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Dietary habits of Byzantine Kovuklukaya (Sinop, Türkiye): an isolated society or ostracized people?

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Introduction: During the medieval period, cultural attitudes toward leprosy often involved the exclusion of infected people from general society. People suffering from leprosy were often moved to live in separate places such as a specific district, village, or hospital. Such actions are social implications of how the disease and its sufferers were viewed and dealt with. This study aims to investigate whether Kovuklukaya was inhabited by segregated lepers.

Methods: This was conducted by examining diet of individuals using stable isotope analysis of bulk bone collagen and compound-specific amino acids, and integrating these results with osteoarchaeological, pathological, and ethnographic data.

Results: The stable isotope values reveal a terrestrial C₃ diet with little inclusion of C₄ plants. The probable source of the animal protein would appear to be sheep and/or goat. Although there is a broad range of available and potentially exploited and consumed food resources in the Black Sea region, the narrow range of the isotopic values would suggest a similarity in the availability or choice of food resources at the different sites in the region.

Discussion: A wide diversity in consumed food resources of ostracized people from different dwellings may be expected. However, the isotopic values suggest that the people of Kovuklukaya consumed a narrow range of food resources. This similarity may arise from the mountainous environment which may restrict the range of available food resources, but is more probably due to common dietary habits such as the regular consumption of the same kinds of foods, eating from the same pots, etc., in a closely related group, kin, or family. The homogeneous characteristic of the dietary habits at Kovuklukaya supports the premise that Kovuklukaya was a small mountain village or hamlet rather than a leper colony.

KEYWORDS

bone collagen, amino acids, stable isotopes, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, leprosy, mobility

1 Introduction

Human mobility can include the temporary or permanent movement and migration of people for ecological, economic and social reasons (De Haas et al., 2019; Manning and Trimmer, 2020). Throughout history, human mobility, migration, and relocation have occurred in various forms and for diverse reasons, including escaping famine or war and seeking better living conditions (Manning and Trimmer, 2020; De Haas et al., 2019). It is also one of the central focuses of historical and bioarchaeological research

(among the others, Killgrove, 2010; Gregoricka, 2021; Eckardt and Barta, 2010). It is known that during the Roman and Byzantine Empires, large-scale forced migrations were commonly conducted by governing authorities to achieve political, economic, or military objectives, which, in turn, reshaped the demographics and settlement patterns across vast regions (Killgrove, 2010; Gregoricka, 2021; Eckardt and Barta, 2010). Human mobility and migration to seek out better living conditions can be a deliberate action performed with intention. However, in some cases, human mobility may be the result of forceful deposition, either directly or indirectly as a consequence of particular actions or circumstances (Killgrove and Montgomery, 2016; Turton, 2003; Patterson, 2018). Direct and forced movement of individuals or groups of people can be the result of the direct orders and actions of an authority. Indirect involuntary movement of individuals or groups of people may also be in response to social pressure. An example of such movements is observed in the treatment of people with leprosy (Fernández and Ribón, 2018). These, often stigmatized, individuals are often forcibly relocated, or feel obliged to relocate themselves in response to social ostracization, to areas outside of the main areas of settlements, sometimes in specially designated areas or institutions such as leper colonies and hospitals (Manchester, 1984; Miller and Nesbitt, 2014).

Leprosy is an infectious disease resulting in particular lesions of the peripheral nervous system, skeleton and skin surface as a result of chronic infection of *Mycobacterium leprae* and *Mycobacterium lepromatosis* bacteria (Roberts and Buikstra, 2019; Britton and Lockwood, 2004; Lockwood and Suneetha, 2005; Maymone et al., 2020). Studies on the transmission of leprosy have discovered that individuals in close contact, especially within family and household units have an increased risk of infection and person-person transmission (Bratschi et al., 2015; Moet et al., 2006). After a lengthy incubation period (sometimes years), the bacteria then attack the skin, mouth, eyes, and nervous system. The infection then results in a chancre, ulceration of the skin, that often spreads to the nasopharyngeal area, resulting in a nasopharyngeal defect, the destruction of the intranasal bones, and antemortem loss of anterior teeth. Concentric destruction of tubular bones in infected phalanges of the hands and feet is also observed, and in severe cases almost all fingers and toes can be lost.

Due to the severity of the sequelae of the disease, a stigmatization and exclusion of individuals with this disease from society developed which has been observed and recorded throughout history and it had an impact on societies and social life. Special centers for the care and treatment of leprosy patients were developed in Europe and the Near East in the Middle Ages, such as *leprosarium*, *katagogion*, and *ptochotropheion* (Manchester, 1984; Miller and Nesbitt, 2014). During the Byzantine, Seljuk and Ottoman periods in Anatolia, special settlements were built for lepers (*miskinhane* in Turkish) and they earned a living by begging in the nearby cities (Başer, 1992).

Leprosy has been encountered in the skeletal population of the small mountain settlement of Kovuklukaya (five out of 36 individuals, 14%), and marks one of the most interesting pathological conditions of this community. Analysis of the disease, and its particular strain, in the Kovuklukaya population suggests that it may possibly be the oldest example encountered in Europe

(Rubini et al., 2014; Donoghue et al., 2015a,b; Sevkar, 2023). Considering the remote location of the settlement among the mountains together with a high incidence of leprosy relative to the population size raises questions about what kind of population structure was in place at Kovuklukaya. Was it a community of ostracized people or simply a small and isolated insular population? It is therefore important to understand the structure of the population to determine whether the existence of leprosy in the Kovuklukaya community originates from within itself as a result of it being an isolated mountain village or from the gathering or relocation of individuals with leprosy that were excluded from their original settlement.

One of the best ways to understand this is to obtain data on mobility in, and of, the community, which can be analyzed in different ways. Archaeogenomic analysis of humans and also microbes are one method. aDNA studies in the populations of Anatolia and beyond have demonstrated that there are generally very few genetic outliers in prehistoric populations (Altınışık et al., 2022; Skourtanioti et al., 2020). Furthermore, in homogeneous or similar genepools aDNA analyses do not allow for sufficiently focused examination of individual life histories as can be observed in individual osteobiographies. Isotopic analyses provide the opportunity to address, on a finer scale, the presence of residential changes throughout the life course of an individual.

Strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) isotope analyzes can allow us to examine the mobility of individuals, particularly movement and migration occurring during and after childhood and identifying adult individuals who died and were buried in a different (geological) location to that of their birth and early childhood (Taylor et al., 2020). Another way that can help us to differentiate non-local individuals are stable isotopes of carbon and nitrogen. Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope analyses of human bone collagen offer valuable insights into the dietary practices and environmental contexts of past populations, including those affected by leprosy. Unlike strontium or oxygen isotopes, which are commonly associated with mobility and geographic origin, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ provide information on dietary composition and, indirectly, aspects of mobility. $\delta^{13}\text{C}$ values primarily reflect the consumption of plants following C_3 or C_4 photosynthetic pathways and marine resources, which may also relate to environmental or geographic conditions (DeNiro and Epstein, 1978; DeNiro, 1985). In contrast, $\delta^{15}\text{N}$ values are linked to trophic level, reflecting the relative contribution of animal protein in the diet and potentially indicating differential access to food resources (Schoeninger and DeNiro, 1984; Müldner and Richards, 2005).

Isotopic studies of leprosy populations or individuals with skeletal markers of leprosy from different regions have revealed diverse dietary patterns, suggesting significant variability in access to, and consumption of food resources (Bayliss et al., 2004; Linderholm et al., 2014; Roffey et al., 2017; Brozou et al., 2019, 2021). This heterogeneity contrasts with findings from non-leprosy populations, where individuals buried in the same cemetery/burial location often exhibit more uniform dietary signatures, reflecting shared access to similar food resources within the population (Itahashi et al., 2019, 2021). Dietary differences inferred from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses can therefore contribute to broader discussions of the social and economic status of leprosy individuals,

as well as potential evidence for geographic origin or mobility, particularly when considered alongside other archaeological and bioarchaeological evidence.

In addition to leprosy, the skeletal population from the Early Byzantine Kovuklukaya Cemetery have many unusual pathological conditions that were observed and recorded on their bones and teeth; for example, tumors, non-alimentary abrasion of the teeth, non-metric traits such as *palatine torus*, and activity-related ontogenetic skeletal defects such as *os acromiale* and spondylosis (Erdal, 2004, 2008; Eroğlu and Erdal, 2008, 2004). The studies by Erdal (2004, 2008) and Eroğlu and Erdal (2008, 2004) provide a comprehensive osteoarchaeological perspective on the Early Byzantine Kovuklukaya community. Erdal (2004) focused on the general health and pathologies, revealing evidence of metabolic stress, infectious diseases, and activity-related skeletal changes, which reflect the challenges of a labor-intensive agropastoral lifestyle. Erdal (2008) highlights the occupational use of teeth as tools, identifying non-alimentary wear patterns that suggest craft-related activities and a division of labor within the community. Eroğlu and Erdal (2004) examined the prevalence of *torus palatinus*, linking it to genetic and environmental factors, including dietary habits and masticatory stress. Expanding this analysis, their 2008 study situates Kovuklukaya within a broader Anatolian context, demonstrating how subsistence strategies and dietary shifts influenced oral stress patterns over time. These pathological conditions help us to understand occupational activities, the genetic familial relationships among the individuals, and the population structure of the Kovuklukaya agropastoralists. In addition, the size of the cemetery and the number of people buried there have broadened our understanding of Byzantine life and mortuary practices by providing a comprehensive body of evidence for the osteoarchaeological analysis. By evaluating the values from stable carbon and nitrogen isotope ratio analysis obtained from bulk human bone collagen and compound-specific amino acids together with osteoarchaeological findings, this study aims to determine whether the Kovuklukaya community is merely an isolated mountain village with a close relationship between individuals, or whether it is a settlement founded as a result of the segregation of individuals with leprosy from other settlements and from society as a whole.

2 Isotopes background

Stable isotope analysis of bulk bone collagen, a widely used method in bioarcheology, relies on carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) as isotopic markers to provide direct evidence of dietary protein sources. However, the period of dietary information captured depends on the turnover rate of the specific bone analyzed. For rib samples, which have a relatively higher turnover rate compared to other skeletal elements, the isotopic data likely reflect dietary intake during the last 5–10 years of an individual's life (Richards et al., 2003). The basic principles behind the technique are that the stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) vary between both different food types and different consumers, and accumulate in human bodies with some fractionation due to their trophic levels.

$\delta^{13}\text{C}$ is represented as the stable carbon isotope ratio of the sample to relative to a standard; in the case of carbon this is limestone (belemnite) from the Pee Dee Formation in South Carolina, called PDB and the values are usually presented by per mil and calculated using the following formula.

$$\delta^{13}\text{C}(\text{‰}) = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}} - (^{13}\text{C}/^{12}\text{C})_{\text{PDB}}}{(^{13}\text{C}/^{12}\text{C})_{\text{PDB}}} \right] \times 1,000$$

The measurement of stable carbon isotope ratios from bulk bone collagen can distinguish between plants employing either the C_3 and C_4 photosynthetic pathways that were either consumed directly by a human, or through the consumption of an animal that fed on these plants (DeNiro and Epstein, 1978; DeNiro, 1985). C_3 plants include temperate grasses, all trees and shrubs, all fruits and nuts and cultivated roots and tubers, which constitute the majority of flora of Europe and Anatolia (Richards et al., 2003) and have $\delta^{13}\text{C}$ values ranging from $\sim -35\text{‰}$ to -26‰ (Ambrose, 1986, 1993; DeNiro, 1987; Katzenberg and Waters-Rist, 2018). C_4 plants are plants and grasses, of which sorghum and millet are the most commonly encountered in societies with developed post-Bronze Age agriculture, which have $\delta^{13}\text{C}$ values around -12‰ (Schoeninger and Moore, 1992). Besides, aquatic and marine plants often have higher $\delta^{13}\text{C}$ than terrestrial C_3 plants, because diffusion of CO_2 in water is slower than that in the atmosphere (O'Leary, 1981). $\delta^{13}\text{C}$ values can also be used to investigate the proportion of marine and terrestrial protein sources (Schoeninger and DeNiro, 1984). Furthermore, it is known that there is small isotopic fractionation of around $+0.5\text{‰}$ to 2‰ of $\delta^{13}\text{C}$ between body tissues and the diet with predation and assimilation; i.e., from animal to animal, animal to human (see DeNiro and Epstein, 1978; Spohnheimer et al., 2003).

Similarly, stable isotope ratios of nitrogen, $\delta^{15}\text{N}$, are represented relative to the standard AIR (Ambient Inhalable Reservoir) which is atmospheric N_2 and the values are also presented per mil and calculated using the following formula.

$$\delta^{15}\text{N}(\text{‰}) = \left[\frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}} - (^{15}\text{N}/^{14}\text{N})_{\text{AIR}}}{(^{15}\text{N}/^{14}\text{N})_{\text{AIR}}} \right] \times 1,000$$

Because $\delta^{15}\text{N}$ values of bulk bone collagen of consumers are 3‰ to 5‰ higher than that of their diet, within the same environment the $\delta^{15}\text{N}$ values of each organism vary depending on their trophic levels (Schoeninger and DeNiro, 1984; Müldner and Richards, 2005). Therefore, the $\delta^{15}\text{N}$ values of animal bone collagen is a useful indicator to understand prey–predator relationship for animals and humans (Schoeninger and DeNiro, 1984). Marine and freshwater environments produce higher $\delta^{15}\text{N}$ values than terrestrial ones, with approximate values for consumers of freshwater and marine resources potentially being around $16\text{‰} \pm 2\text{‰}$ and $12\text{‰} \pm 2\text{‰}$, respectively (Budd et al., 2013). Thus, $\delta^{15}\text{N}$ values can be useful for determining the input of marine and freshwater resources (Schoeninger and DeNiro, 1984; Ambrose, 1986; Minagawa and Wada, 1984; Katzenberg and Waters-Rist, 2018). Furthermore, a contribution of legumes into an animal's dietary habits tend to be exhibited as lower $\delta^{15}\text{N}$ values in the bulk bone collagen, due to the fact that legumes, as nitrogen fixers, tend to have naturally lower $\delta^{15}\text{N}$ values than non-leguminous

plants (Keegan, 1989). $\delta^{15}\text{N}$ values can also be used to determine breastfeeding patterns due to the trophic level difference between mother and the breastfed children, with the children exhibiting $\delta^{15}\text{N}$ values about 2‰ to 3‰ higher values than the mother's (Katzenberg et al., 1996; Dupras and Tocheri, 2007).

The $\delta^{15}\text{N}$ of bulk bone collagen for omnivores, such as humans, can be ambiguous when their animal-based food intake is estimated quantitatively, because $\delta^{15}\text{N}$ values vary across primary producers, which are the baseline members of the food web. To quantitatively estimate the consumption of animal-based foods of ancient humans, compound-specific nitrogen isotope analysis of individual amino acids is useful (Naito et al., 2013; Styring et al., 2015; Itahashi et al., 2017, 2019, 2021). The analysis of compound-specific nitrogen isotopes of individual amino acids has been used as a more precise method of estimating animal food intake (animal protein consumption rate). Because this approach is based on a difference in the trophic isotope discrimination of two common amino acids (glutamic acid and phenylalanine), the trophic position (TP) of an organism can be estimated with more precision without knowing the prey's values as a baseline (Ohkouchi et al., 2017). In the transition from prey to consumer, the $\delta^{15}\text{N}$ value of glutamic acid ($\delta^{15}\text{N}_{\text{Glu}}$) increases by $+8.0\text{‰} \pm 1.1\text{‰}$, whereas that of phenylalanine ($\delta^{15}\text{N}_{\text{Phe}}$) increases by only $+0.4\text{‰}$ (Chikaraishi et al., 2010). The difference in the $\delta^{15}\text{N}_{\text{Glu}}$ and $\delta^{15}\text{N}_{\text{Phe}}$ values ($\Delta^{15}\text{N}_{\text{Glu-Phe}}$) of terrestrial vascular plants has been reported to show a consistency in values with less variation (Ramirez et al., 2021), and the $\Delta^{15}\text{N}_{\text{Glu-Phe}}$ value for animals increases with each step up the food chain from primary producers onwards (Tejada et al., 2021). Therefore, the TP and the animal protein consumption rate of an individual can be estimated from $\Delta^{15}\text{N}_{\text{Glu-Phe}}$ values. Several different equations have been proposed to calculate the TPs in terrestrial ecosystems (TP_{ter}) from $\Delta^{15}\text{N}_{\text{Glu-Phe}}$; however, this study uses the equation in Chikaraishi et al. (2010) for terrestrial ecosystems:

$$\text{TP}_{\text{ter}} = [(\Delta^{15}\text{N}_{\text{Glu-Phe}} + 8.4)/7.6] + 1$$

When the TP is estimated with compound-specific nitrogen isotope analysis of individual amino acids, a TP_{ter} of 2 indicates a hypothetical terrestrial herbivorous diet, whereas a TP_{ter} approaching 3 indicates a diet that is more purely carnivorous (i.e., almost exclusively input of animal protein). In previous studies, it was shown that herbivorous livestock such as sheep, goats, and cattle in Neolithic Turkey had a TP_{ter} of 2 (Itahashi et al., 2019, 2021).

Stable carbon and nitrogen isotopic analysis from bulk bone collagen and compound-specific nitrogen isotopes analysis were combined in this study to allow for a better comprehension of dietary patterns and how individuals with leprosy compare to other members of the community, for example, if they are outliers.

3 Materials and methods

3.1 Site

Kovuklukaya is one of the best-studied Byzantine cemeteries in the Black Sea region. Located 300 m northeast of the modern village

of Çulhalı, 10 km from the town of Boyabat in Sinop Province (Figure 1). The village (of Kovuklukaya) is 650 m above sea level and situated among high mountains. The nearest Black Sea coast is approximately 100 km away. A branch of the Kızılırmak River, called Gökirmak, passes through Boyabat town.

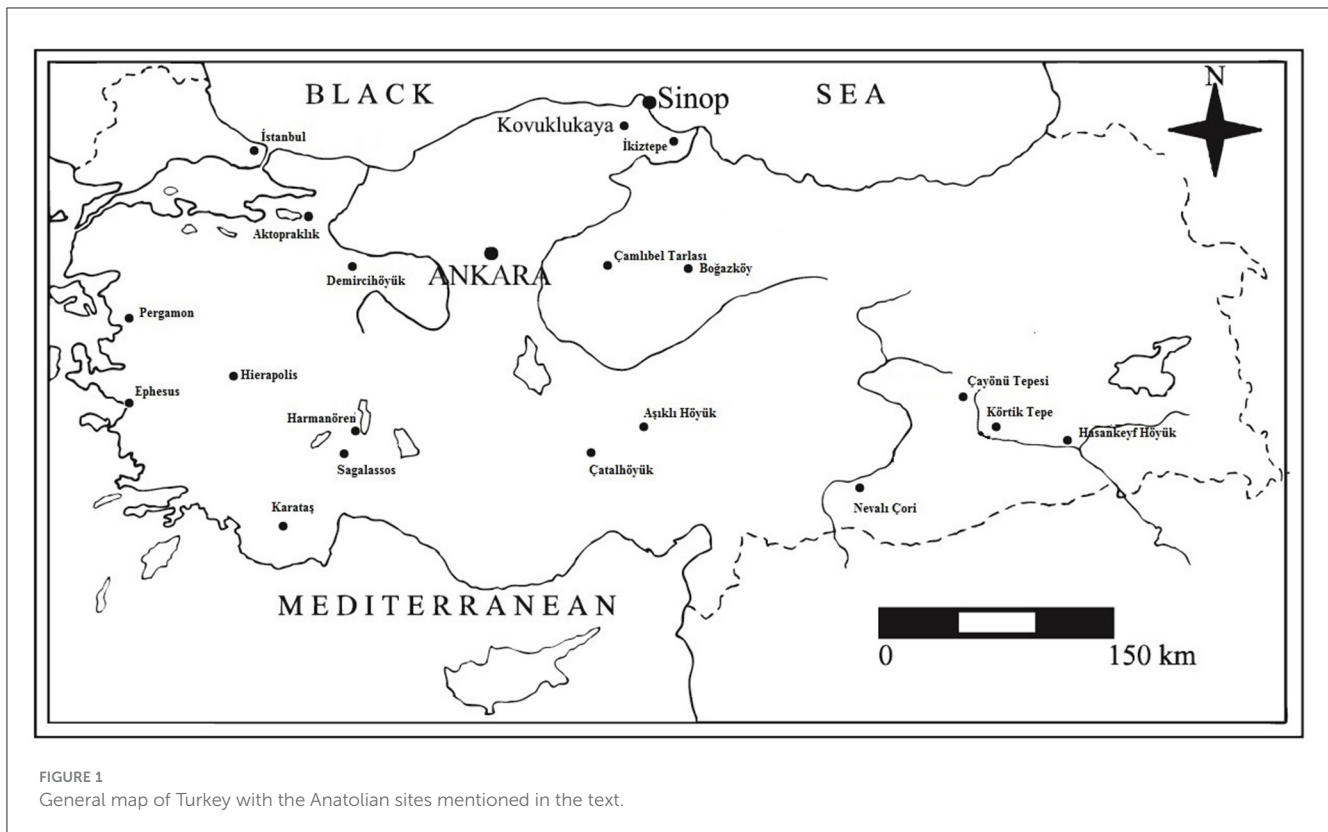
A rescue excavation was carried out in 2002 by Sinop Museum. This rescue excavation revealed that the western side of the mound was inhabited during the Late Chalcolithic and Early Bronze Age. A Byzantine cemetery was located at the top of the mound, which measures approximately 70 x 80 m (Özcan et al., 2003; Dönmez, 2005).

A total of 25 simple pits and cist graves contained single and multiple inhumations of 36 individuals (17 males, 15 females, and 4 sub-adults) (Erdal, 2004, 2008). The positioning of the bodies reflects burial customs that are frequently encountered in Christian graveyards in Anatolia (e.g., Yalman, 1994; Erkanal and Özkan, 1999; Erdal, 2012), characterized by the placement of the corpse in an extended supine position with the head oriented toward the west and legs to the east; hands are left crosswise at the level of the chest or abdomen. A crucifix (the only grave good) gathered from one of the burials (KK'02 M13) allowed the dating of these burials to the Byzantine period. The skeletons are dated $1,175 \pm 35$ years *cal.* BP by radiocarbon dating (i.e. 770–970 2σ CE). This calibrated age range falls at the end of first millennium CE, placing it firmly in the Byzantine period (Erdal, 2008).

The bioarchaeological examination of the well-preserved skeletons was performed at the Hacettepe University Skeletal Biology Laboratory (Husbio_L). Estimation of age-at-death (following methods outlined in Krogman and İşcan, 1986; Meindl and Lovejoy, 1985; Loth and İşcan, 1989; Ubelaker, 1989; Buikstra and Ubelaker, 1994) and the sex (following methods outlined in Acsádi and Nemeskéri, 1970; Krogman and İşcan, 1986; Buikstra and Ubelaker, 1994) of all individuals (except for the children) were previously conducted by Erdal (2004). Age categories were assigned to the individuals in the sample population following those outlined in Buikstra and Ubelaker (1994). These are as follows: child (3–15 years old), young adult (20–34 years old), middle adult (35–49 years old), and old adult (≥ 50 years old). Pathological conditions of the dental and skeletal remains of the Kovuklukaya population were reported by Erdal (2004, 2008), Rubini et al. (2014), and Donoghue et al. (2015a,b) following standard and established criteria (Larsen, 1985, 1997; Mann and Murphy, 1990; Lovell, 1997; Aufderheide and Rodriguez-Martin, 1998; Ortner and Putschar, 1985; Ubelaker, 1989; Buikstra and Ubelaker, 1994; Ortner, 2003).

3.2 Paleopathological study

Human remains from Kovuklukaya were studied for skeletal morphology and non-metric traits, demographic pattern, skeletal malformations, and skeletal and dental pathologies (Erdal, 2004, 2008; Eroğlu and Erdal, 2008, 2004). Paleopathological studies suggest that five individuals from a total of 36 excavated and examined individuals had possible skeletal signs of leprosy. One of these individuals, KK'01 SK 9/1, was a 4–5 months-old infant, who had evidence of subdural infectious on the inner surface



of the occipital bone. aDNA studies on this infant indicated that this individual did indeed have leprosy and a rare example of infantile leprosy (Rubini et al., 2014). Individual KK'02 11/2 has destructive lesions on the hard palate of their maxilla, and individual SK 2/1 has a rounded edge of the nasal aperture as well as slight resorption of the anterior nasal spine and antemortem loss of their maxillary anterior teeth, and slightly developed alveolar resorption. These types of lesions are commonly observed on individuals with leprosy (Møller-Christensen, 1952, 1974; Ortner, 2003; Aufderheide and Rodriguez-Martin, 1998). Other critical pathognomonic lesions associated with leprosy are observed on the oronasal areas. These include a widening of the nasal aperture, rounding on the borders of the nasal aperture, the destruction/resorption of the anterior nasal spine, destruction of the intranasal bones such as the vomer, ethmoid, and inferior nasal concha, antemortem loss of maxillary incisors and resorption of the premaxillary area, and destruction and perforation of the hard palate. All of these bony changes, known as “facies leprosa” or rhinomaxillary syndrome (Møller-Christensen, 1952, 1974; Ortner, 2003; Aufderheide and Rodriguez-Martin, 1998) are the pathognomonic appearance of leprosy on the cranium. These primary lesions together with concentric destruction of the distal phalanges of the hands and feet and periostitis on the tibia and fibula bones were observed on individual SK 20/1 and SK24/1 (Erdal, 2004; Sevkar, 2023).

Only individual SK20/1 produced a high coverage of the leprosy genome (Monot et al., 2009; Rubini et al., 2014; Donoghue et al., 2015a,b; Sevkar, 2023). Individual SK 24/1 had skeletal lesions associated with leprosy, however, PCR genomic analyses of this individual were not conclusive for the disease.

3.3 Isotopes analysis

Rib fragments were sampled from twenty-one individuals of different ages and sexes (Table 1). Samples were prepared and measured for stable isotope and compound-specific amino acid analyses at the Laboratory of Radiocarbon Dating of the University Museum at the University of Tokyo, Japan. Bulk bone collagen samples were extracted from the bones based on improvements of previously established protocols (Longin, 1971; Yoneda et al., 2002). First, the bone fragments were cleaned of external contaminants by brushing and ultrasonic cleaning. Humic acid and fulvic acid were removed by soaking in 0.2M NaOH for 8 h, and the samples were then washed with Milli-Q water. The cleaned and freeze-dried bone samples were then ground to a fine powder. Hydroxyapatite was removed from the powdered bone by reacting with 1 M HCl in cellulose tubes overnight at 4°C. The remains were heated in Milli-Q water at 90°C for 12 h to extract the gelatine, and the dissolved gelatine was then filtered and freeze-dried to obtain bulk bone collagen samples.

The stable isotope values of the bulk bone collagen samples were determined by an elemental analyzer-isotope ratio mass spectrometer (EA-IRMS; Flash 2000 EA coupled to a Delta V advantage IRMS, Thermo Fisher Scientific). The analytical precision (1 σ) based on replicate analyses of reference alanine was <0.2‰ based on USGS-40 and also were checked in each measurement by ten replicate analyses of reference alanine and histidine (SI Science Co., Ltd). The purity of the collagen samples was evaluated on the basis of the carbon and nitrogen content (C% and N%, respectively) in the extracted bulk bone collagen samples. The acceptable C/N atomic ratio follows the range given

TABLE 1 Description and stable isotopes values of Kovuklukaya samples.

Skeletal number	Sex	Age group	Age	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	C %	N %	C/N	$\delta^{15}\text{N}_{\text{Glu}}$	$\delta^{15}\text{N}_{\text{Phe}}$	$\Delta^{15}\text{N}_{\text{Glu-Phe}}$	TP _{ter}
SK 1/1	Female	Old adult	50.5	-19.2	9.1	41	14	3.3	15	12	3	2.5
SK2/1	<i>Female</i>	<i>Old adult</i>	65	-19.1	9.5	44	16	3.3	n.a.	n.a.	n.a.	n.a.
SK 2/2	Male	Old adult	50	-19.2	9.0	44	16	3.2	14	10.8	3.1	2.5
SK 3/1	Male	Middle adult	37.25	-18.9	8.8	40	14	3.3	12.8	10.5	2.3	2.4
SK 7/1	Male	Old adult	59	-19.2	9.2	40	14	3.3	13.6	10.9	2.7	2.5
SK 7/2	Male	Middle adult	37.25	-18.5	9.9	39	14	3.3	14.4	11.4	3	2.5
SK 10/1	Unknown	Child	4.75	-19.2	8.0	42	15	3.3	n.a.	n.a.	n.a.	n.a.
SK 11/2	<i>Male</i>	<i>Old adult</i>	48.25	-19.0	8.6	41	14	3.3	13.1	10.6	2.5	2.4
SK 12/1	Female	Young adult	28	-19.2	8.3	14	4	3.8	n.a.	n.a.	n.a.	n.a.
SK 13/2	Male	Middle adult	39.5	-18.8	8.9	36	13	3.1	13.5	11.2	2.3	2.4
SK 14/1	Male	Young adult	15.5	-18.8	8.4	40	14	3.2	13.9	11.5	2.4	2.4
SK 5/1	Male	Old adult	50.5	-18.9	8.9	42	15	3.2	13.4	11.9	1.5	2.3
SK 15/1b	Female	Old adult	49	-19.0	9.3	41	14	3.3	14.2	11.6	2.6	2.5
SK 18/1	Female	Old adult	46	-18.6	10.2	40	14	3.3	14.7	11.6	3.1	2.5
SK 18/2	Female	Old adult	49	-19.0	9.1	42	15	3.3	13.2	10.3	2.9	2.5
SK 20/1	<i>Female</i>	<i>Middle adult</i>	40.5	-19.2	8.6	40	15	3.2	n.a.	n.a.	n.a.	n.a.
SK 22/1	Female	Old adult	55.5	-19.4	8.9	43	15	3.3	13.3	10.8	2.5	2.4
SK 24/1	<i>Male</i>	<i>Middle adult</i>	37.25	-18.9	8.8	38	14	3.3	13.3	11.4	2	2.4
SK 23/1	Male	Old adult	56	-19.2	8.1	44	16	3.3	13.9	10.8	3.1	2.5
SK 21/1	Male	Old adult	50.5	-18.8	8.9	40	14	3.2	13.6	11.2	2.4	2.4
SK 17/2	Male	Middle adult	39.5	-19.1	8.8	41	15	3.3	12.8	10.4	2.4	2.4

Bold: failed quality criteria. Italic: individuals with leprosy. n.a., = not analyzed.

in DeNiro (1985) of 2.9-3.6 and extracted collagen yields were >1% (Ambrose, 1993). When a bulk bone collagen sample meets these quality criteria, it is considered to be unaffected by diagenesis and acceptable for further analysis.

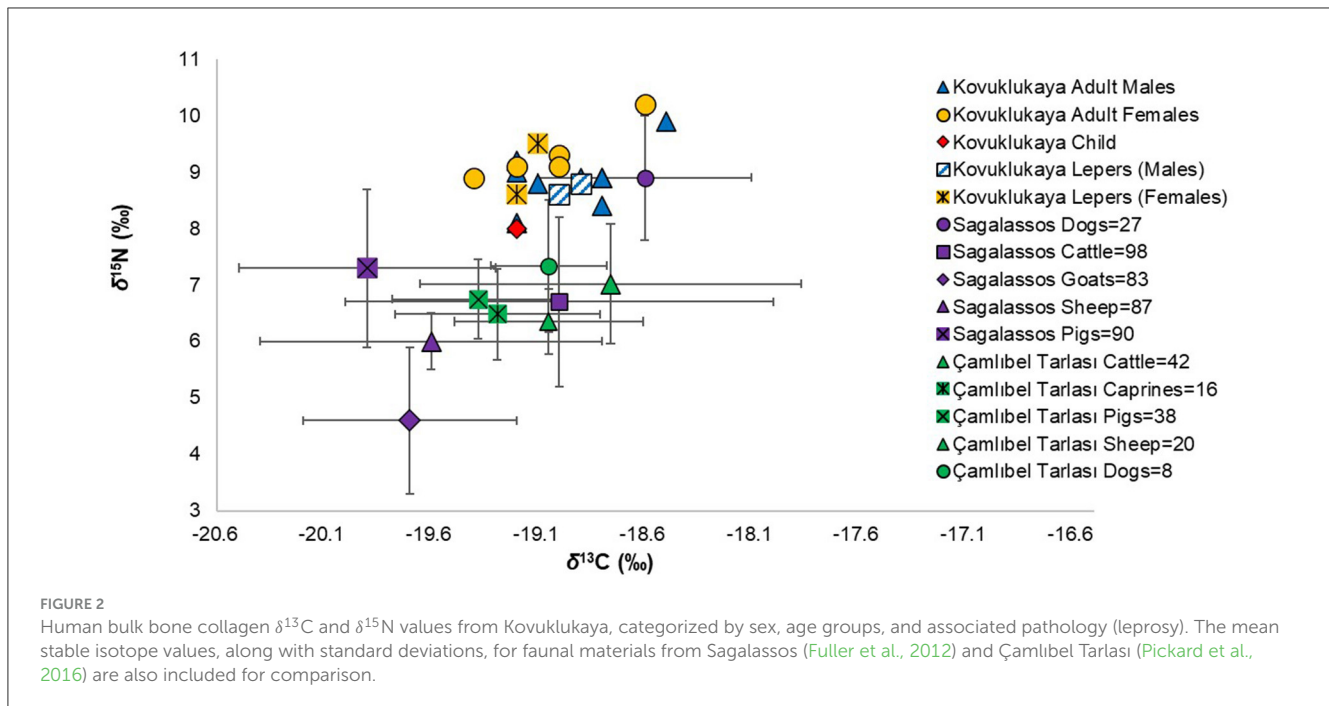
Seventeen of the bulk bone collagen samples (from the same number of distinct individuals) were derivatized for compound-specific nitrogen isotope analysis of individual amino acids. Amino acids were extracted from ~ 2 mg of the bulk bone collagen sample by hydrolysis with 12 M HCl at 110°C for 12 h. After this, derivatization was performed for isotopic analysis following the method outlined previously in Chikaraishi et al. (2010). The hydrolyzed samples were reacted with 1:4 (v/v) thionyl chloride:2-propanol at 110°C for 2 h, followed by treatment with 1:4 (v/v) pivaloyl chloride:dichloromethane at 110°C for 2 h.

The $\delta^{15}\text{N}$ values of the amino acids were determined by gas-chromatography-combustion-isotope-ratio mass spectrometer (GC-C-IRMS; GC IsoLink II coupled to a Delta V advantage IRMS, Thermo Fisher Scientific) at the University Museum of the University of Tokyo (Itahashi et al., 2019). Standard mixtures of amino acids (SI Science Co., Ltd) with known $\delta^{15}\text{N}$ values were analyzed after every five runs. The analytical precision (1σ) for replicate analyses of the reference amino acids was < 0.5‰ for samples containing amino acids.

No animal bones were recovered from the cemetery. In order to define an isotopic baseline of the possible food resources consumed by the Kovuklukaya population, the faunal isotope values from the Late Chalcolithic site of Çamlıbel Tarlası (north-central Anatolia, sharing a similar ecology with Kovuklukaya and in relatively close proximity to it, Pickard et al., 2016), and the Classical Hellenistic to Middle Byzantine site of Sagalassos (southwest Anatolia, dated to the same time period as Kovuklukaya, Fuller et al., 2012) were used as approximate references (see Figure 1 for the sites' location). While these proxies provide valuable comparative data, it is important to recognize the limitations of using non-local faunal sources. If our aim was to demonstrate intra-population diversity, the lack of faunal remains would be a significant limitation, as human dietary diversity is usually inferred from the diversity of domestic species consumed. However, if the isotopic data show homogeneity, the lack of local faunal analysis is not a significant limitation. The main problem for this study is that the small sample size analyzed by stable isotopes may affect the wider applicability of these results.

3.4 Statistics

Statistical analysis includes descriptive statistics (mean and standard deviation) and a *t*-test ($p < 0.05$) to detect differences



in subgroups, such as between leprosy and non-leprosy individuals and between males and females. The Shapiro-Wilk test was used for evaluation of normality.

4 Results

Only one (SK12/1, a young adult female individual, C/N atomic ratio of 3.8) individual out of the 21 sampled individuals did not produce well-preserved collagen (DeNiro, 1985; however, see also Guiry and Szpak, 2021) and this individual was excluded from further analysis (Table 1). The results of the analysis are reported in Table 1 and plotted in Figure 2.

The adult $\delta^{13}\text{C}$ values from human bulk bone collagen samples in the Kovuklukaya population ($n = 20$) range from -19.4‰ to -18.5‰ , with a mean value of $-19.0\text{‰} \pm 0.2\text{‰}$ (Table 2, Figure 2). The $\delta^{15}\text{N}$ values for adults range from 8.0‰ to 10.2‰ , with a mean value of $9.0\text{‰} \pm 0.5\text{‰}$ (Table 2, Figure 2).

The mean $\delta^{13}\text{C}$ values from the bulk bone collagen for the females ($n = 7$) and males ($n = 12$) are $-19.1\text{‰} \pm 0.3\text{‰}$ and $-18.9\text{‰} \pm 0.2\text{‰}$, respectively (Table 2, Figure 2). The $\delta^{15}\text{N}$ mean values from bulk bone collagen are $9.2\text{‰} \pm 0.5\text{‰}$ for females and $8.9\text{‰} \pm 0.4\text{‰}$ for males (Table 2, Figure 2). Regarding the male and female subgroups, the female individuals have a more negative mean value for $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$ than the males but these are not statistically significant; $t = -1.21$, $p = 0.24$ for $\delta^{13}\text{C}$ and $t = 1.75$, $p = 0.10$ for $\delta^{15}\text{N}$. The $\delta^{13}\text{C}$ values of leper individuals ($n = 4$) range between -18.9‰ and -19.2‰ , and their $\delta^{15}\text{N}$ values are between 8.6‰ and 9.5‰ (Table 2, Figure 2). There is no statistically important difference between the means of the leper group ($n = 4$) and adult individuals with no signs of leprosy ($n = 15$); $t = -2.111$, $p = 0.56$ for $\delta^{13}\text{C}$ and $t = 2.11$, $p = 0.58$ for $\delta^{15}\text{N}$.

Although the stable isotope values are generally clustered together and have an observably narrow range, two individuals,

SK18/2 (an old female) and SK7/2 (a middle adult male) have slightly higher values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ than the others (Table 1, Figure 2). Individual SK10/1 belongs to a child (around 4 years old) who has a bulk bone collagen $\delta^{15}\text{N}$ value of 8.0‰ which is lower than the population mean.

Following compound-specific amino acid stable isotope analysis, the $\delta^{15}\text{N}_{\text{Phe}}$ values for the 17 sampled humans from Kovuklukaya range from 10.3‰ to 12.0‰ , and the $\delta^{15}\text{N}_{\text{Glu}}$ values range from 12.8‰ to 15.0‰ (Table 1, Figure 3). The TP_{ter} values of the humans range from 2.3 to 2.5, with the average value being 2.4 ± 0.1 .

5 Discussion

Dietary habits can be influenced by culture, disease, mobility, and environment. Being a stigmatized disease, stable isotopic analysis of individuals with leprosy can offer a good chance to investigate these potential effects on dietary habits. Cultural attitudes toward leprosy in the past often resulted in the exclusion of infected people and, furthermore, with them having different dietary habits from the general society that they were excluded from, as a result of them being forced to live in separate places such as distinct and separate districts, villages, or hospitals (Manchester, 1984; Miller and Nesbitt, 2014). *Miskinhane* or leprosariums were often located outside cities, sometimes in isolated areas (Başer, 1992; Manchester, 1984; Miller and Nesbitt, 2014). Kovuklukaya, which is located between the mountains in the Central Black Sea region could potentially be considered as such a place where ostracized people from different parts of the region resided. Hypothetically, this could then have the effect of heterogeneity in dietary habits of the individuals due to their movement and mobility—i.e., due to the averaging effect on the stable carbon and nitrogen values in bulk bone collagen (Hedges et al., 2007;

TABLE 2 Published mean stable carbon and isotope ratio values, standard deviations (SD), and range from Turkey.

Site/period	Carbon (‰)			Nitrogen (‰)			Reference
	X	SD	Range	X	SD	Range	
Çamlıbel Tarlası/Late Chalcolithic	−18.9	0.4	0.8	8.5	1.3	3.1	Pickard et al., 2016
Bogazköy							
Iron Age	−18.9	0.5	1.8	7.5	1.7	7.3	Pickard et al., 2017
Hellenistic	−18.5	0.4	1.5	9.2	0.7	2.1	
Roman	−18.7	0.5	1.8	8.9	1.1	2.1	
Sagalassos							
Classical-Hellenistic	−19.4	0.4	-	9.7	0.7	-	Fuller et al., 2012
Late Imperial	−19.2	0.2	-	10.1	0.7	-	
Middle Byzantine	−19.0	0.3	-	9.1	0.9	-	
Total	−19.0	0.3	-	9.2	0.9	-	
Ephesus/2nd–3rd C. AD							
Gladiators	−18.9	0.4	1.6	9.3	0.8	3.6	Lösch et al., 2014
NG Male	−19.0	0.2	0.9	9.4	0.6	1.7	
NG Female	−18.9	0.5	1.2	8.9	1.1	2.4	
Total/including three infants	−18.9	0.4	1.8	9.3	0.8	3.8	
Pergamon/Roman and Late Byzantine	−18.4	0.3	-	9.9	0.8	-	Propstmeier et al., 2017
Hierapolis/Roman and Early-Mid Byzantine	−19.3	0.5	2.1	9.8	0.8	3.5	Wong et al., 2017
Kovuklukaya/Early Byzantine							
All Females (n = 7)	−19.1	0.3	0.8	9.2	0.5	1.9	Present study
All Males (n = 12)	−18.9	0.2	0.7	8.9	0.4	1.8	
All Adults (n = 19)	−19.0	0.2	0.9	9.0	0.5	2.1	
Lepers (n = 4)	−19.0	0.1	0.3	8.9	0.4	1.5	
Non-lepers (n = 15)	−19.0	0.3	0.9	9.0	0.5	2.1	
Total (including one child)	−19.0	0.2	0.9	9.0	0.5	2.2	

Katzenberg and Waters-Rist, 2018) their isotopic values would reflect their dietary habits (or quality of diet) of the places where they came from.

5.1 Food sources of Kovuklukaya

The bulk bone collagen $\delta^{13}\text{C}$ values (Table 1, Figure 2) have very little diversity (a range of only 0.9‰). As a result, the values of the individuals cluster closely together and suggest a predominantly terrestrial, C_3 -based diet for the Kovuklukaya people. This is in some ways expected since C_3 plants constituted the main source of plant input in dietary habits in Anatolia throughout the Holocene (Richards et al., 2003; Rao et al., 2012; Pickard et al., 2016). In addition, as extensively reviewed by Bourbou and Richards (2007), the historical sources from the Byzantine world suggest that dry-field crops such as wheat (the most important raw material of bread), barley, oats, rye, and rice, along with plant products like oil and wine, and vegetables, fruits, wild greens and nuts were the important C_3 sources of the Byzantine diet. Interestingly, nine

individuals produced slightly more positive values than -19.0‰ (accepted as a threshold value in Anatolia to determine C_4 input to human diet—see Fuller et al., 2012; Pickard et al., 2016). This would suggest, as an overall pattern, that there was little input of C_4 plants in the dietary habits of the population, either by direct or indirect consumption through an animal vector. However, non-alimentary factors such as physiology, nutritional stress, and metabolic diseases may alter the isotope values as well (Fuller et al., 2005; Reitsem, 2013; Olsen et al., 2014). Osteological data demonstrates that the majority of the Kovuklukaya skeletal population were over the age of 45 when they died, and the frequency of osteoporosis in the population was 38.7%; 23.5 % for males and 57.1% for females (Erdal, 2004). Eight of the nine individuals with the most positive $\delta^{13}\text{C}$ values have age-at-deaths varying between 37 and 56 years old, whilst the other one was 15 years old at death (Table 1). Therefore, it might be possible to say that the presence of pathologies in the population may be affecting the bulk bone collagen stable isotope values. However, this is only a cautious statement as the other individuals with $\delta^{13}\text{C}$ values more negative than -19.0‰ are also adults (with the exception of one child). Pickard et al. (2016) also

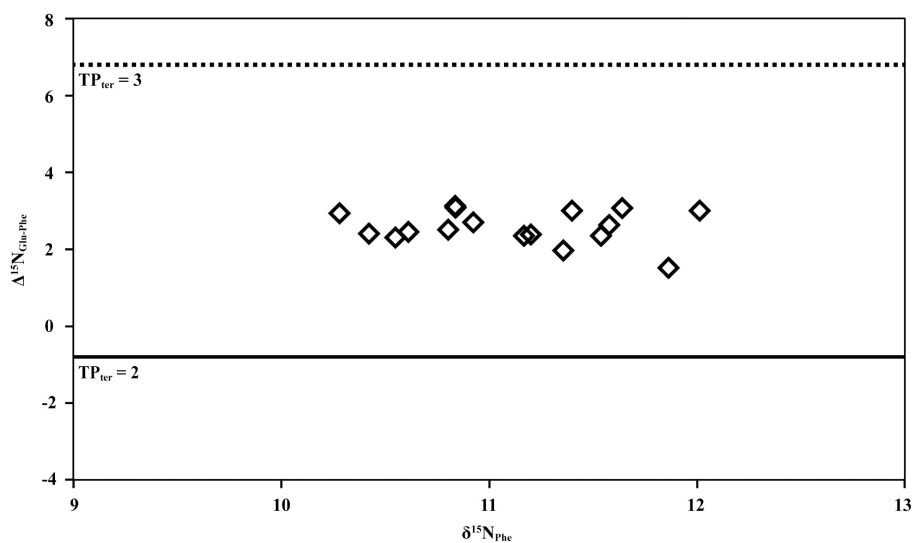


FIGURE 3
The $\delta^{15}\text{N}_{\text{Phe}}$ and $\Delta^{15}\text{N}_{\text{Glu-Phe}}$ of humans from Kovuklukaya.

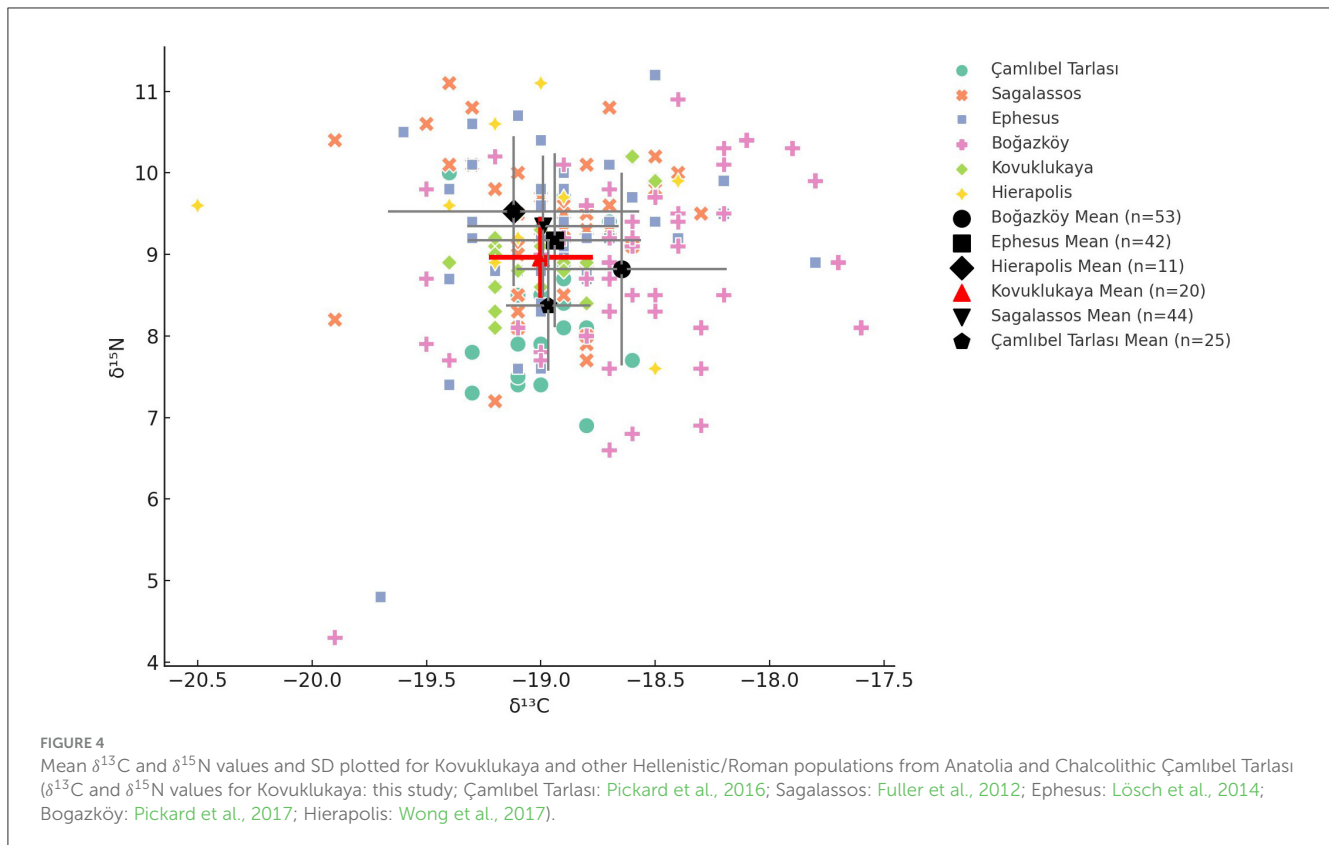
considered that non-alimentary factors may lie behind the more positive $\delta^{13}\text{C}$ values for the individuals from Late Chalcolithic Çamlıbel Tarlası.

Pickard et al. (2016) also did calculate the contribution of C_4 plants to the adult diet of Late Chalcolithic Çamlıbel Tarlası as being 10.0–11.7% by using a simple linear mixing model (using $-19.0\text{‰} = 100\% \text{C}_3$ and $-7\text{‰} = 100\% \text{C}_4$ input; Fuller et al., 2012). The same model yielded an approximately 9–10% input of C_4 plants to the human diet at Kovuklukaya. The presence of C_4 plants in human and animal diet was reported from various sites and populations around Anatolia dated to different time periods with the help of stable isotopes analysis (Richards et al., 2003; Lösch et al., 2006, 2014; Fuller et al., 2012; Pickard et al., 2016; Propstmeier et al., 2017) and paleobotanical research (Fairbairn et al., 2005). It is not clear whether the inclusion of C_4 plants into the Kovuklukaya dietary habits occurred through direct consumption of the plants or indirectly by C_4 -fed animals, since no animal bones were available from the site for stable isotope analysis. Besides, there is also no paleobotanical evidence for C_4 grasses or shrubs that ovicaprid could have fed on. However, the more positive $\delta^{13}\text{C}$ values were not correlated with higher $\delta^{15}\text{N}$ values (which can be an indication of marine resource input, or in this case significant terrestrial animal protein input) and, therefore, this may rather suggest the direct consumption of C_4 plants such as millet (Prowse et al., 2004; Tafuri et al., 2009; Gregoricka and Sheridan, 2013; Lösch et al., 2014). Although millet is seen as less desirable for human diet under normal conditions (Prowse et al., 2004), it became an important cultivated crop in Anatolia beginning in the Early Byzantine Period (Fuller et al., 2012). Textual sources suggest the importance of millet in the Byzantine diet varied according to region and economic status (Bourbou and Richards, 2007).

Compared to the Anatolian sites of Roman Ephesus (mean $\delta^{15}\text{N}$ value of 9.3‰, Table 2, Figure 4; Lösch et al., 2014) and Middle Byzantine Sagalassos (mean $\delta^{15}\text{N}$ value of 9.1‰, Table 2, Figure 4; Fuller et al., 2012), the adult $\delta^{15}\text{N}$ mean value of

Kovuklukaya ($9.0 \pm 0.5\text{‰}$) is slightly lower, but higher than at Late Chalcolithic Çamlıbel Tarlası ($8.3 \pm 0.8\text{‰}$, Table 2, Figure 4; Pickard et al., 2016) (see Figure 1 for the sites' location). The $\delta^{15}\text{N}$ values are consistent with the $\delta^{13}\text{C}$ values indicating a primarily terrestrial diet, and no inclusion of marine foods. Considering its relatively close proximity to the Black Sea, one might expect an input of marine resources in their dietary habits. The sources mention the consumption of different fish species, preserved fish, and fish eggs, *garum*, etc., in the Byzantine diet with seasonal and regional variations (Bourbou and Richards, 2007). However, the high mountains running parallel to the sea make access to marine sources difficult at Kovuklukaya. On the other hand, stable isotope studies at Anatolian sites such as Roman Ephesus (Lösch et al., 2014) and Neolithic and Chalcolithic Aktopraklık (Budd et al., 2013; Figure 4, see Figure 1 for the site's location), despite their close proximity to sea/fresh water, interestingly, show much lower $\delta^{15}\text{N}$ values than the theoretical marine input value of 12‰ (Richards and Hedges, 1999) as well. Budd et al. (2013, p. 6) argues that as "certain farming communities were turning their backs to the sea/freshwater resources and were mainly exploiting C_3 terrestrial resources as a preferred subsistence strategy." Honch et al. (2013) also reported no marine effect in their isotope values from the Durankulak and Varna I cemeteries (Bulgaria) despite their proximity to the Black Sea.

The Kovuklukaya $\delta^{15}\text{N}$ values also suggest a not insignificant input of animal protein. Actually, the proportion of plant vs. animal protein in diet is generally studied by the comparison of the human $\delta^{15}\text{N}$ values with the associated fauna from the site. As in the Kovuklukaya case, where no animal bones were gathered from the excavation, researchers overcame the situation by comparing the faunal results from other sites (e.g., see Lösch et al., 2014). The average $\delta^{15}\text{N}$ value ($9.0\text{‰} \pm 0.5\text{‰}$) for adults of Kovuklukaya is 2.7‰ higher than the reported herbivore mean value ($6.7\text{‰} \pm 0.9\text{‰}$) of Late Chalcolithic Çamlıbel Tarlası where pollen analysis indicates the presence of dense vegetation in the



mountainous environment (Table 2, Figure 2; Pickard et al., 2016). The Kovuklukaya human $\delta^{15}\text{N}$ mean value is also 2.7‰, 3.0‰, and 4.4‰ higher than the mean values for cattle ($6.7\text{‰} \pm 1.5\text{‰}$), sheep ($6.0\text{‰} \pm 1.5\text{‰}$), and goat ($4.6\text{‰} \pm 1.3\text{‰}$), respectively, from Sagalassos (Table 2, Figure 2; Fuller et al., 2012). The high human mean $\delta^{15}\text{N}$ values at Kovuklukaya, relative to the $\delta^{15}\text{N}$ mean values of herbivores from regionally (Çamlıbel Tarlası) and chronologically (Sagalassos) similar sites, may indicate that terrestrial animal food sources (meat or dairy products) were important staples in the Kovuklukaya dietary habits. In addition, the range of $\delta^{15}\text{N}$ values is less than 3‰, supporting the idea of substantial similarity in the inputs of animal protein in the dietary habits of the inhabitants. There are also two individuals (SK18/1 and SK7/2) with higher $\delta^{15}\text{N}$ values (Table 1). The $\delta^{13}\text{C}$ values of these individuals are close to the population mean ($-19.0\text{‰} \pm 0.2\text{‰}$), suggesting that their nitrogen isotope values are not linked to marine protein consumption. They appear to have consumed higher $\delta^{15}\text{N}$ protein sources (more meat or maybe nonlocal sources) or a lower amount of pulses. The $\delta^{15}\text{N}$ of pulses is close to zero due to their nitrogen fixation process, and an input of legumes into the human diet can mask the actual trophic level of humans and the amounts of consumed animal protein (Larsen, 1997). Byzantine sources revealed that legumes including broad beans, chickpeas, peas, lentils and lupine were important crops in the human diet, and vetch was used as animal feed or for enriching the soil (Bourbou and Richards, 2007). Lösch et al. (2006) detected an isotopic indication of legume consumption in Neolithic Anatolia. An archaeobotanical study conducted at the Early Bronze Age site of İkiştepe (close to Kovuklukaya, see Figure 1 for the site's

location) demonstrated that two species of legumes, namely *Vicia ervilia* and *Lathyrus sativus*, were important crops in the region for either human or animal diet (Zeist, 1998).

Çulhalı village, where Kovuklukaya is located, possesses very hilly terrain among high mountains (the Isfendiya and Ilgaz mountains). Those mountains are covered with forest (pine and oak), and are well known for their high quality of lumber in Anatolia (Başoğlu, 1972). As it is still carried out today, lumber production has been the main occupation activity in the region. The forest vegetation and rugged terrain have also influenced other economic activities. The land around Çulhalı village is more suitable for gardening rather than larger scale crop agriculture. Pastures and meadows cover 4% of the area (Başoğlu, 1972). Therefore, together with horticulture, goats and sheep herding (rather than raising cattle) are other important subsistence activities for the present-day inhabitants. According to the statistics on the number of livestock reported in the 1970s, >100,000 sheep (*Ovis aries*) and goats (*Capra hircus*) were grazing in the vicinity of Boyabat (Başoğlu, 1972). In Anatolia, sheep and goats are raised for their meat, milk, and skins, as well as wool and hair. As in the case of lumber production, the region has been known as a production center for high quality wool; Angora wool, mohair, and skins, which are exported to Istanbul as industrial raw materials (Başoğlu, 1972). It can be said that the ethnographic and environmental data obtained from Boyabat are also consistent with the results from the isotopes of the human bulk bone collagen from Kovuklukaya.

Erdal (2004, 2008) identified evidence on the Kovuklukaya skeletons of subsistence activities that continue to the present day. Based on osteoarchaeological analysis of pathological changes and

traumatic injuries observed in the cranial and postcranial bones, he concluded that the Kovuklukaya population, especially the males, were probably engaged in timber production. In addition, he concluded that yarn production was an important activity for the Kovuklukaya females due to the greater incidence of unusual teeth attrition and wear patterns in the females compared to the males. Yarn production is also supported by dental grooves on the anterior dentition of the Kovuklukaya females (Erdal, 2008). Moreover, high frequencies of lateral and medial epicondylitis on the distal ends of the humeri also suggest activities such as yarn production or timber milling (Spigelman et al., 2012). At the same time, these osteological and dental markers also support the idea of gender-based division of labor: females were yarn producers whilst males were involved with forestry activities such as obtaining lumber, as well as farming. However, despite the clear division of labor evidenced by the skeletal remains, no significant difference in dietary habits by sex was revealed in the stable isotope values from the bulk bone collagen at Kovuklukaya. This similarity could be a result of social group dynamics or family life. However, as Bourbou and Richards (2007) have noted, if there was any difference in diet according to sex/gender, it may have been subtle (e.g., different cuts of meat, different proportions in meals or priority in eating) and not detectable by bulk bone collagen stable isotope analysis. Considering the results of the stable isotope values from the bulk bone collagen, ethnographic, and osteoarchaeological evidence together could suggest the raising of goats and sheep as a subsistence strategy, probably also resulting in these animals (or their ante-mortem food products—i.e., dairy) as a source of animal protein in dietary habits. The same data also supports the idea that the residents of Kovuklukaya were a group that engaged in daily argora-pastoral activities for subsistence and survival, rather than going to nearby settlements and begging to obtain food—as may be the case if they were lepers.

The bulk bone collagen isotope values and skeletal-teeth markers of biomechanical stress identified in the analyses (summarized above) may plausibly be attributed to individuals who were forcibly relocated due to leprosy and subsequently died shortly thereafter. These isotopic and skeletal indicators provide a record of the cumulative dietary inputs and biomechanical stresses experienced by individuals over the course of their lives, thereby reflecting conditions prior to their relocation (Hedges et al., 2007; Katzenberg and Waters-Rist, 2018; Larsen, 1997). In addition, historical evidence reveals the widespread social exclusion experienced by individuals with leprosy, which often necessitated their isolation and confinement in purpose-built hospitals or housing for their care (Manchester, 1984; Miller and Nesbitt, 2014), and can therefore be seen as providing indirect support for previous inferences that values may reflect the previous living conditions of forcibly relocated lepers.

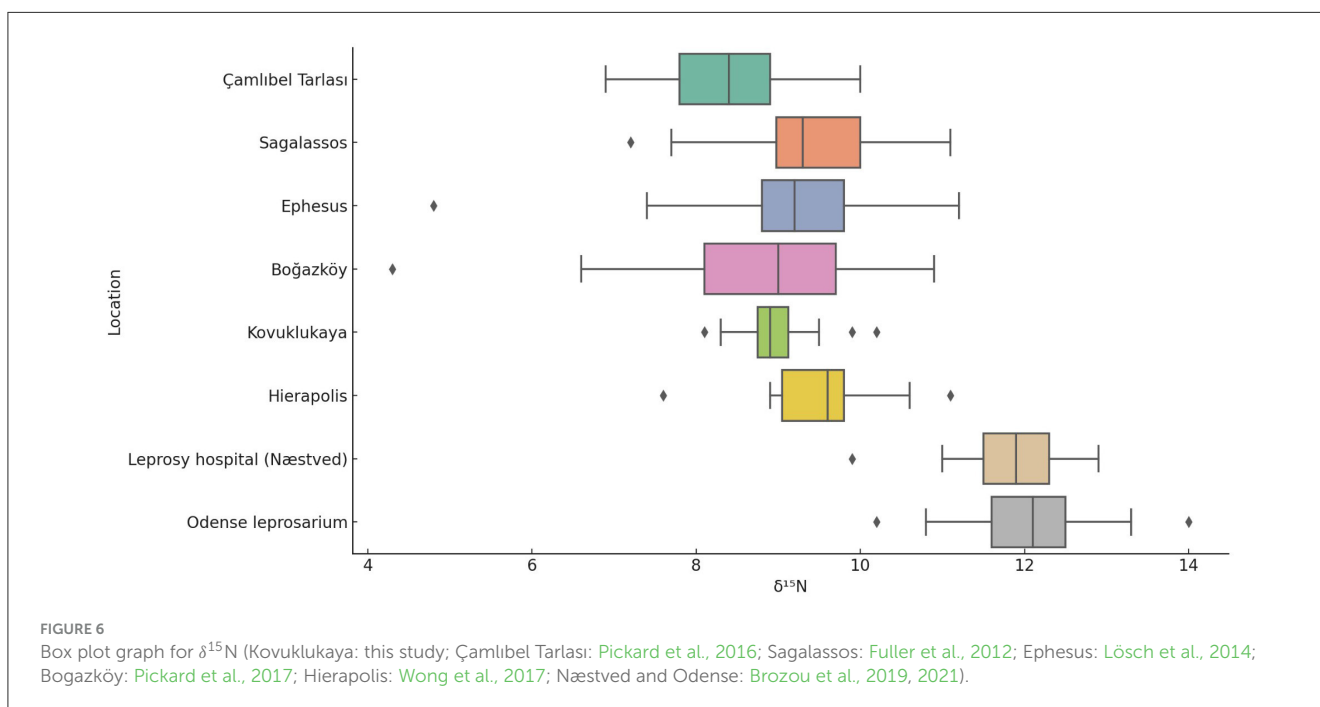
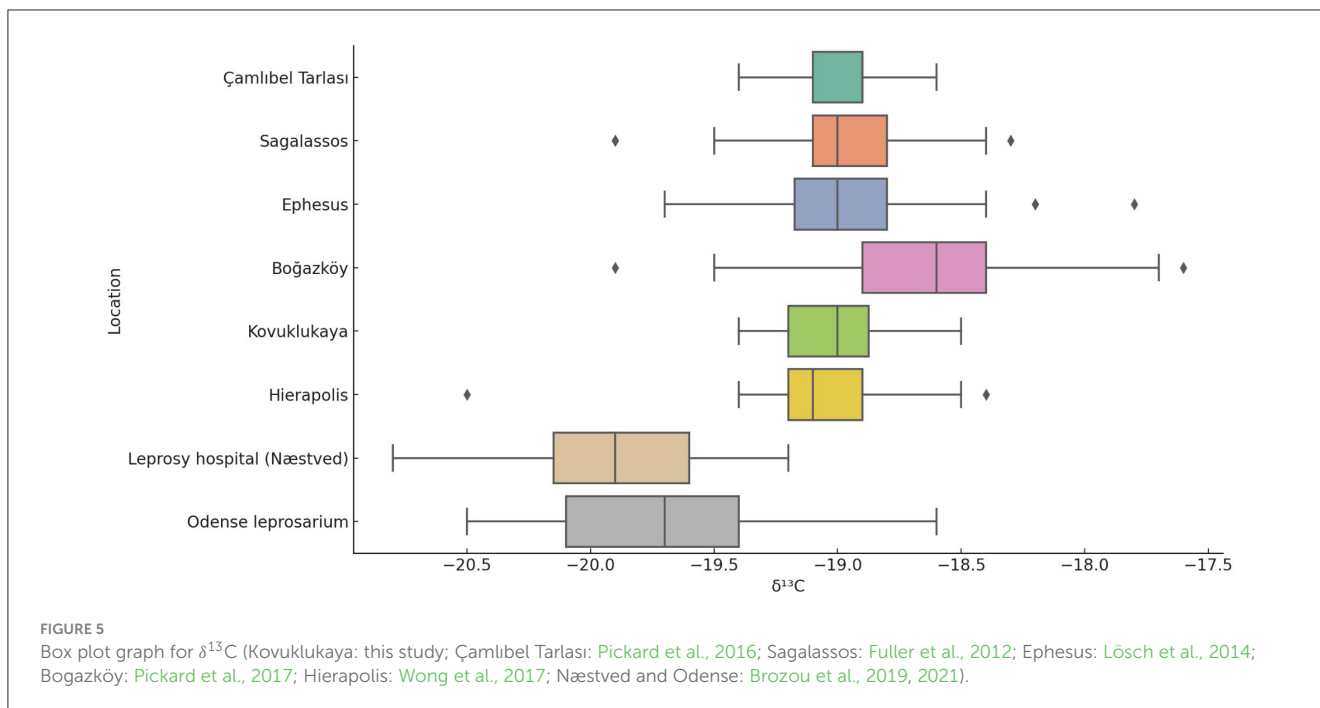
However, individuals who primarily show physical signs of leprosy are condemned, humiliated and ostracized by the community (Fernández and Ribón, 2018). Leprosy typically has a long incubation period, with symptoms appearing 5 to 20 years after infection, depending on host immunity and bacterial load (Britton and Lockwood, 2004; Scollard et al., 2006). Symptoms progress gradually after onset and, in advanced cases, individuals may suffer significant physical disability, including muscle wasting and paralysis, which can affect their ability to

maintain a subsistence lifestyle (Scollard et al., 2006). Because the disease typically remains asymptomatic and does not cause systemic damage, death during the incubation period is rare (Britton and Lockwood, 2004; Scollard et al., 2006). The absence of skeletal evidence of leprosy in 31 of the 36 individuals buried in the Kovuklukaya cemetery refutes the hypothesis that this settlement was a burial site for individuals marginalized by the disease. The absence of pathological markers supports the interpretation that the site was not intended to isolate leprosy patients.

5.2 Isolated society or ostracized individuals

Kovuklukaya has a relatively narrow range of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from bulk bone collagen compared to other settlements in Anatolia and beyond (Table 2). Additionally, the nitrogen isotopic analysis of compound-specific amino acids of the Kovuklukaya humans demonstrated a narrow TP_{ter} value ($2.4 \pm 0.1\text{‰}$) that suggests a relatively homogenous input of animal protein (Table 1, Figure 3). This TP_{ter} value is slightly lower than the average for Neolithic farmers in Turkey reported in previous studies; Çatalhöyük: $2.5 \pm 0.1\text{‰}$ (Styring et al., 2015), Hakemi Use: $2.5 \pm 0.1\text{‰}$ (Itahashi et al., 2019), Aşıklı Höyük: $2.5 \pm 0.2\text{‰}$ (Itahashi et al., 2021) (see Figure 1 for the sites' location). However, the variation of $\Delta^{15}\text{N}_{\text{Glu-Phe}}$ and TP_{ter} was less than those of the Neolithic period populations. The isotopic values of bulk bone collagen and compound specific amino acids suggest that the protein sources in the diet, or the overall dietary quality, of the Kovuklukaya population was consistent and did not match the heterogeneous isotopic values observed in leprosarria from other regions (for example, Brozou et al., 2019, 2021; see Figures 5, 6). These studies were conducted on the individuals from the medieval Danish leprosarium at Næstved and Odense and demonstrated variation in the inputs and sources of protein, reflecting different socioeconomic statuses of the individuals (see Figures 5, 6; Brozou et al., 2019, 2021). Conversely, an isotopic study conducted on individuals from the Neolithic settlement of Aşıklı in Central Anatolia, reported that the stable isotope values of individuals recovered from the same grave were similar and it was hypothesized that this was a result of the individuals in the grave being from one familial/domestic unit and likely closely related (Itahashi et al., 2021). As already explained above, individuals such as SK24/1 and SK 20/1 which have pathognomonic signs of leprosy have $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values that are similar to other sampled individuals from this community; they are not outliers (Table 2, Figure 2). This suggests that their diet was comparable to that of the general population, or at the very least, of similar nutritional quality.

Alternatively, the similarities in the Kovuklukaya stable isotope values and results might be related to the size of the sample, and/or may indicate regular consumption of less diverse food resources as a result of the surrounding mountainous ecosystem of the inland Black Sea region. However, based on present-day statistics (Başoğlu, 1972) and an ethnographic study (Erdal, 2008), together with the geographical characteristics, it is possible to say that the modern inhabitants living in a rich ecology exploit various resources (see above discussion) for their subsistence activities.



The osteological and dental data reveal that the subsistence activities that can be seen practiced today were also practiced by past populations in the region, and suggest the existence of an ecologically rich environment in the past as well. Thus, it is possible to consider that other dynamics are related in conjunction with population structure to produce the observed homogeneity in stable isotope values, rather than factors such as small sample size or a scarcity of resources.

There are some archaeological and anthropological data that suggest population structure characteristics such as small

population size, close relationships between members of a group, and an introverted/closed society for the Kovuklukaya people. The specific characteristics of the cemetery, the mortuary practices and the type of burials allow us to make some inferences about the relationships between the individuals buried there. First, the size of the cemetery (0.56 ha) is relatively small compared to others. For example, Massa (2014) classified the Demircihöyük-Sarikent cemetery, with a size of 0.2 ha, as small in comparison with those of Harmanören (~4 ha) and Karataş (at least 2 ha) among Anatolian necropolises (see Figure 1 for the location of the sites).

Therefore, Kovuklukaya can also be considered as a small cemetery and most probably belonged to a small community rather than a large one. The burial customs of this small group also suggest a close relationship between the individuals. It has been established that nine of the twenty-five graves at Kovuklukaya contain multiple burials (two or three skeletons in each, representing 20 of the total 36 individuals buried there). In addition, in these secondary burials, earlier burials were stacked at the feet of later burials (on the west side). The practice of secondary burials, motivated by the concept of reunification with the ancestors, has been seen as an affirmative mechanism that promotes social cohesion in families or larger groups (Hertz, 1960; Kuijt, 2000; Winter-Livneh et al., 2012; Pearson et al., 2013).

The non-metric traits observed on the skeletons supply more direct information concerning genetic relationship and population structure. The frequency of *palatine torus*, which is described as a paramedian bony protuberance of varying size, form, and extent situated along the median suture of the hard palate (e.g., Bernaba, 1977; Belsky et al., 2003) was observed to be very high (Erdal, 2004; Eroğlu and Erdal, 2004, 2008). It was reported that 15 (60%) of the 25 examined skeletons have these non-metric traits (Eroğlu and Erdal, 2004). Together with other skeletal non-metric traits, the high frequency of palatine torus among Anatolian populations has been considered a result of inbreeding (Erdal, 2004; Eroğlu and Erdal, 2004). The other observed non-metric traits are *os acromiale* and *spondylolysis* (Erdal, 2004). *Os acromiale*, defined as an unfused accessory center of ossification of the acromion of the scapula (e.g., Sammarco, 2000; Edelson et al., 1993), was reported in four male individuals from Kovuklukaya (Erdal, 2004). *Spondylolysis* (separate neural arch, Haukipuro et al., 1978) was observed on the fifth lumbar vertebrae of two male individuals (Erdal, 2004). It is often considered that these non-metric traits are hereditary (Sammarco, 2000; Edelson et al., 1993; Sjøvold, 1984; Haukipuro et al., 1978). *Os acromiale* and *spondylolysis* are also thought to be activity induced anomalies (Stirland and Waldron, 1997). Thus, the presence of these conditions may suggest that the people of Kovuklukaya had similar daily activities and possibly also similar genetics. Additionally, according to biodistance studies conducted on Anatolian populations by using the stepwise discriminant analysis based on cranial measurements (Eroğlu, 2005; Erdal, 2012), Kovuklukaya is accepted as a relatively homogenous group (>90% of the Kovuklukaya individuals are classified together), and this can be considered as further evidence for a genetically isolated population structure at Kovuklukaya. A genetic factor is also considered as the main etiology behind the tumoral formations (Aufderheide and Rodriguez-Martin, 1998) detected on the skull of three Kovuklukaya individuals (10%, Erdal, 2004).

The final pathology that may help us understand family structure is leprosy. *M. leprae* or *M. lepromatosis*, which causes the disease, is a bacterium with a long reproduction interval that multiplies by division, this process lasting between 12 and 14 days (Scollard et al., 2006). It requires intensive contact with different parallels in order to develop independently over a period of 5–10 years. Leprosy contagion generally occurs directly on the nasopharyngeal area via droplets, but it is thought that it can be transmitted through various ways such as sharing the same pillows, clothes, kitchen and eating utensils (such as forks, spoons, and plates) as well as eating and drinking from the same pots (Lewis,

2002; Aufderheide and Rodriguez-Martin, 1998; Rubini et al., 2014; Ortner, 2003; Britton and Lockwood, 2004; Akpolat et al., 2018; Fischer et al., 2010; Bratschi et al., 2015; Moet et al., 2006). Leprosy is thus commonly spread within the family unit or among close relatives, especially between parents and children (Aufderheide and Rodriguez-Martin, 1998). The fact that a 4–5-month-old infant at Kovuklukaya suffered from leprosy (as determined by aDNA analysis), along with four adults in the population, offers additional evidence of close relations among the individuals.

The archaeological evidence indicates that the cemetery belonged to a small group, and the burial customs suggest that members of the Kovuklukaya group were closely related to each other. Additionally, osteological and pathological data suggest that the small community was genetically closed/isolated. Thus, it can be possible to consider the isotopic similarity among the individuals as a reflection of their affinity to the closely related group or the family unit. The homogenous diet of such a group sharing a similar lifestyle (such as eating from the same pot, regular consumption of the same kinds of foods, etc.) likely contributed to the observed isotopic similarity among the individuals at Kovuklukaya.

While familial or kin-based subsistence practices provide one explanation for dietary homogeneity, alternative interpretations should also be considered to provide a more balanced perspective. For instance, dietary uniformity could result from resource constraints rather than familial connections. Despite the ecological richness of the surrounding environment, access to diverse food resources may have been limited by seasonal availability, preservation challenges, or cultural preferences. Additionally, subsistence practices rooted in shared social norms, economic strategies, or communal food distribution systems could also produce dietary homogeneity. For example, reliance on staple crops or a narrow range of domesticated animal products may have constrained dietary variation.

6 Conclusion

The bulk bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from the ribs of adults from the Byzantine cemetery of Kovuklukaya, Sinop, indicate that the diet of this small group relied predominantly on terrestrial sources, namely C_3 plants and animals and/or animal products. The more positive $\delta^{13}\text{C}$ values of some individuals (together with relatively low $\delta^{15}\text{N}$ values) suggest that the direct consumption of C_4 plants also took place to a small extent. There is no indication of an input of marine foods resources. Seemingly, terrestrial animal proteins were as important for the Kovuklukaya diet as C_3 plant sources. Ecological characteristics of the region, dental and osteological data, and relatively high $\delta^{15}\text{N}$ values in comparison with the faunal values from Çamlıbel Tarlası and Sagalassos indicate that the possible source of the animal proteins were goat and sheep. Although the osteological and dental data support a sex-based division of labor, no sex-based distinction was detected in dietary habits according to the isotopic data. The lower $\delta^{15}\text{N}$ and the slightly more positive $\delta^{13}\text{C}$ values of the child in comparison with the adult and female means suggest that the child had already been weaned. The narrow range of the stable isotope values from bulk bone collagen and compound-specific amino acids points to the same animal protein intake in the diet.

The location of Kovuklukaya, situated near a forest on one side and both the inner plains and the sea on the other, provided its inhabitants with access to an ecologically rich environment and diverse food resources. In such a setting, it could be hypothesized that the population would exhibit a heterogeneous nutritional pattern, reflective of the increasing human mobility and dietary diversity characteristic of the medieval period. Furthermore, if the settlement were primarily inhabited by individuals with leprosy, relocated from various regions and excluded from society, one might expect to observe greater variability in stable isotope values and inferred dietary habits due to differing nutritional backgrounds. However, our findings reveal a rather homogeneous dietary pattern among the Kovuklukaya population. This homogeneity likely reflects shared dietary habits shaped by a closely related social group or family structure. Thus, the results of this study refute the hypothesis that Kovuklukaya was a settlement designated for the relocation of individuals with leprosy following segregation. Instead, it is more appropriate to interpret Kovuklukaya as a mountain village where leprosy may have originated within the population itself, among individuals living in close familial or social relationships.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

This study strictly complies with ethical guidelines for the handling and analysis of human skeletal remains. All research was conducted with respect for the individuals represented by the remains, following internationally recognized ethical frameworks, such as those outlined by the World Archaeological Congress (<https://worldarchaeologicalcongress.com/code-of-ethics/>) and the International Council of Museums (<https://icom.museum/en/resources/standards-guidelines/code-of-ethics/>). Permission for analysis was obtained from the appropriate authorities, and efforts were made to ensure that the remains were treated with care, dignity, and cultural sensitivity throughout the study.

Author contributions

KÖ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. YI: Data curation, Formal analysis,

Investigation, Methodology, Visualization, Writing – review & editing. MY: Funding acquisition, Methodology, Writing – review & editing. YE: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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