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Age As A Factor In Inter-tissue Spacing Of Stable Carbon Isotope Values In Juvenile Human Remains From The Dakhleh Oasis, Egypt

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AGE AS A FACTOR IN INTER-TISSUE SPACING OF STABLE CARBON ISOTOPE VALUES IN JUVENILE HUMAN REMAINS FROM THE DAKHLEH OASIS, EGYPT

by

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ABSTRACT

Although stable isotope analysis is routinely utilized in bioarchaeology and relies on principles that are well-understood, there are still fundamental issues that have not been thoroughly investigated. This thesis examines the relationship between inter-tissue spacing of carbon stable isotope values ($\delta^{13}C$) and age in juvenile human remains. Analyses of tissues within the same individual reveal disparate isotopic values for a variety of physiological and biological reasons discussed herein. This project examines the distance between the $\delta^{13}C$ values in bone collagen, skin, hair, and nail, and examines how these distances vary between different age groups, utilizing data collected from 52 well-preserved human remains from the Dakhleh Oasis, Egypt: 28 individuals between the ages of 1 and 4 years, 14 between 5 and 10 years, and 10 between the ages of 11-15 years. The mean carbon isotope values for each tissue were compared across each age group, and used to calculate the differences between each tissue type. Although distances between tissues were found to vary across all age categories, the distances between collagen and hair, collagen and skin, and collagen and nail are all substantially greater in the 11-15 year old category than those in the 1-4 and 5-10 year categories. Possible physiological, developmental and social factors are discussed in an effort to explain this discrepancy.

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CHAPTER ONE: INTRODUCTION

Since its introduction to bioarchaeology in the late 1970s, stable isotope analysis has become a routine procedure in dietary reconstruction of past populations. Although soft tissues have frequently been the subject of studies in a laboratory setting or modern population, in the archaeological context, bones and teeth have been the focus of isotopic research for the obvious, pragmatic reasons of availability. Nevertheless, in the appropriate environmental conditions, tissues like skin, hair and fingernails may be preserved, and have also proved useful for dietary analysis.

The relationships between the isotopic values found in an individual's diet and those found in their tissues, however, are not always clear-cut and straight-forward. Stable isotopes of a given element exist in a ratio of natural abundance in the atmosphere, but as these elements enter and ascend the food chain, the processes of fractionation cause the ratios of isotopes to shift (Hoefs, 2009). The stable isotopes of carbon, ^{12}C and ^{13}C, are incorporated into plants via photosynthesis of atmospheric CO_2, and the ratio of one to the other is altered; the new ratio will depend on what photosynthetic pathway a particular plant utilizes. As the plant is consumed by animals, and those animals are likewise consumed, these fractionation processes continue to alter the basic abundance ratios of ^{13}C to ^{12}C (Hoefs, 2009). Within the tissues of a consumer, further fractionation effects are evident, as different aspects of the diet are incorporated into different tissues. The end result is that within a human body, there is an offset or enrichment factor between the isotopic values in the food the person consumed and those in the body, and there are likewise offsets between different tissues within the same individual.

This inter-tissue spacing of isotopic values is affected by a number of intrinsic and extrinsic factors. These include differing tissue turnover rates, protein composition, and seasonal or even individual diet variation. These processes have been explored in controlled feeding studies using animal models (e.g. DeNiro and Epstein, 1978; Tieszen et al., 1983; Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Warinner and Tuross, 2009), in modern human populations (e.g. O'Connell et al., 2001), and using archaeological human remains (e.g. White, 1991; White and Schwarcz, 1994; Finucane, 2007; Knudson et al., 2007; Williams, 2008; Corr et al., 2009; Raghavan et al., 2010). And yet, questions remain. One question is the extent to which age affects inter-tissue spacing, particularly age in juvenile individuals. Some age categories of juveniles are less prevalent in isotopic studies than adults, owing to their relative rarity at many archaeological sites. Many studies on inter-tissue spacing have either reported no difference between age groups, or have focused only on differences related to the contents of diet, and the variation among different age grades in a population.

To help clarify this issue, the content of this thesis is centered on the question of inter-tissue spacing of $\delta^{13}C$ values, with a focus on variation by age in juvenile human remains. The results indicate not only that there may be significant differences in inter-tissue spacing of isotope values between different age groups, but that these differences may have social or physiological ramifications that have little to do with differential diet content. Bone collagen, hair, nail, and skin $\delta^{13}C$ values from 52 well-preserved human remains from the Dakhleh Oasis, Egypt, originally analyzed by Dr. Tosha Dupras (1999) and Dr. Lana Williams (2008), were examined for this project. Differences among the mean isotope values were calculated for each tissue within each of three age cohorts (1-4 years, 5-10 years and 11-15 years), delineated along

developmental lines (Wheeler, 2009). Individuals in the youngest cohort were in a most precarious position, as it is estimated that two-fifths of children in Roman Egypt did not live beyond the age of five (Bagnall, 1996). At the other end, juveniles between the ages of 11 and 15 were not only experiencing the physiological changes of puberty, with all its myriad challenges, but also the social changes and challenges of incipient adulthood. This project explores the variation in inter-tissue spacing between these groups.

This thesis is presented using a standard thesis format, presenting a literature review in Chapter Two, materials and methods in Chapter Three, results in Chapter Four, discussion of those results in Chapter Five, and a conclusion in Chapter Six. The second chapter begins with a basic overview of the principles of stable isotope research and a concise history of their application in archaeology, with a focus on carbon isotopes and their fractionation processes. Subsequent sections are devoted to the biology of bone collagen, skin, fingernails and hair, and the use of these tissues in isotope research. The section concludes with a discussion of research on inter-tissue spacing of isotopic values.

The Materials section of Chapter Three describes the samples used in this study, which come from the skeletal collection excavated from the Kellis 2 cemetery in the Dakhleh Oasis, Egypt. The hyper arid conditions of the Dakhleh Oasis, located in Egypt's Western Desert, presents a unique opportunity to conduct a study of this nature. Preservation is exceptional, and not only do mummified tissues exist in relative abundance, but unlike many archaeological sites, so do juvenile remains (Tocheri et al., 2005). Chapter Three describes the methods used to chemically process the tissue samples, and those used to analyze the results. The original chemical analyses of the bone samples were carried out by Dupras (1999) at McMaster

University, and the hair, nail and skin samples by Williams (2008) at the University of Western Ontario. Chapter Four presents the results of the inter-tissue spacing analyses of these data from the sample population.

Chapter Five, the Discussion section, examines possible interpretations of the results of the study. First, the overall trends are compared to data from other populations, and then the data from the juvenile sample is compared to data from the adult sample from the Kellis 2 population. The 11-15 year age cohort is discussed in detail due to observed differences between this cohort and the others, and various explanations for this difference are presented. Chapter Six summarizes the conclusions of the project and the broader implications for bioarchaeology, including areas of future research indicated by the findings.

CHAPTER TWO: LITERATURE REVIEW

Stable Isotope Analysis

Take even a cursory glance at the latest publications containing bioarchaeological scholarship, and you will have no difficulty locating several articles that involve isotopic research, used in a wide assortment of geographical regions to unravel an increasingly wide assortment of archaeological questions. The isotopic ratios in bone are a reflection of the diet an individual consumed and the geographic locale in which they lived, and as such they can provide a wealth of information relevant to the archaeological and social context of their lives. Since the first experimental findings on the subject were presented by Vogel and van der Merwe (1977) in their landmark study, "Isotopic evidence for early maize cultivation in New York State," stable isotope analysis of human remains has become a veritable staple of bioarchaeology.

Numerous reviews of the literature have been published on the subject. Many focus on paleodiet studies, the original application and still among the most prominent (e.g. Sillen et al., 1989; Schwarcz and Schoeninger, 1991; Schoeninger and Moore, 1992; Pate, 1994). More recent articles are more comprehensive, covering a wider range of applications and addressing the direction of future research (e.g. Katzenberg and Harrison, 1997; Katzenberg, 2008). Even literature reviews on the recent progresses and expansions in the field of bioarchaeology as a whole typically discuss the growing importance of stable isotope analysis in contributing to our understanding of the human past (e.g. Wright and Yoder, 2003; Larsen, 2006; Knudson and Stojanowski, 2008).

The scope of stable isotope research is vastly increasing, employing an array of elements (e.g. C, N, H, O, Sr, etc.) and utilizing different kinds of preserved human tissue (e.g., hair, nails,

skin, teeth, and bone). This review will focus only on the basic principles of analyzing stable carbon isotopes from bone collagen, hair, nails and skin. This requires some description of the essential biology of these tissues, as well as the chemical principles involved. From there, in accordance with the more specific theme of this project, relevant research on the subject of inter-tissue spacing of isotope signatures will also be addressed.

History and Basic Chemical Principles

'Isotopes' are different forms of the same element which differ in the number of neutrons, thus varying also in mass (Hoefs, 2009). Some of these isotopes are subject to radioactive decay, such as ^{14}C (the number denotes the number of neutrons), which decays over time into ^{14}N (Bowman, 1990). These are also referred to as 'unstable' (Hoefs, 2009). Stable isotopes, by contrast, are those that do not decay over time, but maintain their chemical composition. Carbon, for example, has two stable isotopes, ^{13}C and ^{12}C, in addition to the radioactive ^{14}C (Hoefs, 2009). The majority of elements are composed of more than one isotopic form, but the proportionate abundance of each isotope for each element may differ considerably (Hoefs, 2009). Lighter isotopes – those with fewer neutrons – often outnumber heavier ones to a significant degree (Schoeller, 1999).

Aside from radioactive decay, the primary process which causes variation in the ratio of one isotope to another in an element is isotope fractionation, defined as the "partitioning of isotopes between two substances or two phases of the same substance with different isotope ratios" (Hoefs, 2009:5). As an element in the atmosphere is incorporated into a living organism, for example, the fractionation effect will cause an observable difference in the proportional abundance of different isotopes (Hoefs, 2009).

Stable isotope analysis, which can be considered an area of geochemical research, is used across a number of scientific fields, including environmental and forensic sciences. In bioarchaeology, it has its origins in radiocarbon dating, an earlier application of chemistry in archaeology, which was first introduced in the 1940s (Bowman, 1990). Indeed, much of the methodology developed for radiocarbon dating has subsequently been used in stable isotope analysis, including techniques for isolating collagen (Ambrose and Krigbaum, 2003; Katzenberg, 2008). And as Jonathan Haas commented in his forward to *The Chemistry of Prehistoric Human Bone*, "[w]hat radiocarbon dating did for chronology, bone chemistry studies do for diet" (1989: xviii).

It was in the late 1980s that stable isotope analysis saw rapid development, facilitated by advances in instrumentation making techniques more economically practical (Katzenberg, 2008). In 1989, Douglas Price made the following observation: "It is apparent that the study of archaeological bone chemistry is at a turning point, in transition from an experimental procedure to a major research technique" (1989: xxiv). The abundance of articles which have flourished since then, applying stable isotope analysis to bioarchaeology, appears to confirm this point.

It seems a natural evolution that stable carbon isotopes would be the first seized upon by archaeologists. Carbon from bone collagen was, after all, already subject to scrutiny for ^{14}C dating techniques. The use of carbon isotopes from carbonate, derived from the mineral portion of bone, was first introduced in 1981 and sparked immediate controversy owing to questions about the effects of diagenesis, which can alter isotope values in apatite (Sullivan and Krueger, 1981; Lee-Thorp and van der Merwe, 1991). Although the carbon in collagen and the carbon in carbonate are ultimately derived from diet, different portions of the diet are reflected due to

differing fractionation processes, and therefore the isotopic ratios also differ between collagen and carbonate, which has compelled the exploration of using both sources for a more complete picture of paleodiet (Krueger and Sullivan, 1984; Jim et al., 2004; Kellner and Schoeninger, 2007).

Examination of other tissue types has also been of an area of growing interest. Although earlier studies employing animal models in controlled feeding experiments had compared isotope values between different tissues (e.g. DeNiro and Epstein, 1978; DeNiro and Epstein, 1981), it took rather longer for analysis of soft tissues to be included in an archaeological context, probably owing to the general paucity of such materials. White (1991) was among the earliest to analyze mummy tissues isotopically, in her dissertation on three Nubian populations utilizing muscle, skin and hair (White, 1991). Results showed certain disparities between the carbon enrichment factors for these tissues, an effect also observed in subsequent work using multiple tissues (e.g. O'Connell et al., 2001; Finucane, 2007; Knudson et al., 2007).

Stable Carbon Isotope Analysis

Isotopic data are reported in parts per mil (‰), using the delta notation, where:

$$\delta = \left[\frac{R_{sample} - R_{standard}}{R_{standard}}\right] \times 1000$$

The ratio of the heavy to the light isotope (R) in the sample is compared to that of a standard, which may be either internal to the laboratory performing the analysis, or one of the international standards produced by the International Atomic Energy Agency or the National Bureau of Standards (Schoeller, 1999; Katzenberg, 2008). Isotope ratio mass spectrometry is used to measure the abundance ratios of the stable isotopes of interest (Katzenberg, 2008). This process generates and segregates ions of the material by their mass-to-charge ratio (*m/z*) (Gross, 2004).

As discussed previously, carbon has two stable isotopes: ^{13}C and ^{12}C. The natural abundance of the two stable isotopes is ~98.93% for ^{12}C and ~1.07% for ^{13}C (Hoefs, 2009). The ratio of $^{13}C/^{12}C$ is determined as follows:

$$\delta^{13}C\ ‰ = \left[\frac{\left(^{13}C/^{12}C\right)_{sample} - \left(^{13}C/^{12}C\right)_{standard}}{\left(^{13}C/^{12}C\right)_{standard}}\right] \times 1000$$

Much of the carbon in the human body derives ultimately from atmospheric CO_2, which enters the food chain via the carbon fixation processes of plants (Hoefs, 2009). The first paleodiet study to use carbon isotope ratios from archaeological human bone was an investigation of early maize consumption in prehistoric New York State (Vogel and van der Merwe, 1977). Maize, *Zea mays*, is a C_4 plant, i.e., one utilizing the Hatch-Slack or 4-carbon photosynthetic pathway for carbon-dioxide fixation, rather than the Calvin or 3-carbon pathway (Vogel and van der Merwe, 1977). For this reason, maize tended to return younger radiocarbon dates than contemporaneous materials owing to a high $^{14}C/^{12}C$ ratio (Ambrose and Krigbaum, 2003). These plants also show enriched $\delta^{13}C$ values (an average of -12.5‰) compared with C_3 plants (-26‰ on average), since the C_3 pathway depletes ^{13}C to a larger extent than does C_4 (Vogel and van der Merwe, 1977). It was proposed that, based on these differences between maize and surrounding flora, analyzing the carbon isotope component of bone could be used to determine whether or not ancient populations in certain areas cultivated maize (Hall, 1967).

Vogel and van der Merwe (1977) showed that, compared to the hunter-gatherers, the horticulturalists had higher ^{13}C content and a definite C_4 content to their diet, supporting their hypothesis that maize exploitation could be detected by use of stable carbon isotope analysis. Because the introduction of maize cultivation marked an important point in the agricultural

history of the New World, many studies using similar methods followed examining its developmental patterns, particularly in North America (for review see Schwarcz and Schoeninger, 1991, or Pate, 1994). The cultivation of millet, another C_4 plant, has likewise been studied in China, using the same principles (Weld, 1985; as reported in Schwarcz and Schoeninger, 1991). Human carbon isotope values are also affected by the diets of the animals they consume, so C_4 plants inedible to humans, but consumed by animals in the human dietary food chain, may still be reflected in ^{13}C values of human populations (Schwarcz and Schoeninger, 1991).

Identifying C_4 content in diet is not the only application for carbon isotopes. They have also been used to examine the relative importance of marine versus terrestrial resources in the diet (e.g. Tauber, 1981; Chisholm et al., 1982; Schoeninger and DeNiro, 1984). The ultimate source of carbon for terrestrial plants is atmospheric CO_2, whereas for marine life it is marine bicarbonate (Tauber, 1981; Chisholm et al., 1982). There is a 7-8‰ difference in $\delta^{13}C$ values between the two, with marine plants having elevated $\delta^{13}C$ compared to terrestrial (Tauber, 1981; Chisholm et al., 1982). This difference is likewise seen in animal and human consumers, although Schoeninger and DeNiro (1984) found that there could be considerable overlap between those with terrestrial and those with marine diets. In an environment which favors C_3 plants, populations predominantly exploiting terrestrial C_3 plants may thus be distinguished from populations favoring marine resources (e.g. Tauber, 1981). The obvious difficulty is that both marine resources and C_4 resources will elevate $\delta^{13}C$ values, which in some environments can make results ambiguous (Pate, 1994).

Tissues Used in Analyses

Bone Collagen

Bone is a composite tissue, both organic and inorganic in nature; the organic portion is primarily proteinaceous and composed of Type I collagen, while the inorganic or mineral portion is composed of hydroxylapatite (White et al., 2011). Collagen is the most prevalent protein found in the human body (White et al., 2011). Carbon, nitrogen, hydrogen, oxygen, amino acids and sulfur all contribute to the molecular make-up of collagen (Mays, 1998). It is a component of both bones and teeth, although collagen from the former exists in far more appreciable concentrations. Carbon and nitrogen concentrations are likewise lower in tooth collagen compared to that of bone (Ambrose, 1990). The turnover rate of bone collagen in adults is said to be around 10 years (Stenhouse and Baxter, 1979), although this varies depending on the bone, and it would be perhaps more accurate to say that it is between 3 and 20 years (Pate, 1994). As such, it may take as many as 10-20 years for the isotopic values in bone collagen to equilibrate to a change in diet (Katzenberg, 2008). Bone collagen turnover rates in juveniles are more difficult to come by, although Hedges and colleagues (2007) found that in adolescents between the ages of 10-15, the collagen turnover rate is approximately 10-30% per year, much higher than the 1.5-4% per year observed in adults.

Collagen may be well-preserved in the archaeological record, a fact which has led to the exploitation of collagen for both radiocarbon and stable isotopic studies. Furthermore, the organic portion of bone is less prone to contamination than the mineral portion, and contamination or degradation in collagen is more easily identifiable than in bone mineral (Schurr, 1998). Generally speaking, collagen is unlikely to be present beyond about 50,000 -

100,000 years of age (Fogel et al., 1997). This depends on the environmental conditions, and, in warmer climes, 10,000 years or less is the more likely cut-off point (Lee-Thorp and van der Merwe, 1987).

Still, there is the important question of post-depositional degradation, contamination, and overall structural integrity to consider. How well does the collagen component of an archaeological sample reflect the collagen component of the individual's skeleton as it was in life? There can be much variability in the environmental and other depositional conditions of skeletal remains, and therefore there can be much variability in the preservation of the chemical signatures of the remains. DeNiro (1985) was the first to test the assumption within paleodiet studies that isotope ratios in collagen remain consistent long after death. His findings indicated a need for methods of identifying cases in which alteration in isotope ratios has occurred due to collagen degradation. He observed, as did others afterward (e.g. Ambrose, 1990; van Klinken, 1999), that ratios of C:N could serve as an indicator of preservation quality. Ambrose (1990) and van Klinken (1999) list additional techniques used to detect diagenetic alteration to collagen, including examination of amino acid composition, overall collagen concentrations in whole bone, and the use of infrared spectra. Schoeninger and colleagues (1989) found success in the use of powder X-ray diffractometry as a method of excluding samples too poorly preserved to be suitable for chemical analysis.

Although Schoeninger and colleagues (1989) questioned the reliability of using carbon to nitrogen ratios to detect preservation, Ambrose (1990) observed that highly variable and unreliable results were obtained primarily in cases where the collagen concentrations in the bone were extremely low. Ambrose advocates carbon and nitrogen concentration in the collagen as the

"most useful and unequivocal measure of preservation and reliability of isotopic analysis" (1990: 433). Van Klinken (1999) concurs with this, citing overall collagen concentration, C:N ratios, and $\delta^{13}C$ values as the best means of identifying poorly preserved bone samples.

One further point on the subject is that the difference between contamination and degradation should be acknowledged; the former refers to "diagenetic alteration/breakdown" and the latter to the "presence of exogenous contaminants" (van Klinken, 1999: 688). Which one is more likely to be found on bone can depend upon the environment in which the bone is preserved. For instance, arid climates tend to lead to more degradation, while in temperate climates contamination tends to be a more pressing issue (Van Klinken, 1999). Van Klinken (1999) argues that contamination is generally of less concern for stable isotope analysis than it is for radiocarbon research, rarely causing significant changes to isotope ratios in the bone. However, degradation is a more important consideration, as low concentrations of collagen can alter isotopic signatures.

Soft Tissues

Soft tissues are only occasionally available for analysis in an archaeological context, and in recent years have been sporadically incorporated into bioarchaeological stable isotope research (e.g. White and Schwarcz, 1994; Macko et al., 1999; Schwarcz and White, 2004; Finucane, 2007; Knudson et al., 2007; Williams, 2008; Knudson et al., 2010; Williams et al., 2011). A number of studies have also been conducted using samples derived from living populations, or in controlled feeding studies (e.g. O'Connell and Hedges, 1999; O'Connell et al., 2001; Bol and Pflieger, 2002; Bol et al., 2007). Whereas bone collagen may reflect the individual's diet over a period of years, hair, nails and skin provide a record of diet in the short-

term, generally less than one year prior to death (White and Schwarcz, 1994; O'Connell and Hedges, 1999).

The isotopic examination of hair, nails and skin has also been adopted in forensic investigations, where such tissues are far more likely to be available than in archaeological contexts (e.g. Meier-Augenstein and Fraser, 2008; Meier-Augenstein, 2010).

Skin

Skin is the base component of the integumentary system, the primary function of which is to form a protective barrier between the body and the environment, as well as any hostile organisms (Freinkel and Woodley, 2001; Tallitsch et al., 2006). It also has a role in the regulation of body temperature and in sexual attraction (Freinkel and Woodley, 2001; Tallitsch et al., 2006). Hair, fingernails and toenails also constitute part of the integumentary system and are considered derivative or accessory structures of the skin, along with various glands (Haake et al., 2001).

Skin is composed of an external layer known as the epidermis, and an internal layer called the dermis. Sometimes the hypodermis, the layer underneath the dermis, is also included. Keratinocytes, which produce the structural protein keratin, make up the bulk of the epidermis, or some 90-95% of its cells (Haake et al., 2001). Epidermal cells are produced in the innermost basal layer, the statum germinativum, and are pushed to the surface layer, the statum corneum, over a period of 15-30 days, where they become inert and are subsequently shed (Tallitsch et al., 2006; Scanlon and Sanders, 2007). These dead cells remain for approximately two weeks, forming a protective outer shell of sorts which guards the more sensitive inner layers of the epithelium (Tallitsch et al., 2006; Scanlon and Sanders, 2007).

Of more significance for stable isotope research is the principal protein in the dermis, which is Type I collagen, as in bone (White and Schwarcz, 1994; Haake et al., 2001). The dermis, which acts as a pliable barrier and helps protect against injury, is composed of fibrous connective tissue (Haake et al., 2001). In the dermis reside many sensory receptors and accessory structures such as hair follicles (Haake et al., 2001). The collagen of the dermis makes up 75% of the skin's dry weight, and contributes to its elasticity and tensile strength (Haake et al., 2001). One potential issue in stable isotope research involving skin can be variation in sampling location and techniques, as samples taken at different parts of the body or at varying degrees of thickness may contain different percentages of epidermis, and therefore different ratios of collagen:keratin, which may affect isotopic values (Williams, 2005).

The turnover rate of collagen in human skin is estimated to be around 2-4 months (Finucane, 2007). Babraj and colleagues (2005) report that dermal collagen is synthesized at a rate of ~0.037%/h (in their young adult male subjects), a value similar to results from an earlier study by El-Harake et al. (1998), who found it to be ~ 0.076%/h, or just under 2% per day.

Human skin is rarely preserved in the archaeological record, and as such it has not been used as extensively as bone, or even hair. Nor is it as easily sampled from living human subjects as either hair or fingernails, so it is not often included in these analyses. However, under the conditions which lead to mummification, such as an extremely hot, arid environment or an extremely cold one, skin may be preserved well enough for utilization in isotopic research (e.g. White and Schwarcz, 1994; Finucane, 2007; Corr et al., 2009). It has also been demonstrated that natural mummification does not alter the chemical properties of collagen in skin (Michelin et al.,

1972). As yet, however, no recognized standard exists for determining preservation of skin samples for isotopic research (Williams, 2008).

Nails

Fingernails and toenails are accessory structures which form part of the integumentary system. They are issued from nail follicles nestled in the dermis, and protrude through the epidermis, where they serve the function of protecting the tips of the digits and increasing their dexterity (Tallitsch et al., 2006; Scanlon and Sanders, 2007). At the base, the nail root produces keratinized cells, which form, in an inert state, the rest of the nail (Tallitsch et al., 2006; Scanlon and Sanders, 2007). Keratin is a versatile structural protein, and in addition to skin, nails, and hair, it is also found in hooves, wool, claws, quills and cytoskeletons (Forslind and Lindberg, 2004; Koolman and Röhm, 2005). The amino acid cysteine in keratinized cells contains a remarkably high concentration of sulfur, and these cysteine-sulfur links provide the protein chains with a high degree of mechanical and chemical resistance and stability (Forslind and Lindberg, 2004). Keratin varies in its relative 'softness' or 'hardness,' as one may gather from the list of materials in which it is the primary component. Electrophoresis has been used to analyze dissolved protein in keratinized tissues, and the resulting patterns reveal that nail keratin is more similar in character to epidermal keratin, than to the keratin in hair (Forslind and Lindberg, 2004).

Nail growth has been shown to be affected by a number of factors, including age, sex, health, nutritional status, and even personal habits such as fingernail biting (Gilchrist and Buxton, 1939; Hamilton et al., 1955; Sibinga, 1959; Orentreich et al., 1979; Bean, 1980; Beaven and Brooks, 1994; de Berker and Forslind, 2004). Although inter-individual rates are highly

variable, within an individual nails grow at relatively steady rate each day (Beaven and Brooks, 1994). In adults, the average growth rate of fingernails is reported as 0.1 mm/day (de Berker and Forslind, 2004: 435), or around 3 mm/month (Scanlon and Sanders, 2007:96), and varies from about 0.5-1.2 mm/week (Beaven and Brooks, 1994: 20), assuming that the individual is not suffering from any ailment that might affect nail growth. Beaven and Brooks (1994) report that it takes approximately 3 months in a young adult for the nail to grow from base to tip, and 4-6 months in an older adult, although Hamilton and colleagues' (1955) results indicate that in young adults (~20 years) it is closer to 4 months. In younger children, this time is significantly reduced (Beaven and Brooks, 1994; Hamilton et al., 1955). Nail growth rates respond relatively quickly to metabolic upset, and serious illness or malnutrition can cause a temporary cessation or decline in growth, minutely altering the appearance of the nail (Gilchrist and Buxton, 1939; Beaven and Brooks, 1994). On the other hand, trauma, even nail biting, can stimulate growth, leading to nails growing out thicker and at a slightly faster rate (Beaven and Brooks, 1994). Several studies have also demonstrated a correlation between aging and reduced growth rate (e.g. Hamilton et al., 1955; Orentreich et al., 1979; Bean, 1980). Furthermore, growth rate also differs by sex (Hamilton et al., 1955; Orentreich et al., 1979).

Nails have not been used as often as hair in paleodiet studies, although they have found their way into the archaeological literature to a limited extent (e.g., Williams, 2008). Nails are used more frequently as a component in studies on modern populations (e.g., O'Connell et al., 2001; Fraser et al., 2006; Nardoto et al., 2006; Buchardt et al., 2007), or in a forensic context (e.g., Meier-Augenstein and Fraser, 2008; Meier-Augenstein, 2010). The bulk keratin in adult nails is thought to represent approximately the last six months of the individual's diet, although

estimates vary (O'Connell et al., 2001; Nardoto et al., 2006). Similar to bone collagen, nail preservation for isotopic studies is usually evaluated via atomic C/N ratio (Williams, 2008).

Hair

Hair is also a part of the integumentary system, and like nails it is a derivative structure of skin. In humans, scalp hair serves a limited number of functions, including providing a degree of protection against cold (Scanlon and Sanders, 2007). Hair emanates from hair follicles located in the dermis, extending into the stratum corneum of the epidermis, and is primarily composed of the protein keratin (Robbins, 2002). Although there are several varieties of hair on the human body, this section will focus on scalp hair as the type relevant to the project.

Hair growth has three stages: anagen, catagen, and telogen. Anagen is the active growing phase, catagen is the transitional or involutional phase, and telogen is the resting stage (Messenger and Dawber, 1997; Robbins, 2002). During the anagen phase, hair grows at a stable, steady rate, often replacing an older hair in the telogen phases (Messenger and Dawber, 1997). The anagen phase is often said to last for approximately 1000 days, but it has been demonstrated that it can vary considerably, even down to 550 days (Courtois et al., 1995; Messenger and Dawber, 1997). While a hair follicle remains in the anagen phase for several years, both the catagen and telogen stages are considerably shorter (Messenger and Dawber, 1997; Robbins, 2002). Catagen is a transitional stage, where the base of the hair follicle moves up toward the epidermal surface and metabolic activity decreases, although the hair shaft continues to keratinize (Messenger and Dawber, 1997; Robbins, 2002). Hair takes on a ‘club-shape’, and the cessation of melanization means that the hair lacks pigmentation (Messenger and Dawber, 1997). This phase lasts approximately 2-3 months (Messenger and Dawber, 1997). Telogen is the

resting stage, in which metabolic activity stops. In most instances, a new hair fiber grows to replace the old one, pushing the telogen phase hair shaft toward the skin surface and causing it to be shed, but until this happens the telogen hair may be retained in the epithelial sac for several months (Messenger and Dawber, 1997; Robbins, 2002). If, on the other hand, a telogen phase hair is plucked, it can cause the onset of the anagen phase to advance (Messenger and Dawber, 1997). Complete replacement of all hairs on the scalp will take place, on average, every 3-5 years (Saitoh et al., 1969).

The duration of each phase of the cycle and the distribution of growing phases on the scalp can vary due to a number of factors, both inter- and intra-individually, including age, sex, health, and ancestry (Messenger and Dawber, 1997; Loussouarn, 2001). Furthermore, there is also variation in the percentage of hairs found in each cycle in a given individual. Approximately 80-95% of hair follicles (88% on average) in adults are in the anagen phase, and if telogen hairs make up more than 25% it is usually considered abnormal (Messenger and Dawber, 1997; O'Connell and Hedges, 1999). Hairs in the catagen phase represent only approximately 1-3% of scalp hair, but are not always demonstrable in a clinical setting (Messenger and Dawber, 1997). Variation in these percentages has been demonstrated by both age and sex. Juveniles typically have a higher average anagen:telogen ratio than adults, approximately 90% (Messenger and Dawber, 1997). The distribution also varies by ancestry, as it has also been demonstrated that individuals of African descent have a higher percentage of telogen hairs compared to those of European descent (Loussouarn, 2001). Hair follicles grow independently of one another, meaning that adjacent hairs may be in a different phase of growth; this is referred to as a mosaic pattern (Dawber and van Neste, 2004). For isotopic studies, this can cause a lag of around 0-3

months in ~10% of the isotopic signature if the sample taken includes mixed-growth phases (O'Connell and Hedges, 1999; Williams et al., 2011). For this reason, Williams and colleagues (2011) conclude that phase identification can be crucial for reducing growth cycle error, and gaining a more refined understanding of positional-temporal relationships in sequentially segmented hair.

Adult scalp hair grows at a steady rate of approximately 0.35-0.4 mm/day (Lindberg and Forslind, 2004), or 8-10 mm/month, often given as one centimeter (Myers and Hamilton, 1951; Scanlon and Sanders, 2007). One centimeter of hair, therefore, represents about one month of growth, so a 6 cm sample will represent about 6 months, and so forth (O'Connell and Hedges, 1999). Although the duration of growing phases and the distribution of growing phases on the scalp are variable, the growth rate is remarkably stable, with some exceptions. Myers and Hamilton (1951) compared hair growth rate of their subjects aged 9-11 with an adult sample, and report daily averages of 0.41 mm (for the crown) and 0.37 mm (for the supraear region of the scalp), compared with 0.30 mm and 0.32 mm in their 21-30 year old subjects. It has also been noted that linear growth rate decreases in individuals with thinning hair (Dawber and van Neste, 2004).

Hair growth and hair quality varies over the course of the human life cycle, notably within the age ranges examined for this project. Infants (< 1 year) have very fine hair with a maximum length of around 15 cm (Robbins, 2002). This is replaced after the first year by primary terminal hair – also referred to as prepubertal or children's hair – which is thicker in diameter (~60 µm) and reaches a maximum length of approximately 60 cm (Robbins, 2002). As the timing of hair growth phases is controlled primarily by hormones, it is not surprising that

puberty leads to another major change in hair quality (Robbins, 2002). The onrush of hormones associated with puberty leads to the development of secondary terminal hair, which is coarser, longer and thicker in diameter (~100 µm) than children's hair (Robbins, 2002). The average maximum length is extended to 100 cm, although obviously much variation exists, and many people surpass this length to a considerable degree (Robbins, 2002). After puberty the anagen growth phase of scalp hair shortens, and as a result hair appears both shorter and finer, and has a decreased diameter (Robbins, 2002).

Hair has achieved a greater presence in isotopic research on archaeological samples than either skin or nails (e.g. Macko et al., 1999; Schwarcz and White, 2004; Roy et al., 2005; Knudson et al., 2007; Finucane, 2007; Williams, 2008; Williams et al., 2011). Likewise, numerous isotopic studies on modern human hair have also been conducted, usually related to dietary analysis (e.g., Nakamura et al., 1982; Macko et al., 1999; O'Connell and Hedges, 1999; O'Connell et al., 2001; Bol and Pflieger, 2002; Fraser et al., 2006; Bol et al., 2007; Meier-Augenstein and Fraser, 2008; Meier-Augenstein, 2010; Raghavan et al., 2010; Thompson et al., 2010). When it is well-preserved, hair proteins have been shown to be quite structurally robust. Using X-ray diffraction and infra-red spectroscopy to compare ancient and modern hair samples, Lubec and colleagues (1987) found that the structural integrity of the proteins in hair may be maintained for thousands of years. Similar to bone collagen, hair preservation for isotopic studies is usually evaluated via atomic C/N ratio (Williams, 2008).

Carbon Fractionation and Inter-Tissue Spacing

Typically, the carbon isotope values in the body are not an exact match for those of the diet, nor are those found in one tissue equivalent to those found in another. Differences have

been consistently observed in numerous studies, caused by a combination of intrinsic and extrinsic factors. These are ultimately the result of the processes of fractionation, which are not always well-understood.

DeNiro and Epstein (1978) were among the first to observe within a controlled experimental setting that different tissues within the same organism may be enriched in $\delta^{13}C$ to different degrees. They also found that the spacing of these values varied depending upon the animal's diet. It was suggested, therefore, that "no single tissue can be analyzed in order to determine the carbon isotopic relationship between the animal and its diet. For the purpose of dietary analysis, the determination of the $\delta^{13}C$ values of several tissues from an animal will allow for a better estimate of the $\delta^{13}C$ of its diet than would the analysis of a single tissue" (DeNiro and Epstein, 1978: 501).

As bone is more typically preserved in the archaeological record, focus was placed on the differences observed between the $\delta^{13}C$ in bone collagen and bone carbonate. This difference was observed by Sullivan and Krueger (1981), the first study to isolate the carbonate in bone apatite for paleodietary analysis. They found that the carbon isotope ratios from carbonate were enriched by ~8‰ compared with those derived from collagen. Debate over whether the diagenetic processes affecting bone mineral rendered the material unfit for isotopic analysis (Sullivan and Krueger, 1983; Schoeninger and DeNiro, 1983), was followed by a critical examination of bone mineral diagenesis and the procedures necessary to isolate *in vivo* carbonate from diagenetic carbonate, resulting in more refined methods (Nelson et al., 1986; Thorp and van der Merwe, 1987; Lee-Thorp and van der Merwe, 1991). Krueger and Sullivan (1984) proposed that the reason for the differing isotopic values observed between collagen and carbonate was that the

carbon in each ultimately derived from different parts of the diet. In collagen, the carbon is derived from ingested protein. The carbonate in bone, however, is derived from dissolved bicarbonate in the blood, and therefore is obtained from the carbohydrates and lipids in the diet in addition to proteins, and is a closer representation of the 'whole diet' (Krueger and Sullivan, 1984; Schwarcz, 2002). The isotopic values of each bone tissue reflect the differing sources. This hypothesis is well supported by results from controlled feeding studies (e.g., Ambrose and Norr, 1993; Tieszen and Fagre, 1993).

Isotopic spacing between different soft tissues and between bone and soft tissue has been examined in a number of controlled feeding studies using animal models. Some of these have been conducted with explicitly archaeological analogues in mind (e.g., Hare et al., 1991; Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Jim et al., 2004; Warinner and Tuross, 2009), while other studies derive from ecological concerns (e.g., Tieszen et al., 1983; Hobson and Clark, 1992; Trueman et al., 2005). Few of these contain the particular selection of tissues used in this study, or examine the relationship between inter-tissue spacing and age. Although there are inherent difficulties with applying animal data to humans, nevertheless these experiments have given us a better understanding of the processes of fractionation and the numerous factors that may affect tissue spacing.

As discussed above, a few controlled feeding studies have been used to support hypotheses about isotope fractionation differences between bone carbonate and bone collagen (Ambrose and Norr, 1993; Tieszen and Fagre, 1993; Jim et al., 2004). In addition, other studies have utilized a range of soft tissues not often found in the archaeological record. For example, DeNiro and Epstein (1978) examined multiple tissues of mice, which included hair and various

internal organs, including brain, liver, spleen, etc. They found that the enrichment factor between diet and hair, spleen and brain was positive, up to 1‰, while other tissues were depleted compared to dietary values. They also found that hair and spleen showed considerably more variation than other tissues. They do not offer an explanation for these observations, but state that their results indicate that utilizing multiple tissues for diet reconstruction would produce more robust conclusions than relying on only one (DeNiro and Epstein, 1978). Tieszen and colleagues (1983) observed a similar trend in their multi-tissue carbon isotope analysis of gerbil soft tissues, with hair being more enriched compared to diet than any of the other tissues in the study. They also concluded that tissues with higher metabolic activity and faster turnover would reflect changes in diet more quickly than those with slower turnover rates (Tieszen et al., 1983).

Tieszen and Fagre (1993) utilized a large array of tissues in their diet study on mice, including hair and bone collagen, although their primary focus was on furthering the understanding of fractionation between bone collagen and apatite. Using their results, one may calculate an average difference between hair and bone collagen $\delta^{13}C$ values across the eight diets tested: 2.9‰ ± 0.57, with bone collagen being consistently more enriched than hair. They found that dietary protein was by far the most significant contributor to isotopic values for both tissues (Tieszen and Fagre, 1993). Outside of the laboratory, consistent intra-individual differences between tissues have been observed in animals in the field, such as in Vogel's (1978) study on the diets of large African ungulates. Vogel (1978) observed consistent differences between bone collagen, hide, and flesh. Bone was found to be more enriched than hide by an average of 0.6‰ (Vogel, 1978).

Other studies are conducted using human subjects, such as that by O'Connell and colleagues (2001) utilizing hair, nail and bone samples derived from modern living humans. They examined amino acid composition of the materials as a possible explanation for observed variation in inter-tissue isotope values. Although both are proteinaceous, collagen and keratin differ in their amino acid composition (O'Connell et al., 2001). For example, glycine, an amino acid found in abundance in collagen, but not in keratin, typically displays significant $\delta^{13}C$ enrichment compared to other amino acids (O'Connell et al., 2001). Therefore, collagen would have a higher $\delta^{13}C$ enrichment factor than keratin (O'Connell et al., 2001). Hare and colleagues (1991), in a study involving bone collagen and muscle in pigs, also broke down the proteins in both materials to their basic amino acid components in order to understand observed isotopic patterns.

Similarly, Raghavan and colleagues (2010) examined the amino acid composition of bone collagen and hair in six archaeological individuals from Greenland, and found differences between the two tissues that could account for the 2-4‰ enrichment observed in $\delta^{13}C$ values. Results indicated that while the essential amino acids valine, lysine, and arginine showed similar values for both hair and bone, the essential amino acids phenylalanine and tyrosine (for which phenylalanine is a biosynthetic precursor) displayed a difference. This was also noted in the non-essential amino acids. They suggested that the differences could be the result of hair reflecting short-term seasonal variation in diet, although they were not able to fully resolve the issue of amino acid composition versus turnover rates without a more complete analysis of all amino acids in both tissues (Raghavan et al., 2010).

Corr and colleagues (2009) performed analyses on individual amino acids from the skin and bone in an ancient individual found frozen in a retreating glacier in British Columbia. The proportions of amino acids in both tissues were found to be consistent with the amino acid distribution in collagen. They also found that the $\delta^{13}C$ values found in the skin amino acids were consistently lower than those in bone collagen, and argued that the depletion, taken with the difference in turnover of these tissues, represented a shift in diet from marine to terrestrial resources in the last few months of the individual's life, concurrent with his moving further inland (Corr et al., 2009). Finucane (2007) also found an offset between skin and bone collagen and suggested short-term diet variation as a potential cause, but this explanation was recognized by the author as being somewhat unsatisfactory, given that the offset was observed in each comparison, and it seems unlikely that every individual would have altered their diet in a similar fashion in the last months of their life.

Because bone, hair, fingernails and skin differ in their rate of growth and turnover times, comparisons between bone, which represents an individual's diet over a period of years, and the soft tissues, which represent a period of months, have been used to investigate seasonal and other variations in diet in a population (e.g. White and Schwarcz, 1994; Macko et al., 1999; Dupras, 1999; Knudson et al., 2007; Williams, 2008). Physiological stress in the final months of an individual's life has also been suggested as a potential cause of isotopic discrepancies between bone and tissues with shorter turnover times (Finucane, 2007).

Much of the data on inter-tissue spacing in humans derives from archaeology, as in the studies listed above. For comparative purposes, a selection of results from a number of

archaeological studies is summarized in Table 1, along with studies from two modern populations and one controlled feeding study.

Table 1: Comparison of differences between $\delta^{13}C$ values of tissues across multiple studies

Study	Type	Origin	Δ_{BC-H}	Δ_{BC-N}	Δ_{BC-S}	Δ_{H-N}	Δ_{H-S}	Δ_{S-N}
Tieszen and Fagre, 1993	Controlled Feeding (Mice)	Lab	2.9 ± 0.57*					
Vogel, 1978	Ecological (Ungulate)	South Africa			0.6			
O'Connell et al., 2001	Modern	UK	1.41 ± 0.45	**		0.21 ± .39		
Fraser et al., 2006	Modern	UK				0.55 ± 0.81*		
White, 1991	Archaeo.	Nubia	-0.98		1.05		-2.03	
White et al., 1999	Archaeo.	Egypt					-0.8	
Williams, 2005	Archaeo.	Peru	1.9 ± 1.4	2.0 ± 1.1	0.2 ± 1.0	0.4 ± 1.0	1.6 ± 1.92*	1.7 ± 1.84*
Finucane, 2007	Archaeo.	Peru			0.3 ± 1.84			
Knudson et al., 2007	Archaeo.	Chile	1.7 ± 1.6 & 1.0 ± 0.8†					
Raghavan et al., 2010	Archaeo.	Greenland	2.0 ± 0.24*					

*Calculated for present study
**Bone-Hair and Hair-Nail values represent two separate study groups, so Bone-Nail was not calculated
†For mean of first segment of hair (0-2 cm) and sixth segment (10-12 cm) respectively

CHAPTER THREE: MATERIALS AND METHODS

Materials

The Dakhleh Oasis

The data used in this project were derived from individuals excavated from the Kellis 2 cemetery in the Dakhleh Oasis, Egypt. The Dakhleh Oasis is one of five oases in Egypt's Great Western Desert, and is located approximately 800 km SSW of Cairo (Figure 1). The oasis is approximately 2000-3000 km^2 (Mills, 1979).

Preservation of biological materials recovered from the Dakhleh Oasis is exceptional, owing to the hyper arid environment and conditions of burial. Many of the cemeteries located in the Dakhleh Oasis have proved to be a remarkable source for stable isotope research, since both the preservation and number of individuals recovered present ideal circumstances for relatively large scale isotope studies (Dupras, 1999; Schwarcz et al., 1999; Dupras et al., 2001; Dupras and Schwarcz, 2001; Dupras and Tocheri, 2007; Williams, 2008; Williams et al., 2011; Johns, 2012). It should also be noted that an unusually large number of juvenile remains have been recovered from the Kellis 2 cemetery (Tocheri et al. 2005).

Previous isotopic studies carried out on the skeletal population from the cemeteries of the Dakhleh Oasis have revealed a great deal about diet (e.g., Dupras, 1999), weaning and infant feeding practices (Dupras et al., 2001; Dupras and Tocheri, 2007), migration (Dupras and Schwarcz, 2001), season of death (Williams, 2008) and individual life histories (Johns, 2012).

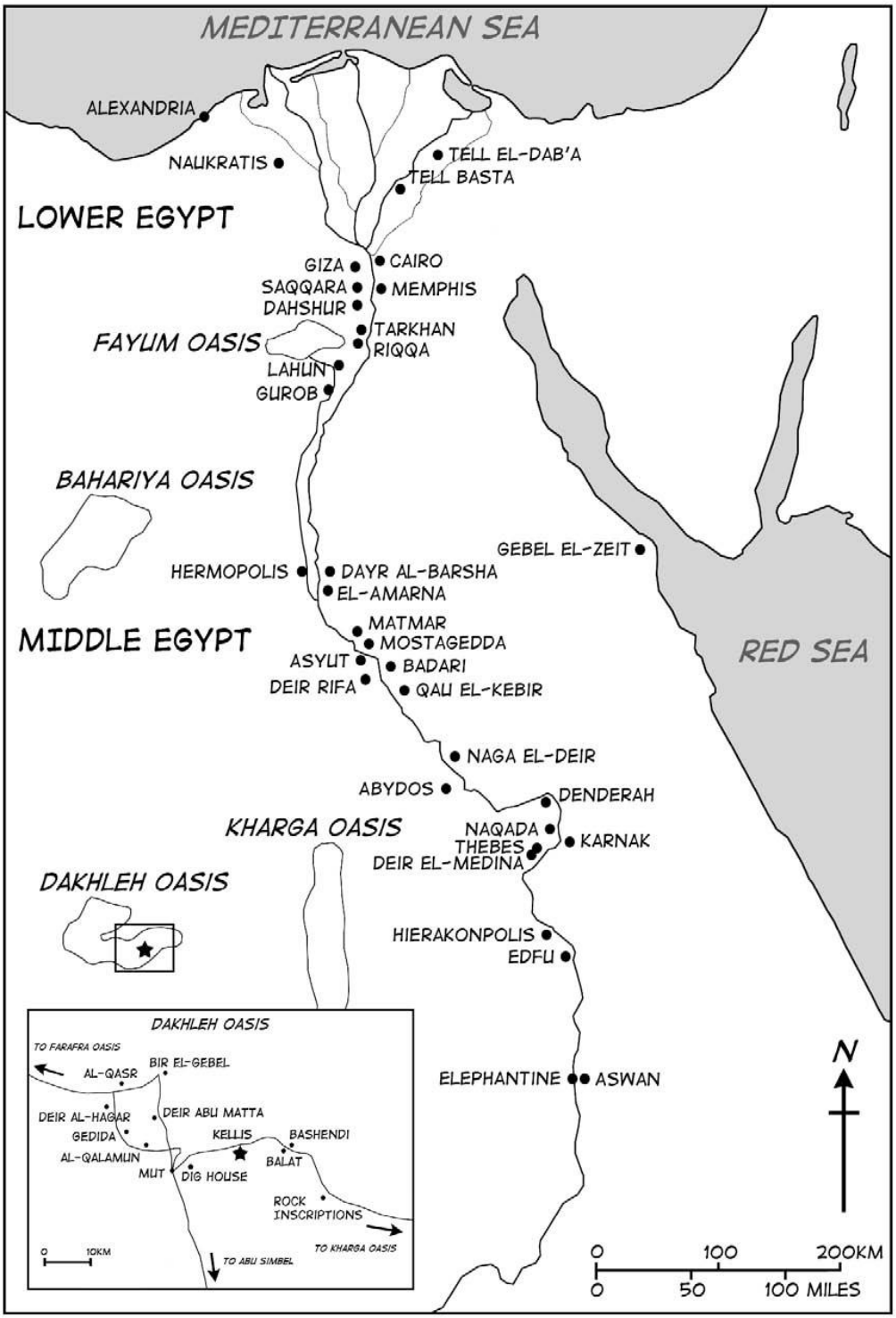

Figure 1: Map of the Egypt showing location of the Dakhleh Oasis with an inset showing the location of Kellis 2 (Map courtesy of Dr. Lana Williams)

Kellis 2 Cemetery

Kellis 2, also called the East Cemetery (Figure 2), is associated with the ancient village of Kellis, a settlement occupied from the Late Ptolemaic into the Roman-Christian period. The archaeological and radiocarbon evidence from the cemetery suggest that it dates to c.AD 50 – AD 450, although this is still under some debate (Fairgrieve and Molto, 2000; Molto 2001). Out of an estimated 3000-4000 graves (Molto, 2002), 765 have been excavated as of 2011 (L. Williams, personal communication).

Of the analyzed skeletal remains, the Kellis 2 demography consists of 64% juveniles and 36% adults (Williams, 2008). The majority of juveniles (39%) are between 41 weeks gestation to one year, representing 25% of the total population. Approximately 14% of the population is between 13 months and 4 years, with approximately 6% between 5 and 10 years, and less than 3% between the ages of 11-15 years. The 11-15 year olds represent the smallest subset of the skeletal population as a whole (Wheeler, 2009).

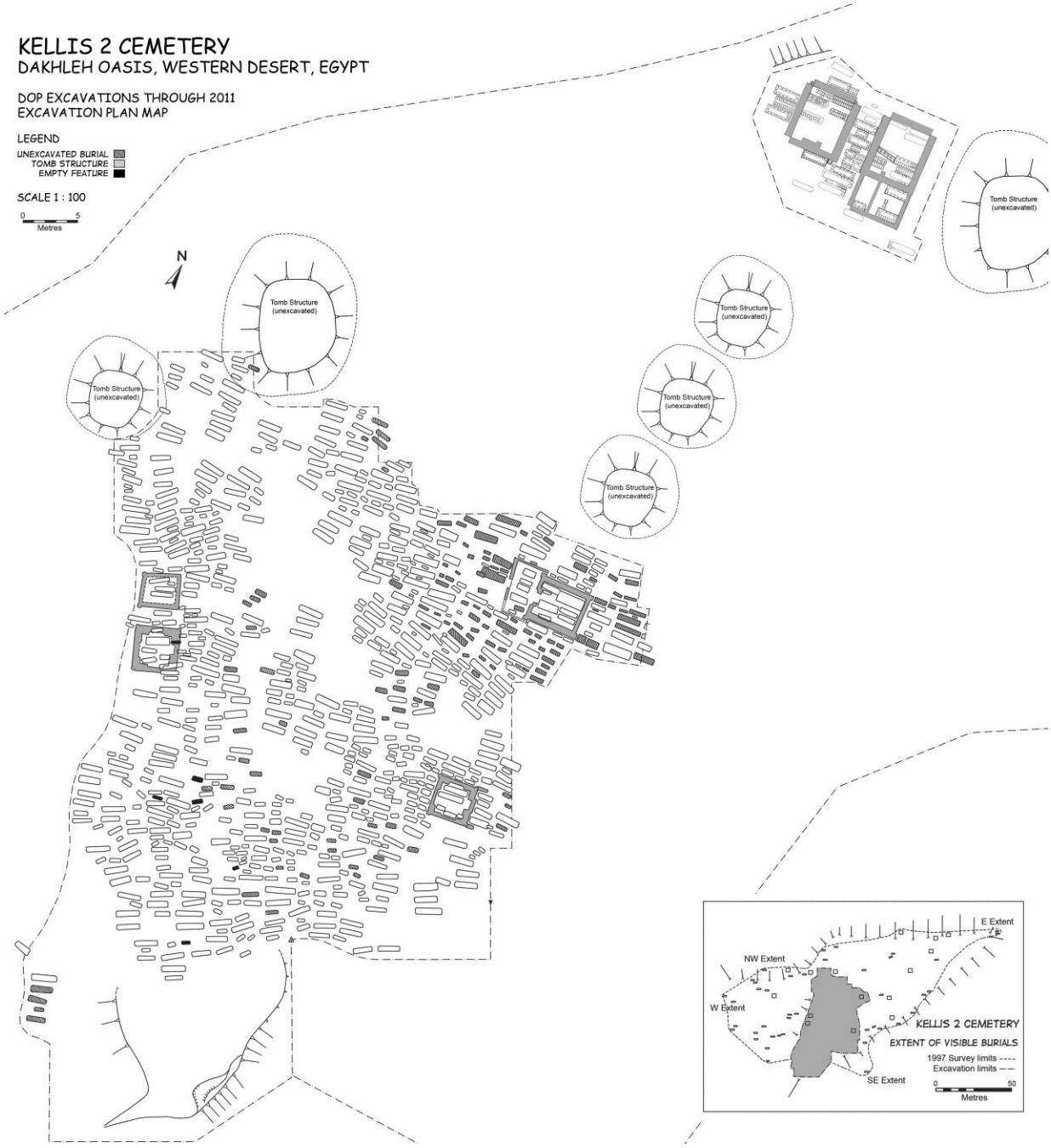

Figure 2: Map of Kellis 2 Cemetery (Map courtesy of L. Williams)

Sample Used in this Study

The burial locations of the individuals sampled for this study are shown in Figure 3. Fifty-two individuals were sampled (Figure 4), representing approximately 7% of the total population and approximately 12% of juveniles recovered. These were divided into three age cohorts (Tables 2-4), using standard osteological methods, delineated along developmental lines: 1-4 years, 5-10 years, and 11-15 years (Wheeler, 2009). Tissues sampled for this project include hair samples from 52 individuals, nail samples from 30 individuals, skin samples from 39 individuals, and bone collagen samples from 12 individuals (Table 5). Tables 2-4 also provide a list of samples obtained from each individual.

Hair samples were taken in 1 cm increments, and a total of 203 hair segment samples were taken for all individuals. For nails, samples were taken from both the proximal and distal ends, and Tables 2-4 indicate which ends were sampled from each individual. A total of 55 nail samples were analyzed. Skin samples were taken primarily from tissue on the cranium (L. Williams, personal communication), and values represent bulk skin samples, 39 in total. All bone samples were collected and analyzed by Dr. Tosha Dupras and all soft tissue samples were collected and analyzed by Dr. Lana Williams, for their respective doctoral dissertations (Dupras, 1999; Williams, 2008).

Although these individuals had survived the most critical period between birth and one year (which is the average age at death for 39% of Kellis 2 juveniles), the individuals in the youngest cohort were still in a precarious position, as it is estimated that two-fifths of children in Roman Egypt did not live beyond the age of five (Bagnall, 1996). This group also encompasses the period of weaning, which began around the age of 6 months and ended at around 3 years

(Dupras et al., 2001). In the oldest cohort, juveniles between the ages of 11 and 15 were not only experiencing the physiological changes of puberty, with all its myriad challenges, but also the social changes and challenges of incipient adulthood. Some of the individuals in this category may have been considered full adults in their community (Bagnall and Frier, 1994), able either to marry or work in the fields, while others would have been on the very cusp of reaching that state.

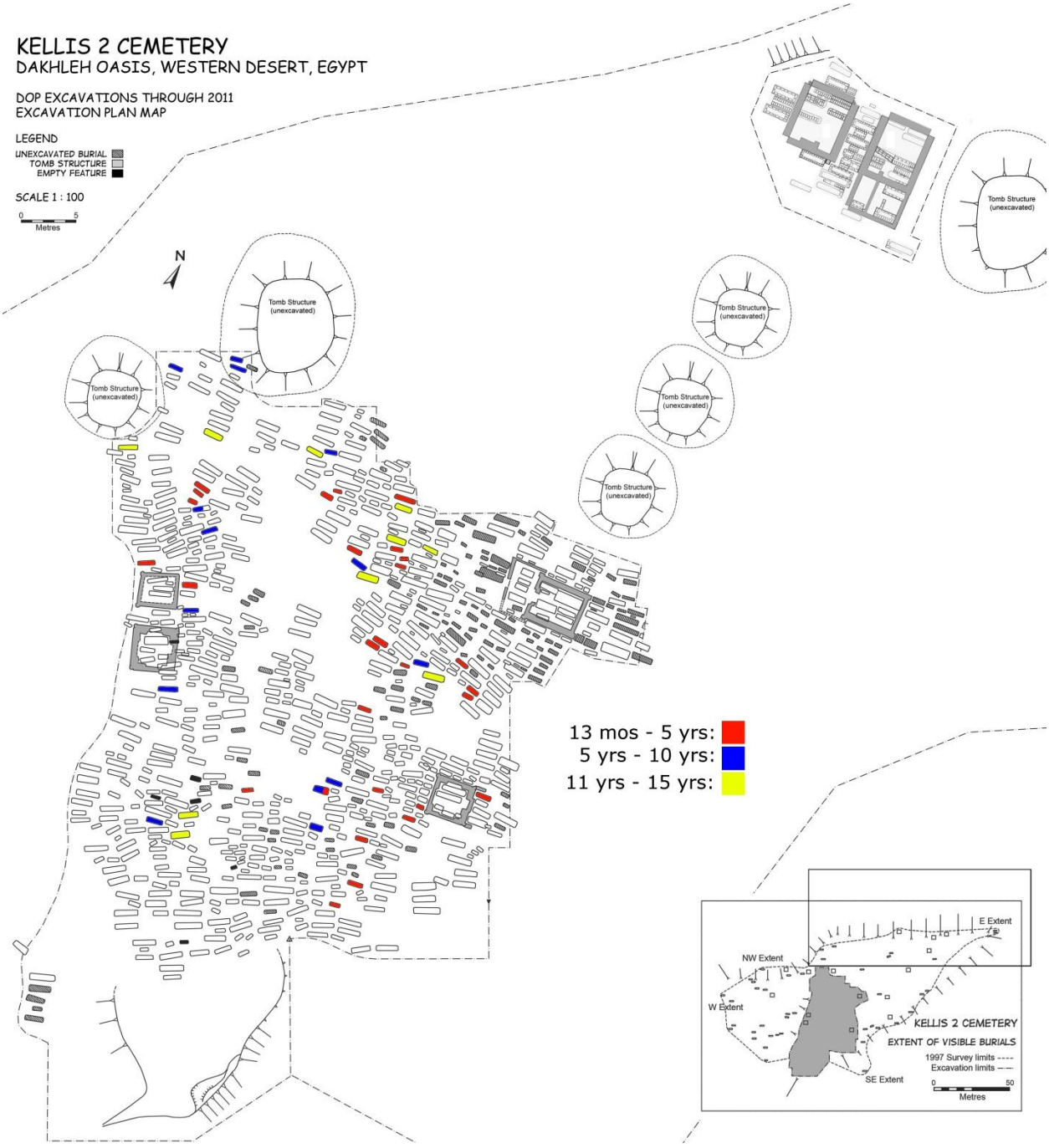

Figure 3: Burial Locations of Individuals Sampled from the Kellis 2 Cemetery (Map courtesy of L. Williams)

Table 2: List of Samples Aged 1-4 Years (n=28)

Burial ID	Age	Hair	Nails	Skin	Bone (Collagen)
23	1.5	2 cm	Prox/Dist	X	
70	1.5	2 cm	Prox/Dist	X	X
71	2	6 cm			X
86	4	2 cm	Prox/Dist		X
108	1.5	1 cm	Proximal	X	X
299	1.25	9 cm	Prox/Dist	X	
323	1.5	1 cm		X	
328	1.25	9 cm	Prox/Dist	X	
330	4	5 cm		X	
349	4	1 cm	Prox/Dist	X	
351	4	1 cm		X	
357	2	1 cm	Proximal	X	
358	2.5	3 cm	Prox/Dist	X	
362	3	2 cm		X	
396	2	7 cm	Proximal		
487	2	5 cm		X	
490	1.5	2 cm		X	
519	2.5	6 cm	Prox/Dist	X	X
534	1.5	8 cm			
560	1.75	1 cm	Proximal	X	
562	1.5	2 cm		X	
579	1.5	6 cm		X	
583B	2.5	7 cm	Prox/Dist		
593A	1.5	4 cm	Prox/Dist		
619	2	1 cm	Prox/Dist	X	
620	2	4 cm	Prox/Dist		
624	1.5	4 cm	Prox/Dist	X	
628	1.5	1 cm	Prox/Dist	X	

Table 3: List of Samples Aged 5-10 Years (n=14)

Burial ID	Age	Hair	Nail	Skin	Bone (Collagen)
24	7	3 cm	Prox/Dist	X	X
49	7	10 cm			X
64	6	2 cm	Prox/Dist	X	X
97	6	6 cm	Proximal		X
163	5	1 cm		X	
258	10	1 cm		X	
336	5	3 cm		X	
360	5	4 cm		X	
370	5	1 cm	Prox/Dist	X	X
374	7	8 cm	Prox/Dist	X	
520	8	3 cm	Prox/Dist	X	
582	10	1 cm	Prox/Dist		
583A	7	2 cm	Prox/Dist	X	
584	5	4 cm	Prox/Dist		

Table 4: List of Samples Aged 11-15 years (n=10)

Burial ID	Age	Hair	Nail	Skin	Bone (Collagen)
149	12	4 cm		X	X
173	12	1 cm			X
239	15	4 cm	Prox/Dist	X	
243	15	4 cm			
260	15	3 cm		X	
263	13	1 cm		X	
288	15	13 cm	Prox/Dist	X	
295	11	8 cm		X	
302	12	3 cm			
468	13	10 cm	Prox/Dist	X	

Table 5: Tissue representation by number of individuals

	Hair	Nails	Skin	Collagen
1-4 years	28	18	22	5
5-10 years	14	9	10	5
11-15 years	10	3	7	2
Total	52	30	39	12

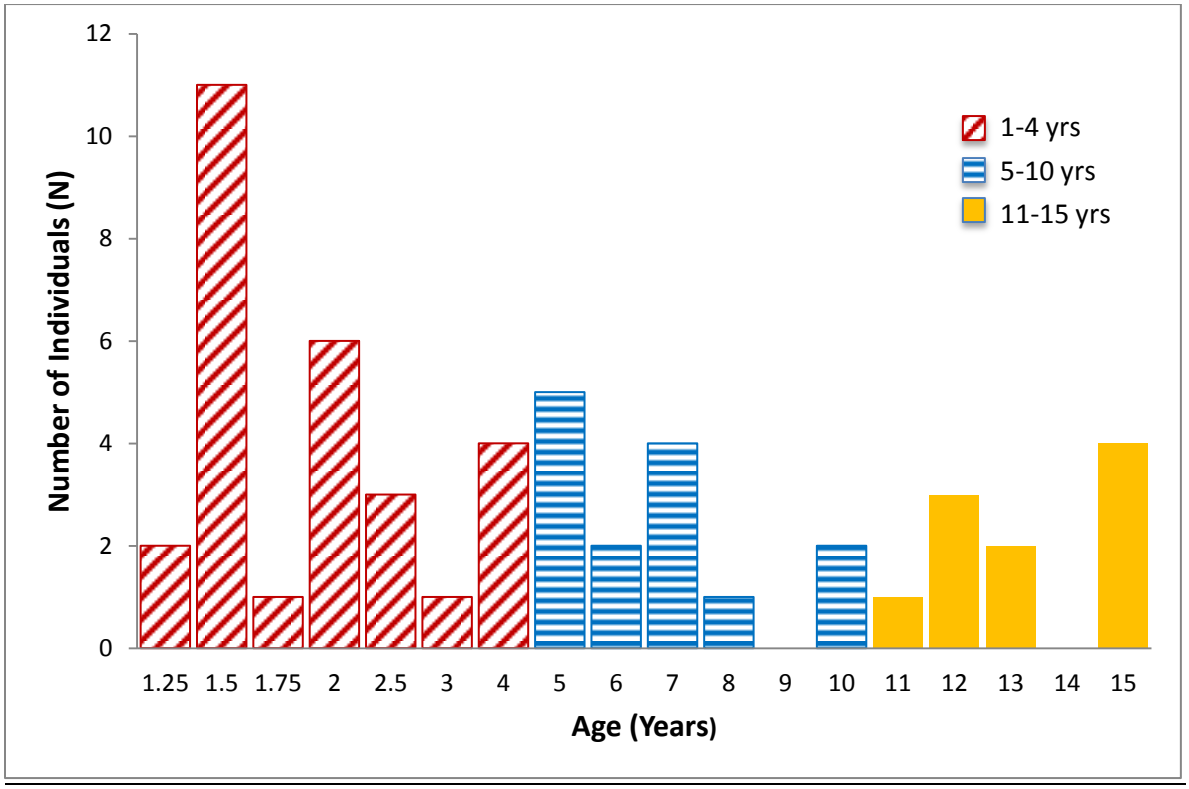

Figure 4: Age Demographics of the Sample

Methods

Isotopic Sample Preparation

The bone collagen samples were processed by Dr. Tosha Dupras at McMaster University, as part of her doctoral dissertation (1999). Samples were prepared using methods described in Longin (1971) and modified by Chisholm and colleagues (1982). For further description of

methods used, see Dupras (1999). Isotopic analyses were performed using a SIRA mass spectrometer. Precision of $\delta^{13}C$ analysis was within 0.08 ± 0.09 ‰. C:N ratios were measured using a Carlo-Erba C,N analyzer, in order to determine collagen preservation. Raw data values of $\delta^{13}C$ analysis are provided in the Appendix (Table 18).

The hair samples were prepared by Dr. Lana Williams (2008) at the Laboratory for Stable Isotope Science (LSIS) at the University of Western Ontario. Scalp sections were rehydrated using methods developed by Grupe and colleagues (1997) and Mekota and Vermehren (2005). For each individual, ~20-25 anagen hairs were collected and cut into 1 cm segments, following methods developed by Williams and colleagues (2011). Isotopic analyses were performed using an automated carbon and nitrogen analyzer with a continuous-flow stable isotope ratio mass spectrometer. Precision of $\delta^{13}C$ analysis was determined to be ± 0.04‰. Accuracy of analysis was a value of -24.03 ± 0.06‰. These results compared well with values obtained through calibration with NBS-22 carbon standard. Raw data values are provided in the Appendix (Table 17).

The nail samples and the samples of naturally desiccated skin were also prepared by Dr. Lana Williams at the LSIS at the University of Western Ontario, following methods outlined in Williams (2008). Isotopic analyses were performed using an automated carbon and nitrogen analyzer with a continuous-flow stable isotope ratio mass spectrometer. Precision of $\delta^{13}C$ analysis was determined to be ± 0.03‰. Accuracy of analysis was determined to be -24.0 ± 0.12‰. These results compared well with values obtained through calibration with NBS-22 carbon standard. Raw data are provided in the Appendix (Table 18).

The samples of naturally desiccated skin were also processed by Dr. Lana Williams at the LSIS at the University of Western Ontario, following methods outlined in Williams (2008). Isotopic analyses were performed using an automated carbon and nitrogen analyzer with a continuous-flow stable isotope ratio mass spectrometer. Precision of $\delta^{13}C$ analysis was determined to be ± 0.03‰. Accuracy of analysis was determined to be -24.0 ± 0.12‰. These results compared well with values obtained through calibration with NBS-22 carbon standard. Raw data are provided in the Appendix (Table 18).

Analysis of Isotopic Data

Means were calculated for the isotope values of the hair samples (in bulk), the nail samples, the skin samples, and the bone collagen samples, both overall and within each of the three age cohorts: 1-4 years, 5-10 years, and 11-15 years. The difference between the means for each tissue was also calculated, and the statistical significance of the observed variation was evaluated using a paired t-test. In addition, mean intra-individual differences between the tissue types, as well as inter-individual effects of age, were evaluated using hierarchical regression.

In addition to examining differences between aggregate means of bulk tissue data, segmented hair and nail data were also considered. The differences were re-calculated and examined using first centimeter (0-1 cm) hair data and proximal nail values to assess any changes occurring in the last month prior to death.

As well as examining the differences in inter-tissue spacing observed between the juvenile age cohorts, in *Chapter Five: Discussion*, the adult data from Kellis 2 were included as a comparison, to further illustrate age as a potential factor in isotopic values. Data from other

studies are also compared, and in some cases calculations were performed on the published data to facilitate better comparisons with results presented here.

Descriptive statistics were calculated using a Microsoft Excel spreadsheet, and all charts and tables were generated using the same program. Other statistical analyses were performed using IBM SPSS Statistics 20.

CHAPTER FOUR: RESULTS

Mean $\delta^{13}C$ isotope values were calculated for each tissue within each of the three age cohorts. The results are reported in Tables 6 and 7, along with the total number of samples for each tissue within each age group, as well as the standard deviation. Although hair samples were taken in 1 cm segments and nail samples include both proximal and distal portions, the n values in Tables 6 and 7 represent bulk samples. In all cases, unless otherwise indicated, hair and nail values reported represent bulk values.

The means are graphically represented in Figure 5. Figure 6 represents the mean, standard deviation, and minimum and maximum values for all age cohorts for all tissues: standard deviation is represented as a box around the mean, while range is indicated by error bars. Results indicate that the mean bone collagen $\delta^{13}C$ values are the most enriched and least variable across all age groups, while nail and skin values are both the most depleted and the most similar to one another in value across all age cohorts.

Table 6: Number of samples, mean $\delta^{13}C$ values, and standard deviation by age group

	1-4 years			**5-10 years**			**11-15 years**		
Tissues	n	$\delta^{13}C$ (‰)	SD	n	$\delta^{13}C$ (‰)	SD	n	$\delta^{13}C$ (‰)	SD
Hair	28	-19.29	0.66	14	-19.68	0.39	10	-19.77	0.74
Nail	18	-20.04	0.69	9	-20.09	0.55	3	-20.42	0.58
Skin	22	-19.88	0.80	10	-20.10	1.30	7	-20.38	1.07
Collagen	5	-18.57	0.63	5	-18.70	0.22	2	-18.28	0.60

Table 7: Number of samples, mean $\delta^{13}C$ values, and standard deviation for all ages

Tissues	n	$\delta^{13}C$ (‰)	SD
Hair	52	-19.51	0.66
Nail	30	-20.09	0.64
Skin	39	-20.03	0.99
Collagen	12	-18.57	0.46

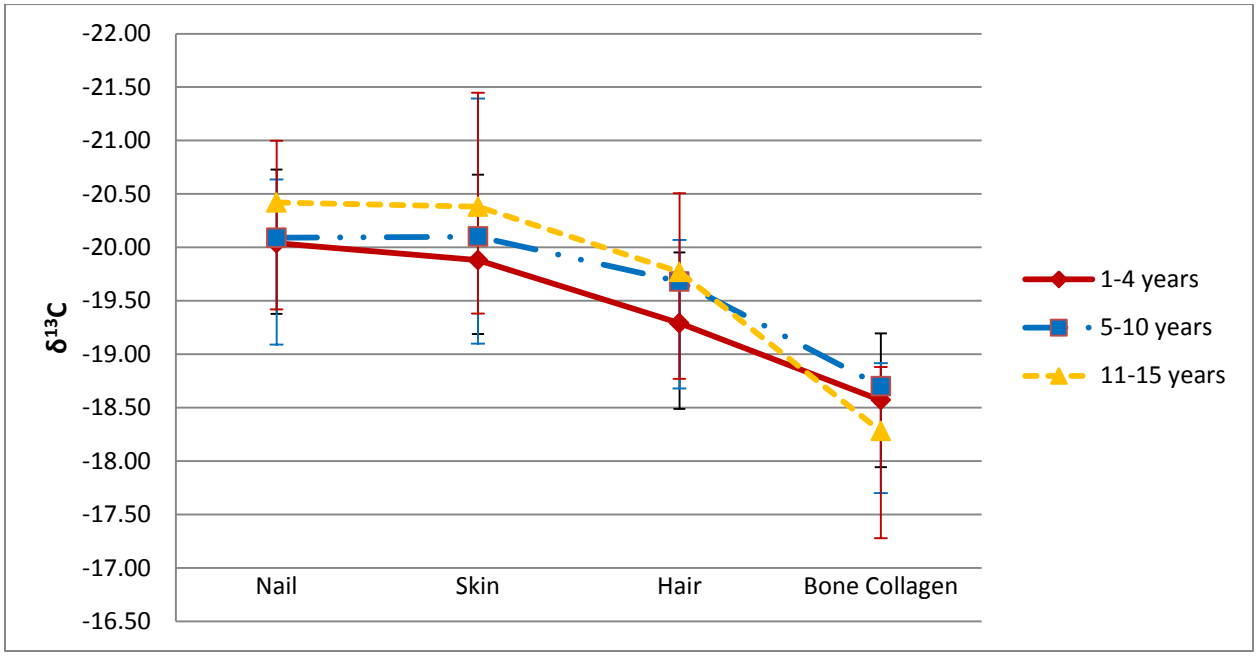

Figure 5: Mean $\delta^{13}C$ (‰) by age category and tissue type.

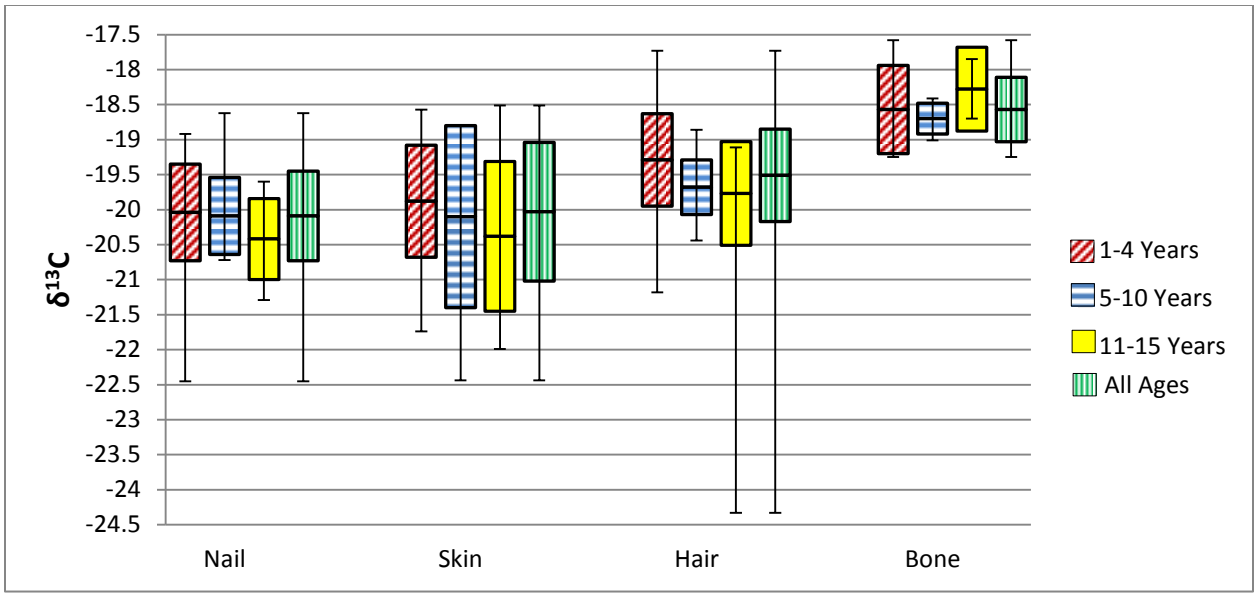

Figure 6: Mean, standard deviation, and range of $\delta^{13}C$ (‰) values for each age cohort within each tissue type

Figures 7-9 display the range of values in more detail, providing a graphic representation of the maximum and minimum $\delta^{13}C$ values, in addition to the mean and standard deviation for each tissue type within each age category. Note that for all graphs, bulk hair and skin values are represented. The exact numbers are given in Tables 8-10. These graphs provide a more nuanced view of these data, and reveal several points of interest. Bone collagen shows the smallest range of isotope values across all age cohorts, as well as the lowest standard deviation in the 1-4 year and 5-10 year age cohorts. The lowest standard deviation in the 11-15 year cohort is seen in nail, which is only ±0.02‰ lower than the standard deviation for bone collagen. The standard deviation across all tissue types is most similar in the 1-4 year age cohort (±0.63‰ - ±0.80‰), whereas the 5-10 year and 11-15 year age cohorts show more variation (±0.22‰ - ±1.30‰ and

±0.58‰ - ±1.07‰ respectively). The standard deviation is highest for skin across all age categories.

1-4 Year Cohort

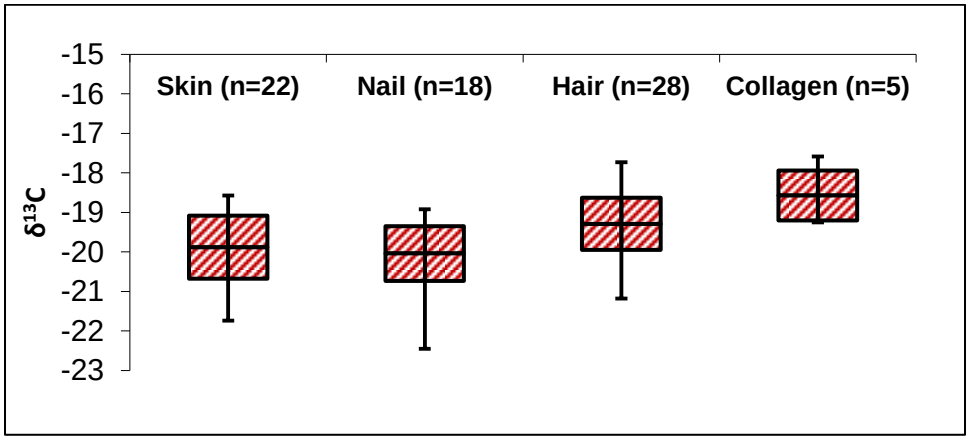

Figure 7: Mean, standard deviation, maximum and minimum $\delta^{13}C$ (‰) values of different tissues in 1-4 year age cohort

As in Figure 6, standard deviation is represented as a box around the mean, while range is indicated by error bars. Table 8 lists the numerical values for the 1-4 year age cohort represented by the graph in Figure 7.

Table 8: Mean, standard deviation, maximum and minimum $\delta^{13}C$ (‰) values of different tissues in 1-4 year age cohort

	Skin	Nail	Hair	Collagen
Max	-18.57	-18.92	-17.73	-17.58
Mean	-19.88	-20.04	-19.29	-18.57
Min	-21.74	-22.45	-21.18	-19.25
SD	0.80	0.69	0.66	0.63

5-10 Year Cohort

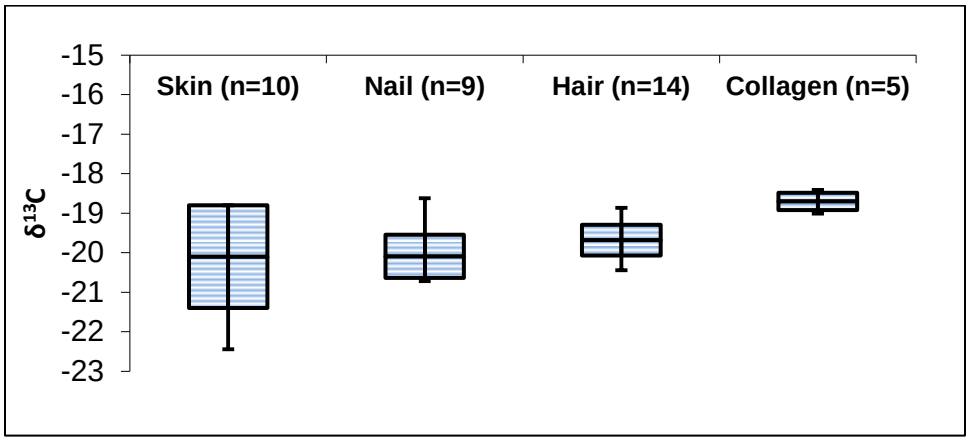

Figure 8: Mean, standard deviation, maximum and minimum $\delta^{13}C$ (‰) values of different tissues in 5-10 year age cohort

As in Figure 6, standard deviation is represented as a box around the mean, while range is indicated by error bars. Table 9 lists the numerical values for the 5-10 year age cohort represented by the graph in Figure 8.

Table 9: Mean, standard deviation, maximum and minimum $\delta^{13}C$ (‰) values of different tissues in 5-10 year age cohort

	Skin	Nail	Hair	Collagen
Max	-18.80	-18.62	-18.86	-18.41
Mean	-20.10	-20.09	-19.68	-18.70
Min	-22.44	-20.72	-20.44	-19.01
SD	1.30	0.55	0.39	0.22

11-15 Year Cohort

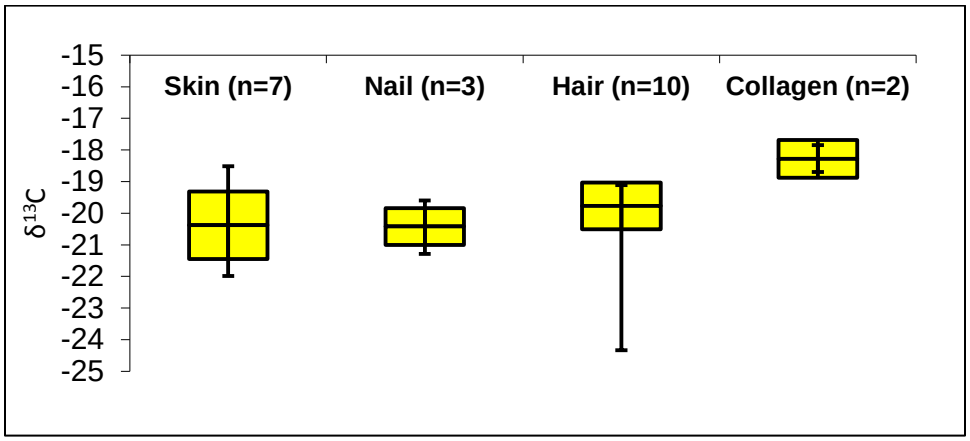

Figure 9: Mean, standard deviation, maximum and minimum $\delta^{13}C$ (‰) values of different tissues in 11-15 year age cohort

As in Figure 6, standard deviation is represented as a box around the mean, while range is indicated by error bars. Table 10 lists the numerical values for the 11-15 year age cohort represented by the graph in Figure 9.

Table 10: Mean, standard deviation, maximum and minimum $\delta^{13}C$ (‰) values of different tissues in 11-15 year age cohort

	Skin	Nail	Hair	Collagen
Max	-18.51	-19.60	-19.11	-17.85
Mean	-20.38	-20.42	-19.77	-18.28
Min	-21.99	-21.29	-24.33	-18.70
SD	1.07	0.58	0.74	0.60

Inter-tissue Spacing

To analyze the spacing of isotopic values between various tissues, the differences between the mean values for each tissue type were calculated, both overall and within each age cohort (Table 11). A paired t-test was performed to evaluate discernible differences between tissues at a 0.05 significance level across all age cohorts. All differences were found to be

statistically significant across the age cohorts, except for the relationship between nail and skin. In Figure 10, it can be readily observed that the spacing between collagen and hair, collagen and skin, and collagen and nail is substantially larger in the 11-15 year old cohort than the spacing between the same tissues in the 1-4 and 5-10 years cohorts.

Mean within-person differences between the tissue types were evaluated using a hierarchical regression pairwise comparison, and the results showed that all differences are statistically significant except those between skin and nail. In addition, hierarchical regression revealed a slight inter-individual effect of age over all tissues types, with older individuals generally having enriched carbon values compared to younger individuals.

Table 11: Differences between mean $\delta^{13}C$ (‰) values of tissues

Tissues	All	1-4 yrs	5-10 yrs	11-15 yrs	t	p-value
$\Delta_{\text{Skin-Nail}}$	0.06 ± 0.18	0.16 ± 0.21	0.01 ± 0.43	0.04 ± 0.47	-1.256	0.336
$\Delta_{\text{Hair-Skin}}$	0.52 ± 0.17	0.59 ± 0.19	0.42 ± 0.41	0.61 ± 0.42	8.959	0.012
$\Delta_{\text{Hair-Nail}}$	0.58 ± 0.10	0.75 ± 0.14	0.41 ± 0.14	0.65 ± 0.26	5.980	0.027
$\Delta_{\text{Bone collagen-Hair}}$	0.94 ± 0.14	0.72 ± 0.29	0.98 ± 0.11	1.49 ± 0.44	-4.702	0.042
$\Delta_{\text{Bone collagen-Skin}}$	1.46 ± 0.21	1.31 ± 0.33	1.40 ± 0.42	2.10 ± 0.59	-6.421	0.023
$\Delta_{\text{Bone collagen-Nail}}$	1.52 ± 0.16	1.47 ± 0.30	1.39 ± 0.16	2.14 ± 0.49	-7.009	0.020

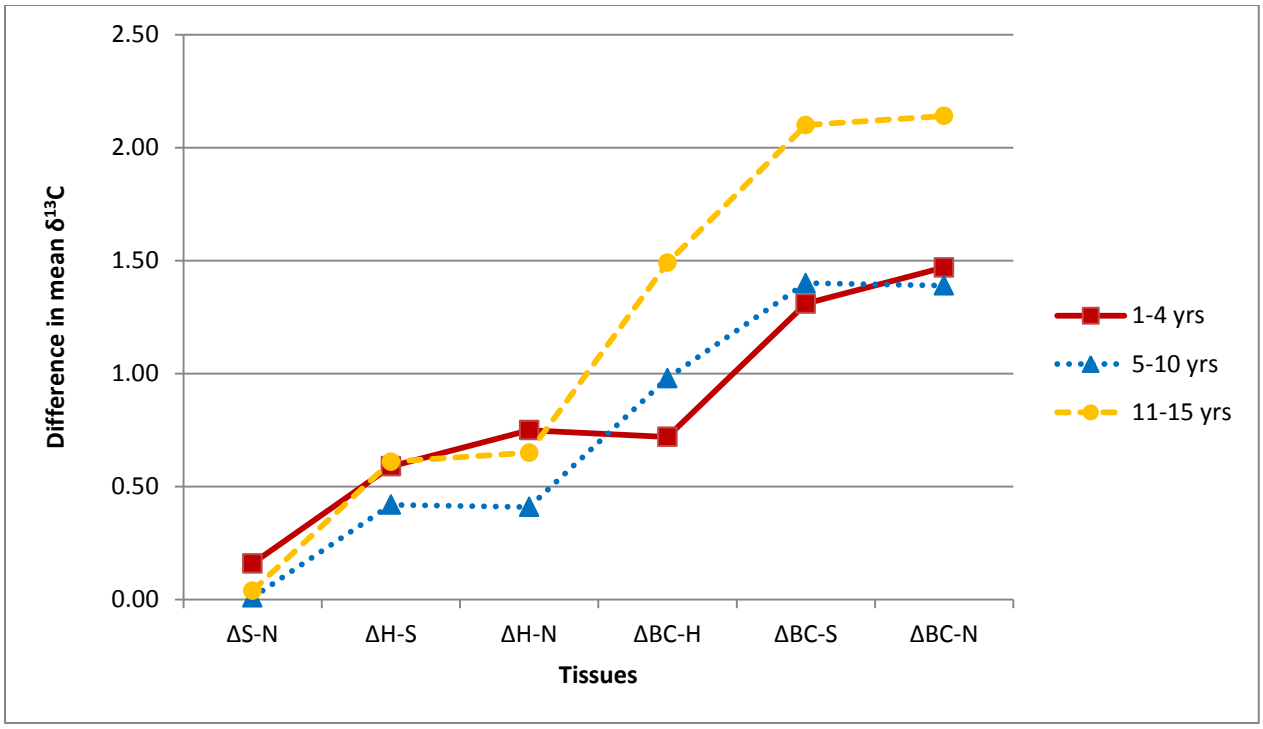

Figure 10: Inter-tissue spacing of mean $\delta^{13}C$ (‰) values in K2 juveniles

The preceding analyses utilize bulk values for all tissues. However, the hair samples were collected and analyzed in 1 cm segments, and for the nail samples, proximal and distal ends were analyzed separately. The first centimeter of hair and the proximal ends of the nails represent a period of time closest to the individual's death (i.e. around 1 month), and may reflect changes related to any conditions which precipitated death, such as illness or malnutrition. As these differences may be reflected in the inter-tissue spacing, a second graph was created using 1 cm hair values and proximal nail values in place of bulk hair and bulk nail values (Figure 11). These distance values are shown alongside those utilizing the bulk data in Table 12. Very little difference can be observed between this graph and that in Figure 10, nor in the numerical values shown in Table 12.

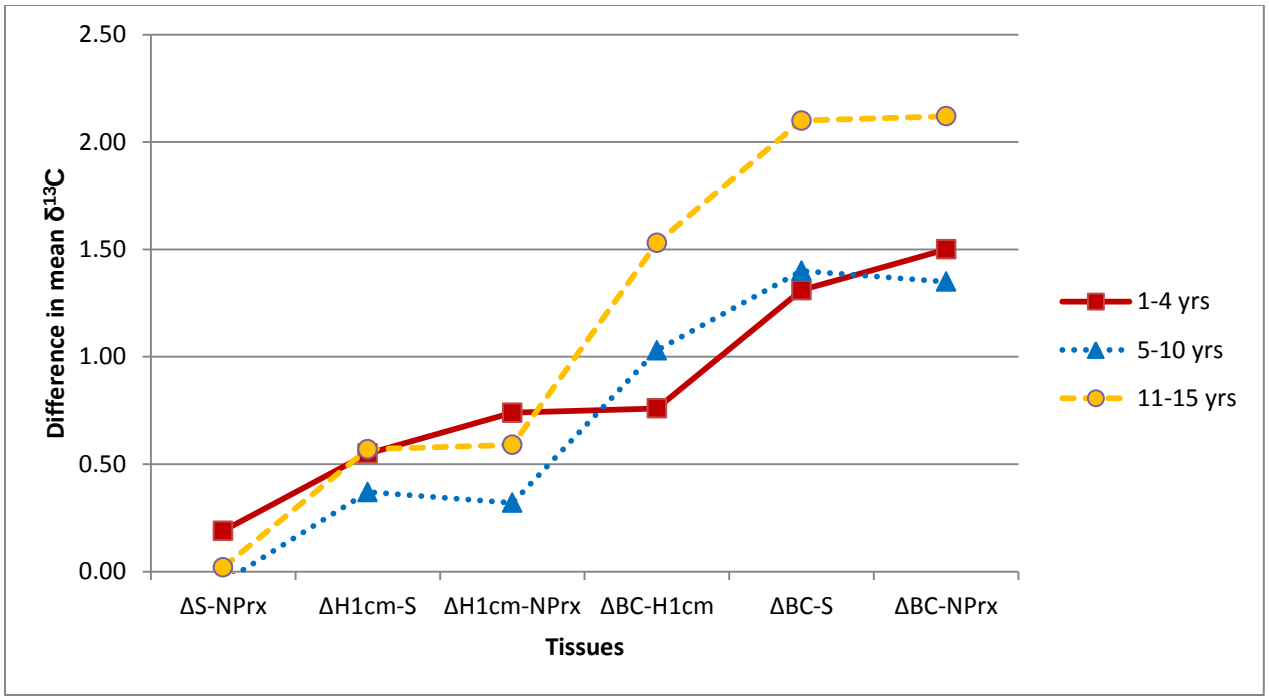

Figure 11: Inter-tissue spacing of mean $\delta^{13}C$ (‰) values (1st cm hair and proximal end of nail)

Table 12: Inter-tissue spacing of all mean $\delta^{13}C$ (‰) values

Tissues	All	1-4 yrs	5-10 yrs	11-15 yrs
Δ_{S-N}	0.06 ± 0.18	0.16 ± 0.21	0.01 ± 0.43	0.04 ± 0.47
Δ_{S-NPrx}	0.07 ± 0.20	0.19 ± 0.25	0.05 ± 0.46	0.02 ± 0.45
Δ_{H-S}	0.52 ± 0.17	0.59 ± 0.19	0.42 ± 0.41	0.61 ± 0.42
Δ_{H1cm-S}	0.50 ± 0.18	0.55 ± 0.22	0.37 ± 0.42	0.57 ± 0.43
Δ_{H-N}	0.58 ± 0.10	0.75 ± 0.14	0.41 ± 0.14	0.65 ± 0.26
$\Delta_{H1cm-NPrx}$	0.57 ± 0.15	0.74 ± 0.22	0.32 ± 0.22	0.59 ± 0.25
Δ_{BC-H}	0.94 ± 0.14	0.72 ± 0.29	0.98 ± 0.11	1.49 ± 0.44
$\Delta_{BC-H1cm}$	0.96 ± 0.16	0.76 ± 0.31	1.03 ± 0.12	1.53 ± 0.45
Δ_{BC-S}	1.46 ± 0.21	1.31 ± 0.33	1.40 ± 0.42	2.10 ± 0.59
Δ_{BC-N}	1.52 ± 0.16	1.47 ± 0.30	1.39 ± 0.16	2.14 ± 0.49
$\Delta_{BC-NPrx}$	1.53 ± 0.18	1.5 ± 0.33	1.35 ± 0.23	2.12 ± 0.47

CHAPTER FIVE: DISCUSSION

There are a number of factors affecting inter-tissue spacing of isotopic values, including differential fractionation effects from the diet (e.g. Krueger and Sullivan, 1984; Ambrose and Norr, 1993), the protein structure of the individual tissues (e.g. O'Connell et al., 2001), and the different turnover rates of the tissues, especially as they relate to dietary seasonality (e.g. Williams et al., 2011). Bone collagen has a slower turnover rate than the other tissues under consideration, and can reflect an average of several years' worth of dietary information, as opposed to hair, nails or skin, which reflects a period of only a few months or weeks (White and Schwarcz, 1994; O'Connell and Hedges, 1999; Williams et al., 2011). This can have a significant effect when diet varies seasonally, as previously shown in the Kellis 2 population from the Dakhleh Oasis (Dupras, 1999; Williams, 2008). In addition, it must be remembered that these short-term tissues represent the individual's last few months of life, and may well have been a time of great stress, either due to disease, malnutrition, or some other contributing factor, and the isotopic values derived from these tissues may reflect dietary changes related to the individual's metabolic condition (Williams, 2008). Another cause of inter-tissue isotopic spacing is in the variability of the basic amino-acid make-up of the proteins and differential fractionation of carbon isotopes in each of these tissues (Corr et al., 2009; Raghavan et al., 2010). Age has rarely been examined in detail as a source of variation in tissue spacing, especially among juveniles, but in this sample the evident disparity between the 11-15 year olds and the two younger age cohorts warrants some discussion. First, however, a comparison of the differences among tissue spacing trends in this population and other studies should be addressed.

Inter-tissue Spacing

The results of this study show a general trend of mean $\delta^{13}C$ values being arranged in this order, starting with the tissue of highest enrichment: bone collagen > bulk hair > skin = bulk nail (see Table 13, next section). There was some variation between the different age categories as to whether skin was more enriched than nail or *vice versa*, but the difference was not determined to be statistically significant at the 0.05 significance level using a paired t-test. The major constituents examined for each tissue are structural proteins (collagen for bone and skin, and keratin for hair and nail), which isotopically represent the protein content of diet, at least primarily (Tieszen and Fagre, 1993).

While short-term variation in diet can account for some differences between tissues with differing turnover rates, several studies have revealed that even when diet is controlled and unchanging, isotopic values show an inherent offset between tissues (e.g. Tieszen et al., 1983; Hare et al., 1991). Tieszen and colleagues (1983) found that hair was consistently more enriched than more metabolically active tissues such as muscle and liver. Bone collagen is likewise found in numerous studies to be more enriched than tissues like hair, skin and nail (e.g. O'Connell et al., 2001; Williams, 2005; Corr et al., 2009; Raghavan et al., 2010) as in the results given here. Given these trends it is difficult to explain why nail values should be as depleted as skin, given that nail grows more slowly than hair, and is not as metabolically active as skin (Tallitsch et al., 2006; Scanlon and Sanders, 2007), although see results are broadly similar to the few other studies that included nail (see Table 14, next section). O'Connell and colleagues (2001) found hair to be slightly more enriched than nail, as did Fraser and colleagues (2006) and Williams (2005).

Although the similarity between hair and nail is accountable, owing to the fact that both are composed of keratin, the similarity between nail and skin is less explicable, since skin is primarily Type I collagen, like in bone. However, differences in sampling technique may be at work here, as the Kellis 2 samples were taken primarily from the cranium, specifically the face (L. Williams, personal communication), where the dermis (the collagen component) is thinner, and therefore a higher percentage may represent epidermis, which is primarily composed of keratin (Tallitsch et al., 2006; Scanlon and Sanders, 2007). Therefore, the similarity between mean nail and skin values seen here may be a byproduct of this. In addition, the hyper arid desert environment in the Dakhleh Oasis may be a factor, as skin is the organ most exposed to the environment and acts as the body's primary barrier against it (Tallitsch et al., 2006; Scanlon and Sanders, 2007).

These tissues vary in their amino acid composition, which also helps account for some of the intrinsic variation between tissues. O'Connell and colleagues (2001) and Raghavan and colleagues (2010) cited this as the primary cause of disparity between bone collagen and hair, while Corr and colleagues (2009) found a similar result when comparing bone collagen to skin.

Cohort Variation

The obvious cohort outlier as far as inter-tissue spacing is concerned is the 11-15 year age cohort, and these individuals will be discussed in detail below. There are, however, other differences between age cohorts which should also be discussed. One would expect to see more variation in diet in the 1-4 year olds than in either of the others, because the individuals in this category would have been at various stages of the weaning process; indeed, the standard deviation of all tissues is consistently over 0.6‰. Standard deviation is also generally high in the

11-15 year age cohort (0.58‰ or greater), while the 5-10 year age cohort has lower values for nail, hair, and bone collagen (0.55‰, 0.39‰, and 0.22‰, respectively). This could be related to growth, as it has been shown in this population that the most pronounced childhood growth spurts occur between birth to five years and again after the age of ten (Wheeler, 2009).

Bone collagen typically has the least variation within each age cohort, which is not unexpected given that bone collagen represents an average of several years' worth of dietary information. Skin, which is arguably the most metabolically active of the soft tissues, consistently shows the highest standard deviation. This is lower in the 1-4 year age category than it is for either of the others, perhaps indicating more short-term variation in diet and lifestyle among the older children. This will be discussed in more detail below, as it relates to the 11-15 year age cohort.

Comparisons

To Other Studies

In certain respects, the results of this study are somewhat at odds with other bioarchaeological projects in which multiple soft tissues were analyzed, although the results are broadly similar to other studies. Very few studies have employed the full set of tissues used here, which makes contextualizing comparisons difficult. However, all four tissues are considered in Jocelyn Williams' 2005 dissertation, so this makes for the most useful comparison. A comparison of her results and those of this study can be found in Table 13, while results from these and other studies are compared in Table 14.

Table 13: Comparison of $\delta^{13}C$ (‰) values from present study to those from Williams (2005)

Tissue	Present Study	Williams, 2005: Juveniles (1-15 yrs)	Williams, 2005: Total Population
Bone Collagen	-18.57 ± 0.46	-10.95 ± 1.21	-11.0 ± 1.1
Hair	-19.51 ± 0.66	-11.97 ± 1.32	-12.9 ± 1.5
Skin	-20.03 ± 0.99	-11.33 ± 1.19	-11.3 ± 1.2
Nail	-20.09 ± 0.64	-13.2 ± 2.25	-13.0 ± 1.4

The results of this study differ from Williams' (2005) in many respects. Results from Williams' Peruvian sample (71% adult, 29% juvenile) indicated that the mean $\delta^{13}C$ values, ranked from most to least enriched, were: bone collagen > skin > hair > nail (2005). This trend is seen in the means for her total population, and in the data from juveniles between the ages of 1-15 years. Interestingly, the distance between hair and nail in Williams (2005) was much greater for the juveniles between 1-15 years than for the population as a whole (1.23 ± 1.4 versus 0.4 ± 1.0‰), while the difference between hair and skin was smaller (0.65 ± 0.6 versus 1.6 ± 1.9), owing to the fact that the mean hair value for the juveniles is more enriched than that of the whole population. The hair and nail distance for the total population was similar to the overall mean presented in this study (0.58 ± 0.65‰) and others (see Table 14). The difference between skin and bone collagen for her juveniles is considerably smaller (0.38 ± 0.6‰ versus 1.46 ± 0.73‰), resulting in the difference in ranking recorded above.

There are several other studies in which bone collagen was found to be more similar to skin, which, like bone, has Type I collagen as its primary constituent (e.g. Vogel, 1978; White and Schwarcz, 1994; Finucane, 2007). These (excluding White and Schwarcz, 1994), can be seen in Table 14. Note that the mean differences from the studies listed here either include both adult

and juvenile data bulked together, or adult data only. The differences between bone and skin values between this and other studies are intriguing. One possible explanation, as mentioned above, is that variation in skin sampling techniques may have an effect; Williams (2005) suggests that variation in the isotopic values of her skin samples may have resulted from variations in the thickness of the epidermis at sampling locations. Williams (2008) took skin samples primarily from the face, where there is less collagen (L. Williams, personal communication).

The difference between skin and nail in this study is small enough to be statistically insignificant, which also lends support to the hypothesis that there may be a higher percentage of keratin in the Kellis 2 skin samples. Unfortunately, few studies compare skin to nail, or include nail at all, so it is difficult to make further comparisons. Other variation between the results presented here and those other studies are likely caused by the significant differences in diet between populations, and even their respective environments.

Table 14: Comparison of δ1^{3}C inter-tissue spacing between this study and others

Study	Δ_{BC-H}	Δ_{BC-N}	Δ_{BC-S}	Δ_{H-N}	Δ_{H-S}	Δ_{S-N}
Present Study	0.94 ± 0.6	1.52 ± 0.6	1.46 ± 0.7	0.58 ± 0.7	0.52 ± 0.8	0.06 ± 0.8
Tieszen and Fagre, 1993	2.9 ± 0.6*					
Vogel, 1978			0.6			
O'Connell et al., 2001	1.41 ± 0.5	**		0.21 ± .4		
Fraser et al., 2006				0.55 ± 0.8*		
White, 1991	0.98		1.05		2.03	
White et al., 1999					-0.8	
Williams, 2005 (All)	1.9 ± 1.4	2.0 ± 1.1	0.2 ± 1.0	0.4 ± 1.0	1.6 ± 1.9*	1.7 ± 1.8*
Williams, 2005 (1-15 Years)	1.03 ± 0.5*	2.25 ± 1.4*	0.38 ± 0.6*	1.23 ± 1.4*	0.65 ± 0.6*	1.88 ± 1.4*
Finucane, 2007			0.3 ± 1.84			
Knudson et al., 2007	1.7 ± 1.6 / 1.0 ± 0.8†					
Raghavan et al., 2010	2.0 ± 0.2*					

Note: All values represent adult or combined adult/juvenile data unless otherwise noted
*Calculated for present study
**Bone-Hair and Hair-Nail values represent two separate study groups, so Bone-Nail was not calculated
†For mean of first segment of hair (0-2 cm) and sixth segment (10-12 cm) respectively

Comparison with the Dakhleh Adult Population

The results of the earlier studies from which the data in this project derives (Dupras, 1999; Williams, 2008), show the same overarching trend in how the mean values line up by enrichment factor: bone >hair > skin = nail. Table 15 shows the mean $\delta^{13}C$ values for the total population and the adult population for all tissue types, with the results of this study for comparison. Table 16 and Figure 12 show the differences between tissues, adding in the mean of

the values for adults for comparison with those of the juveniles. The obvious outliers are in the 11-15 year age cohort, where the differences between bone collagen and skin, bone collagen and nails, and bone collagen and hair, are greater than those for any of the other age cohorts. Likewise, the distance between bone collagen and hair is also greater in this category, although the difference is not quite so pronounced. The 11-15 year olds differ not only from the rest of the juvenile sample, as seen above, but also from the adult members of the population.

Table 15: Mean $\delta^{13}C$ (‰) values compared between juveniles, adults, and total population

Age	Hair	Nail	Skin	Bone Collagen
1- 4 Years	-19.29	-20.04	-19.88	-18.57
5-10 Years	-19.68	-20.09	-20.10	-18.70
11-15 Years	-19.77	-20.42	-20.38	-18.28
Adults (16-60+)	-19.45	-20.18	-20.19	-18.88
Total Population	-19.30	-20.00	-19.80	-18.80

Table 16: Inter-tissue spacing of mean $\delta^{13}C$ values: juveniles and adults

Tissues	1-4 yrs	5-10 yrs	11-15 yrs	Adult
Δ_{S-N}	0.16 ± 0.21	0.01 ± 0.43	0.04 ± 0.47	0.01 ± 0.22
Δ_{H-S}	0.59 ± 0.19	0.42 ± 0.41	0.61 ± 0.42	0.73 ± 0.32
Δ_{H-N}	0.75 ± 0.14	0.41 ± 0.14	0.65 ± 0.26	0.73 ± 0.18
Δ_{BC-H}	0.72 ± 0.29	0.98 ± 0.11	1.49 ± 0.44	0.57 ± 0.07
Δ_{BC-S}	1.31 ± 0.33	1.40 ± 0.42	2.10 ± 0.59	1.30 ± 0.14
Δ_{BC-N}	1.47 ± 0.30	1.39 ± 0.16	2.14 ± 0.49	1.30 ± 0.18

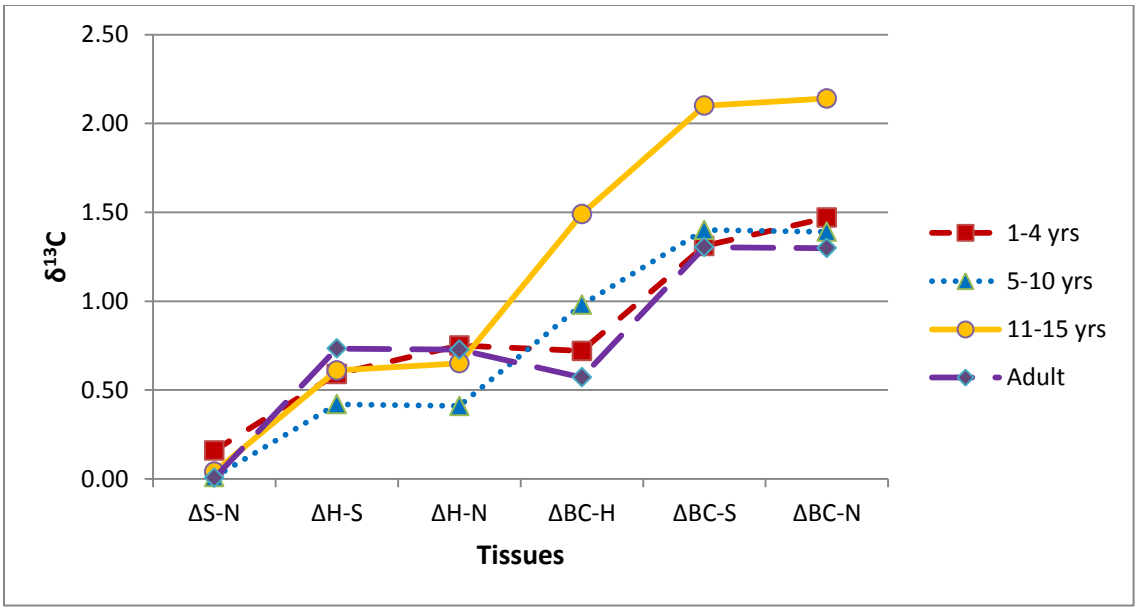

Figure 12: Inter-tissue spacing of mean $\delta^{13}C$ (‰) values: juveniles and adults

11-15 Year Cohort: Adolescence in the Dakhleh Oasis

Why should this group of individuals show such a marked disparity from others in the population? One potentially significant difficulty with these analyses is variation in sample size between tissues and age cohorts, particularly the comparatively small number of bone collagen samples. This is especially unfortunate in the 11-15 year age cohort, in which only two of the individuals have bone collagen values. Ideally, a larger sample size would produce more robust results, and allow for the calculation of individual isotope distance measurements. In addition, this study does not account for sex, owing to the difficulty in skeletal determination of sex in juveniles, and it is conceivable that turnover/growth rates of some of the tissues under consideration may be variable by sex (nail is, as discussed above).

In spite of the relative paucity of 11-15 year olds in this study, this may still qualify as a fair representation of 11-15 year olds from the site as a whole. In fact, the 10 individuals in the

11-15 year old sample represent over half of the total number of 11-15 year old individuals recovered from Kellis 2, as opposed to the 28 individuals in the youngest cohort, who represent only a third of 1-4 year olds recovered from Kellis 2 (Williams, 2008). There are very few 11-15 year olds in the skeletal population as a whole, as this is not an age-group prone to high mortality rates. These were, after all, the individuals who proved themselves robust enough to live past childhood, particularly the critical period prior to the age of about five (Wheeler, 2009). They were also just transitioning into adulthood, although still less likely to be exposed to certain adult risks, such as death in childbirth, as marriages more typically took place in the late teens for women (Bagnall, 1996). However, Wheeler (2009) found a higher incidence of fractures in this age cohort, and suggested that they may represent the increasing incorporation of this age group into the workforce.

It should be noted that the individuals in this cohort (and indeed, all age cohorts) cannot be considered representative of the individuals of this age in the living population. They are representative of the non-survivors only, and given the scarcity of remains in this age group, it would seem that, in general, individuals who reached this age were likely to survive it. Individuals in this age cohort have a high number of non-specific stress markers (e.g. *cribra orbitalia*, enamel hypoplasia, etc.), particularly healed rather than active lesions. Although this is indicative of periods of ill-health or stressful episodes, it could paradoxically indicate general robusticity, as these are episodes which the individuals managed to survive (Wood et al., 1992). One must keep this in mind while examining the potential reasons for the observed differences between this and the other cohorts.

One potential source of the variation could be dietary. Previous studies have not focused on this age group nor have they reflected a change in diet when compared to the 5-10 year olds or the adults. Indeed, the only focus on dietary difference within the population, as it relates to age, occurs within the 1-4 year age cohort, with the weaning process beginning at approximately 6 months and continuing until the age of about 3.5 years (Dupras et al., 2001). Therefore it is not likely that this pattern would have previously been recognized.

The combination of the physiological stressors of puberty and the transition to adulthood may contribute to the inter-tissue spacing difference observed here. It is unfortunate that the potential effects of the physiological changes of puberty on $\delta^{13}C$ values in these tissues do not appear to have been explored in the literature. One can consider growth and turnover rates more generally, however, and it is significant to note that this age group corresponds with the onset of the adolescent growth spurt in this population (Wheeler, 2009).

It is difficult to find a precise estimate for turnover times and growth rates for these soft tissues in juveniles. It is generally acknowledged that fingernail growth rate decreases sharply with age among juveniles, and then levels off as an individual reaches adulthood (Beaven and Brooks, 1994). Generally, in isotope studies, an estimate of 4-6 months is given as the time period represented by a fingernail (e.g. O'Connell et al., 2001), but in most juveniles this is certainly an overestimate (Hamilton et al., 1955; Beaven and Brooks, 1994). Based on the clinical literature, a better estimate for young adults is on the order of 3-4 months, but this may be somewhat less for young teenagers, and the source does not define the age bracket for the category 'young adult' (Beaven and Brooks, 1994). Accurate skin turnover rates are even more difficult to estimate, especially for juveniles, although isotopic studies often give an estimate of

2-4 months (e.g. White and Schwarcz, 1994). This too may be an overestimate, as some of the clinical literature suggests it may be closer to 1-2 months, if skin is synthesized at a rate of approximately 2% per day (El-Harake et al. (1998). This is certainly true in the epidermis, where newly formed cells move from the innermost basal layer, the stratum germinativum, to the exterior layer, the stratum corneum, in 15-30 days and then remain in an inert state for approximately two weeks before being shed (Tallitsch et al., 2006).

The skin and nail isotopic values in these individuals nutritionally represent a brief period of time respectively on the order of 1-2 and approximately 3 months prior to death. This is in contrast to the bone collagen values, which represent a number of years (Pate, 1994). Hedges and colleagues (2007) found that in adolescents of both sexes between the ages of 10-15, the collagen turnover rate is higher, 10-30% per year, versus 1.5-4% per year in adults. This disparity in turnover rates may be related to the relative enrichment of $\delta^{13}C$ observed in the 11-15 year olds in this study, although without comparative turnover rates in the younger age groups this cannot be confirmed as the primary reason for this difference. Wheeler (2009) examined growth in detail among the juveniles of the Dakhleh Oasis, and found that the two greatest increases in long bone length occurred in the first five years of life, and again starting around the age of 10, something she identifies as likely correlating with the adolescent growth spurt. In this sample, the bone collagen values for both the 1-4 year cohort and the 11-15 year cohort are enriched compared to the 5-10 year cohort, and both have a higher standard deviation, which lends support to the hypothesis that the bone collagen enrichment may be correlated with childhood growth spurts.

The reason for the increased difference between bone collagen and nail or skin for the 11-15 year old cohort is that their nail and skin values are depleted compared to other age groups, while the bone collagen mean $\delta^{13}C$ value is enriched compared to other age groups. Hair values are likewise depleted, though not to the same extent. In an individual an enrichment in $\delta^{13}C$ values can indicate an increased protein intake, and, as a corollary, a depletion can be indicative of nutritional stress (Mekota et al., 2006). The slight depletion of short-term $\delta^{13}C$ values for this age group, representing the last few months of their lives, could indicate a period of nutritional stress. The stress undergone by this group in comparison to the others may be correlated with the physiological changes of puberty and the social changes of a transition to adulthood. An examination of the segmented hair data reveals that the mean $\delta^{13}C$ for the 0-1 cm samples (representing the last month prior to death) is -19.81 ± 0.51‰, while the mean for the rest of the hair segments (1cm+) is -19.76 ± 0.79‰. This decrease, though present, is very slight. Looked at individually, it appears that while a slight decrease is evident in some individuals' hair segments (e.g. #260, #239), it is not present in others (e.g. #243, #302). Some of the individuals displaying this decrease also have active cribra orbitalia lesions (e.g. #260, #239), which could support the hypothesis of a stress episode at the time of death (Wheeler, 2009). However, the explanation of nutritional or other physiological stress would not be satisfactory for all individuals in any case, since it is very likely that many deaths in this age cohort are accidental rather than caused by disease or malnourishment. Wheeler (2009) found that out of all the juvenile age cohorts in Kellis 2, 10-15 year olds had the highest incidence of trauma. Indeed, several of the individuals in this sample have multiple fractures (Wheeler 2009), and although none are indicated to be perimortem, it does point to a general prevalence of hazards to these adolescents' physical safety.

Overall, although numerous explanations have been considered, it is evident that none is entirely satisfactory, and multiple factors are likely to be at work. Further studies into the lifestyles of young teenagers in this time period and location may help to shed light on these results. Furthermore, isotopic research with an aim at understanding the ramifications of adolescent growth and development will undoubtedly be necessary for a more refined understanding of this topic.

CHAPTER SIX: CONCLUSION

Although stable isotope analysis has been an active area of research for over three decades in bioarchaeology, it continues to expand, in use and in scope. As it does, our understanding of the minutia of the biochemistry of the human body and its applications in the study of ancient lives continues to grow. Inter-tissue spacing of isotopic values is one area where, although great strides have been made, some of the mechanisms and variables are still obscure.

The focus of this thesis is an examination of age as a factor in inter-tissue spacing, utilizing bone collagen, hair, skin and fingernails from 52 juvenile remains from the Dakhleh Oasis, Egypt. Such a collection is only rarely available for such a study, owing to the relative paucity of well-preserved juvenile remains in an archaeological context. The individuals in the sample were divided into three age groups, and one, the 11-15 year-olds, differed considerably from the other age groups, including adults in the population, in the distance between bone collagen and skin, bone collagen and nail, and bone collagen and hair. The reasons for this are not fully understood, although it is hypothesized that it relates to changing social and physiological circumstances experienced by these individuals at this important transitional phase in their lives. It is evident that numerous factors must be taken into account in considering this question, and finding a definitive conclusion is unlikely given the limitations of both the data and the background literature.

The first limitation to consider is that of sample size, as the outliers in this study also represent the smallest subset of the population. A second limitation is the paucity of historical data regarding this age group, which could be compared to the skeletal and isotopic data. There

are also limitations regarding our current understanding of the relationship between age, growth and development in humans and stable isotope value. Tissue turnover rates in juveniles are also poorly understood. Finally, although bone and to a lesser extent hair are utilized in a number of stable isotope studies, there is also a relative scarcity of studies which utilize nail and skin. A better understanding of skin in stable isotope analysis would help make sense of some of the results, and perhaps lead to better standardization of sampling techniques.

The results indicate that further studies into the effects of age on inter-tissue spacing would be well-advised to better understand the complex intersection of the social, cultural and biological factors which affect the biochemistry of human tissues. More controlled studies utilizing nail and skin in comparison with other tissues would be particularly useful for furthering our understanding of the mechanisms of inter-tissue spacing. More studies involving juveniles are necessary in order to clarify the effects of age and childhood growth on inter-tissue spacing, and isotopic values more generally. In particular, it would be useful to see if similar results for 11-15 year olds would be found in other populations. This could help clarify the question of whether the sociocultural or the physiological factors are of greater influence.

This study has shown evidence that age may have a considerable effect on isotopic spacing between human tissues, and therefore it is crucial that age be taken into account as a factor in studies considering inter-tissue spacing, and isotopic studies in general, especially as our understanding of the subject is currently somewhat lacking. It is not immediately evident what the specific effects of growth and development are on inter-tissue spacing of isotopic values, or to what extent these intrinsic factors are affected by extrinsic social and environmental ones. Further studies are necessary to clarify these issues. However, the basic matter of

importance shown here is that age should definitely be considered as a factor in inter-tissue spacing in stable isotope analysis.

APPENDIX: RAW DATA

Table 17: Hair $\delta^{13}C$ Values for All Age Categories

Burial ID	Age	0-1cm	1-2cm	2-3cm	3-4cm	4-5cm	5-6cm	6-7cm	7-8cm	8-9cm	9-10cm	10-11cm	11-12cm	12-13cm
23	1.5	-21.15	-19.83											
70	1.5	-18.83	-18.55											
71	2	-18.26	-18.09	-17.93	-18.24	-18.92	-19.04							
86	4	-19.80	-19.66											
108	1.5	-19.20												
299	1.25	-19.23	-19.03	-18.74	-18.51	-18.41	-18.57	-18.73	-18.93	-19.29				
323	1.5	-19.54												
328	1.25	-18.78	-18.92	-19.19	-19.87	-20.28	-18.88	-19.16	-19.34	-18.98				
330	4	-17.94	-18.58	-19.35	-19.61	-19.75								
349	4	-19.40												
351	4	-19.67												
357	2	-18.88												
358	2.5	-19.36	-19.64	-19.90										
362	3	-19.75	-19.60											
396	2	-18.98	-19.26	-19.46	-19.70	-19.52	-18.88	-18.38						
487	2	-17.73	-18.14	-18.91	-19.35	-19.09								
490	1.5	-19.19	-19.95											
519	2.5	-19.70	-20.45	-21.18	-20.96	-19.84	-19.66							
534	1.5	-18.91	-19.15	-19.01	-18.97	-18.92	-18.87	-18.82	-18.96					
560	1.75	-19.00												
562	1.5	-20.31	-19.97											
579	1.5	-19.65	-19.15	-18.90	-18.87	-18.70	-18.91							
583B	2.5	-19.78	-19.31	-19.15	-19.42	-19.52	-19.62	-19.41						
593A	1.5	-18.95	-19.12	-20.37	-19.05									
619	2	-20.88												
620	2	-19.39	-20.38	-19.91	-20.65									

Burial ID	Age	0-1cm	1-2cm	2-3cm	3-4cm	4-5cm	5-6cm	6-7cm	7-8cm	8-9cm	9-10cm	10-11cm	11-12cm	12-13cm
624	1.5	-19.41	-19.42	-19.63	-19.62									
628	1.5	-19.53												
24	7	-19.58	-19.49	-19.60										
49	7	-19.50	-19.61	-19.67	-19.77	-19.88	-19.83	-19.95	-20.03	-19.99	-19.68			
64	6	-19.66	-19.63											
97	6	-19.58	-19.56	-19.28	-18.96	-19.27	-19.14							
163	5	-20.23												
258	10	-19.65												
336	5	-19.92	-19.88	-20.08										
360	5	-19.58	-19.20	-19.09	-19.29									
370	5	-19.83												
374	7	-19.17	-19.06	-19.03	-18.86	-19.58	-19.60	-19.78	-20.02					
520	8	-20.18	-20.21	-20.39										
582	10	-19.70												
583A	7	-19.70	-19.60											
584	5	-20.00	-20.43	-20.16	-20.44									
149	12	-20.13	-20.16	-19.98	-19.77									
173	12	-19.73												
239	15	-19.82	-19.29	-19.11	-19.20									
243	15	-19.21	-19.25	-19.46	-19.60									
260	15	-21.08	-20.44	-20.12										
263	13	-19.51												
288	15	-19.60	-19.43	-19.68	-20.12	-24.33	-20.01	-19.89	-19.87	-19.65	-19.67	-19.36	-19.39	-19.70
295	11	-19.53	-19.31	-19.31	-19.32	-19.38	-19.54	-19.70	-19.72					
302	12	-19.67	-19.83	-19.64										
468	13	-19.80	-19.67	-19.65	-19.67	-19.56	-19.64	-19.54	-19.44	-19.86	-19.85			

Table 18: Nail, Skin and Bone Collagen $\delta^{13}C$ Values for All Age Categories

Burial ID	Age	Nail Proximal	Nail Distal	Skin Bulk	Bone Collagen
23	1.5	-20.36	-20.04	-20.25	
70	1.5	-19.51	-18.98	-19.61	-18.86
71	2				-17.58
86	4	-20.38	-20.12		-18.71
108	1.5	-19.01		-19.49	-18.44
299	1.25	-19.61	-19.37	-21.74	
323	1.5			-18.95	
328	1.25	-19.49	-18.92	-19.11	
330	4			-18.57	
349	4	-20.17	-20.76	-20.33	
351	4			-20.18	
357	2	-19.72		-20.35	
358	2.5	-20.24	-20.21	-18.98	
362	3			-20.89	
396	2	-22.45			
487	2			-20.22	
490	1.5			-20.19	
519	2.5	-19.89	-19.92	-19.14	-19.25
534	1.5				
560	1.75	-19.70		-19.43	
562	1.5			-20.88	
579	1.5			-18.91	
583B	2.5	-20.04	-19.81		
593A	1.5	-20.50	-20.66		
619	2	-20.30	-20.34	-20.14	
620	2	-20.03	-19.77		
624	1.5	-19.26	-19.79	-19.67	
628	1.5	-20.66	-21.16	-20.54	
24	7	-19.92	-19.16	-20.56	-18.41
49	7				-18.70
64	6	-19.93	-19.90	-20.03	-19.01
97	6	-18.62			-18.62
163	5			-19.21	
258	10			-19.56	

Burial ID	Age	Nail Proximal	Nail Distal	Skin Bulk	Bone Collagen
336	5			-22.19	
360	5			-18.82	
370	5	-20.09	-20.33	-19.31	-18.74
374	7	-20.59	-20.50	-18.80	
520	8	-20.72	-20.67	-20.10	
582	10	-19.86	-20.04		
583A	7	-20.14	-20.01	-22.44	
584	5	-20.55	-20.51		
149	12			-21.02	-18.70
173	12				-17.85
239	15	-20.79	-21.29	-20.46	
243	15				
260	15			-20.29	
263	13			-19.84	
288	15	-20.17	-20.41	-21.99	
295	11			-20.56	
302	12				
468	13	-20.23	-19.60	-18.51	

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