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Reconstructing Infant Weaning Histories at Roman Period Kellis, Egypt Using Stable Isotope Analysis of Dentition

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ABSTRACT Studies of infant feeding and weaning patterns in past populations that rely on a cross-sectional approach must make the assumption that no infant mortality bias exists. Previous investigations of infant weaning patterns at the Dakhleh Oasis, Egypt, relied on cross-sectional isotope data. In this study, we re-examine this weaning pattern, using a simulated longitudinal approach, which does not require any assumptions regarding potential infant mortality biases. This involves examining the dental isotopic signatures of individuals who survived the weaning process. Stable isotope signatures from juveniles and adults (102 individuals, 297 teeth) were examined to reconstruct the weaning history of those that survived the weaning process. Both deciduous and permanent teeth were sampled. Homogenized enamel and dentin samples were isolated from each tooth and analyzed for $\delta^{13}C_{ap}$ and $\delta^{18}O_{ap}$ from the enamel and $\delta^{15}N_{coll}$ and $\delta^{13}C_{coll}$ from dentin collagen. We investigate differences between *in utero* versus postbirth, preweaning versus postweaning, and juvenile versus adult stable isotope values as reflected in the dentition. A random permutation procedure was used to test for statistically significant differences in stable isotope values between tooth types. Statistically significant differences were observed in all stable isotopes between permanent and deciduous teeth, and between early and later forming permanent teeth in $\delta^{13}C_{ap}$ and $\delta^{15}N_{coll}$ isotopes. These results indicate dietary change between *in utero* and postbirth, and changes occurring during the weaning period. These results provide a more comprehensive picture of infant weaning practices at Kellis and provide further support that complete weaning occurred by 3 years of age. Am J Phys Anthropol 134:63–74, 2007.

Studies of living populations reveal the importance of diet and nutrition in influencing juvenile growth and development, and ultimately, the demographic structure of a population (Delgado et al., 1982; Stuart-Macadam and Dettwyler, 1995). Cultural ideologies regarding infant[1] feeding and weaning practices, as well as access to resources, are key determinants of infant survival; in total, these factors influence overall population growth or decline. Therefore, the study of infant feeding and weaning practices in past populations is recognized as a significant component in the reconstruction of population demography (Herring et al., 1998).

Stable isotope analyses are an established method for determining prehistoric diet and for examining infant feeding and weaning patterns from different chronological and geographical populations (e.g., Katzenberg et al., 1993; White and Schwarcz, 1994; Schurr, 1997; Wright and Schwarcz, 1998; Dupras, 1999; Dittmann and Grupe, 2000; Richards et al., 2002; Prowse et al., 2004, 2005; White et al., 2004; Schurr and Powell, 2005; Williams et al., 2005; Fuller et al., 2006b). At present, investigations of infant feeding and weaning patterns in archaeological samples generally utilize a combination of three stable isotopes: nitrogen, carbon, and oxygen (see Katzenberg, 1992, 2000 and Schwarcz and Schoeninger, 1991, for reviews of the uses of stable isotope analyses in anthropological contexts).

STABLE ISOTOPES AND WEANING PATTERNS

Nitrogen

Nitrogen stable isotopes help determine weaning patterns by illustrating the shift between a reliance on human milk proteins to proteins obtained from solid foods (e.g., Katzenberg and Pfeiffer, 1995; Fogel et al., 1997; Schurr, 1998). Reconstructions of weaning patterns commonly rely on nitrogen isotope analyses from bone

[1]We use the term "infant" to describe the period during which individuals are breastfeeding and experiencing the weaning process. This period may go beyond the age of one, which is typically defined as "infant" (e.g., Baker et al., 2005). Later, we also use the term "juvenile" (Tables 1 and 2) to describe those individuals who have not yet reached skeletal maturity.

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collagen (Katzenberg and Pfeiffer, 1995; Schurr, 1998; Schurr and Powell, 2005). The isotopic composition of nitrogen in collagen, from either bone or dentin, reflects the isotopic composition of dietary proteins. There is a stepwise increase of ~3‰ from one level of the food web to the next; this is known as the trophic level effect (Schoeninger and DeNiro, 1984; Schwarcz et al., 1999). A breast-feeding infant receives its protein from its natural (or surrogate) mother; thus, the $\delta^{15}N$ value of the infant's tissues is ~2–3‰ higher than that of its mother (Fogel et al., 1997).[2] Typically, weaning is gradual as supplementary foods are slowly added to the infant's diet as dependency on breast milk decreases. If $\delta^{15}N$ is measured using bone collagen from a cross-sectional sample, dietary change is detected through decreasing $\delta^{15}N$ values that reach adult values once the weaning process has ceased (Schurr, 1998; Dupras, 1999; Dupras et al., 2001). If $\delta^{15}N$ is measured using dentin collagen, the enriched isotopic signal remains for the entire life of the individual because primary dentin is a biologically static tissue, that once deposited does not change its chemical composition (Hillson, 2003).

Carbon

Carbon stable isotopes help distinguish between diets based on C_3 and C_4 plants[3] (e.g., Katzenberg et al., 1995). C_3 plants, such as wheat, barley, rice, grasses, trees, and most fruits and vegetables, range in $\delta^{13}C$ values from −22 to −33‰, with a mean of −28‰ (Smith and Epstein, 1971). C_4 plants include maize, sorghum, some millets, sugar cane, and tropical grasses. C_4 plants are enriched in ^{13}C in comparison to C_3 plants with values that range from −16 to −9‰, with a mean of −13.5‰ (Smith and Epstein, 1971). The carbon isotope signals of plants are transferred through the food web. There is ~+5‰ difference between plant $\delta^{13}C$ values and those of human collagen (Ambrose and Norr, 1993), and a difference of +11–12‰ between plant $\delta^{13}C$ values and those of human bone and enamel carbonate (Krueger and Sullivan, 1984; Passey et al., 2005). Thus, humans consuming a diet based on C_3 plants have collagen $\delta^{13}C$ values of ~−19‰, and bone/enamel carbonate $\delta^{13}C$ values of ~−12‰. Humans consuming a diet consisting only of C_4 plants have collagen $\delta^{13}C$ values of ~−8‰, and bone/enamel carbonate $\delta^{13}C$ values of ~−1‰. Individuals that consume a mixed diet of both C_3 and C_4 plants have both collagen and bone/enamel carbonate $\delta^{13}C$ values that are intermediate between the earlier values.

Oxygen

Oxygen stable isotopes help detect changes in infant water consumption from breast milk to environmental sources (Wright and Schwarcz, 1998). Oxygen isotopes of enamel apatite carbonate closely reflect the $\delta^{18}O$ values of water in the body. Enamel is a static tissue; therefore, the isotopic signature captured during crown development remains constant throughout an individual's life. Oxygen stable isotope analyses of dental enamel from various modern and fossil animals have shown seasonal changes in water consumption (Koch et al., 1989; Stuart-Williams and Schwarcz, 1997; Balasse, 2002). The weaning process is detectable through shifts in $\delta^{18}O$ because breast milk is significantly enriched in ^{18}O compared with drinking water. Thus, as an infant survives the weaning process, their $\delta^{18}O$ values change because of the inclusion of other water sources in their diet. Once weaning is complete, the values begin to reach similar levels as adults in the population. This "weaning signal" has been documented both in extant and extinct animals (Fricke and O'Niel, 1996; Franz-Odendaal et al., 2003), as well as in archaeological human populations (Wright and Schwarcz, 1998; White et al., 2004; Williams et al., 2005).

Potential mortality bias

Stable isotope analyses often involve sampling a cross-section of individuals to examine patterns of weaning and food consumption in a population. These cross-sectional approaches commonly reconstruct and compare various consumer profiles within the population sample to test a wide range of hypotheses about prehistoric diet. For example, Dupras et al. (2001) used cross-sectional carbon and nitrogen stable isotope data derived from bone collagen to reconstruct and compare the consumer profiles for subsets of a Roman period population from the Dakhleh Oasis, Egypt to investigate infant feeding and weaning patterns. The consumer profile comparisons of the various population subsets suggested that breast-feeding was performed exclusively for the first 6 months of life, after which infants were introduced to weaning foods that were enriched in ^{13}C (e.g., millet, or cow/goat milk from animals fed millet) (Dupras et al., 2001). It was concluded that the weaning process occurred slowly and culminated at ~3 years of age (Dupras et al., 2001).

Wood et al. (1992), however, point out that skeletal populations have an inherent "mortality bias" built into them; that is, the individuals are there for a reason—they died. Therefore, if infant diet is reconstructed from a cross-section of a skeletal sample, then the consideration of a potential infant mortality bias is important. The inherent assumption underlying the use of cross-sectional stable isotope data to investigate weaning patterns is that there is no infant mortality bias affecting the results. This assumption may be unreasonable because infants, in particular, are at the mercy of established customs and ideology concerning nutrition and weaning behavior. For example, an infant that is sick or not thriving may be treated differently than one that is healthy and thriving. Therefore, two infant consumer profiles may exist in one population—one for healthy infants that are more likely to survive the weaning process, the other for sick, nonthriving infants that are more likely not to survive the weaning process. Since the infant age cohorts of a cross-sectional skeletal sample may likely disproportionately represent sick and nonthriving infants, the assumption of no infant mortality bias may be invalid. If this assumption is, in fact, not valid, then the interpretations about infant feeding and weaning practices of past population based on cross-sectional stable isotope data are likely inaccurate.

[2]Stable isotope values are expressed using the δ notation where $\delta = [(R_{sample}/R_{standard}) - 1] \times 1{,}000$. $R = {}^{13}C/{}^{12}C$ for $\delta^{13}C$; $R = {}^{15}N/{}^{14}N$ for $\delta^{15}N$; $R = {}^{18}O/{}^{16}O$ for $\delta^{18}O$. All δ values are expressed in parts per mille (thousand) (‰).

[3]C_3 and C_4 are used to differentiate the physiological pathways, in which plants metabolize carbon from the environment. C_3 plants use the enzyme bisphosphate decarboxylase to fix atmospheric carbon, resulting in a compound with three carbon atoms, while C_4 plants utilize the enzyme phosphoenol pyruvate carboxylase, resulting in a compound with four carbon atoms.

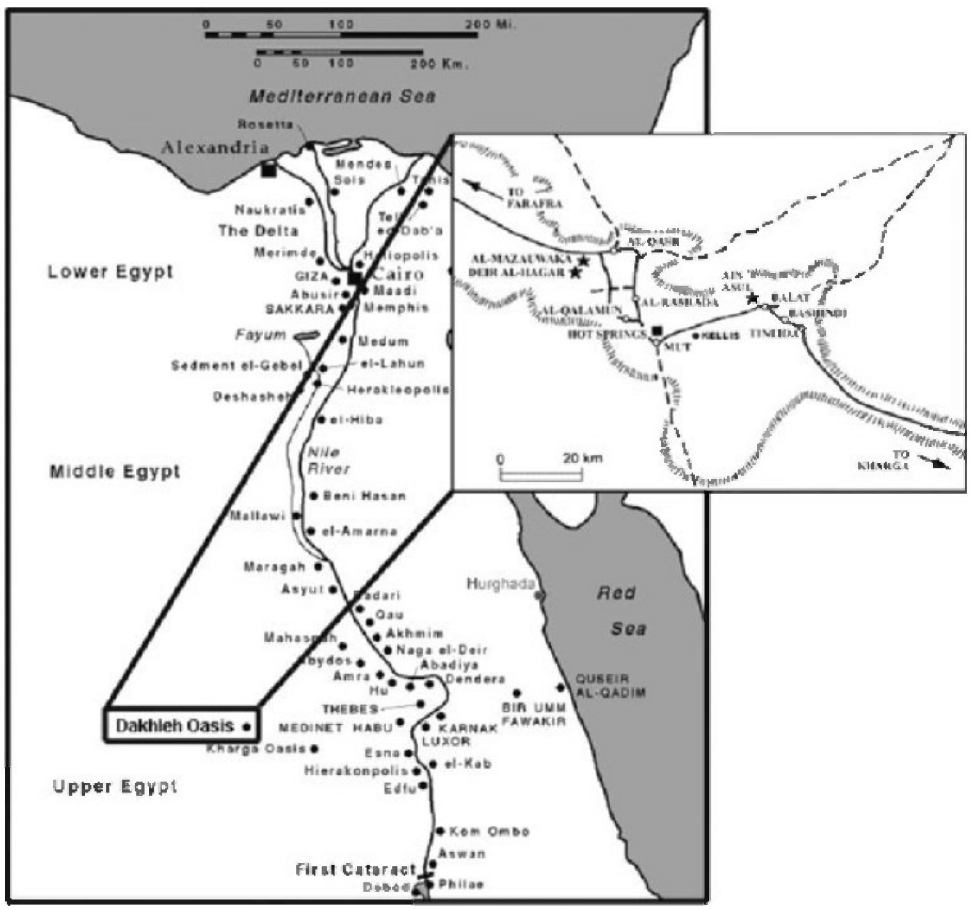

Fig. 1. Map of Egypt showing the location of the Dakhleh Oasis, and inset showing the location of Kellis within the Dakhleh Oasis.

One way to control for the potential infant mortality bias is to examine stable isotope ratios from individuals who successfully survived the weaning process (e.g., Fuller et al., 2003). Dental enamel and dentin are biologically static tissues that do not change their chemical composition after formation; that is, once they are formed they remain the same throughout life. As a result, the isotopic signatures that are captured during tooth formation are expected to remain constant through life. Since the dentition starts to form *in utero* and continues until ~20 years of age, isotopic information can be examined from various teeth representing various stages of an individual's life history, including those that occurred prior to and during the weaning period. Such an approach results in a study of infant feeding and weaning patterns that is essentially free of assumptions regarding potential mortality biases of the infant component of the skeletal sample. Moreover, the examination of the isotopic signatures of consecutively forming teeth from each individual in the study sample simulates a longitudinal study, rather than a cross-sectional one (Wright and Schwarcz, 1998, 1999; Fuller et al., 2003).

Purpose of this study

The purpose of this study is to further investigate infant feeding and weaning patterns during the Roman period at Kellis, Egypt through the reconstruction of individual weaning histories using the simulated longitudinal approach described earlier. Previous research at Kellis has relied on a cross-sectional approach to reconstruct and compare infant versus non-infant consumer profiles to assess the general weaning pattern of the population (Dupras, 1999; Dupras et al., 2001). The main advantages of reconstructing weaning life histories rather than the population weaning pattern in general is that it is free of assumptions regarding infant mortality and we can ask more specific questions about differences in diet that occurred during different stages of the weaning process.

This simulated, longitudinal approach is used on a skeletal sample of individuals ($N = 102$) from the Kellis 2 cemetery in the Dakhleh Oasis, Egypt (Fig. 1) to investigate the following questions. First, by comparing deciduous teeth, which form primarily *in utero*, with permanent teeth, which form primarily after birth, we examine if there is any dietary change that is reflected by the shift from the uterine to postnatal environment. To investigate this question in a cross-sectional study, isotopic analysis would need to be conducted on fetal remains, which in most archaeological contexts is not possible. Second, by comparing permanent teeth, in which 25‰ or more of their development typically occurs before 2 years of age (i.e., I1, I2, C, and M1),[4] with the

[4]I, i = incisor, C, c = canine, P = premolar, M, m = molar; uppercase letters = permanent teeth, lowercase letters = deciduous teeth; number denotes tooth's position.

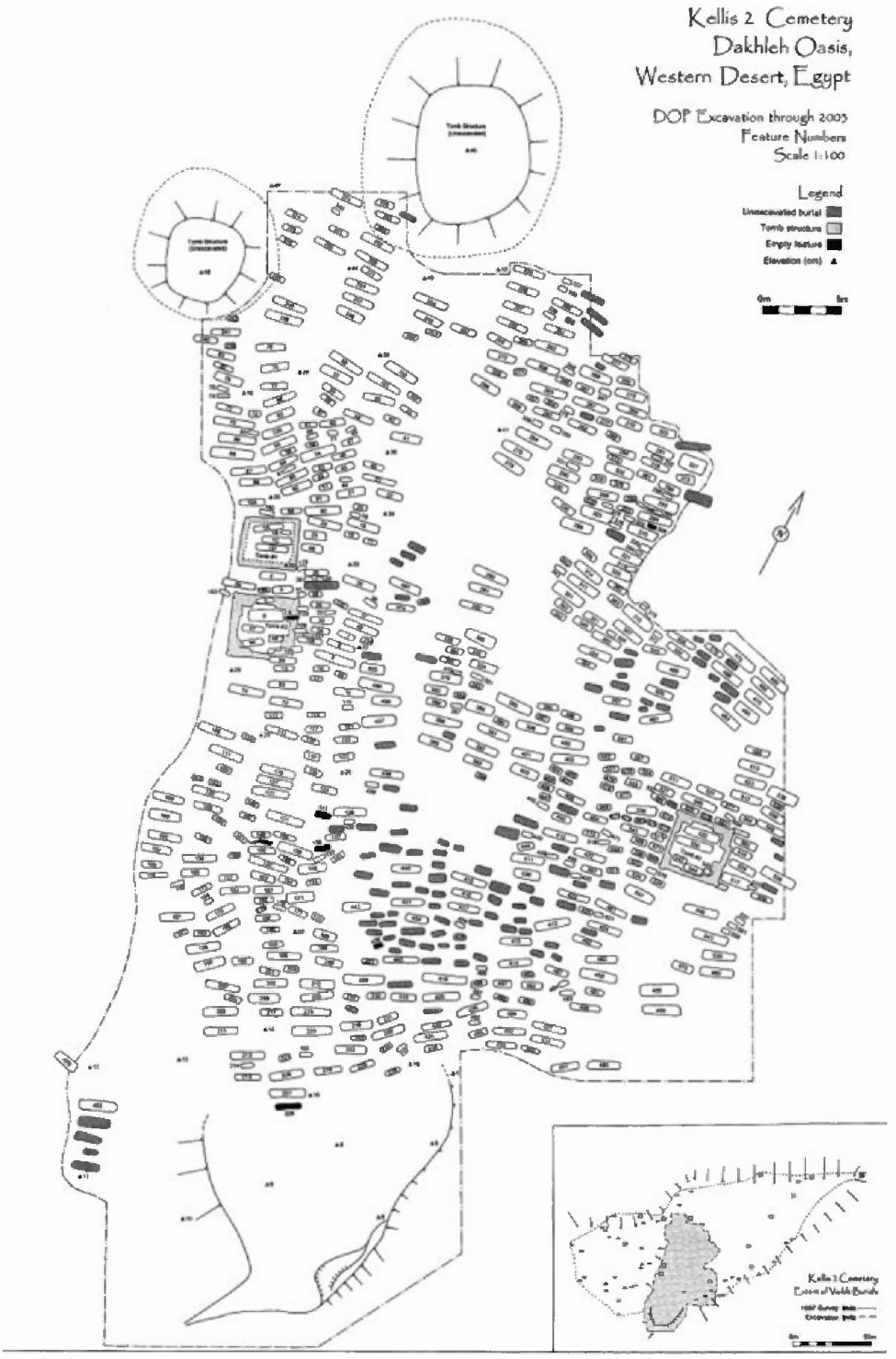

Fig. 2. Map of the Kellis 2 cemetery, illustrating the layout of the cemetery and position of the burials.

permanent teeth that develop almost exclusively after 2 years of age, we examine if there is any changes in diet, which would reflect the weaning process. Lastly, we compare permanent molars belonging to juveniles versus those that belong to adults, to examine the consistency of postweaning diet in Kellis individuals. These analyses significantly extend the previous work of Dupras et al. (2001), and together, provide a more comprehensive and less biased picture of the infant feeding and weaning practices at Kellis.

MATERIALS AND METHODS

The Dakhleh Oasis, Egypt (Fig. 1), has been under archaeological investigation by the Dakhleh Oasis Project (DOP) since 1978. The Dakhleh Oasis is located

~725 km south west of Cairo and is 1 of 5 major depressions in Egypt's Western Desert. The Oasis extends ~80 km east-west and 25 km north-south. It is made up of a depression that is roughly 100 m below the surrounding desert and is bordered by a large escarpment along the northern portion. The Dakhleh Oasis is also noted for seasonal extremes in temperature, varying from 25°C by midday in winter to 40–50°C by midday in summer (Blume et al., 1984; Giddy, 1987). Precipitation is relatively rare, with a mean annual rainfall of 0.3 mm/year. Based on paleoenvironmental studies, these environmental conditions are thought to be the same today as during the Romano-Christian Period (Churcher, 1983, 1993).

The core mandate of the DOP is to better understand the biocultural interaction between humans and such a harsh ecological environment (Mills, 1984). The bioarchaeological component of the DOP consists of ongoing research concerning mortuary treatment, paleodemography and migration, ancient diet and nutrition, skeletal growth and development, changes in health and disease, and paleogenetics (Birrell, 1999; Dupras, 1999; Fairgrieve and Molto, 2000; Molto, 2000, 2001, 2002; Dupras and Schwarcz, 2001; Dupras et al., 2001; Graver et al., 2001; Parr, 2002; Tocheri and Molto, 2002; Maggiano et al., 2003; Stewart et al., 2003; Williams and Dupras, 2004; Tocheri et al., 2005). At present, the majority of this research is focused on the past inhabitants of the Roman period town of Kellis (Fig. 1, inset), which has been under archaeological investigation since 1984 (Hope, 2001).

All individuals sampled for this study were excavated from the Kellis 2 cemetery (Fig. 2), which flanks the eastern portion of Kellis and is one of several common burial grounds thought to be used by the people of Kellis. Within the cemetery, almost all individuals are interred in separate graves and placed in an extended, supine, head-facing west position, with little to no grave goods (Birrell, 1999)—reflecting a Christian style burial (Bowen, 2003). There is some evidence, however, that individuals buried in one part of the cemetery may have also been prepared with resin, reflecting the retention of an earlier pagan burial ritual (Williams and Dupras, 2004). Archaeological evidence currently suggests that Kellis was first occupied during the midfirst century A.D. and abandoned by the end of the fourth century A.D. (Hope, 2001; Bowen, 2003). It is assumed that the adjacent Kellis 2 cemetery was also used during this period; however, radiocarbon dates from skeletal material excavated from Kellis 2 suggest that it may have been used as early as A.D. 100 and as late as A.D. 450 (Molto, 2001; Stewart et al., 2003).

The combined actions of deliberate burial, the arid desert environment, and the high pH level of the burial matrix, result in exceptional chemical preservation of the skeletal material interred in the cemetery (Dupras, 1999; Dupras and Schwarcz, 2001). Juvenile individuals ($N = 27$) displaying a mixed dentition (i.e., both primary and secondary dentition) and adults ($N = 75$) were selected to comprise the study sample. Estimated age-at-death for the juvenile individuals ranged from 3 to 8 years based on standard dental formation methods (Moorrees et al., 1963; Smith, 1991), whereas the adults sampled had completely erupted third molars.

Each tooth has an isotopic signature that reflects a particular period of time during each individual's life. This is because all teeth, whether deciduous or permanent, form at different times, spanning ~20 years of development. Figure 3 shows the root and crown formation of each tooth type, and the order in which teeth are presented on the x-axis represents their location in the dental arcade, mesial to distal. Moreover, most, if not all, of these individuals had survived the weaning process, and therefore represent an appropriate sample with which to compare previously collected cross-sectional data from Kellis 2 infants who did not survive the entire process (Dupras et al., 2001).

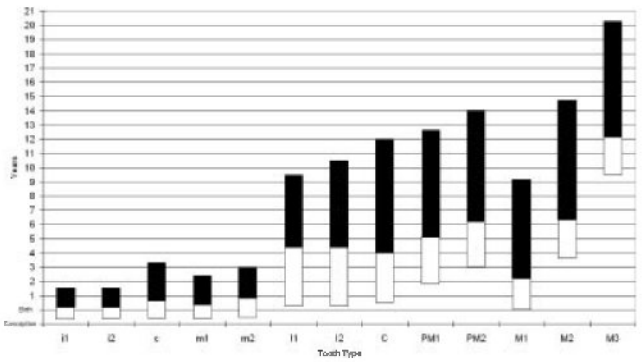

Fig. 3. Dental crown (shown with white □) and root formation (shown with black ■) timing for each tooth type (based on data from Schour and Massler 1940; Lunt and Law 1974; and Smith 1991).

For the juvenile individuals, erupted primary and secondary teeth were removed from the alveoli using forceps, and forming teeth were sampled by removing a small window of bone using a cordless Dremel tool to expose the developing teeth in their crypts. For the adults, molar teeth were removed from the alveoli using forceps. For this analysis, a total of 297 teeth were sampled. The tooth types removed from each individual depended on the respective degree of dental development and postmortem tooth retention. A portion of enamel spanning from cusp to the cemento-enamel junction was separated from the dentin for each tooth then homogenized to a powder using mortar and pestle.

Each sample was soaked in sodium hydroxide (NaOH) to remove the organic component leaving only the hydroxyapatite (method after Wright and Schwarcz, 1998). The hydroxyapatite was reacted with phosphoric acid under vacuum and placed in a 25°C water bath to release the carbonate (CO_3). Dentin samples from the same teeth were homogenized in the same manner as the enamel, and then soaked in 0.5 M hydrochloric acid to remove the inorganic portion. The dentin samples were vacuum-sealed with cupric oxide into Pyrex tubes and combusted at 550°C. Enamel carbon ($\delta^{13}C_{ap}$) and oxygen[5] ($\delta^{18}O_{ap}$) isotope analysis was performed on a VG Optima mass spectrometer, while dentin nitrogen ($\delta^{15}N_{coll}$) and carbon ($\delta^{13}C_{coll}$) isotope analysis was performed on a SIRA mass spectrometer.

Statistical analyses

Differences between the stable isotope means of different teeth or groups of teeth were evaluated for statistical significance using a Randomized Permutation Test (RPT) (Good, 2000). We chose a RPT because the comparisons

[5] $\delta^{18}O_{ap}$ is measured relative to SMOW. We make the assumption that the CO_2 liberated by the phosphoric acid is the same as that between $CaCO_3$ and phosphoric acid (Wright and Schwarcz, 1998).

TABLE 1. Descriptive statistics for $\delta^{18}O_{ap}$ and $\delta^{13}C_{ap}$ from the enamel for each tooth type

		$\delta^{18}O_{ap}$			$\delta^{13}C_{ap}$		
Tooth type	Age group	*N*	Mean	SD	*N*	Mean	SD
i1	Juvenile	9	26.3	0.9	9	−12.6	0.5
i2	Juvenile	12	26.4	1.0	12	−12.4	0.4
C	Juvenile	15	26.4	0.7	15	−12.3	0.5
m1	Juvenile	20	26.4	0.7	20	−12.4	0.5
m2	Juvenile	21	26.3	0.7	21	−12.5	0.5
I1	Juvenile	20	26.0	0.6	20	−12.3	0.4
I2	Juvenile	21	25.9	0.6	21	−12.3	0.4
C	Juvenile	22	25.8	0.5	22	−12.2	0.4
PM1	Juvenile	20	25.9	1.1	20	−11.7	0.5
PM2	Juvenile	15	25.9	1.2	15	−11.8	0.4
M1	Juvenile	18	25.7	0.5	18	−12.6	0.6
M2	Juvenile	11	25.4	0.8	11	−11.9	0.5
Total		204			204		

TABLE 2. Descriptive statistics for $\delta^{13}C_{coll}$ and $\delta^{15}N_{coll}$ from the dentin for each tooth type

		$\delta^{13}C_{coll}$			$\delta^{15}N_{coll}$		
Tooth type	Age group	*N*	Mean	SD	*N*	Mean	SD
i1	Juvenile	6	−17.7	0.3	6	20.6	1.5
i2	Juvenile	6	−18.4	1.1	6	20.6	1.3
c	Juvenile	10	−18.5	0.7	10	20.4	2.4
m1	Juvenile	19	−18.1	0.8	19	20.4	1.1
m2	Juvenile	21	−18.2	0.8	19	19.3	1.4
I1	Juvenile	14	−19.1	1.3	14	19.5	1.2
I2	Juvenile	14	−19.0	1.4	13	19.2	1.1
C	Juvenile	17	−18.8	0.7	17	18.9	1.4
PM1	Juvenile	11	−18.9	0.6	10	18.6	1.0
PM2	Juvenile	7	−18.8	0.7	10	18.0	1.4
M1	Juvenile	21	−18.9	0.8	21	18.0	1.6
M1	Adult	58	−18.7	0.4	58	17.3	1.0
M2	Juvenile	10	−19.1	1.1	8	17.2	0.6
M2	Adult	56	−18.7	0.2	56	17.5	1.3
M3	Adult	27	−18.8	0.3	27	17.7	1.0
Total		297			294		

of interest involve relatively small, unequal sample sizes with unknown distribution properties. The RPT does not require an assumption of normality and is typically robust against differences between the shapes of the sample distributions (Good, 2000).

The RPT was performed as follows:

1. The difference between the means of two groups was calculated;
2. Two samples, equal in size as the original groups, were randomly sampled without replacement from the original data;
3. The difference between the means of the resampled two groups was calculated;
4. Steps 2 and 3 were repeated 999 times;
5. The number of times the difference between the resampled means equaled or exceeded that observed between the original means (Step 1) equaled the probability of observing the difference between the original groups;
6. Since 1,000 permutations do not generate all possible random samples, an alpha value of 0.01 was selected to indicate statistical significance.

RESULTS

Multiple sample analyses (two per sample) were performed to determine the precision of the analyses (i.e., internal reproducibility). Precision for each stable isotope was as follows: $\delta^{13}C_{ap}$‰ ± 0.02‰, $\delta^{13}C_{coll}$‰ ± 0.09‰, $\delta^{18}O_{ap}$‰ ± 0.03‰, and $\delta^{15}N_{coll}$‰ ± 0.25‰. These values are comparable to those reported elsewhere (e.g., Katzenberg et al., 1993; White and Schwarcz, 1993; Wright and Schwarcz, 1998). Descriptive statistics for each stable isotope by tissue and tooth type are summarized in Tables 1 and 2 and shown in Figures 4–7.

Deciduous versus permanent teeth

A statistically significant difference in stable isotope means between deciduous and permanent teeth was observed for all examined isotopes and tissues (Table 3). $\delta^{13}C_{ap}$ values were 0.2‰ higher in the enamel of the permanent dentition ($P < 0.01$) than in deciduous dentition. However, dentin $\delta^{13}C_{coll}$ permanent dentition values were 0.6‰ lower in than deciduous teeth ($P < 0.01$). In the deciduous dentition, enamel $\delta^{18}O$ values were 0.5‰ higher ($P < 0.01$) when compared with the permanent dentition. Dentin $\delta^{15}N$ deciduous values were 2‰ higher than the permanent dentition ($P < 0.01$).

Earlier-forming versus later-forming permanent teeth

Statistically significant differences in stable isotope means between earlier-forming and later-forming permanent teeth were observed for carbon in enamel and nitrogen in dentin (Table 4). The earlier-forming teeth (i.e.,

TABLE 3. Summary of Random Permutation Test for statistically significant differences between sample means (deciduous vs. permanent teeth)

Stable isotope	Tissue	$Mean_{dec}$	$Mean_{perm}$	θ	$\theta' \geq \theta$	*P*
Carbon ($\delta^{13}C_{ap}$)	Enamel	−12.4	−12.2	−0.2	1	0.001
Carbon ($\delta^{13}C_{coll}$)	Dentin	−18.2	−18.8	0.6	0	<0.001
Nitrogen ($\delta^{15}N_{coll}$)	Dentin	20.1	17.9	2.2	0	<0.001
Oxygen ($\delta^{18}O_{ap}$)	Enamel	26.4	25.8	0.5	0	<0.001

$Mean_{dec}$, observed mean for deciduous teeth; $mean_{perm}$, observed mean for permanent teeth; θ, difference between observed sample means; $\theta' \geq \theta$, no. of times a difference between the random sample means equaled or exceeded θ (out of 1,000 random permutations).

TABLE 4. Summary of Random Permutation Test for statistically significant differences between sample means (prewean vs. postwean teeth)

Stable isotope	Tissue	$Mean_{pre}$	$Mean_{post}$	θ	$\theta' \geq \theta$	*P*
Carbon ($\delta^{13}C_{ap}$)	Enamel	−12.4	−11.8	−0.6	0	<0.001
Carbon ($\delta^{13}C_{coll}$)	Dentin	−18.0	−18.0	0.0	359	0.359
Nitrogen ($\delta^{15}N_{coll}$)	Dentin	18.1	17.7	0.4	12	0.012
Oxygen ($\delta^{18}O_{ap}$)	Enamel	25.9	25.8	0.1	300	0.300

$Mean_{pre}$, observed mean for permanent teeth (I1, I2, C, and M1) that begin formation primarily before two years of age; $mean_{post}$, observed mean for permanent teeth (P1, P2, and M2) that begin to form primarily after two years of age; $\theta' \geq \theta$, no. of times a difference between the random sample means equaled or exceeded θ (out of 1,000 random permutations).

TABLE 5. Summary of Random Permutation Test for statistically significant differences between sample means (juvenile vs. adult)

Stable isotope	Tissue	Tooth	$Mean_{juv}$	$Mean_{adult}$	θ	$\theta' \geq \theta$	*P*
Carbon ($\delta^{13}C_{coll}$)	Dentin	M1	−18.9	−18.7	−0.2	139	0.139
Carbon ($\delta^{13}C_{coll}$)	Dentin	M2	−19.1	−18.7	−0.3	62	0.062
Nitrogen ($\delta^{15}N_{coll}$)	Dentin	M1	18.0	17.3	0.7	16	0.016
Nitrogen ($\delta^{15}N_{coll}$)	Dentin	M2	17.2	17.5	−0.3	254	0.254

$Mean_{juv}$, observed mean for juveniles (three to eight years); $mean_{adult}$, observed mean for adults; θ, difference between observed sample means; $\theta' \geq \theta$, no. of times a difference between the random sample means equaled or exceeded θ (out of 1,000 random permutations).

TABLE 6. Summary of Random Permutation Test for statistically significant differences between sample means (juvenile vs. adult)

Stable isotope	Tissue	Tooth	$Mean_{juv}$	$Mean_{adult}$	θ	$\theta' \geq \theta$	*P*
Carbon ($\delta^{13}C_{coll}$)	Dentin	M1	−18.9	−18.7	−0.2	95	0.095
Carbon ($\delta^{13}C_{coll}$)	Dentin	M2	−19.1	−18.7	−0.3	50	0.050
Nitrogen ($\delta^{15}N_{coll}$)	Dentin	M1	17.6	17.3	0.3	160	0.160
Nitrogen ($\delta^{15}N_{coll}$)	Dentin	M2	17.2	17.5	−0.3	277	0.277

$Mean_{juv}$, observed mean for juveniles (3.5–8 years); $mean_{adult}$, observed mean for adults; θ, difference between observed sample means; $\theta' \geq \theta$, no. of times a difference between the random sample means equaled or exceeded θ (out of 1,000 random permutations).

I1, I2, C, and M1) were 0.5‰ higher in $\delta^{15}N$ values ($P = 0.01$) whereas the later-forming teeth (i.e., P1, P2, M2) were 0.6‰ higher in $\delta^{13}C$ values ($P < 0.01$). This latter difference indicates that the differences between deciduous and permanent dentition in respect to carbon in enamel is due to later-forming permanent teeth rather than the entire permanent dentition as a whole.

Permanent molars in juveniles versus adults

Given that each permanent molar forms at approximately the same time in each individual there should not be any significant differences in isotopic values given a fairly homogenous diet. No statistically significant differences in stable isotope means for the first and second permanent molars were observed between juveniles and adults. Although the observed *P*-value for the difference in nitrogen isotope means for the first molar is small (Table 5; $P = 0.016$), if the youngest juveniles in the sample are excluded (e.g., those aged three and a half years and younger), the observed *P*-value increases substantially (Table 6; $P = 0.16$). These data suggest that some of the youngest individuals in the sample (i.e., between 3 and 4 years of age) may have died during the final stages of the weaning process, or shortly after weaning had ceased.

DISCUSSION

The comparisons between deciduous and permanent dentition showed statistically significant differences for each of the stable isotopes. Permanent dentition enamel $\delta^{13}C_{ap}$ mean values were enriched by ~0.2‰ over deciduous dentition (Fig. 4; mean $\delta^{13}C_{ap}$ deciduous dentition = −12.4‰ vs. mean $\delta^{13}C_{ap}$ adult dentition = −12.2‰), while deciduous dentin $\delta^{13}C_{coll}$ mean values were enriched by 0.6‰ (Table 3). Statistically significant differences in mean $\delta^{13}C_{ap}$ values were also observed between earlier-forming (I1, I2, C, and M1), and later-forming permanent dentition (P1, P2, and M2), with the later-forming permanent dentition enriched by 0.6‰ (Table 4).

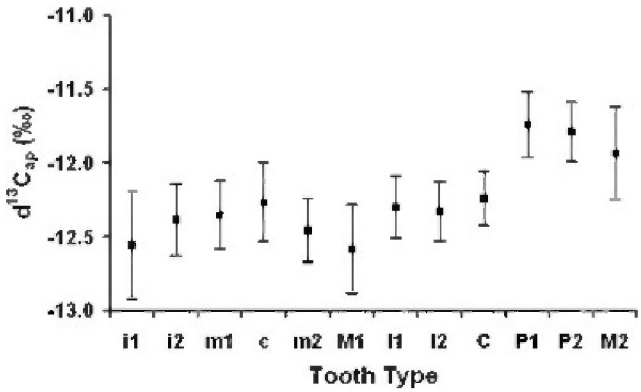

Fig. 4. Mean and 95% confidence limit $\delta^{13}C_{ap}$ values analyzed from enamel for each tooth type. Tooth type, position, and growth stage are denoted by: I, i = incisor; C, c = canine; P = premolar; M, m = molar; uppercase letters = permanent teeth; lowercase letters = deciduous teeth; number denotes tooth's position.

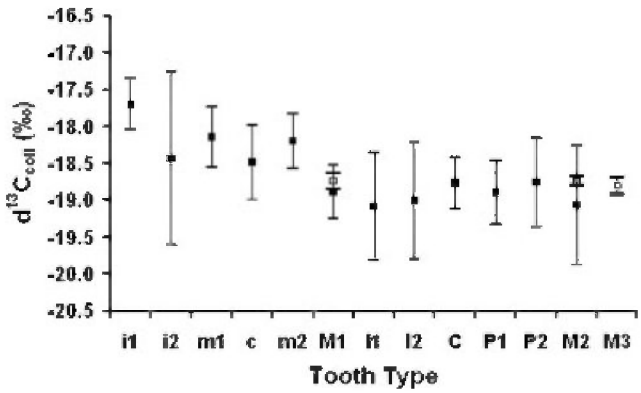

Fig. 5. Mean and 95% confidence limit $\delta^{13}C_{coll}$ values analyzed from dentin for each tooth type. The solid diamonds (■) represent the juvenile individuals, while adult molars are represented by the open squares (□). Horizontal lines representing the 95% confidence limits of the mean are wider for adult molars. Tooth type, position, and growth stage are denoted by: I, i = incisor; C, c = canine; P = premolar; M, m = molar; uppercase letters = permanent teeth; lowercase letters = deciduous teeth; number denotes tooth's position.

When comparing the enamel $\delta^{13}C_{ap}$ data with that of the dentin $\delta^{13}C_{coll}$ (Figs. 4 and 5), the isotope values appear to run in opposite directions through the tooth sequence. This difference is due to the timing and process of dental development between the two tissue types (enamel vs. dentin). Figure 3 shows the timing of dental crown formation for both deciduous and permanent dentition (based on Schour and Massler, 1940; Lunt and Law, 1974; Smith, 1991). The crowns of the deciduous dentition all begin to form during fetal development, with the first and second incisor, and first molar crowns almost completely forming before birth. Thus, the $\delta^{13}C_{ap}$ values of the deciduous teeth largely reflect the fetal environment, most likely mimicking the $\delta^{13}C_{ap}$ values of the mother. Figure 4 shows little isotopic variation between the deciduous and early-forming permanent teeth. The difference in enrichment mainly comes from the late-forming permanent teeth, P1, P2, and M2 (Table 4; Fig. 4). Although significant, the mean enrichment of 0.2‰ in the permanent tooth enamel reflects an average of all permanent teeth. The crowns of all the permanent teeth begin forming after birth, and continue forming until ~7 years of age (the third molar was not included in the $^{13}C_{ap}$ analyses). The $\delta^{13}C_{ap}$ enrichment therefore includes $^{13}C_{ap}$ signals from breastfeeding, weaning, and complete adoption of adult foods. However, examination of Table 4 and Figure 4 clarifies that enrichment begins with the formation of the permanent dentition, but is largely affected by the later-forming permanent teeth, all of which are primarily forming after 2 years of age.

These observed results could signify a greater reliance on foods that are C_4 enriched, such as pearl millet. Pearl millet has been identified as a plant that was grown in the Dakhleh Oasis during this time period (Thanheiser, 1999; Thanheiser et al., 2007). Although not considered the most desirable human food crop during the Roman period, millet was used as fodder for livestock (Darby et al., 1977). Dupras et al. (2001) report significant ^{13}C enrichment in both cows and goats, and this enrichment is accredited to the consumption of millet. As cow and goat milk, along with millet gruel, were recorded as being important weaning foods, these foods may be responsible for the enrichment shown in those teeth forming from birth to ~3 years of age.

More telling is the significant difference in deciduous and permanent dentin $\delta^{13}C_{coll}$ values. Deciduous dentin $\delta^{13}C_{coll}$ values were 0.6‰ enriched over permanent dentin (Table 3; mean deciduous $\delta^{13}C_{coll}$ value = −18.2‰ vs. mean permanent $\delta^{13}C_{coll}$ value = −18.8‰). Deciduous dentin develops after birth during the breastfeeding and weaning period compared with the enamel of the crown, which forms mostly prior to birth. The deciduous roots form around the same time as the crowns of the early-forming permanent teeth, and thus show a similar enrichment level. In most of the permanent teeth (with the exception of M1), dentin starts to form after the third year of development. Therefore, the $^{13}C_{coll}$ enrichment in deciduous dentin most likely reflects the breastfeeding and weaning process. Figure 5 shows that the deciduous teeth are enriched, and that by the time the M1 root starts to develop (after 3 years of age), the mean $^{13}C_{coll}$ value has already decreased to adult values.

Previous research suggests that breast-feeding infants may have up to 1‰ enriched bone collagen $\delta^{13}C$ values in comparison to adults (Dupras et al., 2001; Richards et al., 2002). Dupras et al. (2001) interpret the enriched $\delta^{13}C$ values as a chemical signal of the introduction of weaning foods that are enriched in C_4, such as millet gruel, or milk from cows or goats that were maintained on a C_4 diet. Richards et al. (2002) call this chemical signal the "carnivore" effect of breast-feeding, in which carnivore collagen is enriched in comparison to that of herbivore collagen (Schoeninger and DeNiro, 1984; Bocherens et al., 1995). More recently, it has been shown that modern infants who are exclusively breastfeeding have a 1‰ increase in $\delta^{13}C$ values, adding further support to the "carnivore" effect (Fuller et al., 2006a). Richards et al. (2006) also demonstrate this increase in a population where C_4 foods were not available. While there is clear evidence emerging for a trophic level effect in $\delta^{13}C$, the evidence from Kellis, including the textual evidence, existence of C_4 plants, and cow and goat $\delta^{13}C$ values, should be considered when interpreting $\delta^{13}C$ values in this population. The $\delta^{13}C_{coll}$ enrichment observed in the deciduous dentin from the Kellis sample may be a reflection of both the trophic level effect and breast-feeding practices.

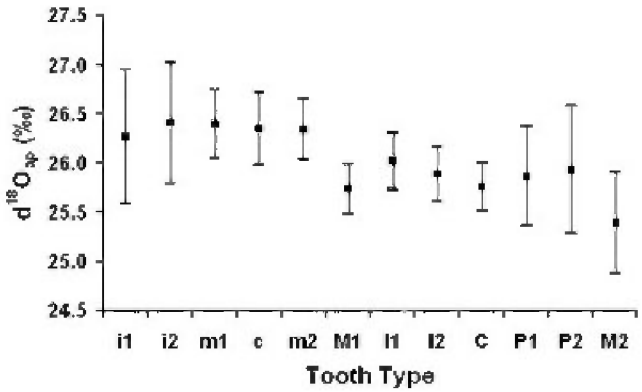

Fig. 6. Mean and 95% confidence limit $\delta^{18}O_{ap}$ values analyzed from enamel for each tooth type. Tooth type, position, and growth stage are denoted by: I, i = incisor; C, c = canine; P = premolar; M, m = molar; uppercase letters = permanent teeth; lowercase letters = deciduous teeth; number denotes tooth's position.

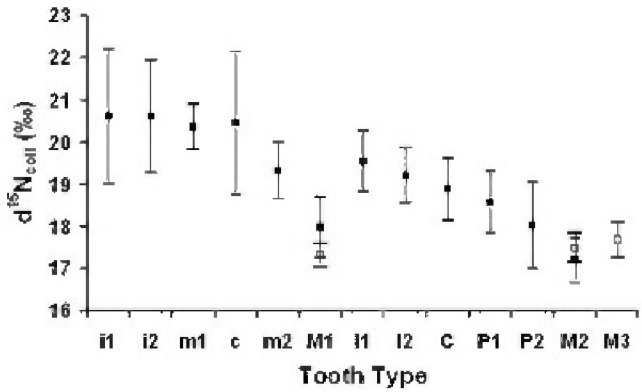

Fig. 7. Mean and 95% confidence limit $\delta^{15}N_{coll}$ values analyzed from dentin for each tooth type. The solid diamonds (■) represent the juvenile individuals, whereas adult molars are represented by the open squares (□). Horizontal lines representing the 95% confidence limits of the mean are wider for adult molars. Tooth type, position, and growth stage are denoted by: I, i = incisor; C, c = canine; P = premolar; M, m = molar; uppercase letters = permanent teeth; lowercase letters = deciduous teeth; number denotes tooth's position.

Statistically significant differences in mean $\delta^{18}O_{ap}$ values were observed between the deciduous and permanent dentition (26.3‰ vs. 25.8‰, respectively), with deciduous dentition being enriched by 0.5‰ (Table 3). The oxygen isotopes ($\delta^{18}O$) of biological tissues are related to the water (H_2O) source that is taken in by that organism (Wright and Schwarcz, 1998). As such, changing $\delta^{18}O$ values in the consumer's tissues reflects any change in water source. During exclusive breastfeeding infants receive their water from their mother's breast-milk; therefore, their $\delta^{18}O$ values reflect those of their mother. The process of weaning is detectable through shifts in $\delta^{18}O$ because breast milk is significantly enriched in ^{18}O in comparison to drinking water. The water source for an infant therefore changes once the weaning process begins. This change is reflected in the infant's tissues. Thus, it is expected that the deciduous dentition should be enriched in $\delta^{18}O$ (Fig. 6). Since the crowns of the deciduous dentition are formed during fetal development and the first year of life (Fig. 3), their isotopic signatures reflect the $\delta^{18}O$ values of the uterine environment and breast milk. Since both of these sources come directly from the mother the isotopic signatures should be enriched in $\delta^{18}O$. The permanent dentition form their crowns after birth, with almost 50‰ of crown formation after the age of two, and as a result, most of the permanent crowns have $\delta^{18}O$ values that reflect both breast-feeding and the adoption of other water sources, with the majority of the signal coming from external water sources.

Deciduous dental dentin $\delta^{15}N$ values were significantly enriched by 2‰ over permanent dental dentin (20‰ vs. 18‰, respectively). Since breast-feeding infants receive their protein from the mother's breast milk, their $\delta^{15}N$ values are ~2–3‰ higher than that of the mother's (Fogel et al., 1997)—a trophic level effect. The mean difference between deciduous and permanent $\delta^{15}N$ values at Kellis reflects a trophic level difference (Fig. 7), and this enrichment clearly indicates breast-feeding. As the dentin of the deciduous teeth forms after birth and is mainly complete by 3 years of age, the enriched isotopic signal reflects dietary protein input during this period of development. In addition, when comparing early versus late-forming permanent dentition (Table 4), the early-forming permanent dentition (I1, I2, C, and M1) were significantly enriched by 0.5‰. Although this is not a trophic level, it suggests a gradual weaning process. Figure 7 shows the enrichment of deciduous teeth and the decreasing $\delta^{15}N$ values during the development of the permanent teeth, until finally reaching adult values. The $\delta^{15}N$ values clearly suggest a reliance on breast milk from birth to ~3 years of age.

CONCLUSIONS

We have examined stable carbon, nitrogen, and oxygen isotopes from dental enamel and dentin from juveniles and adults buried in the Kellis 2 cemetery in the Dakhleh Oasis, Egypt. In comparing deciduous and permanent dentition, significant differences were observed for enamel $\delta^{13}C_{ap}$ (with the permanent dentition enriched over the deciduous teeth), and $\delta^{18}O_{ap}$ (with the deciduous dentition enriched over the permanent dentition). Both dentin $\delta^{13}C_{coll}$ and $\delta^{15}N_{coll}$ in the deciduous dentition were enriched over permanent teeth. Examination of dental formation times suggests that these enrichments are correlated with the weaning process.

The results of this simulated, longitudinal-type study support earlier findings by Dupras et al. (2001), in which $\delta^{15}N$ and $\delta^{13}C$ values from bone collagen showed exclusive breastfeeding for at least 6 months, perhaps followed by an introduction of ^{13}C enriched foods and gradual weaning until 3 years of age. Recent data supporting the trophic level effect of exclusive breastfeeding on $\delta^{13}C$ values must also be considered (Fuller et al., 2006a); however, at present we cannot distinguish between these two possible explanations: 1) the trophic level effect of exclusive breastfeeding on $\delta^{13}C$ values and 2) the possibility that C_4 plants played a role in the weaning process.

Together, the results provide more substantial support for the hypothesis that infants at Kellis were breastfed and weaned slowly until 3 years of age, which is consistent with traditional infant feeding and weaning practices documented by Soranus and Galen, two ancient Greek

and Roman historians (Green, 1951; Tempkin, 1956). Both Galen and Soranus recommended that supplementary foods, such as a mixture of honey and goat milk, should be introduced at 6 months of age, with gradual cessation of weaning occurring until 3 years of age. Historical sources from Egypt also lend support for a slow, gradual weaning period of 3 years (Fildes, 1986; Robins 1993; Donadoni, 1997). For example, Bagnall et al. (2005) translate a papyrus from Berenike dating from approximately A.D. 50 to A.D. 80, in which a mother writing a letter to her son says,

> "[Hikane] to Isidoros [her son, greetings. First of all] I thought it necessary, since the packet boat was putting out to sea, to write... me. I am in Berenike. I wrote you a letter [?but did not receive a] letter. Was it for this that I carried you for ten months and nursed you for three years, so that you would be incapable of remembering me by letter?" (:42).

Dupras et al. (2001) documented that both cows and goats had enriched ^{13}C values. Therefore, if infants were fed milk from either source their isotope values would be enriched. Although it was not possible to get at specific ages for the introduction of particular weaning foods, the longitudinal dental isotope data, in addition to the carnivore effect, could also suggest that individuals were introduced to a food enriched in ^{13}C during the first 2 years of life, and that the weaning process was complete by 3 years of age. The previous cross-sectional study (Dupras et al., 2001) and the present study provide complementary evidence as to the nature and timing of infant diet and the weaning process at Kellis. The comparison of two data sources at Kellis, and previous work by Fuller et al. (2003), suggest that an infant mortality bias may not be a significant problem for stable isotope analyses. Nonetheless, similar studies should be conducted on other human populations to test the validity of this statement.

This study demonstrates the possibility of reconstructing breast-feeding and weaning practices in individuals that lived through the weaning period. It is recognized that using data from homogenized enamel and dentin from whole teeth does not allow for the estimation of exact timing of these processes. One solution to this methodological problem is to use multiple samples from each tooth to represent the different stages of formation. Depending on the tooth, it may be possible to use this method to reconstruct the timing of the weaning process within a single tooth. This approach is possible using laser ablation and has been attempted on non-human teeth (e.g., Cerling and Sharp, 1996).

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