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STABLE ISOTOPE ANALYSIS OF THE MIDDLE HELLADIC POPULATION FROM TWO CEMETERIES AT ASINE: BARBOUNA AND THE EAST CEMETERY

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ABSTRACT

In this paper we report the results of the stable carbon and nitrogen isotope analyses of humans from two Middle Bronze Age cemeteries at Asine, Greece: Barbouna (n=6) and the East Cemetery (n=13). In general, the dietary pattern of adults and juveniles shows a heavy reliance on mainly terrestrial foods; C_3 plants and a varying amount of animal protein (meat, milk or dairy products). The high nitrogen values of some individuals from the East cemetery indicate a substantial consumption of animal protein, although the carbon values show that no detectable amounts of marine foods, or C_4 plants such as millet had been consumed. High nitrogen values as well as the high slaughter age of domestic animals, as found in previous studies point towards a significant utilization of milk and dairy products at Asine. A low increase of nitrogen values in subadults younger than one years of age from Barbouna compared to females at the East cemetery indicates that these children may have been fed breast milk as well as supplementary foods. Therefore, despite the poor preservation and uneven sample size, the Asine isotopic data give us information on diet during the MH period, as well as variation between the members of the community.

KEYWORDS: Stable isotope analysis, Asine, Bronze Age, Middle Helladic, diet, weaning

INTRODUCTION

The reconstruction of prehistoric diets through stable isotope analysis of carbon and nitrogen in human bone collagen has become an important and widespread part of many bioarchaeological and environmental studies. The information gained from osteological analyses and the chemical data derived from the bones have shown to be a fruitful combination complementing each other and contributing towards a more comprehensive knowledge of diet, health and human cultural diversity in past societies. Within the area of Aegean prehistory a growing body of analyses has focussed on material from Greece (Richards and Hedges, 1999; Tzedakis and Martlew, 1999; Papathanasiou *et al*., 2000; Triantaphyllou, 2001; Papathanasiou, 2003; Bourbou & Richards, 2007; Lagia *et al*., 2007; Petroutsa *et al*., 2007; Richards and Hedges, 2008; Richards and Vika, 2008; Triantaphyllou *et al*., 2008). The analysis presented here is part of a broader interdisciplinary research programme, the *Middle Helladic Argolid Project* (on http://www. MHArgolid.nl) whose main aim is to reconstruct social organization and social change during the Middle Bronze Age (or Middle Helladic [MH] period, 2100-1700 BC) in the region of the Argolid, southern Greece. The examination of dietary variation is an important component of this project, and analyses have already been carried out on neighbouring Lerna (Triantaphyllou *et al*., 2008) and Aspis (Triantaphyllou *et al*., in print), while this paper presents the analysis from Asine.

The basic concepts behind dietary reconstructions of past populations have already been extensively reviewed elsewhere (Sealy, 2001; Katzenberg, 2000). In short, different food sources have characteristic stable carbon ($^{13}C/^{12}C$, expressed as $\delta^{13}C$ values) and nitrogen isotope ratios ($^{15}N/^{14}N$, expressed as $\delta^{15}N$ values) which are passed from producer to consumer and accumulate in the human body during the individual's lifetime, with some fractionation. Therefore, measurements of the isotopic composition of human bone collagen will generally reflect the individual's diet, i.e. whether the protein consumed came from terrestrial, marine or freshwater resources, or a combination thereof.

Since atmospheric carbon enters the food chain primarily through the photosynthesis of plants it is possible to differentiate between consumption of C_3-, C_4- or CAM-plants which employs different photosynthetic pathways (Keegan, 1989). Most European plants belong to the C_3 group (e.g. cereals, fruits, nuts and trees) and have quite low carbon values whereas C_4-plants, such as tropical grasses, maize, sorghum and millet have higher carbon values. The CAM-plants (for example pineapple, succulents and cacti) employ a less common pathway and their values are roughly intermediate to C_3 and C_4 plants (Katzenberg, 2000). In Europe where C_4 plants are uncommon, carbon values have often been used to differentiate between terrestrial and marine diets (Schoeninger *et al.*, 1983; Richards and Hedges, 1999). For instance, a predominantly marine feeding pattern would show carbon values around -12‰, whereas a diet based mainly on terrestrial foods would show lower carbon and nitrogen values.

Nitrogen isotopes indicate the trophic level of the consumer, i.e. whether animals or plants were the main protein source. Most plants have low proportions of protein compared to animals (Van Klinken *et al*., 2000). A high nitrogen value will thus indicate that a large amount of the individual's protein intake came from animals, either in the form of meat or dairy products. A further distinction can be made between plants of different $\delta^{15}N$ values: some plants such as legumes take up nitrogen through the atmosphere (i.e. nitrogen fixers) which result in a low nitrogen value, whereas non-legumes plants take up nitrogen from the soil and have higher nitrogen values (Keegan, 1989). The noted difference between trophic levels has also made nitrogen isotopes suitable for analyses of breastfeeding patterns: When children are breastfed they are on a higher trophic level than their mothers. Thus, their nitrogen level will be about 2-3‰ higher than their mother's value. When other foods are introduced their nitrogen values will gradually decrease to become equal to adult values (Fuller *et al.*, 2006).

THE SITE OF ASINE

One of the important and well studied Middle Helladic settlements in the Argolid is Asine, located on the coast near the modern village of Tolo. The Swedish excavations at Asine have been carried out in successive campaigns since the 1920s (see Nordquist & Hägg, 1996 for a review of the excavations). A large number of graves have been excavated in connection to the MH settlement at Asine: within the settlement at Kastraki, among ruined houses on the slope of the nearby Barbouna hill as well as in an unsettled area east of the acropolis (East Cemetery). Here lies the significance of the Asine mortuary data: they allow a comparison between different burial grounds, used perhaps by different segments of the community. Unfortunately, many of the skeletons from the earliest excavations at Kastraki have been lost.

The East Cemetery was used only for burials, and it has been suggested earlier that this cemetery belonged to the elite groups as evidenced by the wealthy graves found there, as well as the existence of a tumulus. It should be pointed out, however, that the differences between the burial grounds may not be as marked as previously suggested; the C14 data have somewhat modified the history of the tumulus (Milka, 2007).

In the 1970s Lawrence Angel examined the skeletons from the East Cemetery and Barbouna. In his report Angel concluded that the Bronze Age population at Asine was less healthy than other Bronze Age populations in Greece (Angel, 1982). He believed that inadequate nutrition and especially a lack of meat protein could have been the primarily responsible factor for the bad health of the population and proposed that bone chemistry was needed to clarify this question. In a later osteological analysis of the subadults from the 1926 excavation of Kastraki, Ingvarsson-Sundström (2008) found an exceptionally high neonatal mortality and a poor growth of children, thus confirming Angel's general interpretation of severe health problems at Asine. The analysis indicated intermittent periods of malnutrition and disease during childhood which could have been linked to feeding practices, such as early supplementation with other foods than breast milk.

A re-examination of the skeletons from the East Cemetery and Barbouna was carried out by A. Ingvarsson-Sundström as a part of the Middle Helladic Argolid Project, whose other main objective was to re-examine MH populations with modern osteological and chemical methods (Voutsaki, 2005; Voutsaki *et al*., 2004; 2005; 2006; 2007). The aim of the stable isotope analysis was to examine whether the Asine diet was poor in animal protein, and if differences existed between the two cemeteries and/or between sexes. It was also the intention that the stable isotopes would clarify the question of breast-feeding customs and shed light on the duration of breast feeding and at what age other foods were introduced.

MATERIAL AND METHODS

A total of 38 bone samples were selected from the 41 osteologically re-examined skeletons from Barbouna and the East Cemetery; 14 samples from the 17 individuals from Barbouna, and 24 samples from the 24 individuals from the East Cemetery. Not all skeletons from the Barbouna cemetery could be sampled because of the poor preservation of three skeletons (two neonates and a juvenile/adult). Ribs were selected for analysis if possible, but since skeletal preservation was generally poor at both cemeteries, other bones occasionally had to be selected as an alternative to ribs.

The age and sex of all individuals had previously been determined through standard osteological methods (Buikstra and Ubelaker, 1994). The skeletal samples belong to individuals of both sexes and ages ranging from newborn to old adulthood (+50 years of age).

Collagen was extracted from the bone samples following a modified Longin (1971) method (Richards and Hedges, 1999) with the addition of an ultrafiltration step (Brown *et al.,* 1988). Collagen extraction and subsequent isotope measurements were made at the Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany. Of the 38 samples initially selected, only 19 samples provided enough well-preserved collagen for analysis (6 of 14 from Barbouna and 13 of 24 from the East cemetery) (*Table 1*). The other 19 samples either had too little collagen preserved or produced a poor C:N ratio. Following DeNiro (1985) only samples with a C:N ratio between 2.9 - 3.6 were considered for analysis. It has been shown that samples outside this range could have been affected by *post mortem* changes which may cause unreliable indications of the individuals' diets. Unfortunately, it was impossible to examine possible chronological differences in diet within the Asine community because of the small sample size. To determine the relative importance of animal vs. plants, the isotope values derived from bone samples from herbivore and omnivore animals from the nearby and contemporaneous site of Lerna have been used as references (Triantaphyllou *et al.,* 2008).

Table 1: Stable carbon and nitrogen isotope values for skeletons from East Cemetery and Barbouna at Asine (Neonate = 0-1 year; Child= 6-12 years; Juvenile= 12-18 years; Adult + 18 years; YA= Young Adult, 18-30 years; PA= Prime Adult, 30-40 years; MA= Mature Adult, 40-50 years, OA= Old adult, +50 years).

Skeleton No.	Cemetery	Age	Sex	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N ratio
44 AS	East	Adult	?	-20.6	9.3	3.6
45 AS	East	MA	M	-19.8	8.5	3.2
47 AS	East	Child	?	-18.7	10.7	3.3
48 AS	East	YA	M	-19.2	9.7	3.2
51 AS	East	Juvenile	?	-19.2	9.2	3.2
53 AS	East	OA	F	-19.0	8.7	3.2
54 AS	East	Adult	M	-19.1	9.3	3.2
55 AS	East	Juvenile	?	-19.1	9.8	3.2
56 AS	East	PA	F	-19.1	10.3	3.2
60 AS	East	YA	M	-19.1	7.9	3.3
61 AS	East	PA	F	-19.8	9.6	3.2
62 AS	East	Child	?	-18.9	8.9	3.3
64 AS	East	Adult	?	-19.0	9.4	3.2
93a AS	Barbouna	Neonate	?	-19.3	9.2	3.3
105 AS	Barbouna	Neonate	?	-18.2	10.2	3.2
106 AS	Barbouna	Neonate	?	-19.0	10.7	3.5
109 AS	Barbouna	Neonate	?	-18.9	10.2	3.3
112 AS?	Barbouna	Neonate	?	-18.4	10.2	3.1
89.324	Barbouna	YA/PA	M	-19.3	8.3	3.3

RESULTS AND DISCUSSION

The dietary pattern of adults and juveniles at Asine shows a heavy reliance on mainly terrestrial foods; i.e. C_3 plants with a varying amount of animal protein (*Fig. 1*). There is no visible addition of marine foods (which is surprising, considering that Asine is located on a promontory); in this case higher $\delta^{15}N$ and $\delta^{13}C$ values would have been observed. The fact that very few fish bones have been found in the animal bone assemblage (Katrin Moberg, *personal communication*) seems to support the results of the stable isotope analysis but it must be emphasised that the sparse fish remains may well be an effect of the recovery methods used; hand collection of bones (which was practised at Asine) typically produce few fish remains whereas water flotation and water sieving usually increase the number of fish bones as well as the number of species (Mylona, 2003). Furthermore, the possibility that low amounts of marine foods of a low trophic level were indeed consumed, but not detected with the methods of the present analysis should not be excluded (Milner *et al*., 2004; Hedges, 2004; cf. Richards and Schulting, 2006).

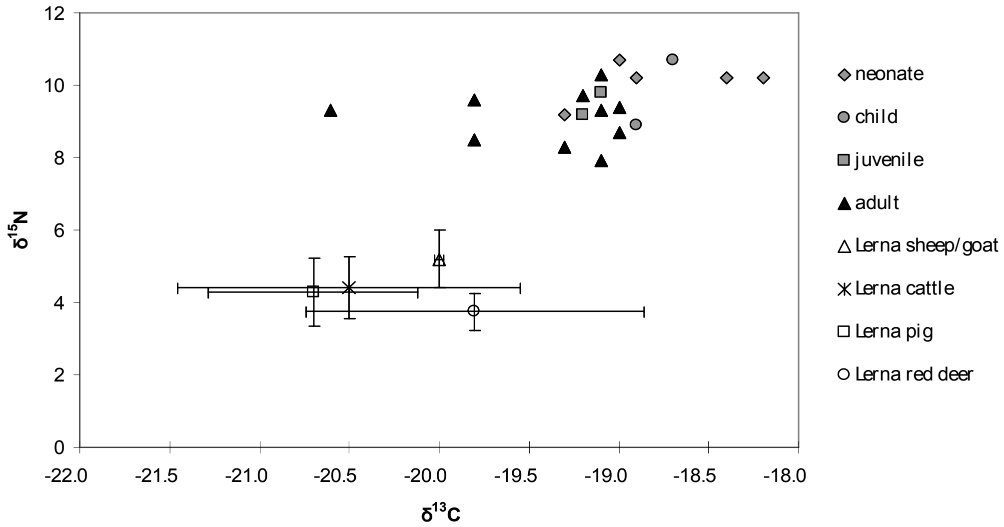

Fig. 1: Plot of bone collagen $\delta^{13}C$ and $\delta^{15}N$ of individuals from Asine and animals from Lerna (means and standard deviations).

Also, there is no evidence that C_4 plants such as millet, or animals fed on C_4 plants were part of the diet in Asine. As can be seen in *Figure 1*, it is nevertheless likely that a majority of the individuals consumed large amounts of animal protein. A study of animal bones from terrace III at Kastraki shows that cattle and pigs played a significant role in the Asine economy, while sheep and goats were also abundant (Moberg Nilsson, 1996).

The nitrogen isotope levels, however, must be interpreted with caution since there are other causes besides ingestion of animal protein that may raise human nitrogen levels. For instance, it has been shown that human working of the soil may disturb the delicate nitrogen cycle between plants and soil and may cause significantly raised values in both (Van Klinken *et al.*, 2000). In addition, the use of animal manure raises the soil nitrogen level considerably and this could affect the human nitrogen values through the food chain (Bogaard *et al.*, 2007; Hedges and Reynard, 2007). Even if these effects may, to some extent, be checked by analyses of plants and animals, archaeobotanical remains are seldom available. There are also indications that periodical nutritional stress can increase human nitrogen levels, meaning that not only diet but also the individual's nitrogen balance can affect the $\delta^{15}N$ values (Fuller *et al.*, 2005). However, it is unlikely that this would show up in bone collagen, which is formed over long time periods.

Yet, the varying stable isotope levels at Asine are by no means exceptional. On the contrary, the stable isotope values at Asine agree well with those seen at other Greek Bronze age sites (e.g. Lerna, Mycenae and Armenoi on Crete) (*Fig. 2*). The majority of the Asine population are comparable to the individuals having high nitrogen values and less negative carbon values. If the comparison is extended to the lavishly furnished burials from the Mycenae Grave Circles, it is interesting to note that the Asine population fits fairly well within the section of values that overlap between the other Bronze Age populations and the elite graves at Mycenae (Grave Circle A, n=9; Grave Circle B, n=6; chamber tombs, n=11)

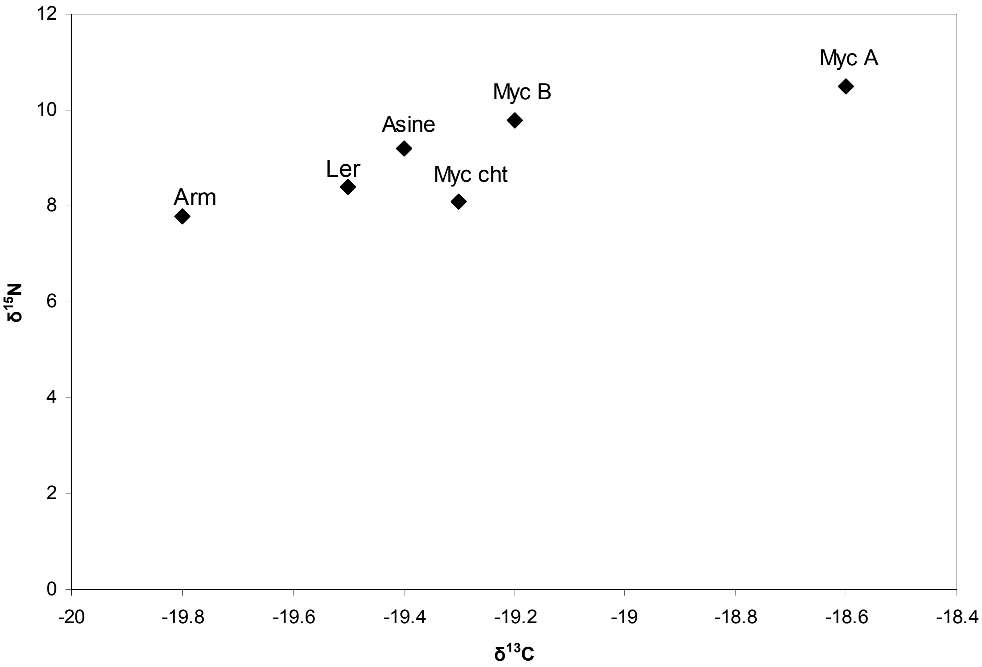

Fig. 2: Stable carbon and nitrogen isotope mean values from Asine in comparison to other Bronze Age populations. Mycenae: Grave circle A (=Myc A), Grave circle B (=Myc B), chamber tombs (=Myc cht), Lerna (=Ler) and Armenoi (=Arm) [Crete].

Turning now to a closer look at the sex specific stable isotope results of the East Cemetery (*Fig. 3*): Since there are only three women represented in this sample, and two individuals could not be determined as to sex, any interpretation of dietary differences between the sexes will necessarily be speculative. However, the material at hand shows that the female mean nitrogen value is higher than the male value, possibly indicating that females at the East Cemetery had more animal protein in their diet than men. On the other hand, the difference is not statistically significant, and it is perhaps more likely that it is an effect of small sample size and quite large individual variation. It is nevertheless interesting to note at least one case where a different burial type and high nitrogen value coincide: The highest nitrogen values of this sample belong to the female and the juvenile buried in one of the two *pithos* (storage jar) graves of the cemetery (Grave 1971-7) (Dietz, 1980): $\delta^{15}N$ =10.3 ‰ and 9.8 ‰ respectively.

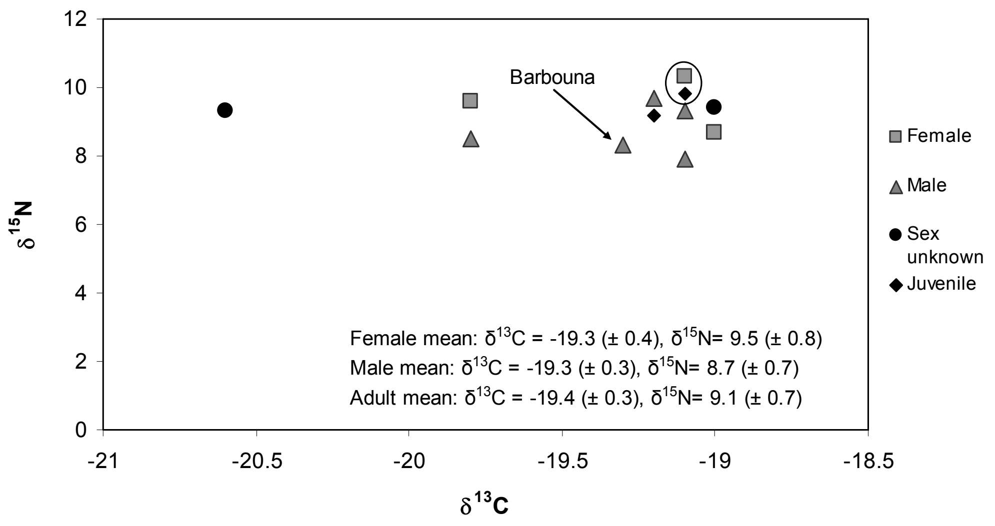

Fig. 3: Plot of nitrogen and carbon isotope values from adults and juveniles at the East cemetery (n=11) and Barbouna (n=1). Circled = individuals from pithos grave 1971-7; means with 1 SD.

Unfortunately, the samples from the second pithos (grave 1971-15), containing two adults did not give any results because the bones did not contain enough collagen for analysis. The majority of the graves of the East Cemetery were cists. While smaller jars were quite frequently used for burials of children during the MH period, the burial of adults in (larger) storage jars is not that common, although parallels are found in neighbouring Argos, as well as in other regions of the southern Greek mainland (Nordquist, 1987; Dietz, 1980). Nordquist has suggested that the presence of this grave type for adult use could have been a result of "marriages" between different elite family groups from the mainland, where an immigrating spouse brought his/her traditional burial customs when moving (into Asine) (Nordquist, 2002). If this suggestion can be accepted, it is not unlikely that the immigrants also brought slightly different dietary habits (along with burial customs). Furthermore, two other burials can be singled out as special from the conspicuous grave finds: grave 1970-12 in which a gold diadem was found and grave 1971-3 containing a large number of vases, a bronze dagger and a limestone pommel (Dietz, 1980). The inhabitants of these graves, a man and an individual of unknown sex also had quite high nitrogen values ($\delta^{15}N$ = 9.3 ‰) possibly suggesting a diet rich in animal protein. However, high nitrogen levels are not only found among the individuals buried with elaborate grave goods: the highest values are found in those individuals whose graves were empty of finds. Possible differences between the populations of the two cemeteries (East and Barbouna) could not be verified since only one adult from Barbouna produced any results in the stable isotope analysis.

As already mentioned, stable isotope analysis has also been used to estimate how long children were breastfed and the time of introduction of solid foods. In exclusively breastfed infants the expected nitrogen enrichment is around 3 ‰ (Katzenberg *et al.*, 1996). When weaning starts, the nitrogen level of the child will gradually decline to the adult value provided that adult and child eat the same type of food. It is not known exactly how long it takes for the isotopic signal of breast milk to reach the bones of a neonate, but a recent study has shown that this process may be fairly rapid; elevated values have been found in very young children (only a few weeks old) when compared to foetal values (Richards *et al*., 2002).

The interpretation of breastfeeding customs in Asine is severely hampered by the small sample size of children, and the lack of children between 1-5 years of age. In this sample all except two older children are younger than one year of age. Furthermore, there are no stable isotope results for the Barbouna females, which implied that the Barbouna children had to be compared to the 3 females of the East Cemetery. Still, a preliminary interpretation of the data at hand shows that the children's $\delta^{15}N$ values are significantly enriched compared to the adults of the sample, but there is no statistically significant difference between the female and subadult means (*Fig. 4*).

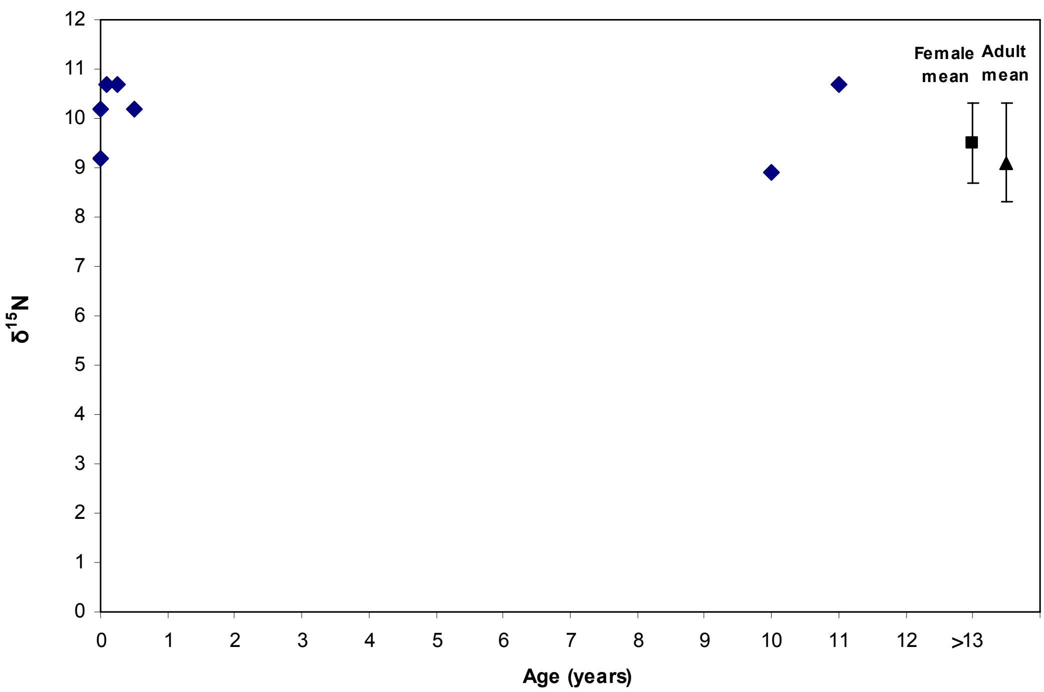

Fig. 4: Nitrogen isotope values of children from Barbouna and the East Cemetery in comparison to female and adult average nitrogen values (minimum and maximum values are indicated for the female and adult samples).

In fact, the children's mean $\delta^{15}N$ value is lower than expected for breastfed children, since it is only 0.5 ‰ higher than the female mean and 1.1 ‰ higher than the general adult mean (whereas the normal increase would have been 2-3‰). Interestingly, a recent study of stable isotope values from fingernail clippings of modern children showed that children receiving breast milk *and* infant formula (based on cow's milk) demonstrate a lower nitrogen increase (1-1.4 ‰) compared to fully breastfed children (Fuller *et al.*, 2006). This differences applied also to the carbon values (0.5 ‰ instead of *c.* 1‰ if fully breastfed). The low increase of the nitrogen and carbon values mirrors the low enrichment seen among the Asine children which are younger than 1 year of age. It is also possible, however, that these isotope values reflect a combination of breastfeeding and foetal diets.

When discussing weaning age, it is also relevant to consider other types of evidence such as data on subadult mortality: the osteological data from Asine indicate a high mortality of children less than 1 year of age. In the Barbouna cemetery 8 of the 17 individuals were children in this age group. It is well known that early introduction of other nutrients than breast milk is potentially risky under poor hygienic circumstances, since it may expose the children to pathogens and nutritional stress (King and Ulijaszek, 1999). Thus, the synergistic effect of infections and early supplementation is associated with high mortality during the first year. Naturally, the possibility that the diet of children dying at an early age may have been different from that of healthy children should not be forgotten. A stable isotope study of teeth from adults could possibly clarify this question. Whether the low enrichment in the children's nitrogen and carbon values indicates that a breast milk diet was supplemented with other nutrients (such as for instance animal milk) has to remain an open question because of the limitations of the sample size already mentioned. It is nevertheless very likely that dairy products formed an important part of the diet at Asine: a study of animal bones from terrace III at Kastraki showed a high slaughter age of the domestic animals usually consistent with a utilization of secondary products, such as milk and wool (Moberg Nilsson, 1996).

CONCLUSIONS

The stable isotope analysis of the skeletons from Asine shows that the diet of the Asine population was similar to that of other Greek Bronze Age populations: there was a heavy reliance on terrestrial foods, namely C_3 plants with a varying amount of animal protein from meat and dairy products. There is no indication that sizeable amounts of marine foods were consumed, and there are no signs of either direct or indirect ingestion of C_4 plants such as millet. Some adults at the East Cemetery seem to have had a lot of animal protein in their diet, and in this respect they are comparable to the individuals from the Grave Circles at Mycenae.

A limitation of the current study is however the modest number of individuals, the low number of sexed adult individuals and the absence of individuals in certain age groups. The question of weaning age of the Asine children is thus problematic. However, there are indications that some children received early supplementation, possibly in the form of animal milk. In this case they may have been exposed to harmful pathogens which could have been a contributory cause to the high mortality of children below 1 year of age.

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