STABLE ISOTOPE ANALYSIS OF HAIR FROM CHRISTIAN PERIOD KULUBNARTI IN SUDANESE NUBIA

by

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Stable Isotope Analysis of Hair from Christian Period Kulubnarti in Sudanese Nubia

Thesis directed by Associate Professor Matt Sponheimer

Stable isotope analysis of hair was used to investigate the dietary patterns of individuals from the Christian period Sudanese Nubian site of Kulubnarti, taking into consideration established patterns of health. Over three decades of research has shown consistent differences between two cemetery populations, believed to represent diachronic periods of use. In this study, carbon and nitrogen isotope ratios were analyzed to reconstruct aspects of diet, while oxygen and hydrogen isotope ratios were analyzed to evaluate the belief that S cemetery dates to the Early Christian period (A.D. 600-850), while the R cemetery dates to the Late Christian period (A.D. 1100-1400). These isotopic data suggest that diets were very similar between the two populations, and therefore did not directly cause the observed differential patterns of stress. Oxygen and hydrogen isotopic data supports the diachronicity of the two cemeteries, an idea which has been called into question by a recent textile analysis.

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CHAPTER ONE

INTRODUCTION

STATEMENT OF PURPOSE

Since 1979, the individuals from two Nubian cemeteries at the ancient site of Kulubnarti have been intensely studied by the excavator, Dr. Dennis Van Gerven of the University of Colorado Boulder, as well as numerous graduate students and colleagues (see Turner et al., 2007; Sandberg, 2006; Van Gerven and Greene, 1999; Sheridan and Van Gerven, 1997; Albert, 1995; Mittler and Van Gerven, 1994; Mittler et al., 1992; Sheridan, 1992; Van Gerven et al., 1990; Cummings, 1989; Burrell, 1988; Van Gerven et al., 1985; Sanford, 1984; Hummert, 1983; Sanford et al., 1983; Van Gerven et al., 1981; Carlson and Van Gerven, 1979). Though a great deal is known from three decades of research, debate continues regarding the temporal relationship between cemeteries 21-S-46 and 21-R-2 (Adams, 1999b). From osteological studies, it is known that individuals of the S cemetery, believed to date to the Early Christian period, A.D. 600-850, were exposed to more stress relative to those from the R cemetery, believed to date to the Late Christian period, A.D. 1100-1400 (Van Gerven et al., 1995). This stress can be inferred from the observation of dental pathologies such as carious lesions, enamel hypoplasias, abscesses, and tooth loss, as well as cribra orbitalia, skeletal growth retardation in childhood, and generalized bone loss (Van Gerven and Greene, 1999). The exact cause of the differential patterns of stress that occurred in the S and R populations is unknown, but may be due to nutritional deficiencies, disease susceptibility, or parasitic infections. Given the ability to quantitatively compare dietary information from the two cemeteries using stable isotope analysis, this investigation is an essential addition to the Kulubnarti research program, the primary goal of which is to determine the role of diet in producing the observed patterns of stress. Although stable isotope analysis has been utilized in previous studies with the Kulubnarti collection (see Turner et al., 2007; Sandberg, 2006), the analysis of hair has not yet been conducted and thus offers a new approach to evaluating differences between the two cemetery populations.

Carbon, nitrogen, oxygen, and hydrogen isotopes were analyzed and patterns of variation were evaluated by cemetery, age, and sex. While carbon and nitrogen isotopes are used to infer dietary information, oxygen and hydrogen isotopes are of particular interest in assessing the diachronicity of the two cemeteries, an ancillary focus of this thesis. Samples were collected from the two well-preserved populations housed in the Department of Anthropology at the University of Colorado Boulder during the summer of 2008.

The following section is intended to provide readers with a clear understanding of the geography and historical context of Nubia during the Medieval Christian period. A thorough discussion of earlier and later cultural horizons will not be presented in this thesis, though it can be found elsewhere (see Edwards, 2004; Adams, 1977; Armelagos, 1968).

NUBIA

Inescapably bound to Egypt, yet possessing its own unique history, Nubia has been "the land between" for thousands of years (Adams, 1977). As inhospitable and remote as it was, the Nile Valley between Aswan and Khartoum provided a dependable route across the Saharan Desert which allowed for the movement of peoples, ideas, and goods. Until the European Age of Exploration opened Africa's coasts in the seventeenth century, the Nubian Corridor was nearly the only point of contact between Egypt and the rest of Africa.

Geographically, Nubia has always been defined by the Nile Cataracts, or shallow areas where boulders protrude from the riverbed. The First and Fourth Cataracts, located at Aswan (now part of Egypt) and Napata, respectively, were the generally accepted boundaries of Nubia in antiquity. The region between the First Cataract and Second Cataract at Wadi Halfa was called Lower Nubia while the land upriver stretching to the Fourth Cataract was called Upper Nubia (see Figure 1.1). In the latter half of the 20th century, dam construction projects elevated river levels, therefore complicating the traditional cataract classification (Van Gerven et al. 1995: 468). However, these projects, chief among them the construction of the high dam at Aswan, were the impetus that brought about the salvage archaeological excavations carried out upriver, including those at Kulubnarti, located between the Second and Third Cataracts, by Van Gerven in 1979. Today, all of ancient Lower Nubia is beneath the waters of Lake Nassar, a creation of the Aswan High Dam.

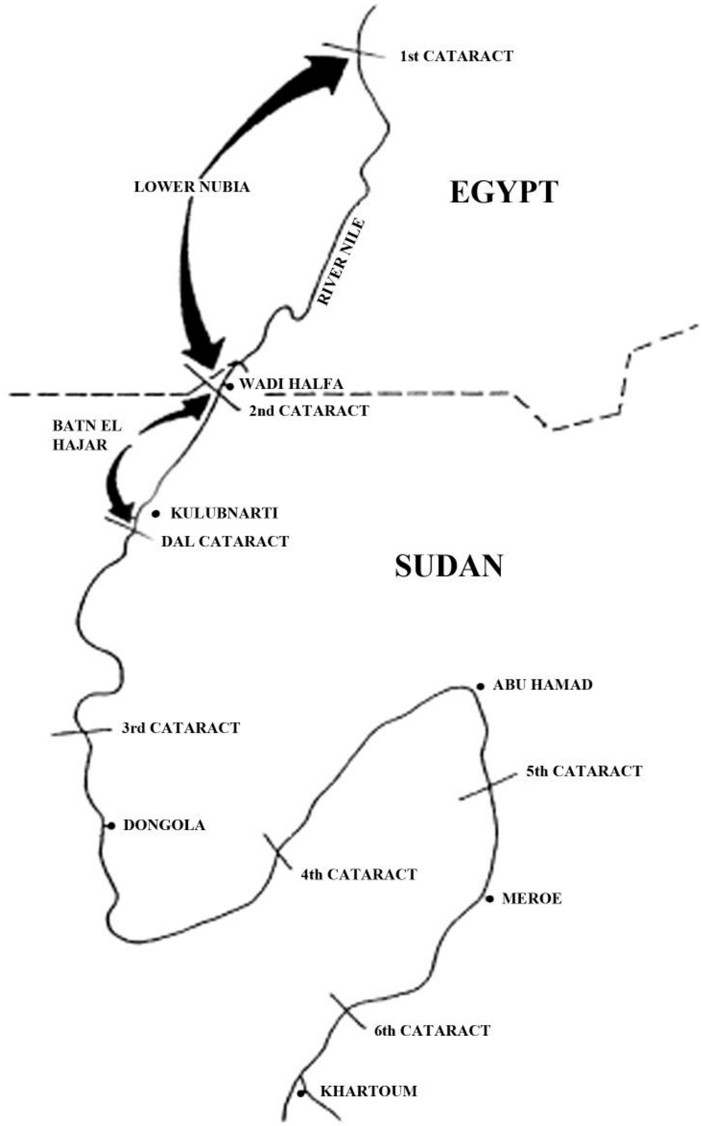


Figure 1.1: Map of Nubia (adapted from Van Gerven et al., 1995).

For all practical purposes, the northern portion of Nubia, from Aswan to Dongola, located south of the Third Cataract, is rainless (Adams, 1977). Beyond a thin swathe of land a couple of miles to the east and west of the Nile, human habitation virtually did not exist in antiquity. The most bleak and inhospitable of all Nubian environments can be found in the

100 mile tract south from the Second Cataract. This region is known as the Batn el Hajar, or "belly of rock." Exposed granite ridges and promontories are interspersed with equally barren alluvial flats, and arable land only occurs in small patches along the river (see Figure 1.2).

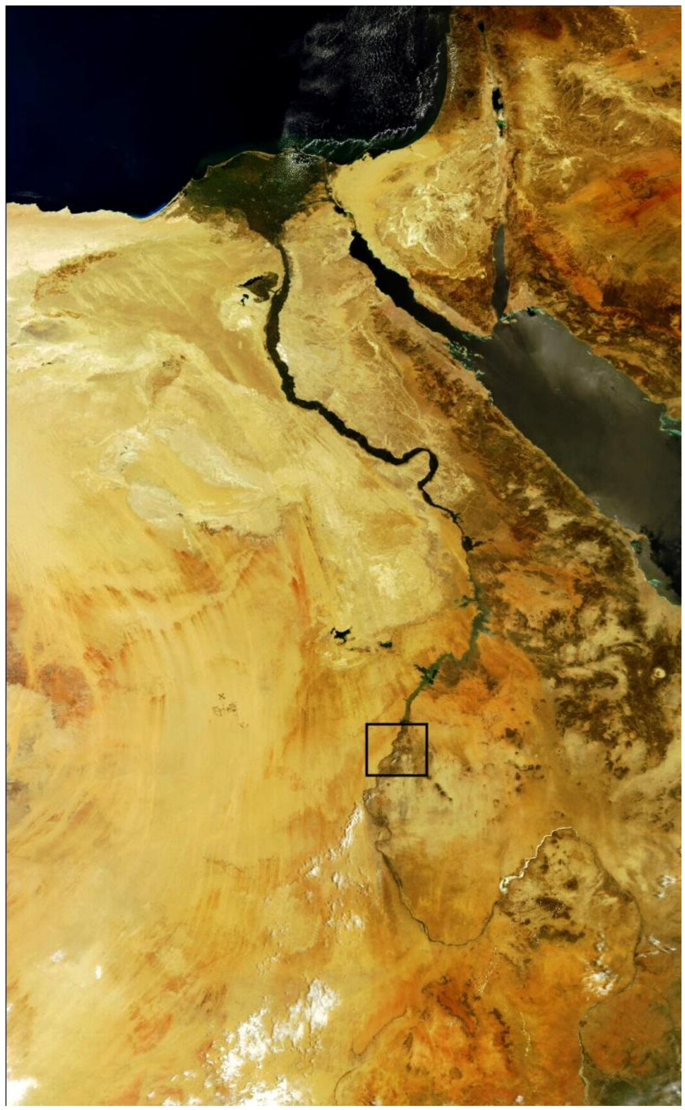


Figure 1.2: The Nile River Valley and the Batn el Hajar (Earth Snapshot, 2011).

The Nile itself through the Batn el Hajar was swift, narrow, and rocky; long distance navigation would have been impossible during seasons of low water, and hazardous at times of optimal conditions. Although population densities would have fluctuated through time to some degree, the Batn el Hajar was never heavily populated due to the ecological constraints imposed by the environment.

It is in this harsh, rugged landscape of the Batn el Hajar that Kulubnarti is located, directly adjacent to the modern village of Kulb (Figure 1.3). Approximately 80 miles upstream from Wadi Halfa, the archaeological site of Kulubnarti is now separated by a portion of the river, thus creating an island roughly three-quarters of a mile in length which would not have existed as such in antiquity except perhaps for a few weeks each year at the peak of the Nile flood season (Adams, 1999a). When this would occur, the island would be divided from the west bank by a narrow channel, otherwise used as farmland during dry seasons.

Figure 1.3: The Island of Kulb in the Batn el Hajar (Google Earth, 2009).

THE CHRISTIAN PERIOD (A.D. 600-A.D. 1500)

For thousands of years of civilization in northeast Africa, Egypt set the political and cultural standard for Nubia; it is therefore not surprising that the eventual spread of Christianity to Nubia was inevitable once it became the state religion of Egypt. In some parts of Nubia, Christianity was established as early as A.D. 543 according to the accounts of John of Ephesus and John of Biclar (Edwards, 2004). The rapid spread of Christianity south of Aswan can be seen archaeologically in the abrupt change from pagan to Christian burial practices in Nubian cemeteries (Adams, 1977).

Less than a century after Nubia embraced Christianity, Egypt was invaded and subjugated by the armies of Islam. Conquest began in A.D 639 and very little resistance was met except in Alexandria, which surrendered by A.D. 641 (Adams, 1977). It was not long after this that the Muslims turned south, penetrating as far as Dongola, the seat of Nubia's Christian kings. According to Arab historians, from whom the majority of what is known about Medieval Nubia derives, the resistance to invasion was greater in Nubia than ever before encountered in the first century of the expansion of Islam (Edwards, 2004). A second invasion was mounted in A.D. 651 and following another indecisive military conflict at Dongola, the Baqt Treaty was formed, in effect granting Nubia the status of a client kingdom of the Islamic Empire (Adams, 1977).

Under the terms of the Baqt, Nubia was in large part left to its own devices as the armies of Islam spread through the remainder of North Africa, Spain, the Byzantine Empire, and Central Asia. In return for its political and religious independence, Nubia was contractually obligated to provide Muslim rulers in Egypt 400 slaves per year, the bulk of which was likely comprised of captives of war (Adams, 1977). In addition to this, Lower Nubia was to be given a special status as a free-trade zone, into which Muslims were permitted to travel, settle, and freely exchange goods with the Nubian Christians. As a result of this economic arrangement, it would appear from archaeological evidence that Lower Nubians enjoyed a significantly higher standard of living than their southern neighbors.

Below the Second Cataract at Wadi Halfa, the rocky and forbidding Batn el Hajar became an important buffer zone—a virtual "granite curtain" separating Islam to the north and Christianity to the south (Van Gerven et al., 1995). As per the Baqt, no Muslims were permitted to travel south of the Second Cataract. It is likely that this aspect of the treaty was relatively easy to enforce, as the barren, rocky landscape was already repellent to most outsiders. Archaeological excavations conducted in the Batn el Hajar indicate that this poor and isolated region served as a refuge for both Egyptian and Nubian Christian populations when the political conflicts began in the north during the thirteenth century which ultimately sealed the fate of Christianity in Nubia (Adams, 1977).

For 600 years, the Baqt determined the course of political and economic relations between Muslim Egypt and Christian Nubia, the delicate balance being maintained as much by geography as by treaty (Van Gerven et al., 1995). Due to institutionalized trade relations with the Islamic world via Lower Nubia, the Classic Christian period, A.D. 850-1100, was an era of great peace and prosperity (Adams, 1977). The fragile neutrality between Muslims and Christians would not last, however.

Although Nubia did not immediately succumb to the same political fate as its conquered northern neighbor, the spread of Islam to Egypt and the Near East forced Nubia into isolation, cut off from the rest of Chistiandom. The Christians of Europe displayed little interest in their Nubian brethren, ignoring the pleas for assistance in staving off Muslim conquest. It is rather astonishing then, that for hundreds upon hundreds of years the Nubians resisted Islamic encroachment until the last Christian church finally collapsed in the fifteenth century (Adams, 1977).

The End of Medieval Nubia

Intensifying persecution of Christians in Egypt following the rise to power of the militaristic Bahri Mameluke dynasty in the thirteenth century led to an increasing amount of conflict against Christians to the south. In Dongola, a series of dynastic intrigues transpired, eventually resulting in a Muslim prince ascending the Nubian throne in A.D. 1323 (Van Gerven et al., 1995). It is at this point in history that Christian control of Nubia came to a halt, though Christianity itself would maintain a tenuous hold in the Batn el Hajar until the end of the fifteenth century.

With the collapse of the Christian kingdom at Dongola, the medieval period of Nubia ended and a new feudal period began. Throughout Nubia, people were forced to revert to a subsistence economy as commerce and manufacturing came to a standstill due to the political and economic turmoil (Van Gerven et al., 1995). Village autonomy increased at this time, and populations in Lower Nubia relocated to a lesser number of larger settlements. In the Batn el Hajar, however, a region that was previously never home to more than a few hamlets, population densities increased with the arrival of those seeking refuge from disturbances to the north. Individuals here experienced a period of even greater economic isolation than before when operating under the Baqt. An examination of the archaeological record during the Classic Christian period shows that imported objects were very common in the Batn el Hajar. In the later feudal age however, less than 10% of artifacts were of foreign manufacture, and those that were found most likely could be attributed to the fairly recent past rather than the Late Christian period (Adams, 1977).

Whereas the Classic Christian period was a time of documented peace and prosperity in Nubia, the decline of Christianity in the fourteenth and fifteenth centuries resulted in the cessation of written history in Nubia. Ultimately, the Islamic conquest of Egypt brought about a dark age in Nubia, and it would be over 400 years until archaeologists would begin studying aspects of the Christian period in the Batn el Hajar.

THE ARCHAEOLOGY OF NUBIA

Interest in medieval Nubia on the part of Western scholars has been relatively recent according to William Y. Adams, who wrote perhaps the most authoritative text on the subject to date, "Nubia: the Corridor to Africa" (1977). Over the past century, the predominant factor determining what and where excavations would occur has been the construction of a series of dams near Aswan. Prior to the building of the first dam, which the British completed in 1902, the Nile River would flood annually during late summer. The high waters associated with floods would transport nutrients and minerals which would enrich the fertile soil along the floodplain and delta. Though this seasonal flooding would make the Nile Valley ideal for farming in ancient times, famine would occasionally occur when abnormally high waters would destroy entire crops, or low waters would create drought conditions. Rapid population growth in northeastern Africa coupled with an increasing desire to control the floods resulted in the construction of subsequently taller dams at Aswan over the course of the twentieth century (Abu-Zeid & El-Shibini, 1997).

The First Archaeological Survey was carried out between 1907 and 1911 in Lower Nubia, just south of what is now referred to as the Low Dam. Initially, those archaeologists involved with this project were completely focused upon mortuary remains almost to the exclusion of other archaeological finds (Adams, 1977). A significant emphasis was placed upon the examination of early phases of Nubian history, particularly following the discovery of zero burial offerings in 1,625 graves in a Christian period cemetery near Shellal, less than five miles south of Aswan. This should not have been unforeseen, as Christian funerary practices did not include the practice of placing offerings in tombs. Nonetheless, as a result of this experience the 900-year phase of Nubian history was almost entirely ignored as Christian Nubians were decided to be “too poor and too recent” to be of any interest to archaeologists (Adams, 1977: 74). Approximately one year after the conclusion of the First Archaeological Survey, an expedition was launched by Oxford University with the intent to study Christian Nubian remains. Ultimately though, the archaeologists chose to focus their efforts on the architectural rather than funerary remains.

During the Second Archaeological Survey, necessitated by the raising of the Low Dam and conducted from 1929 to 1934, attentions were again focused upon mortuary remains in Lower Nubia, though this time in the area closer to the modern Sudanese border at the Second Cataract. As with the First Archaeological Survey, the abundant and well-preserved Christian sites were virtually ignored. Though a great deal was learned about earlier cultural horizons of Nubia, the systematic excavation of Christian Nubia would have to wait a little longer.

The Aswan High Dam

In 1946, the Low Dam at Aswan was nearly over-topped and it was thus decided that a second, taller dam should be constructed. As Egypt’s efforts to raise money for the project progressed, archaeologists began raising concerns that important historical sites were about to be covered by water (Adams, 1977). Consequently, a massive salvage campaign was launched in 1959 by the United Nations Education, Scientific, and Cultural Organization (UNESCO) which would carry on for a full decade. Although the conservation of previously identified monuments was a significant priority, the full length of the Nile Valley throughout Lower Nubia and the Batn el Hajar was surveyed, over 1,000 new sites were discovered, and over 330 of those were excavated (Adams, 1977). This campaign was of an unprecedented scale; more than a million dollars were spent on archaeological work and nearly every developed country in the world participated.

After partnering with the Soviet Union, Egypt began construction of the Aswan High Dam in 1960. UNESCO raced against its completion to save as much cultural heritage as possible. Some of Nubia's most impressive monuments, such as the temples at Abu-Simbel, Philae, Kalbsha, and Amada, were physically disassembled and reconstructed at higher elevations—an immense feat of both archaeology and engineering. When the Aswan Dam reached completion in 1970, a permanent lake, Lake Nassar, was created, flooding the majority of Lower Nubia. Any site either not relocated or thoroughly excavated beforehand was presumably lost forever.

The amount of archaeology that was performed in Nubia in the decade prior to the completion of the High Dam not only exceeded that which was carried out in all previous periods, but is also probably greater than would have been achieved in the next 300 years without the stimulus of the High Dam (Adams, 1977). Never before had such a comprehensive archaeological approach been undertaken in Nubia (Edwards, 2004).

Today, the archaeological knowledge of different parts of the Sudan is still extremely variable. Though some surveys and excavations have been carried out in parts of Darfur more recently, the Egyptocentrism of the past has left its mark, being the largest determinant of where archaeological work was conducted in the twentieth century (Edwards, 2004).

EXCAVATIONS AT KULUBNARTI

As mentioned above, the individuals being studied in this thesis derive from two cemetery populations at the site of Kulubnarti in the Christian period Batn el Hajar. One of the cemeteries was located in a dry wadi (valley) near the west side of the island (21-S-46) and the other was situated on the west bank of the Nile River just opposite of the southern end of the island (21-R-2). Investigations of the two cemeteries of Kulubnarti began in 1969 by a team from the University of Kentucky led by William Y. Adams as part of the massive UNESCO salvage campaign (1970). From other excavations on both the island and mainland, Adams' belief that Kulubnarti may have been the most recent Christian settlement in Nubia was supported. Though excavating graves was not a significant objective of the Kentucky Expedition, the two cemeteries were mapped and 100 graves were actually opened (Adams, 1999a). Lacking a physical anthropologist, the skeletal remains were not studied at this time. However, the excellent preservation of the soft tissues of the Kulubnarti individuals was noted, and presented a unique opportunity for future research regarding diet, disease, and mortality.

Because of the condition of the interments, many of which were naturally mummified by rapid desiccation, it was decided to launch a second expedition to Kulubnarti to excavate as many graves as possible with the intent of detailed laboratory analysis back in the United States. After receiving funding from the National Science Foundation (NSF), Dennis Van Gerven and a joint team from the Universities of Colorado and Kentucky returned to Nubia for a short field season in early 1979 (Van Gerven et al., 1981).

In total, 406 individuals were disinterred between January and March of 1979: 218 from the island cemetery (21-S-46) and 188 from the mainland cemetery (21-R-2). Though an initial survey suggested the two cemeteries were contemporaneous, pottery analysis and architectural features indicate that the island cemetery represents a population from the Early Christian period (A.D. 600-850), while the mainland cemetery likely dates to the Late

Christian period (A.D. 1100-1400) (Van Gerven et al., 1981). In fact, during excavations of the mainland cemetery, a clear transition from Christian burials with an east-west orientation to Muslim burials with a north-south orientation was observed (Van Gerven et al., 1981). This suggests 21-R-2 was in continuous use when the last Christians died at Kulubnarti. Lending additional support to this contention is the fact that as of 1979, the mainland cemetery was still being utilized by the contemporary population (Van Gerven et al., 1995). Within the past decade, however, the diachronicity of the 21-S-46 and 21-R-2 cemeteries have been called into question by an analysis of textiles (Adams, 1999). This more recent research suggests that both cemeteries were in use during the Early Christian period and should be dated to A.D. 600-850. Until more concrete analysis is performed, the question of dating is still very much open to debate.

Although there is some variation in the amount of soft tissue remaining on the skeletal remains, now housed at the University of Colorado, the Kulubnarti individuals were all extremely well-preserved by the dry climate of the Batn el Hajar. Many of the disinterred bodies were found wrapped in the burial shrouds with hair, nails, skin, and organs preserved (Van Gerven et al., 1995). It is because of this remarkable preservation that the analysis for this thesis is possible.

Life at Kulubnarti

Archaeological investigations have demonstrated that Kulubnarti has been continuously occupied from A.D. 550 to the present. By examining ruins at Kulubnarti, archaeologists can glean a better understanding of what Nubian lives were like during the dark age that was brought about by the end of the medieval period and ensuing shift to feudalism. From analyzing the architecture and construction periods, it appears that during the fourteenth century, simple houses were transformed into defensive structures. After this point in time, living quarters did not exist on the ground floor and were only accessed by retractable ladders (Van Gerven et al., 1995). The shifting landscape saw the decline of churches and rise of defensive architectural features. These Christians of Kulubnarti continued life in this manner, largely undisturbed until the Ottoman Turks annexed Lower Nubia in the sixteenth century.

Throughout the Christian period, the people of Kulubnarti practiced small-scale agriculture which was only possible through the extensive use of retaining walls which captured alluvium. Sorghum, millet, barley, beans, lentils, peas, dates, and wheat would have been the crops grown in this area (Adams, 1977). Though domesticated animals were kept, they did not seem to comprise a major portion of the diet (Sheridan, 1992).

THE PRESENT INVESTIGATION

Over the past three decades, extensive research on the skeletal remains from Kulubnarti has established a detailed record of the biological aspects of mortality, nutrition, growth and development, and disease. It has been thoroughly shown that there are significant differences in health between the two cemeteries, 21-S-46 and 21-R-2 (Van Gerven et al, 1981; Hummert, 1983; Van Gerven et al., 1995, Van Gerven & Greene, 1999). Since differences in nutritional status between cemeteries and age and sex categories have been suggested, the stable isotope analysis of hair offers a new approach to evaluating this belief.

CHAPTER TWO

METHODS AND MATERIALS

AN INTRODUCTION TO STABLE ISOTOPE ANALYSIS

Stable isotope analysis is a fairly recent addition to the assortment of analytical techniques for elucidating information about ancient populations, but is becoming increasingly popular within the discipline of anthropology. Chemists and physicists can be credited with most of the refinements to isotopic analysis following the initial discovery of chemical isotopes in the early 20th century (Soddy, 1923; Urey et al., 1932). As the analytical power of stable isotope analysis was gradually realized, a diverse range of fields in addition to chemistry and physics—including geology, zoology, ecology, plant physiology, and paleoclimatology—made abundant use of stable isotope analysis in hypothesis-testing oriented research (Sharp, 2007). For the past couple of decades, anthropologists have been utilizing stable isotope analysis with increasing frequency to address previously unanswerable questions.

In this chapter, principles of stable isotope analysis will be introduced followed by a discussion of how isotopic ratio measurements are expressed and interpreted. Past applications of the stable isotopic analysis of hair in anthropological investigations will also be presented, as well as information regarding the materials which were analyzed for this research.

Isotopes

To begin, isotopes can be defined as different forms of the same chemical element. The isotopes of an element share the same number of protons in the nucleus, and therefore the same atomic number, but differ in the number of neutrons, thus causing different atomic weights. There are 92 naturally occurring chemical elements, 71 of which occur in more than one isotopic form (Meier-Augenstein, 2010). The majority of these isotopes are stable, meaning they do not decay. Those isotopes which are not stable and do undergo radioactive decay are termed radioisotopes.

The various isotopes of an element differ in their abundance in nature; typically, though not always (notable exceptions being Fe and Sr), the lightest isotope of a given element is more abundant than its heavier counterpart(s) (Ehleringer and Rundel, 1988). For a list of isotope abundances in the four elements pertinent to this particular investigation, see Table 2.1.

Table 2.1: Average Terrestrial Abundances of H, C, N, & O Stable Isotopes

Element	**Isotope**	**Abundance (‰)**
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{16}O	99.759
	^{17}O	0.037
	^{18}O	0.204

(Ehleringer & Rundel, 1988)

Fractionation

For the isotopes of a particular element, the chemical and physical properties can vary slightly due to differences in mass. With lighter elements, or those with atomic numbers under 40, these mass differences are relatively large. As a result, molecular reactions and physical processes such as diffusion and vaporization can fractionate, or change the relative proportions of differing isotopes within a substance (Kendall and Caldwell, 1998). For example, in the case of hydrogen, there are two stable isotopes, ^{1}H and ^{2}H, also known as protium and deuterium respectively. Protium is composed of a single proton and zero neutrons whereas deuterium is composed of one proton and one neutron. The difference in mass is quite large between ^{1}H and ^{2}H and therefore a significant fractionation will occur.

In certain reactions that discriminate against the heavier isotope of an element, product molecules are considered to be depleted in that heavy isotope. Conversely, there are chemical and physical processes that can produce molecules which are said to be enriched in the heavier isotope. Isotopic composition provides a way to express the ratio of heavy to light isotopes present within a substance.

Units & Notation

Due to the very small absolute abundances of an isotope in any given material, researchers express stable isotope ratios in the "δ notation" (McKinney et al., 1950). By expressing different isotope abundances relative to an international standard, researchers are able to make the differences found in isotopic ratios between samples more readily apparent (Dawson & Siegwolf, 2007; Sulzman, 2007). In the formula below, R is the ratio of heavy to light isotope:

$$\delta = (R_{sample}/R_{standard} - 1) \times 1000$$

As isotope abundances are often quite small, the δ value is multiplied by 1000 to express differences in units which are easier to use. Thus, delta values are reported in per mil (‰), or parts per thousand. When a δ value is positive, the ratio of heavy to light isotope is higher in the sample being analyzed than in the standard. A negative δ value has the opposite meaning. Delta notation is also very useful because mass spectrometric measurements are more precise when measuring two gases of differing masses rather than a single source gas (Meier-Augenstein, 2010).

Each element has its own international standard to which the ratios of heavy to light isotopes in samples are compared. With carbon ($^{13}C/^{12}C$), for example, the international standard is Pee Dee Belemnite (PDB), a fossil calcium carbonate from the PeeDee geological formation in South Carolina. Though this original standard is no longer available, the International Atomic Energy Agency (IAEA) creates an equivalent carbon standard of a similar isotope value in Vienna known as Vienna Pee Dee Belemnite (VPDB). However, for carbon isotope analyses, the standard is still referred to as PDB (Dawson & Siegwolf, 2007).

For nitrogen ($^{15}N/^{14}N$), the standard is air since nitrogen comprises a fixed 78.08% of the air we breathe. For oxygen ($^{18}O/^{16}O$), the standard is Vienna standard mean ocean water (VSMOW) except when researchers are also determining the $\delta^{13}C$ of a carbonate, in which cases VPDB is used instead. For hydrogen ($^{2}H/^{1}H$ or D/H), the standard is also VSMOW given that the original standard, standard mean ocean water (SMOW), is no longer available (Dawson & Siegwolf, 2007).

Stable isotope ratios of a substance are typically measured using a technique called isotope ratio mass spectrometery (IRMS), which was invented by J.J. Thompson in 1910 and basically separates the atoms or molecules of a sample gas based on their different masses (Sulzman, 2007). Molecules containing the heavy isotopes of an element sort to one end of a collector while those containing the light isotopes are sorted to the other end. Although there are two types of IRMS, dual-inlet (DI-IRMS) and continuous flow (CF-IRMS), the continuous flow method has revolutionized IRMS since becoming commercially available in the late 1980s, and has led to greater use of stable isotope analysis in a variety of disciplines including anthropology (Sharp, 2007). CF-IRMS instruments use a continuously passing, helium carrier gas to sweep the analyte gas into the ion source that sorts the various molecules of a sample gas during analysis. This advantageously allows for sample sizes to be much smaller than with previous equipment which utilized a viscous flow. An additional benefit of CF-IRMS is that an isotope reference gas can be directly introduced into the ion source at virtually any point during analysis, thus situating the sample peak between multiple reference gas peaks of known isotopic composition (Meier-Augenstein, 1997). IRMS instruments can be used to obtain highly accurate and precise measurements of isotopic abundances (with precision of 0.1‰; Meier-Augenstein, 2010), and they are now used for almost every material that can be analyzed due to their efficiency and the smallness of required sample size (Sharp, 2007).

In the following sections, each of the four isotopes relevant to this particular investigation will be discussed, followed by a review of the considerations associated with using hair as a tissue for isotopic analysis.

CARBON ISOTOPES

The use of stable isotopes by anthropologists to reconstruct ancient diets began three decades ago with studies utilizing carbon isotopes. The principle behind this dietary reconstruction is that the isotopic composition of a body tissue is a direct function of an individual's diet (Ambrose, 1993). Although studies of stature, dentition, and both skeletal and dental pathologies have been used in the past by anthropologists to examine diet and nutrition (Hillson, 1979; Larsen, 1995; Hillson, 1997), stable isotope analysis provides a highly quantifiable method to reconstruct aspects of ancient diets.

Since the Industrial Revolution, the atmospheric concentration of $\delta^{13}C$ (-8.0‰) has decreased due to the combustion of large amounts of fossil-fuel derived CO_2. Termed the Suess effect, this reduction of atmospheric $\delta^{13}C$ is a result of fossil fuel CO_2 being depleted in ^{13}C due to fractionation in its formation through photosynthesis (Revelle and Suess, 1957; Bacastow et al., 1996; Gruber and Keeling, 2001). There are two primary photosynthetic pathways utilized by plants in the conversion of CO_2 into plant carbon which affect the carbon isotopic composition of animals consuming these plants (DeNiro and Epstein, 1978). In both of these pathways, photosynthetically fixed carbon is depleted relative to the carbon in atmospheric CO_2.

Trees, shrubs, and many grasses which are adapted to cool growing seasons and shade use the C_3 or Calvin-Benson cycle, whereas tropical grasses which are better adapted to water stress, high temperature, strong sunlight, and low atmospheric CO_2 concentrations use the C_4 or Hatch-Slack cycle (van der Merwe, 1989, 1982; Smith and Epstein, 1971). In the initial phase of photosynthesis, plants that use the Calvin cycle fix CO_2 using the enzyme ribulose bisphosphate carboxylase/oxygenase (rubisco) and create compounds that have three carbon atoms; those that use the Hatch-Slack cycle fix CO_2 using the enzyme phosphoenolpyruvate (PEP) carboxylase and create compounds that have four carbon atoms (Marshall et al., 2007; Farquhar et al., 1989). C_3 plants can vary in their $\delta^{13}C$ values from -20‰ to -35‰ due to differing conditions of water, nutrient, and light availability (also known as the canopy effect; Dawson et al., 2002; van der Merwe and Medina, 1991). In comparison, the C_4 carbon fixation process leads to carbon products that are "heavier" in $\delta^{13}C$ compared to C_3 plants, with values ranging from -10‰ to -19‰ (Dawson and Siegwolf, 2007), and an average of -13‰ (O'Leary, 1988). The large degree of carbon discrimination during photosynthesis in C_3 plants results in an average $\delta^{13}C$ value around -28‰. This is attributed to the slower diffusion of $^{13}CO_2$ relative to $^{12}CO_2$, and rubisco's greater affinity for the more abundant $^{12}CO_2$ (Schlesinger, 1997; Koch et al., 1994).

Isotope ratios are transferred from plants to the animals that consume them. During metabolic processes, the isotopic composition of a food source is fractionated by different factors as ingested elements are incorporated into various body tissues. It has been shown that the fractionation between dietary carbon and the carbon found in hair keratin can vary between +1‰ to +4‰ (DeNiro and Epstein, 1978, Ostrom and Fry, 1993, Sponheimer et al., 2003). Thus, a foodweb based solely on C_3 plants would yield, on average, $\delta^{13}C$ values between -27‰ and -24‰, and a foodweb based solely on C_4 plants would yield, on average, $\delta^{13}C$ values between -12‰ and -9‰.

The disparity of isotope signatures between C_3 and C_4 foods can be clearly seen in Table 2.2, which provides reference values of carbon isotopes for a variety of plants which were likely utilized in Nubia during the Christian Period.

Table 2.2 Carbon Isotope Values of Plants Used in Ancient Nubia.

Scientific Name	**Common Name**	**Plant Part**	**$\delta^{13}C$ ‰**
C_4 plants			
Setaria viridis	millet	seed	-10.82
Digitaria sanguinalis	millet	seed	-10.64
Pennisitum divisum	millet	seed	-12.09
Panicum coloratum	millet	seed	-13.07
Sorghum durra	sorghum	seed	-10.95
Sorghum sudanesnsis	sorghum	seed	-12.38
Mean			-11.65
Standard deviation			±0.9
C_3 plants			
Triticum vulgare	wheat	seed	-26.98
Hordeum vulgare	barley	seed	-22.13
Vigna membranaceae	cowpeas	seed	-25.10
Cajanus cajan	pigeon peas	seed	-27.19
Acacia nilotica	acacia beans	seed	-25.02
Acacia albida	acacia beans	seed	-27.45
Hibiscus esculentus	okra	leaf	-20.98
Eruca sativa	garden rocket	leaf	-30.30
Raphanus sativus	radish tops	leaf	-30.47
Allium sepa	onion	skin	-28.02
Solanum melongena	eggplant	leaf	-28.42
Corchorus olitorius	jew's mallow	leaf	-28.18
Hibiscus sabdariffa	rosella	fruit	-23.31
Balanites aegyptica	date	fruit	-27.10
Mean			-26.50
Standard deviation			±2.9

(White & Schwarcz, 1994)

NITROGEN ISOTOPES

Similar to the way in which carbon isotope analysis has been used in anthropology, stable isotope ratios of nitrogen ($^{15}N/^{14}N$) are frequently examined to elucidate aspects of the dietary history of ancient populations. One of the most significant applications of nitrogen isotopes is the ability to ascertain trophic level position (DeNiro and Epstein, 1981). With each shift up the food chain from plants themselves to herbivores to omnivores to carnivores, $\delta^{15}N$ values of tissues comprised of protein (such as hair keratin) will shift upwards by 3-4‰ (Minagawa and Wada, 1984; Schoeninger and DeNiro, 1984). However, it has been suggested from controlled feeding animal studies that the magnitude of fractionation can increase by over 2‰ if the protein content of the diet is extremely high (Sponheimer et al., 2003). This incremental shift is called the trophic level effect and it is thought to be related to the excretion of urea and other nitrogenous wastes that are enriched in ^{14}N (Koch, 2007).

Human nitrogen isotope values vary primarily because of diet. Though climate, rainfall, and physiological factors such as water stress or pregnancy may have a role, they have not yet been fully quantified, and thus, may make the accurate interpretation of $\delta^{15}N$ difficult (Ambrose, 1991; Hedges and Reynard, 2006). In a study of modern Oxford residents by O'Connell and Hedges (1999), it was shown that differences in magnitude of the nitrogen isotope values of hair keratin did reflect the proportion of animal protein consumed in an individual's diet, but it did not matter whether the protein was obtained from meat specifically, or from other animal products. Therefore, all forms of animal-derived protein from the same animal, such as beef from a cow and its milk—are considered to be isotopically equivalent and are both classified as animal protein when performing isotopic dietary reconstruction.

For information on the $\delta^{15}N$ values of plants that would have been available to the Christian Nubians of Kulubnarti, refer to Table 2.3.

Table 2.3 Nitrogen Isotope Values of Plants Used in Ancient Nubia

Scientific Name	**Common Name**	**Plant Part**	**$\delta^{15}N$ ‰**
C_4 plants			
Setaria viridis	millet	seed	2.84
Digitaria sanguinalis	millet	seed	9.38
Pennisitum divisum	millet	seed	1.05
Sorghum durra	sorghum	seed	8.47
Sorghum sudanesnsis	sorghum	seed	0.47
Mean			4.44
Standard deviation			±4.20
C_3 plants			
Triticum vulgare	wheat	seed	6.51
Hordeum vulgare	barley	seed	7.84
Hibiscus esculentus	okra	leaf	6.71
Eruca sativa	garden rocket	leaf	3.04
Raphanus sativus	radish tops	leaf	1.04
Solanum melongena	eggplant	leaf	3.60
Corchorus olitorius	jew's mallow	leaf	13.09
Hibiscus sabdariffa	rosella	fruit	6.68
Mean			6.06
Standard deviation			±3.66

(White & Schwarcz, 1994)

OXYGEN ISOTOPES

Within anthropology, oxygen isotopic composition ($^{18}O/^{16}O$) is used primarily to study human mobility and the environments in which people lived in the past. As oxygen is one of two components of water molecules, it provides a built-in tracer for water (Sharp, 2007). Just over one-third of oxygen isotopes found in humans are derived from drinking water (O'Brian and Wooller, 2007; Sharp et al., 2003); the $\delta^{18}O$ value within a body tissue is also dependent upon the inputs from atmospheric CO_2 and the O_2 obtained from food sources, as well as outputs of liquid and vapor H_2O and respired CO_2 (Longinelli, 1984; Ayliffe and Chivas, 1990; Ehleringer et al., 2008; Price and Burton, 2011). The $\delta^{18}O$ values of precipitation and other sources of water vary along geographic gradients, with the abundance of ^{18}O and ^{16}O in meteoric water being largely controlled by fractionation processes related to temperature, such as evaporation, condensation, and transpiration (Ehleringer and Dawson, 1992; Dansgaard, 1964; Craig, 1961). There is also a "continental" isotope effect that influences the isotope composition of meteoric waters. As moisture from coastal areas—locations where rain storms often originate—is carried inland, water condenses from the air and leaves the remaining vapor depleted in ^{18}O (Ingraham and Taylor, 1991).

Studies have shown that although the average fractionation between body water and hair is +17‰ (Sharp et al., 2003), the $\delta^{18}O$ of body water has been shown to reflect the mean $\delta^{18}O$ of local meteoric water (Longinelli, 1984). It is therefore possible to distinguish differences in $\delta^{18}O$ between individuals who are obtaining drinking water from separate sources (Ehleringer et al., 2008; O'Brian and Wooller, 2007).

HYDROGEN ISOTOPES

Hydrogen isotopic studies are utilized in a similar manner to oxygen isotope analyses in anthropology and other related disciplines. Along with oxygen, hydrogen is one of the two components of water molecules, thus making it possible to source different pools of water by examining the hydrogen isotopic composition ($^2H/^1H$). Observed δ^2H values of meteoric water can range from +20 to -230‰ across the world, and as with $\delta^{18}O$, values are dependent upon such factors as latitude, altitude, temperature, and distance to open seas (Meier-Augenstein, 2010). Typically, the heavier or less negative δ^2H values are found in coastal/equatorial regions, whereas the lighter or more positive δ^2H values are found in inland/high altitude/high latitude regions (Ingraham and Taylor, 1991).

For humans, the amount of hydrogen in the body is determined by three things: drinking water, water stored in food, and hydrogen which is chemically-bound in food. Only about one-third of hydrogen in human hair keratin is derived from drinking water; the hydrogen bound in food comprises the majority of the hydrogen signal (Sharp et al., 2003). The percentage of hydrogen attributable to drinking water has been found to be closer to 40%, however, when the water used to prepare beverages such as tea and coffee is taken into consideration (Fraser and Meier-Augenstein, 2007). As drinking water and the water people cook with generally originate from the same locale, it would be expected in an archaeological population that the δ^2H values of drinking water and food would be similar (Sharp et al., 2003). This is most likely the case in Nubia, where the largest source of water would be the Nile River. In other situations, where individuals may have access to a diverse range of food and/or water sources, the δ^2H hair signal may not directly reflect that of local meteoric water.

HAIR

Stable isotope analysis may be performed on a number of body tissues including skin, bone collagen, enamel, nails, and hair. In the past, most stable isotope analysis of ancient populations utilized bone collagen, the assumption being that the preservation of bone collagen is such that the original isotopic signal of an organism is retained. However, even under favorable preservation conditions, bone collagen tends to be altered by diagenetic reactions such as hydrolysis, decarboxylation, and deamination (Macko et al., 1999). Hair, composed of the hydrophobic protein keratin, is extremely resistant to diagenesis (Lehninger et al., 1994; Kempson et al., 2003), and it has been shown that very little chemical alteration occurs in hair, even when it has been in the ground for over 5,000 years (Lubec et al., 1987; Macko et al., 1999). When measured by weight, keratin makes up between 65% and 95% of hair fiber.

Another important consideration regarding hair as a tissue for analysis is the fact that it is a static tissue—that is, its isotopic composition does not change over time like that of bone which is in a constant state of remodeling. So, while the isotopic value of a sample of bone tissue may reflect a generalized dietary signal from a period of years, a hair sample can provide a discrete record of isotopic composition (O'Connell and Hedges, 1999). Nutrients are incorporated into a strand of hair only at the follicle; as a hair passes through skin during growth, keratin is synthesized from amino acids in the follicle and the mechanical structure becomes fixed and unchanging, thus preserving a record of an individual's diet at a particular period of time. And, since hair is known to grow at an average rate of approximately one centimeter per month (Zlotkin, 1985), the potential for gleaning aspects of seasonality or dietary change through time is possible by conducting segmental isotopic analysis. Variations in chemical compositions can be measured along the length of a single hair, allowing for changes at a weekly or even a daily level to be assessed (Sharp et al., 2003; Ayliffe et al., 2004).

The normal growth cycle of human hair is comprised of three phases: the long anagen phase in which hair is continuously growing, the short transitional catagen phase, and the intermediate telogen phase in which hair rests (Paus and Cotsarelis, 1999). Though there is a great deal of variation from individual to individual, in the anagen phase, growth can occur for up to five years. The catagen phase generally lasts less than a month, and the telogen phase can last up to four months. Each follicle's growth is independent of the surrounding follicles. This can be problematic if it is assumed that hairs are contemporaneous with one another. In this particular investigation, the bulk analysis of hair from one or more strands from each individual was performed. Whether one strand or multiple strands were used in a sample depended on the length and weight of the available strands. Consequently, the isotopic values obtained in this analysis likely reflect an average isotope signal from two or three months of growth rather than a single week or month.

Hair has not often been the tissue of choice for stable isotope analysis in past anthropological research. However, in more recent years, there have been a number of isotopic investigations in which hair was utilized, especially since hair has been shown to provide a sequential record of dietary changes (Schwerti et al., 2003; Sharp et al., 2003; Cerling et al., 2006). In the section below, a brief review of previous isotopic studies which have utilized hair as a tissue for analysis will be presented.

ANTHROPOLOGICAL APPLICATIONS OF STABLE ISOTOPE ANALYSIS

Reconstructing ancient diets has been an active sphere of research within biological anthropology. While there are many different approaches to this study including the use of archaeological evidence, floral and faunal analyses, paleopathological markers, dental microwear analysis, and chemical elemental analysis of bone, stable isotope analysis provides an alternative method for obtaining direct information regarding the foods people were eating in the past (Ambrose, 1993; Macko et al., 1999; Katzenberger, 2000).

Though it was first posited that stable isotopes could be applied to reconstructing ancient diets in 1967 by Robert Hall at the Cahokia Field Conference (Riddell, 1967), a full decade passed until John Vogel and Nikolaas van der Merwe (1977; van der Merwe and Vogel, 1978) were able to quantify long-term human maize consumption with carbon isotope ratios, thus making clear the relationship between diet and the isotopic composition of animal tissue (Ambrose, 1993). Since then, the applications of stable isotope analysis to furthering anthropological research have expanded dramatically. Beyond dietary reconstruction, stable isotope analysis can provide investigators with a powerful tool for tracking migrations in archaeological populations, as well as understanding the ecological context in which ancient peoples lived (Schwarcz et al., 1991; Dupras & Schwarcz, 2001; Ehleringer et al., 2008).

Dietary Reconstruction

One specific research area in which carbon isotope analysis has been utilized for reconstructing diets is in the detection of the transition to agriculture in past societies. This is possible since domesticates such as maize, millet, and sorghum are C_4 plants. In New World archaeology, identifying populations with dependence on maize agriculture has become important to enhancing our understanding of when and where domestication began, as well as to what degree populations were reliant upon agriculture for subsistence. Although many studies have focused on interpreting carbon isotope values from all over North America and Mesoamerica to document this transition (Bender et al., 1981; Lynott et al., 1986; Buikstra & Milner, 1991; Katzenberg et al., 1993; Reed, 1994; 1999), most have utilized the analysis of bone collagen or dental enamel, as old hair is rarely found without favorable conditions of preservation, such as mummification or freezing.

To evaluate the practicality of using hair as a tissue for stable isotope analyses in archaeological investigations, studies of modern hair from living individuals have been carried out (Minigawa, 1992; Macko et al., 1999; Bol and Pflieger, 2002). Previously, the interpretation of ancient human hair isotope values relied heavily upon comparisons with faunal data and theories developed and refined using laboratory-based controlled feeding animal studies (Jones et al., 1981; Tieszen et al., 1983).

In an investigation by Bol and Pflieger (2002), hair samples from 43 modern humans from SW England, five of which were recent immigrants, were analyzed and differences in $\delta^{13}C$ values were found to reflect the documented diets of those participating in the study. Given the widespread availability of corn-based foods in the United States, the recently arrived American had an obvious C_4 dietary signal that distinguished the individual from the British and other European subjects that displayed a C_3 dietary signal. A similar study performed in England by O'Connell (1996) evaluated the differences in carbon isotope values between vegans, vegetarians, and omnivores. However, no statistically significant differences were found, as all of the individuals being studied had equal, limited access (relative to the United States) to C_4 foods. Nitrogen isotope values were also determined from these individuals' hair, and as expected, true vegans had the lowest $\delta^{15}N$ values with an average around 7‰, while vegetarians were significantly more enriched in ^{15}N, around 9‰, due to their consumption of egg and milk products (O'Connell, 1996). Omnivores also displayed an average $\delta^{15}N$ value of 9‰. Thus, the results from this study were in agreement with the idea that the products of animals such as milk and eggs can be equally treated along with meat as animal protein.

Although hair is not as readily available in the archaeological record as bone, it has been utilized as a tissue for analysis in some ancient dietary reconstructions, often in cases of mummification. Christine White's research on Sudanese mummies from the Wadi Halfa area of Nubia (1993) is particularly interesting given its relatedness to the current investigation. Segmental $\delta^{13}C$ analysis was performed on hair from 14 individuals dating to the Christian and X-Group periods (immediately prior to the Christian period) in an attempt to observe seasonal dietary shifts and to determine the time of year in which most individuals were dying. A +2-4‰ shift toward greater C_4 reliance was associated with summer since maize is typically harvested beginning in June, whereas greater C_3 reliance was associated with winter. From the $\delta^{13}C$ values of hair closest to the scalp and therefore closest to time of death, it was found that the most common season of death appeared to have been summer, which coincides with the greatest amount of climatic, nutritional, and physiological stress for ancient and modern populations (White, 1993). White expanded this investigation to 146 mummies from the Wadi Halfa area with the help of Henry Schwarcz (White and Schwarcz, 1994), and found similar results regarding seasonality with a larger sample size. They also analyzed nitrogen isotopes and found that $\delta^{15}N$ values were consistent with the consumption of herbivorous animals, though over the thousand year span of samples from X-Group to Christian individuals, a 2‰ decline was observed which may indicate decreased animal protein consumption. Although the primary source of nitrogen isotopic variation in humans is diet, it is also possible that other influences such as climate, rainfall, or even physiological differences may affect $\delta^{15}N$ values (O'Connell and Hedges, 1999).

In a more recent investigation, also utilizing hair from mummified individuals, Andrew Wilson and his colleagues (2007) examined samples from four children, the sacrifices of an Inca ritual killing which occurred between A.D. 1430 and 1520 in the south central Andes in Peru. Permafrost conditions preserved their hair, allowing for segmental $\delta^{13}C$ and $\delta^{15}N$ isotope analyses. Results were used to evaluate the belief that prior to sacrifice, individuals would experience an elevation in social status. For two of the children, strong C_4 signals were exhibited closer to time of death, thus indicating an increasing reliance upon maize agriculture. This has been hypothesized to be associated with preparing sacrifices for their long, high-altitude trek (Wilson et al., 2007). Nitrogen isotopic analysis further supported this hypothesis, as at least one of the children experienced a significant increase in the consumption of animal protein in the months prior to death.

Human Mobility

To even a lesser degree than studies which have focused primarily upon dietary reconstruction, there have been very few anthropological investigations of human mobility using hair as a tissue for analysis. In fact, there have been relatively few published studies of $\delta^{18}O$ and $\delta^{2}H$ values of any archaeological human tissue at all (see Stuart-Williams et al., 1996; Wright and Schwarcz, 1999; Müller et al., 2003; White et al., 2004). However, as with carbon and nitrogen isotopes, there have been studies of modern hair which have focused upon the use of oxygen and hydrogen isotopes to determine geolocation, ethnicity, and population movements (Fraser and Meier-Augenstein, 2007; O'Brian and Wooller, 2007; Ehleringer et al., 2008; Thompson et al., 2010). These isotopic analyses are increasingly being used in forensic investigations, but the same principles can be applied to archaeological research in those few cases where hair is preserved.

In a 2003 study by Zachery Sharp and his colleagues, samples of hair from an Incan mummy that was discovered on Mount Aconcagua in Argentina were examined to identify both dietary history and the individual's place of origin. From the segmental analysis of hair, a pattern of seasonality was observed, with the lowest $\delta^{18}O$ and $\delta^{2}H$ values occurring during winter months when $\delta^{13}C$ values were most depleted since maize was not being consumed. The researchers were able to determine that the isotopic values of oxygen and hydrogen indicated the individual obtained his drinking water from lowland sources rather than from higher elevations, even in the weeks immediately prior to death.

These findings were very similar to those noted by Wilson et al. (2007), in the case of the four Inca child sacrifices which were discussed above. In addition to reconstructing diet, oxygen and hydrogen isotopic values were determined to provide information about where the individuals resided in the months preceding sacrifice. It was shown from the hair closest to the scalp in all four children that the trek to high-altitude occurred very rapidly, as $\delta^{18}O$ and $\delta^{2}H$ values were consistently indicative of lower elevation sources until very shortly before death (Wilson et al., 2007).

It is obvious from the above isotopic studies which have analyzed hair to reconstruct aspects of ancient diets, seasonality, patterns of mortality, social hierarchy, as well as human mobility, that the potential for truly unique anthropological research is immense when utilizing stable isotope analysis. In the next section of this chapter, details of the materials selected for this investigation will be provided, and methods of collection and analytical procedures will be presented.

Materials and Methodology

During the spring semester of 2008, hair samples were collected from the Kulubnarti collection at the University of Colorado. An initial inventory was performed, and out of the 406 individuals that were excavated in 1979 from cemeteries 21-S-46 and 21-R-2, almost one-quarter retained some hair. Multiple whole strands of hair, including the root, were taken from all of these individuals, though only 29 samples were chosen for analysis at the University of Bradford in England. Initial preparation of the samples was conducted during July of 2008 by myself, Andy Gledhill, and Khudooma Said Al Na'imi; they were then analyzed in November of 2009 by Dr. Andrew Wilson. Although financial and temporal constraints prevented the analysis of every sample that was initially collected, the selection of an equal mix of the two cemeteries, both sexes, and all age ranges was attempted. In a somewhat fortuitous turn of events, the stable isotope lab at the University of Bradford had previously run hair samples from the individuals of Kulubnarti which were never formally examined by Dr. Charlotte Roberts of Durham University, who collected the samples in 1997. Wilson analyzed hair samples from 50 individuals in 2005 as part of research funded by the Wellcome Trust Bioarchaeology Programme. Though there was a little overlap in the particular samples chosen for analysis, carbon and nitrogen isotopic values were generated for a total of 79 individuals. In cases with multiple carbon and nitrogen results for the same individual, data were averaged. These individuals are marked with an asterisk on Tables 3.1 and 3.2. Hydrogen and oxygen isotopic values were only produced for the 29 samples analyzed more recently.

The materials selected for this investigation consist of 40 individuals from the island (21-S-46) cemetery and 39 individuals from the mainland (21-R-2) cemetery. Ages range from five months in utero to 51+ years. Age at death for sub-adults was determined using dental eruption, and epiphyseal union (Van Gerven and Greene, 1999). Among adults, age at death was first determined by placing individuals into age categories according to changes in the os pubis. These categories were then refined by seriating the remains according to dental wear and degenerative changes on joint surfaces (Van Gerven et al., 1981). For the analysis, individuals were pooled into the following age classes: infants 0-3 years, sub-adults 4-16, and adults 17+. A fourth age class of older individuals 44+ years was ultimately combined with younger adults as there was very little variation noted between mean group isotope values. Although the sub-adult age class extends to 16 years, the oldest individual in this category is 12 years old, and 80% of the sample (n=25) falls between 4 and 7 years of age. After age 12, survivorship dramatically improved at Kulubnarti, and thus very few individuals between 12 and 16 were excavated in 1979 (Van Gerven et al., 1981). Of the 79 individuals included in the analysis, 10 were infants, 25 were sub-adults, and 44 were adults. Because of the high degree of soft tissue preservation, sex was determined by external genitalia and body hair when possible. Pelvic morphology was used when only skeletal tissues were preserved. Sex could be determined for forty-nine individuals out of the 79 selected for sampling, resulting in 25 males and 24 females in the analysis.

Hair samples were prepared for isotopic analysis using standard protocols utilized at the University of Bradford (following O'Connell et al., 2001; Fraser et al., 2006). Samples were soaked in a chloroform/methanol solution overnight, sonicated, and rinsed three times in deionized water to rid the hair of impurities. Afterwards, they were placed in a freeze-drier to eliminate moisture. A microbalance was then used to weigh samples, with the individual strands of hair being cut into small segments. These short pieces of hair were placed into tin capsules and sealed shut to be analyzed using a Thermo Finnigan Delta Plus XL continuous flow mass spectrometer and Flash EA 1112 elemental analyzer for carbon and nitrogen analysis, and Thermochemical elemental analyzer for hydrogen and oxygen analysis. Data are reported in delta notation relative to the internationally recognized standards discussed above.

Interpretive Considerations

It has been shown that the initial effects of isotopic variation within the body caused by a change in diet can be observed in a matter of days (Nakamura et al., 1982). However, following a dietary shift, the transition to an isotopic steady state which is reflective of the new diet will take significantly more time. Most proteins in the body are constantly being resorbed and remodeled, except those of hair, fingernails, and skin.

Every day, the body produces up to five times as much protein as the average daily protein intake. As a result, only a small amount of the amino acids required for protein regeneration within the body is supplied by dietary intake and the remainder is supplied by the body protein pool which is derivative of the breakdown products of other proteins (Ayliffe et al., 2004). This protein pool buffers isotope signatures, thus reducing the effect of short-term fluctuations in the diet (Tieszen et al., 1983). It appears that human hair keratin takes at least 7 to 12 months to approach equilibrium after a shift in diet which would alter the isotopic signature (O'Connell & Hedges, 1999). In this investigation, bulk samples were run rather than segmental analysis. Therefore, this is probably not too much of an issue.

STATISTICAL ANALYSIS

For this thesis, Kolmogorov-Smirnov tests were performed to examine the normality of the distributions for $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and $\delta^{2}H$ values at Kulubnarti. With alpha set at 0.05, all four isotopes were found to be normally distributed ($\delta^{13}C$: $p=0.074$; $\delta^{15}N$: $p=0.804$; $\delta^{18}O$: $p=0.713$; $\delta^{2}H$: $p=0.519$). Therefore, independent Student's t-tests were used to test for significant differences between cemeteries, age, and sex categories.

CHAPTER THREE

RESULTS

CARBON AND NITROGEN

Hair, unlike bone collagen, is not thought to be subjected to the same diagenetic alterations which can eliminate or obscure biogenic isotopic signals. One way to evaluate whether any changes have occurred, however, is to examine the C:N ratios. It has been shown that the acceptable range of C:N ratios in modern hair is between 3.0 and 3.8 (O'Connell and Hedges, 1999). Of the 79 samples that were selected for this investigation, 30 displayed C:N ratios very slightly above the modern observed range of values. However, the degree to which these samples fell outside the range was extremely small, and all samples will therefore be included in this analysis. The average C:N ratio was found to be 3.8 ± .02 and values ranged from 3.5 to 4.2. Using least squares regression, neither carbon nor nitrogen isotope values were found to be correlated with C:N ratios (R^2= .0006 for carbon and .0463 for nitrogen).

The $\delta^{13}C$ and $\delta^{15}N$ values for R and S cemeteries are given in Tables 3.1 and 3.2, respectively. Throughout the remainder of this thesis, means will be presented ± standard error. For both cemeteries combined, the $\delta^{13}C$ mean is -17.8 ± .18‰ and the $\delta^{15}N$ mean is 8.5 ± .18‰. Summary statistics by cemetery, age, and sex are provided in Table 3.3.

Table 3.1: Carbon and Nitrogen Isotope Data from R Cemetery.

Burial	Sex	Age	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N
5		6	-19.1	7.5	3.7
30*	F	34	-17.1	9.2	3.8
41*		3	-15.9	7.9	3.7
44	F	36	-17.4	9.5	3.6
54		11	-17.3	7.7	3.8
67	F	51+	-16.9	9.2	3.6
74		5	-19.0	8.9	3.9
75	F	17+	-16.9	9.7	3.8
80*	F	40	-18.1	9.3	3.8
82*		5	-18.6	7.0	3.9
86	M	6	-18.3	8.9	3.8
93*		10	-18.6	8.8	3.8
94		12	-18.4	6.6	3.9
96*		3	-15.8	8.6	3.7
97	M	44	-19.0	9.2	3.9
98*		4	-15.3	9.4	3.8
99	F	36	-18.8	8.1	3.7
101		4	-17.9	8.3	3.7
102	M	38	-19.2	7.8	3.9
103*		1.5	-18.4	9.6	3.8
104	M	4	-19.2	7.2	3.9
106	F	38	-16.5	7.1	3.9
108*	M	42	-17.8	8.2	3.8
109		birth	-14.7	11.7	3.6
110	M	6	-17.1	6.1	3.7
112	F	45	-17.8	7.6	3.6
117		4	-19.6	8.6	3.7
123*	M	10	-18.9	7.3	3.9
124		5	-19.1	7.0	3.8
129	F	45	-18.5	9.4	3.9
140		4	-19.1	8.1	3.9
141	M	22	-16.8	9.3	3.9
149*	F	31	-15.8	8.4	3.9
153*	M	38	-18.6	6.8	3.9
160	F	17	-16.7	9.0	3.7
162	F	17+	-17.9	10.2	4.0
168*	M	19	-14.4	7.8	3.8
172		5	-19.8	4.4	3.8
175	M	20	-18.5	8.2	3.9

*****indicates averaged data

Table 3.2: Carbon and Nitrogen Isotope Data from S Cemetery.

Burial	Sex	Age	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N
18*	M	21	-16.6	8.6	3.8
26		4	-18.4	9.4	3.7
28		7	-19.7	8.6	3.6
55	M	18	-19.6	9.4	3.9
72		5 months IU**	-15.3	9.2	3.7
78		4	-18.5	9.2	3.6
80*	M	37	-17.6	7.8	3.7
81		3	-15.6	11.7	3.5
83		3	-19.8	7.9	3.6
86*	M	37	-18.8	6.0	3.9
94		infant	-19.8	10.3	3.5
96		5	-16.4	9.7	3.5
99*	M	39	-19.4	7.3	3.8
107*	M	47	-19.4	7.4	3.8
115		3	-20.1	7.0	3.5
121		4	-20.0	6.9	3.6
123		6	-20.6	8.3	3.7
139	F	6	-18.9	7.2	3.6
146	F	42	-13.8	7.9	3.7
168*	M	47	-15.2	8.9	3.7
172	F	51+	-18.4	7.4	3.8
185*	F	31	-19.9	7.5	3.7
186*	F	31	-16.9	7.6	3.6
187*	M	31	-18.3	9.0	3.6
192	M	42	-18.4	9.7	3.7
195		10	-15.2	10.3	3.5
196		3	-15.0	11.9	3.6
197		6	-19.5	8.4	3.5
202*	F	26	-18.6	7.5	3.7
204*	F	19	-17.3	8.9	3.7
206*	M	37	-19.5	10.3	3.7
207	F	49	-18.2	11.0	3.7
211*	M	25	-16.2	9.8	3.8
212*	F	49	-16.5	9.4	3.7
222*	M	25	-16.9	9.0	3.9
223	M	31	-18.6	9.1	3.8
225	M	47	-14.2	10.0	4.2
228*	F	47	-18.9	7.6	3.8
234*	F	51+	-17.3	7.4	3.7
237	F	31	-18.9	9.0	3.7

*indicates averaged data .... **indicates in utero

Variation by Cemetery

Carbon and nitrogen isotope values do not differ significantly between the two cemeteries (Figure 3.1). The average $\delta^{13}C$ value for R cemetery is -17.8 ± .22‰ and the average $\delta^{13}C$ value for S cemetery is -17.9 ± .28‰. The method of Schwarcz et al. (1985) was used to determine the approximate percentage of C_4 plants in the diet:

$$\% \ C_4 = \frac{(\delta h - \delta 3 + \Delta dh)}{(\delta 4 - \delta 3)} \times 100$$

where δ_h = the measured value of the hair sample, Δ_{dh} (the fractionation factor; as discussed previously, this has been observed to vary from +1‰ to +4‰) = +1, δ_3 = -27 (average value of C_3 plants in the Nile Valley; White, 1993), and δ_4 = 11.5 (local isotope value for sorghum and millet, plus a correction factor of 1.5‰ to account for pre-industrial age atmospheric carbon; White, 1993). For both cemeteries, the approximate percentage of C_4 plants in the diet was found to be 26%. No significant differences were found between any age or sex categories when comparing $\delta^{13}C$ values from the two cemeteries. The average $\delta^{15}N$ value for R cemetery is 8.3 ± .21‰ and the average $\delta^{15}N$ value for S cemetery is 8.7 ± .21‰. The only statistically significant difference observed when comparing age and sex categories between R and S cemeteries occurred among the $\delta^{15}N$ values of sub-adults (Figure 3.2). For R cemetery, sub-adults displayed an average nitrogen value of 7.6 ± .32‰, and for S cemetery, sub-adults displayed an average nitrogen value of 8.7 ± .38‰ (p=.05). In almost every category, $\delta^{15}N$ values were lower in R cemetery with two exceptions: females and adults, the latter of which was driven by the female mean. The R cemetery females had a mean nitrogen value of 8.9 ± .26‰ whereas S cemetery females had a mean nitrogen value of 8.2 ± .33‰.

Table 3.3: Summary Statistics for Carbon and Nitrogen Isotopes. Significant differences at α=.05 are bold.

	Count	$\delta^{13}C$ (‰)	Std Dev	$\delta^{15}N$ (‰)	Std Dev
All Individuals					
R	39	-17.8	1.37	8.3	1.29
S	40	-17.9	1.80	8.7	1.34
Student's T		p=.71		p=.14	
Infants					
R	4	-16.2	1.57	9.4	1.66
S	6	-17.6	2.54	9.7	1.99
Student's T		p=.36		p=.86	
Sub-adults					
R	16	-18.5	1.12	7.6	1.28
S	9	-18.6	1.73	8.7	1.13
Student's T		p=.84		**p=.05**	
Adults					
R	19	-17.5	1.21	8.6	0.93
S	25	-17.7	1.65	8.5	1.19
Student's T		p=.63		p=.81	
Males					
R	11	-18.0	1.44	7.9	1.01
S	14	-17.8	1.71	8.7	1.23
Student's T		p=.73		p=.08	
Females					
R	12	-17.4	0.90	8.9	0.90
S	12	-17.8	1.61	8.2	1.15
Student's T		p=.44		p=.12	
R & S Combined					
Infants	10	-17.0	2.23	9.6	1.77
Sub-adults	25	-18.5	1.33	8.0	1.31
Adults	44	-17.6	1.46	8.6	1.08
Males	25	-17.9	1.56	8.4	1.20
Females	24	-17.6	1.29	8.6	1.07

Table 3.3: Continued

	Count	$\delta^{13}C$ (‰)	Std Dev	$\delta^{15}N$ (‰)	Std Dev
Student's T					
I, S-a		**p=.02**		**p=.007**	
I, A		p=.29		**p=.02**	
S-a, A		**p=.02**		**p=.05**	
M, F		p=.50		p=.54	
R Only					
Infants	4	-16.2	1.57	9.4	1.66
Sub-adults	16	-18.5	1.12	7.6	1.28
Adults	19	-17.5	1.21	8.6	0.93
Males	11	-18.0	1.44	7.9	1.01
Females	12	-17.4	0.90	8.9	0.90
Student's T					
I, S-a		**p=.003**		**p=.03**	
I, A		p=.07		p=.18	
S-a, A		**p=.02**		**p=.01**	
M, F		p=.23		**p=.02**	
S Only					
Infants	6	-17.6	2.54	9.7	1.99
Sub-adults	9	-18.6	1.73	8.7	1.13
Adults	25	-17.7	1.65	8.5	1.19
Males	14	-17.8	1.71	8.7	1.23
Females	12	-17.8	1.61	8.2	1.15
Student's T					
I, S-a		p=.39		p=.24	
I, A		p=.87		p=.08	
S-a, A		p=.20		p=.78	
M,F		p=.97		p=.28	

Figure 3.1: Bivariate scattergram comparing $\delta^{13}C$ and $\delta^{15}N$ for R and S cemeteries.

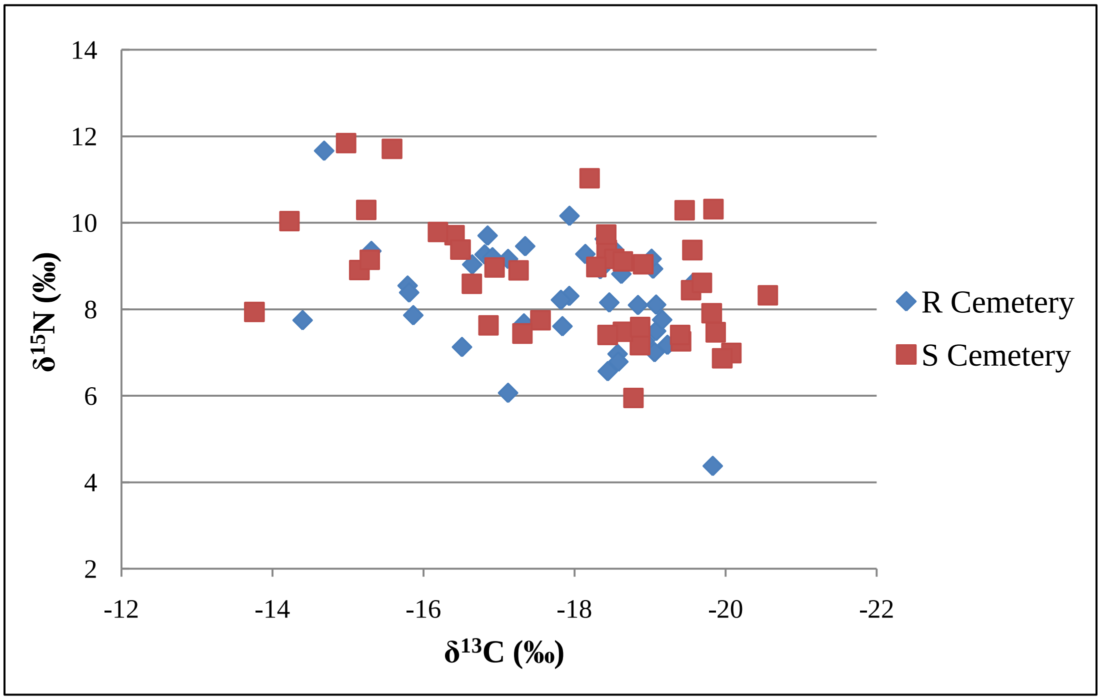
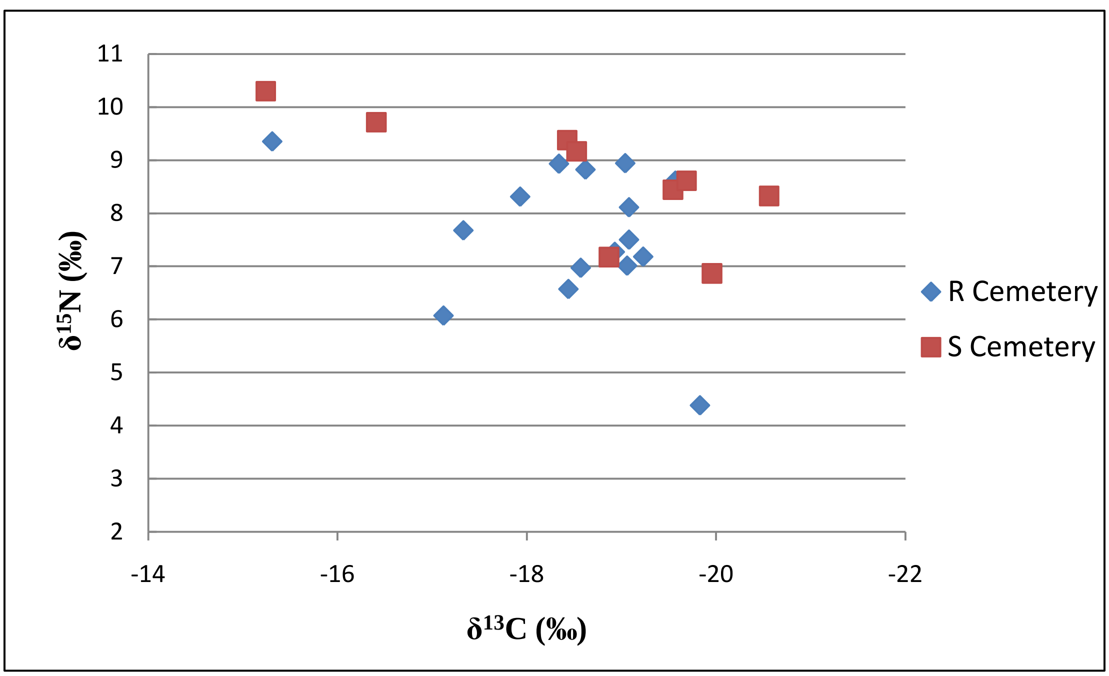


Figure 3.2: Bivariate scattergram comparing $\delta^{13}C$ and $\delta^{15}N$ for sub-adults in R and S cemeteries.



Variation by Age and Sex

When the data from cemeteries R and S are combined, infants are shown to have the most enriched $\delta^{13}C$ and $\delta^{15}N$ values of any age class (Figures 3.3 and 3.4). The mean $\delta^{13}C$ value for infants was found to be -17.0 ± .71‰ and the mean $\delta^{15}N$ value was found to be 9.6 ± .56‰. Conversely, sub-adults displayed the most depleted $\delta^{13}C$ and $\delta^{15}N$ values, with an average $\delta^{13}C$ value of -18.5 ± .27‰ and an average $\delta^{15}N$ value of 8.0 ± .26‰. The 1.5‰ differences in both carbon and nitrogen isotope values between infants and sub-adults were found to be significant (for $\delta^{13}C$, p=.02; for $\delta^{15}N$, p=.007). The calculated percentage of C_4 foods in the infant diet was 28.5% compared to 24.6% in the sub-adult diet. Adults displayed intermediate values, with an average $\delta^{13}C$ value of -17.6 ± .22‰ and an average $\delta^{15}N$ value of 8.6 ± .16‰. Differences between the means of sub-adults and adults were found to be significant (for $\delta^{13}C$, p=.02; for $\delta^{15}N$, p=.05). Only the $\delta^{15}N$ means were found to differ significantly between infants and adults (p=.02). Significant differences were not observed between males and females in either carbon or nitrogen isotope values.

Figure 3.3: Bivariate scattergram comparing $\delta^{13}C$ and $\delta^{15}N$ for infants, sub-adults, and adults.

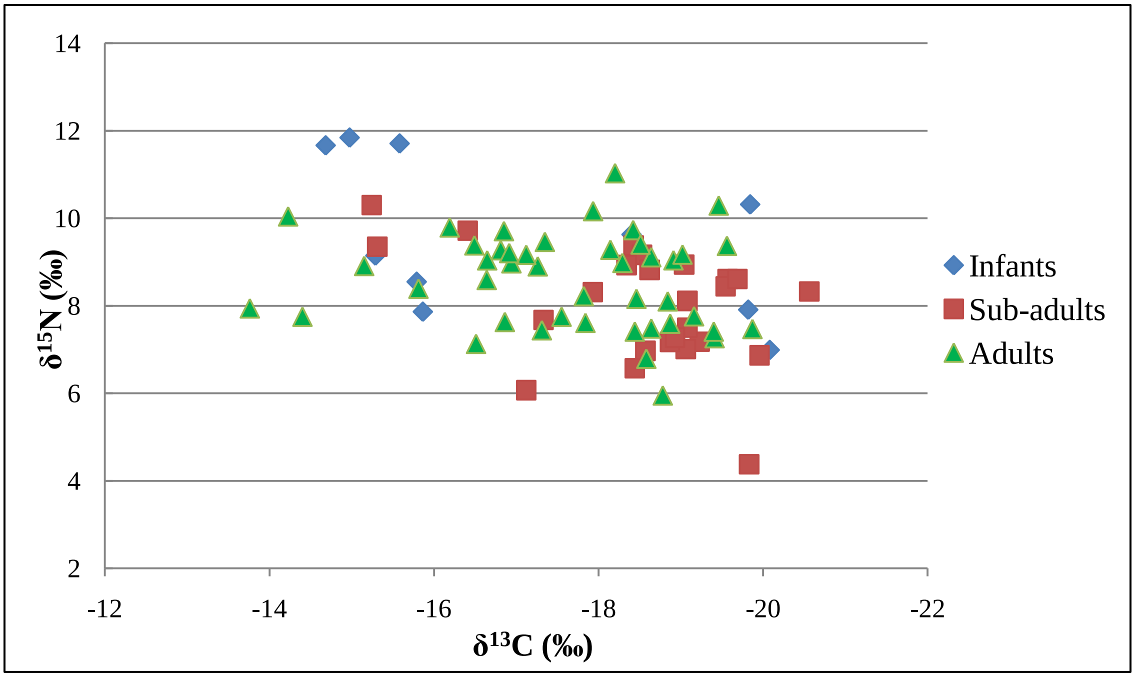
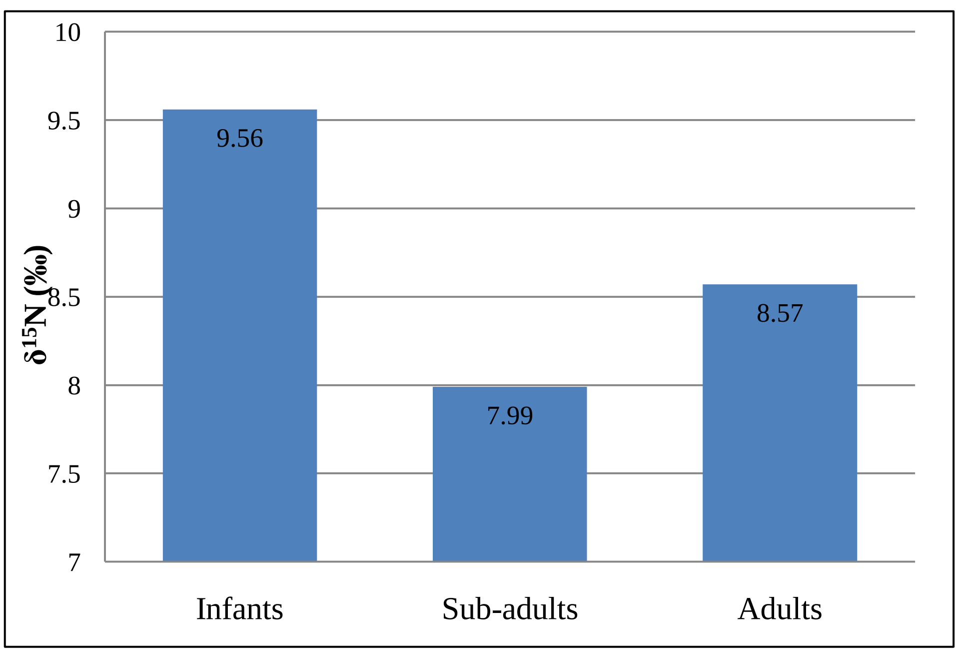
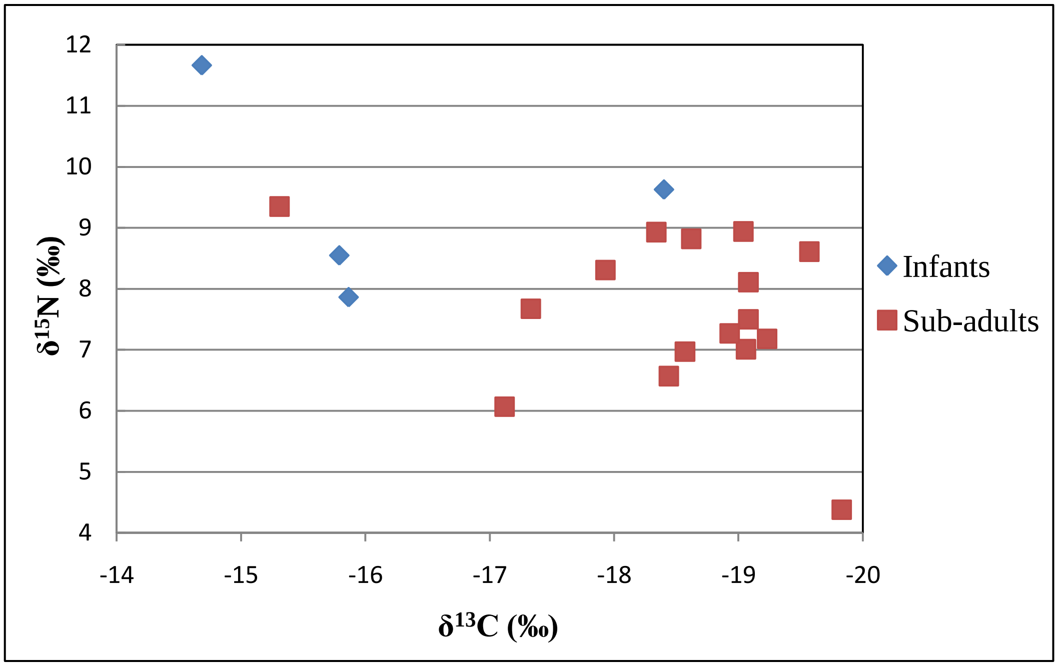


Figure 3.4: Comparison of $\delta^{15}N$ for different age classes.



A similar pattern is retained when cemeteries R and S are analyzed separately, as infants in both cemeteries display the most enriched $\delta^{13}C$ and $\delta^{15}N$ values relative to sub-adults and adults. However, the degree to which infants and sub-adults differ is greater in the R cemetery (Figure 3.5). The average $\delta^{13}C$ value for infants in the R cemetery is -16.2 ± .79‰, and the average $\delta^{15}N$ isotope value is 9.4 ± .83‰. For sub-adults, the mean $\delta^{13}C$ value is -18.5 ± .28 and the mean $\delta^{15}N$ value is 7.6 ± .32‰. Thus, when comparing averages, significant 2‰ and 1.8‰ enrichments were noted among infants in $\delta^{13}C$ and $\delta^{15}N$ respectively, relative to sub-adults (for $\delta^{13}C$, p=.003; for $\delta^{15}N$, p=.03). Infants were consuming 31% C_4 plants while sub-adults were consuming 25%. Sub-adults were also found to be depleted by about 1‰ in both $\delta^{13}C$ and $\delta^{15}N$ relative to adults (for $\delta^{13}C$, p=.02; for $\delta^{15}N$, p=.01). When comparing the males and females of cemetery R, $\delta^{13}C$ values did not differ significantly, but $\delta^{15}N$ values did. Males displayed a mean nitrogen isotope value of 7.9 ± .30‰ and females had a mean nitrogen isotope value of 8.9 ± .26‰ (p=.02). No significant differences were found in any sex or age category in the S cemetery.

Figure 3.5: Bivariate scattergram comparing $\delta^{13}C$ and $\delta^{15}N$ for R cemetery infants and sub-adults.



OXYGEN AND HYDROGEN

As discussed in Chapter Two, oxygen and hydrogen isotope values were determined for 29 rather than 79 individuals. Unfortunately, in this reduced sample size, there are no males and only one sub-adult representing R cemetery. This makes statistical analysis problematic, particularly when looking at R cemetery separately. The $\delta^{18}O$ and $\delta^{2}H$ values for both R and S cemeteries are provided in Table 3.4. For all 29 samples, the $\delta^{18}O$ mean is 20.0 ± .27‰ and the $\delta^{2}H$ mean is -31.4 ± 1.10‰. Summary statistics by cemetery, age, and sex are provided in Table 3.5.

Table 3.4: Oxygen and Hydrogen Isotope Data from R and S Cemeteries.

Burial	Sex	Age	$\delta^{18}O$ (‰)	$\delta^{2}H$ (‰)	H:O
R Cemetery					
44	F	36	19.7	-31.7	3.4
67	F	51+	21.3	-27.3	3.4
75	F	17+	21.2	-30.1	3.2
80	F	40	22.8	-21.3	3.4
93		10	21.8	-25.1	3.4
96		3	19.4	-24.5	3.4
109		birth	19.8	-32.6	3.3
160	F	17	21.2	-28.9	3.4
162	F	17+	21.1	-40.8	3.3
S Cemetery					
26		4	20.6	-29.7	3.3
28		7	20.9	-27.7	3.2
72		5 months IU*	19.1	-36.4	3.3
78		4	20.0	-31.0	3.3
81		3	18.3	-31.2	3.3
83		3	21.9	-26.9	3.3
94		infant	20.5	-31.0	3.4
96		5	19.1	-36.0	3.4
107	M	47	21.1	-31.6	3.2
115		3	20.9	-30.8	3.3
121		4	20.6	-35.5	3.3
123		6	20.3	-34.2	3.3
139	F	6	19.6	-34.4	3.3
168	M	47	17.1	-25.9	3.3
195		10	18.2	-30.5	3.3
196		3	18.6	-32.0	3.2
197		6	21.1	-28.5	3.2
204	F	19	17.8	-34.1	3.3
211	M	25	18.6	-28.2	3.4
225	M	47	17.4	-53.7	3.3

*indicates in utero

Table 3.5: Summary Statistics for Oxygen and Hydrogen Isotopes. Significant differences at α=.05 are bold.

	Count	$\delta^{18}O$ (‰)	Std Dev	$\delta^{2}H$ (‰)	Std Dev
All Individuals					
R	9	20.9	1.12	-29.1	5.68
S	20	19.6	1.41	-32.5	5.86
Student's T		**p=.02**		p=.17	
Infants					
R	2	19.6	.29	-28.5	5.72
S	6	19.9	1.44	-31.4	3.06
Student's T		p=.78		p=.37	
Sub-adults					
R	1	21.8	-	-25.1	-
S	9	20.1	.93	-31.9	3.13
Student's T		-		-	
Adults					
R	6	21.2	.99	-30.0	6.38
S	5	18.4	1.62	-34.7	11.10
Student's T		**p=.006**		p=.40	
Males					
R	-	-	-	-	-
S	4	18.5	1.83	-34.8	12.81
Student's T		-		-	
Females					
R	6	21.2	.99	-30.0	6.38
S	2	18.7	1.32	-34.2	.20
Student's T		**p=.03**		p=.41	
R & S Combined					
Infants	8	19.8	1.20	-30.7	3.60
Sub-adults	10	20.2	1.05	-31.3	3.66
Adults	11	19.9	1.93	-32.2	8.69
Males	4	18.5	1.83	-34.8	12.81
Females	8	20.6	1.51	-31.1	5.73

Table 3.5 Continued

	Count	$\delta^{18}O$ (‰)	Std Dev	$\delta^{2}H$ (‰)	Std Dev
Student's T					
I, S-a		p=.4		p=.74	
I, A		p=.88		p=.65	
S-a, A		p=.67		p=.76	
M, F		p=.06		p=.49	
R Only					
Infants	2	19.6	.29	-28.5	5.7
Sub-adults	1	21.8	-	-25.1	-
Adults	6	21.2	.99	-30.0	6.38
Males	-	-	-	-	-
Females	6	21.2	.99	-30.0	6.38
Student's T					
I, S-a		-		-	
I, A		p=.07		p=.78	
S-a, A		-		-	
M, F		-		-	
S Only					
Infants	6	19.9	1.44	-31.4	3.06
Sub-adults	9	20.1	0.93	-31.9	3.13
Adults	5	18.4	1.62	-34.7	11.10
Males	4	18.5	1.83	-34.8	12.81
Females	2	18.7	1.32	-34.2	0.20
Student's T					
I, S-a		p=.8		p=.7	
I, A		p=.1		p=.5	
S-a, A		**p=.0**		p=.5	
M,F		p=.91		p=.95	

Variation by Cemetery

Cemeteries R and S were found to differ significantly in $\delta^{18}O$, but not $\delta^{2}H$ (Figure 3.6), and the $\delta^{18}O$ and $\delta^{2}H$ data were found to be moderately correlated (Pearson's r=.42), though a stronger correlation would presumably be expected since $\delta^{18}O$ and $\delta^{2}H$ values of meteoric water often exhibit a tight correlation (Craig, 1961). For cemetery R, the mean $\delta^{18}O$ value was 20.9 ± .37‰, while it was 19.6 ± .32‰ for cemetery S (p=.02). Both oxygen and hydrogen isotope values were more enriched in cemetery R; this was driven primarily by the adults, all of which were females. Comparing the $\delta^{18}O$ values of R and S cemetery adults (means of 21.2 ± .40‰ and 18.4 ± .72‰, respectively), a significant 2.8‰ increase was noted in cemetery R (p=.006; Figure 3.7). The range of $\delta^{18}O$ values was not huge (5.7‰), however, the range of $\delta^{2}H$ values was extremely large (32.4‰), with two individuals in particular, S-225 and R-162 displaying hydrogen values considerably depleted below the mean (-53.7‰ and -40.8‰, respectively). However, no significant differences were observed with hydrogen isotope values among any age or sex category between R and S cemeteries.

Figure 3.6: Bivariate scattergram comparing $\delta^{18}O$ and $\delta^{2}H$ for R and S cemeteries.

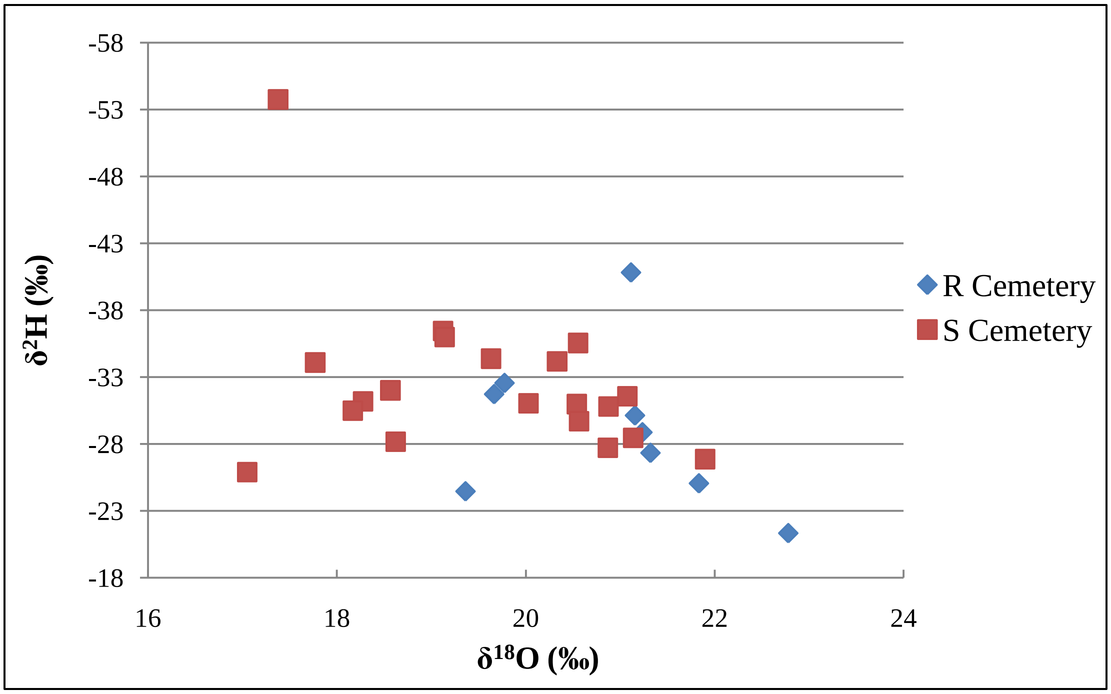
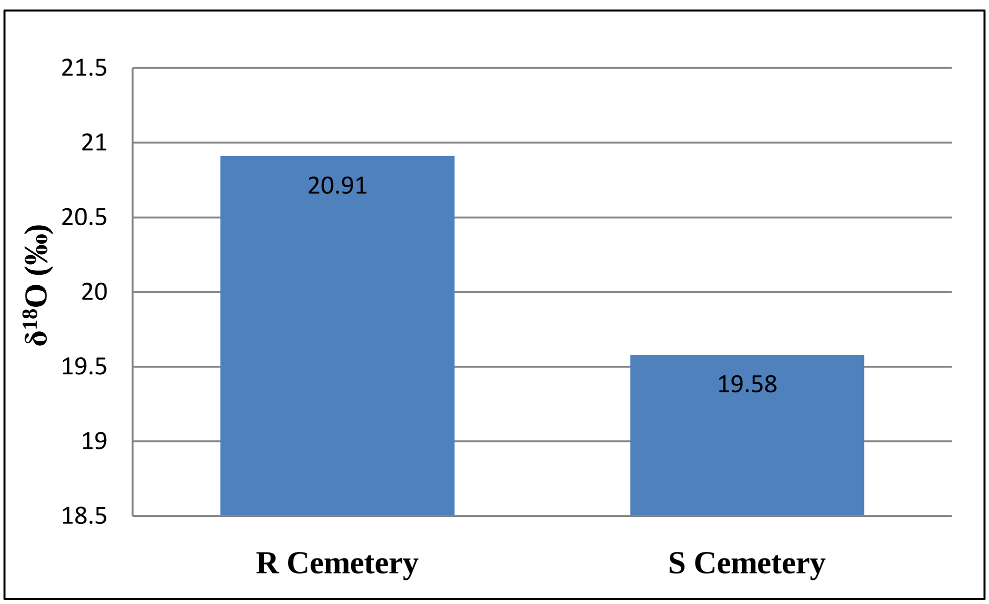


Figure 3.6: Comparison of $\delta^{18}O$ for R and S cemeteries.



Variation by Age and Sex

When the oxygen and hydrogen isotopic data are combined from cemeteries R and S, no significant differences are found among any age or sex category. In fact, the only relationship approaching significance is that of $\delta^{18}O$ values of males and females ($p=.06$). A 2‰ enrichment was noted among females, who displayed an average $\delta^{18}O$ value of 20.6 ± .53‰, while males had an average $\delta^{18}O$ value of 18.5 ± .92‰.

Treated separately, no significant differences were observed among the individuals of any age or sex category in R cemetery. However, thorough analysis was hindered by the fact that there were no males and only one sub-adult in the sample of n=9. In the S cemetery, the only significant difference was found when comparing the oxygen isotope means of sub-adults and adults. Sub-adults displayed an average $\delta^{18}O$ value of 20.0 ± .31‰, a 1.7‰ enrichment over adults with a mean $\delta^{18}O$ value of 18.4 ± .72‰. Although no significant differences were found among hydrogen isotope values of any age or sex category, extremely large standard deviations were observed with the adult and male groups.

CHAPTER FOUR

DISCUSSION

Decades of research on the remains of the Nubian Christians of Kulubnarti have demonstrated well-established patterns of health. Generalized indicators of stress have shown that the individuals of the S cemetery, believed to date to the Early Christian period, A.D. 600-850, were exposed to more stress relative to those from the R cemetery, believed to date to the Late Christian period, A.D. 1100-1400 (Van Gerven et al., 1995). It also appears that the sub-adult years were particularly stressful for the individuals at Kulubnarti. The present isotopic investigation will take the established patterns of health into consideration, and will seek to examine the degree to which the isotope data from hair reflect findings from past research. In particular, information gleaned from the carbon and nitrogen isotopic data may be able to confirm whether observed health differences between cemeteries and age and sex categories have a dietary component, or if there is some other, unknown contributing factor. As the diachronicity of the two cemeteries has also been challenged, oxygen and hydrogen isotopic data may be able to provide support for one view over another.

And finally, since stable isotopic research has previously been conducted on individuals from the Kulubnarti collection (Turner et al., 2007; Sandberg, 2006), the results from the analysis of bone collagen, bone apatite carbonate, and enamel bioapatite will be compared to those from the current investigation.

Carbon and Nitrogen Variation by Cemetery

Upon examining the $\delta^{13}C$ results, no statistically significant differences were noted in any age or sex category. However, a very slight yet consistent depletion was noted among the individuals of the S cemetery relative to those from the R cemetery. With the $\delta^{15}N$ data, one cemetery was not found to be more enriched or depleted than the other in every age or sex category. A significant difference was observed between the sub-adults of R and S cemeteries, with the S sub-adults showing about a 1‰ enrichment relative to the R sub-adults ($p=.05$). As the difference between the two cemeteries is extremely small, it is debatable whether or not diet may have contributed to the differential patterns of stress observed osteologically.

In Nubia, the carbon isotope value of diet can vary due to the shifting availability of C_3 and C_4 plants. Both now and in the past, when temperatures are cooler, wheat, barley, and many fruits and vegetables are consumed. However, in warmer periods, hardier, drought-resistant plants are grown for consumption, such as millet and sorghum, which supplement the staples of wheat and barley (White, 1993). From the isotopic analysis of hair alone, it would appear that the individuals from R and S cemeteries consumed almost identical diets, with the individuals from R cemetery consuming very slightly more C_4 resources than those from the S cemetery. This observed dietary uniformity would likely be expected if Kulubnarti was an egalitarian village devoid of social class differentiation as suggested by Adams about other similar villages in the Batn el Hajar (1977).

It could be argued that as the two cemeteries are believed to date to different time periods, the difference in diet shown by the isotope data would be expected to be much greater. However, it is also quite likely that from the Early Christian period to the Late

Christian period at Kulubnarti, a significant change in diet would not be possible since individuals throughout the entirety of the Christian period would be constrained by the same ecological and cultural parameters that determine food availability.

Carbon and Nitrogen Variation by Age and Sex

When the $\delta^{13}C$ and $\delta^{15}N$ data are combined from cemeteries R and S, more interesting comparisons are seen, particularly between differing age classes. Among the infants, an average $\delta^{13}C$ value was observed of -17.03 ± .71‰, which corresponds to a diet composed of roughly 28.5% C_4 resources, the largest C_4 dietary component of any age or sex category. Sub-adults were found to consume the smallest amount of C_4 plants, with an average $\delta^{13}C$ value of -18.51 ± .27‰ that corresponds to 24.6% C_4 resources. This difference between infants and sub-adults was found to be statistically significant (p=.02), however the actual difference is still quite small. Adults consumed an intermediate amount of C_4 plants relative to infants and sub-adults, with an average $\delta^{13}C$ value of -17.64 ± .22‰, which indicates a C_4 dietary component of 26.9%. Adults and sub-adults were also found to be significantly different (p=.02), though infants and adults were not found to differ significantly.

The fact that infants displayed the most positive $\delta^{13}C$ values may not be unexpected if the weaning process had begun for some of the infants in the sample, as it is plausible that C_4 foods, which can be consumed in soft, pasty preparations such as millet gruel, would be used to supplement breastfeeding. However, it is also entirely possible that C_3 grains could be used as a weanling food, and it might be expected that $\delta^{13}C$ values among infants would be more positive to a greater degree than observed if millet gruel was the preferred weanling food.

Based on the $\delta^{15}N$ data, infants were also the most enriched of any age or sex category, displaying significant increases of 1.5‰ over sub-adults, and 1‰ over adults ($p=.007$ and $p=.02$, respectively). Sub-adults were also observed to have a statistically significant 0.5‰ depletion relative to adults ($p=.05$). The fact that infants have higher $\delta^{15}N$ values relative to both sub-adults and adults is not surprising due to the trophic level effect associated with breastfeeding. In previous isotopic studies, a larger 2-3‰ increase of breastfeeding infants over mothers has been noted (Fuller et al., 2006). However, a very wide range of $\delta^{15}N$ values were observed among the Kulubnarti infants (6.99‰ to 11.85‰), with both the lowest and highest values coming from three-year-olds, the age at which weaning is thought to have occurred at the Roman period site in Egypt where similar foods would likely have been available (Dupras and Tocheri, 2007). This may indicate variability in the timing of weaning, as the lowest value could reflect an individual who is no longer breastfeeding. An alternative explanation for the variability in $\delta^{15}N$ values is that different families fed their children different foods, perhaps a reflection of differing social statuses or access to resources. However, if those individuals at the upper end of the infant age class (0-3 years) were placed into the sub-adult category, a larger enrichment more in line with other research would be seen in the comparison of infants to adults. In the years following weaning, sub-adults probably consumed primarily low protein, cereal gruels and little animal protein, thus resulting in relatively depleted $\delta^{15}N$ values.

When treating the data from the two cemeteries separately, it is evident that the significant differences between age categories discussed above are driven by differences occurring primarily in the R cemetery. No significant differences in $\delta^{13}C$ or $\delta^{15}N$ values were noted in the S cemetery between any age or sex category. It would appear from the isotopic data that the individuals from the R cemetery, believed to date from the Late Christian period, had more overall variability in diet whereas individuals from S cemetery of all ages and both sexes consumed fairly similar diets.

Though males and females were not found to vary significantly in their $\delta^{13}C$ or $\delta^{15}N$ values when the data from R and S cemeteries were combined, a significant increase of 1‰ was observed among the females of R cemetery (p=.02). However, this was driven primarily by the fact that four of the 11 males of cemetery R were sub-adults, while all 12 of the females were adults. When the sub-adult males are omitted to compare only adult males and females, the difference is no longer significant (p=.10). This indicates that the males and females at Kulubnarti shared a relatively similar diet throughout the Early and Late Christian periods. As previous research has demonstrated that female life expectancy exceeded that of males by 19% in childhood, and that females were more likely to survive than males in all age categories (Sheridan and Van Gerven, 1997), isotopic findings suggest there is a physiological rather than a dietary component which explains this greater female resilience at Kulubnarti.

Oxygen and Hydrogen Variation by Cemetery

Based on the $\delta^{18}O$ results, a consistent pattern of R cemetery being more enriched than S cemetery is revealed. Among all individuals from the two cemeteries, adults, and females, this difference is significant (p=.02, p=.006, and p=.03, respectively). However, as there were no males and only a single sub-adult from the R cemetery sample, complete statistical analysis was precluded, and it is evident that the differences among females of the two cemeteries drove the significant differences found in other categories. The ranges of $\delta^{18}O$ values found in each cemetery were fairly similar in terms of size, with R values ranging from 19.36 to 22.78‰ and S values ranging from 17.05 to 21.90‰. Nine individuals of the total S cemetery sample (n=20) had depleted values relative to the most depleted individual of the R cemetery sample (n=9), suggesting there is a real, though perhaps subtle, difference between the two cemeteries. This difference may be related to regional changes in aridity which may have occurred between the Early and Late Christian periods (Iacumin et al., 1996), or else fluctuating Nile river levels.

When looking at the δ^2H values, a pattern similar to that of the $\delta^{18}O$ values is observed, with the R cemetery individuals being consistently more enriched relative to individuals from the S cemetery. With the hydrogen isotope values, however, no significant differences were revealed between R and S cemeteries. What is most interesting about the δ^2H data though is the enormous breadth of values observed within each cemetery. For the R cemetery, values ranged from -21.3‰ to -40.8‰, while in the S cemetery, values ranged from -25.9‰ to -53.7‰. The two individuals exhibiting the greatest depletion in δ^2H values (R-162 and S-225), appear to be outliers when compared to the other individuals within their particular cemeteries. If these two outliers, -40.8‰ and -53.7‰, are removed from analysis, the average δ^2H values for R and S cemeteries become a significantly different -27.2 ± 1.37‰ and -31.3 ± .72‰, respectively (p=.02). Additionally, the ranges of δ^2H values for each cemetery are reduced to a 10‰ span which is conceivably attributable to fluctuating levels of the Nile River which would have been the primary source of drinking water, water used in cooking, and water used for the irrigation of plants.

One possible interpretation of the two outliers is that they were newly-arrived foreigners in Kulubnarti. If this were the case, it might be reasonably expected that these two individuals' $\delta^{18}O$ values would also appear as outliers relative to the other individuals in the R and S samples. This is not true with individual R-162, whose $\delta^{18}O$ value is only 0.2‰ higher relative to the cemetery average. S-225, on the other hand, may in fact represent a foreign presence at Kulubnarti as their $\delta^{18}O$ value is over 2‰ lower relative to the cemetery mean.

A more plausible explanation, however, may be that the hair samples from individuals R-162 and S-225 were degraded, causing a large degree of hydrogen exchange to occur during laboratory sample preparation (Sharp et al., 2003). Carbon, nitrogen, and oxygen isotopes have been found to be more robust and resistant to degradation than hydrogen (O'Connell, 2005). Although these two samples produced $\delta^{13}C$ and $\delta^{15}N$ values in line with the other samples analyzed, the C:N ratios for R-162 and S-225 (4.0 and 4.2, respectively) were also the furthest outside the acceptable range of values established from studies of modern hair (3.0-3.8; O'Connell and Hedges, 1999). Certainly, the $\delta^{2}H$ data from these two individuals should be excluded from analysis, and it may be argued that the same should be done with the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ data from these two individuals.

Although there is not a wealth of archaeological research which has utilized hydrogen isotope studies of hair, it has been found that roughly 10% of the total amount of hydrogen atoms in hair remain exchangeable with water vapor after hair is initially produced (Bowen et al., 2005). Thus, if the same sample of hair was tested in a number of different isotope facilities around the world, results could differ significantly. This is one potential source of error to consider when interpreting the hydrogen isotope data.

Oxygen and Hydrogen Variation by Age and Sex

When the $\delta^{18}O$ and $\delta^{2}H$ data from both cemeteries are pooled, no significant differences are observed between any age or sex category. Interestingly, infants display the least enriched $\delta^{18}O$ values, which is unexpected. Similar to the interpretation of $\delta^{15}N$ values, a trophic level effect has been observed with the $\delta^{18}O$ values of breastfeeding infants (White et al., 2004). A mother's milk is the main source of water for infants, and as a result, an enrichment of 2‰ has been found in infants relative to older age groups. The Kulubnarti females had the most enriched $\delta^{18}O$ values, which also may not be expected as women who are breastfeeding are subject to high water flux rates which result in lower oxygen isotope values (Kohn et al., 1996). When the $\delta^{18}O$ data for the S cemetery only are examined, the one female of reproductive age does show a 2‰ depletion relative to the infant mean, though a sample of one does not yield robust analysis. As significant differences were not found among any age or sex categories, it seems that individuals at Kulubnarti primarily consumed water from the same source, as would be anticipated.

The Diachronicity of Cemeteries R and S

During the Late Christian period in Nubia, the Batn el Hajar experienced a degree of population influx from the north. It is thought that Nile River levels were elevated during the 11th and 12th centuries, and immigrants may have resettled in the south to escape flooded areas (Van Gerven et al., 1995). It may also be possible that population movements were politically motivated, as the spread of Islam caused Christians fleeing persecution to seek refuge in the isolated Batn el Hajar (Adams, 1977). Whatever the exact reasons for immigration, it is likely that Kulubnarti would have been an area where population would have increased. As the majority of people living in the settled areas in Nubia would be obtaining their water from the Nile, it is important to note that $\delta^{18}O$ values vary along the course of the river, with more enriched values being observed downstream and less enriched values being observed upstream in modern times (White et al., 2004). This trend would have been the same during the Christian period, though fluctuating river levels would cause varying isotopic values.

It would be expected that the Late Christian population, thought to be represented by the R cemetery, would have more variability in oxygen and hydrogen isotopes to reflect the new, foreign presence at Kulubnarti. However, in both the R and S cemeteries' $\delta^{18}O$ data, values were fairly uniform in terms of the size of the ranges, with the R cemetery being consistently enriched relative to the S cemetery. The $\delta^{2}H$ data also reflected this trend with the size of ranges being similar, though both had larger variability than observed with the $\delta^{18}O$ data. Since hair was the tissue analyzed in this investigation, it may be that the short period of time of a month or two prior to death represented by a segment of hair was not enough to capture the presence of foreigners at Kulubnarti. Ideally, an isotopic analysis of multiple tissues representative of different stages of life—such as dental enamel, bone collagen, and hair—would be the best way to identify mobility and study life histories within an archaeological population (Katzenberg, 2007; Tykot et al., 1996; Sealy and Schrire, 1995).

Since the difference in the isotopic values of oxygen and hydrogen were consistent across all age and sex categories, the hypothesis that R and S cemeteries are not representative of the same time period is supported by this investigation.

Comparison to Other Stable Isotope Research

Previous isotopic research has been conducted on the Kulubnarti collection which focused on $\delta^{13}C$ and $\delta^{15}N$ ratios from bone collagen, as well as $\delta^{13}C$ and $\delta^{18}O$ from enamel bioapatite (Sandberg, 2006). In a separate study, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values of bone collagen and bone apatite were also analyzed (Turner et al., 2007). In agreement with the isotopic analysis of hair performed in the current study, R and S cemeteries were not found to differ significantly based on the carbon and nitrogen isotope ratios from tissues analyzed previously.

As all of the available isotopic data is in agreement, it is thus very likely that differential patterns of health at Kulubnarti are not related to diet.

Though the isotopic analysis of hair, bone collagen, and enamel bioapatite yielded similar results when reconstructing aspects of diet, differing results were found when comparing the oxygen isotope ratios of hair and enamel bioapatite. Individuals from the R cemetery were shown to be fairly consistently and significantly enriched in $\delta^{18}O$ (and $\delta^{2}H$) from the analysis of hair ($p=.02$), whereas individuals from the S cemetery were found to be significantly enriched in samples of enamel bioapatite ($p=.02$). Although this data suggests a real difference in $\delta^{18}O$ values which could indicate cemeteries R and S date to different time periods, the data from Turner et al. (2007), which benefits from a larger sample size ($n=89$ for oxygen isotope data) than either of the other data sets, indicated there was no significant difference between the two cemeteries, and that an Early Christian period date for both cemeteries is likely. This idea has very recently been confirmed by radiocarbon dating, the results of which were obtained following the conclusion of this investigation.

Other isotopic investigations which have been carried out on Christian period Nubian remains provide logical comparisons for the data generated in this research. Christine White's work at Wadi Halfa, however, is the only other study which utilized hair as a tissue for analysis (1993). The mean $\delta^{13}C$ value at Kulubnarti was found to be less than 1‰ more negative relative to that at Wadi Halfa. However, the sample size in the present investigation is $n=79$, whereas the Wadi Halfa average is derived from an $n=5$, which may be too small of a sample size to make any meaningful comparison. Bone collagen data was also produced from individuals at Wadi Halfa, with an average $\delta^{13}C$ value found to be 1‰ more negative relative to the Kulubnarti hair data (White and Schwarcz, 1994). This is interesting since at Kulubnarti, hair values were lower than those generated from bone collagen due to fractionation differences. It would appear, however, on the basis of carbon isotope values alone, that diets are fairly similar at Wadi Halfa and Kulubnarti.

CHAPTER FIVE

CONCLUSIONS

The stable isotope analysis conducted for this investigation intended to provide an alternative approach to evaluating differences between two cemetery populations from the Christian period site of Kulubnarti in Sudanese Nubia. Since 1979, a large body of research has revealed consistent health differences between cemeteries S and R, once thought to be representative of diachronic, Early and Late Christian period populations. The exact etiology of these differences has remained unknown, though dietary stress has been suggested as one possible cause. Stable isotope analysis allows for the quantitative comparison of aspects of diet between the two cemeteries, and thus can be used to determine the role of diet in producing observed patterns of stress. Resulting patterns of isotopic variability were not found to correlate with the previously established patterns of health. Although some extremely slight dietary differences between cemeteries R and S may have existed as suggested by the isotopic values from hair, it is unlikely that they contributed to the origin of differential health patterns at Kulubnarti.

Even though individuals from the R and S cemeteries were not found to have any significant differences between $\delta^{13}C$ values, a fairly consistent pattern emerged from the data with those from R cemetery displaying more enriched values relative to those from S cemetery. This mirrors the pattern of observed stress markers, however, $\delta^{15}N$ values do not provide such a clear trend. Adults in the R cemetery were found to be slightly more enriched, though the difference was not approaching significance. As the sub-adult years are thought to have been the most stressful critical period for individuals at Kulubnarti (Van Gerven et al., 1981; Van Gerven et al., 1995), adults may have the capacity to reveal the most about conditions at Kulubnarti since they lived through the most stressful period, surviving into their reproductive years. And, as males are thought to experience a heightened susceptibility to stress than females (Sheridan and Van Gerven, 1997), it may be hypothesized that they will reflect differing conditions of health to a greater degree. However, when comparing only adult males from cemeteries R and S (n=7, n=14), $\delta^{13}C$ values are virtually identical (p=.99), which suggests food availability and consumption was very similar among all of the individuals from Kulubnarti. No statistically significant differences were noted in carbon or nitrogen isotope values between males and females, which indicates males and females consumed an isotopically similar diet. Ultimately, if diet was a contributing factor affecting levels of health and disease at Kulubnarti, any difference, even minor, between any age or sex category of individuals between the Early and Late Christian period cemeteries would be expected.

The only significant difference observed between cemeteries R and S occurred among the $\delta^{15}N$ isotopic values of sub-adults. It has been well-established that childhood was a stressful time of life throughout the Christian period at Kulubnarti. It is perhaps unexpected then, that sub-adults from the S cemetery were found to be enriched by 1‰ relative to sub-adults from the R cemetery, and displayed a mean value higher than adults in either cemetery. This may suggest that the sub-adults in the Early Christian period consumed a diet relatively high in protein, which is surprising since lower nitrogen ratios among sub-adults were observed previously at Kulubnarti (Turner et al., 2007; Sandberg, 2006), and also in nearby Wadi Halfa (White et al., 1994). Alternatively, the S cemetery data could also be interpreted as evidence of nutritional stress during growth which has been shown to result in protein catabolism leading to increased $\delta^{15}N$ values (Hobson et al., 1993). This interpretation would be more in agreement with the osteologically observed patterns of stress in cemetery S. Beyond comparing the means between cemeteries or age groups, however, one particularly interesting result which emerged from the nitrogen isotope data from the infants and sub-adults is the large, 6‰ spread in $\delta^{15}N$ values. This is suggestive of something beyond a trophic level shift, perhaps physiological differences, which can produce this wide range of values among the children at Kulubnarti.

Oxygen and hydrogen isotopes were used in this investigation to evaluate the dating of R and S cemeteries. From archaeological excavations and the analysis of the associated material culture, it was originally determined that S cemetery dates to the Early Christian period while R cemetery dates to the Late Christian period. However, a more recent analysis of textiles has suggested the two cemeteries were used contemporaneously in the Early Christian period (Adams, 1999a), an idea supported by isotopic data from Turner et al. (2007). From the isotopic analysis of hair alone though, oxygen and hydrogen isotope data are consistent with the belief that the two cemeteries were used in different periods of time. Since the completion of this thesis, however, radiocarbon dating has shown that the two cemeteries at Kulubnarti are representative of contemporaneous, Early Christian populations.

Overall, the stable isotope analysis conducted for this research project was designed to evaluate the role of diet in producing the observed patterns of health at Kulubnarti. Though it appears that some extremely minor dietary differences may have distinguished the individuals from the two cemeteries, it can be argued that differences were not great enough to produce the differential patterns of health observed osteologically. As it is now known that individuals from cemeteries R and S date to the same time period, and consumed relatively similar diets, perhaps new hypotheses will emerge to explain the osteologically observed differential patterns of stress.

Building on this current investigation, however, an interesting direction for future research with the Kulubnarti collection would be the segmental isotopic analysis of hair to determine how seasonal changes in diet may be related to mortality at Kulubnarti. Obtaining multi-season isotopic profiles on a large number of individuals may be challenging since the majority of the remains do not possess hair longer than a couple of inches. However, there are some individuals in the collection that would permit this type of analysis and the results would be an interesting addition to the large body of research already performed on the individuals from Kulubnarti. It is also clear from this investigation, as well as previous work, that infants and sub-adults yield the most interesting isotopic data and they should therefore be a focus of future studies.

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