

1 **Dietary seasonal variations in the Medieval Nubian population of Kulubnarti as**
2 **indicated by the stable isotope composition of hair**

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14 **Abstract**

15 **Objectives:** The island of Kulubnarti is located in Sudanese Nubia and contains two
16 cemeteries, named R and S, which are dated to AD 550-800. In order to provide more
17 detailed dietary information for this population and examine seasonality of diet, we
18 analyzed the carbon isotope composition of hair samples from both cemeteries.

19 **Materials and methods:** Forty seven separate hair samples from 8 adults, 29 adolescents,
20 7 infants and 3 individuals with unknown age were analyzed. Long hair samples were cut
21 transversely and divided into 2 cm longitudinal segments, to examine temporal variations
22 in the dietary carbon sources.

23 **Results:** The average carbon isotope value for the whole population was -17.95 ‰
24 (SD=1.8). A significant difference between the two cemeteries was found with variances
25 in the amount of C₄ dietary carbon sources consumed.

26 **Discussion:** The results of hair isotope compositions concur with previous soft tissue
27 investigations of Kulubnarti population which suggested that the dietary regimen contains
28 a mix of C₃ and C₄ plant-based sources. A seasonal variation in diet can be inferred from
29 the sequential hair segments of Kulubnarti individuals. These suggest a dietary transition
30 between dominant C₃ plant-based sources in winter to dominant C₄ ones in summer with a
31 small contribution of the non-harvested, alternative, crop.

32 **Key words:** Carbon isotopes, nitrogen isotopes, ancient diet, Kulubnarti, Medieval
33 Sudanese Nubia, ancient hair, seasonality in diet.

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1 **Introduction**

2 Isotopic reconstructions of palaeodiet in ancient populations give insights into
3 patterns of subsistence and resources available and determine variations in the diet among
4 populations. Stable carbon and nitrogen analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) have been widely used in the
5 reconstruction of diet of ancient populations. Most plants consumed by humans use either
6 the C_3 or the C_4 photosynthetic pathway which results in isotopically distinct ranges of
7 $\delta^{13}\text{C}$. For example, C_3 plants have $\delta^{13}\text{C}$ values ranging from -23‰ to -31.5‰ (O’Leary
8 1981, 1988; Kohn 2010), whereas the range of $\delta^{13}\text{C}$ values for C_4 plants is between -11‰
9 and -14‰ (O’Leary 1988; Codron et al. 2005). By measuring $\delta^{13}\text{C}$ in human tissue, the
10 identification of consumed plant type can be deduced, provided that the fractionation
11 between the $\delta^{13}\text{C}$ of the preserved tissue sample and that of the diet is known. It has been
12 assumed that $\delta^{13}\text{C}$ values of human hair are enriched by ~3‰ relative to that of the diet and
13 reflect the protein part of the diet (Nakamura et al. 1982; O’Connell and Hedges 1999;
14 Sealy 2001; Sponheimer et al. 2003). Nitrogen isotopic analysis indicates the sources of
15 protein in the diet, and differentiates between marine and terrestrial diets as well as helps
16 in investigating the ancient weaning practices (Tykot, 2006). In contrast to carbon isotopes,
17 which cannot be used in an investigation of the trophic level, nitrogen isotopic analysis
18 could estimate the proportions of meat to vegetable material in dietary protein. Therefore,
19 $\delta^{15}\text{N}$ values could infer the trophic level of the individual (Ambrose & DeNiro 1986;
20 Hobson 2007). As is the case for carbon, there is a fractionation of about 3-5‰ between
21 the tissue $\delta^{15}\text{N}$ value and that of the consumed diet (Schoeninger, 1985; Ambrose & DeNiro
22 1986; Ambrose, 1991; Sealy, 2001; Minagawa & Wada, 1984; Hedges & Reynard, 2007).

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24 **The Kulubnarti cemeteries**

25 The site where our samples came from is the island of Kulubnarti. This island is situated
26 about 130 Km south of Wadi Halfa in modern-day Sudan (Fig. 1). Kulubnarti is located in
27 the centre of an area known as Batn el-Hajar or “Belly of the rock” that extends between
28 the second and Dal cataracts of the Nile River. The Nile in this region is filled with rocks
29 and rapids separating a succession of tiny islands (Van Gerven et al. 1995). Nubia is one
30 of the most hot and arid regions in the world and the Batn el-Hajar area is one of the most
31 rugged and inhospitable parts of Nubia. These climatic conditions have helped to preserve
32 desiccated natural mummies containing bones as well as soft tissues, including hair, in the
33 Kulubnarti cemeteries (White and Schwarcz 1994). Depending on the Nile and its annual

1 inundation, the Kulubnarti population lived as sedentary agriculturalists producing a range
2 of crops. Their diet consisted mainly of cereal grains, accompanied by small amounts of
3 animal protein (Trigger 1976; Adams et al. 1999; Turner et al. 2007; Basha et al. 2016).
4 The site of Kulubnarti comprises two cemeteries, R & S (Fig. 2), both dating to the
5 Christian period (AD 550-800) as indicated by analysis of burial styles, associated textiles
6 and grave goods (Adams et al. 1999). The S cemetery was situated within an old and long-
7 dry *wadi* near west side of the island. The R cemetery was located on west bank of the Nile
8 just opposite to southern end of the island. Both cemeteries were burial places for people
9 who had lived on the island of Kulubnarti (for more details see Adams et al. 1999). A
10 slightly better health condition of R cemetery individuals has been reported from earlier
11 palaeopathological studies compared to S cemetery individuals (Van Gerven et al. 1981;
12 Hummert 1983; Hummert and Van Gerven 1983; Sandford et al. 1983; Van Gerven et al.
13 1990; Mittler and Van Gerven 1994; Sandford and Kissling 1994; Albert and Greene 1999).
14 It has been urged to investigate dietary profile of the two cemeteries and to examine
15 possible dietary differences that might contribute to health status variation noticed between
16 them.

17 As the Kulubnarti population practiced farming that depended on the Nile and its annual
18 fluctuation, some seasonal variation in subsistence and diet might be expected. The alluvial
19 plains of Batn el Hajar supported the seluka system of agriculture which produced crops
20 depending on annual inundation of the Nile (Adams 1977; Hibbs et al. 2011). Both kinds
21 of plants, C₃ and C₄, were used as crop plants in ancient Nubia; C₃ crops (e.g. wheat, barley)
22 prefer the cooler conditions of the winter season which extended from November to March.
23 C₄ crops (e.g. millet and sorghum) were planted in the summer season but in less quantity.
24 The flood season (damora) extended from August to November, and during this time only
25 the fields that used saqiya irrigation (a method apparently not used by our Kulubnarti
26 population) were able to produce crops (Adams 1977). In addition, the Christian period
27 witnessed the rise of the Nile to one of its highest documented levels which might have
28 affected the farming of the summer C₄ plants at Kulubnarti (Adams 1967; Trigger 1965;
29 Hibbs et al. 2011). Unfortunately, there were no available dietary materials collected from
30 the site to be isotopically analysed. However, local isotopic values for Nubian and Nile
31 valley plants have been reported in White & Schwarz (1994) and used as reference plants.
32 These plants have been thought to be important in ancient Nubian diets as some of them
33 were recovered through an archaeobotanical survey that done by Rowley-Conwy (1989)
34 for the neighbouring area of Qasr Ibrahim. The dietary profile of the Kulubnarti population

1 has been previously investigated using isotope analysis of bone (Turner et al. 2007) and
2 soft tissues (Basha et al. 2016). However, the most appropriate sample to investigate
3 possible seasonal variations in diet is hair. Hair is composed mainly of the protein keratin
4 which is not reabsorbed or modified during life and therefore, records the most recent diet
5 consumed. Hair grows at a rate of about 1cm per month (Saitoh et al. 1969) so, depending
6 on hair shaft length (in cm), the diet of corresponding time before death (in months) of
7 individual's life can be determined. This allows hair to be used in the study of seasonal
8 dietary changes (O'Connell and Hedges 1999). The hair follicles go through different
9 phases during its growth cycle. These phases are recognized as long (average three or more
10 years) growing anagen phase, short (1-2 weeks) transitional catagen phase, and
11 intermediate (3-4 months) resting telogen phase (Saitoh et al., 1970; O'Connell and Hedges
12 1999). Each hair follicle has its independent phase. Consequently, hairs that are located
13 adjacent to each other could not be contemporaneous (O'Connell and Hedges 1999;
14 Williams et al. 2011). It was assumed that hair sample from a healthy individual includes
15 an average of 85–90% actively growing hairs and 10–15% inactive hairs (O'Connell and
16 Hedges 1999). However, the rate of inactive telogen hair could reach up to 39% in some
17 cases (Williams et al. 2011). Therefore, a growth cycle error caused by sampling hair in
18 different growth phases has to be considered (Schwertl et al. 2003; Williams et al. 2011).
19 It has been recommended that sampling at least 25 hairs is minimizing the percentage of
20 obtaining hair in the inactive telogen phase of growth (Mekota et al. 2006). Other authors,
21 however, suggested that sampling of about fifteen hairs could be adequate in reducing the
22 probability of sampling only hair in the resting telogen phase (Williams and Katzenberg
23 2012).

24 Here we report the results of stable carbon isotope analysis of hair samples from both of
25 the Kulubnarti cemeteries. The present study aimed to get more detailed dietary information
26 and investigate dietary differences, if there are any, between S and R cemeteries. In addition
27 to examining the possible seasonality in the diet in the whole Kulubnarti population.

28 **Materials and methods**

29 In 1979 the University of Colorado, in conjunction with the University of Kentucky,
30 excavated the two Christian (AD 550-800) cemeteries, named S & R, of Kulubnarti (Van
31 Gerven et al., 1981; Adams et al. 1999). The number of Mummies/skeletons that uncovered
32 from S cemetery were 215 and 191 from R cemetery, most of them are curated at the
33 University Colorado (Adams et al. 1999). Subsamples of these mummies are preserved at

1 the tissue bank of KNH Centre for Biomedical Egyptology at the University of Manchester.
2 The carbon isotope compositions of 47 hair samples (21 from the R cemetery and 26 from
3 the S cemetery) have been determined. The available hair materials were divided into three
4 age groups: group 1 (infants, 0-3 years), group 2 (children, 4-17 years), and group 3 (adults,
5 18+ years). The sex and age of the Kulubnarti burials have been estimated by Van Gerven
6 et al. (1981) using conventional osteological methods.

7 **Methods:**

8 As hair is composed of 95% keratin, it does not require chemical treatment for
9 protein extraction as is the case for bone or soft tissue samples. Instead, the samples need
10 only a simple cleaning procedure to remove any surface contamination and sebum lipids
11 (Roy et al. 2005). 12-15 hair strands from each individual were cut near the proximal (scalp)
12 end using clean scissors. The cleaning of the hair samples was undertaken following the
13 procedure described by O'Connell and Hedges (1999). Samples were sonicated in 1 ml 2:1
14 v/v methanol: chloroform mixture for 30 minutes then washed three times in 1ml methanol.
15 The whole step was repeated and the samples left to air dry. Long hair strands were divided
16 into 2cm long segments. $\delta^{13}\text{C}$ analyses were carried out at the Natural Environment
17 Research Council (NERC) Isotope Geosciences Facility in Keyworth, Nottinghamshire,
18 U.K. by weighing 0.6 mg subsamples of hair into tin capsules and analyzed using
19 Continuous Flow-Elemental Analysis-Isotope Ratio Mass Spectrometry (CF-EA-IRMS)
20 comprising an Elemental analyser (Flash/EA) coupled to a Thermo Finnigan Delta plus XL
21 isotope ratio mass spectrometer via a ConFlo III interface. All reported isotope ratios are
22 expressed using the delta (δ) notation in parts per thousand (per mil: ‰) relative to a
23 standard:

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$$\delta (\text{‰}) = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000$$
, where R is a ratio of heavy to light isotope (e.g.,
25 $^{13}\text{C}/^{12}\text{C}$)

26 $\delta^{13}\text{C}$ results were reported in per mil (‰) relative to V-PDB. Internal lab standards
27 were used through the run to correct for instrument drift and to normalize the data to
28 internationally accepted standards. Hair carbon isotope ratios were calibrated using an in-
29 house reference material Hair-2 with expected delta values of -20.73‰ (calibrated against
30 CH7, IAEA). The 1σ reproducibility for mass spectrometry controls for the hair analyses
31 were better than $\pm 0.2\text{‰}$. $\delta^{15}\text{N}$ analyses presented here are partly published in Fares et al.
32 (2015).

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2 **Results**

3 The atomic C/N ratio for human hair ranges from 2.9 to 3.8 (O'Connell and Hedges, 1999:
4 415) and was used in this study as an indication of quality and integrity of the ancient hair
5 protein. All hair samples included in the present study had C/N ratios in the range of 3.5 to
6 3.8 which suggests very little diagenetic changes or contamination (Roy et al. 2005;
7 Knudson et al. 2007). The average carbon isotope composition for the whole population
8 was -17.95 ‰ (SD=1.8). We published the nitrogen isotope composition of only adults and
9 subadults in Fares et al. (2015). The average nitrogen isotope value for the whole hair
10 materials including infants is 9.43‰ (SD= 1.4).

11 **Cemetery differences**

12 Shapiro-Wilk tests of normality revealed that the carbon isotope values in both
13 cemeteries were not normally distributed necessitating non parametric statistical analysis.
14 Consequently, Mann-Whitney tests were conducted to compare carbon isotope values
15 between the two cemeteries. A significant difference between the two cemeteries has been
16 found in carbon isotope measurements of the hair samples (U=177, p = 0.04), where R
17 cemetery showed a higher $\delta^{13}\text{C}$ mean=-17.6‰ (SD=1.5) compared to S cemetery = -18.2‰
18 (SD=2.0). We used the nitrogen isotope analysis data of the same hair materials, which was
19 partially published in Fares et al. (2015), in order to generate a plot of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
20 values of hair samples from both Kulubnarti cemeteries as indicated in Figure 3. Each value
21 of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ represent hair sample from an individual and for those who had long hair,
22 an average value from the whole hair sample has been used.

23 **Sex and age differences**

24 Differences in the carbon isotope value by sex could not be examined as there were
25 insufficient sexed individuals to test in both cemeteries; we had only 10 individuals with
26 known-sex, four males and six females. As the number of adults and infants from each
27 cemetery is small, the age differences in the dietary carbon isotope values were examined
28 on the whole Kulubnarti population. A Kruskal-Wallis test showed no significant
29 differences in the carbon isotope compositions among the three age groups, whereas a
30 significant difference was observed in the nitrogen isotope composition (Table 1). After
31 transformation of data to be normally distributed, Post hoc comparisons using the LSD test
32 indicated that the significant nitrogen isotope difference was mainly between the youngest

1 age group (infants) and the children and adolescents where the youngest age group has
2 higher nitrogen isotope values. The total number of the hair samples with known age
3 represented in Table 1 is 44 (19 from the R cemetery and 25 from the S cemetery), as there
4 are 3 individuals with unknown age.

5 **Variations in the carbon isotope composition along the hair shaft**

6 During sampling of the hair materials it was noted that some mummies had long
7 hair strands (>2 cm), in such cases we cut the whole hair bundle (12-15 strands)
8 transversely into 2 cm length sequential segments. Starting from the scalp to the hair tip,
9 each 2cm length segment has been marked and analyzed separately. Unfortunately, long
10 hair strands (>2 cm) were only obtained from 6 individuals (2 infants, 3 juveniles and one
11 individual of unknown age). Table 2 shows the sequential carbon and its corresponding
12 nitrogen isotope values along the hair strands which reflects a varying isotope compositions
13 of diet over more than 2 months according to the hair length. The greatest variation was
14 found in a hair sample from mummy number S72b which showed nearly 8‰ variation in
15 the carbon isotope values over an estimated period of 4 months. Nitrogen isotope values of
16 this mummy, however, did not show much variation. Figure 4 shows shifts in $\delta^{13}\text{C}$ values
17 along the hair shafts from all 6 individuals revealing marked cyclical variations in the
18 samples which had more than two sequential hair segments; each segment is assumed to
19 correspond to ~ 2 months of hair growth. Figure 5 shows the much smaller shifts in the
20 corresponding values of $\delta^{15}\text{N}$ from the same individuals (modified from Fares et al. 2015).

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1 Table (1). Comparison of carbon and nitrogen isotope values of hair samples by age in
 2 Kulubnarti population.

Isotope	Age group	N	Mean isotope values	Mean Rank	Kruskal Wallis Test		
					Chi-Square	df	P
$\delta^{13}\text{C}$	1 (0-3)	7	-17.83	23.06	1.76	2	.415
	2 (4-17)	29	-18.14	21.48			
	3 (>18)	8	-17.41	28.44			
Total		44	-17.95				
$\delta^{15}\text{N}$	1 (0-3)	7	10.54	33.12	8.25	2	.016*
	2 (4-17)	29	9.01	19.03			
	3 (>18)	8	9.81	27.25			
Total		44	9.43				

3 *Indicates significant differences

4 N= number of the individuals

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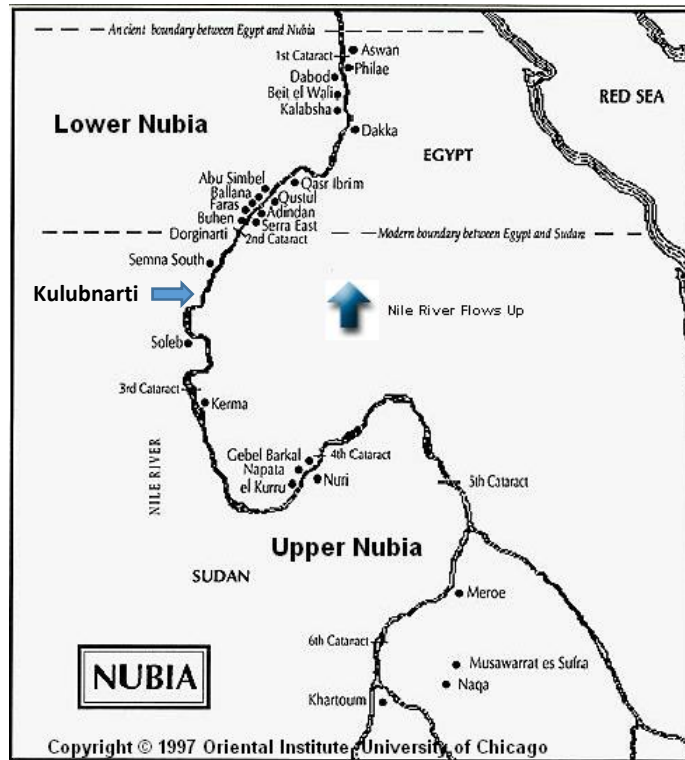
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7 Table (2). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the successive segments along the hair shafts from
 8 individuals with hair longer than 2cm.

Mummy number	age	Hair segments from scalp to hair tip (Each segment = 2 cm).										Mean		variation	
		1 2 months*		2 4 months		3 6 months		4 8 months		5 10 months					
		$\delta^{13}\text{C}$ ‰	$\delta^{15}\text{N}$ ‰	$\delta^{13}\text{C}$ ‰	$\delta^{15}\text{N}$ ‰	$\delta^{13}\text{C}$ ‰	$\delta^{15}\text{N}$ ‰	$\delta^{13}\text{C}$ ‰	$\delta^{15}\text{N}$ ‰	$\delta^{13}\text{C}$ ‰	$\delta^{15}\text{N}$ ‰	$\delta^{13}\text{C}$ ‰	$\delta^{15}\text{N}$ ‰	$\delta^{13}\text{C}$ ‰	$\delta^{15}\text{N}$ ‰
R160	?	-14.8	8.7	-16.2	8.7	-17.9	8.2	-18.8	8.5	-18.2	8.4	-17.2	8.5	3.4	0.3
S124	5	-19.9	7.4	-17.9	8.5	-	-	-	-	-	-	-18.9	8.0	2.0	1.1
S209	1	-18.2	13.9	-18.7	12.4	-	-	-	-	-	-	-18.5	13.2	0.5	1.5
S72b	10	-16.8	8.9	-12.3	8.2	-18.3	8.6	-20.2	8.4	-	-	-16.9	8.5	3.4	0.7
S94b	15	-19.8	10.9	-19.5	10.7	-	-	-	-	-	-	-19.7	10.8	0.3	0.2
S95	11	-19.7	8.1	-19.6	7.7	-	-	-	-	-	-	-19.7	7.9	0.1	0.4

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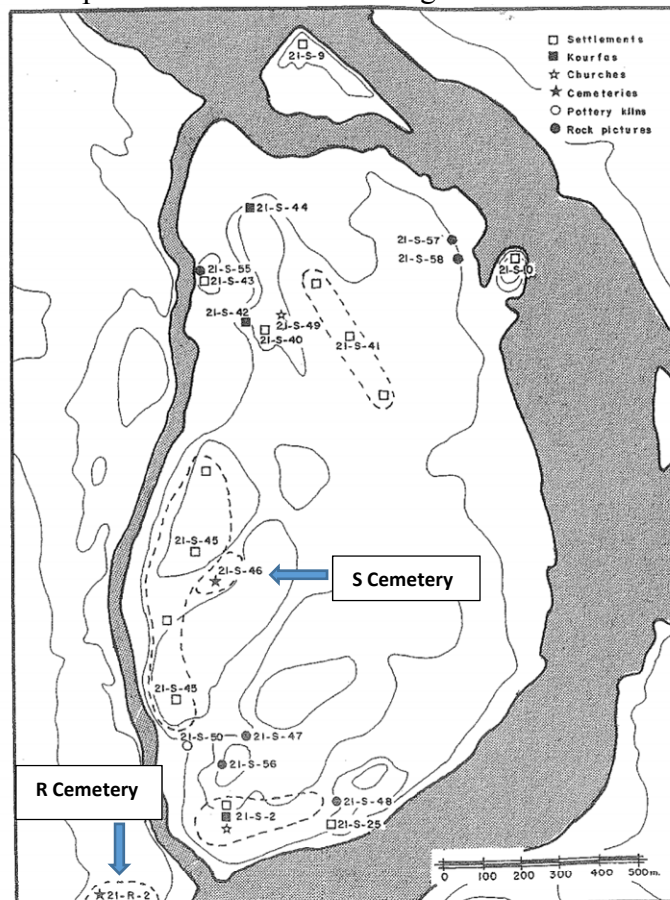
10 *Month before death



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Fig. 1. Map of ancient Nubia showing location of Kulubnarti.



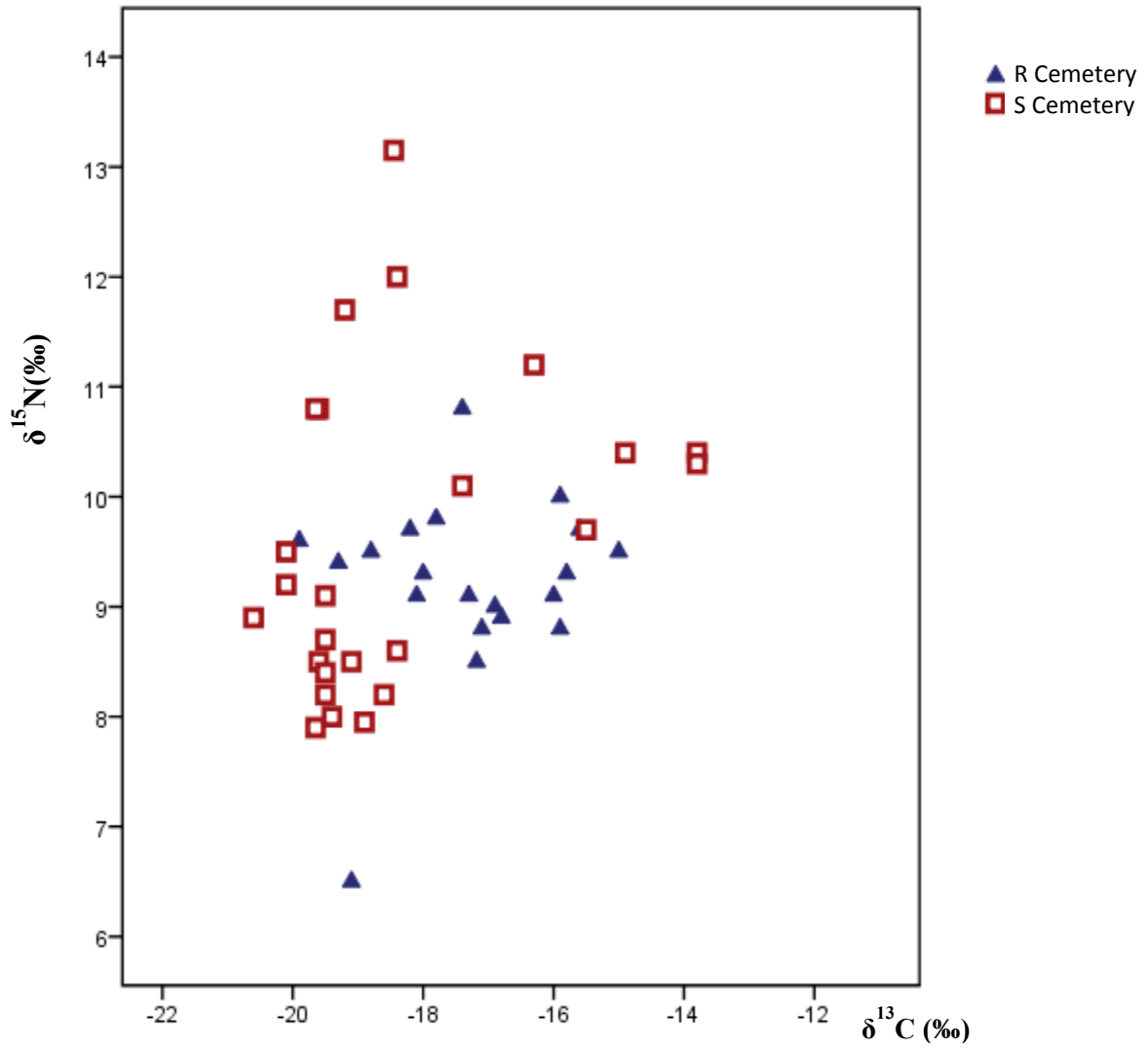
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Fig. 2. Map showing cemeteries on the site of Kulubnarti (Adams et al. 1999)

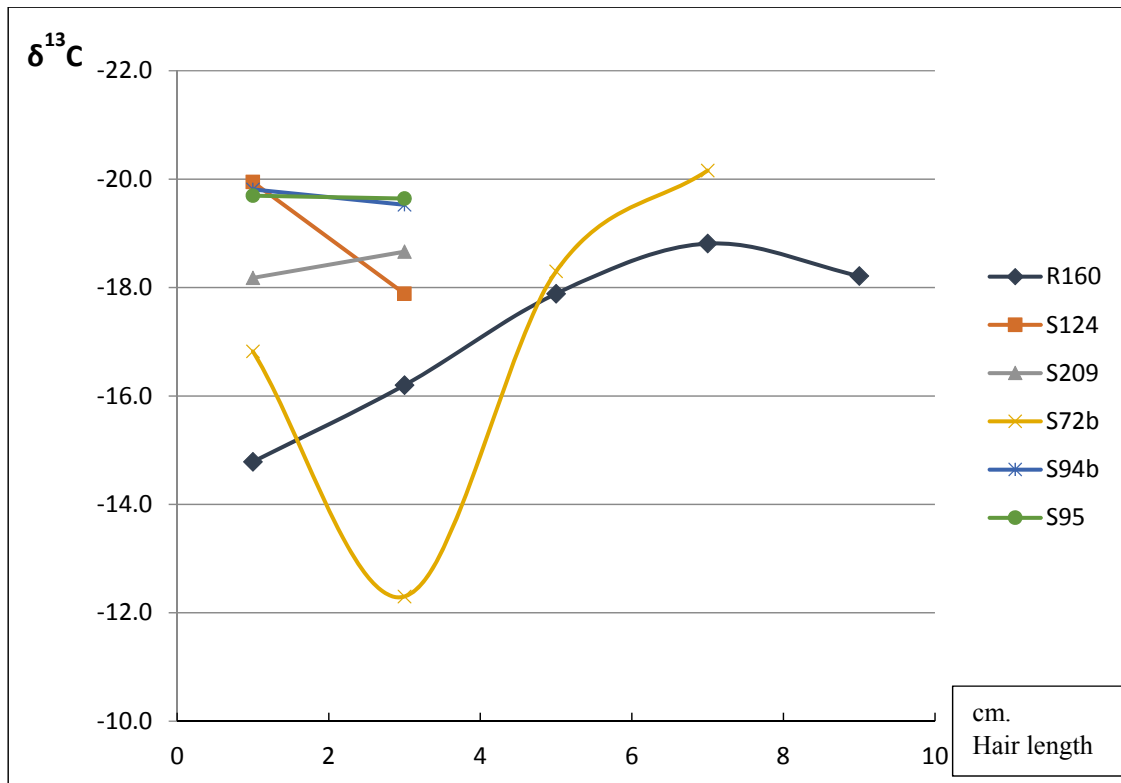
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Fig. 3. Plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data measured from hair samples of both Kulubnarti $\delta^{13}\text{C}$ Cemeteries.

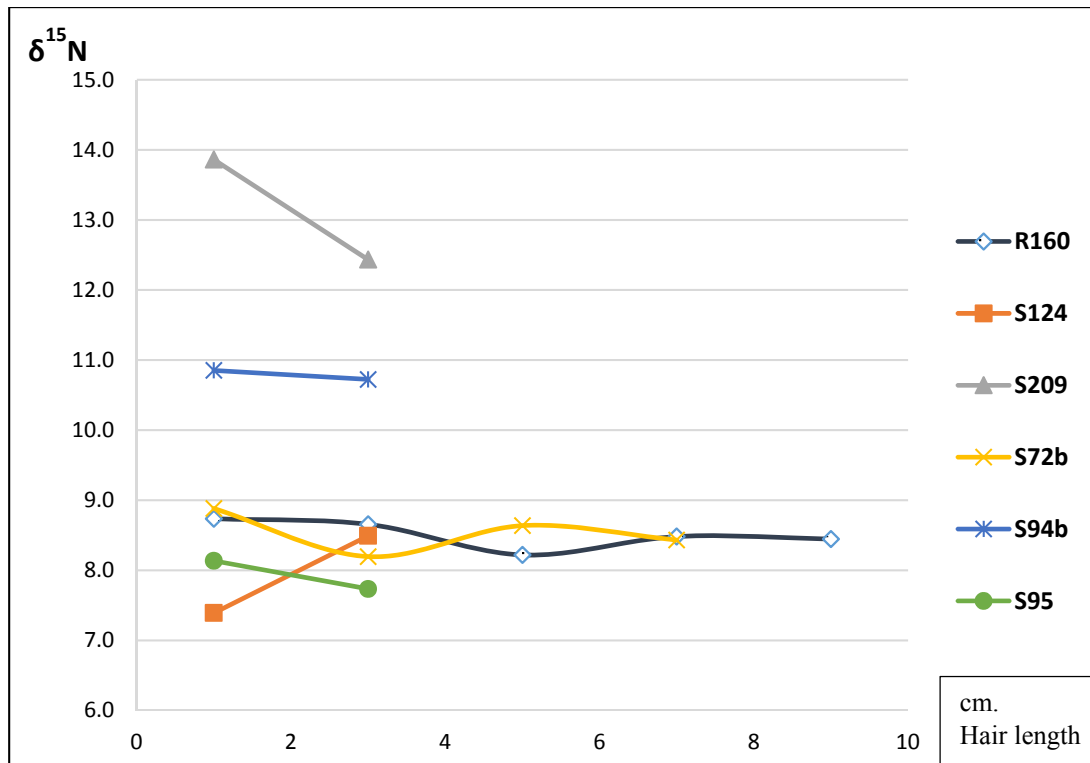


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3 Fig. 4. Shifts in $\delta^{13}\text{C}$ values along the hair shafts from 6 individuals showing the marked
 4 variations in the sequential hair segments, particularly samples R160 and S72b. Each
 5 segment =2 cm corresponded to ~ 2 months. The isotope value has been plotted in the
 6 middle of the length of each segment.

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3 Fig. 5. Shifts in $\delta^{15}\text{N}$ values along the hair shafts from 6 individuals plotted
 4 against the sequential hair segments; each segment =2 cm corresponded to ~
 5 2 months. The isotope value has been plotted in the middle of the length of
 6 each segment (modified from Fares et al. 2015)

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8 Discussion

9 Diet of the Kulubnarti population

10 The mean carbon isotope value of our hair samples = -17.95 ± 1.8 ‰. A consistency
 11 has been observed between the present carbon isotope results of hair samples and previous
 12 studies of bone (-17.4 ± 1 ‰, Turner et al. 2007) and soft tissue (-17.1 ± 0.8 ‰, Basha et
 13 al. 2016) from the same Kulubnarti site. These isotope results suggest a diet containing mix
 14 of C3 and C4 plant sources, mostly C3 ones. Nitrogen isotope composition of the hair
 15 samples from Kulubnarti individuals suggested a dietary protein mainly from plant sources
 16 and less protein from animal sources. However, few individuals that have $\delta^{15}\text{N}$ values
 17 above 10‰ might have consumed more animal protein. Aquatic protein sources are not
 18 evident and the animal dietary protein was mainly from terrestrial sources (Fares, et al.

1 2015). There is also a lack of archaeological evidence of fish consumption (Adams 1977).
2 The results of previous trace elements study and isotope analysis of bone collagen and soft
3 tissues at Kulubnarti (Sandford and Kissling 1994; Turner et al. 2007; Basha et al. 2016)
4 have indicated that dietary animal protein may have made relatively little contribution to
5 the diet of the population as well.

6 **Cemetery differences**

7 Although the previous carbon isotope study of bone collagen did not find dietary
8 differences between the two cemeteries (Turner et al., 2007), both the soft tissue $\delta^{13}\text{C}$
9 results of Basha et al. (2016) along with the present study of hair $\delta^{13}\text{C}$ measurements
10 showed significant differences. Significantly higher $\delta^{13}\text{C}$ values of the R cemetery
11 individuals compared to S ones is observed. This significant difference might be explained
12 as higher consumption of C_4 -based food sources were available to the R cemetery, yet other
13 social or religious factors might be involved. This difference might only appear in the
14 metabolically active soft tissues, including hair, as these tissues register short-term
15 variations in diet in contrast to the long-term averaged isotope values preserved in bone.
16 The lack of any significant differences between the two cemeteries in nitrogen isotope
17 compositions of bone, hair, soft tissues suggested little difference in the type of consumed
18 protein (Turner et al., 2007; Fares et al. 2015; Basha et al., 2016). Although our results
19 suggest the availability of more C_4 plant-based dietary resources for R cemetery
20 individuals, the nitrogen isotope results (Turner et al. 2007; Fares et al. 2015; Basha et al.
21 2016) showed the two cemeteries depended considerably on plant protein with little dietary
22 animal protein and a lack of aquatic protein sources. Hummert (1983) in the study of the
23 cortical bone growth in Kulubnarti children, by calculation of total subperiosteal area,
24 cortical area, medullary area, and percent cortical area at tibia midshaft, suggested a
25 quantitative, but not qualitative, dietary improvement in R cemetery which is supported by
26 the findings of the present study. It is worth noting that recent analysis of ancient
27 mitochondrial DNA showed different haplogroups of individuals from the two cemeteries
28 where, S cemetery inhabitants mostly showed African-based haplogroup whereas, R-
29 cemetery individuals displayed predominance of European and Near Eastern haplogroups
30 (Sirak et al. 2016). Therefore, differences in health status that observed between individuals
31 of the two cemeteries could be related to causes other than dietary or complement to the
32 diet.

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2 **Sex and age differences**

3 Although we cannot examine sex differences in diet using the limited number of
4 hair samples that we have, carbon and nitrogen isotope analysis of bone collagen (Turner
5 et al. 2007) along with results of soft tissue isotope analysis (Basha et al. 2016) showed no
6 dietary differences based on sex of the individuals in this population. Nubian mummies
7 from Wadi Halfa also showed no sex-specific dietary trends based on the study conducted
8 by White (1993) on hair carbon isotope compositions. The lack of significant differences
9 between age groups in $\delta^{13}\text{C}$ values of hair samples is in agreement with results of soft tissue
10 $\delta^{13}\text{C}$ reported for the Kulubnarti population by Basha et al. (2016). On the other hand, the
11 $\delta^{15}\text{N}$ values of juveniles were lower than that of infants and adults. The significantly higher
12 nitrogen isotope values found in the youngest age group might be related in part to the
13 effects of breast feeding as it is well known that nursing children have higher nitrogen
14 isotope values which falls after weaning (Fogel et al. 1989). However, in subadults aged
15 between 4 and 17 years there is a significant depletion in the nitrogen values. Turner et al.
16 (2007) reported that adolescents bone collagen were significantly ^{15}N -depleted relative to
17 adults and infants and explained this depletion as either due to differential growth rates
18 and/or stress events in this group or due to age-specific dietary differences. It is worth
19 noting that the S cemetery adolescents may have been suffered from diseases that might
20 be linked to a diet poor in protein sources such as cribra orbitalia (Mittler and Van Gerven
21 1994). The same depletion of nitrogen isotope values in this age group has been identified
22 in several populations from a wide range of geographical locations and time periods (Turner
23 et al. 2007; Reynard and Tuross 2015: 619) increasing the likelihood that the depletion is
24 due to systematic growth-related metabolic effects. Yet, this might require further study
25 and research.

26 **Seasonal variation at Kulubnarti**

27 Seasonal variations in diet could not be inferred from bone collagen isotope
28 composition because of the slow turnover rate of bone. However, a wide range of isotope
29 values inside each age group was found, suggesting a need for more detailed work to
30 investigate seasonal dietary variations (Turner et al. 2007). A range from -20.6 to -13.8 ‰
31 in the carbon isotope composition of analyzed hair samples has been recorded in the present
32 study (Fig. 3). This markedly wide range is similar to that found in Wadi Halfa (White

1 1993). Seasonal variations in diet have been suggested for the Wadi Halfa population
2 through analysis of carbon isotope compositions of hair samples dated to different cultural
3 periods including the Christian one (White 1993; White and Schwarcz 1994; Schwarcz and
4 White 2004). Additionally, in our Kulubnarti sample a large variability has been found in
5 carbon isotope compositions of the sequential segments of individual hair samples which
6 most probably indicates the presence of seasonal dietary variation in Kulubnarti. White et
7 al. (1999) did not find such seasonal variation in subsistence in a population from Kharga
8 Oasis (dated to AD 400- 700), a community that was roughly contemporaneous to both the
9 Kulubnarti and Wadi Halfa samples. The nitrogen isotope compositions recorded from the
10 S cemetery (range from 7.4‰ to 13.9‰) hair samples showed higher variability compared
11 to R cemetery (range from 5.8‰ to 10.8‰). Such observed wide range of nitrogen isotope
12 values particularly among the S cemetery individuals, could be related to age differences
13 in the type of consumed protein.

14 Another possible interpretation of the wide range noticed in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
15 values, is the presence of non-local immigrants who may have had different diets to those
16 of the indigenous population. Variations in $\delta^{18}\text{O}$ of bone carbonate have been observed
17 between different age groups in the Kulubnarti population which might suggest the
18 presence of immigrants among the population, particularly of adults (Turner et al. 2007).
19 Immigration has already been proposed at Wadi Halfa as well (White et al. 2004).

20 **Variations in $\delta^{13}\text{C}$ values along the individual hair shafts**

21 In the present study $\delta^{13}\text{C}$ values from individuals who had hair longer than 2cm,
22 and therefore represent a period of time more than 2 months, were recorded from the
23 successive segments. Variations of up to 3.9‰ has been found in the $\delta^{13}\text{C}$ values of hair in
24 human subjects in a controlled diet feeding experiment as a result of changing the diet
25 (Huelsemann et al. 2009). From those who had long hair, a high nitrogen isotope value
26 from mummy number S209 has been observed. This mummy represents an infant that was
27 aged 1 years old so, such a high $\delta^{15}\text{N}$ value is expected as a result of breastfeeding (Fogel
28 et al. 1989).

29 The maximum hair length that was available reached 10cm (sample number R160, Fig. 4).
30 Unfortunately, the sex and age of the individual could be not determined. This specimen
31 showed an interesting pattern in the $\delta^{13}\text{C}$ values, where a regular increase in the $\delta^{13}\text{C}$ value
32 from -18.8 ‰ at the segment 6-8cm to -14.8 ‰ at the scalp is observed. This probably
33 indicates a regular increase in the proportion of C_4 plants consumed (either directly or

1 through consumption of products of animals feeding on C₄ plants) for a period of 6 months
2 prior to death. On the other hand, $\delta^{15}\text{N}$ values of the same individual (R160) showed little
3 variance over sequential hair segments which indicate little or no differences in type of
4 dietary protein during the same time period.

5 The next longest specimen of hair was about 8cm for a 10 year old child, sample number
6 S72b, (Fig. 4) which showed a gradual increase in the $\delta^{13}\text{C}$ values from segment 6-8cm to
7 4-6cm, followed by a more pronounced ^{13}C -enrichment to segment 2-4 cm followed by a
8 steep decrease in $\delta^{13}\text{C}$ value from -12.3 ‰ to -16.8 ‰ just near the scalp. Although -12.3
9 ‰ is a relatively high carbon isotope value, similar high values have been found in hair
10 samples from a Christian population at Wadi Halfa (White 1993). However, the most
11 interesting thing here is the abrupt increase from -18.3 to -12.3‰ in a very short time period
12 (2 months) then the quick decrease back to a value of -16.8‰. A similar trend has been
13 observed in Wadi Halfa, where hair samples that have a $\delta^{13}\text{C}$ value less than -16‰ always
14 showed a slow and gradual change whereas those with $\delta^{13}\text{C}$ values higher than -16‰ were
15 characterized by a rapid change and usually showed a sudden variation in the $\delta^{13}\text{C}$ values
16 (Schwarcz and White 2004). However, our sample still shows substantial variation in a
17 short time period. In contrast to carbon isotope composition, the slight variation observed
18 in $\delta^{15}\text{N}$ values along successive hair segments in this specimen (S72b) indicate little change
19 in the type of dietary protein consumed. A possible interpretation of the observed large
20 variation in $\delta^{13}\text{C}$ values is that this 10-year-old child might have had access to a diet from
21 different food sources, as he/she could be a non-local immigrant and the diet had changed
22 substantially as a consequence of the transition from one place to another. However, the
23 immigration in Kulubnarti proposed by Turner et al. (2007) for adults aged over 35 years
24 old at death. The other sequential hair samples were only 4 cm long and showed both
25 directions of transition from C₃ to C₄ based-diet and vice versa. Although variations in the
26 nitrogen isotope compositions along the hair shafts were much less than that of carbon from
27 the same hair sample, both increasing and decreasing of $\delta^{15}\text{N}$ value are still observable
28 along the individual hair shafts (Fares et al. 2015). This might indicate seasonal variation
29 in the diet and transition of the dietary protein between different categories of plants (C₃ &
30 C₄) throughout the year.

31 To sum up, our results have indicated a transition between dominant C₃ dietary
32 sources in winter (Adams 1977) to dominant C₄ dietary sources in summer (Adams 1977)
33 with a small contribution of the non-harvested, alternative, crop was still contributing to
34 the $\delta^{13}\text{C}$ value. However, the mean $\delta^{13}\text{C}$ value along the individual hair shafts points to the

1 overall dominance of the C₃-based food which might indicate a higher relative
2 consumption. Comparable inferences have been deduced at Wadi Halfa where there was
3 dominance of the consumption of the specific C₃ or C₄ crop in its harvested season,
4 however a small percentage of the alternative crop was still contributing to the diet.
5 Furthermore, it was proposed at Wadi Halfa that storage facilities of the crops might be
6 applicable at a small scale as an emergency plans only with the dominant use of the
7 harvested seasonal crop (Schwarcz and White 2004). Similar proposition might be
8 applicable to the Kulubnarti population as well.

9 **Conclusion**

10 The present study has investigated diet of the Medieval Nubian population of
11 Kulubnarti using carbon isotope analysis of hair samples from mummies of two cemeteries.
12 The carbon isotope values suggest a diet contains both C₃ and C₄ plant sources. The results
13 showed no age differences in the dietary carbon sources consumed by the Kulubnarti
14 population. Cemetery differences have been observed which might indicate a significantly
15 higher consumption of C₄-based food sources amongst the R cemetery individuals
16 comparing to S ones. It is possible that seasonal variation in crop growing was the cause of
17 carbon isotope variations along the hair of some individuals, representing a transition from
18 dominant C₃ plants in the winter to dominant C₄ in the summer. The results also suggest
19 that the individuals at Kulubnarti might have practiced a little food storage activity.

20 **Acknowledgments**

21 The authors would like to thank the Cultural Affairs and Missions section of the Egyptian
22 Ministry of Higher Education for the financial support in conducting this research.

23 **Conflict of Interest**

24 The authors declare that they have no conflict of interest.

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