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Journal of Archaeological Science 33 (2006) 1493–1504

Journal of Archaeological SCIENCE

http://www.elsevier.com/locate/jas

A palaeodietary investigation of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) in human and faunal bones from the Copper Age cemeteries of Varna I and Durankulak, Bulgaria

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Received 10 October 2005; received in revised form 18 January 2006; accepted 1 February 2006

Abstract

Stable isotope analyses have been applied to human and faunal bone collagen from the Varna I and Durankulak cemeteries to explore palaeodietary adaptations in the Neolithic and Eneolithic (Copper Age). The results suggest both populations primarily utilised terrestrial, C_3-based diets, despite their proximity to the Black Sea. The wider $\delta^{15}N$ range of the Durankulak humans likely indicates the differential utilisation of terrestrial meat sources, which is probably related to the degree to which primary and/or secondary ovicaprid products were consumed, particularly since ovicaprid $\delta^{15}N$ values differ from other herbivores. The isotopic distribution of Varna I reflects a linear relationship between $\delta^{15}N$ and $\delta^{13}C$, suggesting that a minority of individuals enriched in both isotopic parameters supplemented their diets with marine resources. These burials include the well known 'chieftain' (burial 43) and show notable material wealth by way of grave goods. At the population level, however, there is no significant correlation between stable isotope values and material wealth at Varna I, a fact with implications for theories regarding emergent social/economic hierarchies in Balkan prehistory. Five burials at Durankulak were found to have relatively enriched $\delta^{13}C$ and $\delta^{15}N$ values with respect to the rest of the population. These burials reflect a prominently marine-based or mixed terrestrial C_3-based diet that included C_4 inputs, possibly from millet, for which the limitations of stable isotope analysis on bulk collagen are not able to differentiate. AMS dating has shown that these burials belong to a much later period.

Keywords: Stable isotope analysis; Palaeodiet; Carbon; Nitrogen; Bulgaria; Neolithic; Eneolithic

1. Introduction

The accidental discovery of the Varna I cemetery (henceforth referred to as Varna) over 30 years ago revealed an astonishing assemblage of goldwork, the sheer quantity and elegance of which rivalled prominent but much later finds in Mesopotamia and Egypt [14,43,44]. The volume and diversity of material culture at Varna has widely been interpreted as proof of social complexity [43], statehood [42], and the existence of widespread inter-regional exchange networks in the East Balkans [53]. To some, Varna has even been the focus of a discourse related to the birth of European Civilization (e.g. [32]). Whether or not these connections are tenable, Varna most certainly represents the earliest known mass concentration of gold artefacts in the world [14,26,43,44].

Unlike most Eneolithic (Copper Age) cemeteries in the East Balkans, Varna is not associated with a settlement. Thirteen pile dwellings have been located near the Varna Lakes, which are allegedly coeval with the cemetery [32], but their relevance to the site is not firmly established. It is therefore difficult to link the Varna burials to a domestic or agricultural context that may provide direct archaeological evidence of the regional diets and subsistence strategies of humans in antiquity. The situation is compounded by a lack of comprehensive

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0305-4403/$ - see front matter
doi:10.1016/j.jas.2006.02.002

excavation reports, which has posed a formidable barrier to archaeological analysis [12]. Stable isotope analysis is a viable, direct method of investigating human and faunal diets from preserved biological remains [2,30,41,46]. In light of evidence suggesting the emergence of social differentiation during the Eneolithic (see below), we aimed to investigate whether stable isotope analysis could shed light on the potential relationship, if any, between the diet and status of individuals in the East Balkans during this period.

To the north of Varna is the Eneolithic cemetery of Durankulak, an impressive site that contains over 1000 inhumations, making it one of the largest concentrations of prehistoric burials in Europe [51]. The cemetery is associated with a tell settlement that exists as an island in the western side of Lake Durankulak, a coastal lagoon with a continuous sediment history dating back to the Neolithic [6,7,36]. While the Neolithic settlement on the lake-shore is mostly comprised of pits and the plentiful deposition of artefacts, the Eneolithic settlement phases of the tell contain stone-walled architecture and other novel phenomena that are hallmarks of the late Hamangia and Varna cultures and evidence of settlement differentiation [5,13,52,54]. The Durankulak tell site is the only extensively excavated Eneolithic settlement on the Black Sea littoral and provides useful domestic information that may aid in the reconstruction of human dietary trends during the Neolithic and Eneolithic in this region.

The aims of this study are twofold: (1) to obtain stable isotope data that comparatively assesses the diets of Eneolithic humans at Varna and the Neolithic and Eneolithic humans at Durankulak, providing the first evidence of this kind for the East Balkans; and (2) to explore the ability of stable isotope analysis to relate dietary trends to the inferred social status of prehistoric humans.

1.1. The archaeology of Varna

The Varna cemetery is located in north east Bulgaria, slightly inland of the Black Sea and north of the Varna Lakes (Fig. 1). Archaeological excavations have uncovered approximately 280 burials, which are generally classified into three groups: (i) inhumations (155 burials), (ii) cenotaphs (56 burials), and (iii) disturbed burials (70 burials) [12,13,32]. The number and variety of mortuary goods amongst inhumations varies greatly. Many inhumations are sparse, accompanied only by pottery fragments, whereas others are incredibly abundant. Burial 43, for instance, contained 990 gold artefacts that together weighed 1.5 kg. Amongst other items, the buried human was associated with numerous gold necklaces, earrings, appliqués, bracelets, *Spondylus* bangles, a copper adze with gold rings, and a gold penis sheath. In light of its material wealth, burial 43 has been characterised as everything from a chieftain to a king [32]. Regardless of this individual's precise status, the material culture is exceptional even by Varna standards.

Cenotaph burials lack human remains, the absence of which cannot be explained by differential weathering, post-depositional decay or physical disruption—aspects clearly present in disturbed burials [32,44]. Cenotaphs are inferred to be a deliberate and symbolic act of reverence for individuals who died away from the Varna cemetery [13]. In most instances, rings, diadems, beads, bracelets, and other decorative items are placed in anatomical positions on clay masks [43,44]. Overall, this group of burials consistently contains the largest, most diverse collections of grave goods.

The central cluster of Varna burials is referred to as "the core", which contains a group of symbolic and particularly wealthy inhumations, including burial 43 and a number of

Fig. 1. Location of Varna and Durankulak, Bulgaria.

cenotaphs [18]. Twelve of the most abundant burials are located here, all of which contain bone figurines, *Dentalium* shells, and exceptionally long flint ''superblades'' [11]. The ''core'' lacks female or child burials and may be interpreted as a strong case of male gender bias [11]. Some burials outside the ''core'' do not contain grave goods, although most do, and children and females are represented. Despite the lack of stratigraphic information at Varna, the recent AMS dating of human and faunal bones suggest it was a single-period burial site that was in use for approximately 150 years in the mid-fifth millennium BCE [26]. Isotope samples were taken from a variety of spatial contexts to ensure they were representative of the population.

1.2. The archaeology of Durankulak

The Durankulak tell settlement is located on an island in the western side of Lake Durankulak, a large lagoon separated from the Black Sea by a 100–200 m strip of sand in northeastern Bulgaria (Fig. 1). Stonewalled architecture is used during the early and late Eneolithic—a characteristic phenomenon of the late Hamangia culture and evidence of settlement differentiation [13]. The Neolithic and Eneolithic cemetery is located on the mainland, approximately 400 m west of the Eneolithic tell, near the Neolithic settlement. AMS radiocarbon dates from a variety of Hamangia- and Varna-age burials suggest the Durankulak cemetery was in use for at least 500 years, from the early- to mid-fifth millennium BCE [28]. Excavations have uncovered over 1200 burials, details of which have been published in full [51]. The Durankulak burials vary in the abundance and scope of grave goods, including pottery, stone-based materials, and metal, though not to the same extent as Varna. Over 800 skeletons have undergone physical anthropological investigation at the Bulgarian Academy of Sciences, Sofia [60].

2. Materials and methods

Bones were prepared using a modified protocol of Bronk Ramsey et al. [8]. Briefly, 0.50–0.75 g of bone were shot-blasted with aluminium oxide and crushed to a coarse powder before undergoing demineralisation in 0.5 M HCl for 48 h at <10 °C, or until CO_2 ceased to evolve. The samples were rinsed with deionised MilliQ water, placed in sealed tubes containing a pH 3 HCl solution and gelatinised at 75 °C for at least 48 h. The supernatant was subsequently filtered with a 5–8 mm Ezee® filter (Elkay Laboratory Products) and the soluble gelatine freeze-dried for 48 h. Insoluble residues were discarded.

Bulk collagen from each sample was weighed in triplicate to between 2.0 and 3.5 mg in tin capsules. Isotopic analyses were conducted using an automated carbon and nitrogen analyser and a continuous-flow isotope-ratio-monitoring mass spectrometer (cf-irm-ms; ANCA Roboprep coupled to a 20/20 mass spectrometer or a Carlo Erba carbon and nitrogen elemental analyser coupled to a Europa Geo 20/20 mass spectrometer). Conventional replicate measurement errors of nylon standards were $\pm0.1‰$ for $\delta^{13}C$ and ±0.3 for $\delta^{15}N$.

2.1. Bone condition and sample selection

Many skeletal remains were in poor condition at Varna due to acidic soil conditions. Their friable condition caused problems in the definition of grave floors at the time of excavation and frequently rendered age and sex determination problematic. At Durankulak, most excavated burials were intact, yet many bones were fragile due to wet preservation conditions, causing some to fragment upon removal.

Most samples from Varna and Durankulak were taken as whole bone. In the absence of long bones, which were preferentially sampled, identifiable non-long bone elements were given priority. Highly fragmentary bones were sampled only when necessary. In such cases, fragments were chosen on the expected probability of yielding collagen.

Palaeodietary reconstructions function on the premise that isotopic signatures of food sources are passed along food chains and register in consumer tissues [49]. It is therefore essential for human stable isotope values to be interpreted with reference to potential food sources (e.g. plants and animals). The bone collections at the Bulgarian Academy of Sciences, Sofia, and the Regional Museum of History, Varna, were large but heavily biased toward human remains. As such, a supplementary collection of unpublished faunal bones from the Durankulak tell settlement was sampled at the Dobritch Historical Museum, Bulgaria. The bones were catalogued, well preserved, and most were taxonomically identifiable; this collection served as the regional baseline of faunal isotope values. While the additional samples were representative of the most abundant faunal species in the Durankulak cemetery (*Equus asinus hydruntinus*, *Bos* sp., and ovicaprids), they lacked a number of species that were reported to have been found at the settlement, including the European hare (*Lepus eurpaeus*), the common dolphin (*Delphinus delphis*), the common porpoise (*Phocoena phocoena*), wild boar (*Sus scrofa*), bear (*Ursus arctos*), and otter (*Lutra lutra*), among others [50]. A lack of preserved macrofossils precluded the analysis of plant remains. Available palynological reports from Durankulak lake settlements have provided some evidence of barley (*Hordeum* sp.), wheat (*Triticum* sp.), and trace amounts of millet (*Panicum miliareum*), and lentils (*Lens esculenta*) during the late Neolithic and Eneolithic [7].

2.2. Sample integrity

We assessed the state of bone preservation using a combination of C:N ratios, collagen yield, and minimum weight yields of carbon and nitrogen. Collagen with a C:N ratio of 2.9–3.4 (akin to modern bone) was believed to yield reliable $\delta^{13}C$ and $\delta^{15}N$ values [17,46]. According to Hedges and van Klinken [24], bone of ''good preservation'' contains $>20\%$ of the original collagen (based on a modern value of 200 mg collagen/g of dry bone). Although a high collagen yield cannot ensure that bone protein is uncontaminated it can improve the chances of obtaining biogenic signals over bones with little collagen [55]. Samples were accepted here if they were ≥1% wt% collagen. Minimum weight yields of 0.5 mg for carbon and 0.2 mg of nitrogen, per combusted sample (2.0–3.0 mg total weight), were also applied to ensure sample integrity within the analytical limits of the mass spectrometers.

Triplicate isotope values were averaged unless otherwise stated. Where a triplicate sample measurement did not meet the minimum analytical thresholds of carbon and nitrogen or failed due to analytical machinery malfunction, duplicates were averaged. Single measurements of small yet acceptable collagen were occasionally reported.

3. Results and discussion

Basic sample descriptions and results of stable isotope analysis are presented in Tables 1–4.

3.1. Animal isotope values

The herbivorous mammals (*Bos* sp., *C. elaphus*, *Equus* sp., ovicaprids) fell within the range of terrestrial C_3 consumers and compared well to Neolithic [31,47], Eneolithic, and later Bronze and Iron Age contexts in the Black Sea region [40]

Table 1
Faunal stable isotope data from the Durankulak settlement and the Varna cemetery

Sample	Species	$\delta^{13}C$	$\delta^{15}N$	C:N	Measurements[a]	Wt. % collagen
DA25	Badger (*Meles meles*)	−18.3	10.0	3.2	3	10.1
D11.2	*Bos* sp.	−20.2	5.9	3.2	3	6.7
D3u3.1	*Bos* sp.	−19.6	7.2	3.3	3	13.1
D3u3.2	*Bos* sp.	−19.4	5.9	3.2	3	11.1
D4.4	*Bos* sp.	−19.8	5.5	3.3	3	13.8
D4.6	*Bos* sp.	−19.8	6.3	3.2	3	11.0
D8a	*Bos* sp.	−19.2	5.9	3.2	3	12.2
DA3	*Bos* sp.	−19.7	5.9	3.2	3	6.7
D4.5	Canid	−18.9	8.8	3.2	3	9.1
DA21	Canid	−18.8	10.4	3.2	3	8.2
DA24	Canid	−18.4	8.5	3.2	3	12.5
D11.1	Deer (*Cervus elaphus*)	−20.1	5.3	3.2	2	6.7
D3u3.5	*Equus* sp.	−19.5	6.4	3.3	3	7.7
D488a	*Equus asinus hydruntinus*	−20.6	3.8	3.3	1	2.6
DA27	*Equus asinus hydruntinus*	−20.9	3.5	3.2	1	2.6
DA30	*Equus asinus hydruntinus*	−20.8	3.5	3.2	3	3.4
DA33	*Equus asinus hydruntinus*	−20.7	3.7	3.2	3	3.0
DA22	Fox (*Vulpes vulpes* or *Vulpes corsac*)	−18.5	7.8	3.2	3	17.9
DA23	Fox (*Vulpes vulpes* or *Vulpes corsac*)	−19.7	8.2	3.2	3	17.0
D3u3.3	Sheep (*Ovis aries*)	−19.3	8.1	3.3	3	4.0
D4.3	Sheep (*Ovis aries*)	−19.2	5.8	3.2	3	11.7
D971a	Ovicaprid	−19.4	7.3	3.3	1	3.4
V33a	Ovicaprid	−17.7	10.2	3.3	3	4.3
DA1	Ovicaprid	−18.5	10.1	3.2	2	5.8
DA20	Marine turtle (unidentified)	−14.1	14.8	3.3	2	4.1

[a] Designates the number of isotope measurements that met the criteria of sample integrity; see section 2.2. Acceptable values are averaged per individual.

Table 2
Stable isotope and grave good data for Varna humans

Sample no.	$\delta^{13}C$	$\delta^{15}N$	C:N	Measurements[a]	Wt. % collagen	Grave good no.	Grave good types
V8	−19.1	10.2	3.2	3	4.7	6	2
V11	−19.3	10.4	3.2	3	9.1	940	7
V16	−19.5	9.2	3.2	3	3.8	1	1
V17	−19.4	9.7	3.2	3	4.9	0	0
V20	−19	10.3	3.2	3	2.9	0	0
V25	−18.8	10.4	3.2	3	5.1	2	2
V28	−19.1	10.9	3.2	3	8	2	2
V30	−20	9.1	3.3	2	2.6	7	2
V32	−19	10.2	3.2	3	7.4	4	3
V34	−19.1	10.4	3.2	3	11.5	163	7
V38	−19.4	10.2	3.2	3	6.6	0	0
V42	−18.9	10.1	3.2	3	7.1	1	1
V43	−18.5	11	3.2	2	4.8	1013	25
V44	−19.1	10.8	3.2	3	5.2	0	0
V45	−19.1	10	3.2	3	5.2	6	3
V46	−19.4	9.5	3.3	3	3.2	6	5
V47	−19.3	9.9	3.2	3	5.9	3	2
V50	−19.6	10.1	3.2	3	3.2	2	1
V51	−18.5	10.9	3.2	3	4.3	16	7
V58	−19.1	10.4	3.3	3	4.2	26	2
V67	−19.6	10.6	3.2	3	3.6	9	5
V69	−19	10.4	3.2	3	6	2	2
V71	−19.7	9.6	3.3	2	2.3	111	9
V72	−18.8	10.4	3.1	2	6.3	2	2
V87	−19.7	9.1	3.2	1	1.2	3	3
V94	−19.3	10	3.2	2	2.2	1	1
V99	−19	9.7	3.2	3	3.4	65	5
V111	−19	11.3	3.2	3	3.9	11	6
V117	−19.8	10.2	3.2	3	2.9	9	5
V118	−19.1	10	3.3	3	2.1	2	1
V126	−19.7	8.7	3.2	3	4.9	5	3
V127	−19.1	10.2	3.2	3	4.6	2	2
V129	−19.6	9.7	3.2	3	10.3	1	1
V148	−19.5	9.2	3.2	3	4	3	3
V151	−19.5	9.9	3.2	3	4.9	13	8
V158	−18.9	9.8	3.3	1	1.8	203	10
V160	−19	11	3.2	3	3.2	2	1
V171	−19.4	10.3	3.3	3	3.5	5	3
V174	−19.2	9.4	3.2	3	3.2	5	2
V179	−19.5	9.4	3.3	3	6.7	67	8
V190	−19.3	9.3	3.3	3	3.3	20	6
V197	−19.3	10	3.2	3	2.6	1	1
V214	−19.8	9	3.4	1	1.3	2	2
V215	−19.8	10.8	3.2	2	9.5	5	3
V225	−19.8	9.1	3.2	3	13.3	1	1
V234	−19.4	9.9	3.2	3	2.6	1	1
V249	−19.4	9.9	3.3	6	6.3	11	4
V251	−20	9.2	3.4	2	1.5	5	4
V252	−18.9	10.5	3.2	3	8.5	3	3
V253	−19.1	10.4	3.3	3	3.3	10	7
V255	−18.6	10.4	3.2	2	4.8	5	5
V256	−19.5	10.2	3.3	3	5.4	6	3
V258	−19.2	9.7	3.3	3	4.6	2	2
V260	−19.7	9.3	3.2	2	1.3	2	2
V265	−19.5	10.2	3.2	2	5.8	Unknown	Unknown

[a] Designates the number of isotope measurements that met the criteria of sample integrity; see section 2.2. Acceptable values are averaged per individual.

Table 3
Stable isotope data for Durankulak outliers

Sample no.	$\delta^{13}C$	$\delta^{15}N$	C:N	Measurements[a]	Wt. % collagen	Sex
D4.2	−16.1	11.1	3.2	2	8.8	F
D27	−15.5	12.2	3.2	2	5.8	F
D32	−15.7	10.1	3.2	3	11.2	M
D101	−16.1	9.6	3.2	3	17.2	F
D229	−16.7	12.3	3.2	3	7.5	Child

[a] Designates the number of isotope measurements that met the criteria of sample integrity; See section 2.2. Acceptable values are averaged per individual.

(Fig. 2, Table 5). The mean $\delta^{15}N$ values of herbivorous fauna, excluding ovicaprids, are depleted (3.6–6.4‰) in comparison with the omnivores/carnivores (8–10‰), reflecting a trophic level effect of approximately 3‰, a well-attested phenomenon in terrestrial ecosystems (e.g. [37,48].

The $\delta^{15}N$ value of the Bronze Age turtle is substantially enriched over all other samples, attesting to the numerous trophic levels in marine environments [44]. The enriched $\delta^{13}C$ value (−14.1‰) is likely the result of dissolved carbonate and bicarbonate carbon sources for photosynthesis in marine foodwebs [33] in the Black Sea. This value is comparable to humans (∼−12 to −13‰) and animals (dog: ∼−12 to −14‰) who consume substantial quantities of marine resources [10,15].

The $\delta^{13}C$ (−20.7 to −20.1‰) and $\delta^{15}N$ values (3.5–3.8‰) for *E. asinus hydruntinus* are unusually depleted in comparison to the remaining terrestrial herbivores. This wild species, better known as the 'European wild ass', inhabited South Asia and southern regions of Western Europe until it became extinct in the early Holocene [9,39,50,58]. *E. asinus hydruntinus* persisted for longer periods in the Eastern Balkans and the remains from Durankulak appear to be the latest yet discovered. *E. asinus hydruntinus* is found in domestic and burial contexts, suggesting it was hunted for food as well as ritual. Its extinction was probably caused by a combination of ecological and human-induced pressures. Mild climatic conditions and increased forest cover likely reduced the steppe environment in which *E. asinus hydruntinus* likely thrived [9,20], while intensified hunting during the Neolithic and Eneolithic added further selective pressures [50].

E. asinus hydruntinus $\delta^{15}N$ values are approximately one trophic level below horse, deer, and cattle. In general, horse $\delta^{15}N$ values are comparatively depleted, especially relative to domesticated ruminants, and this is ascribed to their digestive physiology. However, here the $\delta^{15}N$ depletion for *E. asinus hydruntinus* appear especially marked. Considering the depleted $\delta^{13}C$ values, *E. asinus hydruntinus* probably maintained a stable, low protein diet of C_3 grasses. The distinctive isotope values may be because *E. asinus hydruntinus* exploited environmental niches or food resources that other herbivores did not, by choice or because of hunting pressures. Whatever the case, it did not experience long-term dietary stress (see [27]) or occupy arid to dry fringe-environments (see [3,19,21]), which might be expected if heavily hunted, for we would expect to observe highly enriched $\delta^{15}N$ values. With limited data, causative explanations of *E. asinus hydruntinus* extinction are premature; further archaeological and palaeoecological data are required to address this issue.

Most ovicaprids (3–4 individuals) have enriched $\delta^{15}N$ values for herbivores, falling within range of dog and fox. The observed pattern was not restricted to North East Bulgaria but was attested at regional, inland sites in Western Siberia, Neolithic Çatalhöyük in South-Central Turkey, and the northern Black Sea coast in later periods (Table 5). However, the Durankulak ovicaprid $\delta^{15}N$ values are depleted by 2‰ compared to those at contemporaneous inland, Ukranian sites. Differing climatic and agronomic conditions may account for the elevated $\delta^{15}N$ values at Durankulak. Collagen $\delta^{15}N$ values are in some cases known to increase with decreasing rainfall, and to become depleted with increased temperature/aridity [3,21] and resulting water stress [19]. In the broader context, however, there is little evidence for high temperatures and/or low rainfall in the region, especially since other fauna do not appear to be affected in this way. The stable isotope values of the remaining fauna are within ranges typically observed in temperate, well-watered environments. In addition, this would have had to have been a long-term process in order to isotopically register in bone collagen, a tissue with a slow turn-over rate [2,34]. It has recently been noted from isotopic studies of hair keratin in sheep from Turkey that grazing in contained areas is correlated with enriched $\delta^{15}N$ values relative to open, rural contexts, which might be due to natural manuring and the increased nitrification of soils from urine and faeces [23]. It is possible that the elevated $\delta^{15}N$ values of the Durankulak ovicaprid collagen may similarly be explained as the result of controlled grazing in areas adjacent to or on the tell settlement. Currently, the few reported values here represent the sum total of $\delta^{15}N$ values for the Black Sea region and the elucidation of ovicaprid $\delta^{13}C$ and $\delta^{15}N$ variability requires the analysis of more samples from a variety of environments.

3.2. Human isotope values from Varna and Durankulak

The Varna $\delta^{13}C$ values range from −20.0 to −18.5‰, with a population average of -19.3 ± 0.3‰ ($n = 55$), and $\delta^{15}N$ values ranging from 8.7 to 11.3‰ with an average of 10.0 ± 0.6‰. The vast majority of human $\delta^{15}N$ are enriched by at least 3‰ over non-ovicaprid terrestrial herbivores (∼5–6‰), exhibiting a trophic level effect and demonstrating the importance of terrestrial meat sources in the diet. The Durankulak humans show a wider spread in $\delta^{15}N$, ranging from 7.6 to 11.5‰, with a population average of 9.3 ± 0.8‰ ($n = 78$), while the $\delta^{13}C$ values closely resemble Varna and range from −19.8 to −18.6‰, averaging -19.1 ± 0.3‰. Although the $\delta^{13}C$ values of both populations are slightly enriched over humans from inland systems in continental Europe, which are usually between −20 and −21‰ (see [56]), the difference is primarily attributable to climatic variations. Comparative database studies of wood, charcoal, and bone samples from European archaeological sites have shown that $\delta^{13}C$ values tend to become enriched in a north to south direction, following the climatic/temperature cline that

Table 4
Stable isotope data for Durankulak humans

Sample no.	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N	Measurements[a]	Wt. % collagen	Sex
D20	−19.1	9.3	3.2	2	1.4	M
D34	−19.1	9.3	3.2	3	3.1	M
D86	−19.4	7.9	3.2	3	2.9	M
D128	−18.8	9.6	3.1	3	3.9	?
D193	−18.6	10.5	3.2	2	9.3	M
D450	−19.8	9.7	3.2	3	1.6	F
D460	−19.6	10.0	3.2	3	2.0	F
D462	−18.9	9.3	3.2	3	7.7	F
D465	−19.6	9.5	3.2	3	2.4	F
D483	−18.9	9.0	3.2	2	7.6	M
D484	−19.2	8.6	3.2	3	2.6	M
D485	−19.1	8.9	3.2	2	5.8	M
D488	−18.9	10.1	3.2	1	4.0	M
D491	−19.5	8.2	3.2	2	3.9	M
D512	−19.6	9.9	3.2	3	2.6	M
D527	−19.3	9.8	3.2	3	6.2	F
D532	−19.0	11.5	3.2	3	4.5	F
D536	−19.1	10.1	3.2	1	4.2	M
D553	−19.4	10.4	3.2	3	6.6	F
D563	−19.2	8.4	3.2	3	2.5	F
D569	−18.9	8.0	3.2	3	3.7	F
D588	−19.2	10.7	3.2	3	5.5	F
D595	−18.6	11.1	3.2	2	3.6	M
D596	−19.3	10.0	3.2	3	9.2	F
D602	−18.9	10.4	3.2	2	5.3	M
D606	−19.0	10.6	3.2	2	4.2	M
D607	−19.3	9.0	3.2	3	8.5	M
D609	−19.7	9.7	3.3	3	7.8	M
D614	−18.9	9.2	3.2	3	4.4	M
D619	−19.2	8.2	3.2	3	4.5	M
D621	−19.6	10.0	3.2	2	4.2	M
D622	−19.4	9.8	3.2	2	6.0	M
D629	−19.6	7.6	3.3	4	4.1	M
D630	−19.0	8.8	3.2	2	3.3	M
D631	−19.5	9.0	3.4	3	3.0	M
D635	−18.9	9.9	3.2	3	5.0	M
D636	−18.8	9.3	3.2	2	4.5	M
D643	−19.3	10.5	3.2	2	3.5	M
D645	−19.6	9.4	3.3	3	3.1	F
D651	−18.9	10.0	3.2	3	5.3	M
D678	−18.8	8.8	3.2	2	8.2	M
D693	−18.7	8.3	3.2	1	3.6	M
D697	−18.8	8.6	3.2	2	4.5	F
D706	−19.3	9.0	3.2	2	6.7	M
D713	−18.9	8.2	3.2	3	2.6	?
D714	−19.1	8.4	3.2	2	3.1	F
D723	−19.0	9.0	3.2	3	6.1	M
D730	−19.2	8.2	3.2	1	10.2	F
D731	−19.3	11.0	3.2	3	4.0	F
D734	−18.6	9.0	3.2	3	7.0	F
D741	−19.2	9.2	3.2	1	2.0	F
D747	−19.4	10.0	3.2	1	4.6	F
D751	−18.8	9.7	3.2	3	3.5	M
D758	−19.0	8.8	3.2	3	4.7	M
D761	−19.1	8.8	3.2	2	3.1	F
D768	−18.8	9.0	3.2	1	5.7	M
D770	−19.2	8.5	3.2	3	3.1	M
D772	−18.9	8.8	3.2	1	8.2	M
D773	−18.8	8.9	3.2	3	2.6	F
D777	−19.2	8.6	3.3	3	2.7	M
D789	−19.0	9.0	3.2	3	6.9	M
D792	−19.0	9.0	3.2	3	3.2	M
D794	−19.0	9.8	3.2	2	3.7	M
D811	−18.9	8.4	3.2	3	2.4	M
D812	−19.1	8.9	3.2	2	15.7	M
D813	−19.0	7.8	3.2	3	5.3	M
D815	−18.7	9.0	3.2	3	6.8	M
D817	−19.2	8.2	3.2	4	3.5	M
D825	−18.9	9.7	3.2	3	7.3	M
D826	−19.1	9.5	3.2	3	1.5	?
D864	−19.2	9.4	3.3	3	1.8	F
D865	−19.0	8.7	3.3	3	4.4	Infant
D869	−19.5	9.2	3.2	1	3.8	M
D897	−19.3	7.9	3.2	3	3.7	F
D898	−19.1	11.2	3.2	2	6.2	F
D939	−19.0	10.1	3.2	3	3.0	M
D994	−19.7	9.6	3.2	1	1.9	M
D1026	−19.0	9.3	3.2	3	5.7	?

[a] Designates the number of isotope measurements that met the criteria of sample integrity; See section 2.2. Acceptable values are averaged per individual.

increases toward the Mediterranean and, apparently, the proximity to large water bodies like the Black Sea [56,57]. On the whole, it is clear that the Varna and Durankulak humans primarily utilised terrestrial, C_3-based diets with varying proportions of meat, particularly in the case of Durankulak.

It is interesting, however, to note differences in the isotopic distributions of Varna and Durankulak (Fig. 3). Whilst the $\delta^{15}N$ vs $\delta^{13}C$ distribution of Durankulak is roughly circular, with no systematic correlation between the observed variables ($R = 0.012$, $p = 0.92$), the isotope distribution of Varna reflects a linear relationship ($R = 0.61$, $p < 0.001$) with a $\delta^{15}N$:$\delta^{13}C$ gradient of approximately 1:1. Although the $\delta^{13}C$ values are characteristic of terrestrial, C_3-based diets, the observed $\delta^{13}C$ vs $\delta^{15}N$ relationship of Varna is similar to that observed in populations where marine protein made significant contributions to the diet [48,59]. Linear trends with $\delta^{15}N$:$\delta^{13}C$ gradients of ~2:1 have been observed between European Neolithic populations, which primarily consumed terrestrial food resources, and earlier Mesolithic populations, which mostly utilised marine resources (e.g. [35,45]), which tends to support basic assumptions about the preferential routing of dietary protein to collagen (as opposed to de novo synthesis) when the intake of dietary protein is high [41]. It is well known that marine food sources are relatively protein-rich and have elevated $\delta^{13}C$ and $\delta^{15}N$ values with respect to terrestrial food [41]. Controlled feeding studies on rats [4] and pigs [30] have shown that collagen $\delta^{13}C$ and $\delta^{15}N$ values tend to reflect those of dietary protein in high-protein diets. Thus, where marine diets are utilised in any quantity, it would be expected that dietary protein be directly routed to collagen and, depending on the degree to which marine foods were consumed, collagen should show elevated $\delta^{13}C$ and $\delta^{15}N$ with respect to terrestrial, C_3-based diets. In contrast, although we would expect enriched $\delta^{15}N$ values for high protein terrestrial diets (with no marine protein), we would not anticipate a significant increase in $\delta^{13}C$ compared to terrestrial diets of similar composition but of lower protein intake [22].

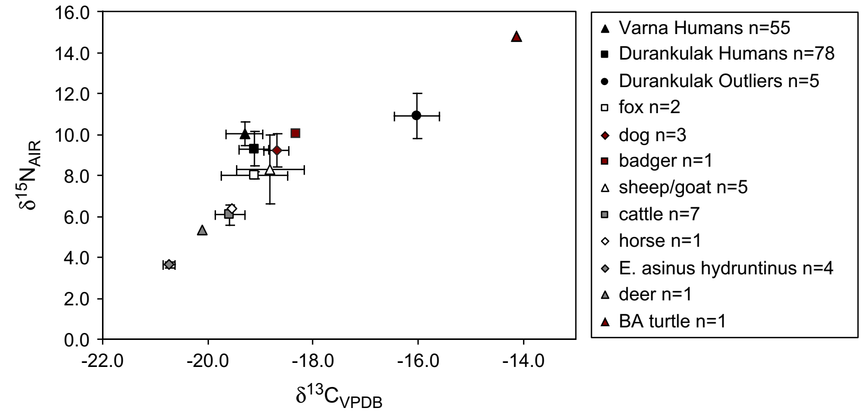

Fig. 2. Human and animal bone collagen isotope data from Varna and Durankulak, north east Bulgaria. All means expressed as ±1σ.

The isotope values of humans at Varna reflect a continuum of dietary protein sources ranging from primarily terrestrial C_3 protein to those that included a combination of mostly terrestrial C_3 protein and a detectable component of marine resources. The former is exemplified in individuals that are depleted in both $\delta^{15}N$ and $\delta^{13}C$ beyond the 1σ range (representing approximately nine individuals or 17% of the sample population; Fig. 4). The latter includes individuals with $\delta^{15}N$ values close to or greater than 11‰, especially those that have $\delta^{13}C$ values beyond 1σ of the population mean (burials 43 and 51). On the whole, however, the consumption of marine protein was apparently minor and

Table 5
Comparative stable isotope data from regional and/or contemporary fauna

Species	*n*	Region	Age	$\delta^{13}C$ (‰)	S.D. $\delta^{13}C$	$\delta^{15}N$ (‰)	S.D. $\delta^{15}N$	Reference
Cattle	2	N. Black Sea Coast	6000 BP	–20.6	N/A	4.2	N/A	[31]
Bos taurus	6	Ukraine[a]	Eneolithic	–20.8	0.5	6.6	1.0	[40]
Bos sp.	7	NE Bulgaria	Eneolithic	–19.6	0.3	6.1	0.5	This study
Equus caballus	6	Ukraine[a]	Eneolithic	–20.6	0.1	3.6	0.2	[40]
Equus sp.	1	NE Bulgaria	Eneolithic	–20.1	N/A	5.1	N/A	This study
E. asinus hydruntinus	4	NE Bulgaria	Eneolithic	–20.8	0.04	3.6	0.1	This study
Deer	1	N. Black Sea Coast	6000 BP	–19.7	N/A	5.8	N/A	[31]
C. elaphus	5	Ukraine[a]	Eneolithic	–21.5	1.0	6.2	1.4	[40]
C. elaphus	1	NE Bulgaria	Eneolithic	–20.1	N/A	5.3	N/A	This study
Ovicaprid	9	Ukraine[a]	Eneolithic	–20.6	0.8	5.8	0.6	[40]
Ovicaprid	7	Urals/W. Siberia	L. Bronze/ E.Iron Age	–19.0	0.9	7.9	1.6	[40]
Ovis	13	Çatalhöyük, Turkey	Neolithic	–18.1	1.3	8.7	1.6	[47]
Sheep	7	Between Black and Caspian Seas[b]	1700–3750 BP	–18.3	1.2	9.1	2.0	[31]
Ovicaprid	5	NE Bulgaria	Eneolithic	–19.1	3.0	7.8	1.6	This study

[a] From the sites of Bugor and Bil'shivtsi, c. 500 km north west of the Black Sea.
[b] Between the Don and Volga rivers.
Shaded rows denote data collected from this study.

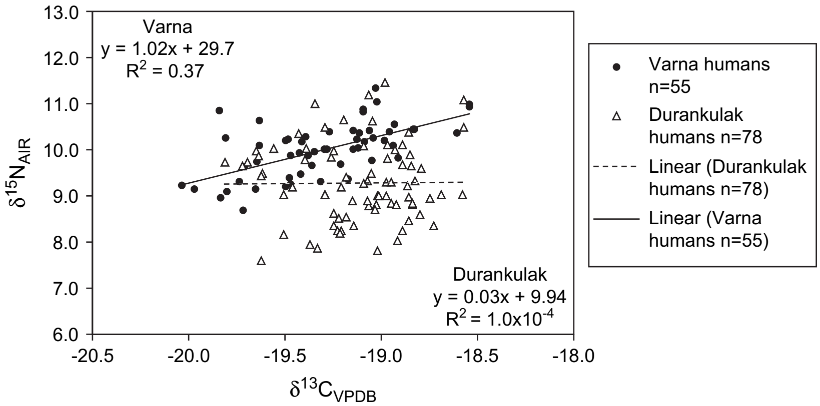

Fig. 3. Human stable isotope data for Varna I and Durankulak. Note the relative distribution of each population.

supplemental to the diet. If individuals were consuming significant amounts of marine protein we would expect radiocarbon offsets between co-interred human and terrestrial animals. Recent research suggests there is a ^{14}C reservoir effect in the Black Sea of about 415 years [1]. The AMS dating of select ($n = 3$) human–terrestrial animal pairs at Varna suggest there is a low probability of offset from true age due to reservoir effects [26], suggesting little dietary protein was derived from marine resources, despite the close location of Varna to the Black Sea.

There is no significant linear relationship between $\delta^{15}N$ and $\delta^{13}C$ values at Durankulak. The observed spread of $\delta^{15}N$ and $\delta^{13}C$ values is likely due to the differential intake of terrestrial, C_3-based protein. About 23% of the Durankulak humans (18 individuals) have lower $\delta^{15}N$ values than the most depleted individual at Varna and are enriched by less than 3‰ over *Bos* sp., the most appropriate isotopic benchmark for terrestrial herbivores.

Although terrestrial meat made important contributions to the diet of these individuals, it did not comprise as much of the overall diet as it did with individuals that exhibited relatively enriched $\delta^{15}N$ values, such as burials D532 (11.5‰), D643 (10.5‰), and D731 (11.0‰). The $\delta^{15}N$ of the latter are upwards of 2–3‰ more enriched than ovicaprids, suggesting their diets were likely supplemented with terrestrial meat and/or secondary products (e.g. milk, cheese) from sheep/goat in addition to terrestrial herbivores and C_3 plants. The $\delta^{13}C$ values of these individuals are close to the population mean and are consistent with terrestrial C_3 protein sources, suggesting their $\delta^{15}N$ values do not reflect marine protein consumption. It is important to note, however, that a small number of Durankulak humans may have utilised marine resources, such as burials D193 and D595. The isotopic signatures of these individuals overlap with the outlying burials at Varna (V43 and V51) and exhibit enriched $\delta^{13}C$ and $\delta^{15}N$ values.

3.3. Isotopes and social stratification at Varna

A central objective of this study was to determine whether a relationship existed between the diet of specific individuals at Varna, as inferred from bulk collagen isotope values, and their associated material culture, as expressed by the number and diversity of grave goods, which may be a reflection of status in prehistory [43,44]. At the population level, the correlation between $\delta^{13}C$ values and the number or types of grave goods per burial was not statistically significant (Table 6). The relationship between $\delta^{15}N$ and grave good variables was also not statistically significant.

In specific cases, however, there are correlations between stable isotopes and material culture. The obvious examples are burial 43, which contains the largest (1013 artefacts) and

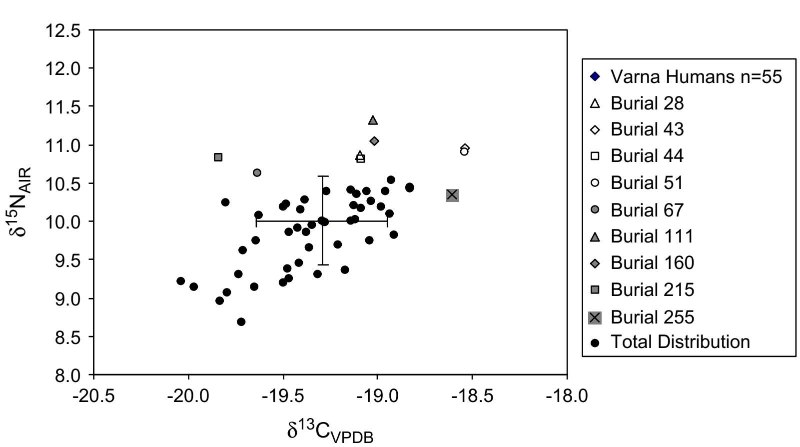

Fig. 4. Stable isotope distribution for Varna humans. Select burials are highlighted.

Table 6
Linear Regression Results of Stable Isotope Data vs Grave Good Variables

Variables	R	p
$\delta^{13}C$ vs Grave Good Number	0.241	0.079
$\delta^{13}C$ vs Grave Good Types	0.263	0.055
$\delta^{15}N$ vs Grave Good Number	0.218	0.113
$\delta^{15}N$ vs Grave Good Types	0.196	0.155

most diverse (25 types) collection of artefacts at Varna, and burial 51, which reflects moderate material wealth (16 artefacts of seven different types). Burials 43 and 51 are the only to show substantial enrichments *in both* $\delta^{13}C$ ($>2\sigma$ above the mean) and $\delta^{15}N$ (Fig. 4). In these cases, the relationship between the isotope signatures and the material wealth of the individuals could be a reflection of their status in antiquity. In the context of the previous discussion, the humans in burials 43 and 51 likely consumed more marine protein than the rest of the population, suggesting long-term dietary differences do correlate with the material wealth and inferred status in special cases. These exceptions, however, do not contravene the finding that most materially rich burials (burials 11, 34, 58, 71, 99, 151, 158, 179, 190, 249, 253) have $\delta^{13}C$ and $\delta^{15}N$ values that are much closer to the population mean (see Table 2). It is also clear that the isotopes of the unique Varna burials overlap with individuals at Durankulk (Fig. 3). Interestingly, these Durankulak burials lack material wealth; D193 contained the fragments of a ceramic vessel, as did D595 in addition to *E. asinus hydruntinus* teeth in the grave fill [51]. These inconsistencies are difficult to interpret despite the relative lack of disparity in material wealth between most Durankulak burials. It may be that the unique Durankulak individuals consumed the same diet as the Varna outliers or simply consumed more terrestrial meat than most at Durankulak and the bulk isotope data cannot differentiate between the two. In either case, the Durankulak data cannot be explained on the basis of inferred status.

3.4. Stable isotopes and the spatial clustering of burials at Varna

Recent chronometric evidence suggests the Varna cemetery was a single-phase site [26,28]. In light of the spatial clustering of graves and the differential allocation of grave goods, we attempted to determine whether stable isotope values were related to the spatial patterning of burials. The distribution of stable isotopes showed significant overlaps between burial clusters, suggesting the overarching diet of each group was quite similar (Fig. 5). It is interesting to note, however, that the population mean of the ‘‘core’’ (area E) is, in a similar way burials 43 and 51 are to the rest of the population, enriched in both $\delta^{13}C$ and $\delta^{15}N$ relative to the other sample groups. The ‘‘core’’ of the Varna cemetery contains most of the materially wealthy inhumations (but also a minority of materially poor ones), including the vast majority of cenotaph burials. Again, a subtle relationship may be reflected between the diet and inferred status of this group in the past.

It is clear that no direct and unambiguous connections can be drawn between diet, as inferred from stable isotope data, and status, as inferred from the material wealth of burials at Varna and Durankulak. This may be due not only to the limited resolution of stable isotope data but also to the theoretical complexities of status—be it social, political, religious, or economic—and its assignation to prehistoric humans on the basis of material remains. Although individuals must have maintained a sufficient local connection to have warranted their burial at Varna or Durankulak, it is possible that some of the populations were originally from distant regions or were local in origin but travelled to other regions throughout their lives (e.g. individuals of prominent economic/political standing or long distance specialists sensu Helms [25]). Some of the observed isotope variation at Varna and Durankulak may thus be due to the consumption of food from a variety of geographic regions or the exposure to climatic or environmental influences that were different from the local area. This might especially be expected at Varna, which is not affiliated with a settlement. However, it is the Durankulak burials—which are associated with a settlement—that show greater isotopic variability. The Durankulak cemetery is much larger and was in use for much longer than Varna; it is possible that the observed isotopic variability is the result of dietary changes that are not currently apparent due to the limitation of the few radiocarbon dates (19) to separate the isotope data into smaller temporal units.

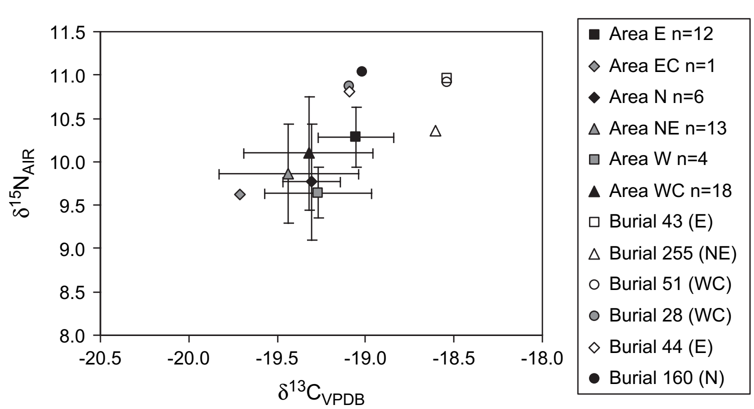

Fig. 5. Varna human isotope data with respect to burial distribution. E, east; NE, north east; WC, west central; N, north.

3.5. Isotopic outliers at Durankulak

Five individuals at Durankulak (D4.2, D27, D32, D101, D229) showed unusually enriched $\delta^{13}C$ values (~4‰) compared to the rest of the population (Fig. 2). AMS results indicate that three of the outliers (D4.2, D27, D32) date to a much later period (~1000 ^{14}C BP; see [28,29]) than the rest of the cemetery. The dated outliers represent the only group of late burials at the site and are likely contemporaneous with the undated samples (D101, D229) in light of their isotopic similarity. The systematic $\delta^{13}C$ enrichment of these individuals may be attributed to one factor or a combination of factors. The collective $\delta^{13}C$ and $\delta^{15}N$ data ($\delta^{13}C_{mean} = -16.0 \pm 0.4‰$, $\delta^{15}N_{mean} = 10.9 \pm 1.1‰$, $n = 5$) suggest marine resources made significant contributions to the diet, more so than in the Neolithic and Eneolithic periods.

Millet consumption, however, cannot be ruled out. Millet is, in essence, the only widespread C_4 plant in Europe that has contributed to the human diet, which could lead to enriched $\delta^{13}C$ bone collagen values. According to archaeological and palaeodietary studies, millet was an important component of the human diet by the Late Bronze or Iron Age [38], and palynological evidence suggests small amounts of millet may have been present at Durnankulak as early as the Neolithic [7]. Since the $\delta^{15}N$ signatures of the medieval Durankulak outliers ($\delta^{15}N_{range}$: 9.6–12.3‰) are only marginally enriched over the Eneolithic population ($\delta^{15}N_{average}$: 9.3‰), they could have maintained a mixed terrestrial/marine diet where a portion of the terrestrial diet included millet. At the level of bulk collagen, however, the influence of C_4 plants and marine resources cannot be resolved. Compound specific stable carbon isotope analysis of single amino acids (e.g. [16]) may be able to resolve this problem.

On the basis of (1) relatively young AMS radiocarbon dates and (2) strikingly different stable isotope data to the rest of the population, the burials in question appear to be recent. It is clear, however, that some contain Hamangia-age material culture [51]. There are two possible scenarios to explain this dilemma. First, in a small number of medieval cases, the mourners placed a few examples of much earlier material culture, which was abundant on or near the surface of the medieval settlement, into the graves of their own newly dead, perhaps to memorialise the associations of the deceased with the long history of their village. The second possibility is that the Copper Age sherds were accidentally incorporated into the medieval grave through the digging of the grave-pit.

Table 7
Stable isotope data by temporal grouping of human burials, Durankulak

Temporal Group	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Hamangia I-II	25	−19.1 ± 0.2	9.4 ± 0.8
Hamangia II-III	1	−18.6	10.5
Hamangia III	37	−19.1 ± 0.3	8.9 ± 0.7
Hamangia IV	11	−19.4 ± 0.3	10.0 ± 0.7
Varna Age	3	−19.2 ± 0.1	9.9 ± 0.8

3.6. Sex-based considerations

There was close similarity between the stable isotope values of the male and female individuals at Durankulak (female: $\delta^{13}C_{mean} = -19.3 \pm 0.3‰$, $\delta^{15}N_{mean} = 9.5 \pm 1.0‰$, $n = 25$; male: $\delta^{13}C_{mean} = -19.1 \pm 0.3‰$, $\delta^{15}N_{mean} = 9.2 \pm 0.8‰$, $n = 48$), suggesting there were no obvious, sex-based differences detectable in the overarching diet of these groups. The sexes of Varna humans have not yet been assigned.

3.7. Lack of diachronic changes in diet at Durankulak

Todorova [51] has assigned a relative sequence to the Durankulak burials on the basis of their material culture. We evaluated our stable isotope results with reference to each group. The isotope values were indistinguishable from each other (Table 7), suggesting little to no dietary change between the Neolithic and Eneolithic periods. It should be noted, however, that AMS radiocarbon dating has uncovered several problems with this typological framework, making firm conclusions premature [28].

4. Conclusions

Stable isotope data from Varna and Durankulak suggest humans at both sites maintained terrestrial, C_3-based diets where protein was mostly derived from terrestrial sources, with a predominance of animal products. The correlated distribution of isotopes at Varna, however, is comparable to populations where marine resources made measurable contributions to the diet. The isotopic data are consistent with a minority of the population utilising some marine resources. Burials 43 and 51 possessed substantial material wealth compared with all other burials as well as exhibited enriched $\delta^{15}N$ and $\delta^{13}C$ values. The isotopic data from Durankulak suggest the population utilised an almost exclusive terrestrial, C_3-based diet with differential inputs of terrestrial meat sources. Individuals with relatively enriched $\delta^{15}N$ values probably consumed primary or secondary ovicaprid products in addition to other terrestrial resources whilst those with lower $\delta^{15}N$ mostly consumed non-ovicaprid protein sources.

Despite correlations between stable isotope data and material culture in select cases (e.g. burials 43 and 51 vs other Varna burials; the "core" vs other areas at Varna), there is no convincing evidence for an overarching trend involving diet and status at the population level at Varna. Although this does not refute the possible wealth and social stature of certain individuals in the Chalcolithic, it implies that long-term dietary trends and differential access to unique or isotopically distinct food resources were not strongly connected with such positions. In any case, it is important to note that some of the observed isotopic variation at Varna may be due to environmental influences associated with residential differences in the buried population. Without a firm connection to a settlement, the buried population may have included individuals with non-local diets, particularly since collagen turns over slowly.

All five isotopic outliers at Durankulak are intrusive to the cemetery and had diets that were considerably different from the rest of the population. The enriched $\delta^{13}C$ values indicate the consumption of marine resources, but it is possible that millet was also an important part of their diet. Stable isotope evidence suggests there were little to no diachronic or sex-based differences in diet at Durankulak.

Acknowledgements

The authors would like to thank H. Todorova, V. Slavchev, B. Dimitrova, and Y. Yordanov for their collaboration on this project. The research underpinning this article formed part of Noah Honch's MSc at the University of Oxford. John Chapman and Bisserka Gaydarska acknowledge the financial support of the British Academy for travel funding; John Chapman thanks the Bulgarian Academy of Science for travel and subsistence within Bulgaria. Noah Honch acknowledges Lincoln College, Oxford, for his travel and subsistence costs. We are grateful to Todor Dimov for providing access to the animal bones at the Historical Museum of Dobritch, and to Sonia Dimitrova for her helpful assistance. We would also like to thank Nikolai Spassov for access to the Durankulak faunal collection at the National Museum of Natural History and to Kalin Dimitrov for drilling the animal bones.

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