Using Oxygen Isotopic Values in Order to Infer Palaeoclimatic Differences between Northern and Central-Southern Greece

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Abstract
Even though isotopic analyses have been extensively implemented on human skeletal remains for the purpose of dietary reconstruction, less attention has been given to the ingested water and thus to the investigation of palaeoclimatic conditions. In particular, oxygen isotopic fingerprinting has never been applied on human skeletal remains from Greece for the abovementioned purpose before. The basic aim of the present study is to compare climatic conditions from two ancient populations, deriving from two different ecological locations; Edessa (Greek Macedonia; 2nd-4th c. AD) and Thebes (Sterea Hellas, 13th-14th c. AD). Oxygen values in Edessa are at -7.69 ±1.13 ‰ and -9.18 ±1.88 ‰ for tooth enamel and bone apatite respectively. On the other hand, oxygen signals in Thebes are at -5.8 ±2.16 ‰ and -9.23 ±1.3 ‰ for the enamel and bone apatite respectively. The utility of oxygen isotopic signatures for the purpose of palaeoclimatic investigation lies on the fact that the ratio of 18O to 16O of meteoric precipitation, expressed as δ18O per mill (‰), relative to the international standard (vSMOW) varies geographically by temperature, humidity, evaporation, distance to the sea, altitude and latitude. Therefore, results as expected, point out that Edessa do presents more negative enamel isotopic values in relation to Thebes, however the noted difference is not observed for the bone apatite samples. The lack of bone apatite differentiation between sites could be attributed to cultural diversity (particularly in Thebes), shift in dietary habits due to migration or social status, climatic fluctuations within each site or to possible diagenetic alteration of bone apatite samples.

1. Introduction
Oxygen isotope ratios of human skeletal and tooth remains have been used in order to reconstruct palaeoclimatic conditions, to study mobility patterns of ancient populations [i.e. 1, 2 ] and assess infant feeding practices (i.e. 3]. Apart from the collagen component of the bone; which mostly reflects the dietary habits of past populations mainly through carbon and nitrogen isotopic values, oxygen isotopic signature from the inorganic part of the teeth and bones principally reflects the isotopic composition of the ingested water [i.e. 4, 5, 6, 7]. Until recently, only two studies have implemented stable oxygen signature in populations from [8] or of Greek origin [9], albeit mostly for the purpose of identifying ‘foreigners’, and less for environmental reconstruction. Thus, the present study is the first to compare palaeoclimatic conditions through oxygen isotopes from bone apatite and tooth enamel in two ancient populations from Greek territory. Two different ecological locations have been chosen, Roman Edessa (2th-4th c. AD) in Greek Macedonia and Medieval Thebes (13th-14th c. AD) in Sterea Hellas (Boeotia) (figure 1).
2. Oxygen isotopes of carbonate in bone apatite and tooth enamel

The utility of oxygen isotopic signatures for the purpose of palaeoclimatic investigation lies on the fact that the ratio of $^{18}$O to $^{16}$O of meteoric precipitation, expressed as $\delta^{18}$O per mill (‰), relative to the international standard (vSMOW) varies geographically by temperature, humidity, evaporation, distance to the sea, altitude and latitude. The oxygen isotopic values decrease in colder climates (cold season, high altitude and latitude, further inland), while in warmer conditions (coastal locations, warmer season, low altitude and latitude) they increase (i.e. Dotsika et al., 2010; Lykoudis et al., 2010). The $\delta^{18}$O in mammal teeth and bones depends on the oxygen isotopic ratio of oxygen sources, such as the ingested meteoric water, oxygen from consumed food and atmospheric $O_2$. However, due to the fact that oxygen isotope values of atmospheric $O_2$ are fairly constant (Kroopnick and Craig, 1972), primarily the consumption of liquid water and secondly food contribute mostly to $\delta^{18}$O values of body water (Longinelli and Peretti-Palladino, 1980). In addition, drinking water differs from that of precipitation by 0.3-0.8 ‰ (Daux et al., 2008). Moreover, while oxygen signal in bone represents the water and food consumed during a wider period of time (i.e. isotopes in bone collagen represent approximately the last 10-20 years of life), oxygen values in tooth enamel of permanent teeth reflect the food and water consumed earlier in life, as tooth enamel is formed during only a limited part of an organism's life (Loftus and Sealy, 2012). Therefore, it is expected that climatic differences between colder and warmer climates will be also reflected in archaeological populations. Hence, the basic purpose of the present study is to assess possible climatic differentiations between two different regions of Greece, one representing the Northern part of the country (i.e. Edessa) and the other the Central- Southern one (i.e. Thebes) (figure 1).

![Figure 1. Map of Greece showing the locations of the archaeological sites under study (Edessa and Thebes) in relation to Athens (capital of Greece)](image-url)
3. Results and Discussion
For the needs of the present study, 35 bone samples and 27 teeth were analyzed; 19 long bone fragments (femoral) from Edessa and 16 from Thebes, while 16 teeth (canine or 2nd molar) from Edessa and 11 from Thebes. The isotopic analyses were conducted at the Stable Isotope and Radiocarbon Unit of the Institute of Nanoscience and Nanotechnology, of N.C.S.R Demokritos, in Athens, Greece. The protocol followed for the bioapatite extraction was according to Bocherens et al., (1996). Powdered bone samples (200 mg) were soaked in 2-3% sodium hypochlorite (NaOC1) for 24 hours, to oxidize organic residues. Samples were then rinsed with distilled water, and treated with 1M acetic acid-Ca acetate buffer for 12 hours, in order to remove exogenous carbonate. The teeth samples underwent the procedure of cleaning and powdering before the analyses, following the trend of non-chemically treating tooth enamel (i.e. Martin et al., 2008).

The oxygen values (bone apatite and tooth enamel) are presented in figure 2. Moreover, in Table 1 the statistical comparisons between sites are presented. In Edessa, the enamel oxygen values range between -5.7‰ to -9.2‰, whereas in Thebes between -3‰ to -9.1‰. The enamel mean difference between the two sites is almost 2δ and statistically significant (Table 1). In contrast, the bone apatite mean difference between sites is not statistically significant.

| Table 1. Statistical comparisons between sites |
|-----------------------------------------------|
| Oxygen isotopes   | Site  | N  | Mean (‰) | Std. Deviation (‰) | T-Test | p-value |
| δ¹⁸Oen             | Edessa | 16 | -7.69     | 1.13               | -2.98   | 0.006   |
|                   | Thebes | 11 | -5.8      | 2.16               |         |         |
| δ¹⁸Oap             | Edessa | 19 | -9.18     | 1.88               | 0.08    | 0.933   |
|                   | Thebes | 16 | -9.23     | 1.30               |         |         |

Figure 2. Oxygen enamel isotope values vs. bone apatite values for both sites
The annual precipitation rate is at 500-600 mm for Boeotia in general and the distance to the sea is 215 meters for Thebes. The mean annual temperature for Boeotia is between 16-18 °C. In addition, $\delta^{18}O$ value for spring waters in Thebes is between -5.5 to -6.5‰ (Dotsika et al., 2010). On the other hand, Edessa’s distance to the sea is at 320 meters. The mean annual precipitation rate is at 500mm; however it exceeds 750mm in regions of higher altitude (mountainous areas). The oxygen isotopic signature for spring waters in Edessa varies from -6.9 to -7.3‰ (Dotsika et al., 2010). The mean maximum temperature in winter is between 8-10 °C, whereas the minimum between -1 to -0.5 °C. In the summer, the mean maximum temperature is at 28 °C, while the minimum between 10-13 °C. Therefore, the mean annual temperature for Edessa is approximately at 12 °C.

Based on the information presented above, more negative oxygen values are expected in Edessa in relation to Thebes, as Edessa’s climate is colder and the Macedonian town presents a higher altitude and a higher precipitation rate in relation to Thebes. In addition, the $\delta^{18}O_{\text{spring water}}$ difference between Edessa and Thebes is approximately at 1-1.5‰. Enamel results seem to verify the expected pattern, contrary to the ones deriving from the bone apatite samples. In particular, Thebes’ enamel and bone apatite results display a wider variability (Figure 2). The noted variability could be attributed to the possible existence of several immigrants, as Thebes was occupied by the Franks and Catalans during the 13th and 14th centuries AD. On the matter of fact, it is documented that Jews of Spanish origin lived in Thebes during that period as well as Armenians (Michael et al., In Prep.). On the other hand, it should not be neglected that bone apatite is frequently diagenetically altered; while enamel apatite fingerprint basically remains unaltered (i.e. Lee-Thorp and van der Merwe, 1991; Koch et al., 1997). Thus, it is quite possible that enamel values reflect more accurately the isotopic signals of the populations under study. Another factor that should be taken into consideration involves climatic fluctuations within each site. Thebes’ population covers a time period of approximately 100 years, whereas Edessa’s population a time period of 200 years. Thus, for example it is quite possible that shifts between more open or forested habitats could have taken place in both environments. Leaf water is relatively enriched in $^{18}O$ in relation to liquid water in plant roots (Gonfiantini et al., 1965). In addition, the aforementioned enrichment is more intense in warmer and more arid conditions (i.e. Ometto et al., 2005), and when foliage is more exposed to sunlight (Quade et al., 1995). Hence, animals that occupy open environments ingest more positive oxygen isotopes in relation to animals which occupy cooler and moister forested habitats, and the abovementioned differentiation can also affect humans that consume animals.

4. Conclusions

Concluding, preliminary oxygen results deriving from enamel and bone apatite samples do point out the expected climatic differentiation between a Northern (i.e. Edessa) and a Central-Southern (i.e. Thebes) site, and this is the first study where such differentiation is presented regarding two ancient populations from Greek territory. Furthermore, even though, the factor of diagenetic alteration for bone apatite samples should not be overlooked; Thebes’ strong isotopic variability can be mostly attributed to the existence of several immigrants both from regions with the same or even warmer climate in relation to Thebes (i.e. Spain), and from areas with colder conditions (i.e. Armenia). Finally, our future goals involve a more detailed palaeoclimatic reconstruction as well as an investigation of geographic origins for both the individuals deriving from Roman Edessa and medieval Thebes.

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