Stable Isotope Forensics for Predicting the Lifestyle and Environmental Exposure of Unidentified Humans

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Abstract. In this study the relation of the ^{18}O isotope from meteoric waters and from teeth enamel of samples from Crete was investigated. ^{13}C and ^{15}N isotopes **measurements** were conducted on hair samples from Crete. The isotopic analysis was conducted in order to determine diet and residence patterns.

1. Introduction

Isotopic fingerprint, in conjunction with the biological information from the skeleton, and the epigenetic fingerprint can aid in the investigation of missing persons by identified the geographic region from which a person is originating and can provide information on the lifestyle diet and socio-economic status of unidentified humans. For this purpose, we conducted isotopic analysis of ^{13}C and ^{18}O of teeth enamel, ^{13}C and ^{15}N of hair and ^{18}O of spring water originating from the island of Crete (Greece).

2. Sampling and methods

In this study water (spring and bottle waters) of Crete (Greece) were collected (15 and 2 samples respectively)in order to provide the relation between ^{18}O of water and ^{18}O of teeth of known human'ssamples.15 teeth and hair samples from the Crete Island were analyzed in order to determine the diet choices and residence pattern.

The isotopic composition of hydrogen (^{2}H), oxygen (^{18}O), carbon (^{13}C) and Nitrogen (^{15}N) was measured in Stable Isotope Unit, Institute of Nanocience and Nanotechnology, NCSR Demokritos (Athens, Greece) on a continuous flow Finnigan DELTA V plus (Thermo Electron Corporation, Bremen, Germany) stable isotope mass spectrometer according to the procedures described in [1, 2]. The results are expressed in standard delta notation (δ) as per mil (‰) deviation from the standard VSMOW as: $\delta = ((R_{sample} - R_{standard})/R_{standard})*1000$ where Rsample and Rstandard = $^{2}H/^{1}H$ or $^{18}O/^{16}O$ or $^{13}C/^{12}C$ ratios of sample and standard respectively. Measurement precision, based on the repeated analysis of internal standard waters, was 1.5, 0.05, 0.5 and 0.5% for $\delta^{2}H$, $\delta^{18}O$, $\delta^{13}C$, and $\delta^{15}N$ respectively.

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2.1 A subsection interpretation of isotope data from human remains

The C, N, H, O isotopic composition of human tissue will not match exactly that of the consumed food and water. This is due to isotopic fractionation process: The differing kinetic and thermodynamic properties of isotopes, due to biological, chemical and metabolic reactions, are responsible for modifications in isotopic composition of the 'light' bio-elements (carbon, hydrogen, oxygen, sulfur).

Carbon isotope ratios of human tissues provide dietary information, specifically about the photosynthetic pathway of plant material. Plants use three different photosynthetic pathways important for human nutrition, characterized by distinctive isotope fractionations of carbon from CO_2 in the atmosphere to starch: C3, C4, and CAM (Crassulacean Acid Metabolism) which imparts different $^{13}C/^{12}C$ ratios to plant tissues. C3 plants (Calvin–Benson cycle: atmospheric CO_2 is fixed through the reductive pentose phosphate pathway), include bushes, temperate shrubs and herbs, most trees and domesticates such as wheat as well as grasses, that are favored by cool growing seasons indicating cool/moist climate and/or high altitude. Modern C3 plants have an average $d^{13}C$ (VPDB) value of -26 ± 5‰ versus PDB (Pee-Dee Belemnite, SC, USA) and typically range from -20‰ (open areas exposed to water stress) to -35‰ (closed canopy). In the C4 plants (Hatch-Slack, C4-dicarboxylic acid pathway) the ^{13}C values are on average about -13.0 ± 5‰ but generally range from -9‰ to -19‰. C4 plants includes arid-adapted grasses and domesticates such as maize and sugar cane, as well as a few desert shrubs and herbs and are common in tropical, subtropical and temperate climates dominated by warm summer rainfall. The ^{13}C values of CAM plants (e.g., agave, pineapple) range between the end members of C3 and C4 plants demonstrating an adaptation capacity in keeping with their environmental conditions.

Carbon is ingested by human directly as vegetal and indirectly as animal products. The carbon isotopic composition of tissues (like human hair or bone collagen) reflects the isotopic composition of the diet with a slight offset of 1‰–3‰ [3, 4].

Nitrogen isotope ratios of animal tissues reflects the quality and quantity of protein consumed [5]. This is due to isotopic fractionation as nitrogen moves from lowest to highest trophic levels, resulting in progressively higher $\delta^{15}N$ values in animals relative to plants or animals lower on the food chain [6]. The isotopic value into the tissues of the consumer present an increase of 3‰ per for each trophic level. Carbon, nitrogen isotope ratio analyses are often used in determining whether or not an individual has changed dietary habits.

The isotopic compositions of hydrogen and oxygen isotopes reflect natural processes in the hydrological cycle. The isotopic ratio [(R = $^2H/^1H$ or $^{18}O/^{16}O$; reported as δ^2H or $\delta^{18}O$, where δ = ((RsampleRstandard)/Rstandard) 1000)] of fresh water varies greatly and systematically across the earth as a result of the spatially and temporarily variable climatic patterns, which govern the delivery of precipitated water to geographic regions. Strong trends in δ^2H and $\delta^{18}O$ occur with increases in latitude, altitude, and continentality and these patterns are relatively well known and documented as maps of precipitation stable isotope ratios [7-9]. So, locally the isotopic composition of precipitation is primarily controlled by regional scale processes: it is greatly influenced by the provenance of wet air masses, the trajectories of the water vapor transport over the continents, their possible partial condensation in continental areas [10] and in general the average rainout history of the air masses [11]. A rather complicated pattern has been observed in the Mediterranean basin, due to intense air-sea interaction processes and the contribution of sea vapor to moisture-depleted continental air masses. Warmer climates generally have higher δ^2H and $\delta^{18}O$ values of precipitation, while colder, higher latitude locations have lower values. These spatial variations can be displayed graphically as isotope landscapes, or isoscapes [12].

The local signals resulting from this predictable water isotope fractionation are propagated through plants and animals and can be recovered from tissues (hair, tooth enamel, or bone) providing geolocation information. The recovered signals will be characteristic of a range of isotopically similar locations or iso-regions. When an individual moves from one iso-region to another the modification of isotopic values of drinking water influences the isotopic composition of the body water and are used to identify the residence patterns. Consequently geographic movements, for one location to a new location, can be reconstructed by analyzing small segments along the length of hair to provide a record of the last month or from tissues representing different periods of an individual's life (tooth vs. bone vs. hair).

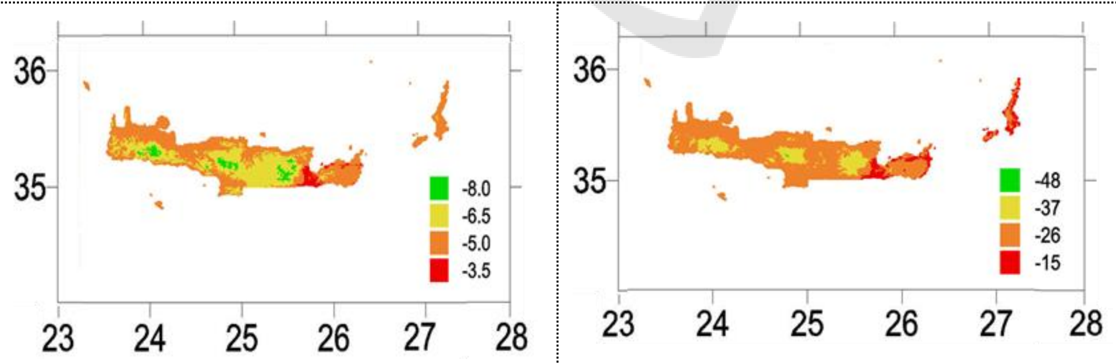
2.2 Results

The use of isotope analysis in modern forensic work would not be possible without of pioneering work in the fields of geology, hydrogeology, anthropology, archaeology, ecology and plant physiology [7-9]. Especially, the oxygen and hydrogen isotope analysis of water leads to construction of the first isoscapes" maps, setting the base for the isotope forensics studies.

In this study, we present the oxygen isotope composition of spring waters from Crete aiming to evaluate the spatial variability of spring water composition. The high resolution map of the spatial distribution of spring water $\delta^{18}O$ and $\delta^{2}H$ (Figure 1) should provide important information for isotope forensic studies. To obtain an overview of the spatial distribution of spring water $\delta^{18}O$, $\delta^{2}H$ we constructed gridded isotopic data sets with a resolution of 30″ × 30″ (approximately 1 km × 1 km) using the methodology of Bowen and Wilkinson [13]. In order to achieve the highest possible resolution we used the GTOPO30 data set maintained by the United States Geological Survey (USGS, 2008). The oxygen and deuterium isotope values of spring waters range between −8.0‰ to −3.5‰ and −48.0‰ to −15.0‰ respectively for Crete Island. The equation of Crete spring water Local Meteoric Water Lines (LMWLCS) is given below and has a slope of 6:

$\delta^{2}H = 6*\delta^{18}O + 6.5$

Generally, an isotope relationship between $\delta^{2}H$ and $\delta^{18}O$ with a slope of about 8 is normally observed for precipitation [14] water. A slope of 6 is attributed to waters with a significant rate of evaporation relative to input [14-16]. Also the weighted mean D-excess values, of 6.5 is not included between the ranges from 10 for global precipitation to 22 for the eastern Mediterranean area [17, 18]. This decrease in LMWL slope and D-excess value in relation to the meteoric water ($\delta^{2}H = 8.7*\delta^{18}O + 19.5$, [7-9]) observed in spring waters across Crete confirms the evaporation of ground water. Possible cause for this enrichment is the partial evaporation of water before the infiltration, the infiltration of recycled irrigation water and evaporation of soil water.



(a) (b)

Figure 1.Spatial distribution of spring water $\delta^{18}O$ (a) and $\delta^{2}H$ (b) of the island of Crete.

To predict potential origins from the $\delta^{18}O$ value determined for the tooth enamel, we first converted carbonate measurements to equivalent phosphate data [19] and then predicted drinking water [20] from the phosphate data. In order to achieve that, several equations from international literature may be used, which practically convert the $\delta^{18}O$ of the carbonate component of the bioapatite ($\delta^{18}O_C$) to $\delta^{18}O$ of the phosphate component ($\delta^{18}O_P$) and finally to $\delta^{18}O$ of water ($\delta^{18}Ow$). As so, the $\delta^{18}O_C$ (vPDB) values need to be converted into $\delta^{18}O_C$ (vSMOW) using the established equation:

$$\delta^{18}OvSMOW=1.03091* \delta^{18}O\ vPDB+30.91[21]$$

According to [19] the relationship between $\delta^{18}O_P$ and $\delta^{18}O_C$ values is expressed by the equation $\delta^{18}O_C=1.015(\pm0.043)* \delta^{18}O_P+8.79(\pm0.79)$, resulting through studies on bone and tooth samples of modern mammals.

For the conversion of $\delta^{18}O_P$ in $\delta^{18}O_w$ Hoppe [20] suggested an equation that combines the structural oxygen with the consuming water: $\delta^{18}O_P=21.28(\pm0.51)+[0.68(\pm0.04)*\delta^{18}O_w]$.

The $\delta^{18}O$ of enamel from the teeth samples of Crete ranges between -9.1‰ and -3.2‰. With the exception of the most negative value (-9.1‰) the other isotopic data suggest that the individual drunk tap water from the region of Crete where they passed all their lives. The most negative isotopic value can be explained by the consumption of bottled waters from Crete ("Zaros" between -8.2‰ and -7.9‰, and "Nera Critis" between -9.2‰ and 8.8‰ for the ^{18}O).

Isotopic values of ^{13}C of hair samples indicate the contribution of plant types to the diet. Also, the knowledge of the specific plants makes it possible to interpret the contribution of different types of animal protein to the diet, as animals that eat these plants will be isotopically similar [22]. Carbon and Nitrogen isotopes can also be used in order to determine the contribution of terrestrial versus marine proteins to the diet. All the above may aid in predicting region of origin or residence patterns where cultural dietary patterns characterize a particular region like in our case the Mediterranean diet [23, 24]. The measured values of ^{13}C range between -19‰ and -21.3‰ and for the ^{15}N between 8‰ and 8.6‰. These values from the hair samples originating from Crete are very similar to the omnivore (Figure 2) values of humans but the lower ^{15}N value indicates that the percentage of vegetable consumption in their diet is significant.

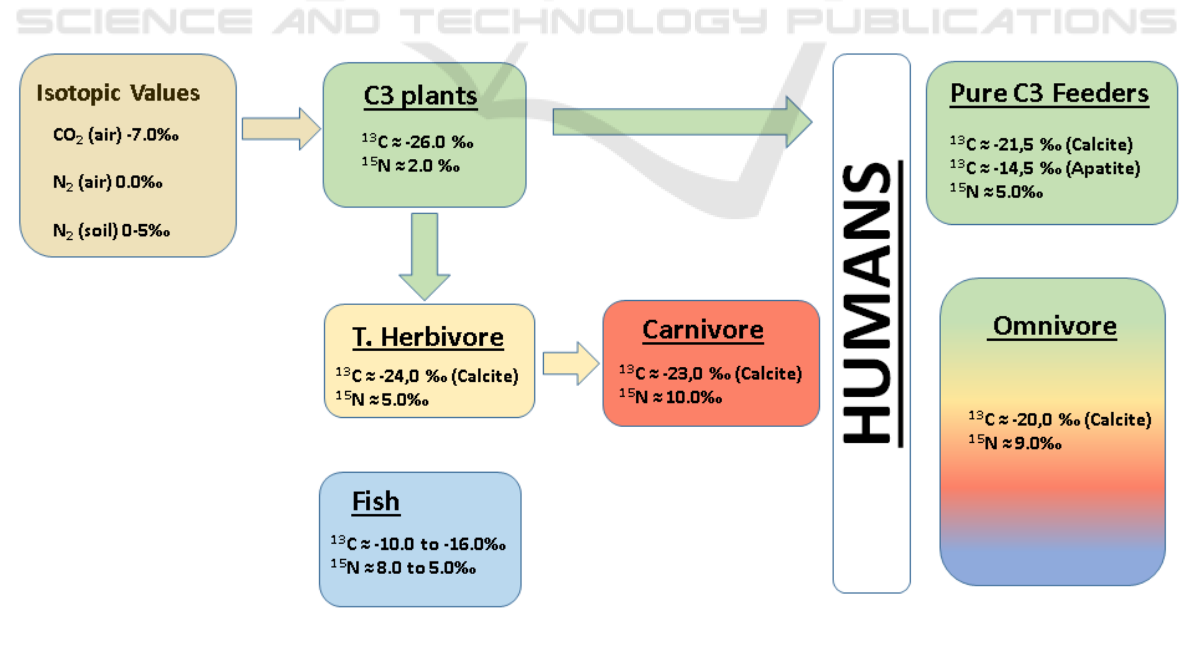


Figure 2. Typical Isotope values of the feeding chain.

3. Conclusions

The oxygen isotopic composition of the meteoric water is well correlated with the oxygen isotopic composition of the teeth enamel in the teeth samples originating from the Island of Crete and potentially can discriminate the provenance. The hair samples from the same area indicate omnivore food consumption with significant contribution of vegetables that can be related to the Mediterranean diet.

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