Archaeological and Anthropological Sciences (2026) 18:151
https://doi.org/10.1007/s12520-026-02506-5

RESEARCH

Age and sex-related differences in diet in hellenistic-early roman sicily: stable isotope evidence ($\delta^{13}C_{collagen}$, $\delta^{13}C_{carbonate}$, $\delta^{15}N$) from the settlement of Menainon

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Received: 6 November 2025 / Accepted: 14 May 2026 / Published online: 2 June 2026

Abstract

During the Hellenistic-early Roman period (4th −1st BCE), Sicily played a major role in shaping sociopolitical developments in the Mediterranean. However, despite its experience of major social, economic, and demographic change, including major events such as Greek colonization and Roman expansion, aspects of past life, including diet and nutrition, in Sicily during this period, remain largely unexplored. This paper employed samples from 50 adult individuals (25 females, 25 males) and applies bulk collagen, tooth enamel and dentine-incremental stable isotope analysis ($\delta^{13}C_{collagen}$, $\delta^{13}C_{carbonate}$, $\delta^{15}N$) as a proxy for dietary patterns in Hellenistic-early Roman Menainon (Sicily) to test the existence of age- or sex-based differences inferred from historical sources. The results from the bulk analysis show limited differences between individuals of different sex and adult age groups, whereas significant differences were found between early life (dentine) and adulthood (bone). Meanwhile, incremental dentine data showed variable patterns among all individuals, regardless of sex and age interval. Our results suggest dietary access was more equitable across age and sex than previously indicated from written sources, where biological sex did not play an important role in access to foodstuffs, but age affected to some degree dietary patterns. This provides a glimpse of everyday rural life at the edge of expanding colonies and empires.

Introduction

Mentions of Sicily's great scale of cereal production and its related importance to Rome can be found in the historical sources (e.g. Cicero, Pliny) referring to it as "frumentaria provincia" (Soraci 2011). Under Roman protection and with heavy taxes on grain, Syracuse became one of the richest states in the Mediterranean, remaining loyal to Rome until 215 BCE. After its fall in 211 BCE, Syracuse would serve as the local capital, and the taxation system adopted by

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Hieron II, later known as Lex Hieronica, was maintained and applied to the rest of the island (Serrati 2000a, b, Erdkamp 2011). Prior to Roman expansion and conquest, Sicily had also experienced Greek colonization in the form of numerous city-states (*poleis*) established along its eastern and southern coasts. Meanwhile, the island's western region saw the establishment of Phoenician colonies reflecting its role as a crossroads between the Greek and Punic sphere of influence, at least until the first Punic war. The significance of Sicily to Mediterranean socioeconomic dynamics and land use between the Iron Age and Classical period is not in doubt (Giangiulio 2010; De Angelis 2016; Sciortino 2025). However, how the wider dynamic socioeconomic context influenced its inhabitants remains poorly understood. In particular, while there is much documentation relating to the cities of Syracuse, Messana, Centuripe, and Segesta (Barone et al. 2005; Biondi 2013; Olivito 2014; Evans 2016), much less is understood about more rural settings, despite accommodating the largest part of the population in ancient times (Wilson 2011; Taylor 2013).

An individual's access to dietary resources in antiquity was influenced by their role held within the society. Specifically, the consumption of food was likely affected by the general level of involvement of the individual in its gathering, preservation, and preparation or by their ability to purchase local and imported products (Purcell 2003). Both were linked to social status, which changed according to age and sex. The hebdomadal system, adopted by Greeks and Romans, divided early life in phases of 7 years each (early/late childhood and adolescence), each with its own socioeconomic status (Beaumont 2020). Moreover, in ancient Rome, women were commonly excluded from blood sacrifices and meat preparation, suggesting a likely more limited access to its consumption (Purcell 2003; Dupont 2015). Similarly, women were excluded from the most important banquet in Greek society (symposium) (Dupont 2015). Differential access to dietary resources might have resulted in different types of malnutrition, hampering proper growth and development (Lieberman 2019), resulting in the disruption of the homeostatic state (e.g. physiological stress) (Temple and Goodman 2014). However, historical sources tend to focus almost exclusively on the lives of wealthy men, and little can be found on women and children across all social classes or the larger mass population (Dalby 2015). Such a bias has major impacts on historical understandings of social dietary distinctions in antiquity, especially among lower classes, leaving a crucial research gap still open.

In recent years, bioarchaeology has started examining more systematically dental diseases and stable isotopes as proxies for dietary patterns during the Greco-Roman period. One of the aims has been to investigate potential age and sex-based differences to support or refute documentary sources. In some cases, bioarchaeological studies have shown results that agree with historical evidence, but in other cases, they challenge them, stressing the context-specific nature of dietary divisions and the need to expand such research (Prowse et al. 2005; Martyr et al. 2018; Mardini et al. 2023; Moles 2023). Moreover, the studies exploring dietary patterns in Italy and Greece during the Hellenistic-Roman period, focused mainly on urban settlements, leaving the diet of the majority of people, living in rural settlements largely unexplored. Given food's role in the development of power relations and social hierarchies, but also in shaping individual and group identities (Hastorf and Foxhall 2017; Mintz and Du Bois 2002; Pilcher 2012), this study examines stable isotopes ($\delta^{13}C$, $\delta^{15}N$) as a proxy for dietary patterns in the Hellenistic-early Roman settlement of Menainon, eastern Sicily. The aim is to explore potential differences between male and female individuals as well as between early life (childhood, adolescence) and adulthood at an intra site level. Given that Menainon was a rural settlement, and its inhabitants were likely engaging, mainly but not exclusively, in agricultural activities, we might hypothesize that social differences in diet between the sexes and age groups were more muted than is portrayed in the historical records.

Approaches to diet in hellenistic-early roman world – isotopic approaches

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses are commonly used in archaeology to examine diet (Lee-Thorp 2008) in early life (e.g., dentine, enamel) as well as during different parts of adulthood (e.g. ribs vs. long bone) (Hedges et al. 2007; Beaumont & Montgomery 2015; Fahy et al. 2017). Whether in bone or dentine, human collagen is a protein that incorporates stable carbon and nitrogen isotopes through metabolic mechanisms associated with dietary intake (Lee-Thorp 2008). Both carbon isotopic ratios (expressed as $\delta^{13}C$) and nitrogen isotopic ratios ($\delta^{15}N$) of collagen largely represent the protein portion of an individual's diet, although $\delta^{13}C$ also reflects a minor component of lipids and carbohydrates (Fernandes et al. 2012). These isotopic proxies can be employed in archaeological populations to identify the consumption of different food groups. In particular, the joint interpretation of $\delta^{13}C$ and $\delta^{15}N$ enables reconstruction of reliance terrestrial versus marine resources, and/or the consumption of C4 plants or C4-fed terrestrial animal products, when the environment is C3 or vice versa. C3 plants that are commonly consumed in ancient European populations were common cereals (wheat, barley, rye, oat), fruit, nuts, and vegetables. While C4 edible plants would have primarily been millet species or sorghum. The level of $\delta^{13}C$ in C3 terrestrial plants spans between −24 and −36‰ (with a global average of −26.5‰), whereas

C4 plants have a lower variability, with a global average of −12.5‰ (Lee-Thorp, 2008). To improve dietary precision, $\delta^{13}C$ can also be measured in the carbonate component of tooth enamel or bone bioapatite. This proxy reflects a full carbon dietary mix of protein, lipids, and carbohydrates, therefore offering comparatively more details on direct consumption of C3 versus C4 plants (Fernandes et al. 2012). Some food sources may possess overlapping isotopic values. For instance, freshwater fish typically present ^{15}N-enriched values similar to marine resources, but also ^{13}C-depleted signals more alike terrestrial foods, hence complicating dietary reconstruction.

Isotopic analyses have been applied to study sex- and age-based differences in diet during the Greco-Roman period. No differences in the diets of males and females were observed in Knossos (Moles et al. 2022), Edessa (Dotsika and Michael 2018) and the Roman sites of Casal Bertone and Castellaccio Europarco (Killgrove and Tykot 2013). However, Craig et al. (2009) found differences in the dietary patterns of male and female individuals in the Roman site of Velia. Their results suggested a greater quantity of ^{15}N-enriched food (e.g., meat, fish) in male diets. However, this pattern was not absolute, and a small number of female individuals showed comparable results to those of the males. The sex-related differences observed in the site of Velia could be the result of the relatively higher involvement of men in meat and fish procurement, but also of differentiations of social status. Similar patterns were found for the people of Isola Sacra (Prowse et al. 2005), where female individuals were found to have consumed a greater quantity of terrestrial C_3 food sources, whereas male individuals consumed a greater variety of foodstuffs, with an important addition of marine resources. Moreover, male individuals were also found to consume a greater amount of marine resources compare to females in the sites of Pompeii (Pate et al. 2016) and Herculaneum (Martyn et al. 2018). Finally, differences between age groups were only observed in the sample from Isola Sacra, where adult individuals had greater values of $\delta^{15}N$ compared to subadults, suggesting that other factors such as occupation, could have played an important role in food access (Prowse et al. 2005). An important initiative exploring dietary patterns in the semi-mountainous settlement of Pontokomi-Vrysi (Greece) in Roman times (1st – 4th CE), examined dental diseases, dental wear (Vergidou et al. 2021) and isotopic evidence (Vergidou et al. 2025). Both studies found no significant difference in the dietary patterns between sex and age-at-death. Critically, however, the majority of these studies have been performed in urban contexts, meaning that social status related dietary differences remain still largely unexplored in rural settings.

Materials and methods

The site

According to Diodorus, the ancient settlement of Menainon (MENAINΩN) was formally established in 459 BCE by Ducetious, the last Sicel general opposing the Greek domination over the Sicels' territory (Agostinetti 2012; Evans 2016). Shortly after, Menainon fell under the control of the Greek colony of Syracuse and remained under its sphere of influence until at least 212 BCE (Maniscalco 2012), when the whole island became the first conquered Roman province (Serrati 2000a, b). The surrounding area was likely employed in farming activities, as it still is today (Castronovo 2015). The development of the modern town, which rose on the same site, prevented major archaeological investigation, except for the cemetery located in the St. Ippolito area (Maniscalco 2012) (Fig. 1).

The cemetery in the St. Ippolito area

The cemetery associated with Menainon (37.2688° N, 14.6916° E, WGS84) was investigated between 1986 and 1988 by the Soprintendenza BB. CC. AA. of Catania as part of rescue excavations. By the end of its final campaign, 209 tombs were excavated, and the recovered grave goods dated them to between the 4th c. BCE and the 1 st c. CE. (Midolo 2012). All contextual information used in this paper was extracted largely from fragmentary excavation diaries, as

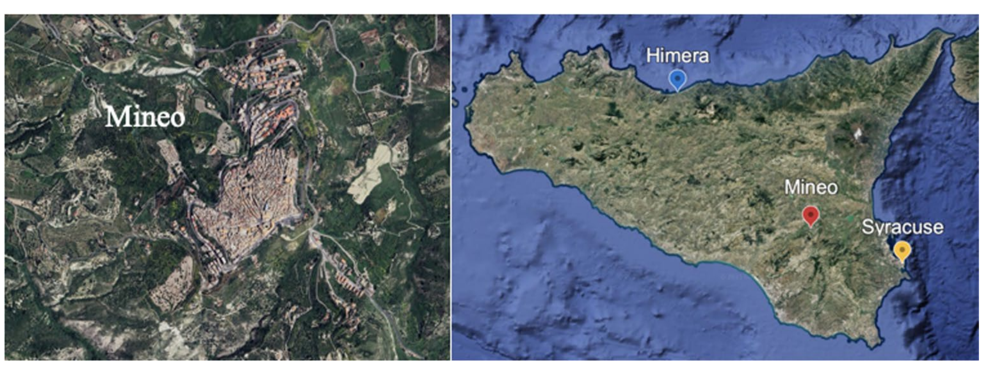


Fig. 1 Map of Sicily showing the location of Menainon (left); Modern town of Mineo and other relevant sites (right) Source: Google Earth (accessed on 29/12/2025)

the cemetery and its material culture remain unpublished, and the original excavator has passed away. Based on available information, most of the burials consisted of inhumations, while primary and secondary cremations were attested in a few cases. The majority of the deceased were placed inside tombs in a supine position or on one side; sometimes they were prone with their face looking toward the east or south. The most common tomb type was a pit dug in soil, sometimes rock-lined or covered by tiles. Rock-cut tombs were also used. Iron nails were recovered in some tombs, suggesting the presence of wooden coffins. In some cases, the tomb location was marked on the surface by cylindrical rocks or transport amphorae. Some of the recovered epitaphs, on squared blocks of rock, specified the name of the deceased (e.g. ΘΕΟΔΩΡΕ) and a few words, generic compliments, such as "ΧΡΗΣΤΕ ΚΑΙ ΑΜΕΜΠΤΕ" or "ΧΡΗΣΤΕ ΧΑΙΡΕ" (Maniscalco 2005). The recovered grave goods included pottery vessels, either associated with banquets or for personal use, as well as objects in metal (Midolo 2005). Among the tombs with grave goods, the most common pottery vessels included unguentaria, transport amphorae, plates, kylikes, lekythoi, oinochoes, and skyphoi. Other grave goods consisted of oil lamps, mirrors, needles, fibulae, rings, and strigils. A bronze coin was often found in one of the hands and in some cases in both hands of the deceased.

The sample

Among the 209 individuals excavated, only 166 (relative chronology 4th-1st c. BCE), could be physically located and examined in previous studies (see Caruso and Nikita 2024a, b, c, 2025). Among these, 50 individuals were selected for isotopic analysis. The samples included in this study are presented in detail in Supplement 1 and consist of 50 adults (28 Young Adult, 20–35 years old; 17 Middle Adult, 35–50 years old; 3 Old Adult, 50 + years old; 2 Adult, 20 + years old), 25 females and 25 males. Sampling was random in terms of contextual information but ensured equal representation of males and females, and a reasonably large sample of young and middle adults, which are the most common age groups in the assemblage. Moreover, preservation state was taken into consideration, ensuring both ribs and multiple teeth were sufficiently preserved. For each individual, a rib fragment and a second permanent molar was sampled; in only two cases (individuals 59 and Q3), second molars were unavailable, and third molars were sampled instead. Moreover, one sheep bone associated to a burial was sampled as the only available faunal species from this site. Therefore, faunal isotopic results of the fauna associated to the Sicilian site of Himera (6th − 5th BCE; 37.9744° N, 13.8238° E, WGS84) was also used as reference (Reitsema et al. 2020). Finally, 12 individuals (10 second molars and two third molars) were also sampled for incremental dentine analysis to explore dietary patterns with a greater chronological resolution of 1 year or less (Beaumont et al. 2013, 2014).

Sex and age-at-death estimation

Sex estimation was performed employing a variety of morphological traits as well as metric standards. In order of importance and according to the overall preservation, we examined first the pelvis, specifically the ventral arc, subpubic concavity, and medial aspect of the ischiopubic ramus, the shape of the greater sciatic notch, and the presence or absence of a preauricular sulcus (Buikstra and Ubelaker 1994). Second, the nuchal crest, mastoid process, and supraorbital margins were examined on the cranium (Walker 2008). Last, for individuals who did not preserve sufficiently the pelvis and the cranium, we employed the metric standards described by Selliah et al. (2020) and Spradley and Jantz (2011), developed respectively on Italian and American collections. Age-at-death was estimated by examining the morphology of the pubic symphysis (Brooks and Suchey 1990) and the auricular surface (Lovejoy et al. 1985), the stage of closure of the cranial sutures (Meindl and Lovejoy 1985), and the sternal rib end morphology (Verzeletti et al. 2010, 2013). Adult individuals were then assigned to one of the following age groups: young adult (20–35 years old), middle adult (35–50 years old), old adult (50 + years old), and adult (20 + years old); the latter expressed cases where it was not possible to reach any more accurate age estimate. The summary of the employed skeletal element examined for sex estimation and age-at-death estimation is given in Table 1.

Additional skeletal parameters

Acknowledging the complexity of the factors affecting one's dietary patterns, beyond sex, additional comparisons were performed employing a variety of skeletal markers linked to different aspects of the daily life of the inhabitants of Menainon. Specifically, potential differences in the

Table 1 Summary of the employed skeletal element used for sex and age-at-death estimation

	Skeletal element		
	1 st choice	2nd choice	3rd choice
Sex estimation	Os Coxae	Cranium	Long bones
Age-at-death estimation	Os Coxae	Rib	Cranium

values of $\delta^{13}C_{collagen}$ and $\delta^{15}N$ were explored in association with dental diseases (carious lesions, dental calculus), physiological stress markers (cribra orbitalia, porotic hyperostosis, linear enamel hypoplasia), and short stature. Carious lesions are traditionally associated with higher consumption of carbohydrates (Hujoel and Lingström 2017), whereas the presence of dental calculus is associated with a protein-rich diet but also with carbohydrate consumption (Innocenti et al. 2022). Physiological stress during childhood is often associated with malnutrition and infectious diseases (Steckel 2005), while short stature, defined as a value that falls within the first quartile of the stature distribution, per sex, of the group under study (Marklein et al. 2016; Zedda et al. 2021), is also considered an overall indicator of poor health that resulted in growth stunting (e.g. Steckel 1995). Physiological stress was linked to nitrogen enrichment/depletion (D'Ortenzio et al. 2015), making those markers relevant for interpreting isotopic results. Supplement 2 gives a brief account on the Greco-Roman diet and how it may have led to nutritional stress.

Bone sampling and treatment for collagen extraction

Collagen extraction and stable isotope analysis were performed at the Max Planck Institute (MPI) of Geoanthropology in Jena (Germany), following the steps described in Riccomi et al. (2024). Specifically, about 1 g of clean bone sample was demineralized in 10 ml of 0.5 M HCl solution. The test tubes were covered in aluminum foil and placed inside the fridge, and the samples were routinely checked every 48 h, changing the acidic solution until full demineralization was reached. The whole process took between 5 and 14 days, depending on the sample. Fully demineralized samples were rinsed three times in ultra-pure water (MilliQ®), placed in 10 ml of pH3 HCl solution, and then left to sit on heating blocks at 70 °C for 48 h. Once the gelatinization of the samples was completed, the content of each test tube was filtered using Ezee™ filter separators, covered with parafilm (with small holes), and placed in the freezer for 24 h. Finally, the samples were placed and left in the freeze dryer for 48 h, repeating the process until no liquid was left inside the test tube.

Tooth sampling and pre-treatment for collagen extraction and enamel bioapatite

The external surface of the teeth was cleaned using a sand blaster before collecting enamel powder with a Dremel® Micro drill. About 10 mg of enamel powder was collected for stable isotopes analysis. The samples reserved for bulk collagen extraction were further drilled, removing as much enamel as possible before starting the demineralization phase. Demineralization, gelatinization, and freeze drying of dentine tissues were done following the steps described above for the bone samples.

The teeth reserved for incremental dentine analysis were cut in half longitudinally using a precision saw blade installed on a Dremel® Micro drill. The resulting half tooth was then partially demineralized in 0.5 M HCl solution for about 5–10 days. Once the samples were sufficiently soft, they were cut into ~1 mm horizontal sections (starting from the crown) using a scalpel, following the steps described in method 2 of Beaumont et al. (2013). Each section was then labelled accordingly and placed back in HCl solution to complete the demineralization stage, which took an additional 5–14 days depending on the sample. Consecutive sections were merged if they could not be cut individually or if they broke into thinner/thicker sections.

The enamel powder was pre-treated by adding 1 ml of 0.1 M acetic acid, vortex, and letting it rest for 10 min. After, the test tubes were placed in the centrifuge for 2 min, and the whole process was repeated 3 additional times with MilliQ® water instead of acetic acid. Lastly, the samples were sealed with parafilm (poking small holes), placed in the freezer for 24 h, and then in the freeze-dryer for another 24 h. The freeze-drying process was repeated until the samples were completely dry (Snoeck and Pellegrini 2015; Ventresca et al., 2018).

Incremental dentine analysis

Incremental dentine analysis of the second permanent molar was employed to reconstruct the isotopic profile of male and female individuals during childhood and early adolescence (2.5–15.5 years old). The age assignment model and the Bayesian model OsteoBioR (https://github.com/Pandora-IsoMemo/OsteoBioR), described in detail by Cocozza et al. (2021, 2025), were used to model isotopic values of each increment into standardized age intervals of 6 months, making them directly comparable within and across individuals. The age estimation, the matrix (renewal rates and isotopic measurements), and the results of the model are given in Supplement 3.

Isotopic measurements

All stable isotope measurements were performed at the Stable Isotope laboratory of the Max Planck Institute of Geoanthropology in Jena (Germany). Between 0.3 and 0.7 mg of collagen was weighed in duplicate into tin capsules which were then combusted in a Thermo Scientific Flash 2000 Elemental Analyzer coupled to a Thermo Delta V Advantage Mass Spectrometer. The stable isotopic measurements of bone and dentine collagen is the ratio between heavy and light isotopes ($^{13}C/^{12}$ or $^{15}N/^{14}N$) measured as δ values in parts per million (‰) calibrated using a two-point calibration between a series of International Standards (IAEA-N-2 Ammonium Sulfate: $\delta^{15}N=+20.3\pm0.20‰$, USGS40 L-Glutamic Acid: δ13C = −26.389±0.04‰, $\delta^{15}N$ = −4.5±0.1‰, IAEA-CH-6 Sucrose: δ13C = −10.49±0.03‰, UREA Isotopic Working IRMS Standard (C13-N15): $\delta^{13}C$ = −36.54; $\delta^{15}N$ = −2.35 and an in-house laboratory standard (fish gelatin, $\delta^{13}C$ = −15.7 and $\delta^{15}N$ = 13.9). Analytical error was studied through the repeated measurement of the in-house fish gelatin standard (n=20, ± 0.10‰ for $\delta^{13}C$ and ±0.10‰ for $\delta^{15}N$).

3–3.5.5 mg of the pre-treated enamel powder was weighted in duplicates in glass vials, and $\delta^{13}C/\delta^{18}O$ values were measured using a Thermo Gas Bench 2 connected to a Thermo Delta V Advantage Mass Spectrometer. The resulting $\delta^{13}C$ and $\delta^{18}O$ values were calibrated (two-point calibration) using International Standards (IAEA NBS 18: $\delta^{13}C$ = −5.0±0.03‰, $\delta^{18}O$ = −23.2±0.10‰; IAEA 603: $\delta^{13}C$=+2.5±0.01‰, $\delta^{18}O$ = −2.4±0.04‰, IAEA CO8: $\delta^{13}C$ = −5.8±0.03‰) and international carbonate standard (USGS44: $\delta^{13}C$ = −42.1). Analytical error was studied through the repeated measurement of an in-house equid carbonate standard (n=24, ± 0.10‰ for $\delta^{13}C$, ± 0.20‰ for $\delta^{18}O$). This work only includes the results for $\delta^{13}C_{carbonate}$, while the $\delta^{18}O$ will be presented in a different study focusing on mobility.

The overall preservation of the collagen was assessed using several criteria based on the results from modern bones. The content of nitrogen (%N) was found to be between 5.5% and 17.3%, while carbon (%C) was between 15.3% and 47% (Ambrose 1990). The atomic ratio between carbon and nitrogen (C: N) was found to be acceptable, between 2.9 and 3.6. Finally, the collagen yield was used only for the bone samples, and it represents the amount of collagen extracted in relation to the sample's initial weight, with acceptable values above 1% (DeNiro 1985; Van Klinken 1999). Only results that met the criteria described above were included in the present analysis, while failed and excluded results are shown in Supplement 4.

Statistical analysis

Differences in the $\delta^{13}C$ and $\delta^{15}N$ results from bone, dentine, and enamel between males and females were tested using the Mann-Whitney or T test, depending on the results of the Shapiro-Wilk normality test (Shapiro-Wilk: < 0.05=Mann-Whitney, > 0.05=T-test). The $\delta^{13}C$ and $\delta^{15}N$ for bulk bone and dentine were also examined in pairs for the same individuals to test juvenile/adult differences using the Wilcoxon test. The effect size r was calculated for Mann-Whitney and Wilcoxon tests using the following formula $r=Z/\sqrt{N}$, and interpreted as small (<0.1), medium (~0.3), and strong (>0.5) effect. Instead, the Cohen's D was used to estimate the effect size of the T test, and it was interpreted as small (~0.2), medium (~0.5), and strong (~0.8). The software used for all comparisons was PAST version 4.13 (Hammer et al. 2001), and the significance level was set at α=0.05.

Results

Bulk analysis

All results are given in Supplement 1, while the statistical comparisons are given in Supplement 5. In total, 50 (out of 51) bone collagen samples successfully met the requirements of %N, %C, C:N, s.d and collagen yield. This also includes the results from the sampled sheep bone. The collagen yield was between 1.22% and 14.88%. 38 (out of 40) dentine collagen samples met the requirements of %N, %C, C:N ratio, and s.d. All 50 enamel bioapatite samples met the requirements of s.d (Table 2).

The average $\delta^{13}C_{collagen}$ value in humans from Menainon is between 0.1–0.3‰ higher than the local sheep and 1.7–1.9‰ higher than the average value from the Himera's fauna, whereas $\delta^{15}N$ values are between 2.3–3.3‰ and 1–2.1.1‰ higher than the local sheep and the reference from Himera, respectively. Figure 2 shows the results of $\delta^{13}C_{collagen}$ and $\delta^{15}N$ for male and female individuals for the bulk bone collagen samples. On average, male individuals show slightly higher values of $\delta^{13}C_{collagen}$ and $\delta^{15}N$ compared to females ($\delta^{15}N$ m. 9.61‰, f. 9.06‰; $\delta^{13}C_{collagen}$ m. −18.27‰, f. −18.36‰), but the difference was not found to be statistically significant, and the effect size was small in both comparisons (r=0.193, r=0.123, respectively). The results from the bulk dentine collagen between males and females are shown in Fig. 3. Average values of $\delta^{13}C_{collagen}$ and $\delta^{15}N$ are higher in males; however, the difference is again small and is not statistically significant ($\delta^{15}N$ m. 10.2‰, f. 9.8‰; $\delta^{13}C_{collagen}$ m. −18.14‰, f. −18.42‰). The effect size is small for both comparisons (r=0.074, r=0.104).

Also, individuals that had results from both dentine and bone samples, were compared with paired test showing significant difference only for $\delta^{15}N$ (N=37, w=551, z=3.01, p-value=0.003), with a strong effect size (r=0.495). Figure 4 shows the distribution of the results for $\delta^{13}C_{carbonate}$ between females and males (left) as well as the results $\delta^{13}C_{collagen}$ and $\delta^{13}C_{carbonate}$ (right). Overall females and males show very little difference with average values of $\delta^{13}C_{carbonate}$ −11‰ and −11.3‰ respectively. When compared statistically, the result was not significant and the effect size was small (r=0.147). The

Table 2 Stable carbon and nitrogen results from bone/dentine collagen and enamel bioapatite

Individual	Bone				Dentine				Enamel	
	$\delta^{15}N$ (‰ - AIR)	s.d.	$\delta^{13}C_{collagen}$ (‰ - VPDB)	s.d.	$\delta^{15}N$ (‰ - AIR)	s.d.	$\delta^{13}C_{collagen}$ (‰ - VPDB)	s.d.	$\delta^{13}C_{carbonate}$ (‰ - VPDB)	s.d.
Female										
S1	9.1	0.0	−18.5	0.0	11.3	0.1	−18.7	0.0	−10.3	0.2
B2	9.0	0.0	−18.5	0.1					−11.6	0.2
43	8.5	0.0	−18.6	0.1	9.2	0.1	−18.8	0.1	−11.8	0.2
A1	8.8	0.1	−18.1	0.0	9.1	0.1	−18.7	0.0	−9.1	0.2
N3	9.7	0.1	−17.4	0.0	9.1	0.0	−18.3	0.0	−10.6	0.2
58	10.1	0.0	−18.8	0.0					−11.8	0.2
Z	8.9	0.2	−18.6	0.0	9.6	0.1	−18.3	0.3	−12.1	0.2
D1	9.6	0.0	−18.3	0.3	11.0	0.0	−17.9	0.0	−10.0	0.2
M3	7.4	0.1	−19.2	0.2	8.3	0.1	−18.8	0.0	−11.5	0.2
117	8.2	0.0	−18.3	0.1	9.1	0.1	−18.2	0.2	−11.0	0.2
53	9.2	0.1	−17.7	0.0	8.4	0.0	−18.8	0.1	−11.3	0.2
Z2	9.5	0.1	−18.6	0.2	9.6	0.1	−18.6	0.0	−11.3	0.2
125					9.5	0.0	−18.4	0.3	−8.4	0.1
Q1	9.5	0.3	−18.0	0.1	8.0	0.1	−18.7	0.0	−10.8	0.3
109	9.5	0.0	−17.6	0.1					−10.2	0.2
H1	9.9	0.1	−18.0	0.0	10.2	0.0	−18.3	0.3	−11.5	0.2
113	8.8	0.0	−17.9	0.0	9.3	0.0	−18.5	0.1	−9.6	0.2
O3	8.5	0.0	−18.0	0.1	9.4	0.0	−18.4	0.1	−11.7	0.2
R3	9.1	0.0	−18.6	0.1	9.6	0.0	−18.1	0.1	−12.0	0.1
41	8.3	0.1	−18.5	0.1					−12.2	0.1
S2	9.8	0.0	−18.4	0.2	10.1	0.0	−18.7	0.0	−9.6	0.1
71	5.4	0.1	−18.7	0.0	14.3	0.1	−17.8	0.0	−11.7	0.1
36	12.6	0.1	−19.2	0.0					−11.7	0.2
90	9.1	0.1	−18.4	0.0	12.3	0.1	−18.0	0.1	−11.2	0.1
E2	8.9	0.0	−18.6	0.0	9.0	0.1	−18.4	0.0	−11.9	0.2
Average	9.1	0.1	−18.4	0.1	9.8	0.1	−18.4	0.1	−11.0	0.2
Male										
N2	10.1	0.0	−18.3	0.3					−9.7	0.1
14	9.7	0.1	−18.0	0.1	8.4	0.0	−19.2	0.1	−12.1	0.2
8	12.3	0.1	−17.5	0.0	12.8	0.0	−18.2	0.3	−13.5	0.2
G1	9.7	0.0	−18.1	0.1					−11.3	0.2
G1?	9.3	0.0	−18.5	0.1	10.5	0.0	−18.4	0.3	−13.6	0.1
P1	9.7	0.1	−18.6	0.1					−11.6	0.2
80	8.8	0.0	−18.5	0.2					−11.0	0.2
32	8.6	0.0	−18.3	0.2	8.8	0.0	−18.5	0.0	−11.5	0.1
45	11.9	0.1	−17.2	0.0	13.9	0.1	−17.0	0.0	−10.8	0.2
59	9.3	0.0	−18.2	0.1	10.5*	0.0	−17.8*	0.0	−11.9*	0.2
18	9.3	0.0	−18.5	0.0	9.2	0.0	−18.9	0.1	−11.4	0.2
30	12.5	0.0	−18.5	0.0	15.3	0.0	−17.8	0.0	−12.6	0.2
L3	9.8	0.1	−18.4	0.3	10.6	0.1	−18.4	0.0	−11.5	0.2
119	8.9	0.0	−18.0	0.0	9.3	0.1	−18.0	0.3	−9.8	0.2
B3	10.1	0.1	−18.3	0.1	10.8	0.0	−15.1	0.0	−6.7	0.2
P3	9.1	0.0	−17.9	0.1	8.3	0.0	−18.8	0.0	−10.4	0.2
123	8.1	0.1	−18.4	0.1					−12.0	0.2
27	10.0	0.0	−18.6	0.0					−11.1	0.2
G3	9.0	0.0	−18.6	0.3	7.9	0.2	−18.1	0.1	−10.6	0.2
Q3	9.1	0.0	−18.5	0.2	9.5*	0.1	−18.7*	0.0	−11.2*	0.2
T3	9.9	0.0	−18.2	0.0	10.2	0.0	−18.2	0.0	−11.1	0.2
E3	9.1	0.2	−18.5	0.1	9.3	0.0	−18.9	0.0	−11.5	0.2
44	8.8	0.2	−18.1	0.0					−12.7	0.1
F3	8.6	0.1	−18.1	0.1	8.5	0.0	−18.3	0.1	−11.9	0.2
82	8.8	0.0	−18.9	0.1	9.7	0.2	−18.2	0.0	−11.9	0.2
Average	9.6	0.1	−18.3	0.1	10.2	0.1	−18.2	0.1	−11.3	0.2
Fauna										
Sheep	6.8	0.1	−18.5	0						

*Results from third molar

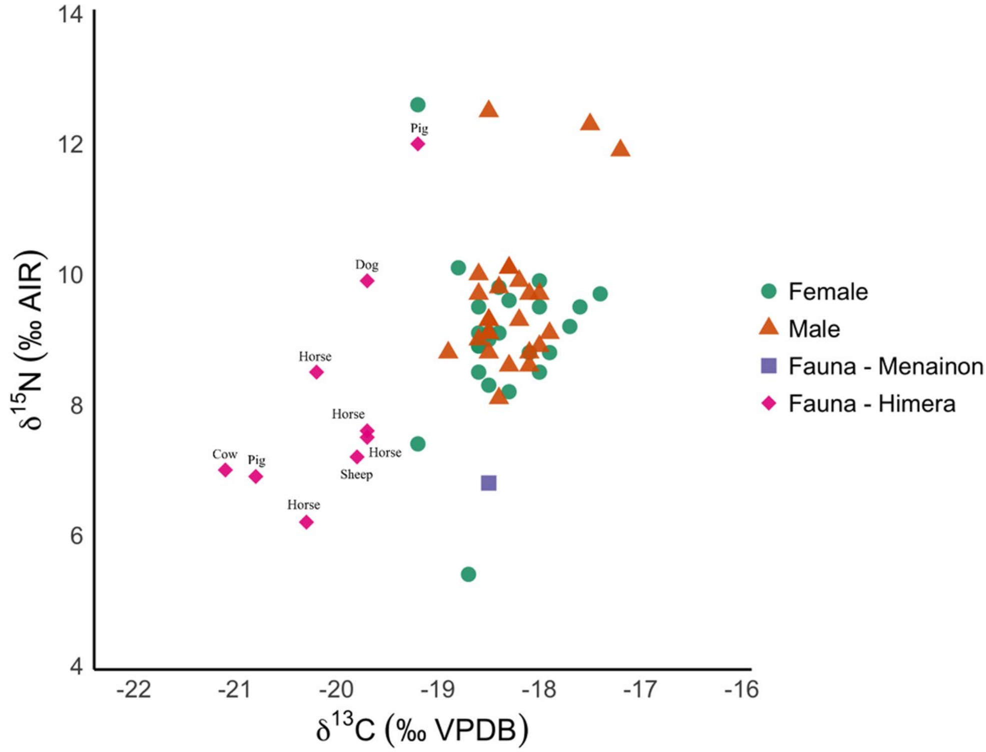


Fig. 2 $\delta^{13}C_{collagen}$ and $\delta^{15}N$ results for bone collagen between male and female individuals, one sheep as well as the fauna from Himera

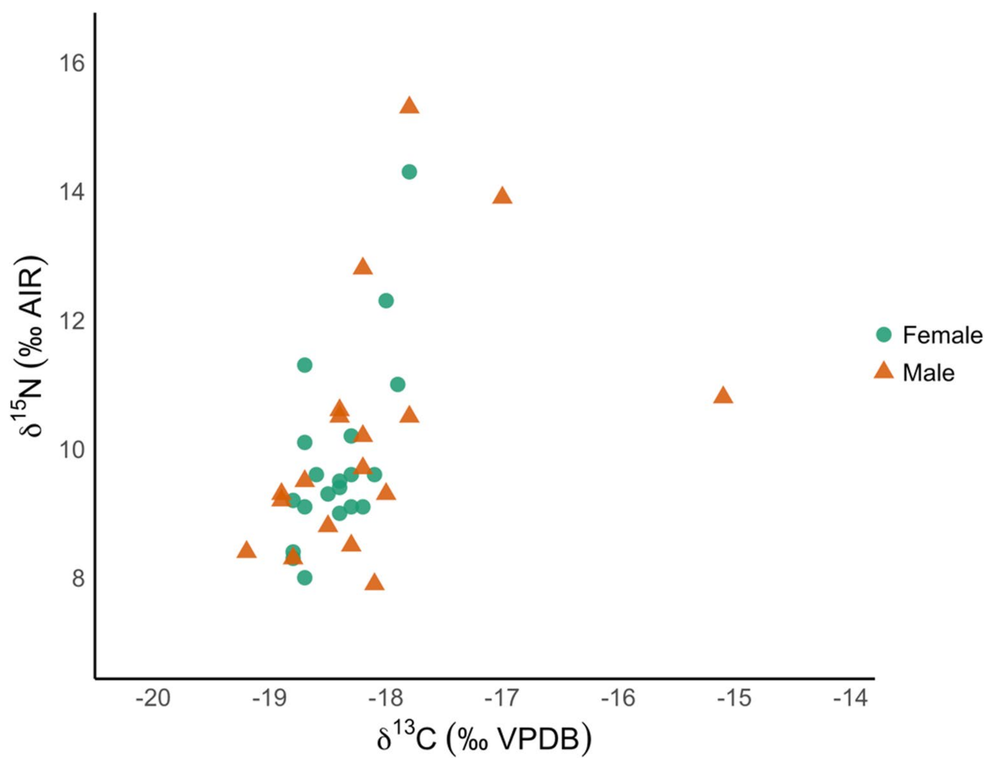


Fig. 3 $\delta^{13}C_{collagen}$ and $\delta^{15}N$ results for bulk dentine collagen between males and females

results for bone collagen between individuals of different adult age groups are shown in Fig. 5. Average values of $\delta^{15}N$ (YA = 9.58‰, MA = 8.95‰, OA = 9.53‰) are similar between younger and older adult groups, while they are lower in middle adult individuals. However, the difference was not statistically significant, and the effect size for the different comparisons was always small (Cohen's D = < 0.1). Meanwhile, average values of $\delta^{13}C_{collagen}$ were similar for all different age groups (YA = −18.34, MA = −18.31, OA = −18.31).

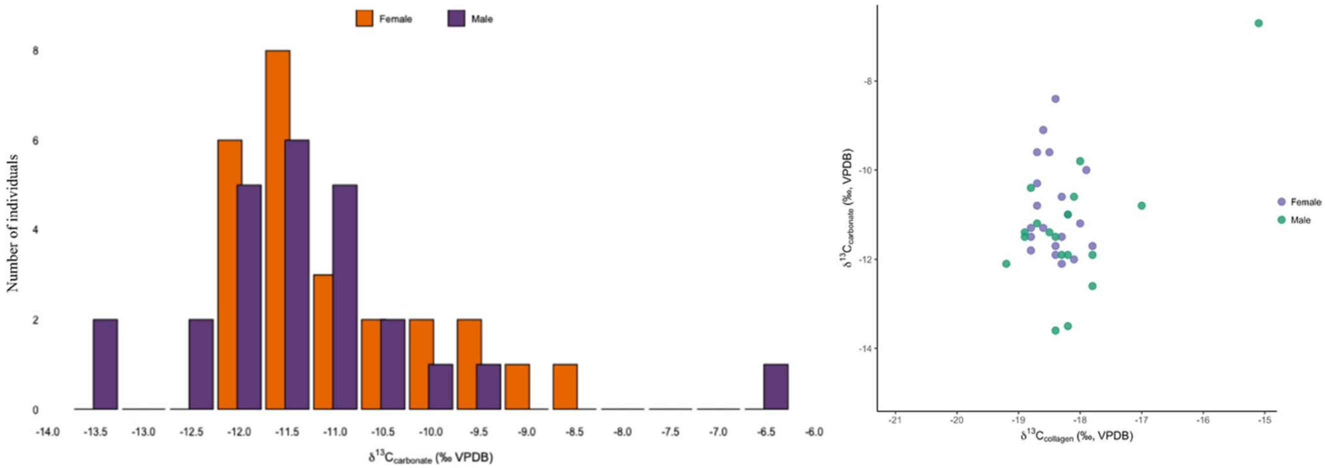
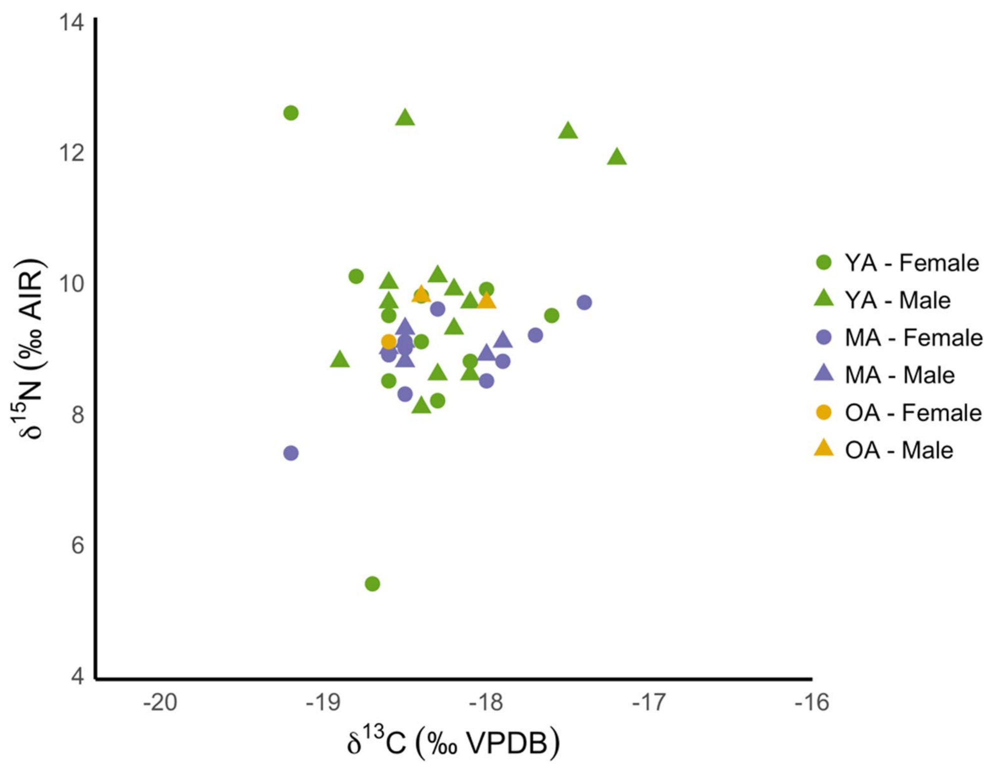


Fig. 4 Results of $\delta^{13}C_{carbonate}$ between Females and Males (left), and results of $\delta^{13}C_{collagen}$ and $\delta^{13}C_{carbonate}$ (right)

Fig. 5 $\delta^{13}C$ $_{collagen}$ and $\delta^{15}N$ results for bone collagen between sexes and adult age groups



Additional comparisons

The results of $\delta^{13}C_{collagen}$ and $\delta^{15}N$ for individuals with and without carious lesions and dental calculus are shown in Fig. 6. Overall, the average value of $\delta^{15}N$ is lower (p. 9.51‰ a. 9.07‰) for individuals who did not exhibit any carious lesions, whereas the values for $\delta^{13}C$ are comparable in the two groups (p. −18.31‰ a. −18.33‰). No difference reached statistical significance. Similar results were obtained for the individuals with and without dental calculus, where both $\delta^{15}N$ (p. 9.41‰ a. 9.05‰) and $\delta^{13}C_{collagen}$ (p. −18.29‰ a. −18.43‰) showed no significant difference and a small effect size in both cases. Figure 7 shows the results of $\delta^{13}C_{collagen}$ and $\delta^{15}N$ between individuals with present/absent physiological stress markers and short stature. The results for $\delta^{15}N$ do not show any significant difference between the two groups, with mean values higher for the group without physiological stress markers (p. 9.53‰ a. 10.8‰), and a small effect size. The mean values for $\delta^{13}C_{collagen}$ were similar, but the two groups were found to be statistically different ($U=96.5$, $Z=2.1489$, p-value$=0.032$, $r=0.349$). Comparable values were found when analysing

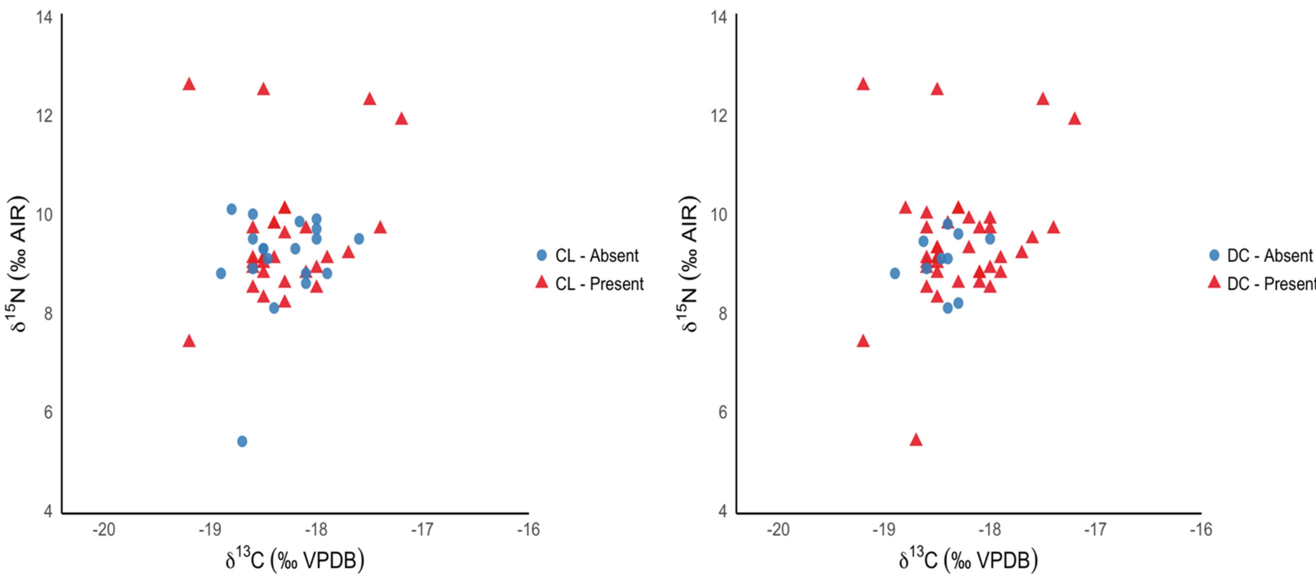


Fig. 6 $\delta^{13}C_{collagen}$ and $\delta^{15}N$ results for bone collagen between individuals with/without carious lesions (CL) (left), and with/without dental calculus (DC) (right)

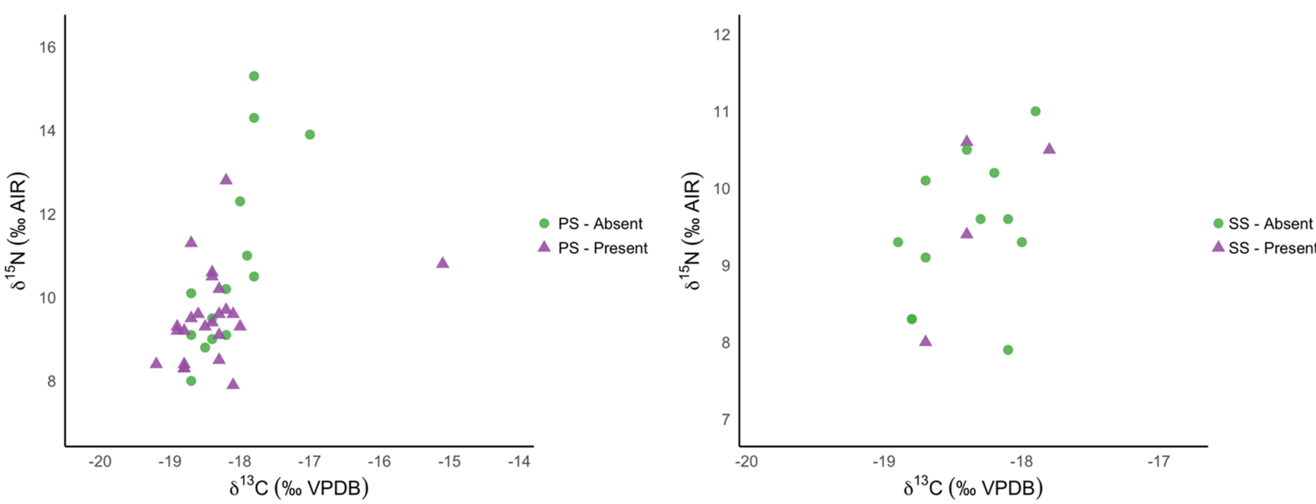


Fig. 7 $\delta^{13}C_{collagen}$ and $\delta^{15}N$ results for bulk dentine collagen between individuals with/without markers of childhood physiological stress (PS) (left), and with and without the short stature (SS) (right)

individuals with/without short stature for both $\delta^{15}N$ (p. 9.61‰ a. 9.44‰) and $\delta^{13}C$ (p. −18.33‰ a. −18.40‰), and the comparisons never reached statistical significance.

Incremental dentine analysis

Some of the dentine increments, at least one per individual, had to be excluded or produced no results (e.g., no collagen, high s.d., single measurement). Overall, a minimum of 47% (8/17) and a maximum of 94% (16/17) of the total number of increments per individual were employed in this analysis. Bayesian modelled $\delta^{13}C_{collagen}$ and $\delta^{15}N$ estimates for the second molars are shown in Fig. 8 for female and male individuals (left and right, respectively). The results for the third molars of two male individuals are shown in Fig. 9. In case of missing measurements for a given age interval, the point shown on the plot represents the median value between the next available measurements. This is required to run the Bayesian estimation, and it is also marked on the plot by a greater range in the 95% credibility intervals.

During early childhood, considerable changes in $\delta^{15}N$ can be seen for the female individual 36 and the male G1. By contrast, the other females and the males, P1 and N2, show little to no variation. Males 80 and 44 show a small increase in the value of $\delta^{15}N$. Little to no variation is shown for the values of $\delta^{13}C_{collagen}$ in all individuals. During late childhood, little to no variation is overall shown for $\delta^{15}N$, with the exception of female 41, showing an increased value, and males G1 and 44, showing an increase and decrease, respectively. The values of $\delta^{13}C_{collagen}$ increased for the female individuals 109 and 41, but decreased for male 44. Finally, in the early adolescence period, the value of $\delta^{15}N$ rose for the females 58, 41, B2, and dropped for the males G1 and 80. In contrast, values of $\delta^{13}C_{collagen}$ dropped for the females 58 and 109, whereas little variation was shown in all male individuals. The results from the two third molars showed little variation during the adolescence period for individual Q3 in both $\delta^{13}C_{collagen}$ and $\delta^{15}N$. In contrast, individual 59 showed a small decrease in $\delta^{15}N$ over time and a spike in $\delta^{13}C$ between late childhood and adolescence. In both individuals, little variation was shown at the beginning of adulthood.

Discussion

Overall, our results contradict expectations based on the historical literature, suggesting far more homogeneous diets across society than often described for the Hellenistic/Roman period. $\delta^{13}C$ values support an overall reliance on terrestrial C_3 resources, with some minimal input of C_4 cereals and/or marine resources. This pattern is consistent for both adulthood and childhood, with the sole exception of a male individual during early life. Despite the current lack of evidence in support for their consumption in Sicily during the Hellenistic and early-Roman periods (e.g., Stika et al. 2008; Reitsema et al. 2020), C_4 plants, such as millets, were particularly suitable for cultivation in this region, and could have been used in emergency situations or in periods of food shortage due to their high resilience, fast growing, low water effort and high yields properties (Weber and Fuller 2008). In addition, millet could have been consumed indirectly since it was commonly used as fodder for animals (e.g., fowl), raised for their meat or byproducts (e.g., eggs, milk) (Heinrich et al. 2021). This is further supported for Menainon by the results from $\delta^{13}C_{carbonate}$ showing some variation in distribution across the sample under examination. However, this result should be taken with caution as the reference was not developed for the Mediterranean region. Additionally, the results from the local sheep, seems suggesting a mixed C3-C4 foddering or consumption of wild C4 plants.

$\delta^{15}N$ values indicate diets reliant on plant and animal resources, with a few exceptions for both bulk dentine and bone tissues. However, the results from dentine samples could be potentially affected by breastfeeding and weaning practices. In addition, the introduction of marine fish and/or freshwater fish could be responsible for the variation in $\delta^{13}C_{collagen}$ and $\delta^{15}N$ (Hedges and Reynard 2007). A few individuals show higher values of both $\delta^{13}C_{collagen}$ and $\delta^{15}N$, suggesting a potential addition to their diet from marine resources (e.g. fish). Menainon had no direct access to the coast, and some studies from Syracuse showed limited fish consumption during the first millennium BCE (Tanasi et al. 2017; Tanasi and Greco 2023). Nevertheless, the recovery of transport amphorae suggests Menainon was part of a trading network potentially importing marine resources while exporting agricultural products. Changes in $\delta^{15}N$ values could also be due to the use of animal fertilizer in cereal farming, which would raise $\delta^{15}N$ values at the base of the food chain (Szpak 2014). Animal manuring was highly considered by the Romans (Semple 1928; Corti et al. 2013), and it was most likely employed by Sicilian farmers (Pritchard 1972).

Bulk analysis

Our data from bulk tissues do not support a differential access to dietary resources between females and males. Instead, they show little variation within the sample, with just a few exceptions. The results for $\delta^{13}C$ showed minimal to no variation between early life and the different adult age groups, supporting equal access to resources throughout life, in agreement with the claimed scale of agricultural production (Serrati 2000a, b).

Some differences can be found for $\delta^{15}N$, which showed greater variability during early life and young adulthood, whereas MA and OA show a smaller range of values. Moreover, $\delta^{15}N$ values progressively decrease between early life and YA, and between YA and MA. Besides diet, certain pathological conditions and physiological stress have been shown to impact isotopic values (Reitsema and Holder 2018). Specifically, changes in $\delta^{13}C$ and $\delta^{15}N$ were observed in cases of nutritional stress and food deprivation (DeWitte et al. 2025). For example, an increase in $\delta^{15}N$ values was found to be correlated with very low Body Mass Index (BMI), while $\delta^{13}C$ values were found to be correlated with a general lack of calories from food intake (Neuberger et al. 2013). Similarly, D'Ortenzio et al. (2015) found alterations in $\delta^{15}N$ values linked to different physiological stressors, such as infections, injuries, or even pregnancy, while values of $\delta^{13}C$ were less affected. In the present study, no pattern was observed in relation to childhood physiological stress. For example, in samples examined for dentine increments, 3 females and 4 males exhibited lesions of cribra orbitalia and porotic hyperostosis while showing very different isotopic signatures over time. However, our isotopic results might not reflect the exact age interval of interest when the lesions formed, since enamel hypoplasia occurred before the period under study, and O'Donnell et al. (2023) found that cribra orbitalia develop between 6 months and 6 years old for females, and

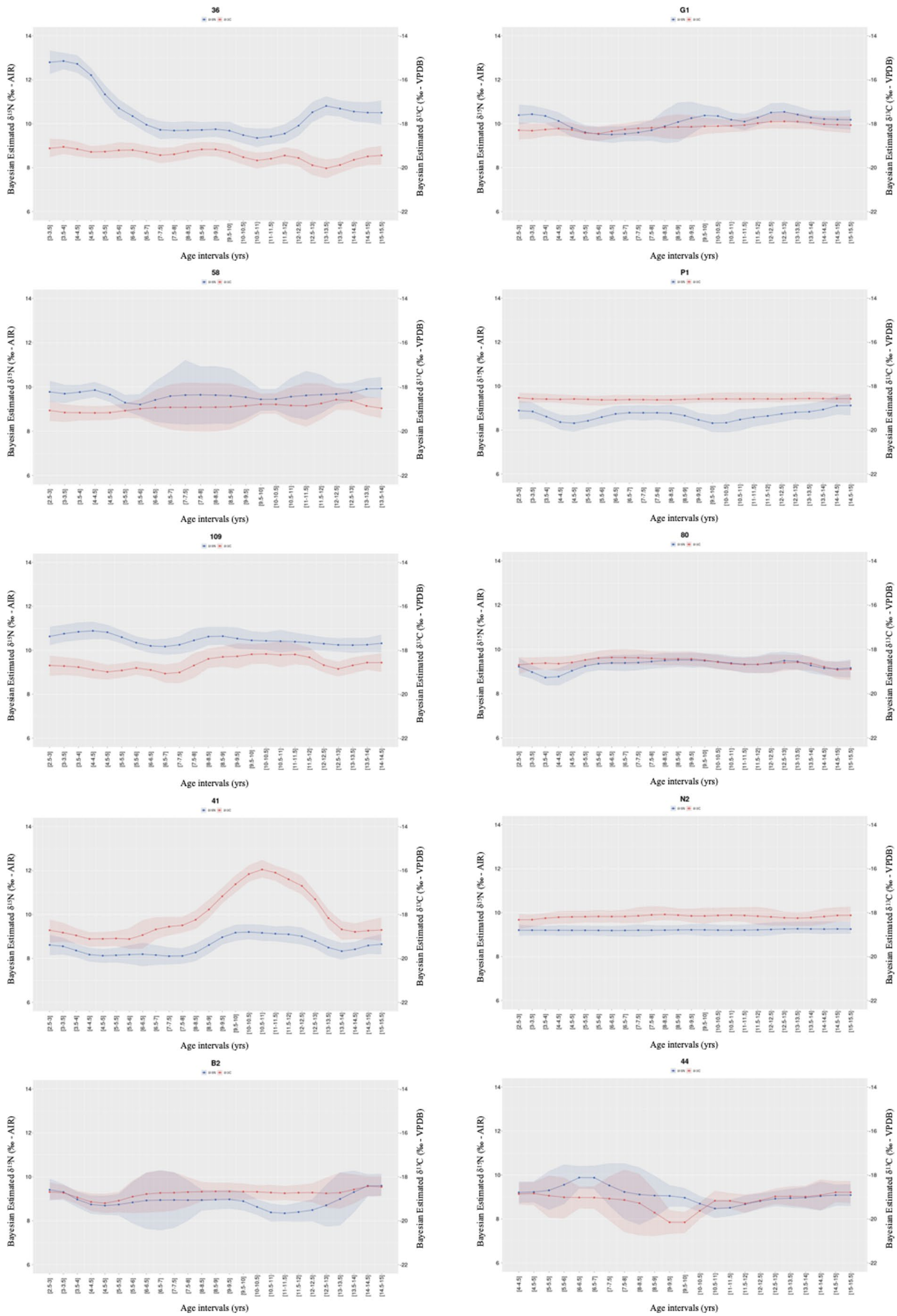

36
G1
58
P1
109
80
41
N2
B2
44
Bayesian Estimated δ15N (‰ - AIR)
Bayesian Estimated δ13C (‰ - VPDB)
Age intervals (yrs)

Fig. 8 Bayesian temporal modeling of M2 incremental dentine $\delta^{13}C_{collagen}$ and $\delta^{15}N$ between female (left) and male (right) individuals. N.B. The greater range of C.I. is the result of imputed/missing values

8 years old for males. Nevertheless, individuals exposed to various stressors (e.g., malnutrition) during childhood were more likely to have experienced hardship during their entire early life rather than during specific isolated events only.

A previous study, examining dental diseases and dental wear in Menainon, found some differences between sexes in certain age groups, but with no clear pattern nor direction (Caruso and Nikita 2024a, b, c). This is not uncommon. Moles et al. (2022) and Moles (2023), reported different results between observations of dental diseases and stable isotopes for the site of Knossos. However, it is acknowledged that pathological lesions have a multifactorial and complex etiology, and that they are not the exclusive result of diet (Marklein et al. 2019). This is clearly shown on the present study, where the results for the individual with carious lesions contradicted the expected pattern. The results of the present analysis are unlikely to have been biased by sample selection. The sample included in this study reflected individuals buried in different tomb types (e.g., pit, rock-cut), burial positions (e.g., supine, prone), as well as with diverse grave goods. In fact, some of the individuals were found with no grave goods, while others were recovered with a variety of objects in different combinations (e.g., pottery, metal objects, personal items). One of the individuals was also found with a gold coin inside his mouth. The sample also included individuals with and without dental disease and childhood physiological stress markers. Finally, the demographic composition of the sample included a balanced number of individuals per sex and age group. Given these premises, the lack of differences in our results, when examining the bulk tissues, is even more striking, as it seems that no aspect related to social status might have affected access to dietary sources.

Incremental dentine analysis

A general lack of sex-based differences is also supported by the results of the incremental dentine analysis, which explored, with a greater resolution, dietary changes throughout childhood and adolescence. While differences are minimal, however, the direction of the subtle differences is highly variable for different individuals, with values of $\delta^{15}N$, for example, spiking during early childhood, or later childhood, or simply remaining constant during the whole early life phase. The interpretation of these patterns is difficult, with many potential factors affecting the changes. For instance, the higher values of $\delta^{15}N$ in early childhood might have been caused by prolonged breastfeeding/weaning. Anastasios et al. (2025) found that Clazomenian children, living in the Greek colony of Abdera, completed their weaning at the age of ~ 6 years old. Some other evidence for prolonged weaning can be found in Bourbou et al. (2013), for the Byzantine period in Greece, where weaning was completed by the age of 3 or 4 years old at the latest. However, the results from those studies might not be as reliable, since they employed bone tissue instead of dentine increments. In fact, in most other instances, weaning was completed much earlier by the age of 2 years old (e.g., Killgrove and Tykot 2013; Ganiatsou et al. 2022; Cocozza et al. 2025) in agreement with ancient historical sources (Fulminante 2015; Baker 2017). Instead, the results for $\delta^{13}C$ show less variability except for 3 individuals, where the values spiked in males, and dropped for the female. Of particular interest is the change showed by individual 41 at around puberty, where the simultaneous changes in $\delta^{13}C_{collagen}$ and $\delta^{15}N$ could be due to the addition of marine resources. A similar pattern, was observed in some individuals at around the age of 7 in Roman Bainesse, with the authors suggesting the addition of freshwater resources linked to a change in social status (Cocozza et al. 2021).

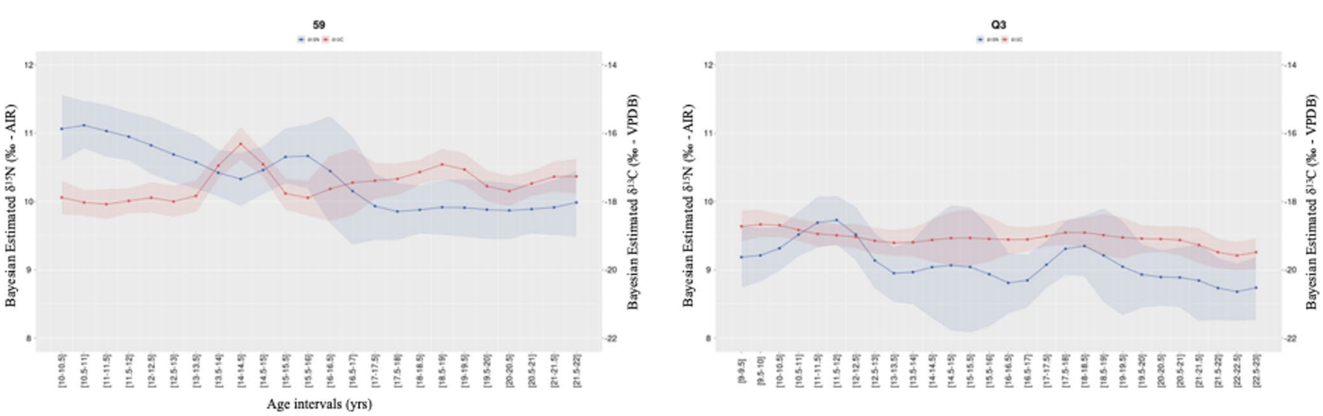


Fig. 9 Bayesian temporal modeling of M3 incremental dentine $\delta^{13}C_{collagen}$ and $\delta^{15}N$ for two male individuals. N.B. The greater range of C.I. is the result of imputed/missing values

Final remarks

Taken together, the results of the present study suggest the existence of a more equitable community compared to the one described in historical sources, with similar dietary patterns, where neither biological sex nor age appears to have played an important role in the access to food. However, we are unable to comment on different aspects linked to the quantity or the quality (e.g. cut) of accessed food as potential source of inequalities. Nevertheless, it is possible that the people of this community were similarly involved in farming activities, given equal access to resources. Similar conclusions were also drawn by the results of previous multi-proxy analyses investigating mechanical stress (Caruso and Nikita 2024b), physiological stress (Caruso and Nikita 2024c), as well as biological kinship (Caruso and Nikita 2025) at the site of Menainon. Nevertheless, the distinctive patterns emerging between the bulk and dentine incremental analyses stress the need for further investigation. More systematic efforts should also be made to collect more evidence on the specific type of food consumed in this community (e.g., microscopic analysis of dental calculus) and wider archaeobotanical and zooarchaeological research in the region. Overall, the lack of chronological data per tomb, and the isotopic baseline are the major limitations of the present study. The baseline from the site of Himera used as a reference in this study, might not accurately reflect the fauna from Menainon due to differences in the environment (e.g. coastal, inland) or subsistence practices. Specifically, Himera and Menainon might have adopted different agricultural/herding strategies and/or differential access to aquatic resources, resulting in different values of the baseline. The result from the sheep seems pointing to this direction but further studies are needed. Additionally, the sample under examination spans over four centuries, influenced by major historical events and shifts in power (Serrati 2000a, b). Moreover, we are unable to exclude any potential changes to the baseline, that might have occurred during the examined chronological period that could have affected the results of the present study (Solomon et al. 2008; Possamai et al. 2021). Finally, given the multicultural background of the island during this period, where Greeks, Romans, Italians, Sicels, Sikans, Elymians, and Phoenicians interacted dynamically with each other, mobility might have played an important role, and thus it should be explored employing a combination of strontium and oxygen isotope analysis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12520-026-02506-5.

Acknowledgements We wish to thank the Soprintendenza BB. CC. AA. of Catania without whom this study would not have been possible, and specifically Rosalba Panvini, Laura Maniscalco, and Michela Ursino for issuing the study permit and for granting us access to this material. We also thank Dr. Angelos Hadjikoumis for helping in the identification of the animal bone from Menainon. The work presented in this paper was performed under the Erasmus+ Traineeship scheme funded by the European Union and it was co-founded by the Max Planck Society and the MetaMobility project. Metamobility is implemented under the programme of social cohesion "THALIA 2021-2027" co-funded by the European Union, through the Research and Innovation Foundation [EXCELLENCE/0421/0376]. EN's contribution was supported by the Dan David Prize.

Author contributions Conceptualization: AC, EN, PR; Methodology: AC, SH, ES, CC, JI; Data curation: AC, SH, ES, JI; Investigation: AC, CC, EN, PR; Writing original draft: AC; Writing - review and editing: All authors; Funding acqusition: AC, EN, PR; Project administration: AC, EN, PR; Supervision: JI, EN; PR.

Funding The work presented in this paper was performed under the Erasmus+ Traineeship scheme funded by the European Union and it was co-funded by the Max Planck Society and the MetaMobility project. Metamobility is implemented under the programme of social cohesion "THALIA 2021–2027" co-funded by the European Union, through the Research and Innovation Foundation [EXCELLENCE/0421/0376].

Data availability All data are given in the manuscript tables, and raw data are given as supplementary material.

Declarations

Competing interests The authors declare no competing interests.

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