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Investigating childhood diet and early life history events in the archaeological record using biogeochemical techniques

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INVESTIGATING CHILDHOOD DIET AND EARLY LIFE HISTORY EVENTS IN THE ARCHAEOLOGICAL RECORD USING BIOGEOCHEMICAL TECHNIQUES

by

PAUL A. SANDBERG

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This thesis entitled:
Investigating childhood diet and early life history events in the archaeological record using biogeochemical techniques
written by Paul A. Sandberg
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The final copy of this thesis has been examined by the signatories, and we Find that both the content and the form meet acceptable presentation standards Of scholarly work in the above mentioned discipline.
Sandberg, Paul (Ph.D., Anthropology)

Investigating Childhood Diet and Early Life History Events in the Archaeological Record Using Biogeochemical Techniques

Thesis directed by Associate Professor Matt Sponheimer

Stable isotopic approaches to studying childhood diet in prehistory have become common in bioarchaeology and have provided researchers with a means to reconstruct life history patterns such as weaning in past human populations. These methods, however, typically rely on isotopic patterning in bone collagen by age-at-death, and lack the resolution to reconstruct weaning in much detail, much less the process in individuals that survived childhood.

In this dissertation, I refine two high-resolution intra-tooth stable isotope techniques for investigating childhood diet and the weaning process in the archaeological record; laser ablation analysis of tooth enamel, and serial micro-sampling of dentine. As these methods are relatively new, their potential relies on an understanding of how best to employ them for archaeological applications. To this end, I addressed a number of methodological issues, including sampling locations within teeth and choice of tooth type. This study was conducted on human remains from Kulubnarti, a Medieval Nubian community which provides an ideal setting for the methodological development of these techniques. This research also explores a number of potential applications to anthropological questions.

The results suggest that: (1) tooth types (first molars and canines) record stable isotopic patterning differently in both enamel and dentine, (2) sampling locations within the thickness of enamel produce isotope profiles with different characteristics, (3) enamel records considerable carbon and oxygen isotopic variability, which may be used to study seasonality in water sources
and agricultural practices, and perhaps seasonality of birth, (4) dentine records carbon and
nitrogen isotopic variability that is likely linked to the weaning process, which can be used to
compare weaning behavior between individuals that survived the process and those that did not,
and (5) both dentine and enamel intra-tooth profiles may be used to study the interaction between
childhood diet, the weaning process, morbidity events, and mortality.
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CHAPTER 1
INTRODUCTION

1.1 Overview

The purpose of this dissertation is to develop and expand upon new high-resolution stable isotope techniques for reconstructing human diet and life history in the archaeological record. Specifically, this research tests the utility of two new sampling and analytical methods for generating high-resolution intra-individual stable isotope profiles in dental tissues. High resolution profiles of carbon and oxygen stable isotope ratios were generated for tooth enamel using laser ablation – gas chromatography – isotope ratio mass spectrometry (LA-GC-IRMS), and high resolution profiles of carbon and nitrogen stable isotope ratios were generated for dentine using a sequential serial sectioning method and conventional isotope ratio mass spectrometry. The ultimate goals of this dissertation are to assess the degree to which these methods yield information regarding dietary change during infancy and childhood, and particularly to ascertain if we can more accurately pinpoint the timing and nature of the weaning process and aspects of seasonality in prehistory.

Before this can be accomplished, several methodological questions concerning sampling strategies, tissue types, and tooth positions are addressed. This research is conducted on human remains from the site of Kulubnarti, a Medieval Nubian archaeological site (~500-800 AD) in the Nile Valley of present day Sudan. Previous research on the biology and health of this population provides a rich context for the methodological development and application of these techniques.
1.2 Background and Rationale

Early childhood health and nutrition are important determinants of a population’s demographic structure through their effects on growth and development, fecundity, morbidity, and mortality (e.g., Vitzthum, 1994; Stuart-Macadam and Dettwyler, 1995; Katzenberg et al., 1996). Indeed, peak human mortality coincides approximately with weaning age, which is therefore the most critical bottleneck in human life history. The duration of breastfeeding is unique among human life history variables in that it is only one directly modified by culture (Dettwyler, 2004). Cultural beliefs and practices also dictate the quantity and quality of supplementary foods during weaning and post-weaning diets (Launer and Habicht, 1989). A more in-depth understanding of this crucial life history stage in past populations is an important goal in the study of both biological and cultural adaptation. One approach to reconstructing weaning behavior and childhood diet in the past is stable isotope analysis.

Stable isotope analysis has become an important tool for studying diet in human prehistory (Schwarcz and Schoeninger, 2011) and has refined the study of early life history in the archaeological record by providing a more direct measure of the weaning process, most notably through the relationship between nitrogen isotope ratios in bone collagen and age at death (e.g., Katzenberg et al., 1993; White and Schwarcz, 1994; Schurr, 1997; Richards et al., 2002; Fuller et al., 2006b; Jay et al., 2008; Pearson et al., 2010). The average age at weaning in a skeletal assemblage is estimable because nitrogen isotope ratios, for example, are higher in breastfeeding infants than they are in adults (Fogel et al., 1989; Fuller et al., 2006a). Therefore, the age at which individuals attain adult nitrogen isotope values is presumed to be the age at which weaning was complete.
Recent attention has been given to dental tissues because they contain a record of isotopic changes associated with diet during infancy and childhood, regardless of age at death. This is because tooth enamel and dentine, once formed and fully mineralized, do not undergo biological turnover. By measuring the isotopic composition of teeth that form at different ages, researchers have been able to assess childhood diet in individuals who survived the process, thus avoiding potential mortality biases of cross-sectional analyses (e.g., Wright and Schwarcz, 1998; Dupras and Tocheri, 2007). While these intra-tooth sampling methods are welcome advances for the study of weaning, they still lack adequate spatial precision to pinpoint the chronology of dietary supplementation and weaning in humans and are therefore silent on a number of important issues. Subtle differences in weaning behavior among individuals or between groups that may have a significant impact on growth and development, morbidity and mortality, and fecundity may go undetected with these conventional isotope analyses.

By sequentially sampling enamel and dentine along the axis of growth, a record of isotopic change associated with diet from infancy through childhood becomes available within single individuals. Detailed records of dietary and seasonal changes have been obtained in large mammal tooth enamel (e.g., Fricke and O’Neil, 1996; Stuart-Williams and Schwarcz, 1997; Fricke et al., 1998; Gadbury et al., 2000; Passey and Cerling, 2002; Balasse et al., 2002; Zazzo et al., 2005; Stevens et al., 2011) and dentine (e.g., Balasse and Tresset, 2002; Zazzo et al., 2006). Intra-tooth stable isotope analysis has only recently been applied to human enamel (Wright, 2012) and dentine (Fuller et al., 2003; Eerkens et al., 2011; Beaumont et al., 2012), and several authors have expressed the need for a greater number of samples per tooth to increase the temporal resolution of the reconstructions, particularly for enamel (e.g., Dupras and Tocheri, 2007; Wright, 2012).
Laser ablation stable isotope analysis provides a means to address these problems by substantially increasing the temporal resolution of intra-tooth isotope profiles in enamel. Laser ablation makes it possible to measure the carbon and oxygen isotope composition of very small amounts of enamel, allowing the generation of time-series isotope profiles in human teeth with much higher resolution than conventional methods permit (Passey and Cerling, 2006). Laser ablation has the additional advantage of being relatively non-destructive, making it particularly well-suited for precious museum specimens and very small teeth (Passey and Cerling, 2006; Sponheimer et al., 2006). Furthermore, the time expended in sample preparation for conventional solution isotope analysis of enamel is eliminated with laser ablation, enabling the analysis of a large number of samples in relatively little time. While this method has been applied to modern and fossil mammal teeth (e.g., Cerling and Sharp, 1996; Passey and Cerling, 2006), including those of early hominins (Sponheimer et al., 2006), it has not yet been applied to human archaeological material or to questions concerning human life history, and several methodological questions about how best to accomplish this remain unanswered.

Intra-tooth stable isotope analysis of dentine is another method that promises to increase the resolution of the weaning process and early childhood diet in humans. Initial serial sampling of dentine by Fuller et al. (2003) has recently been improved upon by increasing the number of samples obtained from single teeth from three or 4 to ten or more (Eerkens et al., 2011; Beaumont et al., 2012). These studies demonstrate that detailed records of isotopic change associated with the weaning process are preserved in human dentine. This opens a door to studying the effects of weaning behavior on mortality. Neither study, however, compared their intra-tooth profiles to stable isotope data from individuals that died during the process. These
studies were also limited to molar teeth, and it is unknown whether other early forming permanent teeth record isotopic variability in the same way.

In this dissertation, high-resolution intra-tooth profiles are created in human enamel using laser ablation stable isotope analysis, and in dentine using new serial sectioning methods. Both methods permit the analysis of very small amounts of material and much more fine-grained spatial control than conventional methods, and, when used in conjunction, may allow us to reconstruct early life history parameters and aspects of seasonality with a level of detail previously unrealized in bioarchaeology. Before the promise of these methods can be fully realized, there are a number of methodological issues to be addressed. Due to the complex developmental geometry and mineralization processes of enamel formation, different sampling strategies may result in isotope profiles with different characteristics (Balasse, 2003; Zazzo et al., 2005; Tafforeau et al., 2007).

In addition, enamel mineral and dentine collagen record different aspects of diet (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004), and it has yet to be shown how high-resolution stable isotope profiles in these two tissue types differ within single individuals or single teeth. To this end, I conducted a number of comparisons between sampling approaches, tooth types, and tissue types to explore how sampling protocol and choice of tooth type and tissue may affect our ability to make interpretations of archaeological interest. This research also utilizes more conventional stable isotope data to generate expectations for the high-resolution analyses, and to evaluate the effects of mortality bias on reconstructions of early life history.

This research is conducted on human remains from the site of Kulubnarti in Sudanese Nubia. The Kulubnarti remains have been the subject of a long-term research program that has
generated a wealth of data regarding demography and health (e.g., Van Gerven et al., 1995). Mortality profiles and patterns of skeletal indicators of stress point to high levels of early childhood stress, most likely associated with the weaning process. However, the relationship between weaning stress, morbidity, and mortality has only been indirectly established, as variability in weaning behavior is difficult to ascertain with traditional methods. The high-resolution stable isotope data generated in this dissertation provide a means to evaluate these relationships directly.

In addition, previously published and unpublished stable isotope data for Kulubnarti show a high degree of variability (Turner et al., 2007; Glasgow, 2011), providing a situation where we might expect variability in the intra-tooth profiles. For example, stable carbon isotope data suggest that the Kulubnarti community cycled between $C_3$ staples in the winter (e.g., wheat, barley, vegetables) and $C_4$ crops in the summer (millet and sorghum), much as they do today (Daffala, 1969; Adams, 1977). Furthermore, the Nile underwent a dramatic annual flood in the past as it does today (Arkell and Ucko, 1965; Daffala, 1969; Adams, 1977; Hassan, 1981; Eltahir, 1996; Jiang et al., 2002), which may impart oxygen isotope variability in growing teeth (see Chapter 4). These factors suggest that intra-tooth carbon and oxygen isotope variation is likely.

1.3 Chapter Outline

The next three chapters provide the necessary background for the remainder of the dissertation. Chapter 2 introduces stable isotope analysis with a focus on reconstructing diet and life history in the archaeological record. It will also introduce high-resolution stable isotope techniques, and identify the space this dissertation inhabits in the anthropological literature. This
chapter will also discuss the benefit of a high-resolution, longitudinal stable isotope approach, particularly in comparison to more conventional cross-sectional analyses. Chapter 3 introduces the site of Kulubnarti. It reviews the biological anthropology research that has been conducted on this collection and provides the setting for the application of the methods used in this dissertation. Chapter 4 reviews the conventional, “bulk tissue” stable isotope research that has been conducted on the Kulubnarti remains, incorporating unpublished data (Sandberg, 2006; Glasgow, 2011) and a previously published dataset (Turner et al., 2007). This chapter provides the isotopic expectations for the high-resolution analyses.

The next two chapters present the high-resolution analyses. Chapter 5 presents the intra-tooth stable isotope data for enamel. The sampling scheme, laser ablation methodology, and results are provided, along with a discussion of the methodological implications and possible interpretations concerning Kulubnarti. The methodology, results, and discussion of the dentine high-resolution analysis are presented in Chapter 6.

Chapter 7 reviews the major findings of this research. This chapter also includes a discussion of how the enamel and dentine results differ and complement one another. The chapter ends by highlighting paths for future research.
CHAPTER 2
STABLE ISOTOPE ANALYSIS

2.1 Chapter Overview

Stable isotope analysis has become a common tool in a variety of scientific fields including anthropology, ecology, paleontology, and the earth sciences. This chapter provides the requisite background to stable isotope analysis and positions this dissertation within anthropological method and theory. It begins with an introduction to stable isotope basics, including definitions, symbols, general measurement procedures, and the principle of isotope fractionation, and then briefly discusses carbon, nitrogen, and oxygen isotope systems in the environment and in animal tissues.

I then discuss the use of stable isotope analysis in archaeology, focusing on the most common applications and those most germane to this dissertation, namely weaning and childhood diet. This chapter then introduces high-resolution stable isotope analysis, and describes how the dissertation draws upon research from other fields. I conclude with a discussion of how this dissertation contributes to current methodological and theoretical trends in biological anthropology.

2.2 Stable Isotope Basics

Each chemical element is defined by the number of protons in its nucleus. For example, carbon has six protons, nitrogen has seven, and oxygen has eight. The number of accompanying neutrons in the nucleus of any given element can vary however, and these different versions of each element are called its isotopes. Isotopes are referred to by their mass number, the total
number of protons and neutrons in the nucleus. Carbon, for example, has three isotopes, \(^{12}\)C, \(^{13}\)C, and \(^{14}\)C. \(^{13}\)C and \(^{14}\)C have seven and eight neutrons, respectively, and are thus “heavier” than \(^{12}\)C, which has 6 neutrons. \(^{12}\)C and \(^{13}\)C are both stable, meaning that they do not undergo radioactive decay, and they account for the majority of carbon on Earth (98.89% and 1.11%, respectively) (Hoefs, 2004). \(^{14}\)C is exceedingly rare (1x10\(^{-12}\) %) and radioactively decays into stable nitrogen (\(^{14}\)N) at a known rate, thus enabling radiocarbon dating (Libby, 1955). Stable isotope analysis is concerned with the relative abundance of non-radioactive isotopes of a given element in a particular substance (e.g., precipitation, soil, plants, and animal tissues).

The chemical behavior of an element is largely determined by its electron structure, and the physical properties of an element are determined by the mass of the nucleus. Differences in mass, particularly among the light elements, affect reaction rates and bond energies, which can lead to isotope effects, whereby isotopes partition among particular phases or chemical substances during chemical reactions (Sharp, 2007). Fractionation is the term used to refer to the process by which products become enriched or depleted in a heavy isotope, and is also used to refer to the magnitude of the shift in the relative abundance of isotopes in reactants and products.

The stable isotopic composition of a compound reflects a history of fractionation processes and thus can be used to learn about the conditions under which the compound formed. The isotopic composition of plants, for example, is the product of fractionation associated with photosynthesis and environmental conditions (Farquhar et al., 1982, 1989), while the isotopic composition of animal tissues is primarily a function of diet and metabolic processes (see below) (e.g., DeNiro and Epstein, 1978, 1981). For example, differences in the isotope ratios of food items at the base of food-webs are passed on to consumers and recorded in their tissues with varying degrees of fractionation during tissue synthesis (e.g., DeNiro and Epstein, 1978, 1981;
Tieszen et al., 1983; Lee-Thorp et al., 1989; Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Fry, 2006; Crawford et al., 2008; Ben-David and Flaherty, 2012). An understanding of fractionation (how isotopes are partitioned) and mixing (how isotopes are combined from different sources) allows researchers to reconstruct aspects of diet and environment by measuring the stable isotope composition of various animal tissues (Fry, 2006).

Stable isotope ratios are expressed relative to internationally accepted standards using the δ-notation in permil units (‰), or parts-per-thousand. Using carbon as an example, δ^{13}C (‰) = \left(\frac{[^{13}C/^{12}C]_{\text{sample}}}{[^{13}C/^{12}C]_{\text{standard}}} - 1\right) \times 1000. For δ^{15}N, the ratios are between ^{15}N and ^{14}N, and for δ^{18}O, the ratio is between ^{18}O and ^{16}O. Positive or negative δ-values signify that the sample has more or less of the heavy isotope relative to the light isotope than the standard. The standard for carbon isotope ratios is Pee Dee Belemnite (PDB), a marine fossil (Belemnitella americana) from the Pee Dee formation of South Carolina. PDB has relatively more ^{13}C than most of the terrestrial biosphere, and therefore terrestrial plants and animals tend to have negative δ^{13}C values. While the PDB standard has been exhausted, there are other available standards that have been calibrated to PDB and can be used as a substitute, one of which is Vienna PDB (VPDB) (Coplen, 1994). The standard for nitrogen isotope ratios (^{15}N/^{14}N, δ^{15}N) is AIR (available inhalable reservoir) and the standard for oxygen isotope ratios (^{18}O/^{16}O, δ^{18}O) can be either PDB or SMOW (standard mean ocean water).

Stable isotope ratios are measured by mass spectrometry (see Sharp, 2007). For δ^{13}C and δ^{15}N measurements of organics, samples are combusted to produce CO₂ and N₂ gas and introduced into a mass spectrometer. The gas is ionized by electron bombardment, focused into a beam, and subjected to a curved magnetic field which forces the lighter molecules to split from the heavier ones and form beams of distinct masses. Ion detectors collect these beams and
record their intensity in voltage, which is proportional to the abundance of each isotope in the sample.

For conventional $\delta^{13}C$ and $\delta^{18}O$ measurements of tooth enamel, CO$_2$ gas is liberated from structural carbonate by phosphoric acid hydrolysis, collected and isolated via cryogenic distillation, and then introduced into a mass spectrometer. $\delta^{13}C$ and $\delta^{18}O$ data of third molar tooth enamel measured in this way are presented in Chapter 4 as part of the Kulubnarti “bulk” tissue stable isotope dataset.

Laser ablation liberates CO$_2$ as well, but incorporates oxygen from all of the oxygen-bearing phases in enamel, which include phosphate (PO$_4^{3-}$) and hydroxyl (OH$^-$) ions in addition to carbonate ions (CO$_3^{2-}$) (see Section 2.7.1). This means that enamel $\delta^{18}O$ values need to be adjusted when comparing data generated by laser ablation and conventional acid hydrolysis. Laser ablation will be discussed in more detail later in this chapter and in Chapter 5.

2.3 Stable Isotopes in Nature

2.3.1 Carbon Isotopes

Most plants (dicot trees, shrubs, temperate grasses) follow the C$_3$ photosynthetic pathway, named for the 3-carbon molecule into which CO$_2$ is initially fixed by the enzyme RuBisCo during the Calvin-Benson cycle. CO$_2$ fixation by RuBisCo discriminates against atmospheric $^{13}$CO$_2$ (-8.0‰) to produce tissue $\delta^{13}C$ values averaging ~-27‰, with values most commonly between -23‰ and -31.5‰ (O’Leary, 1981, 1988; Kohn, 2010).

Most tropical grasses and some sedges have a derived cellular structure (Kranz anatomy) which enables them to concentrate CO$_2$ around RuBisCo to minimize photorespiration and increase photosynthetic efficiency. This alternative photosynthetic pathway is named C$_4$ for the
4-carbon molecule into which CO$_2$ is initially fixed by the enzyme phosphoenolpyruvate (PEP carboxylase) before being delivered to the Calvin-Benson cycle. CO$_2$ fixation by PEP carboxylase discriminates less strongly against $^{13}$C and the subsequent fixation by RuBisCo does not further discriminate against $^{13}$C because all of the delivered CO$_2$ is converted. This results in tissue $\delta^{13}$C values between -11 and -14‰ (Figure 2.1) (O’Leary, 1988; Codron et al., 2005).

Many succulents follow a third photosynthetic pathway, crassulacean acid metabolism (CAM) (Kluge and Ting, 1978), and have intermediate values, or values that are comparable to those of C$_4$ plants (Osmond et al., 1973; Winter, 1979; O’Leary, 1981, 1988). While most domesticated crops are C$_3$, several important staples follow the C$_4$ pathway, including maize, millet, sorghum, and sugar cane.

Carbon isotope variation among C$_3$ plants is controlled largely by environmental factors that influence leaf stomatal conductance and photosynthetic rate, which in turn affect the

![Figure 2.1: Histograms of carbon isotope ratio ($\delta^{13}$C) for C$_3$ and C$_4$ plants (from Cerling et al., 1997).](image)
magnitude of carbon isotope discrimination during photosynthesis (Farquhar et al., 1982, 1989). Stomatal conductance and photosynthetic rate respond strongly to water availability, temperature, and light conditions. The $\delta^{13}C$ values of $C_3$ plants growing in cooler and wetter areas tend to be lower than those growing in warmer, more water stressed environments (Tieszen, 1991; Kohn, 2010). Higher $\delta^{13}C$ values are also found with increasing altitude (~1-3‰/km) (Korner et al., 1991; Sparks and Ehleringer, 1997).

$C_3$ plants growing in forests under dense canopy cover display $\delta^{13}C$ values that are lower than those growing in more open areas due to lower light intensities (Ehleringer et al., 1986) and incorporation of $^{13}C$-depleted $CO_2$ produced by decaying leaf litter (Medina and Minchin, 1980). This “canopy effect” also produces a carbon isotope gradient with height, such that vegetation growing near the ground is more $^{13}C$-depleted than vegetation in the canopy (van der Merwe & Medina, 1989). On average, forest floor vegetation $\delta^{13}C$ values are ~5‰ lower than canopy top vegetation (e.g., Medina and Minchen, 1980; Medina et al., 1991), but the range can exceed 10‰ (e.g., Cerling et al., 2004).

Plant parts also vary in carbon isotope composition with non-photosynthetic tissues (fruit, seeds, stems, wood) typically containing more $^{13}C$ (~1-2‰) than leaves (Codron et al., 2005; Cernusak et al., 2009). Variation can also exist among leaves of the same plant, with young leaves being $^{13}C$-enriched relative to mature leaves (Cernusak et al., 2009). Canopy and plant-part effects are unlikely to play a major role in the carbon isotope values of agricultural communities such as Kulubnarti, but are important to keep in mind if one wishes to make comparisons to populations living in different environments and utilizing different subsistence strategies (e.g., hunter-gather groups).
The carbon isotope ratios in animal tissues reflect those in foods plus some additional fractionation (DeNiro and Epstein, 1978), such that, for example, grazing animals in C4 biomes can easily be distinguished from browsing animals via the $\delta^{13}C$ values of their tissues (Vogel, 1978). Lower $\delta^{13}C$ values have been detected in forest dwelling animals due to the canopy effect (Ambrose and DeNiro, 1986; van der Merwe and Medina, 1991; Cerling et al., 2004). This effect has also been observed in humans (van der Merwe and Medina, 1991).

The carbon isotopic compositions of different animal tissues are fractionated with respect to dietary inputs. The carbon isotope composition of bone and tooth apatite reflects that of whole dietary carbon with relatively high fidelity (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004), but there is considerable variation among species in the amount of diet to apatite isotope fractionation. Enrichments range from $\sim$9-10‰ for rodents (Ambrose and Norr, 1993; Tieszen and Fagre, 1993) to $\sim$14‰ for ruminants (Cerling and Harris, 1999), with most mammals averaging $\sim$12-13‰ (Krueger and Sullivan, 1984; Lee-Thorp et al., 1989; Passey et al., 2005). Interspecific differences are likely caused, in part, by differences in digestive physiology, such as methanogenesis in ungulates (Hedges, 2003; Passey et al., 2005). Diet to enamel carbon isotope enrichment in primates has been reported at $\sim$13‰ (Cerling et al., 2004) and an enrichment of $\sim$12-13‰ may be appropriate for humans as well. The uncertainty in this value for humans is not a significant problem here, because this dissertation focuses on patterns within and between individuals of a single site.

In contrast, the isotopic composition of proteinaceous tissues, such as bone collagen and hair, is largely controlled by that of dietary protein (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; O’Connell and Hedges, 1999). Collagen has $\delta^{13}C$ values that are typically $\sim$5‰ higher than dietary protein, but the magnitude of diet to collagen fractionation can vary due to the
macronutrient composition of the diet as well as the nutritional requirements (Schwarcz, 1991; Podlesak and McWilliams, 2006). Hair, a tissue comprising primarily the protein keratin, has $\delta^{13}C$ values ~3‰ higher than dietary values and, like collagen, also reflects the protein component of the diet to a significant extent (Nakamura et al., 1982; O’Connel and Hedges, 1999; Sponheimer et al., 2003a; Petzke et al., 2005).

The combustion of fossil fuels has decreased the $\delta^{13}C$ value of atmospheric CO$_2$ by ~1.5‰ since the late 19th century, and an offset must be taken into account when comparing $\delta^{13}C$ values of modern humans or other animals to older specimens (Friedli et al., 1986; Long et al., 2005).

2.3.2 Nitrogen Isotopes

Nitrogen isotope variation among plants and animals is more complex than carbon isotope variation. Geographic patterns in $\delta^{15}N$ values of soils and plants are controlled by environmental factors that affect fractionation during the nitrogen cycle, by influencing nitrogen availability, water availability, and the pathways and magnitude of nitrogen loss (Amundson et al., 2003). Soil $\delta^{15}N$ values tend to exceed atmospheric N$_2$ (0‰) because $^{14}N$ is preferentially lost from soil via leaching, ammonia volatilization, and denitrification. Ecosystems in which $^{14}N$ loss is high relative to plant-soil nitrogen cycling have high soil and foliar $\delta^{15}N$ values, and have been characterized as having “open” nitrogen cycles (Austin and Vitousek, 1998). On a global scale, nitrogen isotope ratios of plants generally increase with decreasing precipitation and increasing temperature, reflecting increasing openness of the nitrogen cycle, (Heaton et al., 1987; Handley et al., 1999; Amundson et al., 2003; Hartmann, 2011). Open nitrogen cycles (and high $\delta^{15}N$ values) can also occur, however, in tropical forest environments where nitrogen is highly
available or in excess (Martinelli et al., 1999). Heavy natural grazing or anthropogenic habitat alteration via domestic grazing, cultivation, and use of organic fertilizers can elevate $\delta^{15}$N values in soils and plants (Bateman and Kelly, 2007; Aranibar et al., 2008).

Leguminous plants tend to have lower $\delta^{15}$N values than non-legumes because they derive nitrogen from symbiotic bacteria that directly fix atmospheric nitrogen with no modification of the $^{15}$N/$^{14}$N ratio in air (0‰) (Virginia and Delwiche, 1982). The amount of nitrogen derived from N$_2$-fixation in legumes, however, is subject to environmental influences, and thus legumes do not always have significantly lower $\delta^{15}$N values than non-N$_2$-fixing plants (Kohl et al., 1980; Shearer et al., 1983; Handley et al., 1994).

The nitrogen isotope composition of animal tissues reflects that of diet (DeNiro and Epstein, 1981). Like plants, there is a tendency for herbivore $\delta^{15}$N values to correlate negatively with rainfall (e.g., Heaton et al., 1986; Sealy et al., 1987; Cormie & Schwarcz, 1996; Grocke et al., 1997), but this relationship is non-linear and driven largely by the arid end of the spectrum (e.g., Pate and Anson, 2008). This pattern was originally posited to result from physiological mechanisms in animals adapted to arid environments (Sealy et al., 1987; Ambrose, 1991], but recent research suggests that variable foliar $\delta^{15}$N may explain more of the variation in herbivore $\delta^{15}$N than previously thought (Murphy and Bowman, 2006; Crowley et al., 2011; Hartman, 2011). However, these studies do not exclude the possibility of physiological influences on nitrogen isotope fractionation.

Animal tissues are $^{15}$N-enriched relative to diet, and a step-wise $^{15}$N-enrichment of ~3-5‰ with trophic level is well documented (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984). However, diet-tissue nitrogen isotope discrimination is also highly variable, and additional factors influence nitrogen isotope variation in animals.
(reviewed in Martinez del Rio et al., 2009). In a controlled study, for example, herbivores fed identical diets had diet-hair discrimination factors that varied by 3.6‰, equivalent to a trophic level in many cases (Sponheimer et al., 2003b).

Lower nitrogen isotope discrimination between diet and tissue has been observed with increasing trophic level as protein quality (i.e. biological value, Robbins, 1993) increases (Roth and Hobson, 2000; Robbins et al., 2005). However, this pattern is not universal, particularly within single taxa or trophic levels, as higher nitrogen isotope discriminations have been observed with increasing dietary protein quantity (e.g., Bearhop et al., 2002; Pearson et al., 2003; Sponheimer et al., 2003b; Vanderklift and Ponsard, 2003; but see Balter et al., 2006), and no effect occurred in rats fed diets of varying protein content (Ambrose, 2000). Much of this discrepancy may result from the degree to which dietary protein satisfies, exceeds, or falls short of protein requirements, which causes variability in nitrogen balance and rates of nitrogen assimilation and excretion (McCutchan et al., 2003; Sponheimer et al., 2003b,c; Vanderklift and Ponsard, 2003; Robbins et al., 2005; Barboza and Parker, 2006).

If a diet is extremely low in protein, an animal may enter negative nitrogen balance and begin to catabolize its own tissues. Nutritionally stressed birds have high δ¹⁵N values (Hobson and Clark, 1992; Hobson et al., 1993; Voigt and Matt, 2004; Cherel et al., 2005), but this effect likely emerges only beyond a certain threshold (Kempster et al., 2007), and in only some cases (Ben-David et al., 1999). Relatively high δ¹⁵N values may not always be observable during fasting or episodes of nutritional stress, however, due to variation in rates of tissue synthesis among different organs during periods of inadequate dietary protein intake (Hobson et al., 1993; Cherel et al., 2005). As a result, some tissues may become ¹⁵N-enriched while others may not (e.g., Doucett et al., 1999). Hair appears to reflect periods of nutritional stress in humans. This
is evidenced by higher hair $\delta^{15}N$ values in people suffering from anorexia nervosa (Mekota et al., 2006) and pregnant women undergoing episodes of weight loss (Fuller et al., 2005). There is also evidence that $\delta^{15}N$ is elevated in humans suffering from other pathological conditions, such as those resulting in osteopenia and osteomyelitis (White and Armelagos, 1997; Katzenberg and Lovell, 1999).

Nitrogen isotope discrimination between diet and tissue may also decrease with higher protein assimilation relative to protein loss (i.e. during growth) (Hobson et al., 1993; Fantle et al., 1999; Trueman et al., 2005; Warinner and Tuross, 2010; but see Ponsard and Averbuch, 1999). Pregnancy, lactation, and weaning also contribute to nitrogen isotope variation in animals, but not always consistently (see Section 2.6.2) (Fogel et al., 1989; Jenkins et al., 2001; Fuller et al., 2004, 2006a). Thus, $\delta^{15}N$ values in humans and other animals are the product of the complex interplay among dietary, environmental, and physiological variables, many of which remain poorly understood. Nonetheless, recent models have made progress elucidating the mechanisms that drive diet-tissue nitrogen isotope discrimination (e.g., Martinez del Rio and Wolf, 2005; Hedges and Reynard, 2007).

2.3.3 Oxygen Isotopes

The oxygen isotope composition ($\delta^{18}O$) of animal tissues reflects that of body water, which is ultimately controlled by a complex interplay between climate, geography, diet, and physiology (Longinelli, 1984; Luz et al., 1984; Alyliffe and Chivas, 1990; Bryant and Froelich, 1995; Kohn, 1996; Kohn et al., 1996). The primary sources of body water oxygen are atmospheric O$_2$, drinking water, water in food, and to a lesser extent, oxygen bound in the macronutrients of food. Atmospheric O$_2$ is relatively constant, and thus does not cause
variability in the oxygen isotope composition of animal tissues (Dole et al., 1954). The isotopic composition of meteoric water, on the other hand, is subject to temperature and precipitation effects and is thus highly variable (Dansgaard, 1964).

Isotopically light water (H₂O¹⁶) evaporates more readily than heavy water (H₂O¹⁸), resulting in geographic and seasonal patterning in the isotopic composition of precipitation and other meteoric waters. Precipitation becomes depleted in ¹⁸O with distance from the ocean, and rivers become enriched in ¹⁸O with distance from their sources (Craig, 1961). Evaporative enrichment also elevates the δ¹⁸O of leaf water relative to meteoric water, and animals that obtain a high proportion of their water from leaves therefore have higher δ¹⁸O values than animals that must frequently drink (Kohn et al., 1996; Sponheimer and Lee-Thorp, 2001). These differences mean that the oxygen isotope values of some animals more closely mirrors local waters, while those of others are sensitive to climatic factors that affect evaporation, such as aridity (Levin et al., 2006).

While tissue δ¹⁸O correlates with that of body water, it is fractionated with respect to drinking water (Longinelli, 1984; Luz et al., 1984). As a result, breast milk has higher δ¹⁸O values than drinking water, and nursing infants therefore have higher δ¹⁸O values than their mothers (Wong et al., 1987; Roberts et al., 1988; Bryant and Froelich, 1996). Animal δ¹⁸O is also controlled by the mechanism of water loss (Wong et al., 1988; Sponheimer and Lee-Thorp, 2001) and the magnitude of total water flux (O’Grady et al., 2010), which can both be considered a function of body size (Bryant and Froelich, 1995). The sensitivity of animal δ¹⁸O values to this suite of variables makes them ideal for tackling a number of questions about climate and animal ecology in the past (Koch, 1998). Most of the stable oxygen isotope research on archaeological humans utilizes δ¹⁸O values to reconstruct the weaning process (e.g., Wright and Schwarcz,
1998; Durpas and Tocheri, 2007), or to identify individuals of non-local origin (e.g., White et al., 1998, 2002; Evans et al., 2006).

2.4 Tissue Turnover

The isotopic compositions of different tissues reveal aspects of diet, habitat, and climate at different time scales with respect to an animal’s life span (Phillips and Eldridge, 2006; West et al., 2006). Given its slow rate of turnover, the isotopic composition of bone collagen represents an average of many years. Hedges et al. (2007) estimated turnover rates in human femoral midshaft collagen using bomb 14C content at 5-15% per year for adolescents and 1.5-4% per year for adults. The isotopic composition of hair represents dietary protein intake over a much shorter time scale, on the order of weeks or months (depending on the animal and sampling protocol) (Jones et al., 1981; O’Connell & Hedges, 1999; Ayliffe et al., 2004).

Much like hair, dental tissues are metabolically inert once deposition and mineralization processes are complete, and thus their isotopic compositions represent dietary inputs over the formation period of the tooth. Dental development in humans begins with the development of the deciduous dentition in utero and ends with the closure of the root apex of the third permanent molar around age 20 (Hillson, 1996). Because dental tissues do not turnover, they have the additional advantage of retaining time-series dietary input information allowing incremental analyses to be performed and profiles of isotopic change to be generated (see Section 2.7.2) (e.g., Koch et al., 1989; Fricke and O’Neil, 1996; Balasse et al., 2001; Passey and Cerling, 2002; Stevens et al., 2011).
2.5 Diagenesis

An important issue with any stable isotope study is diagenesis, the process by which original biogenic signals are obscured or even replaced by post-depositional chemical and structural changes and molecular loss (e.g., Nelson et al., 1986; Wang and Cerling, 1994; Nielsen-Marsh and Hedges, 2000a,b). This is a much more significant problem for specimens of greater antiquity than those being analyzed in this dissertation, but diagenesis can vary greatly by site, and the issue will be briefly addressed here.

Bone undergoes structural and chemical alteration over time through processes that are largely determined by hydrological conditions of the depositional environment (Nielsen-Marsh and Hedges, 2000a). During burial, bone is subject to a series of processes including microbial attack, ionic exchange, and mineral leaching and infilling (Collins et al., 2002; Hedges, 2002). Through these processes, bone apatite can be severely altered and collagen breaks down and is ultimately lost. Both the addition of exogenous materials and the removal of the original biological material adversely affect the isotopic composition of both bone collagen and apatite (e.g., Nelson et al., 1986; Ambrose, 1990; Wand and Cerling, 1994; Wright and Schwarcz, 1996).

Several quality control measures are used to assess the preservation of bone collagen and the reliability of $\delta^{13}$C and $\delta^{15}$N values. The two most commonly employed are the C:N ratio and collagen yield (DeNiro, 1985; Ambrose, 1990). Collagen with a C:N ratio outside the range of 2.9-3.6 and a yield of <2% is considered unreliable for stable isotope analysis (DeNiro, 1985; Ambrose, 1990). Enamel is less susceptible to diagenesis because of its large crystal size and low porosity (LeGeros, 1981). While enamel is not immune to diagenetic alteration (e.g., Sponheimer and Lee-Thorp, 1999a; Schoeninger et al., 2003), it often retains biogenic patterning.
for millions of years (e.g., Lee-Thorp and van der Merwe, 1991; Quade et al., 1992; Bocherens et al., 1996; Sponheimer and Lee-Thorp, 1999b; Lee-Thorp, 2000; Zazzo et al., 2000).

2.6 Stable Isotope Analysis in Archaeology

2.6.1 Archaeological Applications

Stable isotope analysis was applied to an archaeological question for the first time by Vogel and van der Merwe (1977) and van der Merwe and Vogel (1978). By analyzing the carbon isotope composition of bone collagen from New York and the U.S. Eastern Woodlands, these researchers demonstrated a rapid shift in subsistence from a purely C$_3$ diet, to one dominated by maize, a C$_4$ domesticate in the space of about 200yrs. This not only established the utility of the technique, but demonstrated how useful it could be for directly testing hypotheses that are difficult to test with other forms of archaeological evidence.

This approach was soon applied to archaeological populations in other parts of North America (e.g., Schwarcz et al., 1985; Buikstra et al., 1987, 1988; White and Schwarcz, 1989; Ezzo, 1993; Katzenberg et al., 1995; Wright and White, 1996). The carbon and nitrogen isotope composition of bone collagen was also found to distinguish populations that consumed marine foods, which are $^{13}$C and $^{15}$N-enriched relative to terrestrial trophic chains (Tauber, 1981; Chisholm et al., 1982; Schoeninger et al., 1983; Sealy and van der Merwe, 1986; Lubell et al., 1994; Richards and Hedges, 1999; Richards et al., 2006). Carbon and nitrogen isotope analysis of bone collagen has also revealed differences in diet by social status and sex, which speaks to cultural and political conditions that limit or grant access to certain foods by specific groups of people (e.g., White et al., 1993; Ubelaker et al., 1995; Privat and O’Connell, 2002; Reitsema and Vercellotti, 2012).
Oxygen isotope ratios in enamel and bone apatite have been used to identify non-local individuals in archaeological populations, which aids in reconstructing levels of human mobility and patterns of migration (e.g., Dupras and Schwarcz, 2001; Evans et al., 2006; White et al., 1998, 2002, 2004; Buzon and Bowen, 2010). Recent attention has been given to combining data from multiple isotopes and tissues to gain additional power in dietary reconstruction (e.g., Sealy et al., 1995; Kellner and Schoeninger, 2007; Froehle et al., 2012; Loftus and Sealy, 2012).

Another major focus of stable isotope research in archaeology, and one that is particularly relevant to this dissertation, is the study of weaning and childhood diet in past populations. This research focus began with a study by Fogel et al. (1989) that showed that breast feeding infants have higher $\delta^{15}$N values than their mothers. The following section introduces these studies.

### 2.6.2 Weaning Applications

Infants are at a higher trophic level than their mothers when breast milk is their sole nutrient source, and as such their $\delta^{15}$N is $\sim$2-3‰ higher than that of their mothers (Fogel et al., 1989; Fuller et al., 2006a). This led to numerous applications to reconstruct weaning duration in archaeological populations (Katzenberg et al., 1993; White and Schwarcz, 1994; Katzenberg and Pfeiffer, 1995; Schurr, 1997, 1998; Herring et al., 1998; Wright and Schwarcz, 1999; Dupras et al., 2001; Mays et al., 2002; Richards et al., 2002; Fuller et al., 2003, 2006b; Schurr and Powell, 2005; Clayton et al., 2006; Fuller et al., 2006b; Dupras and Tocheri, 2007; Turner et al., 2007; Jay et al., 2008; Pearson et al., 2010; Nitsch et al., 2011).

The great interest this new approach generated in anthropology is not surprising because early childhood health and nutrition are also important determinants of a population’s demographic structure through their effects on growth and development, fecundity, morbidity,
and mortality (Knodel and Kintner, 1977; Stuart-Macadam and Dettwyler, 1995; Katzenberg et al., 1996; Vitzthum, 1994). Peak human mortality coincides with weaning and therefore overcoming this period of stress is one of the most important hurdles in human life history (Katzenberg et al., 1996). This is due largely to the exposure of infants to pathogens in extra-maternal food and water sources and the concurrent reduction in the immunological protection against exogenous pathogens provided by breast milk.

Lactation also plays an important role in suppressing ovulation, and the cessation of breastfeeding coincides with a return to fecundity (Ellison, 1995). As such, the timing and duration of the weaning process has a strong effect on fertility and inter-birth interval. The process of milk production and the nutritional content of milk are highly buffered against environmental perturbations yet the low relative daily cost of lactation leaves room for variation in the behavioral adaptations necessary to meet these costs in diverse environmental and social contexts (Ellison, 2001; Dufour and Sauther, 2002). The duration of breastfeeding and the types and quality of supplementary and post-weaning diets are shaped by cultural beliefs and practices, and socioeconomic forces, and thus display considerable variation within and between populations (Launer and Habicht, 1989; Stuart-Macadam, 1995; Quandt, 1995). Modern human life history is also unusual among primates in that human infants are weaned at a relatively young age (Kennedy, 2005). Chimpanzees, for example are weaned at approximately 5 years of age, while modern humans in non-industrial, non-western societies wean much earlier, most commonly between ~2.5 years and 3 years, and western societies typically breastfeed for an even shorter duration (~6-9 months) or not at all (Dettwyler, 1995). Understanding how and why humans developed this unique life history strategy is significant goal of anthropology (e.g., Bogin and Smith, 1996; Mace, 2000; Kennedy, 2005; Humphrey, 2010).
The nitrogen isotope approach to reconstructing weaning usually relies on the relationship between bone collagen $\delta^{15}$N and age at death. $\delta^{15}$N values typically begin higher than adult values, followed by a period of decline. The age at which they reach adult $\delta^{15}$N values is usually interpreted as the cessation of breastfeeding or the completion of weaning. There is some evidence that $\delta^{13}$C is also elevated in breastfeeding infants due to a trophic effect (Fuller et al., 2003, 2006a). Elevated $\delta^{13}$C values in infants and children in parts of the world with C$_4$ agriculture are sometimes interpreted as the inclusion of grain-based gruel in the diets of weanlings (e.g., Wright and Schwarcz, 1999).

Interpreting weaning from bone collagen, however, is not straightforward. There is considerable variation in $\delta^{15}$N among infants and young children (e.g., White and Schwarcz, 1994; Dupras et al., 2001; Fuller et al., 2006b; Jay et al., 2008; Nitsch et al., 2011). Variability in diet and nutritional status among mothers, physiological effects associated with growth rate, and the nutritional adequacy of milk and supplementary foods could all be playing a role.

There is also a particular danger in reconstructing a population’s weaning behaviors from individuals that died during breastfeeding or the weaning process, especially considering the close association between the dangers surrounding weaning and infant mortality. The problem of mortality bias is the reality that a skeletal assemblage is made up of those that died and therefore may not be representative of the living population. This effect is probably the most severe for infants and young children.

Mortality bias, and other potential problems associated with interpreting how a population lived from its skeletal remains has been dubbed the osteological paradox (Wood et al., 1992). The analysis of dental tissues circumvents these potential pitfalls, because teeth preserve information about childhood diet in individuals who survived the weaning process.
(Wright and Schwarcz, 1998, 1999; Dupras and Tocheri, 2007). Wright and Schwarcz (1998) found higher δ¹⁸O values in first molar enamel than in later forming premolars and third molars, which they interpreted as evidence of weaning from ¹⁸O-enriched breastmilk to isotopically lighter sources of water. Dupras and Tocheri (2007) found a similar pattern between deciduous and permanent teeth, but the teeth were not analyzed as pairs from the same individuals. In both studies the magnitude of change in δ¹⁸O was small (<1.0‰).

The interpretation of δ¹³C in enamel and dentine in these studies is complicated by the fact that each population had C₄ staples, which may or may not have been included in supplementary foods during weaning. Both show an increase in δ¹³C values of enamel between early and later forming teeth, but this shift was also small (<1‰). Much of the noise in the data is probably because the formation times of the teeth overlap. The effects of seasonality in δ¹³C and δ¹⁸O inputs could also be playing a role. Sampling methods that achieve higher temporal resolution by generating stable isotope profiles along single teeth are now available and are just beginning to be used in archaeology (Eerkens et al., 2011; Beaumont et al., 2012). The next section discusses high resolution stable isotope methods in enamel and dentine.

2.7 High Resolution Stable Isotope Analysis

2.7.1 Intra-tooth Enamel Analysis

Intra-tooth stable isotope analysis of mammalian tooth enamel has proven to be a useful tool for studying short-term and seasonal changes in climate, diet, and physiology in the past (Fricke and O’Neil, 1996; Kohn et al., 1998; Wiedemann et al., 1999; Gadbury et al., 2000; Balasse et al., 2002; Zazzo et al., 2002; Franz-Odendaal et al., 2003; Nelson, 2005; Martin et al., 2008a; Stevens et al., 2011; Souron et al., 2012). Comparisons of dental developmental
schedules and δ^{18}O records of seasonality in enamel have also enabled determinations of seasonality of birth in sheep (Balasse et al., 2003, 2012; Blaise and Balasse, 2011) and weaning (Fricke and O’Neil, 1996; Frans-Odendall et al., 2003). The teeth of large mammals such as equids, bovids, and cervids, are large enough to facilitate sequential sampling using a handheld drill while achieving enough resolution to see smooth, sinusoidal changes associated with seasonality (e.g., Blaise and Balasse, 2011).

Laser ablation is a relatively new technique that enables carbon and oxygen isotope measurements of enamel in situ (Cerling and Sharp, 1996; Sharp and Cerling, 1996; Sharp et al., 2000) and recent methodological improvements have reduced the necessary sample size enough to enable intra-tooth profiling of very small teeth like those of rodents and rabbits (Passey and Cerling, 2006). The method is also ideal for measuring valuable museum specimens where minimizing sample destruction is imperative. Laser ablation has been used to study variability in early hominin teeth (Sponheimer et al., 2006), but its use is not yet widespread.

One significant problem with the interpretation of intra-tooth isotope profiles in enamel is isotope attenuation. The timing and rate of enamel deposition can be determined with great accuracy through microstructural studies (e.g., Dean and Scandrett, 1996; Reid and Ferrel, 2006), but the process by which it mineralizes is more difficult to ascertain because it is progressive, discontinuous, and does not always proceed parallel to depositional fronts (Robinson et al., 2981; Suga, 1982). The isotopic composition of mature enamel reflects this period of mineralization, rather than solely the initial timing of deposition (Balasse, 2002; Passey and Cerling, 2002). In an experimental study with cattle, Balasse (2002) found that an abrupt diet switch from C_3 to C_4 foods was recorded in enamel that was laid down as much as six months before the switch actually occurred. Furthermore, the diet switch appeared to be much more gradual in the isotope
profile than it actually was. Models have been developed that successfully retrieve the primary input signal from stable isotope profiles in enamel in ever-growing teeth (Passey and Cerling, 2002; Passey et al., 2005), but these models are not suitable for human teeth which have different growth and maturation parameters. Sampling strategy and location also affect the degree to which stable isotope profiles are attenuated.

Because of its relatively high initial mineral content, enamel close to the EDJ may retain more of the primary input signal than enamel closer to the surface (Balasse, 2003; Zazzo et al., 2005; Tafforeau et al., 2007). Zazzo et al. (2005) found that the isotopic composition of enamel at the EDJ of experimental steer teeth more closely approximated the input signal than enamel sampled in other locations, even if the input signal was irregular (non-sinusoidal).

Wright (2012) recently performed intra-tooth stable isotope analysis on human permanent first molars and canines, but only obtained three samples per tooth. She found that the intra-tooth patterns were similar to intertooth patterns from a previous study at the same site (Wright and Schwarcz, 1998), but were more variable and less straight-forward to interpret, and argued that seasonality may be playing a role, particularly for $\delta^{18}$O. She noted that an increased number of samples per tooth would be helpful in evaluating weaning behavior from stable isotope profiles (Wright, 2012).

2.7.2 Intra-tooth Dentine Analysis

Sequential sampling of dentine has also been performed on animals to reconstruct short-term dietary changes and weaning behavior (Koch et al., 1989; Hobson and Sease, 1998; Balasse et al., 2001; Balasse and Tresset, 2002; Zazzo et al., 2006). Dentine forms as a series of stacked cones, and is thus susceptible to cross-sampling of histological features, causing a reduction in
temporal resolution (Balasse et al., 2001; Zazzo et al., 2006). Dentine serial sections were first applied to archaeological humans by Fuller et al. (2003). They found a clear pattern of decreasing $\delta^{15}$N in three successive sections of deciduous second molar dentine, and interpreted the result as indicative of complete weaning at $\sim$2 years of age. No consistent pattern was found in permanent canines, largely because their sampling strategy averaged most of the dentine formed during the first several years of life.

Higher temporal resolution has been recently achieved (Eerkens et al., 2011; Beaumont et al., 2012). Eerkens et al. (2011) obtained 5-10 samples per first permanent molar and found variability in the patterns of $\delta^{15}$N among the teeth. In comparison to adult $\delta^{15}$N values, they argued that this was indicative of variable weaning practices at the site (prehistoric California). Taking a similar approach, Beaumont et al. (2012) also found variability in $\delta^{15}$N patterning, which may be related to variable weaning behavior or nutritional stress associated with famine in 19th century London. Very little variability was observed in the $\delta^{13}$C profiles, save for one individual in the London sample who likely began consuming C$_4$ or marine foods. These studies represent a significant increase in our ability to reconstruct dietary change in the lives of individuals who survived childhood. Neither study, however, compared the intra-tooth data to cross-sectional data or to markers of stress to investigate the potential role weaning played in mortality and morbidity patterns.

### 2.8 Direction of this Research

Human teeth can be a rich source of information about dietary change throughout a substantial portion of the human life span. While recent research has advanced our ability to coax valuable information about the past from the teeth of animals, much remains unknown
regarding how to best accomplish this in humans. Accordingly, there are several methodological questions that need to be addressed. In this dissertation, I use laser ablation stable isotope analysis to generate high resolution $\delta^{13}C$ and $\delta^{18}O$ profiles in archaeological human teeth for the first time.

I take advantage of the great spatial control of the system to compare stable isotope profiles at the EDJ and at the outer enamel surface (OES) to evaluate how these profiles differ in human teeth. One expectation is that the two profiles are similar at a mean level, but the EDJ profiles have more variability. Because first molars begin forming at birth, these teeth provide an excellent opportunity to explore the interplay between breastfeeding, weaning, and seasonality. I also use a sampling method similar to Beaumont et al. (2012) to generate high resolution profiles of $\delta^{13}C$ and $\delta^{15}N$ in dentine. As we will see in the next two chapters, the community of Kulubnarti had both C$_3$ and C$_4$ cultigens. One question to be addressed is how this is manifest in $\delta^{13}C$ profiles in human dentine. I will also compare the high resolution profiles to a large cross-sectional dataset to explore the relationship between mortality and weaning behavior.

Another approach of this dissertation is to compare two different tooth types from the same individuals, permanent first molars and canines, to investigate how profiles generated in each type correspond to one another. These comparisons will provide insights to guide future research. The next two chapters introduce the site of Kulubnarti (Chapter 3) and present previously published and new “bulk tissue” stable isotope data for the site (Chapter 4). These chapters provide some expectations for the high resolution analyses.
CHAPTER 3
BACKGROUND TO KULUBNARTI

3.1 Chapter Overview

This chapter locates the site of Kulubnarti in space and time, provides the archaeological context of the Kulubnarti, and describes what is known about the demographics and health of this population from an extensive body of biological anthropology research. The most salient features of the Kulubnarti human remains are high levels of subadult mortality and stress and consistent health differences between two distinct communities at Kulubnarti. This chapter highlights several lines of evidence that converge on these conclusions; mortality profiles, patterns of cribra orbitalia, the distribution of linear enamel hypoplasia, and patterns of osteopenia at Kulubnarti.

3.2 Introduction

The archaeological site of Kulubnarti is an island located on the Nile River in Sudanese Nubia approximately 80 kilometers south of Wadi Halfa and the border with Egypt. It was first surveyed, mapped, and excavated in 1969 by William Adams and coworkers from the University of Kentucky at the end of the UNESCO Nubian Salvage Campaign of 1959-1969 ahead of the construction of the Aswan High Dam (Adams, 1970 in Van Gerven et al., 1981).

Kulubnarti, meaning “island of Kulb,” was a small hamlet on an island across from the west bank of the modern village of Kulbincoing, which now sits at the southern edge of Lake Nubia (Lake Nassar in Egypt). Before the Aswan High Dam was built, Kulubnarti was an island
for only a short period each year, when the Nile reached its peak flood height. For the remainder of the year, Kulubnarti protruded into the Nile from the west bank.

Excavations in 1969 revealed that Kulubnarti was a Christian Period settlement that was likely occupied beginning around 550 C.E., when Nubia initially adopted Christianity. The site appears to have been continuously occupied to the present and may have been home to some of the last Christians in Nubia. Two cemeteries, one on the island and another on the west bank, were excavated in 1979 by Dennis Van Gerven and coworkers from the Universities of Colorado and Kentucky. Four hundred six individuals were disinterred and are housed at the University of Colorado. The following sections describe Nubia and the subregion where Kulubnarti is located, the time period, and the human remains in more detail.

3.3 Geographic and Archaeological Context

3.3.1 Nubia

Nubia is the land of the six cataracts (rapids) of the Nile between Aswan in Egypt and the modern Sudanese capital of Khartoum at the confluence of the Blue and White Nile Rivers. As with much of the Nile river valley in North Africa, Nubia stretches only as far from the Nile as there is arable land to till, which is no more than one or two kilometers, and is often much less (Adams, 1977). Nubia is traditionally broken up into upper and lower halves. Lower Nubia is the region between the First and Second Cataracts in southern modern-day Egypt. Upper Nubia extends south from the Second Cataract into Sudan (Figure 3.1).

Nubia has been referred to as “the corridor to Africa” because the Nubian Nile was the only connection between the cultures of the Mediterranean and greater Eurasia and those of sub-
Figure 3.1: Map of Nubia showing the Nile, the location of the six cataracts, and major sites, and the location of Kulubnarti. Lower Nubia is the region between the 1st cataract and the 2nd cataract near Wadi Halfa. The Batn el Hajar is the region between the 2nd and Dal cataracts. Upper Nubia (not labeled) is the region of the Nile between the Dal cataract and Khartoum, located at the confluence of the Blue and While Niles (not labeled) at the bottom of the map.

Saharan Africa for most of prehistory (Adams, 1977). Until the development of the trans-Saharan caravan trade in the last millennium B.C.E, Nubia was the only link between these two worlds and remained the major connection until sea routes became used in the seventeenth century. The narrow green ribbon of the Nile Valley was a passageway of people, goods, and ideas and the cultural and ethnic character of the Nubian people has been influenced by this long history of cultural movement and exchange.

As the southern neighbor to Egypt, the Nubian people have been described as the distinctly non-Egyptian people south of Aswan who have had close political, cultural, and
economic ties with Egypt since the Neolithic (Adams, 1977). As a people between two worlds, the study of Nubian culture and biology throughout prehistory traces the scientific history of changing paradigms in physical anthropology and archaeology from racial determinism and biological diffusion to biocultural adaptation and in situ evolution (Carlson and Van Gerven, 1979; Armelagos and Van Gerven, 2003).

3.3.2 The Batn el Hajar

The site of Kulubnarti is located in the Batn el Hajar, or “belly of rock”. This region extends for 100 miles from the Second Cataract south to the Dal Cataract. Here, the Nile courses through unnavigable granite rapids rather than the sandstone characteristic of Lower Nubia to the north and Upper Nubia to the south, and thus the Batn el Hajar acts as a natural barrier between the two regions. Adams described the Batn el Hajar as the most barren and forbidding of all Nubian environments (1977:26). Temperatures can vary by 17°C in any season and reach a mean high of 32°C in July and a mean low of 16°C in January.

With no appreciable rainfall, agriculture today is only possible on alluvium gradually exposed as the Nile recedes from its annual flood in a process called seluka cultivation (Zarroug, 1991). Retaining walls were often built to capture alluvium. The small channel separating the sometime island of Kulubnarti and the mainland is farmed in this manner (Adams et al., 1999). In other parts of the Nubian Nile Valley, small fields are irrigated with a hand or animal powered water wheel called a saqia or a lever and bucket system called a shaduf, but the steep and rocky river banks in the Batn el Hajar make this form of irrigation problematic and rare. As such, the Batn el Hajar is sparsely populated today. Archaeological evidence suggests that it never supported large populations, and the villages it did support were rather impoverished (Adams,
Indeed, there is little natural or cultivated vegetation for long tracts of the Nile in this region.

For the small hamlets of the Batn el Hajar today, the summer crops are sorghum and millet, both C₄ plants, and are harvested in June. The main harvest of the winter crops occurs in April and consists of barley, wheat, legumes, lentils, peas, and dates (C₃ plants), and there is a mixed summer/winter crop harvest in October (Daffala, 1969). Domesticated animals such as goats, cattle, sheep, and pigs are kept in small numbers (Adams, 1977), but animal meat is uncommon in the diet (May, 1961). Archaeological data from this region suggests that the agricultural and climatic conditions have remained relatively unchanged for over 1000 years (Adams, 1977).

The Nile provides the only source of drinking water, which is often cooled and stored in clay basins called zeers (Adams, 1977). The annual flood of the Nile begins in July and reaches its peak in September (Eltahir, 1996), but also fluctuates widely in flood levels over annual and much longer time scales (Hassan, 1981; Jiang et al., 2002). For example, Jiang et al. (2002) found that the period AD 759-848 had significantly lower flood levels than the preceding period AD 622-758.

3.3.3 Kulubnarti during the Christian Period

Christianity arrived in Nubia from Egypt by about 550 C.E., and by the 7th century Upper and Lower Nubia were united as the Christian Kingdom of Makouria (Adams, 1977). Egypt did not remain Christian for long however, and by the early 7th century Egypt succumbed to Arab Islamic conquest. Arab armies penetrated south to Dongola, the capitol of Christian Nubia, but
were defeated. A treaty known as the *Baqt* was enacted in 651 C.E. which established Lower Nubia as a free-trade zone with Egypt (Hasan, 1967).

The geography of the *Batn el Hajar* made it a natural and political buffer zone between the two kingdoms. During the 11th and 12th centuries, the *Batn el Hajar* experienced an influx of northern populations, but the reason for this migration remains unclear (Adams, 1977). In the 13th century, a group called the Mamelukes took power in Egypt and created a period of political and religious upheaval. At this point, Christian villages were common in the *Batn el Hajar* which may have acted as a refuge area for Christian communities from Lower Nubia. This transition is evidenced by a shift in the architecture style in the region from one dominated by Medieval churches to one characterized by defensive castles and two-story houses (Adams, 1977). In 1323, the Christian King at Dongola was replaced by a Muslim prince and Christianity was no longer the official religion of Nubia. However, Christianity continued in the extreme isolation of the *Batn el Hajar* until perhaps the 16th century (Adams, 1977).

Archaeological evidence suggests that Kulubnarti was occupied continuously from approximately 550 C.E. to the present, and was likely one of the last Christian villages in Nubia (Adams, 1977; Adams et al., 1999). Artifacts diagnostic of the Early Christian, Classic Christian, and Late Christian Periods were found in various habitation sites and church ruins across the island and west bank. A Late Christian Period castle that is still in use sits atop a rocky outcrop on the mainland (Figure 3.2). Archaeological details can be found in the work of Adams (1977) and Adams and colleagues (1999).

The human remains from Kulubnarti originated from two cemeteries excavated in 1979, one located on the island and the other on the mainland next to the modern village. The island
cemetery (21-S-46, the “S” cemetery) was dug into a dry wadi and began with pre-Christian graves before transitioning to Early Christian style graves, which were identified on the basis of orientation, body positioning, and burial shroud textiles. 218 individuals were disinterred from this cemetery. The mainland cemetery (21-R-2, the “R” cemetery) was located adjacent to a Classic Christian Period domed church as well as an Early Christian walled settlement. Islamic type graves are also found at one end of the R cemetery. 188 individuals were excavated from this cemetery.

The original interpretation of the available archaeological data was that the two cemeteries were used successive periods of time, although overlapping in time to some degree (e.g., Van Gerven et al., 1981; Van Gerven et al., 1995). Subsequently, a diachronic scheme was employed to interpret the consistent differences in health between the cemeteries in terms of political and economic forces in greater Nubia (e.g., Van Gerven et al., 1981; Van Gerven et al., 1995). However, analysis of textile remains from the graves, and a small sample of preliminary radiocarbon dates, suggest that both cemeteries were used during Early Christian times (Adams
et al., 1999; unpublished data). This creates the possibility that the S cemetery represents an underclass, perhaps of landless laborers, that experienced greater stress and illness than their mainland counterparts. The next section will focus on several lines of evidence that establish a high degree of childhood stress and the health differences between the two communities.

3.4 Biological Anthropology of Kulubnarti

Extensive research on these remains over the last thirty years has provided a detailed record of mortality, nutrition, growth and development, and disease at Kulubnarti. Multiple lines of evidence support the conclusion that the S cemetery population experienced a higher degree of stress (malnutrition, parasitism, infectious disease) than the R cemetery population (Van Gerven et al., 1981; 1985, 1990a, 1990b; Hummert, 1983; Sandford et al., 1983; Hummert and Van Gerven, 1984; Moore et al., 1986; Mittler et al., 1992; Mittler and Van Gerven, 1994; Sandford and Kissling, 1994; Mulhern and Van Gerven, 1997; Sheridan and Van Gerven, 1997). This section will review select data on mortality, cribra orbitalia, linear enamel hypoplasia, and osteopenia.

3.4.1 Mortality

By analyzing percent survivorship and mean life expectancy across age categories based on life tables, Van Gerven et al. (1981) showed that the S cemetery population experienced higher mortality and lower average life expectancy than the R cemetery population between birth and age 14. From age 14 onward, the pattern reverses and the R cemetery experiences higher mortality and lower life expectancy. Peak probability of dying before adulthood occurred in both cemeteries at age 4 years (Van Gerven et al., 1995). Although this overall pattern could
represent a lowering of fecundity in the R cemetery population, the high correspondence between cribra orbitalia, an independent measure of morbidity (see Section 3.3.2), and probability of dying in childhood supports the interpretation that the differences in mortality reflect higher infant and childhood survival in the R community (Van Gerven et al., 1981).

3.4.2 Cribra Orbitalia

Cribra orbitalia is a type of porotic hyperostosis confined to the roof of the eye orbit. It appears as an area of pitted, porous bony growth, and is produced by hypertrophy of the marrow cavity between the inner and outer tables of bone. Although non-anemia conditions are known to cause cribra orbitalia (e.g., Wapler et al., 2004), iron-deficiency anemia was long thought to be the cause of the lesion (e.g., Carlson et al., 1974). This is because iron deficiency is unfortunately a ubiquitous problem in the third world, especially where reliance on cereal grains is high and animal protein intake is low. Furthermore, cereal grains are not only low in iron, they can inhibit iron uptake (Rheinhold, 1982). Iron deficiency is also exacerbated by parasitism and weanling diarrhea, which is partly responsible for high levels of childhood mortality in present day Africa (e.g., Stoltzfus et al., 2000; Brabin et al., 2001).

A recent study, however, found that the iron-deficiency hypothesis is problematic because it cannot cause the necessary marrow hypertrophy that creates the lesion (Walker et al., 2009). Walker et al. (2009) found that megaloblastic anemia, particularly B$_{12}$ deficiency, was a much more likely culprit, and that this type of anemia is related to malnutrition, parasitism, and weaning in the same manner as iron-deficiency. While the Kulubnarti cribra orbitalia analysis was conducted when the iron-deficiency hypothesis was *en vogue* (Van Gerven et al., 1981;
Mittler and Van Gerven (1994), the distribution of the lesion by age at death and between the cemeteries still signals stress associated with weaning.

Ninety-four percent of the S cemetery children (birth-12 years) display the lesion while 82% of the R cemetery children are affected (Van Gerven et al., 1981). While the greater frequency of cribra orbitalia in the S population indicates a higher degree of childhood stress for the S community, the overall prevalence of the lesion compared to a population from Wadi Halfa 80 km north (32%) demonstrates the more severe chronic character of subadult stress at Kulubnarti (Carlson et al., 1974).

Mittler and Van Gerven (1994) showed that the highest frequency of cribra orbitalia occurred at age 4 years and active lesions were present in individuals as old as 12 years. The occurrence of active lesions throughout these ages suggests a synergy between malnutrition and parasitism which implicates both weaning behavior and childhood diet as contributing factors. Furthermore, individuals with cribra orbitalia had a mean life expectancy 15.5 years below those without the lesion (Mittler and Van Gerven, 1994), suggesting that childhood stress has long-lasting effects on health outcomes.

### 3.4.3 Linear Enamel Hypoplasia

Linear enamel hypoplasias (LEH) are growth disruptions in tooth enamel caused by systemic stress events during the formation of the dentition (Goodman and Rose, 1990). Research on modern children, and those from well documented historical contexts, has demonstrated a link between nutritional stress, weaning, and the timing of LEH (Goodman et al., 1987, 1992; May et al., 1993; Zhou and Corruccini, 1998; Saunders and Keenleyside, 1999; but see Corrunccini and Townsend, 2003). Because the timing of the growth perturbation can be
estimated by the position of the LEH on the crown, LEHs are useful measures of recurrent stress loads, and serve as an indirect estimate of the age of weaning in a population.

The presence of LEH is virtually universal in both cemetery populations at Kulubnarti (Van Gerven et al., 1990a). Peak occurrence of LEH by age estimate is between 3-5yrs, with slightly later LEHs more frequent in the S cemetery. The number and spacing of LEHs per tooth differ as well, with the S community experiencing longer periods of hypoplasia formation and fewer periods of recovery than the R cemetery. This mirrors the mortality data in that the probability of dying is higher for longer in the S cemetery (Van Gerven et al., 1995).

3.4.4 Osteopenia

Osteopenia refers to age-related bone loss commonly among females, and serves as a measure of stress in adults. At Kulubnarti, bone loss, as measured by percent cortical area, is more severe among S cemetery pre-menopausal females than their R cemetery counterparts (Van Gerven et al., 1990b). Van Gerven et al. (1990b) argue that this pattern likely reflects a higher degree of nutritional stress during pregnancy and lactation in the S community.

Although males do not show bone loss with age, S cemetery males have less bone than males from the R cemetery. Taken together, these may data represent a continuation of childhood stress into adulthood and may hint at the long-term health effects of poor nutrition, disease, and parasitism associated with weaning and childhood diet.

3.5 Conclusion

This chapter provided the geographical and archaeological background to the site of Kulubnarti and its human remains. The subsistence strategy practiced at Kulubnarti in the Early
Christian period is likely similar to that practiced in the region today (Adams, 1977), which includes both C_{3} and C_{4} staples that are seasonally cycled with some overlap in autumn. The Nile begins to flood just after the summer harvest of the C_{4} crops, and peaks coinciding with mixed harvest. The Nile is the only source of drinking water for the Kulubnarti community, but many people probably use water storage devices to let the silt settle and to keep the water cool.

The biological analyses of the Kulubnarti remains demonstrate a strong pattern of childhood stress and a consistent difference between the two cemeteries. The skeletal measures of stress reviewed in this chapter reflect periods of stress earlier in life, and in the case of LEH, the timing of those stress events can be estimated. When viewed together, peak probability of dying, peak LEH occurrence, and peak incidence of cribra orbitalia all converge at 4 years of age (Van Gerven et al., 1995). These patterns point towards weaning behavior and diet as causative factors in both mortality and morbidity experienced at Kulubnarti. Stable isotope data provide another tool to study the Kulubnarti remains, particularly in terms of dietary variability by age-at-death.
CHAPTER 4
STABLE ISOTOPES AT KULUBNARTI

4.1 Chapter Overview

This chapter presents previously measured and new stable isotope data for bulk tissues at Kulubnarti. A variety of tissues have been analyzed, including bone collagen, bone apatite, enamel, and hair (Sandberg, 2006; Turner et al., 2007; Glasgow, 2011). Stable isotope ratios of carbon, nitrogen, and oxygen have been measured in multiple tissues. The data from these analyses provide the isotopic setting and expectations for the intratooth analyses in Chapters 5 and 6. This chapter includes descriptive statistics and basic interpretations for each isotopic system as well as discussions of the interpretive implications of data from various tissue types, including differences in turnover rates and metabolic routing. I then discuss how the stable isotope compositions vary by age at death, because this is the most important variable for the high-resolution analyses in Chapters 5 and 6.

4.2 Carbon Isotopes

Carbon isotopes have been measured in bone collagen (δ\(^{13}\text{C}_{\text{coll}}\)), bone apatite (δ\(^{13}\text{C}_{\text{ap}}\)), third molar enamel (δ\(^{13}\text{C}_{\text{enamel}}\)), and hair (δ\(^{13}\text{C}_{\text{hair}}\)). Descriptive statistics are given in Table 4.1. All bone collagen and bone apatite data are for ribs. Collagen preservation at Kulubnarti is generally good and most samples had C:N ratios between 2.9-3.6 and adequate collagen yields (DeNiro, 1985; Ambrose, 1990). In the rare instances that samples had C:N ratios outside this range or produced low collagen yields, the data were removed from the analysis. The
proportional contribution of C₄ foods (%C₄) is estimated using a linear mixing model¹ between C₃ and C₄ endmembers of modern Nubian agricultural plant foods (Table 4.2) (White and Schwarcz, 1994). Endmember δ¹³C values are adjusted -1.5‰ to account for the ¹³C-depletion of atmospheric CO₂ caused by the combustion of fossil fuels over the last century (Long et al., 2005). Diet to tissue carbon isotope fractionation factors of 5.0‰, 12.0‰, and 3.2‰ were used for bone collagen, bone and enamel carbonate, and hair, respectively (Lee-Thorp et al., 1989; Ambrose and Norr, 1993; Sponheimer et al., 2003a; Passey et al., 2005). The %C₄ calculations should be treated as rough estimates given the uncertainty in diet-tissue enrichment factors and the actual carbon isotope composition of foods grown at Medieval Kulubnarti. The mean δ¹³C values for these tissues all reflect a mix of C₃ and C₄ derived carbon, ranging from ~25% C₄ in bone carbonate, to ~50% C₄ for enamel and hair. Bone collagen δ¹³C (and δ¹⁵N) values from the

Table 4.1: Descriptive statistics for carbon isotope data for various tissues and estimated %C₄.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>Range</th>
<th>Estimated %C₄*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>61</td>
<td>-17.7</td>
<td>0.9</td>
<td>-20.2</td>
<td>-15.6</td>
<td>4.7</td>
<td>35±6[^a, b]</td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>140</td>
<td>-17.6</td>
<td>1.2</td>
<td>-19.7</td>
<td>-11.8</td>
<td>7.9</td>
<td>36±8[^c]</td>
<td></td>
</tr>
<tr>
<td>Collagen Total**</td>
<td>173</td>
<td>-17.6</td>
<td>1.1</td>
<td>-19.7</td>
<td>-11.8</td>
<td>7.9</td>
<td>36±7[^a, b, c]</td>
<td></td>
</tr>
<tr>
<td>Enamel</td>
<td>31</td>
<td>-8.8</td>
<td>1.9</td>
<td>-12.2</td>
<td>-4.4</td>
<td>7.9</td>
<td>48±13[^#]</td>
<td>a, b</td>
</tr>
<tr>
<td>Bone apatite</td>
<td>71</td>
<td>-11.8</td>
<td>0.9</td>
<td>-13.6</td>
<td>-7.4</td>
<td>6.3</td>
<td>28±6[^c]</td>
<td></td>
</tr>
<tr>
<td>Hair</td>
<td>79</td>
<td>-17.6</td>
<td>1.6</td>
<td>-20.6</td>
<td>-13.8</td>
<td>6.8</td>
<td>47±11[^c]</td>
<td>d</td>
</tr>
<tr>
<td>Δ¹³C enamel-collagen</td>
<td>24</td>
<td>9.2</td>
<td>2.2</td>
<td>5.0</td>
<td>14.9</td>
<td>9.8</td>
<td></td>
<td>a, b</td>
</tr>
<tr>
<td>Δ¹³C bone ap-collagen</td>
<td>34</td>
<td>5.7</td>
<td>0.8</td>
<td>3.9</td>
<td>7.45</td>
<td>3.6</td>
<td></td>
<td>c</td>
</tr>
</tbody>
</table>

* see text for formula and diet-tissue fractionation factors. Plant baseline data are from White and Schwartz (1994) and is adjusted -1.5‰ to account for the fossil fuel effect.
[^a] overrepresents protein component of the diet
[^#] reflects total diet

References: a: this study; b: Sandberg, 2006; c: Turner et al., 2007; d: Glasgow, 2011

Table 4.2: Carbon and nitrogen isotope composition of Nubian plants. Data from White and Schwarcz, 1994.

¹ %C₄ = [(δ¹³C_col – δ¹³C₃ endmember - f) / (δ¹³C₄ endmember * δ¹³C₃ endmember)]*100, where f is the diet-tissue fractionation factor.
unpublished data (Sandberg, 2006) and Turner et al. (2007) are generally in good agreement and the datasets have been combined. The $\delta^{13}C_{coll}$ data in Turner et al. (2007) have a statistically indistinguishable mean, but are slightly more variable (-17.6±1.2‰) than the unpublished collagen data (-17.7±0.9‰) (Sandberg, 2006). The Turner et al. (2007) dataset is drawn from a sample with a broader range of age at death and includes more infants, three of which have very high outlying $\delta^{13}C$ values (-11.8 to -12.9‰). Taken together, the carbon isotope data suggest a slightly higher reliance on C$_4$ crops (millet and/or sorghum) at Kulubnarti (35% average C$_4$ input) than at Wadi Halfa, a site with a contemporaneous community at the northern

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common name</th>
<th>Plant part</th>
<th>$\delta^{13}C$ (‰)</th>
<th>$\delta^{15}N$ (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$_4$ plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Setaria viridis</em></td>
<td>millet</td>
<td>seed</td>
<td>-10.8</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Digitaria sanguinalis</em></td>
<td>millet</td>
<td>seed</td>
<td>-10.6</td>
<td>9.4</td>
</tr>
<tr>
<td><em>Pennisetum divisum</em></td>
<td>millet</td>
<td>seed</td>
<td>-12.1</td>
<td>1.1</td>
</tr>
<tr>
<td><em>Panicum coloratum</em></td>
<td>millet</td>
<td>seed</td>
<td>-13.1</td>
<td>-</td>
</tr>
<tr>
<td><em>Sorghum durra</em></td>
<td>sorghum</td>
<td>seed</td>
<td>-11</td>
<td>8.5</td>
</tr>
<tr>
<td><em>Sorghum sudanensis</em></td>
<td>sorghum</td>
<td>seed</td>
<td>-12.4</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td></td>
<td><strong>-11.65</strong></td>
<td><strong>4.4</strong></td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td></td>
<td></td>
<td><strong>0.9</strong></td>
<td><strong>4.2</strong></td>
</tr>
<tr>
<td>C$_3$ plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Triticum vulagare</em></td>
<td>wheat</td>
<td>seed</td>
<td>-27</td>
<td>6.5</td>
</tr>
<tr>
<td><em>Hordeum vulgare</em></td>
<td>barley</td>
<td>seed</td>
<td>-22.1</td>
<td>7.8</td>
</tr>
<tr>
<td><em>Vigna membranaceae</em></td>
<td>cowpeas</td>
<td>seed</td>
<td>-25.1</td>
<td>-</td>
</tr>
<tr>
<td><em>Cajanus cajan</em></td>
<td>pigeon peas</td>
<td>seed</td>
<td>-27.2</td>
<td>-</td>
</tr>
<tr>
<td><em>Acacia nilotica</em></td>
<td>acacia beans</td>
<td>seed</td>
<td>-25</td>
<td>-</td>
</tr>
<tr>
<td><em>Acacia adbida</em></td>
<td>acacia beans</td>
<td>seed</td>
<td>-27.5</td>
<td>-</td>
</tr>
<tr>
<td><em>Hibiscus esculentus</em></td>
<td>okra</td>
<td>leaf</td>
<td>-21</td>
<td>6.7</td>
</tr>
<tr>
<td><em>Eruca sativa</em></td>
<td>garden rocket</td>
<td>leaf</td>
<td>-30.3</td>
<td>3</td>
</tr>
<tr>
<td><em>Raphanus sativus</em></td>
<td>radish tops</td>
<td>leaf</td>
<td>-30.5</td>
<td>1</td>
</tr>
<tr>
<td><em>Allium sepa</em></td>
<td>onion</td>
<td>skin</td>
<td>-28</td>
<td>-</td>
</tr>
<tr>
<td><em>Solanum melongena</em></td>
<td>eggplant</td>
<td>leaf</td>
<td>-28.4</td>
<td>3.6</td>
</tr>
<tr>
<td><em>Corchorus olitorius</em></td>
<td>jew's mallow</td>
<td>leaf</td>
<td>-28.2</td>
<td>13.1</td>
</tr>
<tr>
<td><em>Hibiscus sabdariffa</em></td>
<td>rosella</td>
<td>fruit</td>
<td>-23.3</td>
<td>6.7</td>
</tr>
<tr>
<td><em>Balanites aegyptiaca</em></td>
<td>date</td>
<td>fruit</td>
<td>-27.1</td>
<td>-</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td></td>
<td><strong>-26.5</strong></td>
<td><strong>6.1</strong></td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td></td>
<td></td>
<td><strong>2.9</strong></td>
<td><strong>3.7</strong></td>
</tr>
</tbody>
</table>
edge of the Batn el Hajar (~10-25% C₄, White and Schwarz, 1994; Schwarz and White, 2004). Unlike Kulubnarti, the topography at Wadi Halfa facilitated saqia irrigation techniques, which presumably enabled the communities there to cultivate larger, more productive, fields of C₃ crops such as barley and wheat (Armelagos, 1968; Edwards, 2004).

\[ \delta^{13}C_{\text{hair}} \] values at Kulubnarti, and Wadi Halfa, are higher than \[ \delta^{13}C_{\text{coll}} \] values, and when corrected for diet-tissue fractionation, reflect higher C₄ input on average (up to ~75%, Glasgow, 2011). This is likely due to differences in turnover between these tissues and the amount of time a sample of each represents. Depending on its length, the isotopic composition of hair likely reflects sub-annual dietary composition, on the order of weeks or months (O’Connell and Hedges, 1999). Hair will therefore record seasonal variation in C₃ and C₄ inputs as it grows. Bone collagen, on the other hand, turns over slowly and reflects the average isotopic composition of diet over many years before death (Hedges et al., 2007). White (1993) and Schwarcz and White (2004) measured \[ \delta^{13}C \] values incrementally along hair strands from Wadi Halfa and found that hair close to the scalp was commonly \(^{13}C\)-enriched, suggesting that the majority of the individuals in her sample died during the summer when C₄ crops are harvested. They also found that the carbon isotopic composition of hair oscillated seasonally, suggesting ~75% C₃ consumption in the winter to ~75% C₄ consumption in the summer. It is not surprising then that some hair \[ \delta^{13}C \] values at Kulubnarti would also reflect variable proportions of C₄-derived carbon than bone collagen.

Enamel \[ \delta^{13}C \] values (Figure 4.1) also reflect greater reliance on C₄ foods on average than collagen at Kulubnarti and are more variable (49±13% vs. 36±6%). Differences in metabolic routing are likely to be partly responsible for this divergence. The isotopic composition of proteinaceous tissues such as bone collagen and hair keratin over-represents dietary protein,
while bone and tooth mineral reflect a well-mixed signal of carbon from all of the dietary macronutrients (protein, carbohydrates, lipids) (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004). C₄ crops are common fodder for domesticated sheep and goats in Lower Nubia today (Bacon, 1948; May and McLennan, 1970 in Schwarcz and White, 2004), and may also have been in the past (e.g., Dupras et al., 2001; Copley et al., 2004), suggesting that dietary protein sources may have been ¹³C-enriched. However, the higher proportion of C₄-derived carbon evident in the δ¹³C enamel hints at the possibility that the dietary protein source was not as ¹³C-enriched as total dietary carbon, or at least not consistently so at Kulubnarti.

While an attempt was made to sample enamel from the entire crowns of third molars, the possibility exists that some seasonal effects are present. This possibility gains support from the broad range of δ¹³C enamel values. The range of enamel δ¹³C values is quite large (-12.2‰ to -4.4‰, range=7.9‰), with estimated C₄ contributions as low as ~25% to as high as ~75%, a pattern that mirrors the hair δ¹³C data from Kulubnarti (Glasgow, 2011) as well as Wadi Halfa (Schwarcz and White, 2004). It is also possible that the individuals in the sample lived up to several hundred years apart, and the variability evident in the δ¹³C enamel values is a product of changes in seasonal agricultural yields over time.

Bone carbonate carbon isotope data from Kulubnarti (-11.8±0.9‰; Turner et al., 2007) are lower and less variable than the enamel data and suggest less C₄-input on average (~28%C₄) than the other tissues analyzed. With one obvious outlier removed (-7.4‰), the bone carbonate δ¹³C mean is even lower and the range is halved. There is a small but significant relationship between δ¹³C ap and δ¹³C coll measured in the same individuals (R²=0.14, N=34, P<0.05), but no such relationship exists between δ¹³C ap and δ¹³C enamel values (R²=0.06, N=9). The disconnect in
$\delta^{13}C_{ap}$ and $\delta^{13}C_{enamel}$ values is possibly the result of diagenetic or pretreatment effects in the bone apatite (Koch et al., 1997; Lee-Thorp and van der Merwe, 1991; Luftus and Sealy, 2012). There is also no relationship between $\delta^{13}C_{coll}$ and $\delta^{13}C_{enamel}$ values measured in the same individuals (Figure 4.1, $R^2<0.01$, $N=25$). In a study comprising several different archaeological populations in Southern Africa, Luftus and Sealy (2012) found a close correspondence between $\delta^{13}C_{coll}$ and $\delta^{13}C_{enamel}$ ($R^2=0.71$) and a collagen-enamel offset of $3.8\pm1.0\%$.

An important factor to consider when comparing the carbon isotope composition of different tissues is that, depending on age at death, $\delta^{13}C_{enamel}$ values could represent diet decades removed from the $\delta^{13}C_{coll}$ and $\delta^{13}C_{ap}$ values. The isotopic composition of bone (collagen and apatite) reflects dietary inputs averaged over many years before death due to bone growth, remodeling, and turnover; and, in this case, third molar enamel reflects diet during the formation of the tooth (~8-12 years of age) (Hillson, 1996). Large differences in diet between enamel and collagen $\delta^{13}C$ values could signal either that an individual immigrated into the community or changing agricultural conditions over time brought about by long-term fluctuations in the flow and flood of the Nile (e.g., Hassan, 1981; Jiang et al., 2002; Eltahir, 1996).

The numerical spacing between $\delta^{13}C$ values for bone apatite and collagen in single individuals (often called apatite-collagen spacing and denoted as $\Delta^{13}C_{ap-coll}$) is another way to explore the relationship between the carbon isotope compositions of these tissues and the macronutrient sources of carbon (e.g., Lee-Thorp et al., 1989; Harrison and Katzenberg, 2003; Clementz et al., 2009)$^2$. Terrestrial mammalian trophic guilds tend to segregate along the $\Delta^{13}C_{ap-coll}$ axis such that carnivores have lower spacing ($4.3\pm1.0\%$) than omnivores ($5.2\pm0.8\%$) and

---

$^2$ The numerical spacing, $\Delta$, is used here to maintain consistency with the archaeological literature. Isotope enrichment, denoted as $\varepsilon$, is a more accurate measure of the difference between two $\delta$ values because it is not scale dependent (Cerling and Harris, 1999). The two measures are similar when isotopic differences between two tissues,
herbivores (6.8±1.4%; Lee-Thorp et al., 1989). The mechanism behind this pattern is likely a mix of dietary macronutrient differences and diet-tissue isotope enrichment differences associated with physiological or metabolic adaptations among the guilds (Hedges, 2003).

Kulubnarti has a wide range of $\Delta^{13}C_{ap-coll}$ values (5.7±0.8‰, range=3.9-7.2) (Turner et al., 2007). These values could represent highly variable dietary macronutrient compositions among individuals. Since apatite-collagen spacing relates in part to protein consumption, one might expect this measure to be correlated negatively with $\delta^{15}N_{coll}$. There is a significant negative relationship between $\delta^{15}N_{coll}$ and $\Delta^{13}C_{ap-coll}$ (Figure 4.2, $R^2=0.22$, $P=0.005$, $N=33$). This lends or between diet and tissue, are small; but they diverge by ~0.5‰ when the difference between two tissues approaches ~20‰.
support to the interpretation that the two measures reflect some degree of access to high-protein foods, and that access to these foods varied widely in the population. The range of \( \Delta^{13}\text{C}_{\text{enamel-coll}} \) values is even larger (5.0 to 14.9), and reaches values uncommon for other animals (Lee-Thorp et al., 1989; Clementz et al., 2009), a finding that is difficult to explain in terms of dietary composition, but again, it must be kept in mind that the two tissues differ in formation times.

### 4.3 Nitrogen Isotopes

Nitrogen isotope ratios have been measured in bone collagen and hair. Descriptive statistics are given in Table 4.3. \( \delta^{15}\text{N}_{\text{coll}} \) values in the published (Turner et al., 2007) and unpublished datasets do not differ and have been combined. The Turner et al. (2007) \( \delta^{15}\text{N}_{\text{coll}} \) values have a slightly higher mean and range (10.4±1.4‰ vs. 10.0±1.0‰) because their dataset
includes several more very young individuals with high $\delta^{15}N_{\text{coll}}$ values. Adult mean values (individuals >20 years of age) are not appreciably lower than the entire dataset because individuals under the age of 20 years have both higher and lower $\delta^{15}N_{\text{coll}}$ values than adults (see section 4.6, Figure 4.6).

Table 4.3: Descriptive statistics for $\delta^{15}N$ of collagen and hair.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>Range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>61</td>
<td>10.0</td>
<td>1.0</td>
<td>7.3</td>
<td>12.7</td>
<td>5.4</td>
<td>a, b</td>
</tr>
<tr>
<td>Collagen</td>
<td>130</td>
<td>10.4</td>
<td>1.4</td>
<td>7.3</td>
<td>14.8</td>
<td>7.5</td>
<td>c</td>
</tr>
<tr>
<td>Collagen Total*</td>
<td>166</td>
<td>10.4</td>
<td>1.3</td>
<td>7.3</td>
<td>14.8</td>
<td>7.5</td>
<td>a, b, c</td>
</tr>
<tr>
<td>Hair</td>
<td>79</td>
<td>8.5</td>
<td>1.3</td>
<td>4.4</td>
<td>11.8</td>
<td>7.5</td>
<td>d</td>
</tr>
</tbody>
</table>

* combined datasets, means used for duplicates

References: a: this study; b: Sandberg, 2006; c: Turner et al., 2007; d: Glasgow, 2011

Figure 4.3: Relationship between $\delta^{15}N_{\text{coll}}$ and $\delta^{13}C_{\text{coll}}$. The three outlying values on the right are less than 1.5 years old ($R^2=0.27$, $P<0.0001$, $N=166$). Removing these values does not appreciably improve the relationship.
The Kulubnarti community has similar $\delta^{15}N_{coll}$ values to other communities in the Nile Valley. White and Schwarcz (1994) report $\delta^{15}N_{coll}$ values from Wadi Halfa around 10.5‰ during the Christian Period, and speculate that caprines could be a good candidate for a protein source. However, it is difficult to reconstruct human diet without comparative faunal $\delta^{15}N$ values. $\delta^{15}N_{coll}$ and $\delta^{13}C_{coll}$ values are correlated (Figure 4.3, $R^2=0.27$, $P<0.0001$, $N=166$), but there is considerable variation around the regression line. $\delta^{15}N_{coll}$ varies by 3.8‰ in adults over age 20. This might suggest that protein sources varied among adults, or possibly that high-quality protein resources were available to some but not others.

### 4.4 Oxygen Isotopes

Oxygen isotope ratios have been measured in enamel (Sandberg, 2006) and bone apatite (Turner et al., 2007). Descriptive statistics are presented in Table 4.4. Similar to carbon isotopes,

<table>
<thead>
<tr>
<th>Tissue</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>Range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enamel</td>
<td>31</td>
<td>-2.0</td>
<td>2.4</td>
<td>-7.3</td>
<td>1.4</td>
<td>8.7</td>
<td>a, b</td>
</tr>
<tr>
<td>Bone apatite</td>
<td>71</td>
<td>2.9</td>
<td>0.8</td>
<td>1.5</td>
<td>5.2</td>
<td>3.7</td>
<td>c</td>
</tr>
<tr>
<td>Hair</td>
<td>29</td>
<td>21.2</td>
<td>1.5</td>
<td>18.1</td>
<td>24.0</td>
<td>5.9</td>
<td>d</td>
</tr>
</tbody>
</table>

* Enamel and bone apatite values are from carbonate and both are vs. PDB
Hair values are vs. SMOW

References: a: this study; b: Sandberg, 2006; c: Turner et al., 2007; d: Glasgow, 2011

Oxygen isotope ratios in enamel are much more variable than in bone apatite (carbonate). The range of $\delta^{18}O_{enamel}$ is 8.7‰. Paired $\delta^{18}O_{enamel}$ and $\delta^{18}O_{ap}$ values are not related ($R^2=0.03$, $P=0.66$, $N=9$), and the means of the two datasets are significantly different from one another.
(Wilcoxon, P<0.001). The mean difference is difficult to interpret and may be related to
diagenesis or pretreatment effects in the bone apatite. The potential reasons for the difference in

\[
d_{18O}^{enamel} = -7.718006 - 0.6532048 d_{13C}^{enamel}
\]

Figure 4.4: Relationship between \( \delta^{18}O_{enamel} \) and \( \delta^{13}C_{enamel} \) \((R^2=0.26, P<0.01, N=31)\).

variability are the same as those for \( \delta^{13}C \) values in enamel and bone apatite. It is possible that
there is a seasonal bias in the enamel due to incomplete sampling of the entire crown. The range
in \( \delta^{18}O_{enamel} \) values could also reflect the presence of migrants, perhaps from elsewhere along the
Nile (White et al., 2004; Buzon and Bowen, 2010). \( \delta^{18}O_{hair} \) values show a large range (5.9‰).
This may be the result of seasonality in the oxygen isotopic composition of Nile water.

The Nile has relatively high \( \delta^{18}O \) values relative to other rivers due to evaporative
enrichment as it courses through an arid environment, and to the effects of agricultural irrigation
return flows that have also been subject to evaporation (Ingraham et al., 1998). The Nile’s
primary tributary, the Blue Nile, has an average \( \delta^{18}O \) value of -1.5‰ and water sampled from
near Cairo has a mean of +3.8‰ (Ingraham et al., 1998). The annual flood of the Nile is driven by tropical monsoon precipitation over the Ethiopian highlands, where the headwaters of the Blue Nile are located. Precipitation during July-August monsoonal rains (July-August) near Addis Ababa is $^{18}$O-depleted ($\delta^{18}$O=−2-3‰ ) relative to rainfall in the other seasons (Levin et al., 2009). Therefore it is likely that the Nile has lower $\delta^{18}$O values during the flood and higher values when the flood recedes.

The Nile is the only source of drinking water at Kulubnarti. Water storage may contribute to the variation in $\delta^{18}$O values in hair and enamel through further evaporation. The length of growing seasons and seasonality in the types of foods eaten throughout the year may also play a role. Nile levels also vary over longer scales and long-term variation in flood levels has been linked to El Nino (Eltahir, 1996) and other global process (e.g., Jiang et al., 2002). It is possible that changing Nile flood levels during the Early Christian period drive some of the variability in $\delta^{18}$O values at Kulubnarti.

$\delta^{18}$O$_{\text{enamel}}$ and $\delta^{13}$C$_{\text{enamel}}$ share a weak but significant negative correlation (Figure 4.4, $R^2=0.26$, $P<0.01$, $N=31$), but each is highly variable.

4.5 Variation by Age

Age-related variation in carbon, nitrogen, and oxygen isotope values at Kulubnarti reflects aspects of diet and physiology that may be linked to stages of life history and nutrition. This section presents the relationships between age-at-death and $\delta^{13}$C$_{\text{coll}}$, $\delta^{15}$N$_{\text{coll}}$, and $\delta^{18}$O$_{\text{ap}}$ values (Figures 4.5-4.8). Age is treated as a continuous variable, and is also reduced to the following ordinal age classes: infant (0-3yrs), child (4-12yrs), adolescent (13-19yrs), and adult ($\geq$20yrs). At a mean level, $\delta^{13}$C$_{\text{coll}}$ values decrease rapidly from the first to the second year, and
then continue to decrease more gradually over the first six years of life by ~2‰ (Figure 4.5, lower portion). It does not reach typical adult values (-17.5±0.7‰) until approximately age 20. The mean for δ15Ncoll (Figure 4.6, lower portion) follows a slightly different pattern through the first five years. The δ15Ncoll mean begins at a high value for the first two years, then rapidly declines from age three to age five by ~3‰. From age five to about age 20, the δ15Ncoll mean is ~1‰ lower than the mean adult value (10.4±0.1‰). Figure # displays the mean lines for both δ15Ncoll and δ13Ccoll and illustrates their divergent patterns in the first five years.

This pattern is similar to that seen in living mother and infant pairs (Fuller et al., 2006a). Fuller et al. (2006a) showed that fingernail δ13C and δ15N values of exclusively breast-fed infants increased rapidly after birth (by ~1‰ and ~2-3‰, respectively), and they argued that this was evidence of a trophic shift in carbon. They also found that δ13C values declined to maternal values more quickly than did δ15N values during the weaning process. The authors suggested that this pattern may result from the consumption of weanling foods that are carbohydrate-rich, while breastmilk continued to be the primary nitrogen source. This suggests that δ15N can be used to monitor the duration of weaning process and δ13C to identify the timing of initial supplementation (Fuller et al., 2006a).
Figure 4.5: Relationship between $\delta^{13}C_{coll}$ and age at death. Scatterplot (above) and box plot with mean line (below). The box plots are by age at death category with progressively more ages included in each category. The box plots simplify the data so that the trend is more readily evident.
Figure 4.6: Relationship between $\delta^{15}\text{N}_{\text{coll}}$ and age at death. Scatterplot (above) and box plot with mean line (below). The box plots are by age at death category with progressively more ages included in each category. The box plots simplify the data so that the trend is more readily evident.
The Kulubnarti data seem to mirror this pattern. Using the means of each age at death cohort, this would imply that supplementation began sometime during or before the first year of life, and that weaning was complete around age five. However, this interpretation is problematic. As in other cross-sectional weaning studies, there is considerable scatter in the Kulubnarti data, so it is clear that not every individual was bound to follow this pattern had they survived.

The $\delta^{15}$N$_{coll}$ range is 4.9‰ for infants (0-3yrs) and 5.6‰ for children between four and 12yrs. Weaning behavior is variable in modern populations (Stuart-Macadam and Dettwyler, 1995), but it is unclear whether the variation we see by age-at-death is a product of unsuccessful weaning practices or whether it characterizes the population as a whole. The skeletal and dental lesions that connote serious illness associated with the weaning process (e.g., linear enamel hypoplasia, cribra orbitalia) are almost universal at Kulubnarti. The illnesses that cause early childhood mortality do so by subjecting children to a suite of physiological problems that may affect the isotopic composition of developing tissues (e.g., Katzenberg and Lovell, 1999; Cherel et al., 2005; Mekota et al., 2006; O’Grady et al., 2010). Slowed growth rate, altered water flux, malnutrition, and tissue wasting are all associated with parasitism and diarrhea which are in turn associated with an inability to balance immunological protection and adequate nutrition during weaning.

Another interesting feature of these data is that both $\delta^{15}$N$_{coll}$ and $\delta^{13}$C$_{coll}$ are lower than adult values between the ages of five and 20. This may be the result of weanling foods of poor-protein quality such as C$_3$-based weanling gruels, and low-quality resources or insufficient quantities of protein throughout adolescence. This pattern has been observed in $\delta^{15}$N$_{coll}$ at Wadi Halfa (White and Schwarcz, 1994) and elsewhere (e.g., Shurr, 1997; Mays et al., 2002; Richards
et al., 2002; Jay et al., 2008; Nitsch et al., 2011), but with a rise to adult values at an earlier age. Reliance on low-quality gruel for an extended period of time after weaning may have increased the risks of iron-deficiency anemia, deficiencies in micronutrients such as B₁₂, other nutritional deficiencies, and the risk of infection for these children (Rheinhold, 1982). This weaning and post-weaning period (~4-7yrs) corresponds to the highest probability of dying before age 33, peak occurrence of cribra orbitalia, and the highest frequency of linear enamel hypoplasia (Van Gerven et al. 1995). This is suggestive of a strong relationship between weanling diets, the timing of the weaning process, morbidity, and mortality. However, other factors affect nitrogen isotope variation that must be kept in mind.

Nitrogen isotope variation is also influenced by nitrogen balance (see Chapter 2) which may affect nitrogen isotope discrimination during growth (see Martinez del Rio et al., 2009). While negative nitrogen balance (i.e., malnutrition) can elevate δ¹⁵N values because body proteins that are ¹⁵N-enriched relative to diet can be catabolized for energy, further elevating δ¹⁵N values in growing tissues (e.g., Hobson et al., 1993; Voigt and Matt, 2004; Cherel et al., 2005), positive nitrogen balance (e.g., high protein diet, or during growth) can result in lower δ¹⁵N values, possibly due to direct routing of dietary proteins to growing tissues, bypassing the processes that result in the preferential loss of ¹⁴N (e.g., Hobson et al., 1993; Gaye-Siessegger et al., 2004; Robbins et al., 2005; Trueman et al., 2005; Warinner and Tuross, 2010). These processes, however, may not be consistent (e.g., Ponsard and Averbuch, 1999; Roth and Hobson, 2000) and it is still unclear how growth affects nitrogen isotope discrimination, and thus differences by age.

Fuller et al (2004) found that healthy women showed declining δ¹⁵Nₚ₉ᵦ values throughout pregnancy, presumably due to positive nitrogen balance and growth, and that women suffering
nutritional stress and restricted weight gain during pregnancy displayed rising $\delta^{15}N_{\text{hair}}$ values (2005). Waters-Rist and Katzenberg (2010) found that a growth effect on nitrogen isotope values was not evident in the bone collagen of juveniles and adults, or in faster growing bone relative to slower growing bone (epiphyses vs. diaphysis). It is possible that the slow growth and turnover of collagen masks a possible growth effect. Nitrogen balance clearly influences diet-tissue nitrogen isotope discrimination, but in ways that are not yet clearly understood.

Nonetheless, it is important to keep in mind that weaning behavior is one of many factors at play in the cross-sectional analyses presented here and the high-resolution analyses presented in the following chapters.

Figure 4.7: Overlay plot of the mean lines for $\delta^{15}N_{\text{coll}}$ and $\delta^{13}C_{\text{coll}}$ by age at death in years.
Figure 4.8: Relationship between $\delta^{18}$O$_{ap}$ and age at death. ($R^2=0.20$, $P<0.0001$, $N=70$).

$\delta^{18}$O$_{ap}$ values are weakly correlated with age at death, but are highly variable (Figure 4.8). No clear weanling related pattern is evident in these data, but there does seem to be a tendency towards lower values later in life.

4.6 Expectations for high resolution analysis of dental tissues

The stable isotope data presented in this chapter suggests that much can be learned by applying high-resolution stable isotope analysis to these remains. This section identifies some questions that will guide the intratooth analyses. Carbon, nitrogen, and oxygen isotope data generated from bone collagen, tooth enamel, bone apatite, and hair reveal that human diet at Kulubnarti was complex. Inter-individual variation in the average consumption of C$_3$ and C$_4$ crops is large, and whole dietary carbon varies more strongly than carbon from protein sources.
Nitrogen isotope variation suggests that there is variability in dietary protein quality, protein quantity, or health among individuals at Kulubnarti. Oxygen isotope variation is substantial, particularly in enamel, pointing to either a large seasonal effect at Kulubnarti or the presence of migrants. Carbon and nitrogen isotope variation is clearly related to age-at-death, providing potential evidence of early supplementation and prolonged breastfeeding and also the possibility of a distinct diet for children and adolescents. Much of this variability could be due to changes in diet over several hundred years of occupation at Kulubnarti.

How much of this variation will we see within single individuals? Will we see more carbon variability in the enamel than the dentine? Are the potential δ\textsuperscript{13}C changes that have been linked to weaning evident in either the enamel or dentine, or will δ\textsuperscript{13}C variability in the diets of mothers and/or supplementary foods obscure such a pattern? Will δ\textsuperscript{15}N behave as it does in the cross-sectional analyses, and do we see survivors dip to the low δ\textsuperscript{15}N values that appear to characterize the post-weaning period? What is the magnitude of δ\textsuperscript{15}N change in dentine sections of single individuals? Is it possible to monitor the shift in water source from breastmilk to external sources in the δ\textsuperscript{18}O values of enamel, or are there seasonal effects that are large enough to overprint this effect? How does the variation in δ\textsuperscript{18}O within individuals compare to the variation Kulubnarti as a whole? These are the questions we turn to now in Chapters 5 and 6.
CHAPTER 5
HIGH RESOLUTION ENAMEL ANALYSIS

5.1 Chapter Overview

This chapter presents the laser ablation intratooth enamel analyses. This study addresses some methodological questions associated with sampling location and tooth choice. It begins with a description of the methods. This section includes a description of the sample, the initial preparation of the teeth, the laser system set-up, sampling locations and analysis, and the procedure used to estimate age for the data points in each profile. Results and discussion follow. The results show differences in stable isotope profiles based on sampling location and tooth type. Any weaning signal in $\delta^{13}$C or $\delta^{18}$O appears to be overprinted by a seasonal signal. Implications for Kulubnarti diet and seasonality and suggestions for future research are discussed.

5.2 Methods

5.2.1 Sample Selection

One of the goals of this study is to determine how tooth choice might affect high-resolution stable isotope profiles. Permanent first molars and canines from five individuals were chosen for this analysis (Table 5.1). First molars are the first permanent teeth to develop and begin forming around birth (Hillson, 1996). This makes them a desirable choice for studies interested in the earliest record of diet. Deciduous teeth develop in utero, but usually enter the archaeological record because of an early age-at-death. First molars are also the first permanent teeth to wear, however, and in many agricultural communities, much of the enamel has worn away by adulthood. This is potentially problematic, because another of this study’s goals is to
avoid mortality bias by measuring early childhood diet in adults. Kulubnarti has a very high degree of dental wear, but there are cases where molars abscessed and fell out early enough in life such that the corresponding upper or lower molar was no longer in occlusion and did not continue to wear. Permanent canine teeth were also chosen because they begin forming within the first year of life, and because they have relatively long cusps and roots, potentially providing more material for both enamel and dentine analyses than other teeth. All of the teeth selected for this study were relatively unworn and appeared to be well preserved. The five individuals in this study died between the ages of 21 and 31yrs. Older individuals tended not to have both an unworn first molar and an unworn canine.

Table 5.1: Sample description.

<table>
<thead>
<tr>
<th>Burial No.</th>
<th>Sex</th>
<th>Age at Death</th>
<th>Tooth Type</th>
<th>Position</th>
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</thead>
<tbody>
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<td>24</td>
<td>Canine</td>
<td>Mandibular</td>
</tr>
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<td></td>
<td></td>
<td>First Molar</td>
<td>Maxillary</td>
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<tr>
<td>R81</td>
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<td>24</td>
<td>Canine</td>
<td>Maxillary</td>
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<td></td>
<td></td>
<td></td>
<td>First Molar</td>
<td>Maxillary</td>
</tr>
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<td>Female</td>
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<td>Canine</td>
<td>Maxillary</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>First Molar</td>
<td>Maxillary</td>
</tr>
<tr>
<td>S18</td>
<td>Male</td>
<td>21</td>
<td>Canine</td>
<td>Maxillary</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>First Molar</td>
<td>Maxillary</td>
</tr>
<tr>
<td>S213</td>
<td>Female</td>
<td>24</td>
<td>Canine</td>
<td>Mandibular</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>First Molar</td>
<td>Maxillary</td>
</tr>
</tbody>
</table>

5.2.2 Initial Preparation

The sampling scheme necessitated that the teeth be sectioned in half to expose the innermost enamel and to portion off a section of root for the dentine analysis. The initial preparation was conducted at the Research Laboratory for Archaeology and the History of Art at Oxford University with Dr. Julia Lee-Thorp. Tooth dimensions were measured and the presence,
location, and size of any enamel defects (e.g., linear enamel hypoplasia) were recorded. Any calculus present on the surface of the teeth was removed with a razor blade and saved for future analysis. The exterior of each tooth was cleaned and any surface staining was removed using an aluminum oxide sandblaster. The teeth were then embedded in Herculite II, a high-strength gypsum molding material similar to plaster of Paris, and cut longitudinally using a water-cooled slow-speed Buehler Isomet saw fitted with Struers diamond cut-off wheel. The canines were cut once to produce mesial and distal halves. The molars were cut into three portions such that the three root radicals were left intact beneath three portions of crown. One half of each canine and one portion of each molar were set aside for the dentine analysis (Chapter 6), and the remaining portions of the molars were archived for future research.

5.2.3 Laser Ablation System

The enamel analysis was conducted using a laser ablation – gas chromatography – isotope ratio mass spectrometry system at the Department of Earth and Planetary Science of Johns Hopkins University with Dr. Ben Passey. The laser system incorporates features that were developed to optimize the analysis of very small enamel samples (Passey and Cerling, 2006). The laser is a Photon Machines Fusions CO₂ laser that emits radiation at a wavelength of 10.6 μm. Short bursts (0.01-0.02s) of low power radiation (~5 Watts) ablate samples in a glass chamber. CO₂ gas is liberated from the sample by the ablation events and is carried by helium gas into an extraction line where it is purified by way of cryogenic removal of potential contaminants such as organics and water vapor. The semi-automated extraction line was custom built by Dr. Passey. The CO₂ is then focused and introduced into a gas chromatography column (Poraplot Q, 25m length, 0.32m inner diameter, held at 70°C), and introduced into a Thermo 253
mass spectrometer via a ConFlo interface. Data are normalized to the VPDB scale via injections of an internal CO$_2$ standard calibrated to the international standard NBS-19 ($\delta^{13}$C=1.95‰, $\delta^{18}$O=-2.19‰ PDB). The CO$_2$ standard is injected into the extraction line and treated in the same manner as sample CO$_2$ generated with the laser.

Samples are loaded onto a platform inside the sample chamber and subjected to helium flow to purge the chamber of CO$_2$. Outgassing of CO$_2$ from sample surfaces can take between a few hours and overnight, or much longer for large porous samples (Passey and Cerling, 2006). The sample platform inside the chamber rotates to allow irregularly shaped samples to be appropriately positioned underneath the laser. The laser moves on an x-y-z stage which allows for precise spatial control of the laser bursts without moving the sample chamber.

During analysis runs, in-house enamel standards that have been measured repeatedly with the conventional phosphoric acid hydrolysis method are measured periodically to monitor for possible isotope effects related to laser ablation. Blank runs are performed periodically between sampling events to characterize the background signal and to monitor the behavior of the chamber through time. Sample yields are monitored to ensure that they exceed the size of the background at least tenfold. Charring and smoke are indicative of the combustion of organics which are $^{13}$C-depleted relative to inorganic carbon. These data are ignored and blanks are run until the background returns to acceptable levels.

Laser ablation and conventional phosphoric acid hydrolysis produce $\delta^{13}$C and $\delta^{18}$O values that have a 1:1 relationship (Passey and Cerling, 2006). There is, however, an offset for both isotopes. Passey and Cerling (2006) calculated that offset to be -0.3±1.1‰ for $\delta^{13}$C, and -6.4±0.7‰ for $\delta^{18}$O. There was more variability in the offset for fossil enamel, especially for $\delta^{18}$O. The larger offset in $\delta^{18}$O results from the mixing of the oxygen-bearing phases of enamel
during laser ablation. The phosphoric acid method liberates CO$_2$ from the carbonate fraction of enamel alone, which accounts for ~6% of the oxygen in enamel apatite. Phosphate accounts for ~90%, and hydroxide accounts for ~3% (Elliot, 1997). Enamel phosphate $\delta^{18}O$ is approximately 9‰ lower than carbonate $\delta^{18}O$ (Bryant et al., 1996; Iacumin et al., 1996). For the purposes of this dissertation, there is no need to alter the laser values, but comparisons between laser and conventional values in the future would have to take this into account.

5.2.4 Sampling Location and Analysis

Intratooth $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ profiles were created in enamel adjacent to the enamel-dentine junction (EDJ) on all five first molars and one canine. Profiles were also created close to the outer enamel surface (OES) on these same six teeth (Figure 5.1). These OES profiles sample enamel adjacent to the surface of the tooth to a depth of about 200µm. Intratooth profiles were created on the OES of the remaining four canines. These OES profiles are truer to their name in that the laser scans were made on the actual outer surface of the teeth, not on a cross-sectional surface. However, for the canine of one individual (R81), deeper enamel was sampled within the grooves of previous sampling paths made by a drill. Laser scans were made horizontally across the growth axis of the teeth and the distance between sequential scans varied. This was done to evaluate how spatial scale on the outer enamel translates to stable isotope variation.

Before analysis, tooth surfaces were briefly cleaned by abrasion with a handheld drill fitted with a diamond-tipped burr, swabbed with methanol to remove particulates, and left to air dry. Newly loaded sample chambers were allowed to purge overnight. The laser settings were kept constant for all of the analyses (line of spots setting, 8-9% power, 0.02s duration, 150µm spot size, 130µm spot spacing). For the EDJ profiles, each analysis consisted of four spots. The
Figure 5.1: Photograph of a cross-sectional surface of a first molar of individual S18. The lingual surface is to the right, buccal is to left, and the occlusal surface is at the top. The dentine horn is visible at the center top. The red line is 1mm. The laser scans can be seen running from top to bottom on the right side of the picture. The OES profile is at the far right and the EDJ profile is to the left of it. Four test scans can be seen on the left portion of the tooth. Charring is evident one of these test shots, as it was positioned too close to the dentine. Each scan is 500-600μm long and ~200μm wide and consists of four laser shots in succession.

OES profiles of the four canines without EDJ profiles consisted of scans with varying numbers of spots.

5.2.5 Age Estimation

Intratooth stable isotope data are usually plotted by distance from the cuspal tip or the cervix. To facilitate comparisons between teeth and sampling locations, and to enable an age-based analysis of the isotopic patterns, I estimated the chronological age that each measurement represented. In an attempt to estimate age with more accuracy, I used dental development data from a recent microstructural study to create growth curves for first molars and canines. Age of tooth formation data from Reid and Dean (2006) were used to create average chronological
trajectories of dental development for first molars and canines. Using enamel microstructural features, Reid and Dean (2006) calculated ages (in days) of cusp completion and attainment of each successive decile of tooth development from the cuspal tip to the cervix along the lingual surface of anterior teeth and along several cusps of the molars. They present results for populations from Northern Europe and Southern Africa. Given that there are some differences in the dental development rates between these populations, and that Nubia is equidistant from both, I combined the means of these data into a general estimate of dental development. The advantage of this approach is that it incorporates the non-linearity of crown extension rates into the model. The rate of enamel development accelerates and decelerates over the course of tooth formation. Ignoring this has been shown to skew age estimations for linear enamel hypoplasia (Reid and Dean, 2000; Ritzman et al., 2008).

Images were taken of each tooth with the laser-mounted camera before and after sampling and were uploaded into the program ObjectJ. Curvilinear measurements were taken from the cuspal tip to the cervix along the OES, and from the dentine horn to the cervix along the EDJ. In cases of minimal wear, the location of the cuspal tip was estimated. The distance between the midpoint of each scan and the dentine horn was measured for EDJ profiles, and the distance between the midpoint of each scan and the cuspal tip was measured along the OES profiles. These points were then fit to the appropriate growth curve by percentage attainment of maximum length, and age estimates were interpolated.

5.3 Results

The laser $\delta^{13}$C and $\delta^{18}$O values for an internal enamel standard (black rhinoceros) were -12.9±0.4 and 20.8±0.8 (N=8) for $\delta^{13}$C and $\delta^{18}$O, respectively. Previous phosphoric acid
hydrolysis measurements were -12.7±0.2 and 28.7±0.5 (N=15) for δ\textsuperscript{13}C and δ\textsuperscript{18}O, respectively (Passey and Cerling, 2006).

5.3.1 EDJ Profiles

Intratooth profiles of δ\textsuperscript{13}C and δ\textsuperscript{18}O measured at the EDJ of five first molars and one canine are presented in Figures 5.2-5.7. Descriptive statistics are given in Table 5.2.

Table 5.2: Descriptive statistics for EDJ stable isotope profiles

<table>
<thead>
<tr>
<th>EDJ profiles</th>
<th>Tooth</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burial</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R26</td>
<td>M1</td>
<td>6</td>
<td>-10.3</td>
<td>0.9</td>
<td>2.2</td>
<td>25.8</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
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<td>M1</td>
<td>5</td>
<td>-11.5</td>
<td>0.7</td>
<td>1.7</td>
<td>25.1</td>
<td>1.2</td>
<td>2.8</td>
</tr>
<tr>
<td>S109</td>
<td>M1</td>
<td>5</td>
<td>-10.1</td>
<td>0.9</td>
<td>2</td>
<td>25.3</td>
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<td>M1</td>
<td>9</td>
<td>-11.0</td>
<td>1.1</td>
<td>3.4</td>
<td>25.4</td>
<td>0.6</td>
<td>1.8</td>
</tr>
<tr>
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<td>-11.1</td>
<td>0.7</td>
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<td>1.0</td>
<td>2.4</td>
</tr>
<tr>
<td>S18</td>
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<td>10</td>
<td>-11.1</td>
<td>0.9</td>
<td>2.8</td>
<td>24.0</td>
<td>0.8</td>
<td>2.6</td>
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</tbody>
</table>

Figure 5.2: Overlay plot of δ\textsuperscript{13}C\textsubscript{ap} and δ\textsuperscript{18}O\textsubscript{ap} profiles measured at the EDJ by age estimate in years for a first molar of individual R26.
Figure 5.3: Overlay plot of $\delta^{13}\text{C}_{\text{ap}}$ and $\delta^{18}\text{O}_{\text{ap}}$ profiles measured at the EDJ by age estimate in years for a first molar of individual R81.

Figure 5.4: Overlay plot of $\delta^{13}\text{C}_{\text{ap}}$ and $\delta^{18}\text{O}_{\text{ap}}$ profiles measured at the EDJ by age estimate in years for a first molar of individual S109.
Figure 5.5: Overlay plot of $\delta^{13}\text{C}_{ap}$ and $\delta^{18}\text{O}_{ap}$ profiles measured at the EDJ by age estimate in years for a first molar of individual S18.

Figure 5.6: Overlay plot of $\delta^{13}\text{C}_{ap}$ and $\delta^{18}\text{O}_{ap}$ profiles measured at the EDJ by age estimate in years for a first molar of individual S213.
Figure 5.7: Overlay plot of $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ profiles measured at the EDJ by age estimate in years for a canine of individual S18. Note that the different age scale for the canine compared with the first molars.

The first molar EDJ profiles display between 1.7‰ and 3.4‰ variation in $\delta^{13}C_{ap}$ and between 0.9‰ and 2.8‰ variation in $\delta^{18}O_{ap}$. The ranges for the canine EDJ profile are 2.8‰ and 2.6‰ for carbon and oxygen isotopes, respectively. The pattern of change through time in $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ appears cyclical, with the two isotopes slightly out of phase with one another. $\delta^{13}C_{ap}$ tends to change direction before $\delta^{18}O_{ap}$. In all but one first molar (R81), $\delta^{13}C_{ap}$ values are relatively high in the earliest forming enamel and decline to lower values towards the first year of life. $\delta^{18}O_{ap}$ begins at higher values as well, but falls to lower values later than $\delta^{13}C_{ap}$.

Individual R81 shows the opposite pattern. The first molar EDJ profiles begin close to birth and end between enamel forming between 2.5yrs and 3yrs. The EDJ profile in the canine of individual S18 begins closer to age 1 and terminates around age 5. It shows a similar pattern of
fluctuation with $\delta^{13}C_{ap}$ changing course before $\delta^{18}O_{ap}$. At a mean level, there are no differences among the profiles in $\delta^{13}C_{ap}$, but there are differences in $\delta^{18}O_{ap}$ (ANOVA, $F_{4,25}=2.8$, $P<0.05$).

5.3.2 OES Profiles

Intratooth profiles of $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ measured at the OES of the five molars are presented in Figures 5.8-5.12. OES profiles for the five canines are presented in Figures 5.13-5.17. Because enamel prisms extend upward and outward from the dentine horn to the OES, the OES profiles begin with later forming enamel than the EDJ profiles. Unlike the first molar profiles, the sampling locations of the canine OES profiles relative to the cuspal tip and cervix vary among teeth. In some cases, the laser scans are very close together and in others farther apart. The age and isotope ratio axes were kept constant across this set of figures. Descriptive statistics are given in Table 5.3.

<table>
<thead>
<tr>
<th>OES profiles</th>
<th>Tooth</th>
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<th>Range</th>
<th>Mean</th>
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<td>0.3</td>
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<td>24.8</td>
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<td>0.6</td>
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</tr>
</tbody>
</table>

Table 5.3: Descriptive statistics for OES stable isotope profiles
The molar and canine OES intratooth profiles show inconsistent patterns. In the first molar OES profiles for individuals R81 (Figure 5.9) and S18 (Figure 5.11), for example, $\delta^{13}\text{C}_{\text{ap}}$ and $\delta^{18}\text{O}_{\text{ap}}$ change is related, but for individual S109, carbon and oxygen isotopes appear to be antiphase in both the molar (Figure 5.10) and canine (Figure 5.15). OES carbon and oxygen isotope profiles for the canine of individual S213 (Figure 5.17) also appears to be antiphase. Some of the OES profiles appear cyclical, but others do not. These profiles also differ in the amount of variation they display. The $\delta^{13}\text{C}_{\text{ap}}$ profile for R26’s canine (Figure 5.13) drops 4.5‰ in the space of about one year, while the profile for the canine of R81 varies by only 1.4‰. Among first molars, there are significant differences among the $\delta^{13}\text{C}_{\text{ap}}$ means (ANOVA, $F_{4,22}=4.3$, $P=0.01$). Individual S109 has the highest $\delta^{13}\text{C}_{\text{ap}}$ mean among the molars (Tukey-Kramer HSD, $P<0.05$). There are also significant differences among molar $\delta^{18}\text{O}_{\text{ap}}$ means (ANOVA, $F_{4,22}=10.7$, $P<0.0001$).

![Figure 5.8: Overlay plot of $\delta^{13}\text{C}_{\text{ap}}$ and $\delta^{18}\text{O}_{\text{ap}}$ profiles generated at the OES by age estimate in years of a first molar of individual R26]
Figure 5.9: Overlay plot of $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ profiles generated at the OES by age estimate in years of a first molar of individual R81.

Figure 5.10: Overlay plot of $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ profiles generated at the OES by age estimate in years of a first molar of individual S109.
Figure 5.11: Overlay plot of $\delta^{13}$C$_{ap}$ and $\delta^{18}$O$_{ap}$ profiles generated at the OES by age estimate in years of a first molar of individual S18.

Figure 5.12: Overlay plot of $\delta^{13}$C$_{ap}$ and $\delta^{18}$O$_{ap}$ profiles generated at the OES by age estimate in years of a first molar of individual S213.
Figure 5.13: Overlay plots of $\delta^{13}\text{C}_{\text{ap}}$ and $\delta^{18}\text{O}_{\text{ap}}$ profiles generated at the OES by age estimate in years for a canine of individual R26.

Figure 5.14: Overlay plots of $\delta^{13}\text{C}_{\text{ap}}$ and $\delta^{18}\text{O}_{\text{ap}}$ profiles generated at the OES by age estimate in years for a canine of individual R81.
Figure 5.15: Overlay plots of $\delta^{13}\text{Cap}$ and $\delta^{18}\text{O}_{ap}$ profiles generated at the OES by age estimate in years for a canine of individual S109.

Figure 5.16: Overlay plots of $\delta^{13}\text{Cap}$ and $\delta^{18}\text{O}_{ap}$ profiles generated at the OES by age estimate in years for a canine of individual S18.
5.3.3 EDJ and OES Comparisons

EDJ and OES profiles are plotted together in Figures 5.18-5.22 for the five molars and the canine of individual S18. Tests of unequal variance (Levene, F-test) find a significant difference in variance between the two sampling locations in only one case, the first molar oxygen isotope profiles of individual S213 (Barlett, P<0.05; F-test, P<0.05). There are, however significant differences between the means. With the data pooled, OES profiles have significantly lower δ^{13}C_{ap} values (t=5.4, P<0.0001) and δ^{18}O_{ap} values (t=2.7, P<0.005) than do the EDJ profiles. This is not always consistent, however. Individual S18 has significantly lower δ^{13}C_{ap} and δ^{18}O_{ap} values in the OES profiles of both the first molar (mean δ^{13}C_{ap} difference=1.3‰, t=2.77, P<0.01; mean δ^{18}O_{ap} difference=0.8‰, t=3.1, P<0.005) and canine (mean δ^{13}C_{ap} difference=1.4‰, t=3.02, mean δ^{18}O_{ap} difference=1.1‰, t=3.1, P<0.005). The δ^{13}C_{ap} means are
significantly lower in the first molar OES profiles of both R81 (mean difference=1.2‰, t=2.74, P<0.02) and S213 (mean difference=1.7‰, t=4.0, P<0.005), but mean δ¹⁸O_ap means between the two sampling locations are indistinguishable for these individuals. For individual R26, it is the δ¹⁸O_ap mean that is lower for the OES (mean difference=1.5‰, t=6.6, P<0.0002) and the δ¹³C_ap means are indistinguishable. Only S109 has indistinguishable EDJ and OES profiles for both isotopes. The pattern of lower OES carbon and oxygen isotope values does not appear to be related to especially high values at early ages in the EDJ profiles, as the EDJ profiles also tend to have high values at later ages than those represented by the OES profiles as well.

The pattern and rate of isotopic change in the two profiles do not correspond well. In some cases, the isotopic variability in the OES profile appears to be out of phase with the EDJ (S213 and S18 molars).

Figure 5.18: Relationship between δ¹³C_ap and δ¹⁸O_ap profiles generated at the EDJ and OES by age estimate in years for a first molar of individual R26.
Figure 5.19: Relationship between $\delta^{13}\text{Cap}$ and $\delta^{18}\text{Oap}$ profiles generated at the EDJ and OES by age estimate in years for a first molar of individual R81.

Figure 5.20: Relationship between $\delta^{13}\text{Cap}$ and $\delta^{18}\text{Oap}$ profiles generated at the EDJ and OES by age estimate in years for a first molar of individual S109.
Figure 5.21: Relationship between $\delta^{13}\text{C}_{ap}$ and $\delta^{18}\text{O}_{ap}$ profiles generated at the EDJ and OES by age estimate in years for a first molar of individual S18.

Figure 5.22: Relationship between $\delta^{13}\text{C}_{ap}$ and $\delta^{18}\text{O}_{ap}$ profiles generated at the EDJ and OES by age estimate in years for a first molar of individual S213.
Figure 5.23: Relationship between $\delta^{13}\text{Cap}$ and $\delta^{18}\text{O}_{\text{ap}}$ profiles generated at the EDJ and OES by age estimate in years for a canine of individual S18.

5.3.4 First Molar and Canine Comparisons

For each individual, the relationship between first molar and canine $\delta^{13}\text{Cap}$ and $\delta^{18}\text{O}_{\text{ap}}$ profiles is plotted in Figures 5.24-5.29. The OES profiles are used for each individual. For individual S18, the relationship between EDJ profiles of the first molar and canine is plotted in Figure #. Note that the scale varies by individual, both for isotope values and age estimate.

There is overlap in the timing of enamel formation between these two teeth types, but the intertooth comparisons reveal that the profiles do not always correspond in value or pattern of change.

With the data pooled, first molars have higher $\delta^{13}\text{Cap}$ (mean difference=0.7‰, t=2.7, P<0.01) and higher $\delta^{18}\text{O}_{\text{ap}}$ (mean difference=1.1‰, t=5.5, P<0.0001) values on average than canines, but this pattern is not expressed in every individual. Mean $\delta^{18}\text{O}_{\text{ap}}$ values are
Figure 5.24: Relationship between first molar and canine $\delta^{13}C_{\text{ap}}$ and $\delta^{18}O_{\text{ap}}$ profiles by age estimate in years for individual R26.

Figure 5.25: Relationship between first molar and canine $\delta^{13}C_{\text{ap}}$ and $\delta^{18}O_{\text{ap}}$ profiles measured at the OES by age estimate in years for individual R81.
Figure 5.26: Relationship between first molar and canine δ¹³C ap and δ¹⁸O ap profiles measured at the OES by age estimate in years for individual S109.

Figure 5.27: Relationship between first molar and canine δ¹³C ap and δ¹⁸O ap profiles measured at the OES by age estimate in years for individual S18.
Figure 5.28: Relationship between first molar and canine $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ profiles measured at the OES by age estimate in years for individual S213.

Figure 5.29: Relationship between first molar and canine $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ profiles measured at the EDJ by age estimate in years for individual S18.
significantly lower in canines than first molars for three out of five individuals. The mean difference is 1.4‰ for individual S213 and the EDJ comparisons for individual S18, 1.7‰ for the OES comparison of individual S18, and 2.8‰ for individual S109.

Carbon isotope values in the two tooth types appear to more closely approximate one another than δ¹⁸Oap values. S109’s canine profile has lower δ¹³Cap values than the first molar by 3.1‰, but the canine δ¹³Cap profile of R81 has higher δ¹³Cap values (0.7‰) than the first molar. There are no differences in the δ¹³Cap and δ¹⁸Oap values of molar and canine profiles for individual R26 (Figure 5.24). Profiles measured at the EDJ for both tooth types in individual S18 show cyclical patterns that overlap considerably in age estimate, but appear to be out of phase with one another.

The differences between canine and molar δ¹³Cap and δ¹⁸Oap values do not appear to be solely the result of a temporal effect. In other words, the differences do not appear to be driven by earlier forming enamel in the first molar that may have a different isotopic composition than later forming enamel sampled in both teeth. Enamel forming at the same age in different teeth can have stable isotope values that differ by ~1-3‰.

Canine and first molar profiles have equal variances in all cases except one; the δ¹³Cap profile for the canine of individual S109 is less variable than that of the first molar (Levene, P<0.05; F-test, P<0.05).

5.4 Discussion

The results presented here suggest that there are differences in how sampling locations and tooth types record isotopic variability. The profiles also reveal interesting patterns that may
speak to seasonal variability and weaning behavior. The methodological implications will be discussed first, followed by a discussion of the possible interpretations regarding human diet and life history at Kulubnarti.

5.4.1 Methodological Implications

Differences between sampling location and tooth type are likely due to interplay between the complexities of mineralization, sampling strategy, and error in age estimation. They may also be due to variation in enamel chemical composition and differences in the structure and formation parameters of canines and molars. Much of the temporal offset between EDJ and OES profiles of first molars, and between profiles of first molars and canines, is probably due to both the averaging effect of the sampling strategies employed and error in age estimations.

The sampling strategy and age estimation procedure produce a substantial averaging effect on the intratooth profiles at the EDJ and OES. Each laser scan spanned approximately 500μm of enamel parallel to the growth axis of the tooth. Enamel deposition in a permanent first molar takes approximately three years. If, for example, the length of the EDJ is sampled sequentially nine times, as it is in individual S18, each laser measurement represents approximately four months of enamel formation. The other EDJ profiles had fewer measurements, averaging relatively more enamel. The extended period of enamel mineralization adds to the averaging effect through isotopic overprinting, and because mineralization is irregular, it may attenuate the input signal differently in different parts of the tooth (e.g., Balasse, 2003; Zazzo et al., 2005). The age estimation procedure used here ignores substantial inter-individual and inter-populational differences in enamel formation times and thus adds to the
uncertainty (e.g., Reid and Dean, 2006). An obvious way to remove the effect of this age estimation method is to calculate enamel formation times for each individual tooth in the sample.

Isotope profiles measured at the EDJ and OES in the same teeth displayed different patterns. EDJ enamel is deposited with a higher initial mineral content than outer enamel and undergoes a shorter period of maturation (Suga, 1982). This should result in less isotopic overprinting (Balasse, 2003; Zazzo et al., 2005; Tafforeau et al., 2007). This is partly reflected in the data. EDJ profiles tend to appear more cyclical than OES profiles with a faster rate of change. One initial prediction was that the EDJ and OES profiles would be similar at a mean level, but the EDJ profiles would have more amplitude. Contrary to expectations, in only one tooth was the EDJ profile more variable than the OES (δ^{18}O_ap in the first molar of individual S213). Additionally, there are mean differences between the two series in both δ^{13}C_ap and δ^{18}O_ap that are not consistently expressed among the teeth, with OES profiles exhibiting lower δ^{13}C_ap values, δ^{18}O_ap values, or both. It is possible that the distribution of carbonate through the thickness of the enamel has an effect on the isotope ratios.

Carbonate content in bovine enamel, for example, decreases from the inner-most enamel to the enamel surface (Zazzo et al., 2005). Carbonate δ^{18}O is ~9‰ higher than phosphate δ^{18}O in apatites (Bryant et al., 1996; Iacumin et al., 1996). Laser ablation mixes the oxygen bearing phases of enamel, and a higher proportion of carbonate-derived oxygen may cause an increase in the δ^{18}O_ap values at the EDJ. In the molar of individual S18, for example, EDJ measurements produced higher CO₂ yields than OES measurements. Scans alternated between the profiles and the settings and number of laser shots were kept constant, ensuring that effects related to laser settings or adsorbed CO₂ from previous ablation events were held relatively constant for each profile. A higher CO₂ yield could reflect higher carbonate content, but yields were not correlated
with $\delta^{18}O_{ap}$ or $\delta^{13}C_{ap}$ values in this tooth (Figure 5.30). Carbonate content also affects the crystallographic properties of enamel (e.g., LeGeros, 1981), which may cause variation in how the laser interacts with enamel. It is also possible that diagenetic processes were operating differentially on the exterior of the teeth, altering the isotopic composition of the surface enamel more so than interior enamel (e.g., Schoeninger et al., 2003).

Mineralization and chemical differences may also play a role in the discrepancies between the molar and canine profiles within single individuals. The reasons for the differences between tooth types are not clear. More research into the mineralization parameters and chemical composition of human tooth enamel is needed to better understand the effects of sampling location and tooth type on stable isotope profiles.

Figure 5.30: Relationship between $\delta^{18}O_{ap}$ and area for EDJ and OES measurements of the first molar of individual S18. Area is a proxy for CO$_2$ yield
Despite these potential caveats, there are a number of possible interpretations regarding diet, seasonality, and life history at Kulubnarti.

5.4.2 Diet, Seasonality, and Life History

Although the small sample size prohibits population wide interpretations, some initial observations about seasonality, diet, and life history of these five individuals can be made. For this purpose, I will focus on the first molar EDJ profiles because they probably record the most meaningful information. Given the averaging effects of mineralization, sampling procedure, and age estimation, the isotope profiles underestimate the amplitude of the input signal, and there is no way to know at present what the magnitude of this underestimate is. These factors should also increase the wavelength of the measured signal, such that estimates of the length of time represented by an isotopic shift are inflated. In first molars, the period of each EDJ profile approximates 1-1.5 years, suggesting that some of the temporal characteristics of seasonality are present in the data (Figure 5.31). Four of the five carbon isotope profiles begin at high values, descend to their minima at about 0.5 and 1.5 years, and then rise to higher values between about 1.5 and 2.5 years. The oxygen isotope profiles of these same four individuals behave in a similar manner. They start relatively high, descend to minimum values between about 1.0 and 2.0 years, and rise to high values between about 2.0 and 2.5 years. This may represent a weaning signal whereby δ¹³C_

While it is highly likely that these individuals were breastfeeding and beginning supplementation during their first three years, the weaning hypothesis is insufficient to explain
Figure 5.31: $\delta^{13}C_{ap}$ (top) and $\delta^{18}O_{ap}$ (bottom) of EDJ profiles in first molars by age estimate in years.
the magnitude of the shifts in both isotopes. The profiles have ranges between 1.7% and 3.4‰ for $\delta^{13}C_{ap}$, and 0.9‰ and 2.8‰ for $\delta^{18}O_{ap}$. These values exceed the presumed magnitude of a weaning effect for both carbon and oxygen isotopes (~1.0% and <0.1%, respectively) (Wright and Schwarcz, 1998; Fuller et al., 2006a). Seasonality in diet and drinking water source probably play a more important role than weaning in controlling the isotopic composition of early forming enamel in this population.

Seasonality at Kulubnarti is expected to be large for both carbon and oxygen isotopes. $\delta^{18}O$ values measured in hair are variable (5.9‰) (Glasgow, 2011), suggesting substantial variability in drinking water $\delta^{18}O$. The Nile fluctuates yearly with an annual flood, and is the only water source at Kulubnarti for drinking and irrigation. Seasonality in carbon and oxygen is likely transferred from mother to children through breastmilk and then supplementary food and water, masking any trophic effect of the weaning process. This could explain the rise to higher values in the profiles. But if seasonality is masking the smaller effects of weaning, why do four out of five individuals appear to follow a similar trajectory and one (R81) appears to go in the opposite direction?

One possibility is that these data reflect season of birth. Intratooth $\delta^{18}O$ profiles have been used to identify season of birth in sheep (Balasse et al., 2003; Blaise and Balasse, 2012; Balasse et al., 2012). Figure 5.32 shows enamel $\delta^{18}O$ profiles in sheep of known birth season. (Blaise and Balasse, 2012, Fig.2). Winter births are clearly distinct from summer births. In the
Kulubnarti profiles, high δ^{13}C values likely reflect mid-summer when millet and sorghum are harvested, and low δ^{18}O values are probably associated with the flood of the Nile which reaches its peak in September. The relationship between the two isotopes in many of the profiles presented here perhaps reflects this temporal difference between these two yearly events.

It is also possible that amelogenesis affects the isotopic composition of phosphate and carbonate differently, which might contribute to an offset between δ^{13}C_{ap} and δ^{18}O_{ap} profiles. δ^{18}O values of phosphate and carbonate theoretically should have a 1:1 relationship with a consistent ~9‰ offset, but several researchers have observed variability between these two oxygen-bearing phases both between and within species (e.g., Bryant et al., 1996; Iacumin et al., 1996; Fox and Fisher, 2001). These differences have been used as a test of the integrity of fossil bone and enamel apatite, because carbonate is more susceptible to diagenetic processes (e.g., Iacumin et al., 1996; Fox and Fisher, 2001; Zasso et al., 2004; Martin et al., 2008a). Variability in the oxygen isotopic composition of carbonate and phosphate in enamel may be due to physiochemical processes controlling temperature and pH during amelogenesis (Adkins et al.,
2003; Martin et al., 2008a). Pellegrini et al. (2011) found that intra-tooth profiles of carbonate 
$\delta^{18}$O in fossil equid and cervid teeth were slightly attenuated relative to phosphate $\delta^{18}$O values, 
and suggest that the two phases may reflect different source pools of oxygen or variation in the 
timing of phosphate and carbonate incorporation into the enamel crystal lattice. This may 
suggest that variability in $\delta^{18}$O values measured with laser ablation, which mixes all of the 
oxygen-bearing phases, could be partly affected by variability in the oxygen isotope composition 
of coexisting carbonate and phosphate. This effect, if present, might influence how $\delta^{13}$Cap 
profiles and $\delta^{18}$Oap profiles correspond.

Pinpointing a season of birth is also made difficult by the signal dampening and 
attenuation issues previously discussed. The four profiles that follow more or less the same 
pattern perhaps suggest that those individuals were born closer to the summer than to the winter. 
If this type of interpretation could be strengthened, it could open a new door to studying fetal 
programming in the past. Environmental factors that affect the inter-uterine environment can 
have immediate and long-lasting effects on the health of newborns (Hille et al., 2007). Season of 
birth in humans has been linked to low birth weight (Murray et al., 2000; Elter et al., 2004), 
longevity (Flouris et al., 2009), and age at menopause (Cagnacci et al., 2005).

If we can identify specific seasons in the isotope profiles of Kulubnarti, we may also be 
able to study the relationship between seasonality and childhood health. One application, for 
example, would be to explore the association between seasonality and growth insults such as 
linear enamel hypoplasia or microstructural defects in enamel (Franz-Odendaal et al., 2003). 
Figure 5.33 shows the EDJ carbon and oxygen isotope profiles for the first molar of individual 
S18 with a shaded box representing the estimated location of a linear enamel hypoplasia on that 
same first molar. The growth disruption corresponds to a rise in the $\delta^{13}$Cap profile, which
suggests that the morbidity event occurred in the summer. This is not surprising because Nubia experienced high rates of morbidity and mortality in the summer in both recent (Corkhill, 1948; May and McLennan, 1970 in White and Schwarz, 1994) and ancient times (White and Schwarz, 1994). This interpretation has to be taken with caution, however, because the enamel at this point on the canines of S18 corresponds to low δ\(^{13}\)C\(_{ap}\) values. Clearly, a better understanding of how enamel mineralization process work in human teeth is needed before we can make such interpretations with confidence.

**5.5 Chapter Summary**

This chapter presented the results of the intratooth high resolution analyses of enamel. The results of the methodological comparisons indicate that δ\(^{13}\)C\(_{ap}\) and δ\(^{18}\)O\(_{ap}\) profiles vary by
sampling location and tooth type in single individuals. The possible reasons for this involve isotope attenuation caused by mineralization processes, error associated with estimating age, and potential differences in chemical or structural characteristics within and between teeth. The intratooth profiles, particularly those measured at the EDJ, reveal several permil variation in both $\delta^{13}$Cap and $\delta^{18}$Oap and appear to track seasonal shifting in C$_3$ and C$_4$ agriculture at Kulubnarti and possibly the yearly flood of the Nile. Seasonal variation in water storage practices may also influence $\delta^{18}$O$_{ap}$ variation. Weaning effects seem to be overprinted by a seasonal signal, which may provide a way to determine season of birth in humans, potentially offering a new window into health and adaptation in the past. An implication of these data is that the small differences between bulk samples of early and later forming teeth may reflect seasonality and not necessarily weaning. Weaning effects are likely to be more apparent in the nitrogen isotope composition of dentine collagen, which is the subject of the next chapter.
CHAPTER 6
HIGH RESOLUTIONS DENTINE ANALYSIS

6.1 Chapter Overview

This chapter presents the high-resolution dentine analysis. The same first molars and canines that were measured in the enamel analysis are used here. The chapter begins with a description of the methods used to create the dentine sections. Intratooth δ^{13}C_{coll} and δ^{15}N_{coll} profiles are first presented by individual, and then canine and molar profiles are compared. The data are interpreted primarily in terms of weaning behavior and childhood diet and are then compared to the cross-sectional collagen data (see Chapter 4). Other possible factors that may influence the patterning of δ^{13}C_{coll} and δ^{15}N_{coll} and methodological considerations are discussed. The chapter closes with a demonstration of the utility of this high-resolution approach for evaluating the relationship between linear enamel hypoplasia (LEH) and the weaning process.

6.2 Methods

6.2.1 Sectioning Method

The method of sectioning dentine for high-resolution analysis used here is similar to Method 2 in Beaumont et al. (2012), whereby a complete root, or portion of root, is demineralized before being sectioned into smaller samples for analysis. After the teeth were longitudinally sectioned (see Chapter 5, section 5.2), one half of each canine and one root radical of each first molar were demineralized in 0.5M hydrochloric acid (HCl) at 4°C for 72-90hrs. This was sufficient time for the enamel on each sample to dissolve. The roots were removed from the HCl and rinsed before they lost their shape. The partially demineralized roots were cut
with a scalpel into ~1mm transverse sections. Between 11 and 15 sections, each between 5 and 20mg, were obtained from each tooth. Each section was placed in HCl again for 12-24 hours until completely demineralized. The samples were then gelatinized in pH 3 HCl solution at 70°C for 48 hours, filtered, and then lyophilized. Carbon and nitrogen isotope ratios were measured at the Research Laboratory for Archaeology and the History of Art at the University of Oxford, and the Stable Isotope Facility at the University of California, Davis.

6.2.2 Age Estimation

The age of each dentine section was estimated using a linear model of root extension based on tooth initiation and root completion times reported in the literature (Hillson, 1986; Reid and Dean, 2006). Initiation times are birth, 0.55 years, and 0.75 years for maxillary first molars, maxillary canines, and mandibular canines, respectively. Root completion times are 9.5 years and 12 years for molars and canines, respectively. This means that the age estimates for molar and canine profiles overlap for nearly the entire profile.

The dentine sections were approximately equal in width, and each was considered an equal proportion of the total length of the root. The midpoint of each proportion was used to estimate the age that each dentine section represents. If, for example, the dentine of a first molar is sectioned into 13 equal portions, the first section accounts for 7.7% of the total length, which corresponds to an age-range of 0-0.7 years. This first section would be assigned an age of 0.35 years, but would include dentine that formed over much of the first year of life. In addition, dentine grows as a series of cones, and due to the transverse sectioning strategy, each section contains dentine from cones present in preceding and subsequent sections (Eerkens et al., 2011). This produces an averaging effect where the isotopic composition of each dentine section is
partially influenced by earlier and later forming dentine. Furthermore, root extension rates are non-linear and variable (Dean and Scandrett, 1995; Dean and Vesey, 2008).

For these reasons, the age estimates of each dentine section used in this study are ordinal approximations. Given that the first serial sections represent diet averaged over much of the first year of life, and that supplementation to some degree must occur by 6-8 months of age to meet the nutritional demands of young children (Chavez et al., 2000), no $\delta^{13}$C or $\delta^{15}$N values are likely to represent exclusive breastfeeding.

### 6.3 Results

Summary statistics for $\delta^{13}$C and $\delta^{15}$N data are presented in Table 6.1. Carbon and nitrogen isotope profiles for first molars and canines for each individual are shown in Figures 4.1-4.5. The relationship between first molar and canine profiles for $\delta^{13}$C and $\delta^{15}$N for each individual are shown in Figures 4.6-4.10.

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</tr>
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<tr>
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</tr>
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<td></td>
<td></td>
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<td></td>
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</tr>
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</tr>
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</tr>
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6.3.1 Carbon Isotopes

Analysis of variance reveals a significant difference among the $\delta^{13}C_{\text{coll}}$ means of both the first molar profiles ($F_{4,57}=24.1$, $P<0.0001$) and the canine profiles ($F_{4,65}=9.9$, $P<0.0001$). $\delta^{13}C_{\text{coll}}$ profile ranges are between 1.1‰ and 2.9‰ and tests for equality of variance find a significant difference among the variances of the canines (Levene, $P<0.001$; Bartlett, $P<0.001$), but not the molars. On average, the carbon isotope variation in each profile is about 20% of the total variation evident in the rib collagen $\delta^{13}C_{\text{coll}}$ data. Most of the first molar $\delta^{13}C_{\text{coll}}$ profiles begin with a descent from relatively higher values to lower ones over the first 1-4 years of life. The only exception is individual S18, whose first molar profile declines gradually by about 1.5‰ over the first 6 or 7 years. The variation in the carbon profiles is irregular and inconsistent. The first molar profile for individual R81 (Figure 4.2), drops by 3.5‰ in the first three years and then levels off, while the first molar profile for individual R26 (Figure 4.1) stays within about 1‰ over the first decade of life.

6.3.2 Nitrogen Isotopes

The nitrogen isotope profiles also differ at a mean level (ANOVA $F_{4,65}=22.0$, $P<0.0001$). The ranges within each $\delta^{15}N_{\text{coll}}$ profile are much larger than in carbon (2.3-5.0‰), and tests for equality of variance find significant differences among the variances of canines (Levene, $P=0.01$; Bartlett, $P<0.01$), but not for molars. The variability within each $\delta^{15}N_{\text{coll}}$ profile is, on average, about 45% of the total variation in rib collagen $\delta^{15}N_{\text{coll}}$. This is more than twice that of the carbon isotope profiles. All of the nitrogen isotope profiles, except for the canine of individual S18, decline from high to lower values over the first several years, but they do so at different rates. The $\delta^{15}N_{\text{coll}}$ of the canine of individual R26, for example, falls only about 1.5‰ before age
Figure 4.1: Biplots of $\delta^{13}C_{coll}$ and $\delta^{15}N_{coll}$ profiles by age estimate in years for the first molar (top) and canine (bottom) of individual R26.
Figure 4.2: Biplots of $\delta^{13}\text{C}_{\text{coll}}$ and $\delta^{15}\text{N}_{\text{coll}}$ profiles by age estimate in years for the first molar (top) and canine (bottom) of individual R81.
Figure 4.3: Biplots of $\delta^{13}\text{C}_{\text{coll}}$ and $\delta^{15}\text{N}_{\text{coll}}$ profiles by age estimate in years for the first molar (top) and canine (bottom) of individual S109.
Figure 4.4: Biplots of $\delta^{13}\text{C}_{\text{coll}}$ and $\delta^{15}\text{N}_{\text{coll}}$ profiles by age estimate in years for the first molar (top) and canine (bottom) of individual S18.
Figure 4.5: Biplots of $\delta^{13}C_{\text{coll}}$ and $\delta^{15}N_{\text{coll}}$ profiles by age estimate in years for the first molar (top) and canine (bottom) of individual S213.
two and then levels out for several years, while the canine of S109 gradually drops ~5‰ over the first 10-12 years of life.

6.3.3 Canine and Molar Comparisons

The canine and molar comparisons show some interesting patterns (Figures 4.6-4.10). For the most part, they correspond reasonably well. There are no differences in variance in either isotope profile between the tooth types (Levene, F-test, P>0.05). However, there are some differences in $\delta^{13}$C$_{coll}$ and $\delta^{15}$N$_{coll}$ values at a mean level. The first molar of individual R26 has higher $\delta^{13}$C$_{coll}$ values ($t=3.4$, $P<0.001$) and higher $\delta^{15}$N$_{coll}$ values ($t=2.2$, $P<0.02$) than the canine. The canine and molar profiles of individual S109 correspond well in $\delta^{15}$N$_{coll}$, but the $\delta^{13}$C$_{coll}$ profiles appear to oscillate out of phase with one another. The canine and molars are least similar for individual S18, where both carbon and nitrogen isotope values diverge at many points along the profiles.

6.3.4 Comparison to Cross-sectional Data

Each profile is plotted together by isotope and tooth type in Figures 4.11-4.14. In each figure, a simulated longitudinal profile, generated from the rib collagen data by age at death, is plotted on the same graph. In carbon, all of the dentine values for canines and molars fall below the rib collagen mean line before the age of five. For nitrogen, the first molar profiles show that four out of five individuals have higher values close to birth and descend to lower values more quickly than the rib collagen mean line. The first molar dentine $\delta^{15}$N$_{coll}$ profiles also extend above the rib collagen mean line after it levels off at 6yrs. These patterns are less clear in the canine profiles.
Figure 4.6: Relationship between first molar and canine profiles for $\delta^{13}C_{coll}$ and $\delta^{15}N_{coll}$ by age estimate in years of individual R26.

Figure 4.7: Relationship between first molar and canine profiles for $\delta^{13}C_{coll}$ and $\delta^{15}N_{coll}$ by age estimate in years of individual R81.
Figure 4.8: Relationship between first molar and canine profiles for $\delta^{13}C_{\text{coll}}$ and $\delta^{15}N_{\text{coll}}$ by age estimate in years of individual S109.

Figure 4.9: Relationship between first molar and canine profiles for $\delta^{13}C_{\text{coll}}$ and $\delta^{15}N_{\text{coll}}$ by age estimate in years of individual S18.
6.4 Discussion

6.4.1 Tooth Types

The intratooth profiles of canines and first molars diverge from one another in both $\delta^{13}\text{C}_{\text{coll}}$ and $\delta^{15}\text{N}_{\text{coll}}$ in three individuals, but not always consistently (Figures 4.6-4.10). The instances in which they do correspond are characterized by having relatively smooth nitrogen isotope profiles (e.g., S109, R81, and to a lesser extent, S213). When the profiles wobble irregularly, the canines and first molars tend to diverge, with the canine exhibiting more irregular peaks and valleys (e.g., S18, R26, $\delta^{13}\text{C}_{\text{coll}}$ profile of S109). In these profiles, first molar and canine profiles appear to reflect dietary change at different ages. Beaumont et al. (2012) found good correspondence between first and second molar profiles, but there was little variability in the isotope signals, and first and second molars share the same general structure.
Figure 4.11: $\delta^{15}N_{\text{coll}}$ profiles by age estimate in years for first molars. The thick black line is composed of mean $\delta^{15}N_{\text{coll}}$ values for rib collagen in the cross-sectional analysis.

Figure 4.12: $\delta^{13}C_{\text{coll}}$ profiles by age estimate in years for first molars. The thick black line is composed of mean $\delta^{13}C_{\text{coll}}$ values for rib collagen in the cross-sectional analysis.
Figure 4.13: $\delta^{15}N_{\text{coll}}$ profiles by age estimate in years for canines. The thick black line is composed of mean $\delta^{15}N_{\text{coll}}$ values for rib collagen in the cross-sectional analysis.

Figure 4.14: $\delta^{13}C_{\text{coll}}$ profiles by age estimate in years for canines. The thick black line is composed of mean $\delta^{13}C_{\text{coll}}$ values for rib collagen in the cross-sectional analysis.
These differences are likely due to a combination of interrelated factors including differences in tooth structure, sampling, dentine growth rate, and age estimation. Molars have a larger volume of dentine under the crown where the growth front is roughly parallel to the transverse sampling sections. The growth lines become more oblique as the root extends beyond the cementum-enamel junction. Early forming growth lines in canine dentine, on the other hand, are more oblique, owing to its single conical cusp. This means that serial sections of early forming molar enamel are less time averaged than those of canines, because they include less enamel from early and later forming cones (Eerkens et al., 2011). Canines also have a faster extension rate than molars (Dean and Vesey, 2008), which might explain why the canine profiles tend to display more variation than the molars in sections farther down the tooth. These factors, coupled with the linear age estimation technique, probably account for the majority of differences between the isotope profiles. First molars are probably a more ideal choice of tooth for isotopic studies that seek to reconstruct weaning because of their early forming dentine and greater volume.

6.4.2 Life History Interpretations

It is clear that the intra-tooth profiles reveal isotopic variability in both $\delta^{15}N_{coll}$ and $\delta^{13}C_{coll}$ values. While there are a number of possible interpretations of these patterns, weaning behavior likely contributes to much of the variation within and between the profiles. Issues that complicate weaning interpretations are discussed in the next section. Infant $\delta^{15}N$ tracks the weaning process by declining during weaning from higher values to values more similar to their mothers at the cessation of breastfeeding (Fogel et al., 1989; Fuller et al., 2006a). The cessation of breastfeeding can be estimated in the dentine profiles by identifying the point at which $\delta^{15}N_{coll}$
ceases to decline and reaches a plateau. Focusing on first molars, because they contain the earliest forming dentine, there are differences in both the magnitude and timing of nitrogen isotope change with time. Individual S18 displays a rapid shift from 9.6‰ to 8.0‰ between the second and third dentine sections, suggesting a relatively abrupt weaning process at approximately 2 years. This shift (1.6‰) is the lowest shift among the first molars and 8.0‰ is the lowest $\delta^{15}N_{coll}$ value in the dataset. The lower $\delta^{15}N_{coll}$ values of S18 may reflect both a low quality maternal diet and a low quality post-weaning diet. Interestingly, S18 appears to recover, with values rising to 10.8‰ after the eighth year, well within the range of the other post-weaning profiles. It is also possible that S18 migrated into the area from a region with different baseline $\delta^{15}N$ values.

The other four profiles record more gradual weaning processes with greater trophic shifts in $\delta^{15}N_{coll}$. These profiles also begin at least 2.8‰ higher than individual S18. R81 displays the largest and most gradual decline in $\delta^{15}N_{coll}$, from 14.1‰ in the first section to 10.5‰ in the eighth section, around 5yrs. The remaining profiles display $\delta^{15}N$ shifts between 2.4‰ and 3.2‰ in the 5th-6th serial sections, corresponding to weaning completion between about 3yrs and 4yrs of age. One pattern that is particularly striking is the correspondence in slope among the four gradual profiles. This presumably reflects similar degrees in the rate of supplementation among these individuals.

The first molar $\delta^{13}C_{coll}$ patterns are less clear. Four of the five profiles descend to lower $\delta^{13}C_{coll}$ values after the 1st or 2nd serial section by between 0.5‰ and 1.2‰. One profile (R81) falls further with a shift of 2.4‰. In general, the $\delta^{13}C_{coll}$ patterns approximate those of nitrogen, suggesting that that carbon isotope variation in early sections is also related to weaning. Three of the five carbon profiles dip to low values at the same age as their associated $\delta^{15}N_{coll}$ patterns.
(S109, R26, S18), but the other two begin to rise to higher $\delta^{13}C_{coll}$ values in earlier dentine sections than nitrogen (R81, S213). The early decline in $\delta^{13}C_{coll}$ could be reflecting a trophic effect (Richards et al., 2002; Fuller et al., 2003; Fuller et al., 2006a), or the isotopic composition of weanling foods. This pattern is possibly explained by variation in the use of sheep or goat milk as a supplement to breast milk.

Animal milk is likely the most $^{15}N$-enriched weanling food available and may have contributed to the gradual $\delta^{15}N_{coll}$ patterns. Sheep or goat milk may have been $^{13}C$-enriched as well at Kulubnarti. $C_4$ crops are common animal fodder in Nubia today (May and McLennen, 1970 in White et al., 2004), and may also have been in the past (e.g., Dupras et al., 2001; Copley et al., 2004). Unfortunately, the isotopic composition of Kulubnarti fauna is unknown.

### 6.4.3 Longitudinal and Cross-sectional Comparisons

The mean $\delta^{15}N_{coll}$ and $\delta^{13}C_{coll}$ values of rib collagen by age at death are plotted with the intratooth profiles to facilitate comparison of life history patterns between individuals who survived infancy and childhood and those that did not. The advantage of this approach is that one can directly evaluate the relationship between weaning behavior and mortality. The sample size here is small, however, limiting the ability to make inferences at the population level. Nevertheless, comparisons of the longitudinal and cross-sectional data reveal some interesting patterns, and demonstrate the utility of the approach. Complicating factors such as bone turnover are discussed below.

The first molar intratooth nitrogen isotope profiles all reach low values and plateau before the rib collagen mean line (Figure 4.11). One interpretation of this pattern is that the cessation of breastfeeding occurred earlier in these five individuals than the average individual
who died during the process. This is perhaps surprising given the immunological benefit of breastfeeding and the well-known association between breastfeeding and reduced mortality (e.g., Knodel and Kintner, 1977; Holland, 1987; Palloni and Teinda 1986; Briand et al., 1988; Lindsrom and Harhanu, 2000; Jackson and Nazar, 2006). However, breastmilk alone is insufficient to meet the nutritional requirements of infants beyond about 6 months of age, when supplemental foods with adequate nutrition must be included in the infant diet (Wray et al., 1978). Earlier weaning in the survivors at Kulubnarti might imply that their nutritional demands were more adequately met with higher quality complementary foods and that non-survivors relied too heavily on breastmilk for too long and suffered from malnutrition. This interpretation is not without precedent in the present-day world.

Extended breastfeeding is a common response to food shortages in Africa, and has been linked to increased mortality in children (e.g., Cantrelle and Leridon, 1971; Victoria et al., 1984; Fawzi et al., 1998; Lindstrom and Berhanu, 2000). For example, Cantrelle and Leridon (1971) found that the mean age at weaning in children that died during a five year period in Senegal was later than those that survived, suggesting that supplementary foods lacked adequate nourishment to stave off severe malnutrition. Similarly, Lindstrom and Berhanu (2000) found that children in Ethiopia that were breastfed beyond 2 years of age were 1.5 times more likely to die before the age of 3 years, and that those breastfed beyond 4 years of age were 8 times more likely to die before the age of 5 years, than those supplemented and weaned earlier.

At Kulubnarti, early supplementation in the survivors may be evidenced by the initial and gradual decline in $\delta^{15}N_{col}$ values for four out of five individuals. In the cross-sectional analysis, the $\delta^{15}N_{col}$ mean remains at ~12‰ for the first three years, perhaps suggesting that the many of these individuals were not yet receiving supplementation. It is also possible that malnutrition
was playing a role in the high values of the non-survivors, as endogenous protein recycling can elevate $\delta^{15}N$ values (e.g., Mekota et al., 2006). Furthermore, the initial $\delta^{15}N_{\text{coll}}$ values are higher in these four profiles than the mean rib collagen $\delta^{15}N_{\text{coll}}$ value for 1 year-olds (0.5-1.5 year age category), which may indicate mothers with higher-quality diets confer an advantage onto their progeny. Research on modern mothers and infants suggests that maternal malnutrition can adversely affect the immunological qualities of colostrum and breast milk and can result in growth stunting and anemia in infants (Miranda et al., 1983; Aihie Sayer and Cooper, 2007).

Four of five $\delta^{15}N_{\text{coll}}$ first molar profiles also plateau at higher values than those that died suggesting that they may have been weaned onto higher quality diets, such as those that include more animal foods. The inclusion of animal foods during supplementation is associated with reduced risk of anemia (e.g., Neumann et al., 2002), increased growth (e.g., Allen et al., 1991), earlier weaning (e.g., Marquis et al., 1997), and earlier attainment of developmental milestones such as walking (Kuklina et al., 2004). Interestingly, as mentioned earlier, the $\delta^{15}N_{\text{coll}}$ values of individual S18 plateau at an earlier age and at lower values than the other profiles and the rib collagen line, but they rise above rib collagen values between the ages of 6 and 8 years, perhaps signifying increased animal protein.

Higher quantities of the same diet could also account for the higher values after age 5 in the profiles, by elevating diet-tissue nitrogen isotope discrimination (Bearhop et al., 2002; Pearson et al., 2003; Sponheimer et al., 2003b, Vanderklift and Ponsard, 2003). Protein quantity, dietary quality, growth rates, and malnutrition can have variable effects on $\delta^{15}N$, making it difficult to interpret the post-weaning patterns any farther.

The rib collagen $\delta^{13}C_{\text{coll}}$ mean values show a gradual decline over the first 6 years, while the intratooth profiles decline earlier and have lower $\delta^{13}C_{\text{coll}}$ values up to about age 4 years. It is
unclear why survivors would have lower carbon isotope values than the average non-survivor. The lower values could reflect weaning foods with more fruits and vegetables and less reliance on C₄ grains. A weanling diet high in fiber can adversely affect health by inhibiting uptake of micronutrients (Rheinhold, 1982).

While the longitudinal vs. cross-sectional approach clearly has potential to gain insight into the early life histories of survivors and non-survivors and potential links between weaning behavior, diet, and mortality, there are some potential caveats that need to be considered. The isotopic compositions of bone collagen and dentine collagen have different temporal characteristics. The isotopic composition of bone is partly a function of remodeling rate (Hedges et al., 2007). Ribs are a good choice of bone for isotopic studies by age at death because trabecular bone turns-over more quickly than denser cortical bone (e.g., femoral mid-shaft) (ICRP, 1975). The turnover rate for adult rib is ~5% per year, but turnover rates in younger individuals are much faster. 100-200% of the skeleton is turned-over within the first year of life, and this rate drops to 10% per year by age 7 (ICRP, 1975). Thus, bone remodeling dynamics ensure that there will be some time averaging effect, and that this will be age-specific.

Until bone collagen is completely turned over with new tissue, its isotopic composition incorporates a signal from the previous diet (Hedges et al., 2007). Because of this, a cross-sectional approach likely overestimates the age when weaning is complete. Serial section analysis of dentine is also subject to time averaging due to the size of the section and the overlapping oblique growth fronts. The degree to which these factors affect comparisons between the tissues is difficult to constrain, but perhaps it would be prudent to incorporate error into the age estimates so that a statistical argument could be made regarding the likelihood that a dietary change occurred at a certain age. Nonetheless, there are interpretable patterns in the data.
and this approach holds promise for testing a number of hypotheses regarding the interplay between weaning behavior, childhood diet, stress events, and mortality. One of these tests is the relationship between weaning behavior and linear enamel hypoplasia (LEH).

6.4.4 Relationship between Enamel Hypoplasia and Weaning Behavior

Given the relatively early ages of LEH and the well-known risks associated with weaning, the two have been logically linked (e.g., Goodman et al., 1984; Corruccini et al., 1985; Van Gerven et al., 1990a; Ubelaker, 1992; Moggi-Cecchi et al., 1994). Indeed, there is evidence linking childhood malnutrition and LEH which coincide with general patterns of weaning in modern children (e.g., Goodman et al., 1987, 1992; May et al., 1993). However, peak frequencies of LEHs have been found to post-date reported weaning times in some historical populations, suggesting that LEHs may not always reflect stress directly associated with weaning (e.g., Blakey et al., 1994; Wood, 1996).

Furthermore, there are methodological problems with the link between LEH and weaning. Traditional methods of estimating the age of occurrence of LEHs are likely to underestimate the timing of these stress events because they do not take into account the formation time of cuspal enamel, suggesting that the connection between LEH occurrence and presumable weaning times in the archaeological record may be exaggerated (Martin et al., 2008b; Ritzman et al., 2008). There are also inter- and intratooth differences in susceptibility to hypoplasia formation; anterior teeth are more commonly affected than posterior teeth, and the middle third of anterior teeth is more commonly affected than the cuspal or cervical thirds (Goodman and Armelagos, 1985; Wright, 1997).
It is clear that the relationship between the timing of LEH formation and the weaning process is not well constrained and is likely to vary among populations (Katzenberg et al., 1996). Intratooth stable isotope profiles of dentine provide a means to directly test the relationship between LEH and the weaning process. And by extension, these longitudinal analyses provide a means to test the effects of weaning behavior on early mortality and long-term health outcomes in the past, independent of LEHs. In the following paragraphs, the relationship between the timing of LEHs and patterns of intratooth nitrogen isotope variation are presented and discussed.

6.4.5 Correspondence between Weaning and LEH at Kulubnarti

LEHs were present on each tooth used in this dissertation. The location of each LEH was measured. For LEHs that occur as thin singular bands, the midpoint was measured; for wider LEHs, the beginning and end was measured to capture the age range of the stress event. Age of occurrence was estimated using the same method used for estimating the age of each OES laser scan (Chapter 5). As described in Chapter 5, this method utilizes enamel formation data from Reid and Dean (2006) and incorporates both the non-linearity of crown development and the formation period of the cuspal enamel. Thus, the age estimates of the LEHs are more accurate than the traditional chart method of Goodman et al. (1980) or the regression method of Goodman and Rose (1990) (see Martin et al., 2008b; Ritzman et al., 2008).

The approximate timing of LEHs present on either the canine or first molar are plotted on the first molar $\delta^{15}N_{coll}$ profiles of each individual in Figures 4.15-4.19. All but one LEH occur when $\delta^{15}N_{coll}$ values are falling from high initial values to lower values, suggesting that each individual experienced systemic stress during the weaning process. One individual, S213, has a series of hypoplastic bands between the ages of 4 years and 5.5 years, after nitrogen isotope
Figure 4.15: $\delta^{15}$N$_{coll}$ profile of the first molar of individual R26 with the approximate position of LEHs superimposed (blue boxes). The width of each box represents the width of each LEH.

Figure 4.16: $\delta^{15}$N$_{coll}$ profile of the first molar of individual R81 with the approximate position of LEHs superimposed (blue boxes). The width of each box represents the width of each LEH. The last three LEHs are thin, and they are spaced approximately 6 months apart.
Figure 4.17: $\delta^{15}$N$_{coll}$ profile of the first molar of individual S109 with the approximate position of LEHs superimposed (blue boxes). The width of each box represents the width of each LEH.

Figure 4.18: $\delta^{15}$N$_{coll}$ profile of the first molar of individual S18 with the approximate position of LEH superimposed (blue box). The width of each box represents the width of the LEH. No LEH data was recorded for the canine of this individual.
values have begun to rise, suggesting post-weaning stress. It is worthwhile to note that only first molars and canines were considered in this analysis, and therefore the latest possible LEH would be close to ~6 years, the age at which the canine crown is completely deposited. LEHs on later forming teeth were not considered here.

These data demonstrate the utility of intratooth nitrogen isotope profiles in dentine for evaluating the temporal relationship between the weaning process and enamel growth defects indicative of systemic stress. Thus, these methods can be used to study the effects of weaning behavior on childhood morbidity in the archaeological record. In the Kulubnarti sample, for example, it appears as though bouts of serious illness associated with the weaning process itself is most likely responsible for the enamel growth disruptions than post-weaning malnutrition or infection.
In addition, irrespective of LEH, reconstructions of the weaning process in individuals that survived the process can be used to study the effects of weaning behavior on both early mortality (in conjunction with cross-sectional analyses) and long-term health outcomes such as longevity. While distributions of LEHs in archaeological assemblages have been used to investigate the effects of early stress (both in utero and post-natal) on longevity (e.g., Rose et al., 1978; Cook and Buikstra, 1979; Goodman and Armelagos, 1988; Duray, 1996), a link between LEH and the weaning process has always been tentative. The methods and data presented here suggest that weaning behavior can be incorporated into such analyses independent of LEH.

6.5 Chapter Summary

This chapter described the methods used for generating intratooth stable isotope profiles in dentine in first molars and canines, and for estimating the ages of each serial section. The results show that carbon and nitrogen isotope patterning does not consistently correspond between the two tooth types within individuals, particularly when isotopic variability is high. Differences in structure and growth characteristics between canines and first molars, as well as age estimation error, may contribute to this disparity. For studies interested in isotopic variability in the earliest post-natal period, first molars are a better choice of tooth type. However, canines appear to reflect slightly more variability at later ages, perhaps making this tooth type useful for other applications.

Nitrogen isotope patterning in first molars appears to be best explained by weaning behavior, although other factors influencing nitrogen isotope variation, such as starvation, dietary quality, and growth rate, may play a role as well. The $\delta^{15}$N$_{coll}$ profiles of the five individuals in this study reflect various ages of weaning completion (between ~2 years and ~5 years of age),
and the data suggest that all of these individuals weaned earlier than the average non-survivor. These data demonstrate the potential utility of combing longitudinal and cross-sectional reconstructions of the weaning process for evaluating the effects of weaning behavior on mortality in the archaeological record. Although the sample size is small, these results may reflect different patterns of weaning behavior and childhood diet among those that survived early childhood and those that did not at Kulubnarti.

High-resolution stable isotope profiles of dentine also provide a means to test the temporal relationship between weaning behavior and episodes of stress as evidenced by LEHs. All five of the individuals measured in this study exhibited LEHs that coincide in time with declining $\delta^{15}N_{\text{coll}}$ values, suggesting that systemic stress occurred during the weaning process. These methods will allow researchers to study the effects of weaning behavior not only on morbidity events in the past (LEHs), but also on long-term health outcomes such as longevity.
CHAPTER 7
CONCLUSIONS

7.1 Chapter Overview

The purpose of this dissertation was to further develop high-resolution intra-tooth stable isotope techniques for investigating childhood diet and the weaning process in the archaeological record, specifically laser ablation analysis of tooth enamel, and serial micro-sampling of dentine. High-resolution intra-tooth analysis of dentine has been performed on archaeological material only recently (Eerkens et al., 2011; Beaumont et al., 2012), and laser ablation stable isotope analysis has not been applied to archaeological questions about childhood diet. As both techniques are relatively new, several methodological issues were addressed, including sampling locations within teeth and choice of tooth type. This step is necessary before these methods can be applied to archaeological questions with confidence.

It was important that the teeth used for this purpose were likely to show isotopic variability so that potential differences between tooth types and sampling locations might be isotopically visible. Human remains from the medieval Nubian site of Kulubnarti (~500-800 AD) were used in this dissertation because the archaeological context (e.g., Van Gerven et al., 1981; Adams et al., 1999), local geography, biocultural data (e.g., Van Gerven et al., 1995), and published stable isotope data from bone (Turner et al., 2007) suggest that there is isotopic variability at the site, and that there is high childhood stress, suggesting that there may be differences in childhood diet and/or weaning behavior at the site. In addition to published and unpublished bone collagen, bone apatite, and hair stable isotope data (Turner et al., 2007;
Glasgow, 2011), I presented new stable isotope data for bulk third molar enamel to provide a richer context in which to interpret the intra-tooth data.

In the following sections, I highlight the results and implications of the enamel and dentine analyses, and then briefly discuss the potential of using high-resolution enamel and dentine data in conjunction. The chapter ends with a discussion of some questions that this dissertation has raised for future research, and some final thoughts on future applications of these techniques.

7.2 Enamel

Laser ablation stable isotope analysis permits the measurement of carbon and oxygen isotope ratios in very small amounts of enamel, facilitating the analysis of small teeth or precious museum specimens where the degree of sample destruction is a primary concern (Passey and Cerling, 2006; Sponheimer et al., 2006). In this dissertation, I took advantage of this property to create intra-tooth profiles along the growth axis of human teeth for the first time. Previous research indicates the stable isotope patterning in the tooth enamel of animals is attenuated relative to primary input signals due to the complex and protracted process of enamel mineralization (e.g., Balasse, 2002, 2003; Passey and Cerling, 2002; Zazzo et al., 2005). Several researchers have suggested that the isotopic composition of enamel closest to the EDJ might be less affected by attenuation than enamel closer to the OES because of its higher initial mineral content (Balasse, 2003; Zazzo et al., 2005; Tafforeau et al., 2007).

To evaluate the effect of sampling location on stable isotope variation in human enamel, profiles of carbon and oxygen isotopes were generated at the EDJ and close to the OES in five first molars and one canine. Contrary to expectations, with only one exception (the $\delta^{18}O_{ap}$
profile of the first molar of individual S213) the EDJ profiles were no more variable than those measured at the OES. However, at a mean level, the OES profiles had lower isotope values (lower by 0.8-1.7‰ for both isotopes), but the pattern was not consistent. With regard to mean values at the OES, one individual had lower values for both isotopes (S18), two individuals had lower $\delta^{13}C_{ap}$ values but not $\delta^{18}O_{ap}$ values (R81 and S213), one individual had lower $\delta^{18}O_{ap}$ values but not $\delta^{13}C_{ap}$ values (R26), and one individual showed no mean difference in either isotope (S109).

There are also inconsistencies in the canine and first molar stable isotope profiles within single individuals. The canines of three out of five individuals have lower $\delta^{18}O_{ap}$ values than the first molars, and these differences occur in enamel that formed over roughly the same period of time. The $\delta^{18}O_{ap}$ means in these canines are between 1.4‰ and 2.8‰ lower than the first molars.

The reasons for these inconsistencies are unclear at this time, but may have to do with variation in structural and crystallographic properties throughout the thickness of the enamel and inter-individual variation in amelogenesis processes. While estimating age for each isotope measurement was a necessary step to facilitate comparisons between profiles, this is likely a contributing source of error in the analysis, particularly in situations where the patterning is similar but offset along the age axis. What is clear from the methodological aspects of the enamel analysis is that the choice of tooth type and sampling location may greatly affect both the patterning and absolute values of $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ in humans. With regard to the EDJ and OES profiles, another possibility is that the inconsistency is driven by differential diagenetic processes operating on the exterior of the teeth. The comparability in patterning among the EDJ profiles across the teeth may support this notion.
The EDJ profiles of first molars generally show a similar amount of variation and patterns of change that appear cyclical. Although a decrease in $\delta^{18}$O$_{ap}$ and $\delta^{13}$C$_{ap}$ values shortly after birth is consistent with a purported weaning effect (e.g., Wright and Schwarcz, 1998; Fuller et al., 2006a), the values quickly rise again. Furthermore, one individual (R81) shows the opposite pattern, with rising $\delta^{18}$O$_{ap}$ and $\delta^{13}$C$_{ap}$ values shortly after birth. While these profiles capture a period of time when the transition from breastfeeding to solid foods is occurring (~birth-3 years), any weaning effect appears to be overprinted by a seasonal signal. This leads to a number of possible interpretations regarding seasonality in the isotopic composition of source water and the likely seasonal shifting of C$_3$ and C$_4$ crops.

Because Nile river water is likely the primary source of drinking water at Kulubnarti, it is reasonable to propose that the $\delta^{18}$O$_{ap}$ variation evident in the teeth reflects seasonal changes in the isotopic composition of the Nile mediated by the annual flood. Water storage practices may also play a role, particularly if water is stored for longer periods of time in particular seasons. Longer water storage times might have resulted in $^{18}$O enrichment of drinking water via evaporation. Seasonal variation in the oxygen isotopic composition of food may contribute to the patterns as well. The duration between peaks in the EDJ $\delta^{18}$O$_{ap}$ profiles of first molars is longer than a year, but this may reflect input variability on a short timescale (perhaps ~1 year), given attenuation considerations. Furthermore, the magnitude of seasonal effects is likely larger than that evident in the enamel due to isotopic attenuation, but how much larger is difficult to say at this time.

The $\delta^{13}$C$_{ap}$ variation in these profiles may reflect seasonality in C$_3$ and C$_4$ crop harvests, as suggested by the carbon isotope composition of hair from Kulubnarti (Glasgow, 2011) and the nearby site of Wadi Halfa (White and Schwarcz, 1994; Schwarcz and White; 2004). If the
patterns of $\delta^{18}O_{ap}$ and $\delta^{13}C_{ap}$ variation in these teeth are primarily driven by seasonality, then it might be possible to estimate seasons of birth in these individuals, or seasons more strongly associated with episodes of stress as evidence by LEHs, potentially opening a door to investigating the effects of seasonality on morbidity and later health outcomes in the archaeological record. However, this level of interpretation would rely on a firmer understanding of site specific hydrology, seasonality, and subsistence patterns.

7.3 Dentine

Intra-tooth isotopic analysis of dentine in humans was pioneered by Fuller et al. (2003) and expanded upon by Eerkens et al. (2011) and Beaumont et al. (2012). The primary utility of this technique is that it produces age-related profiles of nitrogen isotope variation, which is sensitive to weaning behavior (e.g., Fogel et al., 1989; Fuller et al., 2006a), and can be used to characterize the weaning process in prehistory with higher resolution than conventional bone collagen analyses by age-at-death allows. There are, however, a number of methodological considerations that remain to be addressed. None of the previous studies found much carbon isotope variation in their dentine profiles (with one exception in Beaumont et al. 2012), and neither of the two more recent studies compared their intra-tooth data to bulk collagen data. Fuller et al. (2003) found that the timing of the weaning process reconstructed from intra-tooth dentine serial sections was comparable to cross-sectional analyses, suggesting that mortality bias did not result in two distinguishable patterns. However, the resolution of this analysis was relatively low, with only 3-4 serial samples per tooth.

In this dissertation, intra-tooth stable carbon and nitrogen isotope profiles were created in first molars and canines to explore how these tooth types correspond in single individuals. These
data were compared to bone collagen data to evaluate how longitudinal and cross-sectional stable isotope data might produce divergent interpretations of the weaning process. Bulk stable isotope data from bone collagen and hair from Kulubnarti revealed variability in $\delta^{13}C$ values between individuals and perhaps between seasons (Turner et al., 2007; Glasgow, 2011), suggesting that some degree of $\delta^{13}C_{\text{coll}}$ variability would be found in the dentine profiles. In addition, the dentine profiles created in this dissertation were compared to the timing of LEHs to explore the relationship between weaning behavior and morbidity events at Kulubnarti. The method used to create the dentine intra-tooth profiles was similar to Method 2 in Beaumont et al. (2012).

Results suggest that intra-tooth profiles generated in canines and first molars, plotted by age of enamel formation, do not always correspond, particularly in cases where there is a relatively high degree of variability in $\delta^{13}C_{\text{coll}}$ and $\delta^{15}N_{\text{coll}}$ values. There were no differences in variance between the tooth types, and only one individual showed differences in $\delta^{13}C_{\text{coll}}$ and $\delta^{15}N_{\text{coll}}$ values at a mean level (R26). Much of the disparity between profiles occurred relative to age estimate. The possible reasons for this include the interaction between the sampling method and differences in the structural and developmental characteristics of the two tooth types. The method of estimating the age of each isotopic measurement also introduces some error. First molars are likely a better choice than canines for research interested in isotopic changes associated with the earliest years of life.

The nitrogen isotope variation in the dentine profiles is probably best explained by the weaning process. Interpreted in this way, the data show differences in the weaning process among the five individuals measured in this dissertation. Focusing on first molars, four of the five dentine profiles show a trend of gradually decreasing $\delta^{15}N_{\text{coll}}$ values that plateau at different ages. The magnitude of this change is between 2.4‰ and 3.6‰, which is similar to that found
during weaning in modern infants (Fuller et al., 2006a). Using the estimated ages where the values level out as an indicative of the cessation of breastfeeding, these four individuals completed weaning between the ages of 3 years and 5 years.

The fifth individual (S18) shows a much earlier and more abrupt drop in $\delta^{15}N_{\text{coll}}$ values, suggesting that this individual was weaned earlier, perhaps at the age of 2 years. The profile for S18 also had much lower $\delta^{15}N_{\text{coll}}$ values than the others for the first ~7 years of life, suggesting that this individual has a relatively low quality diet, or perhaps that this individual spent her childhood in a different environment.

The intra-tooth first molar dentine profiles also show early declines in $\delta^{13}C_{\text{coll}}$ values, which may reflect the early stages of supplementation (e.g., Fuller et al., 2006a). The carbon isotope values then fluctuate irregularly, possibly signaling changes in the isotopic composition of the protein component of the diet throughout childhood.

In comparison to the bone collagen stable isotope data, the five individuals reach plateaus in $\delta^{15}N_{\text{coll}}$ earlier than the average individual by age-at-death. One possible interpretation of this pattern is that survivors weaned earlier than non-survivors, a pattern that has been observed in modern children living in areas with periodic food shortages (e.g., Cantrelle and Leridon, 1971; Fawzi et al., 1998; Lindstrom and Berhanu, 2000). However, cross-sectional analyses of bone collagen likely over-estimate the timing of dietary shifts due to bone turnover rates (e.g., Hedges et al., 2007). Furthermore, there are several other factors that can affect $\delta^{15}N_{\text{coll}}$ values in both dentine and bone collagen that cannot be ruled out. Childhood is a time of rapid growth, and, at least at Kulubnarti, a time of high nutritional stress, both of which may impact nitrogen isotope variation (e.g., Hobson et al., 1993; Fantle et al., 1999; Trueman et al., 2005; Mekota et al., 2006). The time period of life that a stable isotope measurement represents, and the factors that
affect nitrogen isotope variation, will have to be better constrained before interpretations like this can carry more weight.

The timing of LEHs on the first molars and canines were plotted on the first molar nitrogen isotope profiles to evaluate the relationship between nitrogen isotope variation and episodes of childhood morbidity. This analysis showed that LEHs typically occurred while $\delta^{15}N_{coll}$ values were falling, suggesting that periods of systemic stress were associated with the weaning process. This demonstrates a potentially powerful application of intra-tooth stable isotope analysis to the study of the synchrony between weaning behavior and LEH occurrence, a relationship that has received considerable attention in the literature (e.g., Goodman et al., 1984; Corruccini et al., 1985; Van Gerven et al., 1990; Ubelaker, 1992; Blakey et al., 1994; Moggi-Cecchi et al., 1994; Katzenberg et al., 1996; Wood, 1996; Ritzman et al., 2008), but has not been directly testable. The approach presented in this dissertation may provide such a test, and may also provide researchers with a means to study the relationship between early life history events and later health outcomes.

7.4 Combining Enamel and Dentine

High-resolution intra-tooth stable isotope analyses of enamel and dentine provide different types of information. It is well established that the carbon isotopic composition of biological apatites reflect that of whole diet while dietary protein is overrepresented in collagen and other proteinaceous tissues (e.g., Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Howland et al., 2003; Jim et al., 2004).

Several studies have utilized the spacing between $\delta^{13}C_{ap}$ and $\delta^{13}C_{coll}$ to investigate differences in the macronutrient sources of carbon, and the potential contribution of animal
protein in the diets of animals and humans (e.g., Lee-Thorp et al., 1989; Katzenberg and Weber, 1999; Harrison and Katzenberg, 2003; Kellner and Schoeninger, 2007; Clementz et al., 2009). These studies focused on bone apatite – collagen spacing because the two $\delta^{13}C$ values represent different aspects of diet during the same period of time. The carbon isotope composition of enamel apatite, however, may reflect diet during a much different life stage than collagen, depending on age-at-death, making the enamel apatite – collagen spacing less useful for dietary studies.

One potential way around this problem is to compare $\delta^{13}C_{ap}$ and $\delta^{13}C_{coll}$ values of enamel and dentine that formed at the same ages. These data suggest that it might be possible to evaluate how carbon isotope apatite-collagen spacing changes over the formation period of the tooth. The use of dentine and enamel in tandem might provide an alternative way to calculate carbon isotope apatite – collagen spacing, and one that does not rely on bone apatite, which is highly susceptible to diagenesis (e.g., Nelson et al., 1986; Wang and Cerling, 1994; Wright and Schwarcz, 1996).

In the Kulubnarti sample, intra-tooth carbon stable isotope variation appears to reflect dietary changes associated with seasonal changes in whole dietary carbon in enamel (e.g., C$_3$ vs. C$_4$ crops) and changes in the protein component of the diet in dentine (e.g., breastmilk vs. extra-maternal foods). These two types of information could be used in tandem to build a more complete picture of early childhood diet. Figure 7.1 shows the EDJ $\delta^{13}C_{ap}$ profile of the canine of individual S18 and the $\delta^{13}C_{coll}$ profile of the same tooth. Putting differences in sampling strategy aside for the moment, it appears from this figure that $\delta^{13}C_{ap}$ and $\delta^{13}C_{coll}$ are tracking different aspects of diet.
Figure 7.1: $\delta^{13}\text{C}_{\text{cap}}$ profile measured at the EDJ of the canine of individual S18, and $\delta^{13}\text{C}_{\text{coll}}$ profile measured in the dentine of the same tooth.

In addition, if specific seasons can be tied to birth or episodes of childhood morbidity using intra-tooth stable isotope analysis of enamel, this information could be used in conjunction with reconstructions of the weaning process and its effects on morbidity, mortality, and later health.

7.5 Final Thoughts

This study has demonstrated the utility of two high-resolution intra-tooth stable isotope methods for addressing archaeological questions concerning early childhood diet and life history. It also has shed light on a number of problems associated with their application. In this sense it has raised more questions than it answered. The potential power of these new techniques is clear, and this dissertation has highlighted some of the areas that require additional research before that promise can be realized. A better understanding of enamel mineralization patterns in
human teeth, for example, would help constrain the ages at which dietary changes take place. Nevertheless, this dissertation has identified some archaeological and biocultural applications for high-resolution stable isotope analysis, and I hope the data herein prove useful for future research. With further development, these methods could be applied to a range of archaeological material. For example it would be interesting to investigate childhood diet and weaning practices in archaeological populations undergoing changes in subsistence, technology, or population size, or during interaction with other cultural groups. These methods might also be applied to more ancient material, potentially facilitating reconstructions of childhood diet, seasonality, and weaning behavior in earlier stages of human biological and cultural evolution.
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## APPENDIX

### Table A.1: Enamel stable isotope data.

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<th>$\delta^{18}\text{O}_{\text{sp}}$ (SMOW)</th>
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