pm 2.38\%$, $\delta^{13}\text{C}_{\text{collagen}} = -9.10 \pm 2.22\%$, and mean $\delta^{15}\text{N}_{\text{collagen}} = 9.22 \pm 0.70\%$. Compared to these farmers, the Heilbron hunter gatherer reflects the ecology of the Highveld where this specific individual lived to a much greater extent than the subsistence strategy of hunting and gathering: mean $\delta^{13}\text{C}_{\text{bone apatite}} = -8.4\%$, $\delta^{13}\text{C}_{\text{tooth enamel}} = -4.4\%$, $\delta^{13}\text{C}_{\text{collagen}} = -11\%$, and mean $\delta^{15}\text{N}_{\text{collagen}} = 10.6\%$.

Late Iron Age farmers encompass the range of values seen in the Early Iron Age, but extend further into more positive values for all four tissues. Mean $\delta^{13}\text{C}_{\text{bone apatite}} = -3.18 \pm 1.76\%$, $\delta^{13}\text{C}_{\text{tooth enamel}} = -2.39 \pm 1.86\%$, $\delta^{13}\text{C}_{\text{collagen}} = -8.17 \pm 1.74\%$, and mean $\delta^{15}\text{N}_{\text{collagen}} = 9.97 \pm 1.92\%$.

5.3.1. $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{15}\text{N}_{\text{collagen}}$

$\delta^{13}\text{C}_{\text{collagen}}$ values are more positive among Late Iron Age agriculturalists (-6 to -12.6‰) than the coastal hunter gatherers (-13.6 to -14.1‰), and EIA farmers (-6.6 to -12.9‰) cover the same range of values as those of the LIA. The larger sample size for LIA farmers is likely responsible for some of the wider range. UCT 3696 from Heilbron (with

⁴¹ This includes both coastal hunter gatherers and the individual from Heilbron, UCT 3685

$\delta^{13}\text{C}_{\text{collagen}}$ of -11‰) falls within the EIA/LIA range, towards the more depleted end of the $\delta^{13}\text{C}$ range (Figure 5-3). There is a wide spread of $\delta^{15}\text{N}_{\text{collagen}}$ across all three groups (from 6.0 to 14.4‰), with the coastal hunter gatherers on the high end (11.5 to 13.3‰), along with some LIA farmers who group in the north of the study area (geographical patterning is discussed further below). The Heilbron hunter gatherer (with $\delta^{15}\text{N}$ of 10.6‰) is also more enriched in ^{15}N than most farmers. Early Iron Age farmer $\delta^{15}\text{N}_{\text{collagen}}$ clusters the most closely, with Late Iron Age farmers falling above and below them. There is no significant difference in collagen-derived isotope values ($\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{15}\text{N}_{\text{collagen}}$) between EIA and LIA populations (single-factor ANOVA, $\delta^{13}\text{C}_{\text{collagen}} p = 0.12$, $\delta^{15}\text{N}_{\text{collagen}} p = 0.50$; $\alpha = 0.05$).

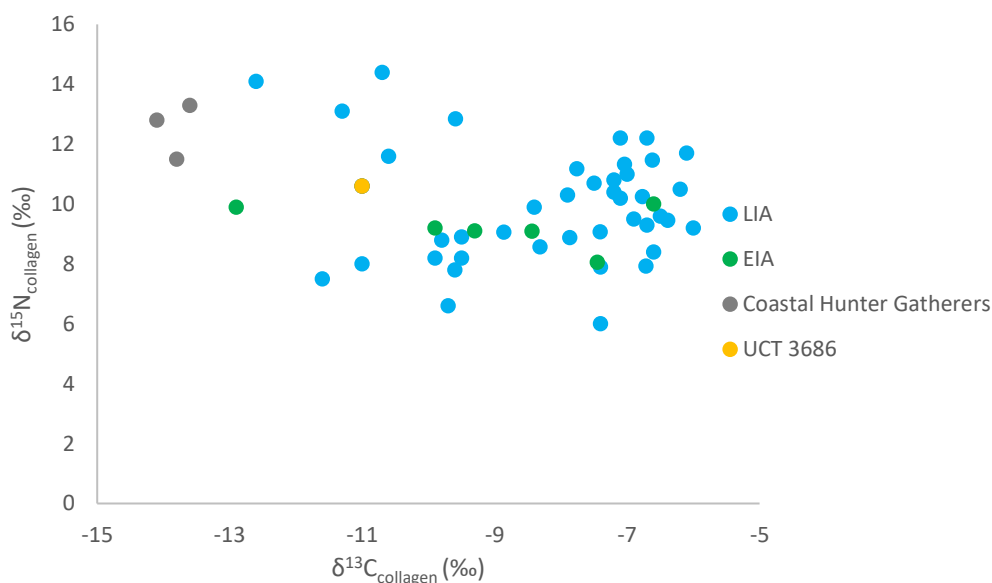


Figure 5-3 $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{15}\text{N}_{\text{collagen}}$ for all archaeological humans

$\delta^{13}\text{C}_{\text{bone apatite}}$ values range from -10.2 to 0.5‰ , with the coastal hunter gatherers being the most negative (-9.7 to -10.2‰) and LIA farmers the most positive (up to 0.5‰) (Figure 5-4). UCT 3686 ($\delta^{13}\text{C}_{\text{bone apatite}}$ of -8.4‰) falls at the negative end of the range. Farmers from the EIA span much of the range of those from the LIA, but no EIA farmers have $\delta^{13}\text{C}_{\text{bone apatite}}$ greater than -2‰ . Bone apatite is significantly enriched in ^{13}C from the Early to the Late Iron Age (single-factor ANOVA, $\delta^{13}\text{C}_{\text{bone apatite}} p = 0.00$, $\alpha = 0.05$).

All three groups show consistent and similar offsets between $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{collagen}}$, as evidenced by the R^2 of the best-fit regression line through all points ($R^2=0.67$). Mean $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ ^{42,43} for all individuals is $4.77\pm 1.42\%$.

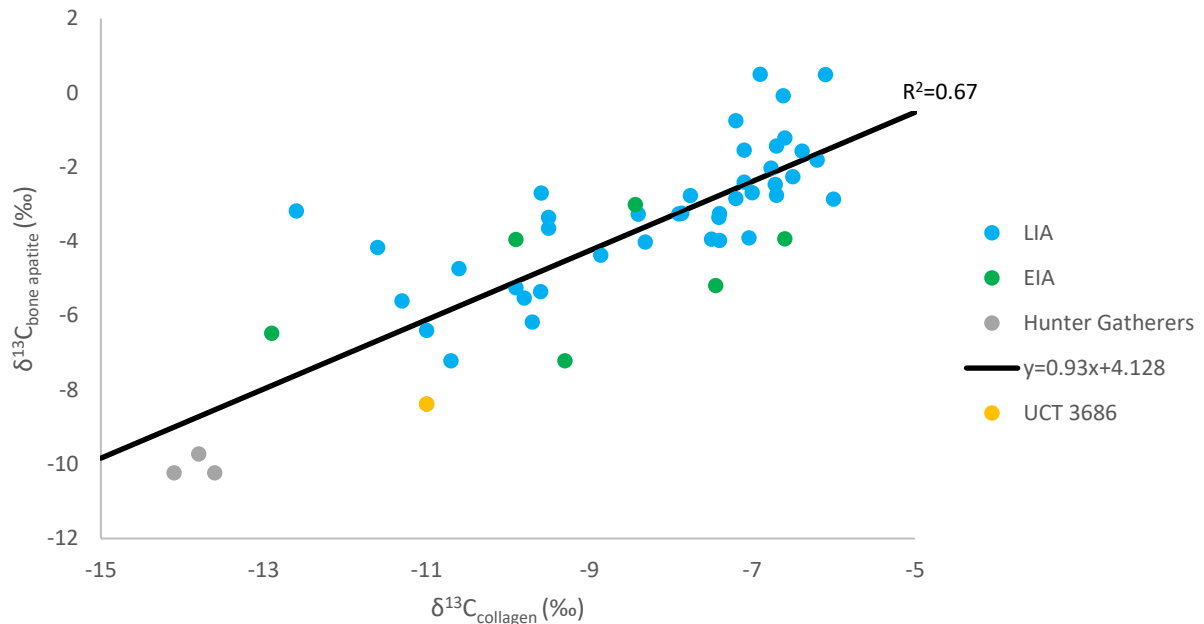


Figure 5-4 $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{collagen}}$ for all archaeological humans

5.3.2. $\delta^{13}\text{C}_{\text{tooth enamel}}$

The trends in $\delta^{13}\text{C}_{\text{tooth enamel}}$ across the different groups are largely the same as those for $\delta^{13}\text{C}_{\text{bone apatite}}$, with a greater overall range of values for all three groups (Figure 5-5). EIA farmers again span most of the range of LIA farmers (overall range for the Iron Age is -7.3 to 0.8‰), but EIA $\delta^{13}\text{C}_{\text{tooth enamel}}$ does not exceed 0‰. The coastal hunter gatherers are the most depleted in ^{13}C (-7.9 to -10.8‰), while UCT 3686 from Heilbron has $\delta^{13}\text{C}_{\text{tooth enamel}}$ of -4.4‰, towards the lower end of the Iron Age farmer range. This is substantially different from the

⁴² Throughout this study, offsets between two tissues are listed in the order they were calculated; i.e. $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ was calculated by subtracting $\delta^{13}\text{C}_{\text{bone apatite}}$ from $\delta^{13}\text{C}_{\text{collagen}}$, $\Delta^{13}\text{C}_{\text{enamel-bone apatite}}$ was calculated by subtracting bone apatite from enamel, etc. The absolute value of all offsets are reported in the text for ease of comparison and conceptualization, although of course most isotopic values are negative.

⁴³ Isotopic spacing is reported here as Δ , the numerical difference between two delta values. Strictly speaking, the correct expression of difference between two delta values is ϵ , isotopic enrichment (Sponheimer and Cerling, 2014). The enrichment (ϵ) between $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$, for example, is calculated as:

$$\left[\left(\frac{1000 + \delta^{13}\text{C collagen}}{1000 + \delta^{13}\text{C bone apatite}} \right) - 1 \right] * 1000$$

Sponheimer and Cerling (2014) point out that ϵ is more accurate than Δ when the values compared differ by larger amounts (e.g. 10‰). The largest isotopic spacing in this data base is found in UCT 3682, with $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ of -8.44‰ and $\Delta^{13}\text{C}_{\text{collagen-apatite}}$ of 9.41‰. $\epsilon^{13}\text{C}_{\text{collagen-enamel}}$ for UCT 3682 is -8.47‰ and $\epsilon^{13}\text{C}_{\text{collagen-apatite}}$ is 9.44‰. These slight differences are much smaller than the natural range of variation in human isotopic values, and so Δ will be used throughout this study.

$\delta^{13}\text{C}_{\text{bone apatite}}$ value for this individual (-8.4‰) and separates it from the rest of the hunter gatherers. $\delta^{13}\text{C}_{\text{tooth enamel}}$ values for the coastal hunter gatherers also span a much wider range of compared with $\delta^{13}\text{C}_{\text{bone apatite}}$ (-9.7 to -10.2‰) but this cannot be statistically assessed with only 3 individuals⁴⁴.

$\delta^{13}\text{C}_{\text{tooth enamel}}$ for LIA farmers (-6.2 to 0.9‰) is significantly more positive than for the EIA (-7.3 to -0.9‰) (Single factor ANOVA, $p=0.00$, $\alpha=0.05$). Mean $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ for all individuals is $5.67\pm 1.66\text{‰}$, which is significantly larger than that of $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ (Mann-Whitney U test, $p=0.0$). R^2 of the best-fit line in Figure 5-5 is 0.57. All three coastal hunter gatherers fall below the line and have a smaller collagen-enamel offset than the farmers and UCT 3686 ($4.15\pm 1.3\text{‰}$ compared to $5.7\pm 1.7\text{‰}$ and 6.5‰).

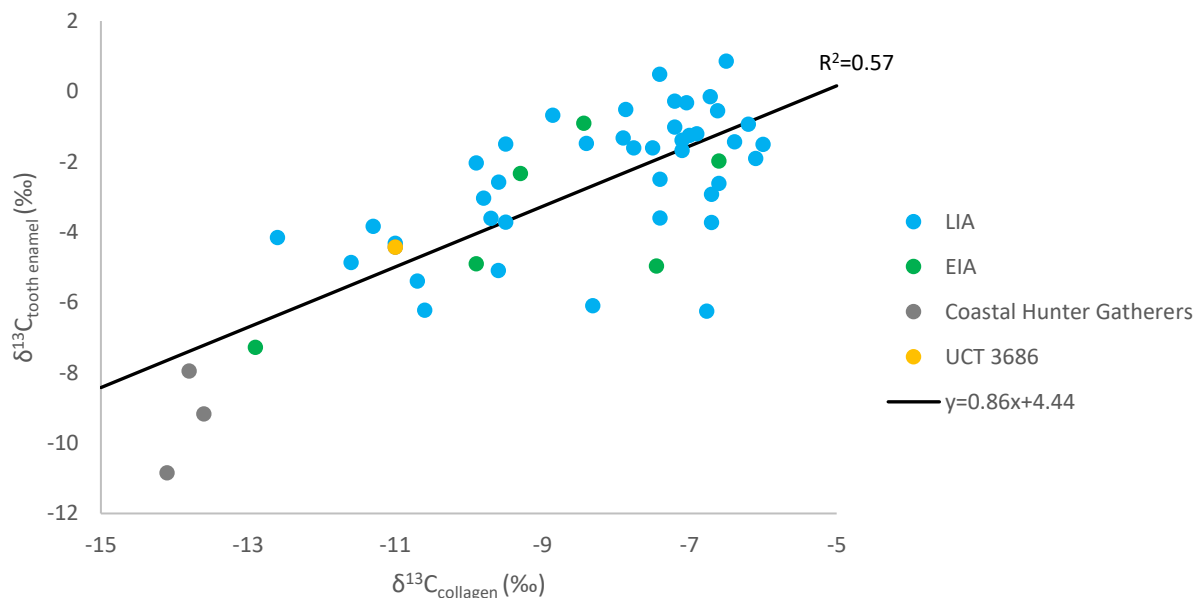


Figure 5-5 $\delta^{13}\text{C}_{\text{tooth enamel}}$ and $\delta^{13}\text{C}_{\text{collagen}}$ for all archaeological humans

5.3.3. $\Delta^{13}\text{C}_{\text{enamel-bone apatite}}$

Mean $\Delta^{13}\text{C}_{\text{enamel-bone apatite}}$ for all individuals is $+1.74\pm 1.19\text{‰}$. No difference in enamel-bone apatite spacing was observed between the hunter gatherers, the EIA, or the LIA agriculturalists (single-factor ANOVA, $p=0.49$).

When regression lines are plotted for each group, slopes close to one indicate the smallest $\Delta^{13}\text{C}_{\text{enamel-bone apatite}}$. The slope of the regression line for LIA farmers is 0.47, for EIA farmers it is 0.65, and for hunter gatherers it is 4. Thus $\Delta^{13}\text{C}_{\text{enamel-bone apatite}}$ is larger for coastal

⁴⁴ Since there are only 3 coastal hunter gatherers, this group is too small to test qualitatively using a test such as the Mann-Whitney U test.

hunter-gatherers than for agriculturalists, but this effect is actually due to the very small number of individuals in this group and is likely spurious.

Very low R^2 values (0.20 for both EIA and LIA regression lines) indicate that the offset between enamel and bone apatite is not consistent from person to person, and this does not pattern between EIA and LIA datasets. The high degree of scatter on both sides of each line illustrate the degree of inter-individual variability in the offset. The R^2 value is higher for hunter gatherers ($R^2=0.66$), but this is confounded by the very small number of observations. Overall R^2 for all individuals is 0.49.

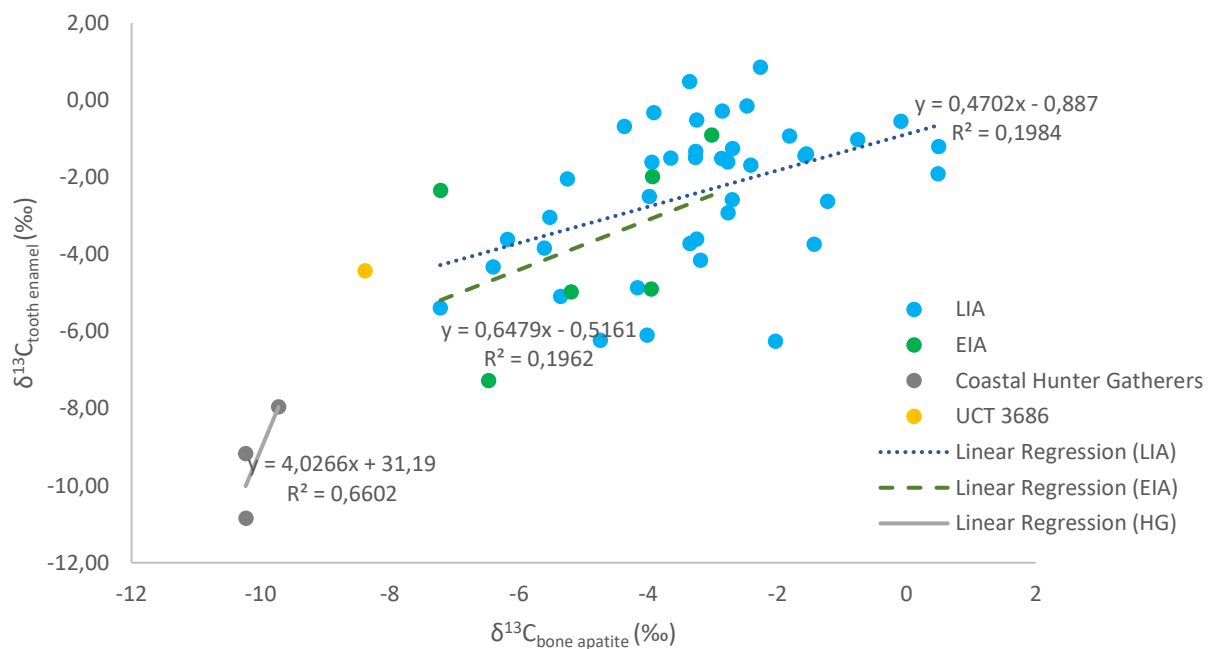


Figure 5-6 $\delta^{13}C_{bone\ apatite}$ and $\delta^{13}C_{tooth\ enamel}$ for all archaeological humans

An important consideration is that the sample sizes for each group in the study differ considerably. LIA farmers form the largest group by far (N=41), while there are only 6 EIA farmers and 4 hunter gatherers. This reflects the large number of known and excavated LIA sites, as well as the theorised population increase between the LIA and the EIA. It also means that the trends identified in the smaller groups (hunter gatherers and EIA farmers) are less robust than those for LIA farmers, and differences between them may be exaggerated by the difference in sample size. Especially in cases where no significant difference has been found between them through traditional ANOVA or Mann-Whitney tests, it is possible that failure to reject the null hypothesis is simply due to a lack of evidence, rather than true equivalence between the groups. Thus, appropriate caution should be exercised when interpreting low-

magnitude differences or similarities, and very large ones (such as the gap between hunter gatherer and agriculturalist $\delta^{13}\text{C}_{\text{collagen}}$) are more likely robust.

5.3.4. $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ and $\Delta^{13}\text{C}_{\text{collagen-enamel}}$

The range in values for $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ and $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ further illustrate the differences between $\delta^{13}\text{C}_{\text{tooth enamel}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$. There is no clear pattern among hunter gatherers, or farmers, and EIA farmers are scattered throughout the entirety of the LIA farmer range. $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ for hunter gatherers does not exceed 4‰, which is midway through the overall range of the farmers. High offsets between mineral (bone apatite, tooth enamel), which records a whole diet signal, and collagen (which largely records protein), suggest a diet with limited protein components, which are isotopically distinct from the rest of the diet. In low protein diets, few of the isotopes from the protein are available for incorporation into the mineral tissue, but if the protein is isotopically similar to the rest of the diet than these offsets will be less pronounced. This will also be the case if plant material is a significant source of protein. The issue of interpreting collagen-biomineral offsets will be explored in more detail in the following chapter.

$\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ for all individuals is 4.77 ± 1.42 ‰, and $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ is 5.67 ± 1.66 ‰ (Figure 5-7). There is no significant difference between the offsets for EIA farmers and hunter gatherers (Mann Whitney U-test, $p=0.08$). Given the high degree of overlap between all three groups, any difference observed between them is likely due to the larger sample size of the LIA farmers. Overall R^2 is 0.05, suggesting that there is virtually no correlation at all between the two offsets, and is even lower than expected given that the correlation between $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{tooth enamel}}$ was 0.2.

One individual, UCT 3682, had anomalously large $\Delta^{13}\text{C}_{\text{collagen-apatite}}$ (9.4‰) and $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ (8.4‰) compared to the LIA and all other groups. These large offsets are driven by relatively negative $\delta^{13}\text{C}_{\text{collagen}}$ (-12.6‰) compared to the LIA average of -8.17 ± 1.74 ‰. This suggests more C_3 protein sources, such as browsing fauna, combined with C_4 non-protein dietary components, in a diet that was relatively low in protein.

UCT 17511, from Pilgrim's Rest, has a $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ of 0.5‰, by far the lowest in the data. This individual has $\delta^{13}\text{C}_{\text{collagen}}$ comparable to other Iron Age farmers (-6.7‰) but more negative $\delta^{13}\text{C}_{\text{tooth enamel}}$ compared to other farmers (-6.2‰). The $\delta^{13}\text{C}_{\text{tooth enamel}}$ value is comparable to that of the coastal hunter gatherers. Such a close resemblance between

collagen and tooth enamel can suggest a resemblance between protein and non-protein food sources. However, since whole diet-collagen spacing is smaller than whole diet-biomineral, it could also suggest a diet with protein that is more enriched in ^{13}C compared to the rest of the diet (making $\delta^{13}\text{C}_{\text{collagen}}$ more positive relative to $\delta^{13}\text{C}_{\text{whole diet}}$ and thus closer to $\delta^{13}\text{C}_{\text{tooth enamel}}$). UCT 17512, a contemporaneous individual from Pilgrim's Rest, has $\delta^{13}\text{C}_{\text{tooth enamel}}$ of -1.4‰ and a comparable $\delta^{13}\text{C}_{\text{collagen}}$ (-6.4‰). There is no available archaeological evidence on this site to assess distinctions between UCT 17511 and 17512 (such as social status), and it is also possible that diagenesis has affected the bone and influenced the isotopic signal.

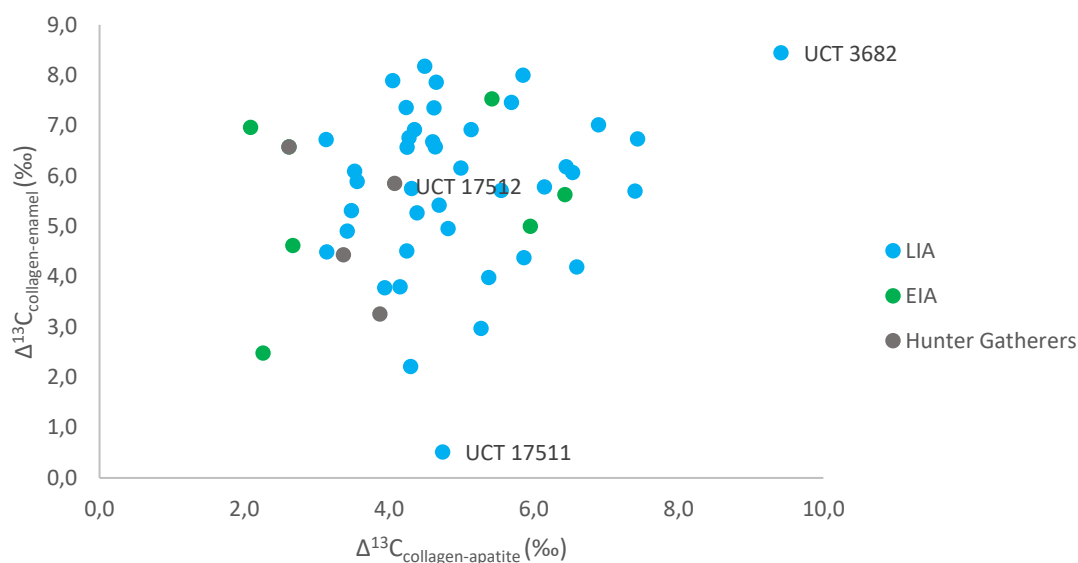


Figure 5-7 $\Delta^{13}\text{C}_{\text{collagen-apatite}}$ and $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ for all archaeological humans

The most notable difference between EIA and LIA farmers, an overall shift to more positive $\delta^{13}\text{C}_{\text{bone apatite}}$ in the LIA, is significant (Mann-Whitney U test, $p=0.03$), although again the differences in sample size ($N=6$ and $N=41$) must be taken into consideration. The range of variation amongst individuals causes a great deal of overlap between the two groups. The significant difference between $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ and $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ observed in the LIA does not exist in the EIA (Mann Whitney U-test, $p=0.23$). The small size of the EIA group compromises the significance of differences between it and the LIA, and it is likely that the difference in $\delta^{13}\text{C}_{\text{bone apatite}}$ is an artefact of this discrepancy. Accordingly, these two groups will be combined as 'IA farmers' for subsequent analyses, capturing a larger sample size overall.

5.3.5. Geographic Patterns

$\delta^{13}\text{C}_{\text{collagen}}$ does not pattern strongly across biomes, with both savanna and grassland individuals falling across the full range of values (see Table 3-1 for the biomes of each site). In both biomes, enriched ^{13}C occurs in individuals with enriched ^{15}N , but there are no other geographical patterns in $\delta^{15}\text{N}_{\text{collagen}}$ (Figure 5-8).

The two individuals from Robinson's Shelter and the two from Kaybar's Cave form a separate cluster of grassland points in the lower-left of Figure 5-8. These individuals have slightly lower $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{15}\text{N}_{\text{collagen}}$ (-9.7 to -11.6‰ and 6.6 to 8.8‰ respectively) than the rest of the grassland farmers (grassland average $\delta^{13}\text{C}_{\text{collagen}}$ is $-8.22 \pm 1.9\%$; average $\delta^{15}\text{N}_{\text{collagen}}$ is $9.18 \pm 1.5\%$). The difference is significant for both $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{15}\text{N}_{\text{collagen}}$ (Mann Whitney U-test, $p < 0$). These two sites are in the most wet regions of the grassland included in this study (Map 3-2); while $\delta^{15}\text{N}_{\text{collagen}}$ is likely demonstrating some environmental effects, increased moisture in this region would have impacted productivity and vegetation in the area and driven a dietary shift. Depleted in ^{13}C , these individuals would have consumed more C_3 resources which were not available in more arid regions.

The coastal hunter gatherers are on the most depleted end of the $\delta^{13}\text{C}_{\text{collagen}}$ range, and have fairly high $\delta^{15}\text{N}_{\text{collagen}}$ as previously discussed. Although on the Indian Ocean coast, the vegetation in this region is largely savanna, so these individuals are indicated in Figures 5-8 through 5-11 as 'coastal savanna hunter gatherers.' They fall closest to a group of five Iron Age farmers from the savanna: all four skeletons in this study that come from Skutwater and one from Wellington Estates. These have the highest $\delta^{15}\text{N}_{\text{collagen}}$ values (11.6 to 14.4‰), and the four from Skutwater have more depleted $\delta^{13}\text{C}_{\text{collagen}}$ (-10.6 to -12.6‰), than the rest of the Iron Age individuals. This suggests a more mixed C_3/C_4 diet overall than the farmers from the other biomes, but environmental factors have a significant influence on $\delta^{15}\text{N}_{\text{collagen}}$.

The individuals from Skutwater are largely separated from the rest of the dataset due to high $\delta^{15}\text{N}_{\text{collagen}}$ (mean = $13.3 \pm 1.3\%$, compared to the overall savanna mean of $10.27 \pm 1.8\%$). The northernmost site in this study, Skutwater is also the most arid today (201-400 mm/year), and aridity effects may be driving ^{15}N enrichment. Isotopic reconstruction of the climate in the region based on archaeological fauna reflect a similar rainfall range, between 350mm and 450mm/year during the period of the occupation of Skutwater (12th century AD) (Smith et al., 2007)⁴⁵. Overall, there is a wide range of $\delta^{15}\text{N}_{\text{collagen}}$ values, and the

⁴⁵ This rainfall interval is as reported in Smith et al. 2007, and falls largely within the 363-707mm/year interval given in figure 2.

roles of aridity and consumption of animal protein cannot be disentangled without further data, including isotopic values from contemporaneous faunal remains and plants and animals from more arid regions.

Individual UCT 16565 from Wellington Estates, with $\delta^{13}\text{C}_{\text{collagen}}$ of -6.4‰ and $\delta^{15}\text{N}_{\text{collagen}}$ of 11.7‰, is more depleted in ^{13}C than the rest of the contemporaneous individuals from that site, but has comparable $\delta^{15}\text{N}_{\text{collagen}}$, suggesting a more mixed diet overall and no difference in animal consumption. Wellington Estates, which is close to Nylsvlei and Boshoff's Farm, experiences comparable mean annual rainfall and temperature, suggesting dietary differences are more impactful than environment on the difference between UCT 16565 and other individuals from the region.

The individual from Fynnlads is very depleted in ^{13}C ($\delta^{13}\text{C}_{\text{collagen}}$ -12.9‰, $\delta^{13}\text{C}_{\text{bone apatite}}$ -7.3‰, $\delta^{13}\text{C}_{\text{tooth enamel}}$ -6.5‰) compared to the other savanna individuals but has comparable $\delta^{15}\text{N}_{\text{collagen}}$ (9.9‰). Compared to the other coastal farmers, whose $\delta^{15}\text{N}_{\text{collagen}}$ values span a wide range, Fynnlads is the upper limit of ^{15}N enrichment, but it is quite depleted in ^{13}C . The proximity of the Fynnlads individual to the coastal hunter gatherers suggests a very mixed diet, and the potential influence of marine resources cannot be ruled out

Farmers from Nanda and Ballito Bay—also sites in the on the coastal savanna—are clustered in both $\delta^{13}\text{C}_{\text{collagen}}$ (-9.3 to -9.9‰) and $\delta^{15}\text{N}_{\text{collagen}}$ (8.2 to 9.2‰), although Nanda is 40 km inland while Ballito Bay is on the coast. They fall within the range of grassland and savanna values, although on the lower end of the $\delta^{15}\text{N}_{\text{collagen}}$ range, suggesting less animal protein in their diets. The individual from Tinley Manor, also from the coast, close to Ballito Bay, is depleted in ^{15}N relative to the others from the coast. The low $\delta^{15}\text{N}_{\text{collagen}}$ value from Tinley Manor was attributed to the heavily glued state of the bones by Ribot et al., who conducted the original collagen analysis (2010).

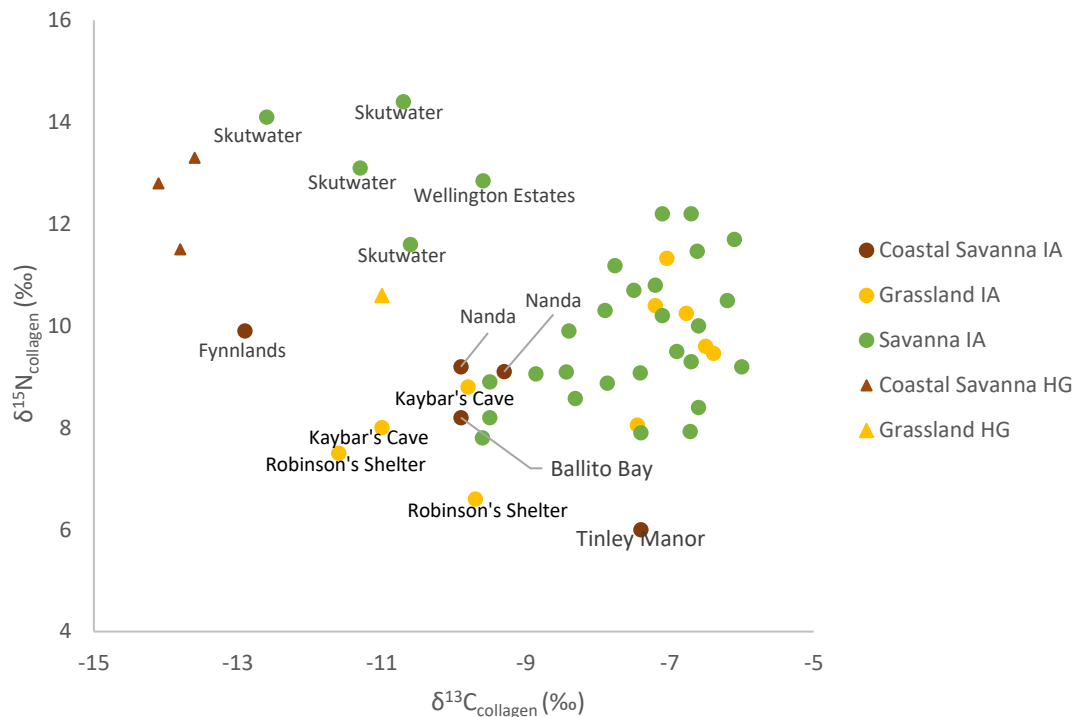


Figure 5-8 $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{15}\text{N}_{\text{collagen}}$, by geographical distribution

Grassland and savanna farmers are intermingled in Figure 5-9, which compares $\delta^{13}\text{C}_{\text{bone apatite}}$ to $\delta^{13}\text{C}_{\text{collagen}}$. Depleted values for the coastal hunter gatherers group in the lower-left corner, and the grassland hunter gatherer from Heilbron is also more depleted in both tissues than most agriculturalists. The coastal farmers are scattered throughout the middle of the data and do not group as closely as in Figure 5-8, suggesting that they differ most from the other farmers in ^{15}N , probably due to consumption of marine resources. The uppermost range of $\delta^{13}\text{C}_{\text{bone apatite}}$, from -2‰ to 2‰, is occupied only by individuals from the savanna.

In Figure 5-9, the individuals from Skutwater and UCT 16565 from Wellington Estates are largely intermingled with the rest of the savanna and grassland farmers, indicating that it is really their nitrogen values that differentiate them. They are, however, on the lower end of the $\delta^{13}\text{C}_{\text{bone apatite}}$ range, in the direction of the hunter gatherers. Fynnlands is again the most negative non-hunter gatherer in $\delta^{13}\text{C}_{\text{collagen}}$ and one of the most negative for $\delta^{13}\text{C}_{\text{bone apatite}}$, falling close to UCT 5392 from Nanda. This result further emphasizes the presence of C_3 elements in the diet of the Fynnlands individual, as both the whole diet signal from bone apatite and the protein-based signal from collagen are depleted in ^{13}C .

UCT 5392 from Nanda and UCT 3683 from Skutwater have nearly identical, very negative $\delta^{13}\text{C}_{\text{bone apatite}}$ (-7.2‰ and -7.2‰ respectively), indicating a mixed whole diet. This similarity, compared to their very divergent $\delta^{15}\text{N}_{\text{collagen}}$ values (9.1‰ and 14.4‰ respectively), highlights the role of aridity in the ^{15}N enrichment of the Skutwater individuals. If the disparity had been due to a high protein diet at Skutwater and a low one at Nanda, more of that protein would be expected to reflect in the whole diet signal, and the Skutwater $\delta^{13}\text{C}_{\text{bone apatite}}$ signal would not be so similar to that of Nanda. While this is most clear in the case of these two individuals, and there is a wide range of $\delta^{13}\text{C}_{\text{bone apatite}}$ at Skutwater (-3.2 to -7.2‰), aridity would have effected everyone living at the site.



Figure 5-9 $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{collagen}}$ by geographical distribution

All geographical regions are less tightly grouped in $\delta^{13}\text{C}_{\text{tooth enamel}}$ (single-factor ANOVA, for $\delta^{13}\text{C}_{\text{bone apatite}}$ $p < 0.0$, for $\delta^{13}\text{C}_{\text{tooth enamel}}$ $p = 0.2$) for all farmers and the savanna hunter gatherer (Figure 5-10). Coastal hunter gatherers remain significantly distinct in $\delta^{13}\text{C}_{\text{tooth enamel}}$ (single-factor ANOVA, $p = 0.0$). Grassland and savanna agriculturalists are intermixed throughout the entire range of $\delta^{13}\text{C}_{\text{tooth enamel}}$, with grassland individuals more scattered overall and falling at the most enriched and depleted ends of the data, and fewer individuals in the middle when compared to savanna. Again, the individuals from Skutwater

have relatively depleted tooth enamel compared to the other farmers, but fall amongst individuals from the grassland. UCT 16565 from Wellington Estates is not distinguished from other savanna farmers on either axis. The individual from Fynnlands is again the most depleted agriculturalist, and the spread in $\delta^{13}\text{C}_{\text{tooth enamel}}$ within the coastal hunter gatherer group makes them very close to Fynnlands. The grassland hunter gatherer from Heilbron lies within the range of values for farmers, although at the negative end of the range. The fact that labelled individuals from Figure 5-9 fall in very different regions of Figure 5-10 indicates the distinction between $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{tooth enamel}}$.

While high $\delta^{15}\text{N}_{\text{collagen}}$ at Skutwater is likely driven by aridity, their depleted $\delta^{13}\text{C}_{\text{collagen}}$ compared to $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{tooth enamel}}$ indicates a diet with distinct protein and non-protein elements. Collagen depleted in ^{13}C suggests protein sources that are more mixed than the rest of the diet, likely browsing animals such as goats or wild fauna. As Lee-Thorp et al. (1993) pointed out, there was a relatively large quantity of faunal remains from wild game found at the site. The large separation between the whole diet and protein signals suggest a relatively low-protein diet, as the C_3 influence of the protein is not apparent in the isotopic value of the bone or enamel.

The shift to C_3 dietary resources amongst the individuals from Kaybar's Cave and Robinson's Shelter (and the clustering of the data points) is also apparent in Figure 5-9, and less so in Figure 5-10, suggesting that the C_3 component of the diet was enough to influence the whole diet. These four individuals have significantly more negative $\delta^{13}\text{C}_{\text{bone apatite}}$ (Mann Whitney U-test, $p=.02$) than the rest of the grassland farmers, but not significantly more negative $\delta^{13}\text{C}_{\text{tooth enamel}}$ (Mann Whitney U-test, $p=0.47$). This example underlines the difference in metabolism and fractionation for bone apatite and tooth enamel, and the inappropriateness of treating them interchangeably as 'biominerals.'

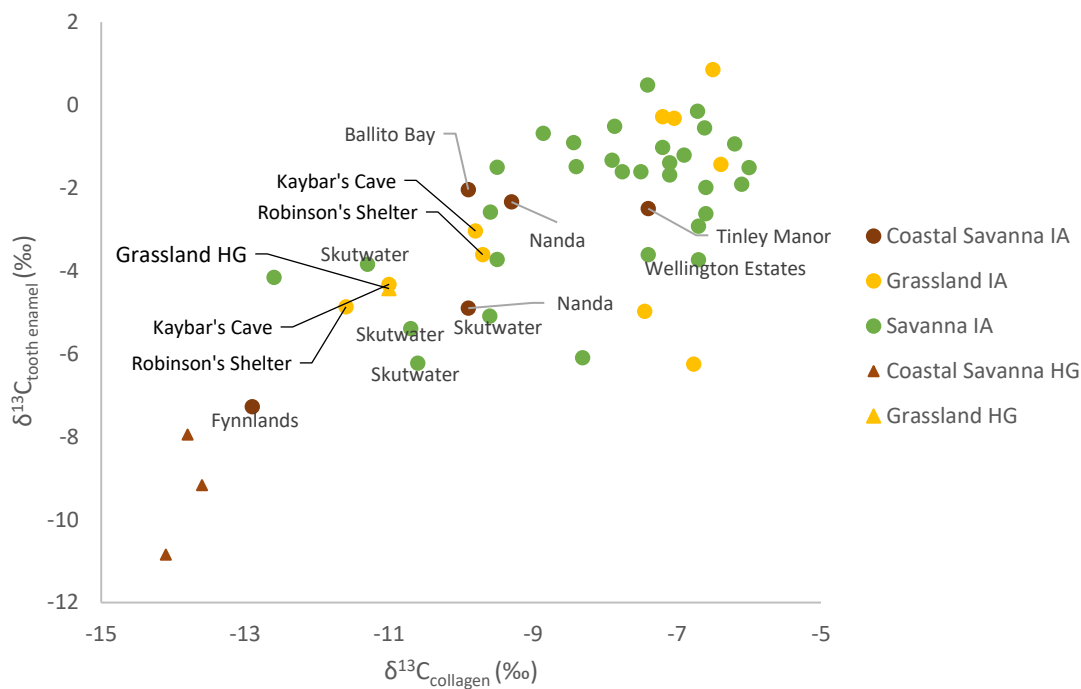


Figure 5-10 $\delta^{13}C_{tooth\ enamel}$ and $\delta^{13}C_{collagen}$ by geographical distribution

Based on Figures 5-8 through 5-10, it is clear that while there is geographic patterning in the data (namely between the coast and the savanna/grasslands), and regions of interest do emerge (such as arid Skutwater), there is no distinct and clear separation between the farmers of different biomes, especially not for carbon.

Figure 5-11, which is a recreation of Figure 5-7 but distinguishes between biomes, reinforces the similarity of carbon isotope values for all individuals across biomes. $\Delta^{13}C_{collagen-apatite}$ and $\Delta^{13}C_{collagen-enamel}$ are fully intermingled for all three biomes, and even hunter gatherers are not distinct from farmers. Individuals, such as UCT 3682 from Skutwater, and UCT 17511 from Pilgrim's Rest, have distinguishing $\Delta^{13}C_{collagen-apatite}$ and $\Delta^{13}C_{collagen-enamel}$, but these effects are not seen in other individuals from these sites and are likely due to dietary differences or preservational issues, as discussed in section 2.6.1. The majority of the grassland farmers have lower $\Delta^{13}C_{collagen-apatite}$ than those from the savanna, but this is not the case for all of them, and this could also be due to the disproportionate number of savanna farmers represented in the data, especially considering the difference is not significant (Mann Whitney U-test, $p=0.3$). The wide range of $\Delta^{13}C_{collagen-apatite}$ and $\Delta^{13}C_{collagen-enamel}$ found throughout this data, which do not pattern by biome or with time (EIA versus

LIA) suggest that there are other, more fundamental causes of variation in $\delta^{13}\text{C}_{\text{tooth enamel}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$ that are discussed in Chapter 6.

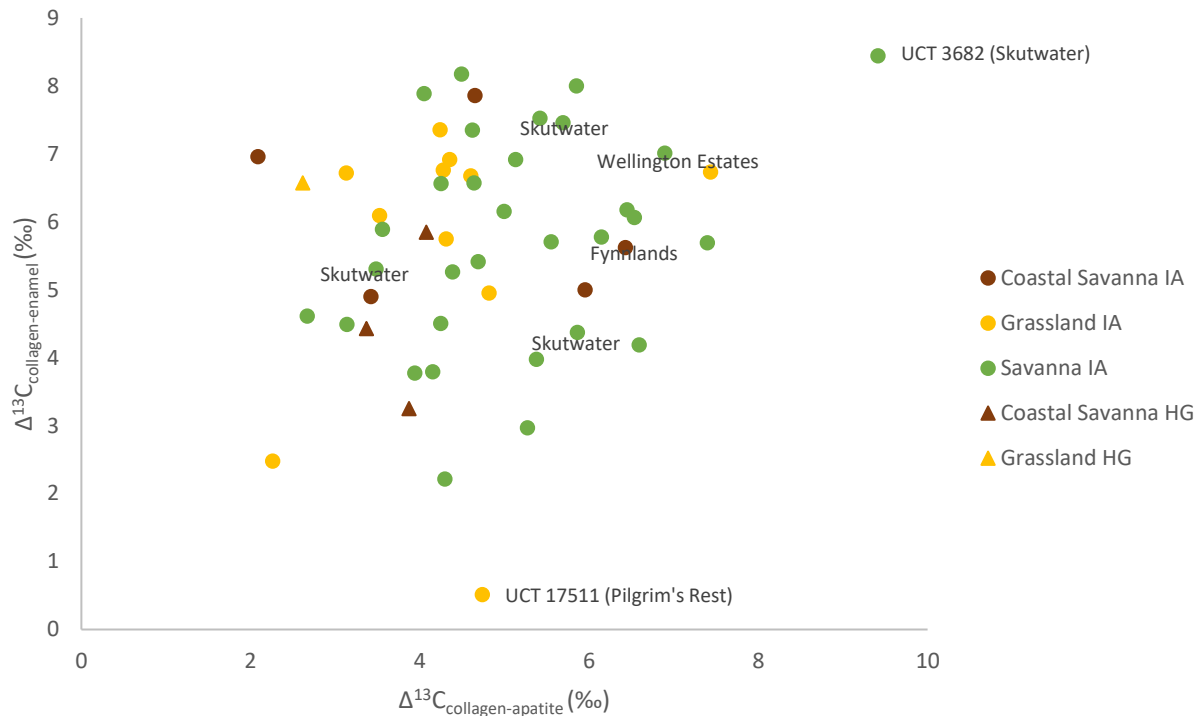


Figure 5-11 $\Delta^{13}\text{C}_{\text{collagen-apatite}}$ and $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ by geographical distribution

Based on the dominance of C_4 grasses in the grassland biome, it was expected that farmers there would fall on the most enriched end of the ^{13}C range for all tissues; however, this is not the case. Although they do fall towards the upper end of the range for enamel, bone apatite, and collagen, they are intermixed with savanna individuals in all three cases, and savanna farmers have higher $\delta^{13}\text{C}$ for bone apatite and collagen. Notably, a group of farmers from the grassland also fall at the most depleted end of the ^{13}C range for all three tissues, which would not be expected if they consumed significantly more C_4 resources. There are more savanna farmers ($N=31$) than grassland farmers ($N=11$) and the difference in sample size should also be taken into consideration when comparing the two groups; this and their overlap suggests that any differences in isotopic value between them are not significant enough to merit separation in subsequent analysis and discussion.

The preliminary results of this study indicate that there are distinct patterns of isotopic differences across tissues between hunter gatherers and Iron Age agriculturalists, but that these do not clearly distinguish between farmers of the Early and Late Iron Ages. As expected, agriculturalists trend towards more C_4 dominated diets as the Iron Age progresses.

There are no clear geographical patterns by biome; distinct data points draw attention to the presence of coastal marine resources or the effects of aridity on collagen isotope values, rather than large-scale geographical trends between the grassland and savanna. Throughout these data, sample size effects are pronounced, and groups have been combined (such as the EIA/LIA and grassland/savanna) for more robust, if more general, analysis and conclusions. There are a number of individuals that clearly illustrate the distinct dietary signals reflected in different bodily tissues, and the consumption of isotopically distinct dietary components. The implications of these distinctions for Iron Age diets, especially the role of maize, are explored through a number of multivariate reconstruction techniques in the following chapter. Chapter 6 also explores the variability in $\delta^{13}\text{C}_{\text{tooth enamel}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$, and the implications of the results of the pre-treatment experiments and this dataset for isotopic analysis of mineralized tissues worldwide.

6. Discussion and Conclusion

6.1. Isotopic analysis of mineral tissues: bone apatite and tooth enamel

6.1.1. The role and effect of pre-treatment

Previous studies of pre-treatment for apatite samples have established the effects of prolonged exposure to reagents on the measured isotopic value, primarily focusing on treatment time in acid (Koch et al., 1997; Lee-Thorp et al., 1997; Garvie-Lok et al., 2004; Smith, 2005; Webb et al., 2014). While some researchers have adopted pre-treatment methods for bone apatite more similar to those for enamel, this is not a common practice, and even in these cases the particle sizes of the bone powder was not accounted for (Smith, 2005; Pestle et al., 2014; Santana-Sagredo et al., 2015).

In the course of this experiment, bone apatite was ground in a SPEX mill and separated into five groups based on particle size. Even brief grinding in the SPEX mill (<1.5 minutes overall) was sufficient to produce bone powder in all five size groups. Since the

majority of authors simply state that bone was “mechanically cleaned and ground,” it is likely that previously studied bone powders contained a mixture of different sizes. In some cases, prolonged grinding times (such as 3 minutes per sample in Garvie-Lok et al. 2004) would have produced bone powders that were close to or as fine as size E (< 25 μm) in this study. This is significant as it was the smallest particles that displayed the most dramatically shifted isotopic values over the course of pre-treatment.

Although not statistically significant, treatment with NaClO had a larger impact on $\delta^{13}\text{C}_{\text{bone apatite}}$ of the size E bone powder group than acid time. NaClO is intended to remove organic material (including collagen) in the sample. $\delta^{13}\text{C}_{\text{collagen}}$ is depleted relative to $\delta^{13}\text{C}_{\text{bone apatite}}$, and if collagen or other organic material had not been completely removed by the NaClO, the $\delta^{13}\text{C}_{\text{bone apatite}}$ could be effected if any of the organic material produced CO_2 when reacted with phosphoric acid. While $\delta^{13}\text{C}_{\text{bone apatite}}$ for size E is much lower than the average for the rib ($-1.38 \pm 0.91\text{‰}$ compared to $-0.56 \pm 0.6\text{‰}$), there is little or no correlation between time in NaClO and $\delta^{13}\text{C}_{\text{bone apatite}}$ ($R^2=0.12$), with some depleted values observed with increasing time in NaClO, but no consistent pattern. This suggests that the NaClO is exchanging isotopes with the sample or reorganizing the mineral in some isotopically relevant way. This is consistent with the findings of Pestle et al. (2014), who also reported that variation in organic removal method caused more of the variation in $\delta^{13}\text{C}_{\text{bone apatite}}$ observed between laboratories than variation in the acid treatment step.

Further exploration of this phenomenon is not possible within this thesis, but would be valuable. Historically, the focus in pre-treatment has been on the effects of acid exposure time and not on the prior step to remove organic materials, which now seems to be of interest.

The possibility of isotopic alteration by the NaClO is also raised by the results from particle size A (>180 μm), which showed the second largest shifts in $\delta^{13}\text{C}_{\text{bone apatite}}$ value (>0.5‰) over the course of the treatments. Although neither time in acid nor NaClO was statistically significant, reaction time with sodium hypochlorite was, as for particle size group E, very close to significance (single factor ANOVA, $p=0.06$, $\alpha=0.05$). In the case of size group A, it is not likely to be recrystallisation in NaClO that causes this shift: with large particles, problems of incomplete homogenization and inconsistent exposure to the reagents are of more concern. Large particles can shield others, either during the reaction (although samples were shaken vigorously every 15 minutes to re-suspend the particulates) or during acid digestion on the gas bench.

Webb et al. (2014) found that pre-treatment (72 hours in NaClO, 4 hours in acetic acid) had no consistent effect on bone apatite $\delta^{13}\text{C}$ values (from -1.7‰ to +0.5‰), which may be explained if particle size is taken into account: bone powder was established to be “<180 μm ” but separated no further. Repeated measurements of this powder would have included grains of different sizes, including very fine ones that affect the analysis through susceptibility to pre-treatment recrystallisation. Treatment times were also significantly longer than those tested here and would have been likely to cause isotopic changes, although the lack of consistency emphasizes that the direction and type of effect remains to be understood. Care should be taken to sieve bone powder and choose a particle size that is both consistent and homogenous, and also robust enough to withstand pre-treatment. This is primarily of concern for studies that did not sieve bone powder, and may have included very fine particles in the analysis along with larger ones, resulting in a ‘composite’ isotopic value that is influenced by more altered fine particles. There is currently no consistent particle size used across laboratories, and so this effect may vary widely from study to study (Pestle et al., 2014).

These results also draw attention to the effects of sodium hypochlorite and the leaching process to remove organics on the final isotopic value of the sample. Where previous focus has been on the acid step, researchers have already begun shortening the originally long exposure times, and this study confirms that the discrepancy between 10 and 15 minutes is not enough to produce meaningful shifts (Sponheimer, 1999; Smith, 2005; Santana-Sagredo et al., 2015). The effects of NaClO exposure on more poorly preserved bone were considered in pre-treatment test 2. Encouragingly, the five poorly preserved human bone samples showed minimal change to $\delta^{13}\text{C}_{\text{bone apatite}}$ across three exposure times in NaClO.

6.1.2. Evaluating $\delta^{13}\text{C}_{\text{bone apatite}}$: diagenesis and metabolism

The use of bone apatite for isotopic analysis has been questioned for decades, dominated by concerns about diagenesis. These results illustrate that carefully controlling the conditions of pre-treatment, especially through sieving and use of a consistent particle size, can yield replicable isotopic measurements. Although mitigating some of the concerns over the effects of pre-treatment itself on bone apatite isotopic value, these data are not enough to fully illustrate that pre-treatment has successfully removed diagenetic material and restored the bone to its unaltered isotopic value. This can only be further investigated by comparing $\delta^{13}\text{C}_{\text{bone apatite}}$ to $\delta^{13}\text{C}_{\text{tooth enamel}}$ and $\delta^{13}\text{C}_{\text{collagen}}$.

Shin and Hedges (2012) proposed that $\delta^{13}\text{C}_{\text{tooth enamel}}$ can be used to confirm the validity of $\delta^{13}\text{C}_{\text{bone apatite}}$, as enamel is more resistant to diagenesis. They compared enamel and bone apatite from archaeological cattle, and reported a difference between the two tissues ranging from 0.62-2.44‰. The enamel-bone apatite spacing is thought to be between 0-2‰ based on animal studies (Cerling et al., 1997a; Passey et al., 2005), and in human studies of adults (France and Owsley, 2012; Loftus and Sealy, 2012; Santana-Sagredo et al., 2015) and juveniles (Webb et al., 2014) it ranges from $+0.6 \pm 1.5\text{‰}$ to $+4.3 \pm 1.2\text{‰}$. In this study, $\Delta^{13}\text{C}_{\text{enamel-bone apatite}}$ was $+1.74 \pm 1.19\text{‰}$. The growing body of work comparing the two mineral tissues, and the concordance between their results, suggests that there is a systematic range of offset between $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{tooth enamel}}$, and that comparison can mitigate concerns around diagenesis of bone apatite.

However, taking into account the reported standard deviations, the range of $\Delta^{13}\text{C}_{\text{enamel-bone apatite}}$ is large. Combining the full range of published offsets, $\Delta^{13}\text{C}_{\text{enamel-bone apatite}}$ could range between -0.9‰ to +5.5‰, and even disregarding the juveniles reported by Webb et al. (2014), could be as high as +4.1‰. The variability of the offset between enamel and bone is further emphasized by the variation in correlation coefficients reported in different studies: R^2 as low as 0.3 (Webb et al., 2014) and as high as 0.74 (France and Owsley, 2012) have been reported (Table 6-1).

These studies include humans from many regions (from North and South America to southern Africa) and time periods (ranging from 8000 BCE to the 19th century AD), so variation is expected. The two highest correlations, 0.74 and 0.62 (France and Owsley, 2012 and Santana-Sagredo et al., 2015, respectively) occur among the most recent samples. Both were selected for their good preservation, and the individuals analysed by Santana-Sagredo et al. (2015) were interred in an extremely arid region where very little diagenesis is expected. However, Webb et al. (2014) also report that their samples were well preserved, and each sample was analysed via FTIR prior to pre-treatment to further establish their quality.

In reference to the low (0.37) R^2 value of $\delta^{13}\text{C}_{\text{tooth enamel}}$ vs $\delta^{13}\text{C}_{\text{bone apatite}}$ in their study, Loftus and Sealy (2012) cite the lack of systematic evaluation of bone preservation at the time of bone apatite analysis (their $\delta^{13}\text{C}_{\text{bone apatite}}$ data is drawn from Lee-Thorp et al., 1989 and Sealy, 1997) and suggest that there may be a residual diagenetic signal. Compared to the results of Webb et al. (2014), however, and those of this study, it appears that $\delta^{13}\text{C}_{\text{tooth enamel}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$ are simply less well correlated than had previously been surmised. Although

it cannot be guaranteed that all diagenetic material was removed from the bone apatite in this study, the consistent results of the pre-treatment experiments and additional step of sieving strengthen the argument that the correlation (or lack thereof) between bone and tooth enamel is real.

The standard deviation of $\delta^{13}\text{C}_{\text{bone apatite}}$ in this study is 2.45‰, and that of $\delta^{13}\text{C}_{\text{tooth enamel}}$ is 2.50‰. Considering the range of locations and times from which the studied individuals derive, if diagenesis were affecting $\delta^{13}\text{C}_{\text{bone apatite}}$ to a greater extent than $\delta^{13}\text{C}_{\text{tooth enamel}}$, the bone apatite values would not be expected to group so tightly and in the same way. Combined with extensive efforts to remove diagenetic material, and eliminate the effects of pre-treatment, it seems clear that $\delta^{13}\text{C}_{\text{bone apatite}}$ is recording real isotopic evidence of diet.

The relatively consistent spacing but widely varying correlation between the two tissues, across geographical regions and levels of preservation, suggests that while there is a definite relationship between them, there are as yet unknown metabolic factors that affect the isotopic values of tooth enamel and bone apatite. Significant differences (Mann Whitney U-Test, $p < 0.0$) between $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ ($4.77 \pm 1.42\%$) and $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ ($5.67 \pm 1.66\%$) in this study emphasize the distinct isotopic signature of each tissue. Furthermore, these two offsets—which are both based on the same $\delta^{13}\text{C}_{\text{collagen}}$ —are not correlated at all ($R^2 = 0.05$).

Because both minerals form from bicarbonates and carbonates in the blood, the possibility of differential fractionation due to metabolic or formation factors has not been thoroughly explored. The fact that enamel apatite is hydroxylated while bone apatite is not does indicate that they undergo distinct formation processes (Pasteris et al., 2004). Webb et al. (2014) propose that additional fractionation occurs during tooth enamel formation: to counteract an acidic formation environment, ameloblasts release bicarbonate ions during tooth development. With greater diffusion and buffering capacity in the liquid formation environment, bone does not release ions during formation and thus may not undergo additional fractionation after it forms in equilibrium with blood bicarbonate. In two studies of pigs fed on controlled diets, Howland et al. (2005) reported $\Delta^{13}\text{C}_{\text{bone apatite-diet}}$ of 8.7 to 12.1‰, while Passey et al. (2005) reported $\Delta^{13}\text{C}_{\text{enamel-diet}}$ of 13.1‰, and attributed the difference to preferential digestion of isotopically distinct dietary components. This seems unlikely to have occurred, however (Warinner and Tuross, 2009), and differential formation processes could explain the bone-enamel offset.

This mechanism would be largely valid across taxa, which synthesize biomineral in comparable ways, and is consistent with enamel-bone apatite spacings largely in agreement with those of humans (e.g. 0-2‰ in various large animals (Cerling et al., 1997a; Passey et al., 2005), 2.3‰ in swine (Warinner and Tuross, 2009)). Comparisons across taxa may not be appropriate, however: Clementz et al. (2007) found both positive and negative $\Delta^{13}\text{C}_{\text{tooth-bone}}$ for various marine mammals. Shin and Hedges (2012) report an offset for archaeological cattle that is near the 0-2‰ range (0.66-2.44‰), but in the opposite direction of the human studies: bone apatite is enriched relative to tooth enamel in their study. There is clearly a case for increased investigation of the formation processes of biomineral in various taxa, and their isotopic ramifications.

The mean $\Delta^{13}\text{C}_{\text{enamel-bone apatite}}$ from Loftus and Sealy falls within the range of values reported in other studies but is the lowest. That dataset is the only one in Table 6-1 in which people's diets contained a significant proportion of protein enriched in ^{13}C (marine foods) and carbohydrates depleted in ^{13}C (terrestrial C_3 plants). This, and the distinctive $\Delta^{13}\text{C}_{\text{enamel-bone apatite}}$, suggest there may be an element of dietary routing occurring that contributes to the separation between $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{tooth enamel}}$.

Table 6-1 Enamel-bone apatite spacing in humans for all published studies. R^2 values indicate the degree of correlation between the two tissues.

Study	$\Delta^{13}\text{C}_{\text{enamel-bone apatite}}$	R^2 value ⁴⁶	Age of samples	Region
Loftus and Sealy, 2012	+0.6±1.5‰	0.37	From 8120 BCE to 1390 AD	Southern Africa (S and W Cape coasts)
France and Owsley, 2012	+1.7±2.5‰	0.74	18 th and 19 th century	North America (E coast) ⁴⁷
Santana-Sagredo et al., 2015	+1.8±2.3‰	0.62	900-1300 AD	South America (Chile)
Webb et al., 2014	+4.3±1.2‰ ⁴⁸	0.30	100 BCE to 1650 AD	South America (Belize)

⁴⁶ R^2 is reported as the correlation coefficient of a linear regression of $\delta^{13}\text{C}_{\text{bone apatite}}$ on $\delta^{13}\text{C}_{\text{tooth enamel}}$, using all published data points. Regressions were performed by the author.

⁴⁷ One group of individuals was excavated in New Mexico, but the authors note that this was merely their place of death and that they all hailed from the east coast.

⁴⁸ This is the only study to examine co-forming bone and enamel from human juveniles. As enamel forms early in life and records dietary signals from that time, while bone records more recent diet, it would be expected that adults would show a larger enamel-apatite spacing. Webb et al. explain the large enamel-bone offset as the result of ionic buffering in the maturation environment of tooth enamel during formation. However, this does not explain the fact that adults show a smaller, rather than larger, offset when compared to the juveniles.

This study	+1.74±1.19‰	0.49 ⁴⁹	600-1800 AD	Southern Africa (N interior, NE coast)
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This study, which comprises the largest (N=51) comparison to date between human $\delta^{13}\text{C}_{\text{tooth enamel}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$, adds to the growing body of evidence that human tooth enamel is enriched in ^{13}C compared to bone apatite from the same body. Although the direction of the offset in humans is consistent across the literature, the magnitude is variable—in many cases, overlapping enough to suggest a species-wide pattern, and in others not. While the effect of diagenesis cannot be entirely ruled out, the range of precautions taken here and by other authors means that the results should not be attributed to preservational issues alone. It seems that $\delta^{13}\text{C}_{\text{tooth enamel}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$ are not directly correlated in humans, and additional work to study the formation process and environment is needed to understand the pathways by which dietary isotopes are incorporated into biominerals.

6.1.3. Pre-treatment variation and a note on inter-study comparison

A final aspect to consider when comparing biomineral isotope values across studies is, of course, the pre-treatment protocol applied in each study. No other studies report the particle size of the bone apatite, and it seems likely that small particles introduced a degree of imprecision into the isotope values (which may contribute to relatively wide standard deviations overall). Bone apatite for individuals analysed in Loftus and Sealy (2012) was prepared using NaClO overnight, and 1.0M acid for several days (Lee-Thorp et al., 1989; Sealy, 1997), and those in France and Owsley (2012) were treated almost as vigorously (NaClO overnight and 1.0M acetic acid for 4 hours). This difference in pre-treatment and the likely effect on the measured isotopic value of the samples makes it impossible to definitively attribute the lower $\Delta^{13}\text{C}_{\text{enamel-bone apatite}}$ reported in Loftus and Sealy (2012) to dietary routing and the distinct diet of the studied population, but the unique diet also complicates straightforward interpretation of the effect of such long pre-treatment times. France and Owsley's $\Delta^{13}\text{C}_{\text{enamel-bone apatite}}$ falls in the middle of those reported in Table 6-1. It is interesting to note that their samples also have the highest correlation between enamel and bone apatite. It is not clear how or if re-crystallisation due to exposure to NaClO would have increased $\delta^{13}\text{C}_{\text{bone apatite}}$, causing it to approach $\delta^{13}\text{C}_{\text{tooth enamel}}$, but it is a possibility. Except for sieving, Santana-Sagredo et al. (2015) followed a nearly identical pre-treatment protocol to that followed in this study, and reported the next highest R^2 value.

⁴⁹ When the three coastal hunter gatherers are not included in the regression, R^2 drops to 0.24.

It is not yet clear the extent to which small particles, mixed in with larger ones and subject to the same pre-treatment protocol, can alter the overall isotopic results; nor is it clear what shift in ^{13}C would be expected due to recrystallization in NaClO. Pestle et al. report that 56% of the variation in $\delta^{13}\text{C}_{\text{bone apatite}}$ between 21 different laboratories, all analysing the same bone, was due to variation in pre-treatment methodologies. What is clear is that both are likely common occurrences in many studies, and care should be exercised when comparing and interpreting datasets that have been derived using different pre-treatment techniques.

6.2. Dietary Reconstruction of Southern African farmers

The isotope results presented in chapter 5 largely align with the expected dietary trends for farmers from the Iron Age of Southern Africa. More positive $\delta^{13}\text{C}_{\text{tooth enamel}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$ suggest that overall diet was based largely on C_4 foods, and $\delta^{13}\text{C}_{\text{collagen}}$ also indicates C_4 protein sources, either plants or animals that consumed them. The wide range of values indicates that the dietary mix varied widely across the region, and the lack of distinction between the EIA and LIA farmers illustrates the heterogeneity of Iron Age diets (Lee-Thorp et al., 1993).

6.2.1. Reconstruction by offset: fractionation factors

Chapter 2 discussed the wide range of fractionation factors that have been used to reconstruct the isotopic value of diet from that of body tissues, and the need to use factors that are as context, diet, and taxon-specific as possible. No previous studies of humans in the summer rainfall zone of South Africa has established such factors, and no study of human populations elsewhere has not relied on previously published values from other datasets. Ribot et al. (2010) relied on qualitative assessment of diets, loosely based on theoretical end-points, to reconstruct the diet of coastal Iron Age farmers, as did Lee-Thorp et al. (1993) when analyzing the collagen of many of the farmers included in this study. Using the full range of postulated human fractionation factors should produce a range that brackets the ‘true’ values of the original diet; compounded with the wide range of factors for each tissue, it is apparent that a great many dietary interpretations are possible. Table 6-2 illustrates the wide range of values that result when such factors are applied to the mean, maximum and minimum values of the study dataset for each tissue.

Table 6-2 Possible $\delta^{13}\text{C}_{\text{whole diet}}$ values based on fractionation factors for all three tissues. Ranges were calculated by adding the standard deviation to the mean and subtracting the smallest fractionation factor (yielding the upper bound); and subtracting the standard deviation from the mean and subtracting the largest fractionation factor (yielding the lower bound). As this study includes a variety of rainfall regimes and some very arid areas, nitrogen fractionation factors should be even

more specific to the environment at the time of the individual's life. Such factors are even more limited and so the dietary information yielded by such reconstruction requires significant additional evidence to provide much insight into diet.

	Factor	Iron Age Mean value	$\delta^{13}\text{C}_{\text{whole diet}}$	Iron Age min value	$\delta^{13}\text{C}_{\text{whole diet}}$ min	Iron Age max value	$\delta^{13}\text{C}_{\text{whole diet}}$ max
$\Delta^{13}\text{C}_{\text{collagen-whole diet}}^{50}$	+3.7 to 6‰	$-8.6 \pm 2.2\text{‰}$ ($\delta^{13}\text{C}_{\text{collagen}}$)	-16.8 to - 10.1‰	-14.1‰	-20.1‰	-6‰	-9.7‰
$\Delta^{13}\text{C}_{\text{bone apatite-whole diet}}^{51}$	+9.4 to +12‰	$-3.8 \pm 2.5\text{‰}$ ($\delta^{13}\text{C}_{\text{bone apatite}}$)	-18.3 to - 10.7‰	-10.2‰	-22.2‰	0.5‰	-8.9‰
$\Delta^{13}\text{C}_{\text{enamel-whole diet}}^{52}$	+10‰	$-2.9 \pm 2.5\text{‰}$ ($\delta^{13}\text{C}_{\text{tooth enamel}}$)	-15.4 to - 10.4‰	-10.8‰	-20.8‰	0.7‰	-9.3‰

The reconstructed $\delta^{13}\text{C}_{\text{whole diet}}$ based on $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$ are remarkably close, although the reconstruction based on bone apatite encompasses a wider range of values. $\delta^{13}\text{C}_{\text{whole diet}}$ reconstructed using $\delta^{13}\text{C}_{\text{tooth enamel}}$ based on non-ruminant animals (Schoeninger, 2014) also overlaps with the other two tissues and is close to the $\delta^{13}\text{C}_{\text{bone apatite}}$ reconstruction when considered from minimum to maximum value. $\delta^{13}\text{C}_{\text{whole diet}}$ reconstructed from $\delta^{13}\text{C}_{\text{collagen}}$ alone could range from -20.1 to -9.7‰⁵³, which spans the C₃-C₄ range of plant values. This interpretation would include a wide range of C₃ and at least 'mixed' protein sources. The interpretation based on $\delta^{13}\text{C}_{\text{bone apatite}}$ alone would be similar, but even wider: from -22.2‰ to -8.9‰, providing even less specific information (and virtually no help in distinguishing between the protein and energy sources of the diet).

Reconstructed $\delta^{13}\text{C}_{\text{whole diet}}$ based on tooth enamel is comparable to that of a diet based on $\delta^{13}\text{C}_{\text{bone apatite}}$. A reconstruction using the generally accepted fractionation factor of +14‰, based on ungulates and ruminants (Passey et al., 2005), would be substantially more depleted than the other two, suggesting a hypothetical diet that is even further from reality: it would range from -24.8‰ to -13.1‰, squarely within the C₃ range of plants. This discrepancy

⁵⁰ Based on ranges given in: Bocherens and Drucker, 2003; Froehle et al., 2010.

⁵¹ Based on ranges given in: Ambrose and Norr, 1993; Jim et al., 2004; Kellner and Schoeninger, 2007; Warinner and Tuross, 2009.

⁵² $\Delta^{13}\text{C}_{\text{enamel-whole diet}}$ has been reported for a wide range of ruminants and ungulates to be +14‰ (Kohn and Cerling, 2002; Hedges, 2003; Passey et al., 2005). Few primates produce methane to assist in digestion the way that ruminants do (humans do not), and studies of non-methane producing animals have suggested that the enamel-diet offset should be closer to 10‰ (Kellner and Schoeninger, 2007; Warinner and Tuross, 2009; Schoeninger, 2014; Sponheimer and Cerling, 2014).

⁵³ Based on the minimum and maximum values from the dataset; based on the average it would still fall largely in the C₃ range, from -16.8 to -10.1‰. The same is true for bone apatite (-18.3 to -10.7‰) and tooth enamel (-15.4 to -10.4‰).

demonstrates that the diet to enamel fractionation factor is smaller for primates (including humans), and again emphasizes the importance using of taxa and even species specific factors (Schoeninger, 2014; Sponheimer and Cerling, 2014).

This exercise in using a wide range of reported fractionation factors reveals the importance of applying factors that are as relevant (taxonomically, geographically, and metabolically) and contextually based as possible. In this case, reconstructions based on factors contained such large uncertainties that the results were not useful, and in some instances were simply wrong. Simple comparisons between raw isotopic values (such as Figures 5-3 and 5-4) were for the large part more informative than these reconstructions, and studies that base dietary interpretations solely off individual fractionation factors, which may not be wholly applicable, should be approached with caution.

6.2.2. The Kellner and Schoeninger simple carbon isotope model

When plotted onto the bivariate carbon isotope model (based on $\delta^{13}\text{C}_{\text{bone apatite}}$ vs $\delta^{13}\text{C}_{\text{collagen}}$) proposed by Kellner and Schoeninger (2007) (see section 2.3.2, Figure 2-1), farmers and hunter gatherers fall between 50-100% C_4 energy (from midway to the most enriched ends of the lines on the Y, or $\delta^{13}\text{C}_{\text{bone apatite}}$, axis) (Figure 6-1). This is consistent with a mix of wild (C_3) and cultivated and wild (C_4) plant foods, and for which there is archaeological evidence of utilization, including legumes and cucurbits (C_3) and sorghum and various types of millet (C_4).

No individuals in this study, farmer or hunter gatherer, fall on the C_3 protein line. The vast majority fall solidly on the C_4 protein line and a few (including the coastal hunter gatherers) overlap with the marine protein line. There are virtually none that fall in the 'mixed protein' middle space between the lines, where Kellner and Schoeninger predicted individuals consuming protein of both types would fall. Although C_4 protein consumption is expected for the Iron Age, the near total absence of any C_3 protein based on this reconstruction is notable.

Even with the spread of domesticated animals, especially cattle (which strongly prefer to graze rather than browse), wild animals would have remained an important fallback food and were consumed throughout the Iron Age (Maggs, 1976; Plug and Voigt, 1985; Van Ewyk, 1987). While some wild fauna such as alcelaphines and hippotragines consume almost exclusively C_4 grass, there are many others that are mixed feeders or browsers and would be expected to record some C_3 signal (e.g. cephalophines and tragelaphines) (Sponheimer et al.,

2003). Small stock, especially goats, were also regularly consumed throughout the Iron Age in the savanna, grassland, and the coast (Mason, 1981; Maggs and Ward, 1984; Voigt, 1984; Voigt and Plug, 1984; Maggs and Whitelaw, 1991), and would also have contributed a more mixed C₃/C₄ signal to the diet of those who consumed them. Goats are mixed feeders, and especially if competing with grazing cattle for grass, will consume browse (Gordon and Prins, 2007). Isotopically, then, they should be more depleted in ¹³C than cattle and other dedicated grazers. Due to their osteological similarity, goats and sheep are often grouped together as ‘ovicaprines’ in faunal analyses, and so the evidence for goat consumption specifically is not definitive. However, it does seem likely that goats were consumed and would be expected to contribute to human isotopic values. African sheep are also more flexible feeders than cattle, and would have contributed a more depleted ¹³C signal to individuals who consumed them as well.

The apparently highly C₄-based protein component of the diets of farmers could also be due in part to plant protein. Sorghum and millet, the staple grain crops of southern African farmers, contain relatively high levels of protein (12% and 16% respectively, compared with 8-10% in maize), and contain all essential amino acids (in both cases, lysine is the limiting amino acid but still present) (Board on Science and Technology for International Development, 1996; Rooney et al., 2004). Consumption of large quantities of such crops would make a solidly C₄ contribution to $\delta^{13}\text{C}_{\text{collagen}}$, alongside that of the animals consumed. If consumed in sufficient amounts, sorghum and millet contain enough protein to provide amino acids for incorporation into mineral tissues as well as synthesis into collagen. When combined with animal protein, diets would contain well above the 12% protein content threshold documented by Jim et al. (2006) to cause $\delta^{13}\text{C}_{\text{collagen}}$ to shift towards $\delta^{13}\text{C}_{\text{biomineral}}$ (reflecting whole diet). The isotopic spacing between these crops and a mixed C₃/C₄ animal protein would be large enough to shift the overall value of $\delta^{13}\text{C}_{\text{collagen}}$ in the direction of C₄ foods, recalling the 50% shift that was caused by the addition of just 5% isotopically different protein in mice (Ambrose and Norr, 1993). These results are consistent with the findings of Froehle et al. (2010), that $\delta^{13}\text{C}_{\text{collagen}}$ is affected by both $\delta^{13}\text{C}_{\text{protein}}$ and $\delta^{13}\text{C}_{\text{whole diet}}$, and the closer those two values are, the more closely $\delta^{13}\text{C}_{\text{collagen}}$ tracks $\delta^{13}\text{C}_{\text{whole diet}}$ (and thus, $\delta^{13}\text{C}_{\text{bone apatite}}$). For monoisotopic diets, $\Delta^{13}\text{C}_{\text{collagen-diet}}$ was around +4‰, while mixed diets ranged from -1‰ to +10‰ depending on how far apart $\delta^{13}\text{C}_{\text{protein}}$ and $\delta^{13}\text{C}_{\text{whole diet}}$ were (Froehle et al., 2010).

The LIA farmers, who were the first to move into the grassland, are the most solidly on the C₄ protein line. This could be due to increased reliance on C₄-grazing cattle and wild fauna in the C₄-dominated grassland, which would be expected to result in more positive $\delta^{13}\text{C}_{\text{collagen}}$. Paired with heavier reliance on C₄ crops, this explains the thick clustering around the upper end of the C₄ line, suggesting that Iron Age farmers' diets were more monoisotopic than implied by archaeological evidence.

It is interesting that they are not more distinguished from farmers in the EIA, who would have encountered more C₃ resources living in the savanna. However, many of the C₃ plant foods that would have been consumed (cucurbits, leafy vegetables, etc) are not very high in protein and would not be expected to influence $\delta^{13}\text{C}_{\text{collagen}}$, especially when C₄ foods are contributing so much protein to the diet. The fact that these C₃ foods do not reflect in $\delta^{13}\text{C}_{\text{bone apatite}}$ would be due to the influence of protein too, as there would be more than enough protein in the diet to contribute isotopes to biomineral.

A handful of individuals do fall either on the marine protein line or just past it, but only two of them are from areas near marine resources (the individual from Fynnlands, indicated in Figure 6-1, and one from Nanda, directly on the marine protein line). The others, from Skutwater (indicated), Rooikrans (on the line) and Wellington Estates (UCT 16565, the individual with unusually high $\delta^{15}\text{N}_{\text{collagen}}$), have the most mixed protein diets of the dataset, although they are still not in the 'mixed' zone proper. Van Ewyk (1987) has suggested that Skutwater may have been a satellite town for K2, and that the residents would have hunted more wild game to compensate for the lack of cattle, paid as tribute to high status individuals at K2. Lee-Thorp et al. (1993) notes a there was a high proportion of wild game found at the site. At Rooikrans, the faunal assemblage included a large number of wild bovids, indicating that hunting was a more important source of animal protein than herding (Plug, 1984). Large numbers of klipspringer (*Oreotragus oreotragus*) and southern reedbeek (*Redunca arundinum*) were present; klipspringer are browsers and thus C₃ consumers, while reedbeek are grazers and are more enriched in ¹³C (Sponheimer et al., 2003; Codron et al., 2005a). While the location of the two Rooikrans data points on the Kellner and Schoeninger lines is slightly to the left of the C₄ protein line, they do not fall where expected for a diet in which protein from a C₃ browsing species was very important. The coastal hunter gatherers cluster on the marine protein line as expected.

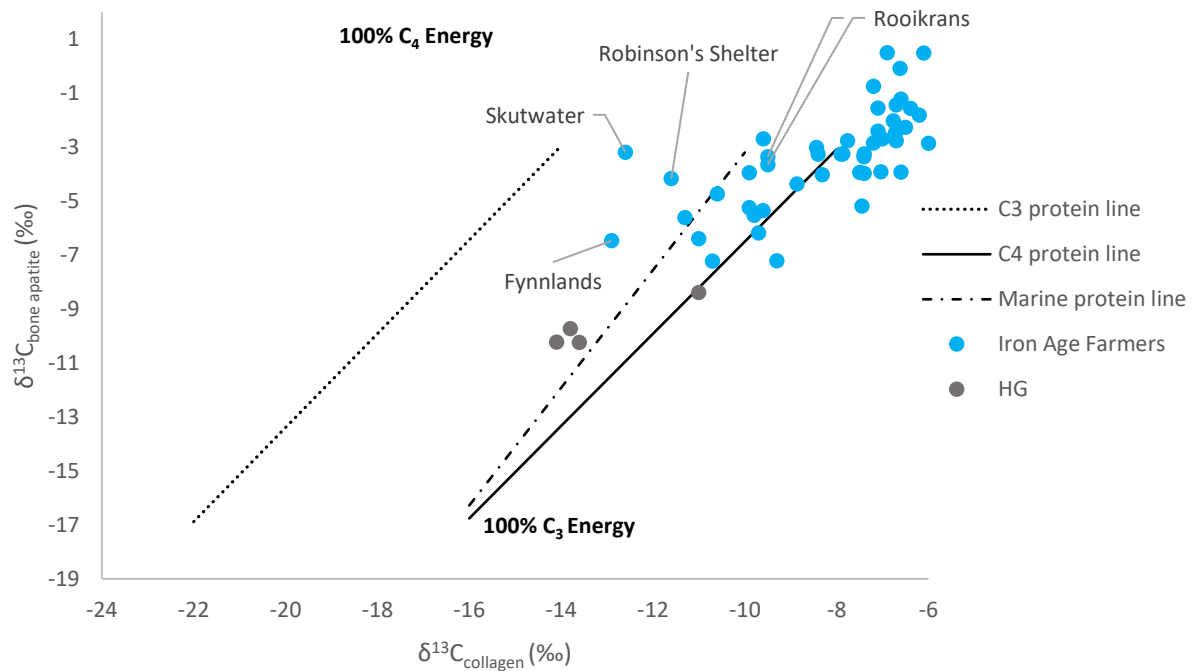


Figure 6-1 Bivariate carbon plot after Kellner and Schoeninger 2007, grouping Iron Age farmers and hunter gatherers along C₃ and C₄ protein lines. Farmers fall between 50-100% C₄ energy as predicted, but farmers and hunter gatherers are more tightly clustered around the C₄ protein line than expected.

6.2.3. The Froehle et al. multivariate carbon and nitrogen isotope model

The multivariate model proposed by Froehle et al. (2012) incorporates $\delta^{15}\text{N}_{\text{collagen}}$ as well as $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$. It is intended to build on the bivariate model described above to provide additional information about protein consumption, especially discriminating between marine and C₄ protein sources, and to provide greater differentiation between isotopically mixed but distinct energy sources. The two discriminant functions—which were derived from cluster analysis based on all the archaeological human data included in Kellner and Schoeninger (2007)—each include all three isotopic values. Function one is primarily driven by $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{collagen}}$, and so is designated the ‘carbon’ function, and function two is designated the ‘nitrogen’ function as it is primarily affected by $\delta^{15}\text{N}_{\text{collagen}}$. The fact that each function comprises all three values highlights the complex relationships between dietary sources, especially when attempting to distinguish between protein sources that affect $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{15}\text{N}_{\text{collagen}}$.

When converted to DFA scores and plotted, the Iron Age farmers fall closest to dietary cluster 2, but extend much further along the X-axis (the ‘carbon’ function) than the data of Froehle et al. (2012) (Figure 6-2). This is expected, as cluster 2 describes a diet that is 70% C₄ energy and >50% C₄ protein. In the Iron Age, F1 values exceed the values of the

original model (7.63 compared to 5.29), with more enriched carbon isotopes and suggest a diet even more heavily C₄ than cluster 2 (the most heavily C₄ diet in the original model). Interpreting the functions is not straightforward, however: cluster 3 (50% C₄ energy and marine protein) actually has a higher F1 score than cluster 2, and also fully overlaps the Iron Age data in terms of F1 scores. The ‘carbon’ function cannot fully distinguish between the two protein sources, and a difference of 20% C₄ energy may not be readily apparent on this axis.

The ‘nitrogen’ function provides further differentiation, as cluster 3 falls much higher up the Y-axis than cluster 2 and no Iron Age individuals are near it—suggesting that it is primarily carbon, rather than nitrogen driving the resemblance to cluster 3. As in Figure 6-1, it appears that Iron Age farmers consumed more isotopically mixed energy sources than traditional reconstructions such as Figure 5-4 and 5-3 suggest, although the function obfuscates a clear interpretation of what increasing or decreasing scores mean in terms of real diet (since less C₄ intake is indicated for values both more positive and more negative than the centroid of cluster 2).

The individuals with the highest F2 scores also have the highest $\delta^{15}\text{N}_{\text{collagen}}$, from Skutwater. On the ‘nitrogen’ axis they fall alongside the coastal hunter gatherers, midway between >50% C₄ protein and marine protein. In terms of the ‘carbon’ axis, the coastal hunter gatherers are nearest to cluster 5 (30% C₄ energy, >65% C₃ protein). This placement is consistent with Figures 6-1 and 5-3, and a diet based on wild plant foods and a mixture of wild and marine animal resources.

If dietary plant protein made a substantial contribution to the high C₄ protein effect seen in Figure 6-1, it would contribute to the high F1 scores of the Iron Age farmers. Function one is driven in part by $\delta^{13}\text{C}_{\text{collagen}}$, which would have been enriched by the high protein content of C₄ crops, even as $\delta^{15}\text{N}_{\text{collagen}}$ stayed relatively low. Clusters 2 and 3 were based on similar $\delta^{13}\text{C}_{\text{collagen}}$ (-11.3‰ and -10.5‰ respectively), compared to the study average of -8.65 ± 2.2 ‰; even if the amount of protein in the diets are similar (and the amount of marine protein is not specified in Froehle et al.), the protein consumed by Iron Age farmers was more enriched in ¹³C than that consumed by the other study populations. The apatite content of function one would also reflect the carbohydrates of such crops. This example illustrates how the use of multivariate models like this one should be combined with more traditional approaches to the interpretation of isotopic data. These models provide useful

comparisons between populations and elicit patterns by expanding the overall dataset, but the absolute values are still needed for dietary information in the context of a specific study population.

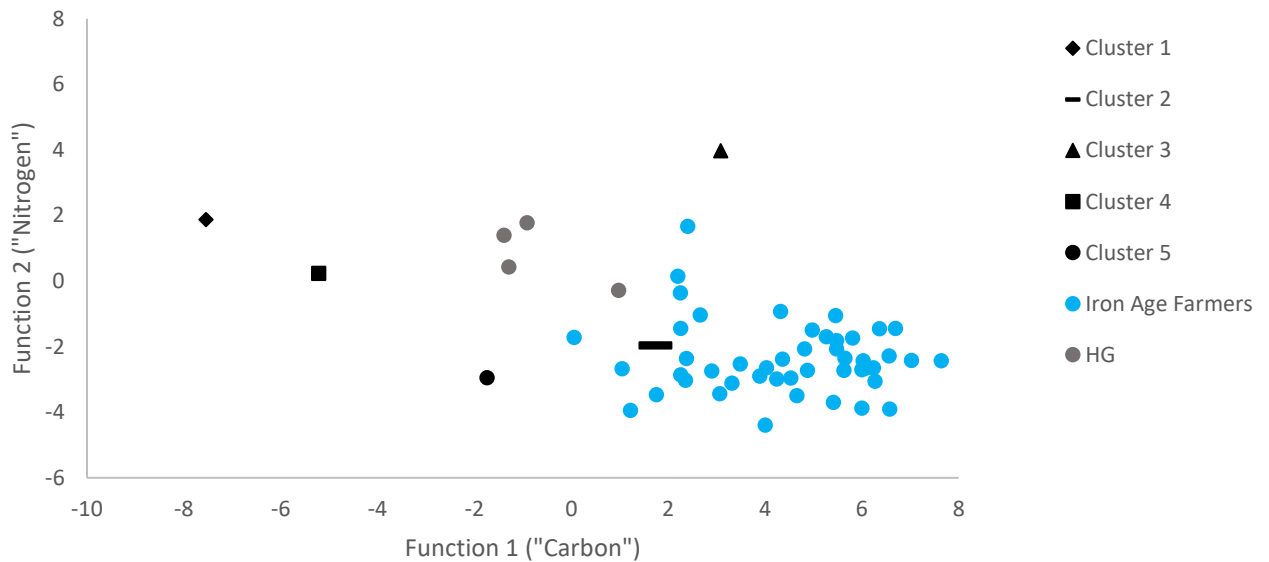


Figure 6-2 Plot of discriminant function analysis scores after Froehle et al. (2012). The black symbols indicate the centroids of the five dietary clusters identified by Froehle et al.; they are described by energy source ($C_3:C_4$) and percent protein. Cluster 1: 100:0, 100% C_3 protein; Cluster 2: 30:70, >50% C_4 protein; Cluster 3: 50:50, marine protein; Cluster 4: 70:30, >65% C_3 protein; Cluster 5: 30:70, 65% C_3 protein. Clusters 2 and 5 have the highest proportion of C_4 energy, and so are most similar to the diet of Iron Age farmers (plotted in blue).

6.2.4. Expanding the multivariate model

Adding the Iron Age farmers to the global dataset used by Froehle et al. (2012) to create the multivariate model dramatically reduces the parsimony of the dietary clusters they identified. The methodology for determining cluster membership was identical to that of Froehle et al. (2012): two methods of hierarchical clustering (the unweighted pair-group method using the average (UPGMA) and Ward's method) were compared to K-means cluster analysis for each given number of clusters (1-6). Froehle et al were contacted to compare the computational syntax used in each case to ensure as close a match as possible in cluster methodologies.

An important note to begin with: the clusters produced during cluster analysis are assigned 'names' (usually numbers) in the order they are derived by the algorithm. Thus, cluster 1 using one method is not the same as cluster 1 using a different method. When

comparing individual membership across methods, then, it is necessary to determine which clusters correspond to which other ones. Clusters are defined by their centroids, which are simply the mean value for each variable (in this case, $\delta^{13}\text{C}_{\text{bone apatite}}$, $\delta^{13}\text{C}_{\text{collagen}}$, $\delta^{15}\text{N}_{\text{collagen}}$) of the individuals in the cluster. As membership changes from method to method, so too does the centroid. Clusters are assumed to be representing the same space (in this case, the same diet) if their centroids are highly comparable. Then the membership of individuals can be compared. For the purposes of this study, a difference of <3‰ was considered comparable, as there is no clear dietary difference at that scale.

While Froehle et al. (2012) reported very high agreement between the methods for 4 and 5 clusters (between 96-97% agreement in each case), this was not the case when the Iron Age data was included. When sorted into 5 clusters, the UPGMA and Ward’s methods classified 66% of cases into the same cluster, and K-means clustering classified 75% of cases and 82% of cases in the same cluster, respectively. When sorted into 6 clusters (a scenario rejected by Froehle et al. 2012 due to lower agreement), the methods produce mixed results: 89% of cases are classified in the same cluster by the UPGMA and K-means methods, and 58% using Ward’s method compared to K-means. The two hierarchical methods overlap in 58% of cases (Table 6-3). When 6 clusters were specified, UPGMA and K-means produced six comparable clusters.

Table 6-3 Cluster analysis for identifying dietary groups, based on Froehle et al. (2012). The number of cases for which both methods agreed is given along with the percentage. N=209 individuals.

5 clusters	K-means	UPGMA
k-means		
UPGMA	75% (157)	
Ward’s	82% (172)	66% (139)

6 clusters	K-means	UPGMA
k-means		
UPGMA	89% (188)	
Ward’s	58% (121)	58% (121)

The reduction in the consistency between clustering methods is driven by the heterogeneity of the Iron Age data. Their overlap with a number of defined clusters is evident in Figure 6-2, and the result suggests that a new model based on this data, along with the North and South American data, would not identify such clear dietary groupings. Some of the difference between these results and those of Froehle et al. (2012) are expected due to

computational changes such as slight changes to the software over the last four years. Cluster analysis is also not inference-based, and the clusters can be interpreted in slightly different ways. To test for the relative magnitude of such discrepancies, the previously published data were re-analysed to recreate Froehle et al.'s result; when this was done, one individual was placed in a different cluster. This suggests that any discrepancy due to computational differences is very low.

The magnitude of this result is surprising, as visually the Iron Age data appears to simply form the sixth dietary cluster that is possible to interpret (C_4 energy and C_4 protein sources), and suggests that reconstructed 'diets' result in more heterogeneous isotope values than previously thought. The relatively high parsimony between the UPGMA and K-means methods when six clusters are specified does at least suggest an additional ' C_4/C_4 ' diet. The discrepancy between this result and that of Ward's method compared with K-means (and between the two hierarchical methods) suggests that Iron Age farmer diets included a wide range of foods, which result in isotopic compositions that fall generally into the category of 'enriched' or ' C_4 ' but are in fact quite different from each other. This is especially true for $\delta^{15}N_{\text{collagen}}$, which is included in this model and which varies widely in the Iron Age data. It is not possible to disentangle the effect of aridity in some cases compared to other factors that could influence $\delta^{15}N_{\text{collagen}}$ with the data available, and Froehle et al.'s model does not interpret the dietary implications of $\delta^{15}N_{\text{collagen}}$ at all, although it is included in the clusters.

6.2.5. Evaluating models: what goes in must come out

Dietary reconstruction from stable isotopes is an important tool for archaeologists and others interested in understanding the behavior and ecology of past humans. Modelling techniques present new and promising approaches that can bring greater nuance to existing datasets, as well as tease apart confounding associations such as the marine/ C_4 protein similarity. They also eliminate the need for fractionation factors, which are taxon-, tissue-, and metabolism-specific and so pose significant challenges when working with archaeological data. Beyond the additional wealth of information that multi-tissue isotopic data presents, collecting such data to expand and utilize such models should become standard practice for isotope studies moving forward.

An important caveat when using any model is that the outputs and valid interpretations depend on the inputs originally used to create the model. The Kellner and Schoeninger lines are derived from the published literature on animal feeding experiments, and are based on results in rats, mice, and swine. As discussed (section 2.4), extrapolating

animal results and physiology to humans should be done judiciously; animal studies also tend to be based on small sample sizes (the entire dataset on which the Kellner and Schoeninger model is based is N=21). Although fed a variety of diets, these animals do not capture the full range of variety in protein and energy source and proportion found in human diets, particularly since the metabolisms of rats and mice are not good analogues for those of humans. Although based on humans, the Froehle et al. model is derived from populations in North America (Ontario, Greater Cahokia, San Nicolas Island) and South America (Tierra del Fuego), and the authors acknowledge that they lack a dietary cluster that is overwhelmingly C₄⁵⁴. As in the case of the bivariate model, individuals eating diets outside the original input diets fall ‘off the graph’ and suggest that such models offer fewer insights for such populations.

These factors do not undermine the value of the model, but valid results are limited to those cases which were covered in the data used to build it. Only two of the 21 diets included in the base data had C₄ protein sources *and* C₄ energy sources, and both were monoisotopic diets⁵⁵ that would not accurately reflect the predominantly C₄ but mixed diets of southern African farmers. None of the archaeological human populations with which Kellner and Schoeninger test their model have diets similar to those studied here: five ate C₃ protein sources only, two C₃ with some freshwater fish, and three depended on marine animal protein. Those populations were selected specifically *because* there was strong archaeological evidence for a monoisotopic protein source, and the authors explicitly acknowledge that no population had been published with both collagen and apatite data that consumed C₄ protein. The excellent fit between those data and the model derived from the animal experiments illustrates the importance of using models to interpret data within the range of the model’s inputs.

This is also the case for the clusters in Froehle et al. (2012), where the inclusion of a much more diverse dataset (the Iron Age farmers) resulted in a significant reduction in the parsimony of the clusters. If the Iron Age farmers had eaten as homogenous a diet as the other populations included in the model data, they likely would have simply formed a sixth dietary cluster. Instead, it appears that this model is less robust for data from more heterogeneous populations. Each cluster identified by Froehle et al. (2012) has a smaller

⁵⁴ For another example of a model based on both experimental animal and human isotope values, see Pestle et al., 2015.

⁵⁵ Diet d4h from Jim et al., 2004 and diet 8 from Tieszen and Fagre, 1993

range of values for $\delta^{13}\text{C}_{\text{bone apatite}}$ than that observed in the Iron Age data (ranging from 2.5-7.7‰, compared to 10.7‰ among the Iron Age dataset). Although the $\delta^{15}\text{N}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{collagen}}$ ranges overlap with those of the Iron Age farmers, the average range within a cluster is substantially smaller: 6.3 and 6.8‰, compared to 8.4 and 8.1‰. This greater variance explains at least part of the failure of the Iron Age agriculturalists to form a sixth cluster.

When compared to the populations that formed the basis of the Froehle et al. (2012) model, the Iron Age farmers have highly variable $\delta^{15}\text{N}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$ in particular. Although comparable to two populations (Southern Ontario and American Bottom), the range of $\delta^{13}\text{C}_{\text{bone apatite}}$ is much higher in the Iron Age data than the other four populations, and $\delta^{15}\text{N}_{\text{collagen}}$ is more variable than all but the Tierra del Fuego population (Table 6-4). This suggests that these two variables may be driving the lack of cohesive clustering. These two isotope-tissue systems are less well understood than that of $\delta^{13}\text{C}_{\text{collagen}}$, and models such as this one may be strongly affected by their complexity. Given the wide range of effects from environment, metabolism, and other factors that affect $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{15}\text{N}_{\text{collagen}}$, it is not surprising that a model built on a limited amount of geographically localized data would not be robust to a diverse dataset from a very different environmental system.

Table 6-4 Ranges (in ‰) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bone collagen, and $\delta^{13}\text{C}$ in bone apatite, from Froehle et al. (2012) and this study. Range = maximum isotope value - minimum value; all data from Froehle et al. (2012) except southern African farmers.

	American Bottom	Cahokia Mound	Illinois River Valley	San Nicholas Island	Southern Ontario	Tierra del Fuego	Southern African farmers
Range, $\delta^{13}\text{C}_{\text{collagen}}$ (‰)	16.2	4.5	10	8.3	12.1	12	8.1
Range, $\delta^{13}\text{C}_{\text{bone apatite}}$ (‰)	11.7	8.4	4.9	5.9	11.5	8	10.7
Range, $\delta^{15}\text{N}_{\text{collagen}}$ (‰)	7.8	4	2.3	5.9	5.2	8.2	8.4

Southern African farmers, with diets outside the range of the model, and more enriched isotope values than any individuals in the base populations, could be assigned artificially monoisotopic protein sources in the linear reconstruction. The lack of very enriched isotope values in the original dataset shifts the model lines to the left, causing the Iron Age farmers to seem more C_4 dependent than was likely the case based on archaeological evidence. Even accounting for the low protein content of C_3 resources, it is

surprising that the data fall largely off the model lines entirely. The fact that most of the data fall above the C₄ and marine protein lines, and $\delta^{13}\text{C}_{\text{bone apatite}}$ in the southern African farmers is much more positive compared with the populations in Kellner and Schoeninger (2007) and Froehle et al. (2012), is a further illustration of how this model is most useful when applied to similar datasets. This effect is also apparent in the multivariate model, where most of the Iron Age data lie far to the right, suggesting a ‘sixth cluster,’ or a diet that was not included in the original model. Interpreting this ‘sixth cluster’ would be especially challenging without external confirmation of dietary elements, since the original five were defined in ‘real diet’ terms by comparing them to bivariate lines of the same animal studies on which Kellner and Schoeninger’s model is based (Froehle et al., 2010).

Interpreting data that lie outside the range of a model should be avoided when possible, and the usefulness of models is limited in new contexts. However, this is not surprising given the enormity of natural variation amongst human diets and between humans themselves; perhaps instead it is encouraging how successful these models can be in many cases.

6.3. Isotopic evidence for dietary change

6.3.1. The Iron Age: Three possible diets for a heterogeneous people

The overarching pattern in the isotopic data indicates that agriculturalist diet varied widely across southern Africa, but became increasingly enriched in ¹³C as the Iron Age proceeded. This enrichment is observable in $\delta^{13}\text{C}$ values of all three tissues, but does not follow the simple upward trajectory expected with the cultivation of cereal crops sorghum and millet, and then maize. In Figure 6-3A, $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{collagen}}$ follow very similar trajectories through time: slight depletion in the later EIA and early LIA, followed by enrichment in ¹³C from the 12th to 16th centuries AD, and then depletion again until the end of the dataset in the 19th century, ending near 6th century $\delta^{13}\text{C}$ values⁵⁶. Reflecting the relative scarcity of EIA skeletal remains, there are no data between the 6th and 12th centuries AD, which certainly affects the shape of the graph, specifically whether there really is a depletion in ¹³C during that interval. $\delta^{13}\text{C}_{\text{bone apatite}}$ is not significantly different in the 6th and 12th centuries (Mann Whitney U-test, $p=0.33$), although $\delta^{13}\text{C}_{\text{collagen}}$ is (Mann Whitney U-test, $p<0$). This reflects the strong influence of C₄ plant foods throughout the Iron Age, but

⁵⁶ Generalized centuries are not the most precise time intervals; however, limiting the data to only individuals with direct radiocarbon dates would have reduced the sample size substantially.

indicates more C₃ protein in the EIA, possibly in the form of wild animals (in the savanna wild animals are more predominantly C₃).

There is considerable scatter around both sides of this curve, and the described trajectory is a visual guide, rather than a statistically significant trend. Considered separately, $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{collagen}}$ are not correlated with time: R² is 0.1 for $\delta^{13}\text{C}_{\text{bone apatite}}$ and 0.08 for $\delta^{13}\text{C}_{\text{collagen}}$. This is in contrast to Gilbert (1995), who plotted $\delta^{13}\text{C}_{\text{collagen}}$ against time and reported an R² value of 0.72. However, Gilbert's data began in the 9th century AD, and without comparable data from earlier in the EIA, the trend could appear much more significant.

Such a low correlation suggests that diet was and remained extremely isotopically variable throughout the Iron Age, and that changes to the diet, such as the introduction of maize, which probably had a substantial impact on the economy and society at the time, did not cause significant isotopic shifts that were recorded in individual tissues. The move into the grassland areas of the Highveld at the beginning of the LIA, probably the most significant socio-economic and ecological change expected to be recorded in the isotopic data, can be seen in the trend towards more enriched collagen and apatite from the 10th century AD onward. The role of this process, and that of the introduction of maize, in interpreting these data for dietary reconstruction is explored further in section 6.3.1.

When comparing the 12th century to the 19th, $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{tooth enamel}}$ are (or are close to) significantly different (Mann Whitney U-test, $p=0.03$, 0.06 respectively), but $\delta^{13}\text{C}_{\text{bone apatite}}$ is not ($p=0.23$) (Figure 6-3B). The 19th century was a period of great socioeconomic upheaval and change across southern Africa, with the arrival of European colonists, a period of war and disruption amongst farmers known as the *mfecane*, and the changing of many ways of life, and a shift and increase in variation for all tissues is not surprising. There is also an increase in variation in $\delta^{15}\text{N}_{\text{collagen}}$ in this period. It is not clear what dietary shift would be reflected in $\delta^{13}\text{C}_{\text{tooth enamel}}$ but not $\delta^{13}\text{C}_{\text{bone apatite}}$; this is another instance of the differences that effect the isotopic value of the two biominerals.

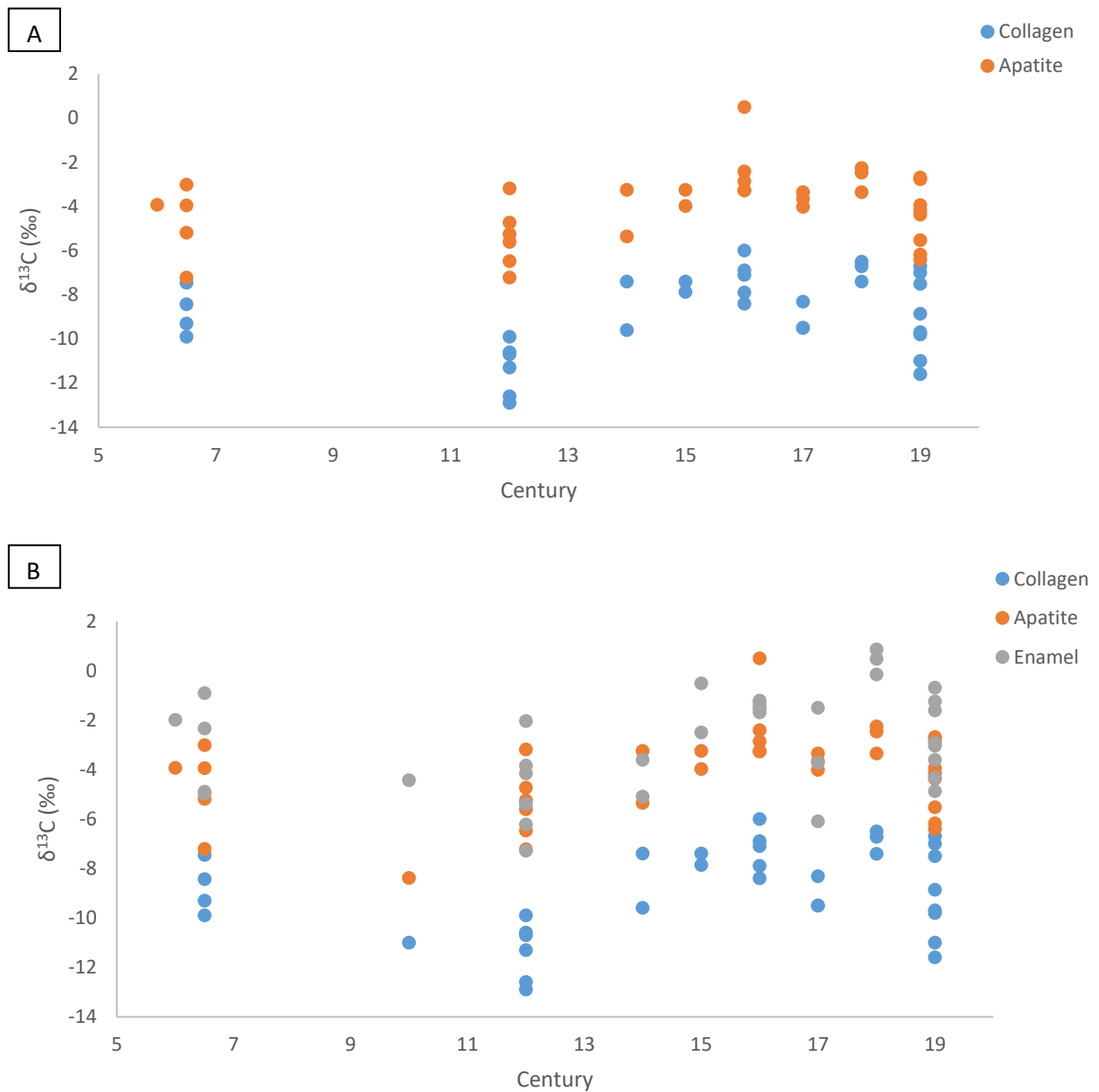


Figure 6-3 Carbon isotope trends throughout time for Iron Age agriculturalists. Circles represent dated individuals that could be attributed to a specific century either through radiocarbon dating or secure site context. B) $\delta^{13}\text{C}_{\text{tooth enamel}}$ is plotted on to the same graph along with $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{collagen}}$ in (A). As noted in the text, $\delta^{13}\text{C}_{\text{collagen}}$ is significantly different in the 12th century compared to the 6th ($\delta^{13}\text{C}_{\text{tooth enamel}}$ is not significant, Mann Whitney U-test, $p=0.17$, nor is $\delta^{13}\text{C}_{\text{bone apatite}}$, $p=0.33$).

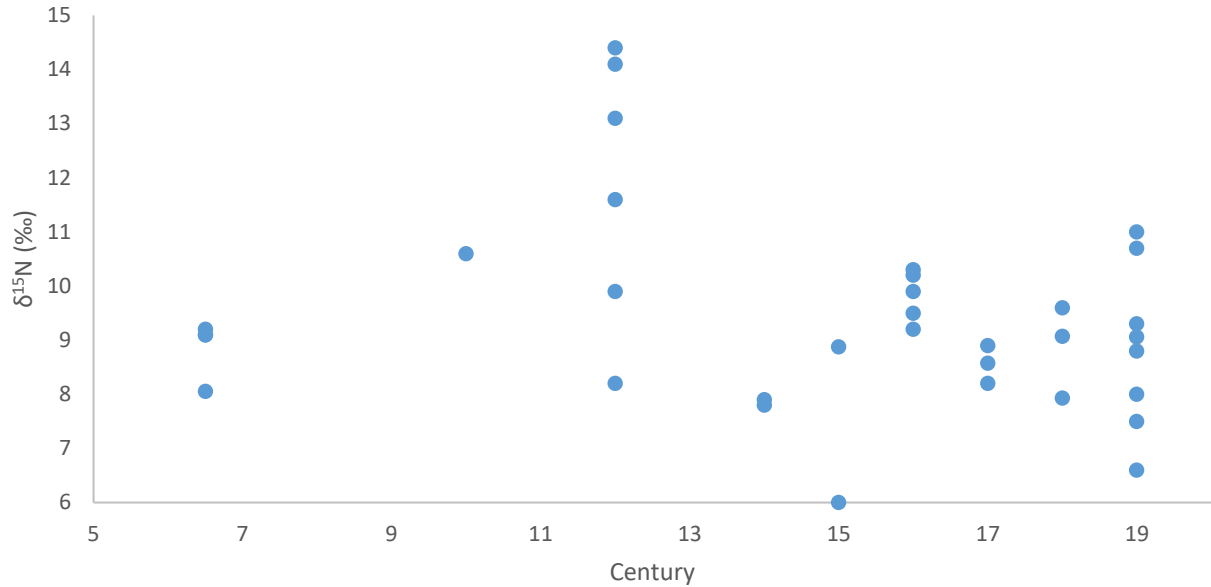


Figure 6-4 Nitrogen isotope trends throughout time for Iron Age agriculturalists. Circles represent dated individuals that could be attributed to a specific century either through radiocarbon dating or secure site context.

As people moved from a diet of C₄ energy and mixed protein sources (even including marine protein) to one dominated by both C₄ energy and protein, relative increases and decreases in δ¹³C values are less interesting than the concordance between bone apatite and collagen. There is no question that these values reflect the presence of C₄ resources as a major component of diet over time, but the synchronous changes to both δ¹³C_{bone apatite} and δ¹³C_{collagen} indicate that protein and non-protein sources were isotopically similar, which suggest several dietary possibilities which are explored in turn below.

The first possibility is that animals (wild and domesticated) grazing on C₄ grasses were a significant source of protein, abundant enough to contribute carbon atoms to carbonate and bicarbonate in blood plasma and cause δ¹³C_{bone apatite} to resemble δ¹³C_{collagen}. In regions where there is no possibility of marine protein consumption, and rainfall is relatively limited (e.g. the Highveld), δ¹⁵N in humans should reflect their animal protein intake by being 3-6‰ more enriched than their food source (Hedges and Reynard, 2007; O’Connell et al., 2012). In Figure 6-4, although there is a spike in δ¹⁵N enrichment in the 12th century AD, there is not a clear trend of enrichment over time, and there is no correlation between time and δ¹⁵N (R²=0.05). The later centuries of the Iron Age are more variable in δ¹⁵N, with some of the highest and lowest δ¹⁵N values (6.6‰ to 11‰). Given these values, the food consumed would fall between 0.6‰ and 9‰, which includes the full range of southern African C₄ plants (from +1 to +6‰) (Heaton, 1987; Swap and Aranibar, 2004; Codron et al., 2005a).

This suggests that $\delta^{15}\text{N}$ is increasing slightly over time, but there is little animal protein in the diet even at the high end of the range.

This is consistent with Gilbert (1995) who reported a reduction in animal consumption over time, but cautioned that the appearance of a reduction was largely due to high $\delta^{15}\text{N}$ in very arid areas. The high $\delta^{15}\text{N}$ values in the 12th century AD are from the Skutwater individuals, and are probably due to aridity in the region. In general, given the unresolved questions around interpreting $\delta^{15}\text{N}$, this trend should be interpreted with caution (Heaton, 1987; Sealy et al., 1987; Murphy and Bowman, 2006; Hedges and Reynard, 2007). Given this, it is not likely that an increase in animal consumption is the source of the similarity between $\delta^{13}\text{C}_{\text{bone apatite}}$ and $\delta^{13}\text{C}_{\text{collagen}}$.

The second possibility is that C_4 cereal crops, which contain many (although not all) essential amino acids, formed the primary component of Iron Age diet, as reflected in $\delta^{13}\text{C}_{\text{bone apatite}}$. The lowest $\delta^{15}\text{N}$ values indicate minimal animal protein, although not all of them are particularly low. Both plant and animal proteins contribute amino acids to the synthesis of proteins such as collagen, and with the current data it is not possible to distinguish which specific dietary component is reflected in collagen. Sorghum and millet, having relatively high protein content, could contribute amino acids directly to collagen that would cause $\delta^{13}\text{C}_{\text{collagen}}$ to resemble $\delta^{13}\text{C}_{\text{bone apatite}}$. Given Figure 6-4, this is likely in many cases with very low $\delta^{15}\text{N}$. Typically, the resemblance of $\delta^{13}\text{C}_{\text{collagen}}$ to $\delta^{13}\text{C}_{\text{bone apatite}}$ would also be interpreted as evidence for a very low protein diet; in this case, the primary plant foods are adequate sources of protein, but since protein (or amino acids for synthesis) and non-protein components are all coming from the same C_4 plant, it is not possible to comment on protein quantity in the diet. This hypothesis is consistent with the $\delta^{15}\text{N}$ range discussed above, as plant protein would not raise $\delta^{15}\text{N}$ the way animal protein would.

The third possibility is a combination of the first two. A diet with elements of both plant and animal protein, both coming from C_4 dominated sources, would be isotopically indistinguishable from the current ^{13}C data. When both protein and mineral tissues reflect isotopically similar resources, the additional dietary information they can provide is more limited than in cases where the sources differ. Combining multiple tissues and isotopes, however, can be the next step to disentangling two isotopically similar resources.

The discriminant function analysis scores calculated by Froehle et al. (2012) represent composite scores of $\delta^{15}\text{N}_{\text{collagen}}$, $\delta^{13}\text{C}_{\text{bone apatite}}$, and $\delta^{13}\text{C}_{\text{collagen}}$, and 98.8% of their variance was

accounted for by two functions, the 'carbon' (F1) and 'nitrogen' (F2) functions. Figures 6-5A and B illustrate that the F1 and F2 scores follow largely the same curves as in Figures 6-3 and 6-4. The exception, however, is that for function 2, dependent mainly on $\delta^{15}\text{N}_{\text{collagen}}$, the post 14th century scores are largely flat: there is an increase in variability as time goes on, but the scores largely remain below -2 (Figure 6-5B). The individuals from Skutwater have the highest F2 scores (Figure 6-5B, indicated) but do not affect the overall flatness of the trend; in fact, removing them would reduce the variation in the data significantly. The dietary clusters with the most negative F2 scores are cluster 5 (30:70 C₃:C₄ energy, >65% C₃ protein) and cluster 2 (30:70 C₃:C₄ energy, >50% C₄ protein) (Froehle et al., 2012). These clusters represent the two diets in Froehle et al. (2012) with the largest C₄ component, and the shift to lower F2 scores is consistent with a diet with high C₄ energy. Higher F1 scores also shift in the direction of cluster 2, and cluster 3 (50:50 energy, marine protein), the two dietary clusters with the protein sources most isotopically similar to C₄ foods.

Although this technique cannot discriminate precisely between plant and animal protein sources, it provides evidence that both were increasing across the Iron Age. Such a complex dietary system, where most components are on the 'C₄ side' of the $\delta^{13}\text{C}$ spectrum, will require a new technique or more detailed understanding of the C₄ isotopic range completely unravel, if indeed that is ever possible. It is interesting to note that shifts in F2, associated with nitrogen (which is largely associated with the consumption of animal protein), are best explained by the energy components of the dietary clusters, and the 'carbon' function (F1) is explained by the isotopic type of the protein in the diet. This underscores the complexity of $\delta^{15}\text{N}$, especially in terms of aridity and the effect of rainfall on plant and animal $\delta^{15}\text{N}$. In general, interpretations of composite values such as these functions, which include $\delta^{15}\text{N}$, should be made carefully, especially since they are based on samples that do not reflect the full range of human isotopic variation. These composite scores, do, however have the advantage of visually encoding all three tissue isotope values and illustrating the effects of multiple dietary shifts (i.e. protein and non-protein sources changing at the same time) which may not be apparent from plotting a single tissue's isotope values. Beginning after the 14th century, the trend towards more negative F2 scores and positive F1 scores is consistent with the shift to the grassland and adoption of more productive C₄ crops such as maize, and the social and economic growth and diversity that accompanied it.

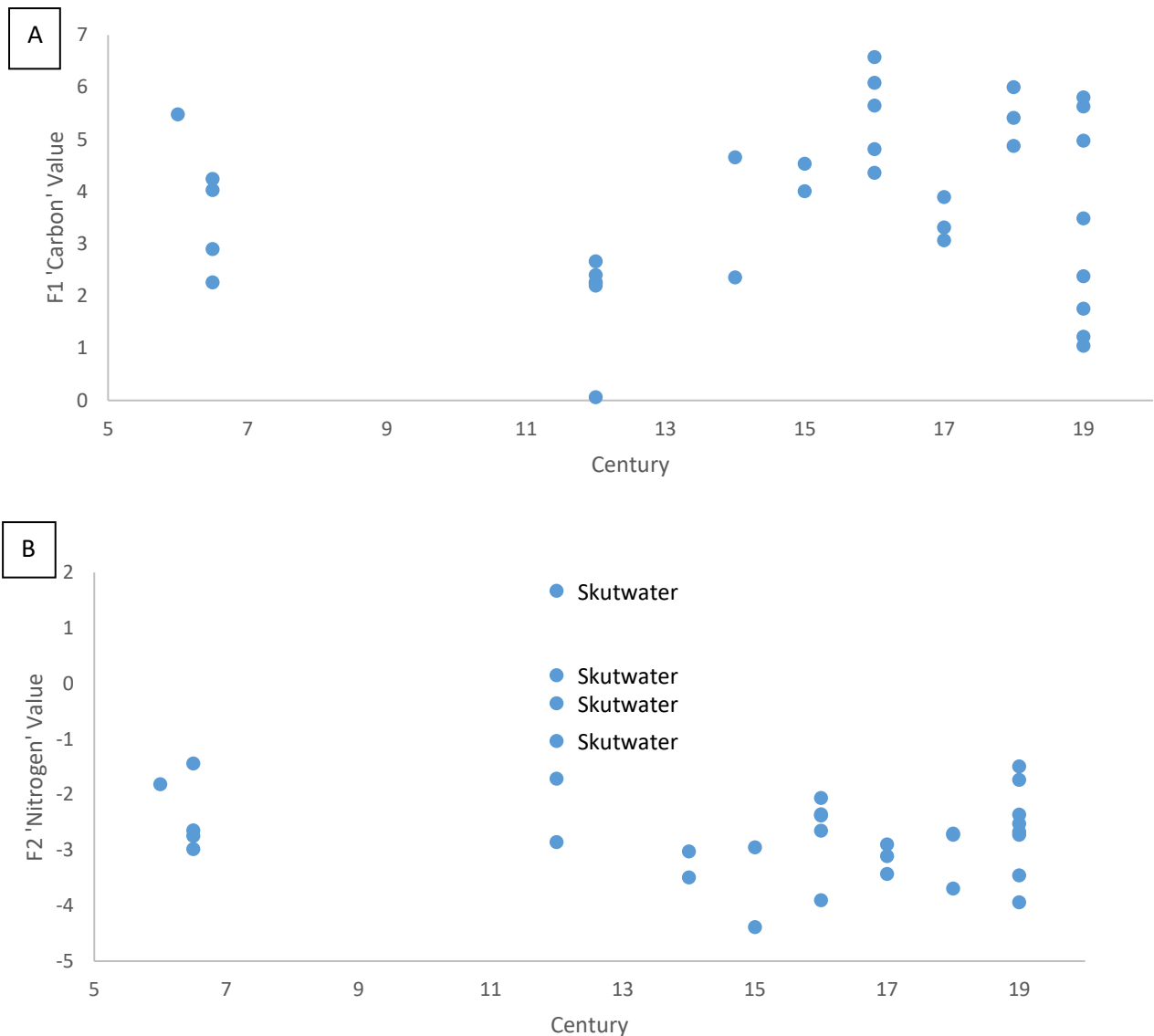


Figure 6-5 Discriminant function analysis scores from Froehle et al. 2012 for data in this study throughout the Iron Age. DFA scores for each data point (as explained in sections 2.3.2 and 6.2.3) are plotted against century. A) the 'Carbon' values (Function 1); Function one is primarily influenced by $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$. B) the 'Nitrogen' values (Function 2); function two is primarily influenced by $\delta^{15}\text{N}_{\text{collagen}}$.

6.3.2. Maize

As discussed in section 3.3.3, direct evidence for maize comes from pollen and maize cobs from approximately 400 years ago. Prior to that, the size and density of the Bokoni settlements circa 16th century have been attributed to the use of more productive crops such as maize (Maggs, 1976). Archaeological evidence for maize is not likely to be apparent until its use is quite prevalent.

Because maize is a more productive crop, the trend in ^{13}C enrichment over time could be attributed to increased consumption as use of sorghum and millet waned. This is apparent in Figure 6-6B, which illustrates that for some individuals, whole diet (as reflected by $\delta^{13}\text{C}_{\text{bone apatite}}$) was especially impacted by the addition of C_4 plant foods. However, Figures 6-

6 A and B also make clear the wide range of agriculturalist diets consumed even after the putative date for introduction of maize in the region. Furthermore, Late Iron Age settlement in the grassland region of the Highveld occurred early in the second millennium, well before the cultivation of maize. Individuals living in this area would have more positive $\delta^{13}\text{C}_{\text{collagen}}$ from consuming both domesticated and wild animals that were primarily eating the abundant C_4 grasses (in this region, mixed protein sources can no longer be as securely attributed to the consumption of wild fauna), and wild, C_3 plants consumed by humans are likely to have negligible protein content. Even before the introduction of maize, $\delta^{13}\text{C}_{\text{collagen}}$ is expected to approach $\delta^{13}\text{C}_{\text{bone apatite}}$, as in Figure 6-3.

It is difficult to disentangle the move to the grassland from the possible adoption of maize in the isotopic data. As the grassland provides excellent natural grazing land, it may be expected that consumption of C_4 animal protein in the form of domestic and wild fauna would increase, enriching $\delta^{13}\text{C}_{\text{collagen}}$ (and possibly $\delta^{13}\text{C}_{\text{bone apatite}}$). If maize did become important, it likely to have led to a rise in $\delta^{13}\text{C}_{\text{bone apatite}}$ as $\delta^{13}\text{C}_{\text{whole diet}}$ shifted. As discussed, however, there is no clear pattern of enrichment in $\delta^{15}\text{N}$ over time, as would be expected with increased animal consumption, although a small increase may be captured in the rise of F2 scores in the multivariate model. In Figure 6-6A, $\delta^{15}\text{N}$ actually appears to be lower overall in the post-maize era, although there are individuals from the pre-maize era that fall both above and throughout the range. This suggests a decrease in animal protein consumption, as lower-protein maize replaces sorghum and millet. After the introduction of maize, absolute protein intake probably decreased, but as both the protein (from plants and animals) and energy are all solidly in the C_4 range, it is not possible to determine more about the protein source split from the data.

The wide range in the post-maize data reflects the larger sample size and wider spread of Iron Age settlements, as well as possibly variation in the extent of maize adoption between communities (individuals still eating millet or sorghum would have higher overall protein intake and even more convergence between their $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$) (Figure 6-6B). Figures 6-7A and B reinforce the hypotheses presented above regarding high reliance on C_4 resources (likely maize) pushing the diet of Iron Age farmers out of the range of any diets previously included in a multivariate model. There is a high degree of overlap between the pre- and post-maize era data, however, and these models cannot differentiate between maize and crops like sorghum and millet without additional isotopic information on the crops themselves. Figure 5-7A also illustrates the more mixed diet of many EIA individuals,

overlapping with the marine protein line—but there are also EIA individuals far the right of the C₄ line, an area for which Kellner and Schoeninger (2007) have not provided an interpretation. Gilbert (1995) reported no increase in dental caries, which are caused by high-sugar dietary components, until the 18th century. She interpreted this as evidence for minimal or gradual adoption of maize throughout the 16th and 17th centuries, well into the occupation of the Highveld.

Although the isotopic evidence does not reveal a specific point for the adoption of maize or a clear distinction from the C₄ environment of the Highveld, the overall trend of ¹³C enrichment is unmistakable. Complementary archaeological, ethnographical, and historical evidence indicate that maize was definitely under cultivation between the 15th and 16th centuries, and the isotopic trends support this claim.

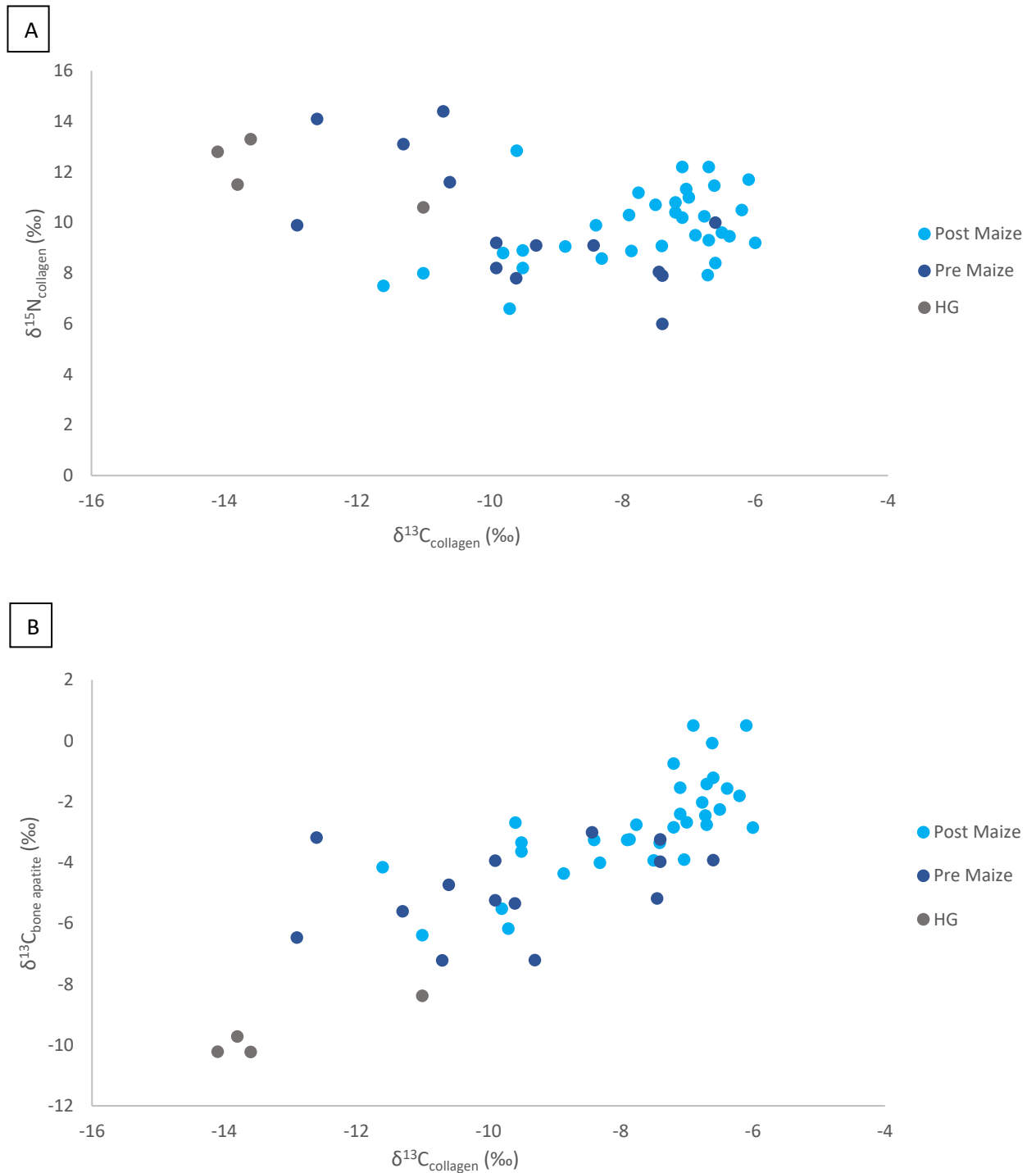


Figure 6-6 Isotopic data for Iron Age individuals before and after the introduction of maize. Circles represent all dated individuals based on radiocarbon dates or secure site attribution, and for these figures, individuals dated in the 16th century AD or later are considered 'post maize.' Individual date attributions can be found in Table 5-4. A) $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{15}\text{N}_{\text{collagen}}$ before and after the introduction of maize in southern Africa. B) $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$.

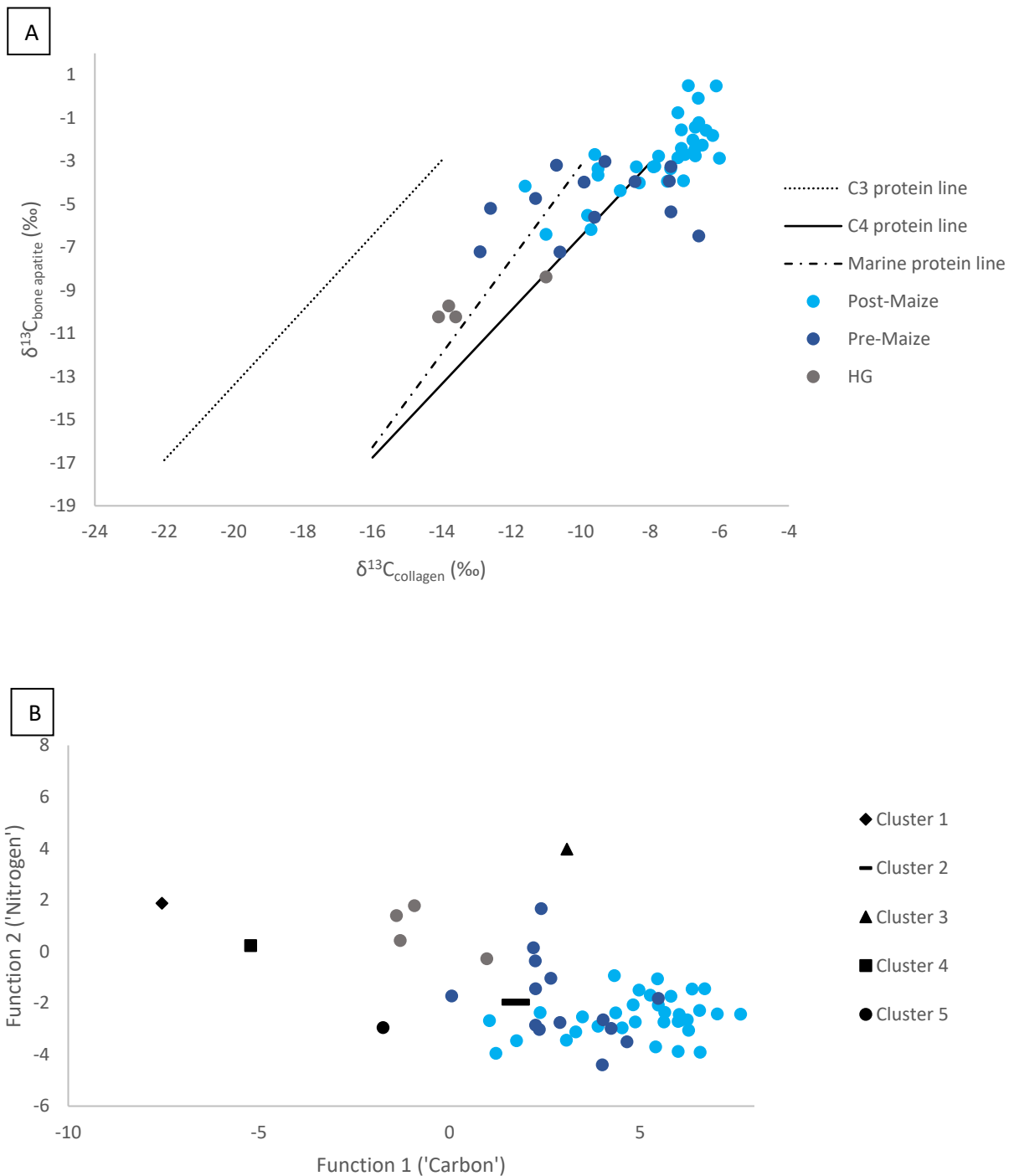


Figure 6-7 Multivariate models of Iron Age data indicating the adoption of maize. A) Bivariate model after Kellner and Schoeninger 2007, B) multivariate model after Froehle et al. 2012

6.4. Collagen-mineral offsets

The wide variety of $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ and $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ among the Iron Age farmers has already been discussed, but the isotopic composition of diets in this region makes it difficult to use these offsets to resolve the issue of animal or plant protein consumption.

Large offsets between collagen and mineral tissues indicate a diet with isotopically different protein and energy sources. Low protein diets result in smaller offsets, as collagen comes to resemble mineral. However, high protein diets, where the converse occurs, can also result in small $\Delta^{13}\text{C}_{\text{collagen-mineral}}$. This is problematic in the case of Iron Age farmers: before the introduction of maize, sorghum and millet contributed significant protein (and carbon isotopes) to bone collagen. In both pre- and post-maize eras at least some animal protein was also C_4 , and diet became more monoisotopic over time. Consequently, collagen-mineral offsets are expected to be small in nearly all cases, and significant socio-economic events may not be reflected. While not consistently small, the $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ and $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ in the study are very inconsistent and unrelated to time ($R^2=0.0$ and 0.1 respectively) (Figure 6-8).

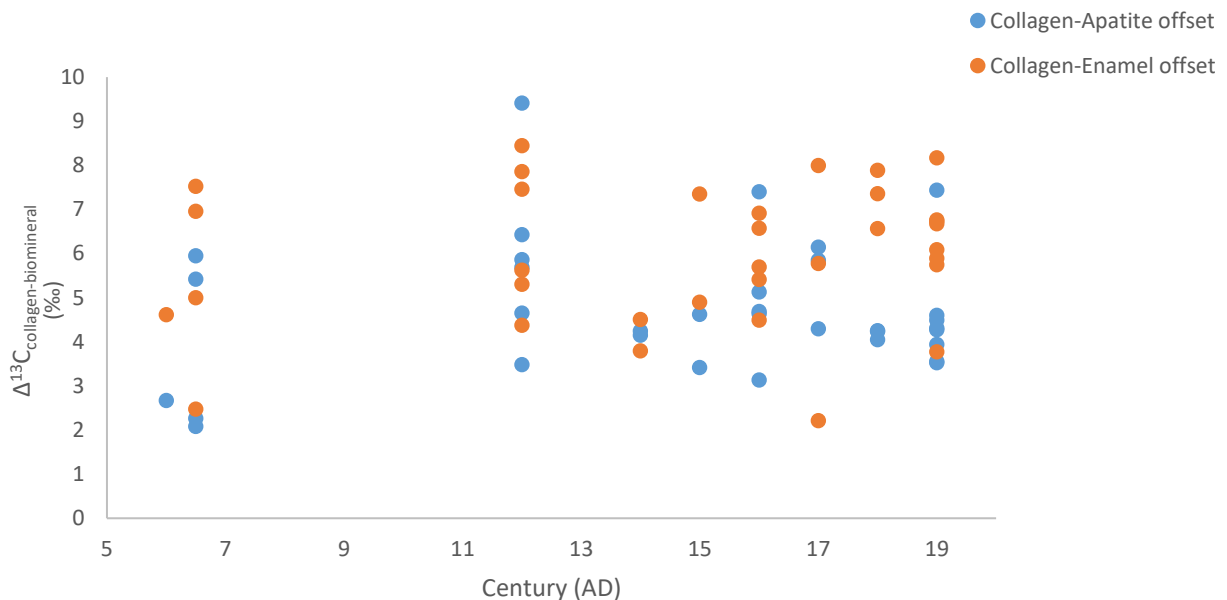


Figure 6-8 $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ and $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ over time.

Notably, there is no clear trend between $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ and $\Delta^{13}\text{C}_{\text{collagen-enamel}}$, reflecting the range of values and lack of correlation discussed in section 2.1.2. The lack of agreement between these two offsets further emphasizes the importance of caution when comparing the two biominerals or isotopic data derived from them, and the need for further study of their metabolism and fractionation. Variability in $\Delta^{13}\text{C}_{\text{collagen-enamel}}$ is reflective of the

overall variation in tooth enamel, and also suggests that enamel even from late-forming teeth like third molars does not convey the same dietary information as bone apatite.

The monoisotopic nature of the Iron Age diet, as well as the protein effects of sorghum and millet, make it difficult to use $\Delta^{13}\text{C}_{\text{collagen-mineral}}$ to reveal additional aspects of diet or resolve outstanding questions. Compared with the individuals from the southwestern Cape studied in Lee-Thorp et al. (1989), $\Delta^{13}\text{C}_{\text{collagen-bone apatite}}$ does not pattern with $\delta^{13}\text{C}_{\text{collagen}}$. Individuals in the southwestern Cape consumed C_3 plant foods and relied heavily on marine resources, mostly high in protein and enriched in ^{13}C . This example illustrates the usefulness of $\Delta^{13}\text{C}_{\text{collagen-mineral}}$ in cases with isotopically distinct protein sources. The wide range of offsets in the data presented here support the observed heterogeneity of Iron Age diets, and illustrate the importance of caution when interpreting one biomineral (tooth enamel) in the context of another (bone apatite).

6.5. Geographic patterning and the heterogeneity of Iron Age diets

As noted in section 5.3.5, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ do not clearly pattern geographically in terms of the grassland and savanna biomes. However, there is a distinct trend of more negative $\delta^{13}\text{C}$ in the south-east of the study region, specifically amongst individuals located along the river valleys of the Indian Ocean Coast. Even though they are substantially inland, these sites are (which tend to be EIA sites) are near rivers and other resources that ensure a diversity of food options, leading to depleted ^{13}C when compared to individuals from the Highveld or more inland savanna regions. The individuals from Skutwater are comparable in $\delta^{13}\text{C}$, in line with the observation that they consumed a more mixed diet, potentially for social reasons (Van Ewyk, 1987). Figure 6-9 A-C illustrates this pattern, showing every Iron Age individual in the study coded by their relative enrichment or depletion compared to the study mean. This effect is visible for $\delta^{13}\text{C}_{\text{collagen}}$, but most pronounced for $\delta^{13}\text{C}_{\text{tooth enamel}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$ (Figure 5-9 B and C), suggesting that in these regions non-protein energy sources were more depleted than the protein dietary elements. This would be consistent with individuals living in a more riverine environment, or the most arid region of the savanna included in the study—in both cases, additional types of plant foods would have been consumed, possibly to supplement the diet in a region less conducive to agriculture (like Skutwater). It is interesting to note that although these two cases probably stem from different dietary conditions (in one case, a greater abundance of alternate resource, in the other a lack of staple foods), they both

manifest as isotopically comparable, reinforcing the importance of local context when making dietary reconstructions.

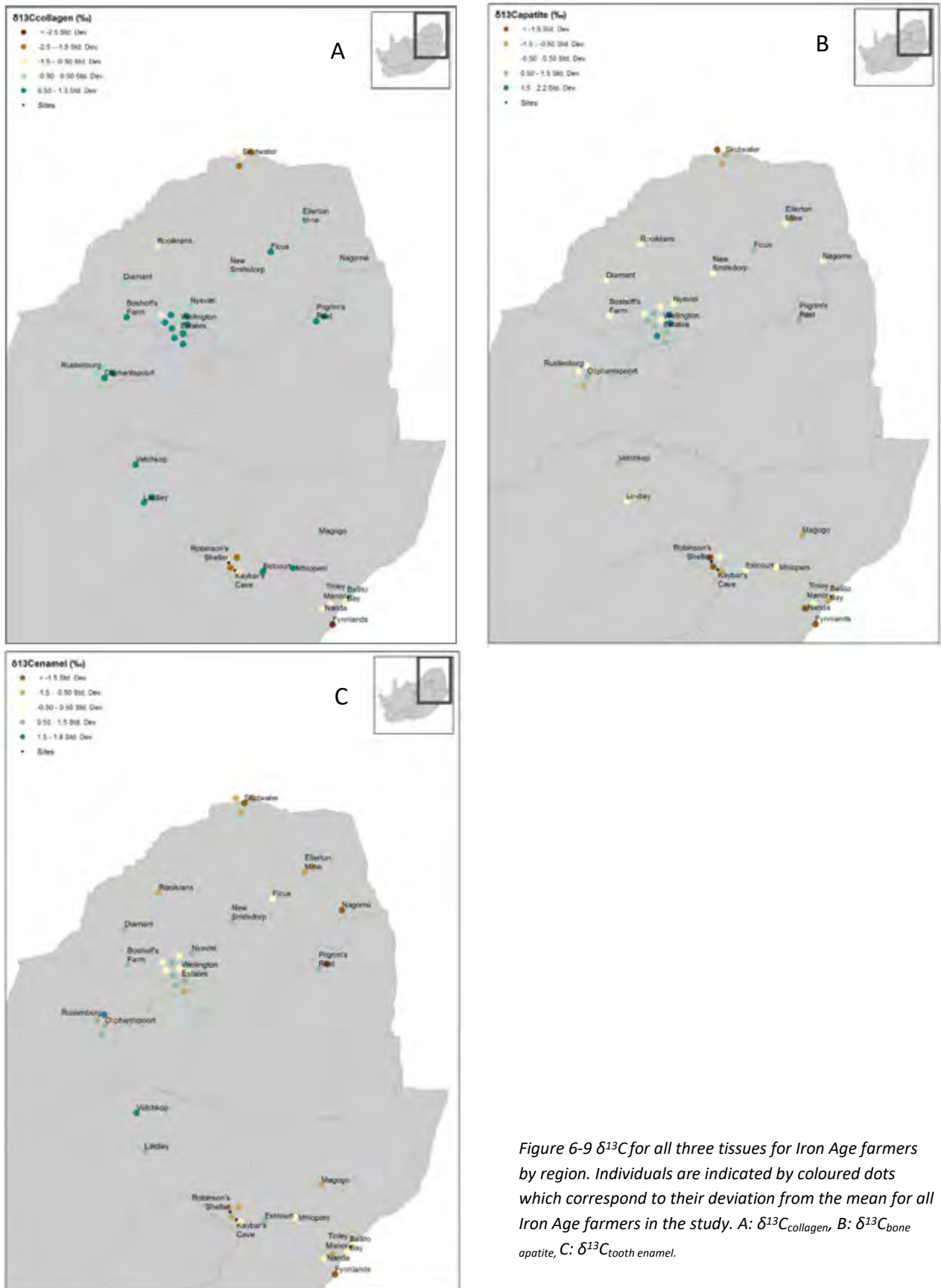


Figure 6-9 $\delta^{13}C$ for all three tissues for Iron Age farmers by region. Individuals are indicated by coloured dots which correspond to their deviation from the mean for all Iron Age farmers in the study. A: $\delta^{13}C_{\text{collagen}}$, B: $\delta^{13}C_{\text{bone apatite}}$, C: $\delta^{13}C_{\text{tooth enamel}}$.



Figure 6-10 $\delta^{15}\text{N}$ for Iron Age farmers by region. Individuals are indicated by coloured dots which correspond to their deviation from the mean for all Iron Age farmers in the study.

$\delta^{13}\text{C}_{\text{collagen}}$ is most positive in the central portion of the study region, consistent with the strongly C_4 environment of the Highveld and an increase in agriculture in that region. This could be consistent with either a substantial portion of dietary protein coming from C_4 plant foods, or animals with a strong C_4 signal.

In the case of $\delta^{15}\text{N}_{\text{collagen}}$, nearly all individuals in the study are either close to the mean or slightly depleted in ^{15}N (Figure 6-10). A notable exception is Skutwater, where as discussed the individuals are substantially enriched, likely due to the aridity of the region. The rest of the most enriched individuals come from Wellington Estates, but since this ‘site’ was not excavated, there is no way to establish a cultural or temporal affinity between them. On the whole this reflects the low overall $\delta^{15}\text{N}_{\text{collagen}}$ values of the study group, and the relative consistency between individuals and sites.

6.6. Conclusion

The Iron Age in southern Africa was a period of socioeconomic change, of geographical shift in settlement area and of population expansion, together with the

utilisation of new resources. The consumption of cereal crops such as sorghum and millet is apparent in the elevated $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$ of farmers when compared to the coastal hunter gatherers, and there is some isotopic evidence for increased reliance on C_4 based foods associated with the spread into the Highveld, and later the adoption of maize. Neither event is associated with an increase in consumption of animal protein, and the close relationship between $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$ may be due to the high protein content of the crops. The expansion to the Highveld, where there are C_4 grazing wild animals, obscures a possible increase in animal protein that is isotopically similar to the energy sources. Energy sources high in protein such as millet and sorghum are challenging to interpret isotopically, and the strict protein/animal-energy/plant interpretation is not viable here. $\delta^{15}\text{N}_{\text{collagen}}$ does not pattern clearly by geography or time, and aridity effects pose significant problems when investigating dietary distinctions across environmental gradients.

There is significant variation in isotope values across the EIA and LIA, and across the various biomes from which these samples were taken. The lack of clear temporal trends suggests that even after such significant events, there remained a wide range of economic and resource utilisation practices across southern Africa, resulting in heterogeneous diets. Despite this, there is isotopic evidence for increasing utilisation of C_4 food resources in the latter half of the LIA, probably from low-protein energy sources like maize.

Understanding of isotope chemistry, metabolic routing, and analytical methods now allows for the interpretation of isotope analysis of multiple tissues, which facilitates more nuanced, detailed dietary reconstructions. Comparing multiple tissue isotopes also illustrates how much more there is to understand about these complex chemico-physical systems and the windows into the past that they offer to researchers. Accordingly, more research and attention should be paid to the preparatory and analytical methods that are used, especially to mitigate the effect of diagenesis on biominerals. Previously published biomineral isotope measurements (especially for bone apatite) may be the result of very different pre-treatments, and so the results may not be comparable. Pre-treatment protocols have a significant impact on the final isotope value and should be carefully compared in all experiments. In particular, particle size is relevant to the isotopic analysis and should be controlled. Refining these pre-treatments and determining better tests for the quality of biomineral analytical results should be a priority, as the wealth of information available from this tissue is very valuable.

Tooth enamel and bone apatite are not only mineralogically distinct, they also reflect different isotopic signals, from different times of life and different metabolic and fractionation processes. The range of reported correlations and offsets between $\delta^{13}\text{C}_{\text{tooth enamel}}$ and $\delta^{13}\text{C}_{\text{bone apatite}}$ emphasizes the need for additional study of the process through which dietary isotopes are incorporated into these biominerals, and our ability to interpret them in archaeological contexts. They should be treated separately, and likely reflect meaningfully different dietary information from which future reconstructions can draw. Just as developing more robust pre-treatment protocols for bone apatite will facilitate inter-study comparisons and expand the global human dataset, more fully understanding the dietary realities that affect these biominerals can reveal whole new lines of evidence into past diets.

Traditional isotopic analysis, including the calculation of collagen-mineral offsets and comparison of multiple tissue isotopes, can be supplemented and expanded using new multivariate modeling techniques. These techniques are based on observed patterns across animal and human populations, and so can overcome many of the challenges of conventional reconstruction. These include the suitability of fractionation factors, the difficulty of defining and accounting for complex, dynamic systems such as metabolism and fractionation, and the problems of applying such factors across species. Model interpretations, which are inherently comparative, should be combined with analysis of the absolute isotopic values and the relationships between isotopes from multiple tissues.

Based on existing datasets, however, these models are only valid to the extent of the original data used to create them, and for the interpretation of similar data. In the case of Iron Age farmers, the data on which the models were based were quite different: archaeological individuals from North and South America, with different diets. Iron Age farmers overlapped with the extreme C_4 portions of each model, but extended far beyond them, providing a clear next step for the improvement of existing models and the development of new ones: larger, more comprehensive datasets from diverse areas, encompassing all isotopically possible diets. As agriculturalists with strongly C_4 -based diets, these farmers form a new and valuable data set to add to the global development of these models and can expand their interpretive power.

Farmers of the southern African Iron Age present an exciting and challenging group to study isotopically because of their diet (C_4 -dominant diets are less studied worldwide) and the monoisotopic nature of their many food resources. By applying multiple isotopic dietary

reconstruction techniques, this study has provided further insight into the heterogeneity of Iron Age diet across both time (the EIA and the LIA) and space (the full range of modern-day South Africa where individuals have been found). It also illustrated both the power and complications of using these techniques in settings with monoisotopic diets or groups who have not been widely included in existing models. Future research should focus on comparing the data collected here from humans to more environmentally specific isotope values for crops and other likely food sources. Future work will also be needed to better understand the processes of fractionation and formation that tooth enamel and bone apatite undergo, and the implications for isotopic work on these valuable mineral tissues.

Sources Cited

- Ambrose S, Norr L. 1993. Experimental evidence for the relationship of the carbon stable isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In: Lambert J, Grupe G, editors. Prehistoric human bone: archaeology at the molecular level. Berlin: Springer-Verlag. p 1–38.
- Ambrose SH. 1986. Stable carbon and nitrogen isotope analysis of human and animal diet in Africa. *J Hum Evol* 15:707–731.
- Ambrose SH. 1990. Preparation and Characterization of Bone and Tooth Collagen for Isotopic Analysis. *J Archaeol Sci* 17:431–451.
- Badenhorst S. 2009. The central cattle pattern during the Iron Age of southern Africa: a critique of its spatial features. *South African Archaeol Bull* 64:148–155.
- Board on Science and Technology for International Development. 1996. *Lost Crops of Africa Vol. 1 Grains*. Washington, D.C.: National Academy Press.
- Bocherens H, Drucker D. 2003. Trophic level isotopic enrichment of carbon and nitrogen in bone collagen: Case studies from recent and ancient terrestrial ecosystems. *Int J Osteoarchaeol* 13:46–53.
- Caut S, Angulo E, Courchamp F. 2009. Variation in discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$): The effect of diet isotopic values and applications for diet reconstruction. *J Appl Ecol* 46:443–453.
- Cerling TE, Harris JM, Ambrose SH, Leakey MG, Solounias N. 1997a. Dietary and environmental reconstruction with stable isotope analyses of herbivore tooth enamel from the Miocene locality of Fort Ternan, Kenya. *J Hum Evol* 33:635–650.
- Cerling TE, Harris JM, Macfadden BJ, Leakey MG, Quade J, Eisenmann V, Ehleringer JR. 1997b. Global vegetation change through the Miocene / Pliocene boundary. *Nature* 389:153–158.
- Cerling TE, Wang Y, Quade J. 1993. Expansion of C4 ecosystems as an indicator of global ecological change in the late Miocene. *Nature* 361:344–345.
- Codron D, Codron J, Lee-Thorp JA, Sponheimer M, de Ruiter D. 2005a. Animal diets in the Waterberg based on stable isotopic composition of faeces. *South African J Wildl Res* [Internet] 35:43–52. Available from: [http://www.colorado.edu/Anthropology/sponheimer/pdfs/Codron et al 05 SAJWR.pdf](http://www.colorado.edu/Anthropology/sponheimer/pdfs/Codron%20et%20al%2005%20SAJWR.pdf)
- Codron J, Codron D, Lee-Thorp J a., Sponheimer M, Bond WJ, de Ruiter D, Grant R. 2005b. Taxonomic, anatomical, and spatio-temporal variations in the stable carbon and nitrogen isotopic compositions of plants from an African savanna. *J Archaeol Sci* 32:1757–1772.
- Codron J, Lee-Thorp JA, Sponheimer M, Codron D. 2013. Plant stable isotope composition across habitatgradients in a semi-arid savanna: implications forenvironmental reconstruction. *J Quat Sci* 28:301–310.
- Craine JM, Elmore AJ, Aida MPM, Bustamante M, Dawson TE, Hobbie E a., Kahmen A, MacK MC, McLauchlan KK, Michelsen A, Nardoto GB, Pardo LH, Peñuelas J, Reich PB, Schuur E a G, Stock WD, Templer PH, Virginia R a., Welker JM, Wright IJ. 2009. Global patterns of foliar nitrogen isotopes and their relationships with climate,

- mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytol* 183:980–992.
- Crowley BE, Carter ML, Karpanty SM, Zihlman AL, Koch PL, Dominy NJ. 2010. Stable carbon and nitrogen isotope enrichment in primate tissues. *Oecologia* 164:611–626.
- DeNiro MJ. 1985. Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. *Nature* 317:806–809.
- DeNiro MJ, Epstein S. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* [Internet] 42:495–506. Available from: http://ac.els-cdn.com.ezp-prod1.hul.harvard.edu/0016703778901990/1-s2.0-0016703778901990-main.pdf?_tid=4a0b5dc4-64e4-11e3-a4f1-00000aab0f27&acdnat=1387041921_16d37a148351724d141b7d179858c029
- DeNiro MJ, Epstein S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta* [Internet] 45:341–351. Available from: http://ac.els-cdn.com.ezp-prod1.hul.harvard.edu/0016703781902441/1-s2.0-0016703781902441-main.pdf?_tid=3208f0b0-64e4-11e3-92e6-00000aab0f26&acdnat=1387041880_3afc907f27eee370e6f16d4fc8b3aa79
- Edwards EJ, Osborne CP, Strömberg CAE, Smith SA, Edwards E, Strömberg CAE, Smith SA, Consortium CG. 2010. The Origins of C4 Grasslands : and Integrating Evolutionary Science Ecosystem. *Science* (80-) 328:587–591.
- Eklom A, Gillson L, Risberg J, Holmgren K, Chidoub Z. 2012. Rainfall variability and vegetation dynamics of the lower Limpopo Valley, Southern Africa, 500AD to present. *Palaeogeogr Palaeoclimatol Palaeoecol* [Internet] 363–364:69–78. Available from: <http://dx.doi.org/10.1016/j.palaeo.2012.08.015>
- Van Ewyk JF. 1987. The Prehistory of an Iron Age Site on Skutwater.
- Fichardt AJ. 1960. Skeletal , Cultural and Faunal Material from the Springbok Flats. *South African Archaeol Bull* 15:23–24.
- France C a. M, Owsley DW. 2012. Stable Carbon and Oxygen Isotope Spacing Between Bone and Tooth Collagen and Hydroxyapatite in Human Archaeological Remains. *Int J Osteoarchaeol* [Internet]. Available from: <http://doi.wiley.com/10.1002/oa.2300>
- Froehle AW, Kellner CM, Schoeninger MJ. 2010. FOCUS: effect of diet and protein source on carbon stable isotope ratios in collagen: follow up to. *J Archaeol Sci* [Internet] 37:2662–2670. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0305440310001895>
- Froehle AW, Kellner CM, Schoeninger MJ. 2012. Multivariate Carbon and Nitrogen Stable Isotope Model for the Reconstruction of Prehistoric Human Diet. *Am J Phys Anthropol* 147:352–369.
- Galloway A. 1936. Some Prehistoric Skeletal Remains From the Natal Coast. *Trans R Soc South Africa* 23:277–295.
- Garvie-Lok SJ, Varney TL, Katzenberg MA. 2004. Preparation of bone carbonate for stable isotope analysis: The effects of treatment time and acid concentration. *J Archaeol Sci* 31:763–776.
- Gilbert C. 1995. Diet and subsistence patterns in the Later Iron Age of South Africa: an

alysis of stable carbon and nitrogen isotopes and the incidence of dental caries.

Gillson L, Ekblom A. 2009. Untangling anthropogenic and climatic influence on riverine forest in the Kruger National Park, South Africa. *Veg Hist Archaeobot* 18:171–185.

Gordon IJ, Prins HHT. 2007. *The Ecology of Browsing and Grazing*. Springer.

Hall M. 1984. The Burden of Tribalism : The Social Context of Southern African Iron Age Studies. *Am Antiq* 49:455–467.

Handley L, Raven J. 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant Cell Environ* [Internet] 15:965–985. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-3040.1992.tb01650.x/abstract>

Hare PE, Fogel ML, Stafford Jr TW, Mitchell AD, Hoering TC. 1991. The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. *J Archaeol Sci* [Internet] 18:277–292. Available from: <http://linkinghub.elsevier.com/retrieve/pii/030544039190066X>

Harrison RG, Katzenberg MA. 2003. Paleodiet studies using stable carbon isotopes from bone apatite and collagen: Examples from Southern Ontario and San Nicolas Island, California. *J Anthropol Archaeol* 22:227–244.

Heaton THE. 1987. The $^{15}\text{N}/^{14}\text{N}$ ratios of plants in South Africa and Namibia: relationship to climate and coastal/saline environments. *Oecologia* 74:236–246.

Heaton THE, Vogel JC, von la Chevallerie G, Collett G. 1986. Climatic influence on the isotopic composition of bone nitrogen. *Nature* 322:822–823.

Hedges REM. 2002. Bone diagenesis: an overview of processes. *Archaeometry* [Internet] 44:319–328. Available from: <http://doi.wiley.com/10.1111/1475-4754.00064>

Hedges REM. 2003. On bone collagen - Apatite-carbonate isotopic relationships. *Int J Osteoarchaeol* 13:66–79.

Hedges REM, Clement JG, Thomas CDL, O’Connell TC. 2007. Collagen turnover in the adult femoral mid-shaft: modeled from anthropogenic radiocarbon tracer measurements. *Am J Phys Anthropol* 133:808–816.

Hedges REM, Reynard LM. 2007. Nitrogen isotopes and the trophic level of humans in archaeology. *J Archaeol Sci* [Internet] 34:1240–1251. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0305440306002214>

Hillson S. 1996. *Dental Anthropology*. Cambridge: Cambridge University Press.

Howland MR, Corr LT, Young SMM, Jones V, Jim S, Van Der Merwe NJ, Mitchell a. D, Evershed RP. 2003. Expression of the dietary isotope signal in the compound-specific d^{13}C values of pig bone lipids and amino acids. *Int J Osteoarchaeol* 13:54–65.

Huffman TN. 1982. Archaeology and Ethnography of the African Iron Age. *Annu Rev Anthropol* 11:133–150.

Huffman TN. 1989. Ceramic, Settlements and Late Iron Age Migrations. *African Archaeol Rev* 7:155–182.

Huffman TN. 1996. Archaeological evidence for climate change during the last 2000 years in southern Africa. *Quat Int* 33:55–60.

- Jim S, Ambrose SH, Evershed RP. 2004. Stable carbon isotopic evidence for differences in the dietary origin of bone cholesterol, collagen and apatite: Implications for their use in palaeodietary reconstruction. *Geochim Cosmochim Acta* 68:61–72.
- Jim S, Jones V, Ambrose SH, Evershed RP. 2006. Quantifying dietary macronutrient sources of carbon for bone collagen biosynthesis using natural abundance stable carbon isotope analysis. *Br J Nutr* 95:1055–1062.
- Kellner CM, Schoeninger MJ. 2007. A Simple Carbon Isotope Model for Reconstructing Prehistoric Human Diet. *Am J Phys Anthropol* 1127:1112–1127.
- van Klinken GJ. 1999. Bone Collagen Quality Indicators for Palaeodietary and Radiocarbon Measurements. *J Archaeol Sci* 26:687–695.
- Koch PL, Tuross N, Fogel ML. 1997. The Effects of Sample Treatment and Diagenesis on the Isotopic Integrity of Carbonate in Biogenic Hydroxylapatite. *J Archaeol Sci* [Internet] 24:417–429. Available from: <http://www.sciencedirect.com/science/article/pii/S0305440396901260>
- Kohn MJ, Cerling TE. 2002. Stable isotope compositions of biological apatite. *Rev Mineral Geochemistry* 48:455–488.
- Krueger H. 1991. Exchange of carbon with biological apatite. *J Archaeol Sci* 18:355–361.
- Krueger H., Sullivan CH. 1984. Models for carbon isotope fractionation between diet and bone. In: Turnlund, J.F. , Johnson PE, editor. *Stable Isotopes in Nutrition*. Washington: ACS Symposium Series 258, American Chemical Society. p 205–222.
- Lee-Thorp J. 2002. Two decades of progress towards understanding fossilization processes and isotopic signals in calcified tissue minerals. *Archaeometry* [Internet] 44:435–446. Available from: <http://doi.wiley.com/10.1111/1475-4754.t01-1-00076>
- Lee-Thorp J a., van der Merwe NJ. 1991. Aspects of the chemistry of modern and fossil biological apatites. *J Archaeol Sci* 18:343–354.
- Lee-Thorp JA, Sealy JC, Morris AG. 1993. Isotopic evidence for diets of prehistoric farmers in South Africa. In: Lambert JB, Grupe G, editors. *Prehistoric human bone: archaeology at the molecular level*. Berlin: Springer-Verlag. p 99–120.
- Lee-Thorp J, Manning L, Sponheimer M. 1997. Problems and prospects for carbonate isotope analysis of very small samples of fossil tooth enamel. *Bull la Soc Geol Fr* 168:767–773.
- Lee-Thorp J, Sealy J. 2008. Beyond documenting diagenesis: The fifth international bone diagenesis workshop. *Palaeogeogr Palaeoclimatol Palaeoecol* [Internet] 266:129–133. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0031018208002356>
- Lee-Thorp J, Sponheimer M. 2003. Three case studies used to reassess the reliability of fossil bone and enamel isotope signals for paleodietary studies. *J Anthropol Archaeol* [Internet] 22:208–216. Available from: http://ac.els-cdn.com.ezp-prod1.hul.harvard.edu/S0278416503000357/1-s2.0-S0278416503000357-main.pdf?_tid=42e41c24-64e5-11e3-b984-00000aab0f6c&acdnat=1387042338_c1ece779c6077a7c6d0c2d8d57695ef7
- Lee-Thorp J, Sponheimer M. 2006. Contributions of Biogeochemistry to Understanding Hominin Dietary Ecology. *Yearb Phys Anthropol* 148:131–148.

- Lee-Thorp JJ, Sealy JJC, van der Merwe NJN. 1989. Stable carbon isotope ratio differences between bone collagen and bone apatite, and their relationship to diet. *J Archaeol Sci* 32:1459–1470.
- Levin NE, Simpson SW, Quade J, Cerling TE, Frost SR. 2008. Herbivore enamel carbon isotopic composition and the environmental context of *Ardipithecus* at Gona, Ethiopia. *Geol Soc Am Spec Pap* 446:215–234.
- Loftus E, Sealy J. 2012. Technical note: interpreting stable carbon isotopes in human tooth enamel: an examination of tissue spacings from South Africa. *Am J Phys Anthropol* [Internet] 147:499–507. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22270879>
- Maggs T. 1976. Iron Age communities of the southern highveld. Pietermaritzburg: Natal Museum.
- Maggs T. 1980. Mzonjani and the beginning of the Iron Age in Natal. *Ann Natal Museum* [Internet] 24:71–96. Available from: <http://content.ajarchive.org/cgi-bin/showfile.exe?CISOROOT=/03040798&CISOPTR=560>
- Maggs T. 2008. The Mpumalanga Escarpment settlements: some answers, many questions. In: Swanepoel N, Esterhuysen A, Bonner P, editors. *Five Hundred Years Rediscovered: Southern African Precedents and Prospects*. Johannesburg: Witwatersrand University Press. p 169–182.
- Maggs T, Ward V. 1984. Early Iron Age sites in the Muden area of Natal. *Ann Natal Museum* [Internet] 26:105–140. Available from: <http://content.ajarchive.org/cgi-bin/showfile.exe?CISOROOT=/03040798&CISOPTR=484> \n <http://hdl.handle.net/10499/AJ464>
- Maggs T, Whitelaw G. 1991. A Review of Recent Archaeological Research on Food-Producing Communities in Southern Africa. *J Afr Hist* 32:3–24.
- Maggs TO. 1994. The Early Iron Age in the extreme south: some patterns and problems. *Azania Archaeol Res Africa* 29/30:171–178.
- Marino B, McElroy M. 1991. Isotopic composition of atmospheric CO₂ inferred from carbon in C₄ plant cellulose. *Nature* 342:127–131.
- Mason R. 1986. Origins of the Black People of Johannesburg and the Southern Western Central Transvaal AD 350-1880. Johannesburg.
- Mason RJ. 1981. Early Iron Age settlement at Broederstroom 24/73, Transvaal, South Africa. *S Afr J Sci* 77:401–416.
- Van Der Merwe NJ, Vogel JC. 1978. ¹³C content of human collagen as a measure of prehistoric diet in woodland North America. *Nature* [Internet] 276:815–817. Available from: <http://www.nature.com.ezp-prod1.hul.harvard.edu/nature/journal/v276/n5690/pdf/276815a0.pdf>
- Minagawa M, Wada E. 1984. Stepwise enrichment of δN along food chains: Further evidence and the relation between δN and animal age. *Geochim Cosmochim Acta* 48:1135–1140.
- Mitchell P. 2002. *The Archaeology of Southern Africa*. Cape Town: Cambridge University Press.

- Morris AG. 1992. A master catalogue: holocene human skeletons from South Africa. Johannesburg: Witwatersrand University Press.
- Mosothwane MN. 2010. Foragers among farmers in the Early Iron Age of Botswana? Dietary evidence from stable isotopes. Unpubl thesis.
- Mucina L, Rutherford MC eds. 2006. The Vegetation of South Africa, Lesotho and Swaziland. Pretoria: Strelitzia 19. South African National Biodiversity Institute.
- Murphy BP, Bowman DMJS. 2006. Kangaroo metabolism does not cause the relationship between bone collagen $\delta^{15}\text{N}$ and water availability. *Funct Ecol* 20:1062–1069.
- Myers L. 1958. New skeletal remains from the Springbok Flats. *S Afr J Sci* [Internet] 54:167–176. Available from: <http://content.ajarchive.org/cgi-bin/showfile.exe?CISOROOT=/00382353&CISOPTR=539\nhttp://hdl.handle.net/10499/AJ38712>
- Nelson BK, Deniro MJ, Schoeninger MJ, De Paolo DJ, Hare P. 1986. Effects of diagenesis on strontium, carbon, nitrogen and oxygen concentration and isotopic composition of bone. *Geochim Cosmochim Acta* 50:1941–1949.
- Neumann FH, Stager JC, Scott L, Venter HJT, Weyhenmeyer C. 2008. Holocene vegetation and climate records from Lake Sibaya, KwaZulu-Natal (South Africa). *Rev Palaeobot Palynol* [Internet] 152:113–128. Available from: <http://dx.doi.org/10.1016/j.revpalbo.2008.04.006>
- Nielsen-Marsh CM, Hedges REM. 1997. Dissolution experiments on modern and diagenetically altered bone and the effect on the infrared splitting factor. *Bull la Soc Geol Fr* 168:485–490.
- Nielsen-Marsh CM, Hedges REM. 2000. Patterns of Diagenesis in Bone II: Effects of Acetic Acid Treatment and the Removal of Diagenetic CO_2 . *J Archaeol Sci* [Internet] 27:1151–1159. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0305440399905381>
- O’Connell TC, Kneale CJ, Tasevska N, Kuhnle GGC. 2012. The diet-body offset in human nitrogen isotopic values: A controlled dietary study. *Am J Phys Anthropol* 149:426–434.
- Passey B, Robinson T, Ayliffe L, Cerling T, Sponheimer M, Dearing M, Roeder B, Ehleringer J. 2005. Carbon isotope fractionation between diet, breath CO_2 , and bioapatite in different mammals. *J Archaeol Sci* 32:1459–1470.
- Pasteris JD, Wopenka B, Freeman JJ, Rogers K, Valsami-Jones E, Van Der Houwen J a M, Silva MJ. 2004. Lack of OH in nanocrystalline apatite as a function of degree of atomic order: Implications for bone and biomaterials. *Biomaterials* 25:229–238.
- Pasteris JD, Wopenka B, Valsami-Jones E. 2008. Bone and Tooth Mineralization: Why Apatite? *Elements* 4:97–104.
- Pellegrini M, Snoeck C. 2016. Comparing bioapatite carbonate pre-treatments for isotopic measurements: Part 2 - Impact on carbon and oxygen isotope compositions. *Chem Geol* [Internet] 420:88–96. Available from: <http://dx.doi.org/10.1016/j.chemgeo.2015.10.038>
- Pestle WJ, Crowley BE, Weirauch MT. 2014. Quantifying inter-laboratory variability in stable isotope analysis of ancient skeletal remains. *PLoS One* 9.

- Pestle WJ, Hubbe M, Smith EK, Stevenson JM. 2015. A linear model for predicting $\delta^{13}\text{C}$ protein. *Am J Phys Anthropol* [Internet] 0:n/a-n/a. Available from: <http://doi.wiley.com/10.1002/ajpa.22743>
- Phillipson DW. 1977. *The later prehistory of Eastern and Southern Africa*. London: Heinemann Educational Books Ltd.
- Plug I. 1984. The faunal remains from two Iron Age sites, Rooikrans and Rhenosterkloof, Central Transvaal. *Ann Cape Prov Museum* 1:201–210.
- Plug I. 1985. Domestic animals during the Early Iron Age in southern Africa. In: Pwiti G, Soper R, editors. *Aspects of African Archaeology, Papers from the 10th Congress of the Pan African Association for Prehistory and Related Studies*. Harare. p 515–520.
- Plug I, Voigt EA. 1985. Archaeozoological studies of Iron Age communities in southern Africa. *Adv World Archaeol* 4:189–238.
- Quin PJ. 1964. Foods and feeding habits of the Pedi. *South African Med J*:969–971.
- Reitsema LJ, Crews DE, Polcyn M. 2010. Preliminary evidence for medieval Polish diet from carbon and nitrogen stable isotopes. *J Archaeol Sci* [Internet] 37:1413–1423. Available from: <http://dx.doi.org/10.1016/j.jas.2010.01.001>
- Ribot I, Morris AG, Sealy J, Maggs T. 2010. Population history and economic change in the last 2000 years in KwaZulu-Natal, RSA. *South African Humanit* 22:89–112.
- Rightmire GP, van der Merwe NJN. 1976. Two Burials from Phalaborwa and the Association of Race and Culture in the Iron Age of Southern Africa. *South African Archaeol Bull* 31:147–152.
- Rooney LW, McDonough C., Waniska RD. 2004. The corn kernel. In: Smith CW, Betran J, Runge ECA, editors. *Corn: Origin, History, Technology and Production*. John Wiley and Sons. p 273–303.
- Russell T, Steele J. 2009. A geo-referenced radiocarbon database for Early Iron Age sites in sub-Saharan Africa: initial analysis. *South African Humanit* [Internet] 21:327–344. Available from: <http://discovery.ucl.ac.uk/49855/>
- Sage RF. 1999. Why C4 photosynthesis? *C4 plant Biol*:3–16.
- Santana-Sagredo F, Lee-Thorp J a., Schulting R, Uribe M. 2015. Isotopic evidence for divergent diets and mobility patterns in the Atacama Desert, northern Chile, during the Late Intermediate Period (AD 900-1450). *Am J Phys Anthropol* [Internet] 156:374–387. Available from: <http://doi.wiley.com/10.1002/ajpa.22663>
- Santoro AE. 2016. The do-it-all nitrifier. *Science* (80-) 351:342–343.
- Schoeninger MJ. 2014. Stable Isotope Analyses and the Evolution of Human Diets. *Annu Rev Anthropol* 43:413–430.
- Schoeninger MJ, Deniro MJ. 1982. Carbon isotope ratios of apatite from fossil bone cannot be used to reconstruct diets of animals. *Nature* 297:577–578.
- Schoeninger MJ, De Niro M. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochim Cosmochim Acta* [Internet] 48:625–639. Available from: <http://ac.els-cdn.com.ezp-prod1.hul.harvard.edu/0016703784900917/1-s2.0-0016703784900917->

main.pdf?_tid=6ecd3c72-64e4-11e3-93b2-00000aab0f27&acdnat=1387041982_f23eb616f8f4ab61044f7731a3fa8079

- Szwarcz HP, Schoeninger MJ. 2012. Handbook of Environmental Isotope Geochemistry. Available from: <http://link.springer.com/10.1007/978-3-642-10637-8>
- Sealy J, Johnson M, Richards M, Nehlich O. 2014. Comparison of two methods of extracting bone collagen for stable carbon and nitrogen isotope analysis : comparing whole bone demineralization with gelatinization and ultra filtration. *J Archaeol Sci* [Internet] 47:64–69. Available from: <http://dx.doi.org/10.1016/j.jas.2014.04.011>
- Sealy JC. 1997. Stable carbon and nitrogen isotope ratios and coastal diets in the Later Stone Age of South Africa: a comparison and critical analysis of two data sets. *Anc Biomol* 1:131–147.
- Sealy JC, Armstrong R, Schrire C. 1995. Beyond lifetime averages: tracing life histories through isotopic analysis of different calcified tissues from archaeological human skeletons. *Antiquity* 69:290.
- Sealy JC, van der Merwe NJ, Lee-Thorp JA, Lanham JL. 1987. Nitrogen isotopic ecology in southern Africa: Implications for environmental and dietary tracing. *Geochim Cosmochim Acta* [Internet] 51:2707–2717. Available from: http://ac.els-cdn.com.ezp-prod1.hul.harvard.edu/0016703787901517/1-s2.0-0016703787901517-main.pdf?_tid=98d28644-64e4-11e3-a353-00000aab0f26&acdnat=1387042053_14b00df6c4ea8af16de1e77bde98b811
- Shin JY, Hedges REM. 2012. Diagenesis in bone and enamel apatite carbonate; The potential of density separation to assess the original composition. *J Archaeol Sci* [Internet] 39:1123–1130. Available from: <http://dx.doi.org/10.1016/j.jas.2011.12.016>
- Smith J. 2005. Climate change and agropastoral sustainability in the Shashe/Limpopo river basin from AD 900.
- Smith J, Lee-thorp J, Hall S. 2007. Climate Change and Agropastoralist Settlement in the Shashe-Limpopo River Basin , Southern Africa : Ad 880 To 1700. *South African Archaeol Bull* 62:115–125.
- Snoeck C, Pellegrini M. 2015. Comparing bioapatite carbonate pre-treatments for isotopic measurements: Part 1-Impact on structure and chemical composition. *Chem Geol* [Internet] 417:349–403. Available from: <http://dx.doi.org/10.1016/j.chemgeo.2015.10.004>
- Somerville AD, Fauvelle M, Froehle AW. 2013. Applying new approaches to modeling diet and status : isotopic evidence for commoner resiliency and elite variability in the Classic Maya lowlands. *J Archaeol Sci* [Internet] 40:1539–1553. Available from: <http://dx.doi.org/10.1016/j.jas.2012.10.029>
- Sponheimer M. 1999. Isotopic Paleoecology of the Makapansgat Limeworks Fauna.
- Sponheimer M, Cerling TE. 2014. Investigating Ancient Diets Using Stable Isotopes in Bioapatites. *Treatise on Geochemistry* [Internet] 14: Archae:341–355. Available from: <http://dx.doi.org/10.1016/B978-0-08-095975-7.01222-5>
- Sponheimer M, Lee-Thorp JA, DeRuiter DJ, Smith JM, van der Merwe NJ, Reed K, Grant CC, Ayliffe LK, Robinson TF, Heidelberg C, Marcus W. 2003. Diets of Southern African Bovidae: Stable Isotope Evidence. *J Mammal* 84:471–479.

- Stott AW, Davies E, Evershed RP, Tuross N. 1997. Monitoring the routing of dietary and biosynthesized lipids through compound-specific stable isotope (^{13}C) measurements at natural abundance. *Naturwissenschaften* 84:82–86.
- Stowe M, Sealy J. 2015. Terminal Pleistocene and Holocene dynamics of southern Africa's winter rainfall zone based on carbon and oxygen isotope analysis of bovid tooth enamel from Elands Bay Cave. *Quat Int*.
- Sullivan CH, Krueger H. 1981. Carbon isotope analysis of separate chemical phases in modern and fossil bone. *Nature* 292:333–335.
- Swap R, Aranibar J. 2004. Natural abundance of ^{13}C and ^{15}N in C_3 and C_4 vegetation of southern Africa: patterns and implications. *Glob Chang Biol* [Internet]:350–358. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2486.2003.00702.x/full>
- Tieszen LLLL, Fagre T. 1993. Effect of diet quality and composition on the isotopic composition of respiratory CO_2 , bone collagen, bioapatite, and soft tissues. In: Lambert J, Grupe G, editors. *Prehistoric human bone: archaeology at the molecular level*. Berlin: Springer-Verlag. p 121–155.
- Trueman CN, Privat K, Field J. 2008. Why do crystallinity values fail to predict the extent of diagenetic alteration of bone mineral? *Palaeogeogr Palaeoclimatol Palaeoecol* 266:160–167.
- Tyson PD, Holmgren K, Thackeray JF. 2002. Changing gradients of climate change in southern Africa during the past millenium: implications for population movements. *Clim Change* 52:129–135.
- Vogel JC, Fuls A, Ellis RP. 1978. The geographical distribution of kranz grasses in South Africa. *S Afr J Sci* 74:209–215.
- Vogel JC, Van Der Merwe NJ, Vogel AC, Van Der Merwe NJ. 1977. Isotopic Evidence for Early Maize Cultivation in New York State. *Am Antiq* [Internet] 42:238–242. Available from: <http://www.jstor.org.ezp-prod1.hul.harvard.edu/stable/pdfplus/278984.pdf>
- Voigt E a. 1984. The faunal remains from Magogo and Mhlopeni: small stock herding in the Early Iron Age of Natal. *Ann Natal Museum* 26:141–163.
- Voigt EA, Plug I. 1984. Happy Rest: the earliest Iron Age fauna from the Soutpansberg. *S Afr J Sci* 80:222–227.
- Wang Y, Cerling TE. 1994. A model of fossil tooth and bone diagenesis: implications for paleodiet reconstruction from stable isotopes. *Palaeogeogr Palaeoclimatol Palaeoecol* [Internet] 107:281–289. Available from: http://ac.els-cdn.com.ezp-prod1.hul.harvard.edu/0031018294901007/1-s2.0-0031018294901007-main.pdf?_tid=16dcee58-64f9-11e3-b589-00000aab0f6b&acdnat=1387050854_ec55a6dff178db7c27643a8b05db0b5d
- Warinner C, Tuross N. 2009. Alkaline cooking and stable isotope tissue-diet spacing in swine: archaeological implications. *J Archaeol Sci* [Internet] 36:1690–1697. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0305440309000910>
- Webb EC, White CD, Longstaffe FJ. 2014. Investigating inherent differences in isotopic composition between human bone and enamel bioapatite : implications for reconstructing residential histories. *J Archaeol Sci* [Internet] 50:97–107. Available from:

<http://dx.doi.org/10.1016/j.jas.2014.07.001>

Wells LH, Dart R a. 1934. A Further Note on Human Skeletal Remains From the Natal Coast. *Trans R Soc South Africa* [Internet] 22:235–243. Available from: <http://www.tandfonline.com/doi/abs/10.1080/00359193409519341>

White CD, Pendergast DM, Longstaffe FJ, Law KR, White CD, Pendergast DM, Longstaffe FJ, Law KR. 2001. Social Complexity and Food Systems at Altun Ha, Belize: The Isotopic Evidence. *Lat Am Antiq* 12:371–393.

Whitelaw G. 1993. Customs and settlement patterns in the first millennium AD: evidence from Nanda, an Early Iron Age site in the Mngeni River valley, Natal. *Natal Museum J Humanit* 5:47–81.

Woodborne S, Hall G, Robertson I, Patrut A, Rouault M, Loader NJ, Hofmeyr M. 2015. A 1000-year carbon isotope rainfall proxy record from South African baobab trees (*Adansonia digitata* L.). *PLoS One* 10:1–18.

Wopenka B, Pasteris JD. 2005. A mineralogical perspective on the apatite in bone. *Mater Sci Eng C* 25:131–143.

Zazzo A, Lécuyer C, Mariotti A. 2004. Experimentally-controlled carbon and oxygen isotope exchange between bioapatites and water under inorganic and microbially-mediated conditions. *Geochim Cosmochim Acta* 68:1–12.

Appendix A: $\delta^{18}\text{O}$ results from bone apatite and tooth enamel and available C:N ratios

UCT Code	Site	Date (Century)	$\delta^{18}\text{O}_{\text{tooth enamel}}$ (‰)	Internal σ	$\delta^{18}\text{O}_{\text{bone apatite}}$ (‰)	Internal σ	C:N ratios of bone collagen ⁵⁷
3682	Skutwater	12	-1.31	0.09	-4.08	0.15	3.8
3683	Skutwater	12	-3.12	0.17	-3.19	0.11	3.5
3684	Skutwater	12	-0.77	0.26	-4.75	0.18	3.5
3685	Skutwater	12	-0.12	0.18	-2.41	0.16	3.8
3686	Heilbron	10	-1.10	0.16	-4.33	0.09	3.4
3690	Lindley (Makwareng)	17 to 19	-1.82	0.16	-4.21	0.20	3.7
3691	Lindley (Makwareng)	17 to 19	-2.74	0.13	-1.81	0.15	3.9
3696	New Smitsdorp	16	-0.36	0.13	-5.41	0.08	3.8
3704	Wellington Estates	16 to 19	-0.68	0.09	-2.80	0.06	3.4
3705	Wellington Estates	16 to 19	-0.44	0.08	-1.65	0.15	3.6
3708	Rustenburg	18	-1.36	0.13	-4.13	0.21	3.4
3710	Oliphantspoort	19	-3.99	0.19	-4.39	0.18	3.4
3711	Oliphantspoort	18	-2.16	0.18	-3.66	0.15	3.2
3712	Oliphantspoort	15	-1.45	0.04	-3.89	0.20	3.3

⁵⁷ For those individuals where C:N ratios are available, see page 58

4148	Nanda	6 to 7	-4.06	0.16	-3.28	0.21	4.2
4152	Oliphantspoort	19	-2.89	0.20	-5.73	0.14	3.9
4171	Nylsvlei	16	-0.79	0.13	-2.56	0.18	3.6
4309	Wellington Estates	16 to 19	-1.09	0.09	-3.93	0.20	3.3
4310	Wellington Estates	16 to 19	-0.51	0.07	-2.79	0.27	3.4
4497	Venus/Estcourt	19	-0.26	0.13	-2.99	0.17	3.8
4913	Vetchkop	18	1.64	0.08	-1.90	0.19	N/A
4914	Kaybars Cave	19	-3.50	0.14	-4.09	0.19	N/A
4915	Kaybars Cave	19	-1.26	0.10	-3.66	0.05	N/A
4916	Robinsons Shelter	19	-2.61	0.14	-1.86	0.17	N/A
4917	Robinsons Shelter	19	-0.87	0.12	-4.04	0.18	N/A
4919	Ellerton Mine	14	-3.61	0.09	-6.71	0.15	N/A
4920	Ellerton Mine	14	-10.99	0.14	-3.31	0.26	N/A
4921	Wellington Estates	16 to 19	-2.83	0.12	-3.23	0.18	N/A
4922	Boshoff's Farm	16	-2.95	0.18	-2.39	0.13	N/A
4923	Wellington Estates	16 to 19	0.54	0.07	-2.15	0.17	N/A
4924	Wellington Estates	16	-1.69	0.08	-2.90	0.15	N/A
4925	Wellington Estates	16 to 19	0.82	0.11	-3.73	0.06	N/A
4926	Wellington Estates	19	-0.32	0.10	-2.93	0.21	N/A
4928	Wellington Estates	16 to 19	-2.07	0.11	-3.10	0.23	N/A
4932	Ficus	16	-2.81	0.06	-3.74	0.17	N/A
4948	Rooikrans	17	-0.54	0.10	-2.61	0.16	N/A
4949	Rooikrans	17	-1.00	0.13	-2.20	0.15	N/A
5387	Diamant	6 to 7	-3.92	0.13	-4.03	0.19	N/A
5392	Nanda	6 to 7	-2.49	0.17	-3.68	0.11	N/A
5395	Tinley Manor	15	-2.06	0.13	-2.98	0.12	3.4
5396	Ballito Bay	12	-2.38	0.13	-5.11	0.20	3.2
5398	Ballito Bay	>2000 BP	-2.29	0.08	-4.30	0.13	3.3
5428	Fynnlands	12	-1.48	0.11	-3.60	0.10	3.4
5431	Illovo Beach	>2000 BP	-3.79	0.17	-4.26	0.16	3.4
10853	Richards Bay	>2000 BP	-2.75	0.15	-4.63	0.13	3.3
16364	Nagome	17	-2.22	0.11	-4.54	0.15	3.3
16365	Mhlopheni	6	-2.61	0.20	-2.21	0.09	3.1
16367	Magogo	6 to 7	-1.79	0.20	-2.91	0.13	3.2
16565	Wellington Estates	16 to 19	-0.49	0.09	-5.29	0.19	3.6
17511	Pilgrim's Rest	LIA	-15.44	0.27	-4.64	0.10	3.2
17512	Pilgrim's Rest	LIA	-3.65	0.13	-2.55	0.17	3.2