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Kontopoulos, Ioannis; Sampson, Adamantios

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PREHISTORIC DIET ON THE ISLAND OF EUBOEA, GREECE: AN ISOTOPIC INVESTIGATION

Ioannis Kontopoulos*[1] and Adamantios Sampson[2]

[1]Department of Archaeology, University of York, York, United Kingdom
[2]Department of Mediterranean Studies, University of the Aegean, Rhodes, Greece

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Corresponding author: Ioannis Kontopoulos (ik620@york.ac.uk)

ABSTRACT

In this study, the subsistence patterns of two prehistoric communities on the island of Euboea were reconstructed using carbon and nitrogen isotopic compositions of human and faunal bone collagen. The Late Neolithic (5300/5200–3300/3200 B.C.) samples were obtained from Tharrounia (human *n*=14, faunal *n*=4), while the Early Bronze Age (2900/2850-2350/2300 B.C.) skeletal specimens derived from the coastal settlement of Manika (human *n*=107, faunal *n*=7).

The average $\delta^{13}C$ value of human isotopic signatures of Tharrounians was consistent with a C_3 terrestrial-based diet. Mean $\delta^{15}N$ value indicated a diet mainly focused on agricultural products with a systematic exploitation of animal protein (i.e. meat and/or milk products), whereas marine resources were not an important component of Late Neolithic diets. With regard to the inhabitants of Manika, $\delta^{13}C$ values indicated that all individuals also had a C_3 terrestrial-based diet. In terms of nitrogen isotope values, these suggested that the majority of the individuals were consuming animal products on a regular basis and in comparatively higher amounts than the Late Neolithic population at Tharrounia. Besides the contributions from animal and plant protein, the distribution of $\delta^{15}N$ values showed that some individuals could have supplemented their diets with small amounts of marine food or their $\delta^{15}N$ values could have been increased as a result of manuring of the crops. Finally, isotopic data pointed out that overall there was a relatively low level of social differentiation as there was little variation in the diet between different groups of individuals in both prehistoric settlements.

KEYWORDS: Palaeodiet, Stable isotopes, Carbon, Nitrogen, Collagen, Late Neolithic, Early Bronze Age, Greece.

1. INTRODUCTION

The nature of prehistoric diet and the subsistence strategies followed by Neolithic (*c.* 7th-4th millennia BC) and Early Bronze Age (*c.* 3rd millennium BC) communities in Greece has proven controversial. Archaeological data, generally, indicates that prehistoric Aegean diet was primarily based on cereals, legumes, and in a lesser extent, on milk products and meat (Bintliff 2012; Halstead 1981, 1987a). Crops consumed during the Neolithic and Bronze Age periods included a variety of cereals (e.g. wheat, einkorn, emmer, barley, millet) and pulses (e.g. lentil, pea, grass pea) (Hansen 1988; Mangafa 1993; Valamoti 2004, 2007; Valamoti et al. 2011). In addition, zooarchaeological evidence primarily suggests that there was no intensive dairying and direct subsistence on livestock, except during periods of crop failure (Halstead 2011; Mee 2011). As a result, the theory of a low meat strategy with a small-scale, intensive husbandry rather than extensive farming is considered the most probable scenario for the prehistoric Aegean communities (Halstead 1989, 2007).

This view, however, has been challenged as archaeological evidence occasionally appeared contradictory. In some prehistoric faunal assemblages the kill patterns for sheep and goats indicated a meat production strategy with heavy culling of juvenile and sub-adult males (Cavanagh 2007; Halstead 1987a, 1996; Mee 2011; Sampson 1993). Moreover, a husbandry that aimed at milk products has also been suggested as a likely prehistoric subsistence strategy (Cavanagh 2007). Nevertheless, relatively little is known about the origins and development of secondary animal products exploitation (Greenfield 2005, 2010).

It can thus be argued that the subsistence patterns followed by prehistoric communities in the Aegean could have varied depending on their social and cultural complexity, the geographic location of the settlement, and the environmental characteristics of the area occupied (Greenfield 2010; Sampson 1993). For that reason, a broader and more balanced subsistence system, which would include the exploitation of a wide range of plants and animals (meat and/or milk products) to avoid shortage of food, should not be excluded (Halstead 1989, 1996).

Consequently, even if it is presumed that cereals and pulses comprised the bulk of the diet (milk consumption and storage of food surplus were the alternative strategy in times of a decrease in annual yields), it is not possible to infer in what relative proportions these available food resources were consumed (Bintliff 2012; Cavanagh 2007; Demoule and Perlès 1993; Halstead 1987a, 1989, 2004, 2007).

In theory, stable carbon and nitrogen isotopic investigations can play a key role in the debate over Neolithic and Bronze Age subsistence strategies in the prehistoric Aegean region. Many isotopic studies have investigated prehistoric diet in the Aegean, offering valuable information thus far. In particular, a few studies have demonstrated that during the Neolithic and Bronze Age periods in Greece there was a C_3 terrestrial-based diet with no significant animal or C_4 plant consumption and no significant marine input irrespective to the location of the site and its proximity to the sea (Lagia et al. 2007; Papathanasiou 2003; Papathanasiou et al. 2000; Petroutsa and Manolis 2010; Petroutsa et al. 2007; Petroutsa et al. 2009; Richards and Hedges 2008; Richards and Vika 2008; Triantaphyllou et al. 2008; Vika 2011).

Accordingly, stable carbon and nitrogen isotope analysis was conducted for the assessment of the relative importance of plant, animal, and marine proteins in the dietary patterns of the Late Neolithic (LN) and Early Bronze Age (EBA)/Early Helladic (EH) communities of Euboea. The main aim of this study was to reconstruct and assess the probable composition of human diet of the prehistoric populations of Tharrounia (LN) and Manika (EH). Additionally, we intended to: a) explore if the dietary habits varied through time and proximity to the sea; and b) investigate dietary variations between different population subgroups defined by various mortuary practices including burial status, grave size, grave orientation and burial locations.

2. ARCHAEOLOGICAL BACKGROUND

2.1. *Late Neolithic Tharrounia*

Hitherto, the Neolithic cave of Skoteini, the settlement, and the cemetery at Tharrounia are the most important relics of prehistoric human occupation on the island of Euboea (Figure 1) (Sampson 1993). These are located in a semi-mountainous area of central Euboea, near the modern village of Tharrounia, at an altitude of *c.*450m (Sampson 1993). The main Neolithic activity in Skoteini cave lasts from the end of the 6th to the end of the 4th millennium B.C. (5300/5200 – 3300/3200 B.C.) (Sampson 1993). Occupation in subsequent time-periods was in short duration (Sampson 1993). The excavations conducted within the cave, however, revealed that no permanent occupation of Skoteini was apparent (Sampson 1993). The Neolithic inhabitants of the area, therefore, were probably using the cave as a temporary residence in cases of calamities, heavy winters, insupportable summer heat, or to store food surplus (Sampson 1993). When the cave was not used for any of the aforementioned reasons, some secondary inhumations likely took place as evidenced by the few scattered human bones found inside the cave (Sampson 1993).

Figure 1. Map of Greece showing the island of Euboea and the location of the Late Neolithic Tharrounia and the Early Helladic Manika.

Regarding the nearby cemetery, which is situated 400m away from Skoteini cave, this was a certain place for burials during the LN II phase (*c.* 4200/4100-3300/3200 BC) (Sampson 1993). Burials were characterized by the absence of grave offerings, a burial custom already noted in other prehistoric sites of the Aegean (Sampson 1993). The graves themselves were partially or even completely destructed, due to later agricultural activities (Sampson 1993). Those that survived had an irregular trapezoidal or a petaloid shape, with limestone slabs placed vertically to line the side walls of the pits and horizontally to cover them (Sampson 1993). Finally, apart from the cave and the cemetery, Neolithic pottery, obsidian, millstones, and grinders were discovered at the settlement with only few building foundations observed (Sampson 1993).

With reference to the Late Neolithic economy, the settlements in southern Greece are usually associated with a more nomadic lifestyle, with more emphasis on animal husbandry and transhumance (i.e. lowland winter grazing vs highland summer pastures) (Demoule and Perlès 1993; Cavanagh 2007; Halstead 1987b; Sampson 1993). At Tharrounia, the examination of the faunal remains suggests that throughout the main occupational phase there was breeding predominantly of sheep/goats (~70%), followed by pigs (~15%) (Kotzabopoulou and Trantalidou 1993; Sampson 1993). Flock size would have been small, but the inhabitants would certainly had tried to extend them (Kotzabopoulou and Trantalidou 1993; Sampson 1993). Animal meat was most likely the primary goal of exploitation (i.e. cut-marks, burning, young males slaughtered), but dairy products seem to have been utilized as well (Kotzabopoulou and Trantalidou 1993). Thus, a reliance on a mixed meat-milk processing strategy rather than intensive carnivory could be a more plausible scheme for marginal Late Neolithic sites, such as Tharrounia (Greenfield 2010; Halstead 2007).

Regarding archaeobotanical evidence, remains of many cultivated and wild species have been recovered (e.g. einkorn, emmer, macaroni wheat, possibly bread wheat, six-row barley, possibly two-row barley, rye, oat, horse bean, pea, grass pea and lentil, fruits and olives) (Mangafa 1993). Hence, plants should have constituted an important component of Neolithic Tharrounians' diet.

2.2 *Early Helladic Manika*

The Early Bronze Age (EBA) or Early Helladic (EH) site of Manika (EH II: 2900/2850-2450/2400 B.C and EH III: 2450/2400-2350/2300 B.C.) was a very important coastal settlement on Euboea island, about 5km from the modern city of Chalkis (Sampson 1988). Manika (Figure 1) was a well-designed EH urban settlement (Sampson 1988). The public buildings, the houses, and the streets discovered revealed a city with exceptional urban planning (Sampson 1988). The city had a strategic position for the control of the north and south Euboean Gulf, with access to two major valleys of central Euboea (i.e. Psachna and Lelandion plains) (Sampson 1986, 1988). In total, the EH settlement covered an area of approximately 50 or more hectares (Sampson 1988). Therefore, it can be assumed that Manika probably had the control of the commercial trade of the region (e.g. obsidian, metals, and agricultural products) (Sampson 1988).

The cemetery of Manika was very close to the settlement and it was divided into two chronological phases: a) an EH II phase (main) and b) an EH III phase (only few graves) (Sampson 1985, 1988). It covered an area of at least 5-6 hectares, containing more than 5000 graves (Sampson 1985, 1988). The graves were chamber tombs consisting of a corridor (i.e. "dromos") and usually one chamber (Sampson 1988). Some of these tombs were used only once, however, many graves were used in different time-periods for new burials (Sampson 1985).

Although only 189 tombs were excavated, these burials provided important evidence with respect to the socio-economic status of the dead (Sampson

1988). Tombs were divided into large-, medium-, and small-sized, indicating some kind of social distinction (Sampson 1988). In addition, while the grave goods were not numerous, the presence of prestigious offerings such as metal objects (e.g. daggers, brooches, tweezers) or specific types of pottery (e.g. bowls, plates), and the total number of the grave offerings, were used to classify the graves as rich (i.e. more than three and/or 'valuable' grave offerings) and poor (i.e. three or less 'invaluable' grave goods) (Sampson 1985, 1988). Nevertheless, the size of the grave and the number of the grave goods were not always inter-related (Sampson 1988).

3. STABLE ISOTOPE ANALYSES AND PALAEODIETARY RECONSTRUCTION

Throughout the last three decades, stable isotope analyses of bone collagen have been proved a valuable tool for dietary reconstruction (i.e. protein content) of past human populations (Ambrose et al. 1997; Chisholm et al. 1983; Sealy and van der Merwe 1985; Walker and DeNiro 1986; Schoeninger and Moore 1992). Using the δ notation, stable isotopes are expressed in parts per thousand/per mil (‰) of the relevant isotope ratio relative to a standard reference material (i.e. PeeDee Belemnite or PDB marine limestone from South Carolina for carbon; and atmospheric N_2 (air) for nitrogen) (Mariotti 1983; Schoeninger and Moore 1992; Schwarcz et al. 1985):

$$\delta^{13}C = \left(\frac{^{13}C/^{12}C_{\text{ sample}}}{^{13}C/^{12}C_{\text{ PDB}}} - 1 \right) \times 1000$$

$$\delta^{15}N = \left(\frac{^{15}N/^{14}N_{\text{ sample}}}{^{15}N/^{14}N_{\text{ AIR}}} - 1 \right) \times 1000$$

In terrestrial ecosystems, carbon derives from atmospheric CO_2 ($\delta^{13}C$ value about -7‰) and carbon isotopic composition of plants is directly related to their photosynthetic systems (Edwards et al. 2010; Farquhar et al. 1982; Latorre et al. 1997; Pagani et al. 1999; Smith and Epstein 1971; Tieszen 1991; Tieszen et al. 1979). Averaged $\delta^{13}C$ values are approximately -26.5‰ for C_3 plants, -12.5‰ for C_4 plants and -19‰ for Crassuleacean Acid Metabolism (CAM) plants (Ambrose 1986; Bender et al. 1973; Chisholm 1989; Farquhar et al. 1982; Latorre et al. 1997; Tieszen 1978; Smith 1972; van der Merwe and Vogel 1978). Nevertheless, depleted $\delta^{13}C$ values can be observed in plants (mainly C_3 type) in dense forests, with $\delta^{13}C$ of canopy leaves usually ranging from about -29‰ to -30.5‰ (Ambrose 1986; Medina and Minchin 1980; van der Merwe and Medina 1989).

Dietarily important C_3 plants include wheat, rice, most vegetables, fruits, all root crops, and nuts, whereas important C_4 plants include maize, millet, sorghum, sugar cane and tropical grasses (Ambrose and DeNiro 1986; Ambrose et al. 1997; Bocherens et al. 2006; Tieszen 1978). As to CAM plants, this category is mainly represented by succulents such as cacti, agaves and euphorbias (Ambrose 1986; Ambrose and DeNiro 1986).

With respect to marine environments, marine organisms have $\delta^{13}C$ values between C_3 and C_4 plants averaging -19‰, as carbon derives from dissolved bicarbonate ($\delta^{13}C$ value ~0‰) (Smith 1972; Smith and Epstein 1971). Freshwater environments, on the other hand, constitute a different and more complex aquatic class (Price 1989).

All carbon isotopic signatures of food resources consumed (e.g. C_3, C_4 plants) pass up the food chain and their $\delta^{13}C$ values are reflected in consumer's tissues (e.g. bone collagen) (DeNiro and Epstein 1978; DeNiro and Schoeninger 1983; Schoeninger and DeNiro 1984). An enrichment of approximately 5‰ of herbivores' bone collagen has been established relevant to local flora consumed (Ambrose and DeNiro 1986; Tieszen 1991). In higher trophic levels, a smaller stepwise enrichment of about 0.5-1‰ has been recorded (Ambrose 1993; DeNiro and Epstein 1978; Schoeninger 1985; Tieszen et al. 1983). Therefore, stable carbon isotope ratios can be used for the distinction of food resources with relatively large differences in their $\delta^{13}C$ values, such as C_3 versus C_4, or terrestrial versus marine diets (Ambrose 1986; Bender et al. 1973; Chisholm 1989; DeNiro and Epstein 1978; DeNiro and Schoeninger 1983; Latorre et al. 1997; Schoeninger 1985; Schoeninger and Moore 1992; Sullivan and Krueger 1981; Tieszen 1978, 1991; Tieszen et al. 1979).

Nitrogen isotope ratios can be used to determine the trophic level within terrestrial ecosystems, assess the level of animal protein in diet (i.e. proportions of aquatic and terrestrial resources), and evaluate weaning patterns in archaeological populations (Ambrose and DeNiro 1986; Ambrose et al. 1997; DeNiro and Epstein 1981; Fuller et al. 2006; Hedges and Reynard 2007; Herring et al. 1998; O'Connell and Hedges 1999; Schoeninger 1985; Schoeninger and DeNiro 1984).

The majority of the terrestrial dietary resources exhibit higher $^{15}N/^{14}N$ ratios than air ($\delta^{15}N$=0‰), while marine plants exhibit about 4‰ higher $\delta^{15}N$ values than terrestrial plants (Ambrose 1986; Ambrose et al. 1997). Recent investigations, however, indicated that depending on the frequency/intensity of manuring and the $\delta^{15}N$ value of the fertilizer, $\delta^{15}N$ values can be significantly raised primarily in cereals (Bogaard et al. 2007; Fraser et al. 2011; Szpak et al. 2012, 2014).

A 3-5‰ stepwise enrichment in $\delta^{15}N$ values has been observed at each successively higher trophic level in terrestrial and aquatic ecosystems (i.e. from plants to herbivores to primary and secondary carnivores) (Ambrose and DeNiro 1986; Bocherens and Drucker 2003; DeNiro and Epstein 1981; Hedges and Reynard 2007; Minagawa and Wada 1984; Schoeninger and DeNiro 1984). Nevertheless, environmental (e.g. rainfall, soil acidity), physiological, and pathological factors may also influence nitrogen isotope composition which can lead to greater stepwise enrichment between trophic levels (Ambrose 1986; Ambrose 1991; Ambrose et al. 1997; Heaton et al. 1986; Katzenberg and Lovell 1999; Lee Thorp 2008).

4. MATERIALS AND METHODS

A total of 132 samples were analysed. The Late Neolithic samples were obtained from Tharrounia skeletal collection (human n=14 and faunal n=4), while the Early Helladic skeletal assemblage was comprised of 107 humans and 7 animals.

The human skeletal remains from Tharrounia were derived from three trenches dug inside the cave, as well as from six burial pits of the nearby contemporaneous cemetery (Sampson 1993; Stravopodi 1993). The human remains from the cave burials were sparse, commingled and quite often unidentifiable, whilst the human remains from the cemetery represented a more demographically balanced collection (Stravopodi 1993). Due to their fragmentary nature, however, sex and age assessment was often problematic as the diagnostic traits were missing (Stravopodi 1993). Severe teeth attrition of the occlusal surfaces was observed, but otherwise the dental health of all Tharrounians was good (Stravopodi 1993); probably indicative of a diet which was not dominated by carbohydrates (Stravopodi 1993).

The human skeletons from Manika were also poorly preserved, with the majority of the long bones missing (Neroutsos et al. 1994; Bartoli et al. 2001). The soil type and the location of the cemetery, which is situated near the coast, probably contributed to the poor preservation of the skeletal remains (Sampson 1988; Neroutsos et al. 1994). Age and sex assessment was not easy due to the preservation of the skeletons. The inhabitants of Manika had good dental health (i.e. low incidence of dental caries and ante-mortem tooth loss) as a result of their good, balanced diet (Bartoli et al. 2001; Neroutsos et al. 1994). Nevertheless, a high degree of tooth wear was frequently observed (Bartoli et al. 2001; Neroutsos et al. 1994).

Bone collagen was extracted from each sample following the procedures outlined in Richards and Hedges (1999). Initially, samples of 1-2 grams bone were taken by using an electric bone saw. The exposed surfaces were cleaned by sandblasting (Al_2O_3) and then bones were powdered. Bone powder was demineralised with ~10ml 0.5M HCl solution for a week at approximately 4° C. Samples were agitated twice daily and acid solution was changed every two days. When demineralisation was completed, the supernatant was drained off and samples were rinsed three times in distilled water. pH_3 HCl was added in the tube and samples were placed in hot blocks at 65-75°C for 48 hours. The supernatant liquor which contains the collagen was filtered off by using Eezee filters and was freeze-dried for 2-3 days in pre-weighed plastic tubes.

Finally, samples were weighed in triplicate in tin capsules (1.0-1.5 mg) and measurements were carried out on a Europa ANCA CHN analyser coupled to a Europa 20/20 continuous flow isotope ratio mass spectrometry with an error of ±0.1-0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. Alanine and Bovine Liver Standards (BLS) were used as reference standards to screen the measurements.

The degree of preservation was evaluated by the calculation of the C/N ratio which should be 2.9-3.6 (DeNiro 1985; Ambrose 1990, 1993; Iacumin et al. 1998). Nevertheless, samples with C/N ratios 3.4 or higher were eliminated as contaminated (Ambrose 1990, 1993; DeNiro 1985; DeNiro and Weiner 1988). Well preserved prehistoric bone should have more than 1% collagen by weight, more than 4.5% carbon by weight and more than 1% nitrogen by weight (Ambrose 1990, 1993; Iacumin et al. 1998; van Klinken, 1999). When the total collagen concentrations were low (i.e. <1%), atomic C/N ratios outside the accepted range, or %C and %N yields low, the residue was rejected as non-collagenous (Ambrose 1990, 1993; van Klinken 1999).

5. RESULTS AND DISCUSSION

5.1 *Faunal Data*

The results from the isotopic analysis of archaeological faunal remains from Tharrounia and Manika were used as a baseline for the better interpretation of the human isotopic values. Samples yielded collagen of good quality for stable carbon and nitrogen isotope analysis, however, two of them (MAN111 and MAN112) were eliminated from the study as AMS Radiocarbon dating indicated that these samples belong to a much later period. Table 1 displays the $\delta^{13}C$ and $\delta^{15}N$ values of the associated fauna from both sites.

The mean $\delta^{13}C$ of herbivorous mammals (i.e. sheep/goat, cattle) was -20.13±0.72‰ (-20.99‰ to -

19.39‰) for Tharrounia, and -20.01±0.40‰ (-20.46‰ to -19.55‰) for Manika. Mean δ^{15}N values of 5.37±1.81‰ (3.75‰ to 7.86‰) and 5.85±1.92‰ (3.39 to 7.48‰) were observed for Tharrounia and Manika, respectively. All herbivorous mammals examined, therefore, fell within the range of terrestrial C_3 consumers. These data also suggest that sheep/goat specimens exhibited high variations in their δ^{15}N values, a phenomenon previously observed in other isotopic studies (Honch et al. 2006; Triantaphyllou et al. 2008; Vaiglova et al. 2014). In general, such variations in δ^{15}N in ovicaprids should be expected and they can be ascribed to controlled grazing near or within the settlement (Honch et al. 2006; Vaiglova et al. 2014). As a final point, different species, or different individuals of the same species raised on the same diets, may exhibit variations in their isotopic fingerprints (e.g. grazer vs browser, water-stressed vs unstressed animals) (Ambrose 1986; DeNiro and Epstein 1986; Vaiglova et al. 2014).

Table 1. Quality indicators, δ^{13}C and δ^{15}N values of fauna from the Late Neolithic site at Tharrounia, and the Early Helladic settlement of Manika.

*Grey shaded samples were eliminated as they belong to a much later period (Radiocarbon dating was performed at the Oxford Radiocarbon Accelerator Unit (ORAU)). Calibration was generated using the Oxcal (v4.1) computer program (Bronk Ramsey 1995).

MAN112 (OxA-V-2382-40): 216±21 uncalibrated in radiocarbon years BP (Before Present - AD 1950) using the half-life of 5568 years. 1646-1955 cal. AD (95.4% probability).

MAN113 (OxA-V-2382-41): 223±22 uncalibrated in radiocarbon years BP (Before Present - AD 1950) using the half-life of 5568 years. 1643-1955 cal. AD (95.4% probability).

Sample	Species	δ^{13}C	δ^{15}N	% Col.	% C	% N	C:N
THA10	Sheep/Goat	-19.72	7.86	11.81	30.76	11.11	3.23
THA12	Sheep/Goat?	-19.39	4.37	7.84	27.58	9.94	3.24
THA13	Sheep/Goat	-20.99	5.50	2.13	22.59	8.06	3.28
THA18	Sheep/Goat?	-20.43	3.75	5.51	25.0	8.90	3.28
MAN91	Sheep/Goat	-19.55	7.26	2.89	21.34	7.52	3.30
MAN111	Sheep/Goat	-19.85	7.48	3.70	18.60	6.46	3.37
MAN112	Sheep/Goat	-16.77	8.87	11.14	34.88	12.71	3.20
MAN113	Sheep/Goat	-16.49	8.57	18.37	40.65	14.95	3.17
MAN114	Sheep/Goat	-20.46	3.39	6.44	30.46	10.96	3.24
MAN115	Cattle	-20.19	5.25	1.87	21.08	7.40	3.32

5.2 *Human Data*

5.2.1 Tharrounia

From the 14 human samples analyzed, only 3 specimens were eliminated due to their poor preservation. The Late Neolithic Tharrounians' mean bone collagen δ^{13}C value was -19.85±0.39‰ (-20.43‰ to -19.17‰), whereas their mean δ^{15}N value was 8.26±0.84‰ (7.10‰ to 9.86‰). These results are consistent with previous findings (i.e. δ^{13}C=-19.99±0.22‰ and δ^{15}N=8.04±0.67‰) (Papathanasiou 2003). A summary of stable carbon and nitrogen isotope values of well-preserved samples obtained from the LN Tharrounia skeletal collection are presented in Table 2, whilst Figure 2 illustrates the LN isotopic data.

The average δ^{13}C value of human isotopic signatures at Tharrounia is consistent with a C_3 terrestrial diet. In terms of the mean δ^{15}N value, this showed an increase of ~3‰ compared with the mean nitrogen isotope value of local domesticate animals. It can be argued, therefore, that the LN population of Tharrounia had a diet mainly focused on agricultural products, with systematic exploitation of animal protein, and no indication for marine food consumption.

Nonetheless, the distribution of δ^{15}N values suggests that some individuals (e.g. THA6, THA14) consumed comparatively more animal protein than others (e.g. THA2, THA17). In any case, this important intake of animal protein pointed out from our isotopic data for the majority of the individuals could be likely even with a stepwise enrichment for nitrogen of about 5‰ (Hedges and Reynard 2007). Increased δ^{15}N values, however, could be a result of crop manuring (Bogaard et al. 2007; Fraser et al. 2011; Szpak et al 2012, 2014; Vaiglova et al. 2014).

In general, in such environment, the subsistence would be more pastoral than agricultural in nature. Thus, either a meat or meat-milk strategy would

Table 2. Summary of the $\delta^{13}C$ and $\delta^{15}N$ values from bone collagen, collagen quality indicators, and archaeological information for humans from the Late Neolithic Tharrounia, Euboea, Greece.

Samples	Location	Element	Age	$\delta^{13}C$	$\delta^{15}N$	% Col.	% C	% N	C/N
THA1	Cemetery	Clavicle	Adult	-20.43	8.07	7.40	29.85	10.74	3.25
THA2	Cemetery	Clavicle	Adult	-20.01	7.72	9.05	33.48	11.97	3.26
THA3	Cemetery	Clavicle	Adult	-20.16	8.79	6.15	27.71	9.77	3.31
THA4	Cemetery	Femur	Adult	-19.92	8.54	2.77	23.41	8.28	3.30
THA6	Cemetery	Humerus	Adult	-19.82	9.38	5.0	27.94	10.01	3.26
THA9	Cave	Fibula	Adult	-19.70	7.64	3.18	15.05	5.20	3.38
THA11	Cave	Ulna	Adult	-19.27	8.43	16.50	37.12	13.31	3.25
THA14	Cave	Femur	Juvenile	-19.62	9.86	7.54	29.57	10.33	3.34
THA15	Cave	Humerus	Adult	-19.17	7.85	17.14	42.25	15.22	3.25
THA16	Cave	Tibia	Adult	-20.15	7.43	2.83	25.89	9.27	3.26
THA17	Cave	Tibia	Adult	-20.11	7.10	4.36	20.84	7.36	3.30

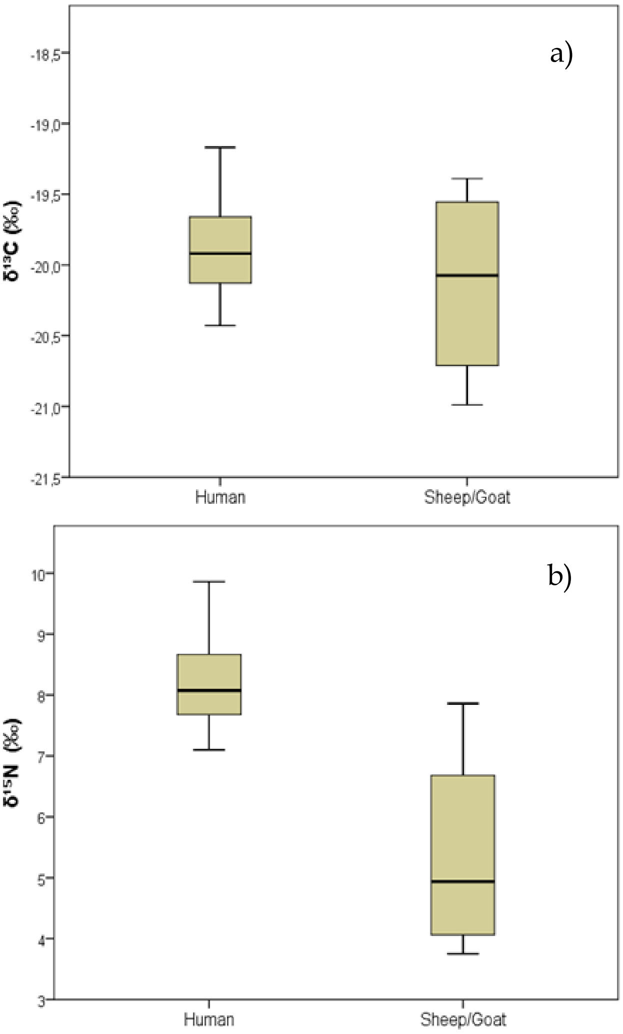

Figure 2. Boxplots displaying a) carbon and b) nitrogen isotope ratios of human and faunal bone collagen from Tharrounia.

have been followed (Greenfield 2010). While meat consumption strategy has been previously suggested as a subsistence pattern for the Late Neolithic Tharrounians, goats and sheep could have been exploited for their milk products as well (Sampson 1993; Kotzabopoulou and Trantalidou 1993). Moreover, the higher survival rates for male goats at Tharrounia suggest that there was probably a combination of a meat-milk orientation (Kotzabopoulou and Trantalidou 1993). Nonetheless, it is very difficult to make any inferences about dairying based entirely on slaughter age profiles (Greenfield 2005).

On the whole, the isotopic data indicated a reliance on both agricultural and animal (i.e. meat and/or milk) products. Regarding animal protein consumption, exploitation of both primary and secondary animal products was probably a more attractive system for a marginal Late Neolithic site of southern Greece (Cavanagh 2007; Greenfield 2010; Kotzabopoulou and Trantalidou 1993; Sampson 1993). Secondary products (e.g. milk) could have been repeatedly extracted from animals throughout their lifetime. Hence, humans could have incorporated animal protein in their diet without slaughtering animals.

5.2.2 Manika

With reference to the human skeletal specimens from Manika, only 17 out of 107 were well-preserved. These had a mean $\delta^{13}C$ value of -19.66±0.29‰ (-20.10‰ to -18.96‰), which is indicative of a C_3 terrestrial-based diet (Table 3; Figure 3). $\delta^{15}N$ values, on the other hand, ranged from 7.70‰ to 10.66‰ with a mean of 9.32±1.04‰ (Table 3; Figure 3). Although $\delta^{15}N$ signatures may have been raised by prehistoric manuring, nitrogen isotopic signatures exhibited an enrichment of ~3.5‰ relative to those of local herbivores (Bogaard et al. 2007; Fraser et al. 2011; Szpak et al. 2012, 2014; Vaiglova et al. 2014).

Table 3. $\delta^{13}C$ and $\delta^{15}N$ from bone collagen, quality indicators, and archaeological information for humans from Manika.

Sample	Element	Age	Status	Grave Size	Grave Orientation	$\delta^{13}C$	$\delta^{15}N$	% Col.	% C	% N	C/N
MAN3	Rib	Adult	Poor	Medium	N-S	-19.49	10.66	1.05	9.02	3.12	3.41
MAN8	Rib	Adult	Poor	Medium	W-E	-18.96	10.25	3.18	27.57	9.89	3.26
MAN9	Rib	Adult	Poor	Small	W-E	-19.7	8.68	1.87	13.55	4.80	3.30
MAN13	Mandible	Adult	Rich	Large	N-S	-20.02	8.64	0.86	15.40	5.44	3.32
MAN14	Humerus	Adult	Rich	Large	NE-SW	-19.72	7.89	10.43	38.73	14.04	3.21
MAN20	Rib	Adult	Poor	Small	NW-SE	-19.60	8.62	4.89	36.18	13.05	3.23
MAN21	Rib	Adult	Poor	Small	W-E	-19.55	10.40	1.66	35.16	12.45	3.30
MAN26	Rib	Adult	Rich	Small	N-S	-19.97	10.31	0.50	26.58	9.0	3.44
MAN31	Rib	Adult	Poor	Small	N-S	-19.25	10.15	2.25	28.34	10.19	3.25
MAN35	Clavicle	Adult	Poor	Medium	N-S	-19.60	9.77	1.46	15.75	5.78	3.27
MAN42	Rib	Adult	Poor	Large	N-S	-19.92	10.16	1.05	36.03	12.24	3.43
MAN43	Long bone?	Adult	Medium	Medium	W-E	-20.10	8.13	2.72	23.22	8.36	3.24
MAN44	Long bone?	Adult	Poor	Small	W-E	-19.70	9.41	1.32	19.73	7.00	3.26
MAN45	Femur	Adult	Poor	Small	W-E	-19.46	10.26	0.96	15.66	5.54	3.31
MAN65	Humerus	Adult	Poor	Small	W-E	-19.78	7.70	1.02	12.61	4.48	3.29
MAN89	Rib	Adult	N/A	N/A	N/A	-19.90	7.77	0.82	11.15	3.88	3.37
MAN95	Rib	Juvenile	N/A	N/A	N/A	-19.55	9.58	2.10	18.00	6.38	3.30

Therefore, it seems likely that the inhabitants of Manika, except C_3 plants, were consuming animal products (meat and/or milk) in high amounts (60-80%) on a regular basis. A meat-milk subsistence strategy for Manika can be further supported by the archaeological evidence, as Early Bronze Age period is considered the time when a major shift in strategies from meat to milk products occurred (i.e. Secondary Products Revolution) (Sherratt 1981; Greenfield 2005, 2010).

Nevertheless, the wide range of human $\delta^{15}N$ values in Manika, undoubtedly, indicates a different utilization of terrestrial sources by its inhabitants. MAN65 and MAN89, for instance, exhibited very low $\delta^{15}N$ values (i.e. 7.70‰ and 7.77‰, respectively), in comparison to other individuals. These isotopic signatures probably suggest that C_3 plants were the staple foods for those individuals. In an EH city of that size, however, a dietary variability should be expected.

Besides the contributions of animal and plant protein, the distribution of $\delta^{15}N$ values indicated that some individuals could have supplemented their diets with marine resources. Although marine fish consumption can be better identified through elevated $\delta^{13}C$ values, individuals with increased $\delta^{15}N$ values and slightly enriched $\delta^{13}C$ values (e.g. MAN8) could have had a sporadic/small input from marine resources (Hedges and Reynard 2007). The population of Manika had access to marine food, most likely throughout the year. Thus, a small contribution of fish in diet could be possible. In case

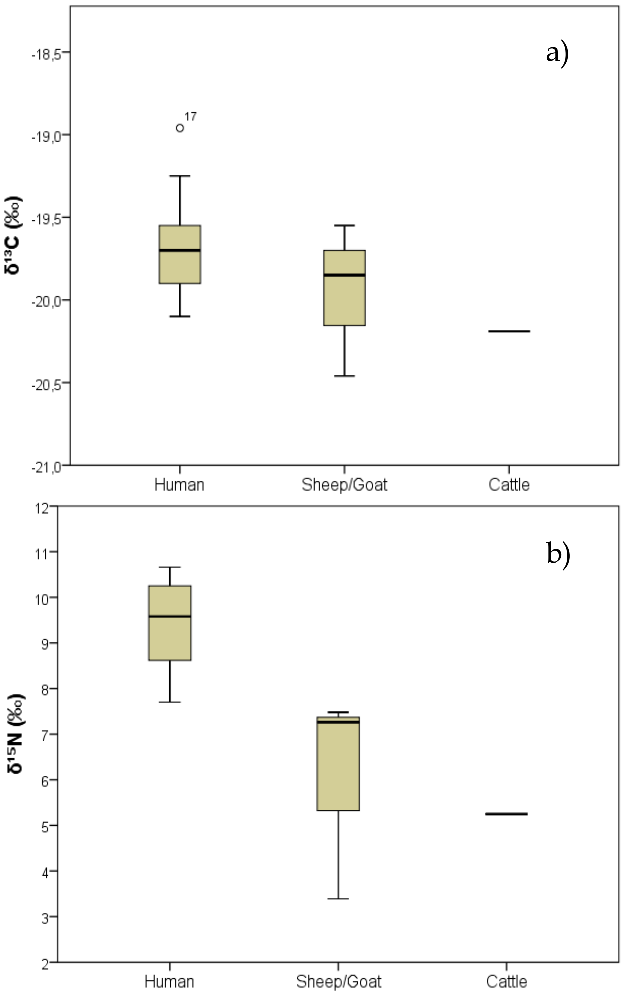

Figure 3. Boxplots showing the distribution of human and faunal $\delta^{13}C$ (a) and $\delta^{15}N$ (b) values from Manika.

there was a consumption of marine fish in Manika by some individuals, then the percentage of animal protein in their diets would be reduced.

This marine input, however, may have been underestimated as the isotopic data from a large number of fish bones from various Mesolithic to Classical period sites in the Aegean suggest a great variability and an overlap with terrestrial resources (Vika and Theodoropoulou 2012). Still, the identification of marine consumption through stable carbon and nitrogen isotope analysis of bone collagen can be fairly problematic (Szpak 2011; Vika and Theodoropoulou 2012). Therefore, this warrants the need for further research.

5.2.3 LN vs EH diet

Comparisons were made between Late Neolithic and Early Helladic human isotopic signatures. The statistical analyses which performed using SPSS Statistics (version 22.0) indicated that there was no significant difference between LN and EH populations in terms of $\delta^{13}C$ values (2-tailed t=-1.475, df=26, p=0.152). Nevertheless, a statistically significant difference was observed in $\delta^{15}N$ values between LN and EH individuals, with the latter group exhibiting enriched $\delta^{15}N$ values (Mann-Whitney U=38, 2-tailed p=0.009) (Figure 4).

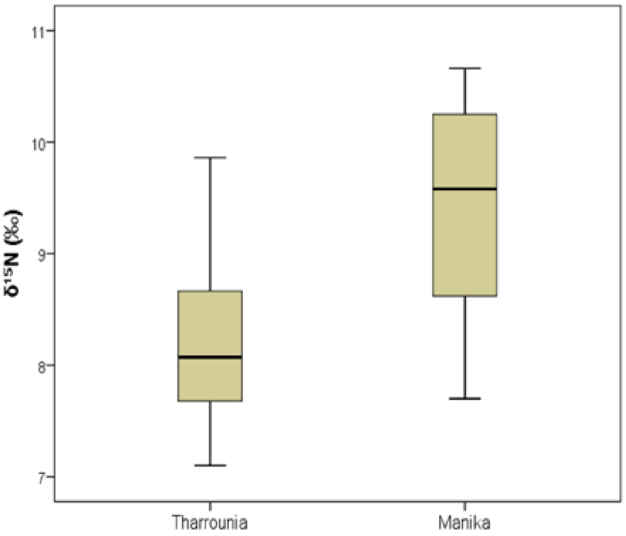

Figure 4. Boxplot displaying the different distribution of $\delta^{15}N$ values for individuals from Tharrounia and Manika.

In Tharrounia, the mean enrichment in $\delta^{15}N$ value observed between human and fauna was approximately 2.9‰, whilst in Manika was around 3.5‰. This indicated a very high proportion of dietary protein consumed from domesticated animals (either primary and/or secondary animal products) for both populations. However, the consumption of more animal protein, the practice or the intensification of manuring of the crops, or a combination of the above, could have significantly increased the average $\delta^{15}N$ value for the inhabitants of Manika. This difference, whatever its cause, pointed out that some changes occurred in the diets and subsistence strategies of the Late Neolithic and the Early Helladic populations on the island of Euboea. However, given the limited number of both faunal and human samples, caution should be exercised when interpreting these isotopic data.

5.3 *Intra-Site Variability*

5.3.1 Tharrounia

An attempt has been made to identify any intra-population differences in stable isotope values at Tharrounia in terms of location (i.e. cave vs cemetery burials). The mean $\delta^{13}C$ value for cave burials was -19.67±0.41‰ (-20.15‰ to -19.17‰), whilst for the cemetery specimens was -20.07±0.24‰ (-20.43‰ to -19.82‰). Concerning mean $\delta^{15}N$ value for individuals recovered from the cave, this was found 8.05±0.99‰ (7.10‰ to 9.86‰), whereas nitrogen isotope values of cemetery burials averaged 8.50±0.64‰ (7.72‰ to 9.38‰).

The isotopic data of the individuals recovered from those two locations, therefore, indicated that both groups consumed a similar type of diet based on terrestrial C_3 plants and some consumption of animal protein (i.e. meat and/or milk produce). T-test conducted for both $\delta^{13}C$ and $\delta^{15}N$ values between cave and cemetery burial groups suggested that there is no statistically significant difference between those two assemblages (2-tailed t=1.912, df=9, p=0.088 for $\delta^{13}C$ and 2-tailed t=-0.867, df=9, p=0.409 for $\delta^{15}N$). Consequently, exploration of the isotopic data revealed that individuals found inside the cave and those buried at the adjacent cemetery had a rather uniform diet with no differential access to food resources.

5.3.2 Manika

The relationship between status and wealth are often related to differential food access, even to the basic resources (Parker Pearson 1999). Hence, the analysis of the variability of the burial practices for the reconstruction of the social organization of a population and the social status (either ascribed or achieved) of the buried individual is of significant importance to archaeology (Parker Pearson 1999). For that reason, diet variation was examined against differences between the EH graves (i.e. number of grave offerings, grave size, grave orientation).

The comparison of human stable isotope values

Table 4. Descriptive statistics of individuals from Manika and t-test comparing isotopic values between different subgroups.

Burial Customs	**$\delta^{13}C$ (‰)**					**$\delta^{15}N$ (‰)**				
	Mean	SD	2-tailed t	df	p	Mean	SD	2-tailed t	df	p
Poor (n=11)	-19.90	0.16	2.205	12	.048	9.64	0.93	1.078	12	.302
Rich (n=3)	-19.55	0.26				8.95	1.24			
Small (n=8)	-19.63	0.22	1.884	9	.092	9.44	1.01	.771	9	.461
Large (n=3)	-19.89	0.15				8.90	1.16			
N-S (n=6)	-19.71	0.31	-.548	11	.595	9.95	0.70	1.307	11	.218
W-E (n=7)	-19.61	0.35				9.26	1.11			

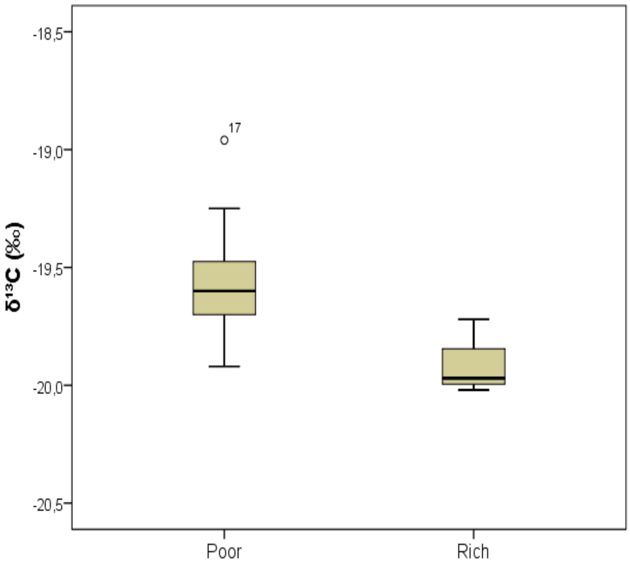

Figure 5. Boxplot showing the distribution of $\delta^{13}C$ values of poor and rich burials at Manika.

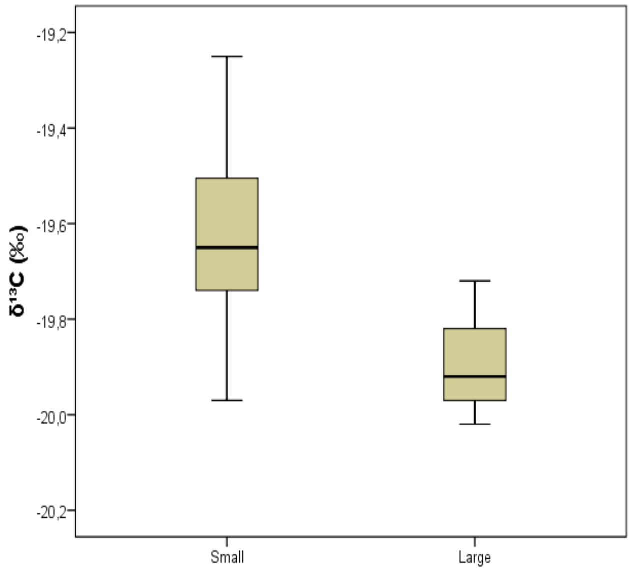

Figure 6. Boxplot demonstrating $\delta^{13}C$ values of individuals buried in small graves, against those of individuals buried in large graves at Manika.

between poor and rich burials suggested that there was probably a status-related dietary variability in carbon (i.e. statistically significant difference) but not nitrogen (i.e. no statistically significant difference observed) at Manika (Table 4; Figure 5). Investigation of dietary variability was also carried out between individuals recovered from small versus large graves. Comparisons between those two groups indicated that there is no statistically significant difference for both $\delta^{13}C$ and $\delta^{15}N$ values (Table 4). However, skeletal remains recovered from small graves exhibited relatively enriched $\delta^{13}C$ values, compared with the individuals from large graves (Figure 6). Human $\delta^{13}C$ and $\delta^{15}N$ values were lastly classified into two broad groups based on the grave orientations (i.e. N-S group included burials facing both N and S orientations; W-E group consisted of graves facing both W and E directions). Nonetheless, similarly to rich/poor and small/large classifications, statistical analysis indicated that there was no significant difference between N-S and W-E oriented graves (Table 4).

Interestingly, these atypical isotopic data suggested that individuals from poor/small graves had increased $\delta^{13}C$ compared with those from rich/large graves, while distributions of their $\delta^{15}N$ values overlapped. If these subgroups had differential access to food, however, this should have been depicted in their $\delta^{15}N$ values as well. Consequently, it can be assumed that there was no clear association between diet and funerary practices, as the distribution of isotope values exhibited an overlap which indicated that diet is likely to have been homogeneous across the population with no differential access to food resources.

6. CONCLUSION

The isotopic data presented in this study documented the dietary habits that occurred at the Late Neolithic Tharrounia and the Early Helladic Manika communities on the island of Euboea, Greece. On average, the measured $\delta^{13}C$ values suggested that C_3 terrestrial-based food dominated both LN and EH diets. A difference of about 3-3.5‰ was observed between mean bone collagen $\delta^{15}N$ values of herbivorous animals and humans from both settlements. Thus, the majority of individuals from those sites consumed significant amounts of primary and/or secondary animal products. In consequence, as animals were far more costly and meat was probably expensive to obtain, a meat-milk strategy could have been followed by those prehistoric communities.

Additionally, human $\delta^{15}N$ values of some EH inhabitants of Manika indicated that these individuals could have had a sporadic/small marine input. Nonetheless, despite proximity to the sea, no individual had $\delta^{13}C$ and $\delta^{15}N$ values that clearly reflected a significant amount of marine protein intake. Accordingly, increased $\delta^{15}N$ values could possibly be the effect of crop manuring.

Statistical analyses performed, indicated that while $\delta^{13}C$ values of LN and EH individuals showed no significant differences, their $\delta^{15}N$ values significantly varied. As a result, a small change in subsistence strategies was indicated by nitrogen isotope values between those two prehistoric communities. Hence, either EH individuals consumed animal protein in higher amounts, or their $\delta^{15}N$ values could have been increased by an intensification of manuring. Nevertheless, as more samples are needed to better understand and compare LN and EH dietary habits, these findings should be cautiously interpreted.

Concluding, isotopic data and statistical analyses also indicated that overall there was surprisingly little dietary variation between different groups of individuals in both settlements. Statistically significant differences observed only in $\delta^{13}C$ values of poor and rich burials at Manika. Therefore, there was a limited social differentiation in these LN and EH communities.

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