Age-related Variation in Isotopic Indicators of Diet at Medieval Kulubnarti, Sudanese Nubia

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ABSTRACT This study compares trends in dietary composition in two large cemetery populations from the site of Kulubnarti (AD 550–800) in Sudanese Nubia. Bone collagen and bone apatite carbonate were analysed to characterise stable carbon, nitrogen and oxygen isotopes. Previous research on these cemeteries has suggested marked differences in nutritional status and health between the populations. Contrary to expectations, there were no significant relationships between any isotopic indicators related to sex or cemetery of burial, suggesting no isotopically-measurable differences in diet. However, collagen $\delta^{13}C$ and $\delta^{15}N$ were significantly related to age, suggesting age-related differences in protein intake or other factors.

Weaning trends are gradual and variable, with the range in $\delta^{15}N$ values exceeding 4% among infants/young children (0–3 yrs) and standard deviations exceeding 1% in collagen $\delta^{13}C$ and $\delta^{15}N$ for both infants/young children and subadults (4–17 yrs). This suggests varied weaning strategies among both populations and variable diets prior to adulthood. Also observed was a distinct range of isotopic carbon and nitrogen values among individuals classified as subadults (4–17 yrs), who are depleted in collagen $\delta^{13}C$ and $\delta^{15}N$ relative to adults. However, both infants/young children and subadults are slightly enriched in $\delta^{18}O$ relative to adults, which suggests the presence of non-local individuals or age-related variation in water sources. While most isotopic studies of age-related dietary trends have focused on reconstructing the weaning process, this study presents findings that indicate tripartite isotopic trends distinguishing infancy, subadulthood and adulthood as separate dietary categories. Broad similarities are evident between the results presented here and those from several earlier studies of smaller populations and to nutritional studies of modern communities. These findings suggest that further research into health disparities at Kulubnarti should focus on non-dietary causal factors, and more generally, that greater attention should be paid to subadulthood in palaeodiet studies. Copyright © 2006 John Wiley & Sons, Ltd.

Key words: Kulubnarti; weaning; subadult; diet; Nubia; isotopes; collagen; apatite

Introduction

Isotopic reconstructions of palaeodiet in archaeological populations constitute an important area of bioarchaeological research, utilising biochemical measures of the relative importance of constituent food types to individual diets (DeNiro & Schoeninger, 1983; Schwarcz & Schoeninger, 1991; Katzenberg, 1992; Schoeninger & Moore, 1992; Ambrose, 1993; Katzenberg et al., 1995; Fogel et al., 1997). Such techniques provide insights into patterns of subsistence and resource
utilisation, and measure inter- and intra-group variation in dietary composition, such as the relative abundance of different forms of protein and carbohydrate in the overall diet (reviewed in Katzenberg & Harrison, 1997).

Palaeodietary analyses using stable isotopes have grown increasingly sophisticated with the refinement of analytical techniques, increased resolution and greater understanding of the complex and variable cycling of stable isotopes in biological systems. Carbon isotopes in bone and tooth apatite, expressed as $\delta^{13}C_{\text{AP}}$, generally reflect the relative abundance of $^{13}$C/$^{12}$C in the overall diet (Ambrose & Norr, 1993). Carbon and nitrogen isotope ratios in bone collagen, expressed respectively as $\delta^{13}C_{\text{COL}}$ and $\delta^{15}N$, primarily reflect the contribution and source of dietary protein. Nitrogen isotopes also reflect trophic-level effects in protein intake associated with the position of organisms in food webs (Lee-Thorp et al., 1989; Ambrose & Norr, 1993; Ambrose et al., 1997). Calculated offsets between apatite and collagen $\delta^{13}C$ ($\delta^{13}C_{\text{AP}} - \delta^{13}C_{\text{COL}}$, i.e. $\Delta_{\text{AP,COL}}$) estimate the relative dependence on animal versus vegetable protein and is used to measure the extent of carnivory in the diet or changes in dietary macronutrients (Lee-Thorp et al., 1989). Early interpretations of these offsets suggested that values of 4% or less indicated diets in which the bulk of carbon was drawn from animal protein, indicating a more carnivorous subsistence pattern, while values of 6–7% indicated a more herbivorous diet, and values intermediate between the two indicated an omnivorous diet (Krueger & Sullivan, 1984). More recent interpretations, however, suggest that dietary differences only account for part of this herbivore–carnivore effect and must be considered along with processes such as methanogenesis and bone formation (Hedges, 2003), pointing to the need to consider metabolic variation across taxa when interpreting offset values. From these premises, carbon and nitrogen isotopes have been used to explore further the trophic-level effects between isotopic signatures in tissues of consumers and their prey (Sullivan & Krueger, 1983), the metabolic effects of water stress (Ambrose, 1991), and human subsistence variation in a wide variety of ecological contexts (Larsen et al., 1992; Pate, 1997; Katzenberg & Weber, 1999; Auferheide & Santoro, 1999, White et al., 2001; Richards et al., 2003; Papatheodoriou, 2003; Yoneda et al., 2004). Oxygen isotopes in bone and enamel apatite, expressed as $\delta^{18}O$, reflect the isotopic composition of sources of consumed water, and can be used to reconstruct seasonality, geographical origin and patterns of migration in several species of animals (Koch et al., 1989; Kohn et al., 1998; Gadbury et al., 2000) and humans (Fricke et al., 1995; White et al., 1998, 2002).

Isotopic markers of dietary composition related to developmental age or assigned sex are useful in discussing less tangible cultural characteristics such as gender variation in diet (Reinhard et al., 1994) and constructions of different life stages within a context of differential subsistence strategies. An example is the distinct range of isotopic values commonly found in the tissues of infants who subsist primarily on protein sources such as breastmilk or herbivore milk during the first months of life. Preserved infant tissues commonly show an enrichment of $\delta^{13}N$ and $\delta^{18}O$ in their skeletal tissues by as much as 3–5% relative to the tissues of juveniles and adults (Jenkins et al., 2001; Bocherens & Drucker, 2003), reflecting trophic-level fractionation effects between the maternal diet, breastmilk and infant tissues. Population-specific weaning trends in humans and other animals can be characterised isotopically as the introduction of supplementary foods through enrichment in $\delta^{13}C$ and the cessation of breastfeeding through depletion of $\delta^{15}N$ as the diet becomes more like that of associated adults (Katzenberg, 1992; Schoeninger, 1995; Katzenberg et al., 1996; Balasse et al., 2001). Recently, $\delta^{18}O$ has been characterised in human enamel carbonate to track changes in water intake associated with the weaning process, as infants are supplemented with water directly after getting their water exclusively from maternal milk during breastfeeding (Wright & Schwarcz, 1998, 1999).

The importance of infant and weanling diets as correlated to short-term and long-term health outcomes is well documented in clinical literature (Cunningham, 1995; Oddy, 2002), and the timing of the process as well as the composition of weaning foods often influences rates of infant morbidity and mortality (McDade & Worthman, 2006 John Wiley & Sons, Ltd. Int. J. Osteoarchaeol. 17: 1–25 (2007)
Isotopic studies of archaeological populations have demonstrated shifts in $\delta^{15}$N and $\delta^{13}$C among the remains of the very young and allowed researchers to estimate the timing and nature of introduced supplemental foods (Katzenberg et al., 1996; Schurr, 1997; Herring et al., 1998; Dupras et al., 2001). Such studies have brought new dimensions to studies of the detrimental effects of dietary change in archaeological populations, measured primarily through analyses of enamel hypoplasia and other dental pathology (Katzenberg et al., 1996).

To that end, this study seeks to identify and describe isotopic weaning trends in two large cemetery populations ($n = 137$) from the Kulubnarti archaeological site in modern-day Sudan (Figure 1). The samples are from riverine cemeteries less than 1 km apart, both dated to AD 550–800 and encompassing the Early Christian phase of the medieval period (Adams et al., 1999). Previous studies have suggested differential nutritional and occupational stress between the two samples, possibly reflecting differential status, based on pathological indicators such as cribra orbitalia and reduced cortical thickness (Mittler & Van Gerven, 1994; Van Gerven et al., 1995). The non-specific nature of these indicators and the number of possible causes means that the extent to which these indicators are the result of variations in diet is unclear. Therefore, this study also seeks to test the hypothesis that the two cemetery populations differed in their dietary

Figure 1. Map of Nubia.

composition, a critical parameter in assessing metabolic stress variation between the two populations. In doing so, this study is the first to directly investigate age-related patterning of dietary composition across both Kulubnarti cemetery samples, which includes large numbers of well-preserved infants/young children, juveniles and adults (Figure 2).

The data generated in this study also reveals isotopic variation at Kulubnarti that suggests dietary patterns among subadults distinct from infants/young children and adults, with several possible interpretations. A less-recognised potential dietary pattern in bioarchaeological literature is the post-weaning, juvenile diet as a separate category, with its own cultural implications and health outcomes. Ethnographic data from extant populations have identified distinct, ‘childhood’ diets in several cultures resulting from differential food allocation within households (Gittelsohn et al., 1998; Luo et al., 2001; Adams, 1977; Graham, 1997; Messer, 1997). It is uncertain as to how household food allocation varies cross-culturally, but it is reasonable to assume that such patterns could have existed in archaic societies as well. Studies of catch-up growth and malnutrition-related health consequences in children (Martorell, 1989, 1995) illustrate the important effects that dietary composition and nutritional status during childhood have on morbidity, mortality and health later in life. Dietary reconstruction among both younger and older subadults in archaeological populations may therefore critically inform population-level analyses of resource access and health outcomes, and such a reconstruction is presented here.

Isotopic markers of age-related dietary changes such as weaning were assessed using analyses of the stable isotopic compositions of carbon and nitrogen in bone collagen ($\delta^{13}$C$_{COL}$, $\delta^{15}$N) and oxygen and carbon in apatite ($\delta^{18}$O$_{AP}$, $\delta^{13}$C$_{AP}$). These values were used to reconstruct individual- and group-level variation in dietary composition and identify predictors of isotopic ranges such as age, sex and cemetery of interment.

![Figure 2. Age distribution of individuals analysed isotopically.](image-url)
The Kulubnarti human remains

The two Kulubnarti skeletal populations were interred in nearby cemeteries located in the hyperarid region known as the Batn el-Hajar, or ‘Belly of Rock’, between the second and Dal cataracts of the Nile River in modern-day Sudan (Figure 1). This marginal region is characterised by poorly-developed soils and massive granite outcrops, with limited riverine maritime activity due to rapids. Despite this inhospitable environment, the Batn el-Hajar has for centuries supported scattered populations based in small villages and hamlets clustered around the region’s few fertile floodplains. The Kulubnarti site encompasses both cemeteries as well as settlements on the western bank of the Nile and on a small island close to the shore, adjacent to the modern village of Kulb (Adams, 1977; Adams, et al., 1999). The two populations are designated 21-R-2, or the mainland population, and 21-S-46, the island population, and are respectively referred to herein as the R and S cemeteries.

Based on associated architectural features and burial styles, Adams (1977) and Van Gerven et al. (1981, 1995) originally regarded the S cemetery sample as chronologically precedent (AD 550–750) to the mainland R cemetery (AD 1000–1550). Recent analyses of burial styles, associated textiles and grave goods, however, suggest synchronicity between the two (Adams et al., 1999), resulting in modified dates of AD 550–800 for both cemeteries. The burials in both cemeteries include minimal adornment or accompaniment, probably reflecting the Christian ideologies of the population, and therefore there is no significant variation in burial styles suggesting differential status (Adams et al., 1999). Interpretations of site features indicate that the two populations engaged in comparable agropastoral subsistence practices, producing millet, sorghum and vegetable crops and grazing...
goats (Adams, 1977). Evidence of cyclical δ\\(^{13}\)C variation from the Nubian site of Wadi Halfa based on human hair (White, 1993), and more recently single osteons (Schwarcz et al., 2004), has been used to interpret seasonal variation in subsistence, characterised by the cultivation of the C\(_4\) crop sorghum during dry seasons and C\(_3\) plants during rainy seasons. Although the current study did not include soft tissue analysis to test whether this seasonal variation was also present at Kulubnarti, the dietary composition of the S and R cemetery populations also may have been affected by seasonally variable subsistence strategies involving shifting consumption of C\(_3\) and C\(_4\) plants. Cyclical dietary variation of this sort would not be readily visible in bone collagen due to the averaging effects of δ\\(^{13}\)C\_\text{COL} and δ\\(^{15}\)N during the slow turnover rate of organic bone components, but among very young individuals with faster overall tissue turnover rates, such variation could be a factor to consider. Additionally, seasonal trends may be delineated in tooth enamel and dentine, which do not remodel once formed and produce isotopic records during the period of tooth development. Future research using dental or soft tissues may address these questions at Kulubnarti.

Because of the substantial number of well-preserved individuals in both cemeteries, it was possible to select a large sample from each with representation of sex and estimated age. Developmental age at death was determined by Van Gerven et al. (1981) for each individual based on seriated inter-individual variation in multiple ageing criteria including stages of dental eruption and epiphyseal fusion. Excellent preservation of the remains, resulting from the hot, arid conditions characteristic of the region, provided many opportunities to determine sex among subadults in addition to adults based on preserved genitalia, pelvic dimensions and/or cranial robusticity.

**Methods**

Ribs were selected from each individual, with preference given to the right third rib whenever possible to control for variation in remodelling dynamics between different bone types (Mulhern & Van Gerven, 1997; Mulhern, 2000). After grinding off surface contaminants from the bone with a Dremel tool, approximately 300 mg of adult cortical bone was removed from the lateral end of the blade with a Dremel tool, and then cleaned twice ultrasonically. Less bone, approximately 80–100 mg, was taken from subadults’ and infants’/young children’s ribs to minimise intrusion. Each sample was then divided for collagen or apatite preparation and analysis. Recent assessments of isolation protocols for archaeological bone collagen (Liden et al., 1995) suggest that methods of extraction using only sodium hydroxide (NaOH) to remove lipids can decrease collagen yields and fail to remove lipid contaminants completely. As the Kulubnarti remains are exceptionally well preserved, effective removal of all non-collagen organic material, including lipids, was a primary concern. Thus, a collagen δ\\(^{13}\)C\_\text{COL} and δ\\(^{15}\)N extraction protocol designed to minimise contaminants was adapted from those discussed by Stafford (personal communication), Liden et al. (1995) and Ambrose (1993). Samples were crushed with an agate mortar and pestle and continually flushed for four hours with a 10:5:1 solution of methanol, chloroform and water in a soxhlet distillation apparatus to remove lipids, then freeze-dried for 18 hours in a vacuum container. This was followed by demineralisation in 0.5 M HCl in annealed glass test tubes at 4°C until translucent in appearance, with periodic replacement of HCl using annealed glass pipettes. Samples were then treated with a 0.2% potassium hydroxide (KOH) solution for 48–72 hours (depending on sample integrity) to remove humic contaminants and then solubised in a 0.05 M HCl solution at 90–100°C for approximately eight hours. The gelatinised samples were filtered through 0.045 μm millipore syringe tips to remove any residual contaminants, and freeze-dried.

Bone apatite carbonate was isolated for δ\\(^{13}\)C\_\text{CAP} and δ\\(^{18}\)O\_\text{AP} characterisation using methods adapted from van der Merwe et al. (1995), Ambrose (1993) and Schoeninger et al. (1989). Samples were powdered in a Spex 6700 freezer/mill, and treated for 24–48 hours with a 2.5% sodium hypochlorite (bleach) solution. Following neutralisation, samples were soaked for 2 hours in a 0.1 M acetic acid solution, rinsed...
to neutral with deionised water and freeze-dried for approximately 24 hours.

Carbon and nitrogen stable isotope compositions of purified collagen samples were analysed on a Carla Erba CNS analyser interfaced with a Micromass Prism Series II stable isotope ratio mass spectrometer at the Center for Isotope Geoscience at the University of Florida, Gainesville. Bone apatite $\delta^{13}$C and $\delta^{18}$O were measured using a Mountain Mass Spec. Multiprep system (single acid aliquot per sample) interfaced with the Micromass Prism. The analytical precision of the mass spectrometer was $\pm 0.20\%$ for $\delta^{15}$N and $\pm 0.14\%$ for collagen $\delta^{13}$C. International and in-house laboratory standards, as well as replicate runs (indicated by multiple collagen and/or isotopic values per individual in Table 1) yielded a standard deviation of $\pm 0.05\%$ for apatite $\delta^{13}$C and $\pm 0.11\%$ for $\delta^{18}$O. Raw data for both cemeteries are presented in Table 1, and measured isotopic values are expressed as per mil (‰) relative to established international standards: atmospheric nitrogen for $\delta^{15}$N and PDB marine carbonate for $\delta^{13}$C$_{COL}$, $\delta^{13}$C$_{AP}$ and $\delta^{18}$O$_{AP}$.

**Results**

Bone collagen and apatite are both susceptible to diagenetic alteration, which can distort biogenic isotopic signals. Percentage yields and carbon:nitrogen (C/N) ratios are commonly used as a test of collagen integrity, since yields below 5% (DeNiro, 1985) and atomic C/N ratios outside a range of 2.9 to 3.6 (DeNiro, 1985; Ambrose, 1993) are indicative of post-mortem chemical alteration. Collagen yields for the Kulubnarti samples were typically above 5% for samples in which yields were determined (64/67). The utility of percentage collagen yield as a criterion for assessing diagenesis, however, was compromised by the potential of sample loss during the isolation process described above as the protocol for isolating collagen was being developed. As the process was refined and sample loss limited, yields were consistently greater than 5% (Table 1). Weight percentages of C and N provided by the elemental analyser were used to calculate atomic C/N ratios for 16 representative collagen samples. C/N ratios ranged between 3.0 and 3.5 with one exception (3.9) (Table 1). Correlations calculated between estimated C/N ratios and isotope values are consistently low ($\rho_{\delta^{13}C, \delta^{15}N} = 0.17$, $\rho_{\delta^{13}C, \delta^{15}N} = 0.13$), indicating that isotopic signatures of the collagen are not linked to relative yields. While C/N ratios were not determined for all samples, ion beam ratios of masses 44 and 29 were used to assess alteration in the samples as they were analysed on the mass spectrometer. Although these ion beam ratios do not directly reflect atomic ratios, since N$_2$ and CO$_2$ gas pulses flow through different capillary systems in the elemental analyser ConFlo interface system, they provide a quantitative indicator of similarity or dissimilarity of C/N ratios in the samples. Kulubnarti runs were assessed for the three days the samples ran and the ion beam ratios were more invariant than the organic standards, indicating that the collagen samples had very similar C/N contents. The 16 samples for which weight percentage C/N ratios were determined were distributed throughout the various runs to provide calibration and indicate that most, if not all, samples are within the acceptable C/N ratio range. The excellent preservation of the Kulubnarti skeletal remains, including their fresh appearance and texture and the presence of preserved soft tissues, suggests a low risk of diagenetic alteration (Dupras et al., 2001). As a test of methodological integrity, a subsample of duplicates was processed and run for 38 individuals, with the majority showing isotopic differences under 1‰ (Table 1).

Shapiro-Wilk tests of normality revealed that while carbonate $\delta^{18}$O is distributed normally for both the S and R cemeteries ($P = 0.095$), neither cemetery is normally distributed by $\delta^{13}$C$_{COL}$ ($P = 0.000$), $\delta^{13}$C$_{AP}$ ($P = 0.000$) or $\delta^{15}$N ($P = 0.006$), necessitating nonparametric statistical analysis for the latter three variables. Consequently, Kruskal-Wallis tests of population equality were performed to estimate the predictive value of cemetery membership, sex and age on variation in isotopic values for apatite carbon and collagen carbon and nitrogen, while a one-way ANOVA (analysis of Variance) was performed to estimate the predictive value of cemetery membership, sex and age on the variation in values for apatite oxygen. These tests revealed no
significant predictive relationship between cemetery membership or sex and \( \delta^{13}\text{C}_{\text{COL}} \), \( \delta^{13}\text{C}_{\text{AP}} \), \( \delta^{15}\text{N} \) or \( \delta^{18}\text{O}_{\text{AP}} \), suggesting little difference in dietary composition between males and females. Consequently, the S and R samples were pooled in subsequent statistical tests. Perhaps more importantly, if cemetery membership (S vs R) was not a significant predictor of either organic or inorganic isotopic values, then this suggests that proportional dietary composition was not significantly different between the two cemetery groups. This contrasts with previous osteological studies that report differential indicators of nutritional status and infectious stress between the S and R cemeteries based on prevalence of cnbra orbitalia (Mittler & Van Gerven, 1994), tibial length (Hummert, 1983; Hummert & Van Gerven, 1983), cortical thickness (Van Gerven et al., 1995) and remodelling dynamics of ribs and femora. The results from this study do not necessarily dispute these interpretations, but they do raise the question of what factors besides overall dietary composition, such as parasite load, chronic infection, absolute portion size or differences in micronutrient content between isotope-ically similar foods, would have differentially affected the two Kulubnarti populations.

As summarised in Table 2, infants/young children are enriched in \( \delta^{15}\text{N} \), \( \delta^{13}\text{C}_{\text{COL}} \) and \( \delta^{13}\text{C}_{\text{AP}} \) relative to both subadults and adults, however, mean \( \delta^{15}\text{N} \) in the combined S and R infant/young child cohort is \( 12.19\% \), only \( 2.46\% \) greater than mean \( \delta^{15}\text{N} \) for the pooled subadults and \( 1.89\% \) greater than mean \( \delta^{15}\text{N} \) for the pooled adults. This is less than the expected \( 3\% \) indicative of a trophic-level enrichment expected for breastfeeding infants/young children, but this can be explained by the variability within each cohort. Infants/young children and subadults have \( \delta^{15}\text{N} \) standard deviations of \( 1.26\% \) and \( 1.12\% \), respectively, and show a range in within-cohort values of over \( 4\% \) (Figure 4). Similar trends are seen in mean \( \delta^{13}\text{C}_{\text{COL}} \) values for the pooled S and R cohorts, where the mean infant/young child value is \( -16.46\% \) and enriched relative to mean subadult \( \delta^{13}\text{C}_{\text{COL}} \) by \( 1.6\% \) and mean adult \( \delta^{13}\text{C}_{\text{COL}} \) by only \( 1.16\% \), but the standard deviation for the pooled infant/young child cohort is \( 1.63\% \) and the range is over \( 6\% \) (Figure 3). Mean \( \delta^{13}\text{C}_{\text{AP}} \) among infants/young children also shows enrichment relative to adults with a high standard deviation (\( 3.90\% \)), however, only two infants/young children have \( \delta^{13}\text{C}_{\text{AP}} \) values, a fact that limits further interpretation.

As seen in Figures 3 and 4, the distribution of \( \delta^{13}\text{C}_{\text{COL}} \) and \( \delta^{15}\text{N} \) values by estimated age at death displayed an interesting patterning of data points for both S and R cemeteries, although no significant correlation was found between \( \delta^{13}\text{C}_{\text{COL}} \) and \( \delta^{15}\text{N} \). There is apparent enrichment among infants/young children and depletion for subadults in both \( \delta^{13}\text{C}_{\text{COL}} \) and \( \delta^{15}\text{N} \). Adult values are intermediate between the infants/young children and subadults. The differences in mean values for \( \delta^{13}\text{C}_{\text{COL}} \) and \( \delta^{15}\text{N} \) between subadults and adults, however, are small. The mean \( \delta^{13}\text{C}_{\text{COL}} \) among the pooled subadults is \( 18.06\% \), only \( 0.44\% \) less than the mean adult value, while the mean \( \delta^{15}\text{N} \) among subadults is \( 9.73\% \), only \( 0.57\% \) less than the mean for adults (Table 2). These small differences in mean isotopic values could suggest little difference in collagen isotope ratios between subadults and adults, with only infants/young children showing a difference. However, the standard deviation in isotopic values is also large for subadults, (SD of \( \delta^{13}\text{C}_{\text{COL}} = 0.80\% \), SD of \( \delta^{15}\text{N} = 1.12\% \)), and as a result mean values are less powerful measures of between-cohort variation by themselves. The wide ranges in the raw distribution of \( \delta^{13}\text{C}_{\text{COL}} \) and \( \delta^{15}\text{N} \) point to overlap between these three clusters, with minimum and maximum values for subadults and adults overlapping with each other and also with minimum values for infants/young children, which can be explained by the substantial range in subadult and adult \( \delta^{13}\text{C}_{\text{COL}} \) and \( \delta^{15}\text{N} \) values. As shown in Figure 3, subadults and adults range within their cohorts by almost \( 3\% \) in values for \( \delta^{13}\text{C}_{\text{COL}} \). Figure 4 shows that subadults range within their cohorts by almost \( 4\% \) in \( \delta^{15}\text{N} \), while adults range by almost \( 3\% \). Taking these wide ranges into account, however, a tripartite distinction is still visible in the raw collagen \( \delta^{13}\text{C}_{\text{COL}} \) and \( \delta^{15}\text{N} \) data, especially in the latter, that merits further exploration.

Based on Kruskal-Wallis tests of population equality, it appears that these three age clusters are significantly distinct in their ranges of \( \delta^{15}\text{N} \) (\( \chi^2 = 52.702, P = 0.0001 \)) and \( \delta^{13}\text{C}_{\text{COL}} \) (\( \chi^2 = 36.763, P = 0.0001 \)). In other words, despite
Table 1. Isotopic data for S and R cemetery individuals.

<table>
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<tr>
<th>Burial ID</th>
<th>Age at death (yrs)</th>
<th>Sex</th>
<th>C/N ratio</th>
<th>% collagen yield</th>
<th>δ¹⁵N (‰)</th>
<th>δ¹³C_COL (‰)</th>
<th>δ¹³C_CAP (‰)</th>
<th>δ¹⁸O (‰)</th>
<th>Δ¹³C_CAP-COL (‰)</th>
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<td>-11.86</td>
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Continues
large individual variations within the three age cohorts, there are clear distinctions between them. However, there was no significant relationship between age category and $\delta^{13}C_{\text{AP}}$ ($\chi^2 = 0.872$, $P = 0.6466$), which is reflected in the more random distribution of values in Figure 5. This suggests that what are significantly different between the three age groupings are isotopic signals of protein intake rather than overall diet.

To explore this possibility further, $\Delta^{13}C_{\text{AP-COL}}$ offsets were calculated for the subset of the pooled S and R groups for whom both apatite and collagen data were generated (Table 2). These offsets (Table 1) also suggest a possible age-related trend in protein intake across the three age categories (Kruskal-Wallis Test of Population Equality, $P = 0.02$), although only two observations were present for the infant/young child age cohort. However, the offsets between the juvenile and adult age cohorts also approaches significance (Kruskal-Wallis Test of Population Equality, $P = 0.07$), further supporting this interpretation. Mean collagen $\delta^{13}C_{\text{COL}}$ and $\delta^{15}N$ values for the three age clusters are clearly different, although with some overlap in raw values (Table 2).

The distribution of $\delta^{18}O_{\text{AP}}$ data for the S and R cemeteries (Figure 6) is more variable across the three age clusters and indicates a general decline of 3–4% with advancing age, a trend that appears slightly more pronounced in the S cemetery sample. A one-way ANOVA shows a significant relationship between age category and $\delta^{18}O$ ($F = 4.5079$, $P = 0.003$), but this decline in oxygen isotopic values across the age categories is too gradual to suggest a weaning trend across the two infant/young child and subadult age cohorts, especially given that there are only two infants/young children in the infant/young child cohort for oxygen values. Furthermore, the range in $\delta^{18}O$ among the subadult cohort is large, exceeding 4%, with a standard deviation approaching 1%. If some enrichment in infant/young child $\delta^{18}O$ due to isotopic enrichment of maternal body water in breastmilk is assumed, then the two infant/young child isotopic values may be assumed to fall within the substantial range in subadult values. The distribution of $\delta^{18}O$ seen in Figure 6 points to the adult cohort as the

<table>
<thead>
<tr>
<th>Burial ID</th>
<th>Age at death (yrs)</th>
<th>Sex</th>
<th>C/N ratio</th>
<th>$%$ collagen yield</th>
<th>$\delta^{13}N$ (‰)</th>
<th>$\delta^{13}C_{\text{AP}}$ (‰)</th>
<th>$\delta^{13}C_{\text{COL}}$ (‰)</th>
<th>$\delta^{13}C_{\text{AP-COL}}$ (‰)</th>
<th>$\delta^{18}O$ (‰)</th>
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<tr>
<td>R176a</td>
<td>30</td>
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<td>9.795</td>
<td>9.795</td>
<td>17.51</td>
<td>17.51</td>
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<td>12.43</td>
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<td>9.25</td>
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<td>17.22</td>
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<td>12.98</td>
<td>12.98</td>
<td>17.22</td>
<td>17.22</td>
<td>-1.91</td>
</tr>
</tbody>
</table>

aAges estimated by Van Gerven (Adams et al., 1999).

bMissing values for carbon, nitrogen and oxygen due to insufficient amounts of extracted material.

**Multiple collagen and/or apatite values given for a single individual indicate replicates.**
potential source of age-related variation, supported by an adult mean $\delta^{18}O$ value of 2.71 ± 0.65‰ that is depleted relative to the subadult mean by 0.63‰ (Table 2). The subadult–adult difference in mean $\delta^{18}O$ within the S group sample is just under 1‰, and while the difference within the R group means is noticeably different, these differences failed to reach statistical significance and are perhaps again due to the wide variation in each cohort.

In sum, the above analyses point to significant relationships between collagen carbon and nitrogen isotopic values and age, including significant differences between juvenile and adult age ranges, but a wide range of values within each cluster. No significant relationship was found between these values and sex or cemetery of interment, suggesting similar dietary patterns or physiological processes between the two populations. Bone apatite carbon isotopic values do not appear significantly differentiated by any of the categorical variables above such as cemetery location or sex. A significant, negative relationship exists between isotopic oxygen signatures and age category that is perhaps due to depletion among the adult cohort rather than enrichment among infants/young children, although limitations in sample size among the infant/young child cohort preclude ruling out the presence of a $\delta^{18}O$ weaning trend.

### Discussion

A number of interesting interpretations can be made regarding the distinct infant/young child, subadult and adult dietary ranges outlined above. The simplest interpretation is that the clustering in $\delta^{15}N$ and $\delta^{13}C_{COL}$ values is due to age-related differences in dietary intake, especially of animal protein. Controlled feeding studies have supported the hypothesis that while apatite $\delta^{13}C$ values reflect overall dietary intake, including carbohydrates, protein and fats, collagen $\delta^{15}C$ and $\delta^{15}N$ primarily reflect consumed protein (Ambrose & Norr, 1993). The lack of statistical significance in apatite isotopic values versus the significance of collagen values suggests that it is dietary animal protein intake, rather than whole diet composition, that varies by age, with

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**Table 2. Mean isotopic values by age cluster for S and R groups**

<table>
<thead>
<tr>
<th>Application</th>
<th>$\delta^{15}N$ (%)</th>
<th>$\delta^{13}C_{COL}$ (%)</th>
<th>$\delta^{13}C_{CAP}$ (%)</th>
<th>$\delta^{18}O$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-S-46 (S group)</td>
<td>0–3 yrs</td>
<td>12.56 ± 1.27 (37)</td>
<td>10.38 ± 0.64 (37)</td>
<td>16.33 ± 1.75 (38)</td>
</tr>
<tr>
<td></td>
<td>4–17 yrs</td>
<td>9.81 ± 1.34 (37)</td>
<td>10.38 ± 0.64 (37)</td>
<td>18.13 ± 1.47 (38)</td>
</tr>
<tr>
<td>21-R-2 (R group)</td>
<td>0–3 yrs</td>
<td>11.73 ± 1.27 (32)</td>
<td>10.96 ± 0.68 (32)</td>
<td>16.46 ± 1.63 (30)</td>
</tr>
<tr>
<td></td>
<td>4–17 yrs</td>
<td>10.63 ± 1.47 (32)</td>
<td>10.63 ± 0.68 (32)</td>
<td>18.06 ± 1.47 (38)</td>
</tr>
<tr>
<td>Total (both S &amp; R)</td>
<td>0–3 yrs</td>
<td>12.19 ± 1.26 (27)</td>
<td>10.63 ± 0.68 (24)</td>
<td>16.46 ± 1.63 (30)</td>
</tr>
<tr>
<td></td>
<td>4–17 yrs</td>
<td>9.73 ± 1.17 (27)</td>
<td>9.73 ± 0.69 (39)</td>
<td>18.06 ± 1.47 (38)</td>
</tr>
</tbody>
</table>

Numbers in parentheses equal the number of observations for each mean value (single observations indicate an individual isotopic value).
subadults eating less of it. Within the infant/young child (0–3 yrs) and juvenile (4–17 yrs) clusters, however, there exists substantial variation. Such wide intragroup ranges suggest several possible causal factors.

Firstly, regarding the infant/young child (0–3 yrs) category, it is possible that the Kulubnarti populations engaged in several weaning practices rather than one uniform standard, which influenced the timing and level of dietary supplementation and the duration and intensity of breastfeeding. These strategies could depend on resource access or individual variation in feeding practices over the duration of the Christian period, which spanned anywhere from 200–900 + years and encompassed the period of occupation at Kulubnarti (Adams et al., 1999). The wide variability in the timing of supplementation, the types of supplementary foods used, and the duration of breastfeeding in modern populations supports this interpretation (Abel et al., 2001; Holman & Grimes, 2003). Anecdotal reports during the 1960–70s, when Kulubnarti and other Nubian sites such as Wadi Halfa were excavated, identified millet gruel as a commonly administered supplementary food for nursing infants, a recipe that could have ancient origins. The use of gruels as weaning foods in ancient populations was hypothesised by Buikstra et al. (1986), and could have been employed at Kulubnarti. However, if gruels made of millet, a C₄ plant, were the primary weaning foods at Kulubnarti, infant/young child $\delta^{13}$C.COL values should be enriched relative to those observed here, which are more indicative of C₃-based supplementary diets. Regarding the observed decline in $\delta^{15}$N, Balasse et al. (2001) demonstrated through controlled feeding experiments that $\delta^{15}$N depletion in collagen reflects not the gradual decline in breastfeeding, but the actual cessation of breastfeeding, unless there is a substantial amount of protein in the supplementary food. If the supplementary diet involved a protein source such as herbivore’s milk, as was suggested at Wadi Halfa.

Figure 4. Stable nitrogen isotopic composition of collagen samples from R and S cemetery groups by age.
by White & Schwarcz (1994) and at the Dakhleh Oasis by Dupras et al. (2001), then perhaps the overall isotopic decline is indicative of a gradual shift from maternal milk as the primary protein source to one that included protein from C3-browser sources. The variation in the infant/young child category, however, suggests equal variability in the supplementary diet, which could include varying contributions of milk and/or flesh from C3 browsers and C4 grazers and of C3 and C4 plants, with varying contributions of carbon and nitrogen from animal and plant sources.

A second possible interpretation specific to the infant/young child category is that the observed ranges are due to variation in physiology affecting metabolism and growth rates or reflecting pre-existing poor health. The category of ‘infant/young child’ in this study spans birth through to three years of age, and individuals classified as infants/young children were probably growing and developing at different velocities depending on their exact age within this larger categorisation. The decline in standard deviations between the three age categories over time, also visible in a declining range in the raw distributions of isotopic values (Figures 3 and 4), supports the suggestion of an overall decline in the rate and variability of bone turnover after infancy and childhood. Such characteristics are difficult to test, due to the inherently cross-sectional nature of skeletal populations and the possible biasing effects of mortality due to acute infectious or nutritional stress that precludes many hard-tissue symptoms (Wood et al., 1992). The large percentage of infants/young children and subadults in both cemetery populations suggests that such acute stress may have had a strong presence at Kulubnarti. However, infants/young children in both cemeteries were interred in ceramic containers, which provided an effective buffer against taphonomic processes and may have resulted in a substantial infant/young child sample by virtue of unusually good preservation.

Finally, seasonal variation in maternal dietary intake, similar to that argued by White (1993) for the Wadi Halfa population, may have also played...
an indirect role in the isotopic variation in the infant/young child age category. Growing infants and young children have markedly higher rates of bone turnover and isotopic incorporation compared with adults and, if breastfeeding, would be more likely to reflect seasonal shifts in the isotopic composition of breastmilk, which in turn would reflect seasonal shifts in the maternal diet. Therefore, the substantial variation in infant/young child isotopic values may reflect differences in the season of individual infant and young child deaths. In essence, infants and young children who died during different times of the year would have skeletal isotopic signatures that reflect their mothers’ seasonally-dependent diets. An interpretation of diverse adult (therefore maternal) diets is supported by the descriptive $\delta^{15}\text{N}$ and $\delta^{13}\text{C}_{\text{COL}}$ data for all individuals aged 18 and older (Table 2), which suggest differential adult dietary intakes and thus potential variability in the isotopic composition of maternal tissues and breastmilk. Bone collagen isotopic data represent long-term averages and thus cannot directly support a suggestion of seasonal variation in the composition of maternal tissues, but the range of isotopic variation among the infants and young children in these populations and the evidence for seasonal effects at Wadi Halfa make this interesting food for thought.

A separate set of interpretations can be advanced regarding the distinct range of isotopic values for individuals aged 4–17 years at death. The Kulubnarti subadult range in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}_{\text{COL}}$ values is slightly but significantly depleted relative to both younger and older individuals, with a mean $\delta^{15}\text{N}$ of $9.73 \pm 1.12\%$ and a mean $\delta^{13}\text{C}_{\text{COL}}$ of $-18.01 \pm 0.80\%$ (Table 2). This could be attributed to a number of possible factors, including differential growth rates or stress episodes, differential dietary intake, and variable levels of water stress. A substantial literature exists that identifies arrested skeletal growth relative to dental or absolute age as a marker of nutritional and/or infectious stress.
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Age-related Isotopic Variation in Nubia

(Martorell, 1983, 1989), a stress response that also affects rates of bone protein turnover and micronutrient metabolism (May et al., 1993, Liu & Barrett, 2002, Martorell, 2002; Robins, 2003). The presence of pathological stress markers such as cribra orbitalia in both cemetery populations at Kulubnarti lends support to the claim that at least some individuals experienced stress during childhood, although the prevalence was much higher in very young individuals and declined in adolescents (Mittler & Van Gerven, 1994). Because of this high stress load indicated at Kulubnarti, the depleted δ15N among subadults could represent protein-poor diets that contributed to an early death. Under this rationale, the higher δ15N among adults could reflect better diets consumed by individuals who survived subadulthood. Even if these differences are skewed by mortality bias, however, this interpretation nevertheless leans towards variation in diet.

Interestingly, while White & Schwarcz (1994) found significant correlations of δ15N with age, including a depletion in values among subadults, they found no such relationship for δ13C COL in the Christian period remains at Wadi Halfa. This suggests that aside from the changes undergone during weaning, there was little variation in dietary isotopic composition across the Wadi Halfa skeletal sample. However, the juvenile δ15N trend found at Kulubnarti is similar to that found by Richards et al. (2002) at the Wharram Percy site in England, by White & Schwarcz (1994) at the Nubian site of Wadi Halfa, and by Katzenberg & Pfeiffer (1995) in Canada. Differences in sample size and the actual shape of the δ15N distribution by age between these data-sets make interpretations of isotopic composition related to growth and development difficult. They do, however, raise interesting questions regarding age-based differences in macronutrient metabolism, and their effects on the isotopic composition of skeletal tissues. It has been suggested that high juvenile rates of growth and development relative to adults would result in altered levels of 15N discrimination in metabolic pathways and subsequent rates of 15N routing to bone collagen, producing depleted values in juvenile bone relative to adults (Hare et al., 1991). However, the factors involved in skeletal growth and development are complex, and the effects of these processes on the incorporation of isotopic signatures in skeletal tissues merit further research.

The age-related trends seen in both δ15N and δ13C COL values at Kulubnarti may be due in part to a marked difference in subsistence options at the site. Unlike Wadi Halfa, the Kulubnarti site yielded no evidence of irrigation, but could have supported the cultivation of C3 staples such as wheat and barley without such technology due to the flood pattern particular to the alluvial plains of the Batn el-Hajar (Adams, 1977: 178–9). However, this region contained fewer floodplains overall, which would make it advantageous to herd C4 grazers such as goats and produce C4 staples such as millet and sorghum due to their higher survivability in hot, dry soils (Ambrose, 1991; Tieszen, 1991). An archaeobotanical survey of common plant remains dated to the Christian period at Qasr Ibrim in Sudanese Nubia (Rowley-Conwy, 1989, in White & Schwarcz, 1994) suggested that sorghum and barley were most prominent among cultigens. An additional contributing factor to δ13C COL levels in the Kulubnarti remains would be the isotopic composition of consumed herbivore tissues such as meat and milk, which would also reflect C4 or C3 plant-based herbivore diets (Dupras et al., 2001).

Taking all of these issues into consideration, the diet at Kulubnarti appears to have been roughly similar to those from nearby contemporary sites, consisting of mostly C3 (barley, legumes, wheat) with some C4 (sorghum, millet) plants and leguminous and/or animal protein. Trace element analyses of hair from the S and R cemeteries have suggested a primarily plant-based diet, suggesting a reliance on legumes such as beans for protein (Sandford & Kissling, 1994). However, the range of δ15N values in adults for both the S and R cemeteries is between 7.5‰ and 11.8‰. The range for most legumes is 0.4–5.0‰ (van der Merwe, 1982; Ambrose, 1993), although δ15N enrichment in arid soils is known to enrich the δ15N of many plants, including nitrogen-fixers such as legumes (Schwarcz et al., 1999). Even taking a 3–5‰ trophic level enrichment into consideration (Bocherens & Drucker, 2003), it is possible that at least some animal protein was included in adult diets to produce the δ15N values observed in their cohort.
However, because low levels of rainfall characterise the surrounding environment, $\delta^{15}N$ values throughout the local foodweb would potentially have been enriched by up to 3%o (Ambrose, 1991), and $\delta^{13}C$ values by approximately 2–3%o (Ambrose, 1993). When one takes into account the enrichment of $\delta^{15}N$ values expected in an arid locale such as Kulubnarti, these adult isotopic ranges could reflect dietary protein intake dominated by leguminous sources. While it is also possible that riverine products such as fish contributed to the diet at Kulubnarti, the range of isotopic values, coupled with ethnographic evidence of long-present fish taboos and a lack of archaeological evidence of fish consumption (Adams, 1977), suggest a more terrestrially-based diet.

With these factors in mind, it appears as though a variable but distinctly age-dependent set of subsistence strategies was pursued at Kulubnarti. Depending on rates of delay between dietary shifts and the incorporation of new isotopic ratios into collagen and apatite, young children were probably completely weaned soon after age three and were consuming a subadult diet after age four. The gradual decline in $\delta^{15}N$ across infants/young children aged six months to four years at death may have reflected supplementation with protein sources such as bean gruel or animal products, the latter of which may have been present at the Wadi Halfa site (White & Schwarcz, 1994). Supplementation with a protein source could create a more gradual $\delta^{15}N$ isotopic decline in infant/young child tissues because those individuals would be shifting to a lower trophic level nonetheless characterised by different protein intakes relative to subadults and adults. Individuals with relatively low values of $\delta^{13}C_{COL}$ and $\delta^{15}N$, which in the Kulubnarti samples were primarily subadults, may have consumed disproportionately less protein or different forms of protein with lower $\delta^{15}N$ values, such as increased consumption of legumes and decreased consumption of milk, blood, meat or other animal protein. While a comparison of the calculated $\Delta^{13}C_{AP-COL}$ offsets between the pooled age cohorts reveals only very small differences between the juvenile and adult age cohorts (less than 1), the relatively small subsample size and slightly larger offset for juveniles, especially among the S group, means that the possibility of differential protein consumption by juveniles should not be ruled out.

This interpretation is complicated by the potential enriching effect of aridity throughout the Kulubnarti food web. If all individuals shared equal access to water sources, however, the differences between the juvenile and adult groupings suggest a subadult dietary pattern characterised by lighter $\delta^{13}C_{COL}$ and $\delta^{15}N$ values. Even if it is assumed that preferential access to potable water was given to juveniles aged 4–17 years in this sample, which would buffer them from water-stress induced $\delta^{15}N$ enrichment compared with adults, such a practice would still constitute a separate, intermediate overall subsistence category.

The implications of these age-related trends are unclear. Modern nutritional and ethnographic research has identified substantial variation in differential intra-household food allocation among numerous populations, with beneficial (Gittelsohn et al., 1997; Graham, 1997) and harmful results (reviewed in Messer, 1997). These studies emphasise not only the variability in intrahousehold allocation strategies, but also the importance of measuring protein and micronutrient intake in addition to overall caloric intake, to more accurately assess overall nutritional status. This is especially salient to this study, as the Kulubnarti data suggest age-related differences specifically in protein intake versus merely the overall diet, allowing an assessment of childhood dietary composition with a more clearly defined set of dietary variables. Furthermore, ethnographic studies point to belief systems centred on definitions of childhood, vulnerability and/or gender bias at various age levels as strong predictors of allocation strategies, perhaps more so than household food security or household economy (Messer, 1997). Applying this to the Kulubnarti data, the lack of predictive value of sex on inorganic or organic $\delta^{13}C$ or $\delta^{15}N$ suggests that, within the resolving power of isotopic discrimination, sex is not a markedly contributing factor to dietary intake and nutritional access. However, the variation between the subadult and adult cohorts suggests a set of dietary practices centred on childhood as distinct from adulthood. The range of isotopic variation
within the subadult cohort suggests that these practices were not narrowly defined or perhaps varied by season or food availability.

An alternate interpretation is that this trend is not indicative of food allocation differentiating juveniles from infants and young children and adults, but is rather an artefact of two mortuary populations, an interpretation that could be extended to the clustering of values in the infant/young child (0–3 yrs) age category. Wright & Schwarcz (1999: 1160) suggested that ages of supplementation and weaning among infants/young children in mortuary samples are earlier than those of survivors, due to the protective effects of prolonged breastfeeding. The very nature of infant/young child and subadult mortuary samples, essentially an assemblage of non-survivors, could mean that the trends seen here represent subsistence strategies that ‘failed’, in the sense that the affected individuals were unable to survive early-to-mid childhood. The adult isotopic range, therefore, may more accurately reflect common diets at Kulubnarti in that such diets permitted survivorship past infancy and subadult-hood. The fact that the infants/young children and subadults in both cemeteries, despite marked intra-group variation, were clearly different at the inter-group level suggests that whether the subsistence patterns were successful or not, they were still distinct.

A final interpretive possibility is that the isotopic distinction between subadults and adults at Kulubnarti is influenced by the presence of non-local immigrants in the adult cohort. As shown in Figure 6, δ18O values in the adult cohort are depleted relative to the infant/young child and subadult cohorts, especially among adults aged over 35 years at death. Moreover, a range of approximately 3‰ within the pooled adult cohort supports the notion that some of the adults interred at Kulubnarti were not from the area, or were and had relocated elsewhere during life. White et al. (2004) posit immigration as a source of δ18O variation at the nearby site of Wadi Halfa, while Dupras & Schwarcz (2001) described similar trends in Egypt, suggesting a greater degree of population movement in the region than previously considered. Residence in an ecologically distinct area would imply subsistence strategies that utilised different foodwebs and different sources of water. It is therefore possible that the presence of non-local immigrants at Kulubnarti was responsible for the differences in isotopic dietary indicators, rather than culturally-mediated dietary variation within a stable, local population. Future research on the Kulubnarti S and R populations will examine bone carbonate δ18O variation in a larger sample of the population in order to address this question more directly.

Conclusion

This study has produced isotopic dietary profiles for two large skeletal populations with known burial contexts and a substantial background of osteological and archaeological research, permitting an in-depth interpretive analysis of palaeodietary trends within a larger framework of behavioural and environmental interaction. Several interesting trends emerged from the isotopic data-set, most notably a distinct age-based patterning of δ15N and δ13C values between infants/young children, juveniles and adults. The causal factors for this tripartite patterning are difficult to identify. However, it is suggested here that a ‘childhood’ diet may have existed at Kulubnarti that consisted of isotopically depleted protein and plant sources relative to the diets of infants/young children, juveniles and adults. The gradual decline in infant/young child δ15N values up to age four also suggests varying degrees of supplementation with high protein foods or prolonged breastfeeding with supplementation.

Also interesting is the lack of significant difference in any isotopic dietary indicator between the S and R cemeteries. This suggests that the
differences in markers of stress and ill-health found between the two populations are due to factors other than diet, at least those aspects of diet such as macronutrient content measurable through isotopic analysis. Future consideration of the substantial data existing from numerous analyses of the Kulubnarti S and R groups must take this into account when examining and interpreting differential experiences of stress and status between the two populations, perhaps focusing on the role of parasitic infection in creating and perpetuating poor skeletal health. In summary, isotopic analyses at Kulubnarti revealed patterning in diet that highlights an unrecognised complexity in dietary composition among the site’s inhabitants, and an unrecognised homogeneity between the two cemeteries. This raises new questions about the effects of diet, physiology, population movement and environmental context on overall health and well-being in medieval Nubia.

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