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Review Articles

Isotopic Perspectives on Past Human Lifestyles: Methods, Applications, and Interpretations in Bioarchaeology

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Abstract

Bioarchaeology integrates osteological and biochemical analyses to reconstruct various aspects of past human lifestyles, including diet, mobility, and social organization. Stable isotope analysis represents a key methodological approach, providing direct geochemical evidence for interpreting dietary habits and geographic origins. This paper reviews the principal methods and interpretative frameworks of isotopic research in bioarchaeology. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes are employed to distinguish dietary sources such as C_3 and C_4 plants, marine versus terrestrial foods, and trophic levels. Strontium ($^{87}Sr/^{86}Sr$) and oxygen ($\delta^{18}O$) isotopes serve as indicators of mobility and provenance, while sulfur ($\delta^{34}S$) and selected trace elements complement dietary and environmental assessments. The study highlights the main biological substrates bone collagen, dentin, and enamel and discusses essential analytical procedures, including IRMS and MC-ICP-MS. Case studies from Türkiye and other regions demonstrate how isotopic evidence contributes to a comprehensive understanding of economic systems and population dynamics in past societies.

Key words: Stable Isotope Analysis, Paleodiet, Paleomobility, Social Structure, Bioarchaeology.

Introduction

Bioarchaeology is an interdisciplinary field that investigates biological and cultural processes in the archaeological record through the analysis of human skeletal remains. By combining biological, cultural, and environmental perspectives, it reconstructs aspects of past human life such as diet, mobility, and social organization [37, 64]. Through these approaches, bioarchaeology provides tangible evidence for understanding environmental adaptations, economic systems, and social hierarchies in ancient societies. Human skeletons thus serve not only as biological entities but also as biographical archives that reflect individual life histories and collective social structures.

Within this framework, stable isotope analysis has become a central methodological tool, offering direct geochemical evidence about diet, geographic origin, and environmental conditions. Isotopes of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), oxygen ($\delta^{18}O$), and strontium ($^{87}Sr/^{86}Sr$) extracted from human tissues enable the reconstruction of dietary patterns, mobility, and ecological contexts [58, 61]. Compared to indirect paleoenvironmental proxies such as sediments or tree rings, isotopic data provide information that can be directly linked to human activities [13].

Carbon and nitrogen isotope analyses of bone collagen and dental dentin allow the identification of dietary sources and trophic levels. $\delta^{13}C$ values differentiate between C_3 and C_4 plants and between marine and terrestrial food resources, while $\delta^{15}N$ values reflect protein intake and trophic position [1, 62]. These isotopic indicators reveal not only nutritional strategies but also broader aspects of economy and environmental adaptation among populations.

The analysis of strontium and oxygen isotopes provides complementary information on geographic origin and mobility. Strontium isotope ratios ($^{87}Sr/^{86}Sr$) mirror local geological signatures, while oxygen isotope values ($\delta^{18}O$) reflect climatic conditions and drinking-water sources [5]. Combined, they allow the identification of individuals who migrated between regions or experienced changes in living environments during their lifetime. Such data are crucial for understanding demographic movements, trade networks, and patterns of interaction within and between ancient communities.

Isotopic investigations also contribute to interpreting social structure and inequality. Variations in diet or mobility may correspond to differences in social status, gender, or occupation. For instance, isotope studies have demonstrated that elite individuals often consumed diets higher in trophic level proteins, while non-local individuals might exhibit distinct isotopic signatures linked to migration and integration processes [35]. When integrated with spatial and burial data, isotope evidence enhances interpretations of community organization and social stratification.

The development of isotope analysis in archaeology has its roots in mid-20th-century geochemical research. Early studies demonstrated that isotopic compositions in biological and geological materials reflected environmental conditions [75]. By the late 1970s and 1980s, pioneering work by Van der Merwe, Vogel, and Ambrose established the use of carbon and nitrogen isotopes in reconstructing human diets [77, 2]. Subsequently, isotopic methods expanded to explore geographic mobility through strontium and oxygen systems [16, 5]. In recent decades, isotope research has moved beyond subsistence and provenance studies to encompass social and cultural interpretations, such as migration, marriage exchange, and inequality [48].

Technological advancements have further broadened the analytical potential of isotope research. High-resolution instruments like Isotope Ratio Mass Spectrometry (IRMS) and Multi-Collector Inductively Coupled Plasma Mass Spectrometry (MC-ICP-MS) enable precise multi-isotope measurements. Incremental sampling methods, meanwhile, permit temporal reconstructions of individual life histories [58]. These innovations have consolidated isotope analysis as an indispensable component of archaeological science.

The present study synthesizes these methodological and interpretative developments, focusing on how isotope analyses inform key bioarchaeological themes diet, mobility, settlement patterns, and social organization. By integrating case studies from Türkiye and other regions, the paper aims to demonstrate how isotopic data can refine archaeological interpretations and illuminate the complex interplay between biological signals and cultural practices. Ultimately, isotope analysis has evolved from a purely methodological technique into an interdisciplinary research paradigm central to the holistic reconstruction of past human lives.

1. Isotopes Used in Human Skeletal Remains

In bioarchaeological research, the analysis of various isotopic systems preserved in human skeletal tissues has been shown to provide valuable insights into past diets, mobility, and environmental interactions. Each element proffers distinct yet complementary information. For instance, carbon and nitrogen isotopes primarily reflect dietary protein sources and trophic levels; oxygen and strontium record geographic and climatic signatures that inform on migration and provenance; while sulfur and lead isotopes contribute additional data on local ecology and exposure to anthropogenic or geological influences. The subsequent sections delineate the principles and interpretive potential of these major isotopic systems in the context of human skeletal remains. **Table 1** provides an overview of the main isotopic systems used in bioarchaeology, illustrating their analytical scope, interpretative value, and major limitations.

1.1. Carbon Isotopes

Carbon isotopes ($\delta^{13}C$) are among the most frequently employed indicators in the reconstruction of past societies' diets. This isotope displays marked variations based on the photosynthetic pathways of plants. C3 plants (e.g. wheat, barley, rice, many fruits and vegetables) are characterised by low δ13C values (approximately –35‰ to –20‰), while C4 plants (e.g. corn, millet, sorghum) have higher values (approximately –14‰ to –9‰). It is evident that these isotopic differences are directly reflected in human and animal tissues; as such, the analysis of the carbon isotope ($\delta^{13}C$) is a powerful tool with which to gain a greater understanding of agricultural strategies and dietary patterns [70, 79]. Furthermore, the analysis of carbon isotopes is of critical importance in the differentiation of marine and terrestrial sources. In terrestrial ecosystems, diets based on C3 plants in particular show lower δ13C values, while marine ecosystems are distinguished by richer carbon isotope values (~ –12‰). Consequently, the analysis of the stable isotope δ13C in human faeces provides a reliable indication of the relative proportion of marine sources in an individual's diet [9, 66]. From an archaeological perspective, the analysis of δ13C data provides insights into not only the dietary preferences of individuals but also the processes of agricultural diffusion, the dietary differences between coastal and inland communities, and the effects of social stratification on diet. For instance, the early adoption of corn cultivation in the Americas has been clearly documented through the use of δ13C analyses [79]. The analysis of δ13C data can provide a biochemical indicator for archaeological research, contributing to a comprehensive understanding of economic subsistence patterns and environmental adaptations.

1.2. Nitrogen Isotopes

Nitrogen isotopes ($\delta^{15}N$) are a fundamental indicator in dietary analysis, particularly for determining trophic levels and protein sources. As protein consumption is directly reflected in bone collagen, the $\delta^{15}N$ values of these bones are of critical importance in distinguishing between animal and plant-based proteins in an individual's diet. As demonstrated in the seminal study by Minagawa and Wada [46], there is an approximate 3–5‰ increase in the values of the delta-15N isotope at each trophic level in the food chain. This results in individuals consuming the meat of herbivorous animals or seafood having higher delta-15N values. Terrestrial ecosystems characteristically exhibit lower δ15N values, in contrast to the elevated levels observed in marine ecosystems, a phenomenon attributable to the extended duration of the food chain [66]. Consequently, the analysis of nitrogen isotopes ($\delta^{15}N$) is imperative in elucidating the extent to which coastal communities are reliant on seafood, as well as the significance of agricultural production and animal husbandry in the diet of terrestrial communities. Furthermore, the use of $\delta^{15}N$ values has been employed to evaluate the impact of cultural factors, including social stratification and gender roles, on dietary habits. For instance, elevated $\delta^{15}N$ levels among elites in certain societies suggest that their diets were rich in animal protein, particularly meat [26]. However, it should be noted that the $\delta^{15}N$ values are sensitive not only to diet but also to environmental factors. Variables such as fertiliser use in soil, climatic conditions, or water stress have been shown to affect the nitrogen isotope composition of plants [6]. These environmental factors can also affect human tissues indirectly. Analysis of nitrogen isotopes ($\delta^{15}N$) is a key method for understanding dietary intricacies, environmental adaptation and agricultural strategy in the past.

1.3. Oxygen Isotopes

Oxygen isotopes ($\delta^{18}O$) have been shown to provide significant insights into environmental conditions, climate variability, and the geographical origins of individuals in bioarchaeological research. In particular, oxygen isotope ratios in phosphate groups found in apatite reflect the isotopic composition of the water consumed by individuals throughout their lives [43, 45]. It is evident that the ingestion of water directly corresponds to the delta-18O values of precipitation. Consequently, deductions can be made concerning the climatic characteristics and geographical disparities in the environment in which the subjects inhabited. For instance, the study found that precipitation exhibited relatively elevated δ18O values at low latitudes and in warmer regions, while the opposite was observed at high latitudes, high altitudes, and in cold climates [11, 23]. In this context, the analysis of oxygen isotopes ($\delta^{18}O$) is a significant tool for determining not only the climatic environments of individuals but also for the study of migration and mobility. As posited by White et al. [81] and Bentley [5], discrepancies in the $\delta^{18}O$ values measured in different teeth or bone tissues of the same individual may suggest that they inhabited disparate geographical regions during their lifetimes. Furthermore, oxygen isotopes are known to reflect not only environmental information, but also the type of water sources; for example, it is well documented that river and lake waters typically carry $\delta^{18}O$ values derived from precipitation, while it is equally well documented that $\delta^{18}O$ values increase in areas close to seawater or in areas exposed to evaporation effects [65]. In bioarchaeology, δ18O analysis complements collagen nitrogen and carbon isotope analysis, helping us understand past climate and human mobility.

1.4. Strontium Isotopes

Strontium isotopes ($^{87}Sr/^{86}Sr$) are a geochemical tool frequently employed in archaeological studies of human and animal remains, particularly in mobility and geographic origin determination studies. The primary rationale for this phenomenon is that the distribution of strontium on Earth is directly related to the age and structure of the underlying geological strata. As Faure and Mensing [20] demonstrated, the $^{87}Sr/^{86}Sr$ ratio varies depending on the age of the rock, with different rock types and geological regions offering distinguishable isotopic signatures. This isotopic difference is transferred from soils to plants and then to human and animal tissues through the food chain. Consequently, the $^{87}Sr/^{86}Sr$ ratios measured in an individual's bones and teeth reflect the geological identity of the geography where they were fed and drank water throughout their life [5]. From a bioarchaeological perspective, it is imperative to recognise that different tissues are indicative of varying periods throughout an individual's lifespan. Tooth enamel, which is formed during childhood, provides permanent information about an individual's geographical origin during their early life. Conversely, bones, which are subject to a continuous process of remodelling, offer insights into mobility during the final 10–15 years of life [59]. This feature facilitates the determination of whether an individual has relocated during different stages of their life by comparing strontium values in teeth and bones. This facilitates a detailed examination of social phenomena, including migration, inter-community mobility of individuals through marriage, the utilisation of trade routes, and even the mobility strategies employed by elite classes. In the domain of archaeological research, strontium isotopes are frequently utilised in conjunction with oxygen isotopes to ensure the attainment of more reliable results. This is due to the fact that strontium analysis alone can be limited in some regions due to the presence of overlapping geological signals. However, when combined with the analysis of δ18O, the geographical origin of an individual can be determined with a greater degree of reliability, taking into account both climatic and geological variability [18]. Furthermore, the use of regional isotope maps, termed "isoscapes," enables the precise determination of the geographical origins of individuals and the timing of their migratory movements to different regions during their lifetimes. The significance of strontium isotopes in archaeology extends beyond the realm of individual mobility, encompassing the broader context of large-scale historical processes. To illustrate this point, consider the mobility of farmers throughout the Neolithic period in Europe, which has been thoroughly documented through 87Sr/86Sr analysis. This method has enabled the reconstruction of interactions with local hunter-gatherer communities and migration routes [5]. Furthermore, the migration of individuals from disparate regions of the Roman Empire to metropolitan areas has been elucidated through enamel strontium analyses [48]. Strontium isotopes have been identified as a scientific tool with the capacity to facilitate comprehension of both the life histories of individuals on a micro level and the processes of social and cultural change on a macro level.

1.5. Sulfur Isotopes

Sulfur isotopes ($\delta^{34}S$) are utilised as a complementary tool in bioarchaeological research to facilitate comprehension of diet and mobility. These elements provide a robust indicator, especially when distinguishing between marine and terrestrial protein sources. The isotopic composition of sulfates in marine environments exhibits higher

$\delta^{34}S$ values in comparison to terrestrial ecosystems; this allows individuals who consume marine products to be distinguished [63]. Furthermore, sulfur isotopes have been demonstrated to reflect regional environmental differences. For instance, individuals residing in coastal regions characteristically exhibit elevated $\delta^{34}S$ values, while those inhabiting inland areas frequently display diminished values. Consequently, sulfur isotopes offer insights into not only dietary patterns but also the geographical origins and potential migratory patterns of individuals [50]. Sulfur analysis in archaeological human remains is typically done on bone collagen. Combined with carbon and nitrogen isotope analyses, it can help show what people ate. $\delta^{34}S$ analysis is special because it can show how much marine protein was eaten. Sulfur isotopes are important for bioarchaeological studies because they can show dietary habits and how people adapted to their environment.

1.6. Lead Isotopes

Lead isotopes (Pb isotopes) represent a potent instrument in the domain of bioarchaeology, employed not primarily for the acquisition of direct dietary information but rather for the elucidation of individuals' geographic origins, environmental exposures, and relationships to human activities such as mining or metalworking. Lead isotope composition is specific to its geological source; Pb isotope ratios in rocks and ores from different regions are distinguishable [47]. This feature enables the measurement of Pb isotope ratios in human and animal remains, thereby facilitating the identification of the environmental sources to which individuals were exposed during their lifetimes and, consequently, their geographical origins. For instance, elevated levels of lead (Pb) and region-specific isotopic signatures have been identified in the bones and teeth of individuals inhabiting areas with intensive mining operations in ancient times [24]. In this context, the use of Pb isotopes in conjunction with strontium and oxygen isotopes in mobility studies has been shown to contribute to the determination of whether individuals migrated or resided in different regions [48]. Furthermore, lead isotopes play a critical role in understanding environmental pollution and toxic exposure in past societies. Lead used in water pipes during the Roman period left detectable isotopic traces in individuals' skeletons, thus providing insight into the discussion of environmental poisoning in ancient societies [60]. In summary, Pb isotope analyses are regarded as a multifaceted research instrument in bioarchaeology, encompassing geographical mobility and human-environment interactions.

1.7. Other Isotopes

In recent bioarchaeological research, the use of hydrogen isotopes (δD or δ2H) has increased. As Ehleringer et al. [14] demonstrate, hydrogen isotopes are essentially linked to drinking water sources and are therefore considered a powerful complementary factor in determining geographical origin. This phenomenon can be attributed to the fact that the δD value of precipitation is subject to variation in accordance with climatic factors, including latitude, altitude, and distance from the coast. The analysis of hydrogen isotope values in biological tissues, such as tooth enamel and hair, has been demonstrated to yield insights into the climate region in which an individual resided. When utilised in conjunction with δ18O, particularly within the context of migration and mobility studies, they facilitate the discernment of climatic and environmental variations.

Another development is the isotope analysis of elements such as calcium ($\delta^{44/42}Ca$) and barium (Ba/Ca ratios). Calcium isotopes have been demonstrated to be directly linked to individuals' mineral metabolism [74], and are utilised, particularly in weaning studies, to track childhood nutrition. Barium, too, has been observed to demonstrate distinct isotopic changes during the transition from breast milk to a normal diet. Studying Ca and Ba isotopes offers new insights into the health, growth and life cycles of historical communities.

Furthermore, the use of zinc isotopes ($\delta^{66}Zn$) has become a significant tool in the reconstruction of dietary habits in recent years. Zinc isotope ratios have been shown to be sensitive to trophic levels and can be used to distinguish between carnivorous, herbivorous, and omnivorous diets [33]. This provides a valuable additional data source, especially when carbon and nitrogen isotopes are limited (e.g., when comparing communities consuming similar resources within the same ecosystem).

It has been established through further research that iron (Fe) and copper (Cu) isotopes are associated with metabolism, nutrition, and health status. For instance, the monitoring of health issues such as anaemia or iron deficiency is facilitated by iron isotopes, while the analysis of differences in the food chain is enabled by copper isotopes [31]. These isotope systems are still in the process of becoming widespread in bioarchaeology; however, due to their direct linkage to human metabolism and health biochemistry, it is anticipated that their utilisation will be more prevalent in the future.

Table 1 Comparative overview of major isotope measurement techniques used in bioarchaeology

2. Sample Types

2.1. Dental Enamel

Dental enamel is a frequently utilised sample type in the domain of archaeological isotope analysis. The primary rationale for this phenomenon pertains to the high mineralisation level and absence of organic matter characteristic of enamel, which renders it highly resistant to decay. This characteristic renders enamel one of the most resistant biological tissues to both diagenetic (environmental change-related) deterioration during burial and post-depositional chemical processes [41]. Consequently, tooth enamel is regarded as a reliable source for the analysis of oxygen ($\delta^{18}O$) and strontium ($^{87}Sr/^{86}Sr$) isotopes. Tooth enamel begins to mineralise during early childhood and does not undergo biological reshaping after this process is complete. This property is attributed to the capacity of tooth enamel to serve as a "fixed record" of the individual's childhood nutrition and environmental conditions, as previously described by Hillson [28]. For instance, while analyses of oxygen isotopes ($\delta^{18}O$) reflect the isotopic composition of drinking water and provide information about the climatic and geographical characteristics of the region where the individual resided [43], strontium isotopes carry traces of the geological strata in the region where the individual resided [5].

Furthermore, analyses of carbonate ($\delta^{13}C$) in enamel provide indirect insights into dietary patterns. The carbonate fraction has been demonstrated to be a reliable indicator of C3 and C4 plant consumption patterns, as it reflects the carbon isotope composition of the nutrients metabolically processed by the individual [39]. However, it should be noted that, in contrast to collagen, carbonate does not directly reflect the

Table 1 Comparative overview of major isotope measurement techniques used in bioarchaeology

Technique	Sample type	Target isotopes / elements	Analytical method	Information provided	Main limitations
Carbon ($\delta^{13}C$)	Bone collagen, dentin, enamel carbonate	$^{13}C/^{12}C$	IRMS	Differentiates C_3 and C_4 plant consumption; distinguishes marine vs terrestrial diets	Requires well-preserved collagen; subject to diagenetic alteration
Nitrogen ($\delta^{15}N$)	Bone collagen, dentin	$^{15}N/^{14}N$	IRMS	Indicates trophic level and main protein source	Influenced by physiological stress and environmental variability
Oxygen ($\delta^{18}O$)	Tooth enamel carbonate and phosphate	$^{18}O/^{16}O$	IRMS	Reflects local drinking water composition and paleoclimate; used in mobility studies	Affected by temperature shifts and water isotope variability
Strontium ($^{87}Sr/^{86}Sr$)	Tooth enamel, bone	$^{87}Sr/^{86}Sr$	MC-ICP-MS	Identifies geographic origin and migration patterns based on local geology	Requires detailed baseline data; post-depositional contamination possible
Sulfur ($\delta^{34}S$)	Bone collagen, hair, keratin	$^{34}S/^{32}S$	IRMS	Differentiates marine and terrestrial protein sources; supports mobility and diet reconstruction	Sensitive to diagenetic and environmental sulfur variation
Lead (Pb isotopes)	Bone, enamel	^{206}Pb, ^{207}Pb, ^{208}Pb	MC-ICP-MS	Traces exposure to metal sources; reflects industrial or geological background	Contamination risk; complex interpretive framework
Trace elements (e.g., Zn, Ca, Ba)	Bone, enamel	Elemental ratios	ICP-MS	Supplements isotope data in dietary and environmental reconstruction	Strongly affected by diagenesis; indirect dietary indicator

protein source, but rather indicates the overall nutritional signal. Consequently, enamel carbonate data are typically evaluated in conjunction with collagen data to perform a more comprehensive dietary reconstruction [2].

2.2. Dentin

Dental dentin is a critical biological record source for examining an individual's life history through isotope analysis. In contrast to enamel, which is predominantly composed of organic components, dentin contains both organic (particularly collagen) and inorganic elements. In this respect, dentin facilitates the analysis of both carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes, thereby revealing the dietary and nutritional patterns of individuals during the periods of childhood and adolescence with high resolution [69, 4]. Dentin samples are widely utilised in the assessment of individuals' dietary habits during early life stages, particularly due to the collagen fraction's capacity to provide reliable information regarding protein-based nutrition. The most significant attribute of dental dentin is its incremental (layered) growth. The accumulation of these tissues during dentinogenesis enables the separate storage of biochemical records reflecting distinct age periods within an individual. Consequently, the analysis of dentin using isotope techniques, such as micromilling or serial sampling, facilitates the tracking of dietary shifts and nutritional transitions across various life stages, from childhood to adolescence [3]. To illustrate this point, the weaning period, alterations in protein sources, or nutritional variations associated with social crises can be traced in meticulous detail through dentin isotope profiles.

Furthermore, dentin has been shown to be a reliable indicator of environmental stresses and nutritional deficiencies experienced during childhood. As posited by Fuller et al. [22], sudden increases or decreases in nitrogen isotope values have been shown to be indicative of metabolic stress or disease processes. Consequently, dentin functions not only as a biochemical archive of diet but also of health and stress factors. Dentin has been shown to be more fragile than enamel in post-burial processes and is more sensitive to diagenetic changes [34]. Samples must be carefully preserved and diagenetic tests conducted as part of dentin analysis. Dentin is a valuable sample type for isotope analysis as it provides unique information about an individual's early life.

2.3. Bone Collagen

Bone collagen is the most commonly used organic fraction in archaeological isotope analyses and is a critical source of information, particularly for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses. It has been determined that collagen constitutes approximately 90% of the organic composition of bone and provides direct information about protein-based nutrition [1]. Consequently, through the analysis of bone collagen, the long-term dietary habits of individuals can be evaluated in detail, as well as the diversity of their food sources. The defining biological characteristic of bone tissue is its continuous remodelling. It is important to note that this process occurs at different rates in different bone types. For instance, the cortical part of long bones exhibits a slow metabolism, while bones such as ribs are subject to more rapid renewal. As a result, the isotope values obtained from collagen usually show the average dietary information for the final 10–20 years of a person's life [27]. This makes collagen a powerful tool for investigating the dietary habits of adults.

Carbon isotopes ($\delta^{13}C$) have been shown to reveal C_3/C_4 plant differentiation and the consumption ratios of marine/terrestrial food sources [12, 66]. Nitrogen isotopes

($\delta^{15}N$) have been demonstrated to reflect trophic level in the food chain and, in particular, animal protein consumption. Consequently, bone collagen is pivotal in comprehending the agricultural strategies employed by communities and their subsistence patterns, encompassing hunting-gathering, pastoralism, and mixed economies. Furthermore, increases in $\delta^{15}N$ values have been shown to indicate weaning processes in children or metabolic changes associated with environmental stress conditions [21]. However, diagenetic processes represent a significant limitation in the analysis of collagen. It is hypothesised that organic molecules may undergo degradation as a consequence of the burial conditions to which they are subjected, with the result that the isotopic signal may be compromised. As a result, quality control standards such as the C: N ratio are often used to judge the preservation state of collagen. In well-preserved samples, this ratio is expected to fall within the range of 2.9 to 3.6 [1, 77]. This criterion enhances the reliability of the obtained isotope data and prevents misinterpretation.

2.4. Appatite Carbonate Samples

Apatite carbonate, the inorganic component of bones and teeth, provides a valuable alternative to collagen for dietary reconstruction, in addition to the information collagen offers in isotope analyses. The carbonate ions embedded within hydroxyapatite crystals reflect the comprehensive traces of the foods consumed throughout an individual's lifetime. While collagen, the organic fraction, essentially represents protein sources, the carbonate portion of apatite reveals a broader dietary spectrum encompassing all macronutrient groups, including carbohydrates and lipids [39, 2]. Consequently, the δ13C values obtained from apatite are pivotal in determining agricultural consumption and different energy sources. A notable benefit of apatite carbonate is its dual presence in both bones and teeth. Bone apatites have been shown to represent the diet of adults in the last decades of their lives on account of the continuous biological transformation (remodelling) they undergo [71]. Conversely, the crystal structure of apatite in tooth enamel undergoes mineralisation during development and remains biologically unchanged thereafter. This feature provides a reliable record of the foods consumed during childhood through enamel apatite [83].

Isotope data obtained from apatite carbonate are generally based on $\delta^{13}C$ analyses. These values provide a collagen-independent control for determining C3/C4 plant consumption ratios and are particularly helpful in understanding the energy contribution of different food groups [41]. For instance, while collagen is indicative of a collagen-protein-rich diet, the δ13C signal from apatite may offer a more precise reflection of the consumption of plant sources such as grains and starch. Consequently, a combined evaluation of these fractions facilitates a more comprehensive interpretation of the diet. Furthermore, it has been demonstrated that apatite is subject to limitations in terms of its resistance to diagenetic changes. In comparison with collagen, it has been shown that the inorganic structure of apatite is more susceptible to such changes, and this is dependent upon the burial conditions [36, 51]. In particular, the processes of dissolution or recrystallization in groundwater have the potential to distort the original isotopic signal of apatite. Therefore, quality control methodologies that verify crystal structure, such as Fourier transform infrared spectroscopy (FTIR), are widely used to ensure the reliability of data obtained from apatite [71].

2.5. Post-cremation Isotope Analysis

Despite the fact that cremation was a widespread component of funeral practices within various communities during both the ancient and medieval periods, it nevertheless imposes considerable constraints on the feasibility of conducting isotope analysis. It has been established that elevated temperatures, ranging from 600 to 900 degrees Celsius, result in the complete combustion and destruction of the organic fraction, including components such as bone collagen. Thus, conventional diet analyses based on carbon and nitrogen isotopes are largely unfeasible [78]. However, it should be noted that not all biochemical information is necessarily lost; the inorganic fraction, namely the carbonate and phosphate components of apatite, is often partially preserved. Appropriate processing methodologies can be used to extract paleoenvironmental and biogeographic insights from both carbon and oxygen isotopes within these structures [83].

Furthermore, the degree of cremation is a pivotal factor in the success of the analysis. It has been demonstrated that bones exposed to medium temperatures (approximately 300–500 °C) may undergo partial changes in isotopic fractions without compromising their structural integrity. In contrast, bones that have undergone high-temperature burning may exhibit microscopic recrystallization, a process that has the potential to distort the original isotopic signal [38]. As a result, investigators must first evaluate the trustworthiness of the information by carrying out mineralogical inspections, such as Fourier Transform Infrared Spectroscopy (FTIR) or X-ray diffraction (XRD), on burned remains [15].

In the domain of post-cremation studies, the isotopes strontium ($^{87}Sr/^{86}Sr$) and oxygen ($\delta^{18}O$) hold particular significance. This phenomenon can be attributed to the fact that these elements are embedded within the inorganic fraction, thereby retaining their traces even after the process of combustion. For instance, strontium analysis continues to serve as a reliable method for elucidating the geographic mobility of individuals; nevertheless, the implementation of comparative controls is imperative in light of the diagenetic effects engendered by cremation [25]. Furthermore, oxygen isotopes have been shown to be a reliable indicator of climatic and geographical conditions. However, it should be noted that the process of bone combustion can result in deviations from the expected values of the delta-18O ratio, primarily due to the loss of water from the bone during this process.

3. Methodological Approaches in Isotope Analysis

The reliability of isotope analyses in bioarchaeological research is contingent on the rigour of the methodological approaches applied. In this context, numerous stages play a critical role, from sampling strategies to laboratory preparation processes, measurement techniques to contamination control. Given that each biological tissue (bone collagen, dental dentin, dental enamel, carbonate fraction of apatite) possesses different biochemical properties, it is essential to select the most appropriate material and method for the research question being addressed [1, 39].

One of the most significant steps in the process of obtaining isotope samples from bioarchaeological material is the evaluation of the preservation status of said samples. In the context of archaeological environments, bones and teeth are subject to diagenetic processes, which have the capacity to degrade organic components and result in deviations from the original biological signals in terms of isotopic values [73].

Before sampling, the collagen's integrity is verified through microscopic examinations, Fourier-transform infrared spectroscopy (FTIR) analyses, and amino acid profile measurements. Similarly, surface cleaning procedures are imperative prior to laser ablation analyses on tooth enamel to remove environmental contaminants [47].

The standard method for carbon and nitrogen isotope analysis involves the extraction of collagen from human and animal bones. The protocols developed by Longin [42] and subsequently modified have enabled the isolation of the organic fraction (collagen) from bone. The process typically involves demineralisation of bone powder, removal of humic acids with an alkaline solution, and subsequent gelatinisation [7]. A carbonate content of less than 1% and a nitrogen expectation of 3–5% are considered to be indicative of well-preserved collagen, which is essential for the preservation of tissue integrity [1].

In the context of inorganic fraction analyses, the carbonate portion of apatite is targeted. Apatite carbonate, while an additional source of carbon isotopes, is more susceptible to diagenetic alteration [68]. In final, results obtained without testing the preservation of the crystal structure using XRD (X-ray Diffraction) or FTIR analysis are not considered reliable.

Dental dentin and enamel are unique in that they provide "time series" for different periods of life. Incremental sampling, utilising micro-milling or laser ablation techniques, facilitates high-resolution tracking of dietary and mobility changes during childhood and adolescence [4]. This methodology facilitates meticulous scrutiny of discrete phases within an individual's life history, including, but not limited to, weaning periods, migratory patterns, and periods of infirmity.

The most common method employed in the analysis of isotopes is Isotope Ratio Mass Spectrometry (IRMS). The measurement of gases (CO_2, N_2, SO_2), obtained from organic fractions, is conducted using an IRMS device. Subsequently, the calculation of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values is undertaken [12]. Thermal Ionization Mass Spectrometry (TIMS) and, more commonly, Multi-Collector Inductively Coupled Plasma Mass Spectrometry (MC-ICP-MS) are utilised for the measurement of strontium, lead, and rare elements [5]. The precision of these methods is such that they can determine $^{87}Sr/^{86}Sr$ ratios with very low error margins (±0.00001).

The most significant methodological challenge encountered in the analysis of archaeological samples pertains to the issue of contamination and diagenetic changes, which are primarily influenced by environmental factors. Carbonates from soil water, humic acids bound to collagen, or elements integrated into the phosphate structure have been shown to distort the original isotopic signal [72]. Therefore, it is recommended that samples undergo chemical pretreatment, be compared with reference modern samples, and that different tissues from the same individual be analysed for cross-checking purposes.

All isotope analyses are reported in accordance with international standards. The δ13C values are then normalised to Vienna Pee Dee Belemnite (VPDB), the δ15N values to Air-N2, the δ18O values to Vienna Standard Mean Ocean Water (VSMOW), the δ34S values to Canyon Diablo Troilite (CDT), and the strontium isotopes to the NIST SRM 987 standard [10]. This standardisation ensures the comparability of analyses performed in different laboratories and forms the basis of methodological reliability.

Isotope Measurement Techniques: The instruments employed in bioarchaeological isotope analysis are contingent on the specific isotope to be measured. Light element isotopes (e.g. carbon, nitrogen, oxygen, sulphur and hydrogen) are typically measured using Isotope Ratio Mass Spectrometry (IRMS), while heavy element isotopes (e.g. strontium, lead, neodymium, calcium and zinc) are measured using Thermal Ionization Mass Spectrometry (TIMS) or the more modern and accurate method, Multi-Collector Inductively Coupled Plasma Mass Spectrometry (MC-ICP-MS) [5, 10].

IRMS (Isotope Ratio Mass Spectrometry): Gas products (CO_2, N_2, SO_2, H2) obtained from organic fractions (collagen, keratin) are ionized, and their isotopic ratios (e.g. 13C/12C, 15N/14N) are measured. The device has been engineered to detect differences at the ppm level, with a documented accuracy of ±0.1‰. Thus, it is the most frequently employed device in paleodiet studies [12].

TIMS (Thermal Ionization Mass Spectrometry): This technique, whilst not the most contemporary, is one that has been demonstrated to be both dependable and effective in measuring the isotopic ratios of heavy elements. The sample is ionised on a heated filament and separated in a mass spectrometer. It has played a pioneering role in strontium isotope analysis, but due to the lengthy sample preparation and low throughput, it has largely been replaced by MC-ICP-MS today [20].

MC-ICP-MS (Multi-Collector Inductively Coupled Plasma Mass Spectrometry): The plasma environment is used to ionize the sample, with different isotope masses being measured simultaneously in multiple collectors. This method provides high precision (±0.00001) for elements such as Sr, Pb, Ca, and Zn and has become indispensable, especially in mobility studies [48]. Moreover, the necessity for reduced sample quantities represents a substantial benefit for archaeological material.

Sample Preparation and Preliminary Preparations: In the domain of isotope analysis, laboratory preparations are of paramount importance for the reliability of the results obtained, in equal measure to the sensitivity of the instruments utilised. In the preparation of collagen samples, a two-step procedure is employed. Initially, demineralization is carried out using hydrochloric acid, followed by the removal of humic acids through the use of sodium hydroxide. In the final stage, gelatinization is performed at temperatures ranging from 58 to 70°C. The preservation of collagen is determined by the %C, %N, and C/N ratio (3.2–3.6) [1, 7]. In the process of preparing apatite carbonate, organic and secondary carbonates are meticulously removed from the sample using a combination of NaOCl and acetic acid. The carbonate-phosphate ratio and crystallinity index are evaluated using Fourier-transform infrared spectroscopy (FTIR). High crystallinity is generally indicative of diagenetic alteration [67]. In the case of strontium/Pb, tooth enamel is micro-milled into powder. The substance is then dissolved in an acid solution, after which Sr or Pb ions are separated using selective chromatographic resins. This step is critical, especially in Sr isotope analysis, to prevent environmental contamination [5].

In recent years, the advent of sophisticated techniques in the analysis of isotopes has resulted in a paradigm shift within the domain of bioarchaeological research, thereby introducing novel dimensions to the field. Laser Ablation (LA-MC-ICP-MS) has been instrumental in facilitating micro-scale incremental analysis of tooth enamel or bone. This methodology enables the identification of fluctuations in migration, disease, or dietary patterns over time [47]. In dual isotope approaches, the combination of δ13C–δ15N provides more reliable results regarding diet, while the combination of δ18O–87Sr offers insights into mobility. The combination of δ34S–δ13C provides insights into the marine versus terrestrial sources of nutrition [62]. New biomarker isotopes, including zinc (Zn) and calcium (Ca) isotopes, have recently emerged as a means to determine predator-herbivore separation or plant/animal protein consumption ratios [32]. This approach enables the execution of more detailed dietary interpretations that extend beyond the scope of classical C–N analyses.

Bioarchaeological Applications

Isotope analysis is a powerful research method that enables the understanding of biological, cultural and social processes through the analysis of archaeological human skeletons. In this context, the primary research areas include dietary reconstruction, determining mobility and migration movements, and analysing lifestyles related to social structure.

It is imperative to recognise that diet constitutes a pivotal parameter for comprehending the economic organisation, environmental adaptations, and social distinctions of bygone societies. Analyses based on carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes have been used to reveal the photosynthetic pathways (C_3/C_4) of the plants consumed by individuals, and thus determine their trophic levels in the food chain. For instance, the analysis of the isotope composition of European Neolithic societies indicates the predominant consumption of C3 plants such as wheat, barley, and legumes [2, 76], while the analysis of the isotope composition of agricultural societies in Central and South America shows that C4 plants such as corn were the primary food source [76]. Conversely, analyses of nitrogen isotopes have facilitated the differentiation of diets derived from marine sources and those originating from terrestrial sources. This provides substantial evidence that these distinctions mirrored social and economic transformations, particularly during the transition from the Mesolithic to the Neolithic era [63].

Strontium ($^{87}Sr/^{86}Sr$) and oxygen ($\delta^{18}O$) isotope analyses are utilised to elucidate individuals' exposure to disparate geographical environments throughout their lifetimes and potential migratory movements. It is evident that data obtained from tooth enamel offers a unique identifier of an individual's geographical origin during childhood. Sr and O isotope analyses conducted on individuals buried near Stonehenge in England have shown that these people came from outside the British Isles in the early stages of their lives, reflecting labour mobility in monumental architecture [19]. Analyses of cemeteries from the Roman and Byzantine periods have revealed the biological traces of inter-imperial migration and urbanisation, thereby contributing to a more concrete documentation of social diversity [47].

Isotope analysis has been demonstrated to reflect not only environmental adaptations but also differences in social structure. When compared with parameters such as dietary differences, gender, age, social status, or ethnic identity, traces of hierarchical structures in past societies can be seen. For instance, isotope analysis of individuals with high social status in Maya societies revealed a greater consumption of corn and seafood compared to individuals with lower status [80]. In medieval European urban societies, significant dietary disparities were observed between monastic individuals and the lay population, a phenomenon attributed to religious directives and social constraints [49].

The examples presented illustrate the effectiveness of isotope analysis as a tool for understanding a variety of phenomena, such as biological processes, social organisation, economic systems, and cultural preferences. As a result, when assessed in an archaeological context with material culture, isotope information allows for a thorough comprehension of the habits of ancient human societies.

The Neolithic Revolution is widely regarded as one of the most significant economic and social transformations in the history of humankind. The transition from hunter-gatherer strategies to agriculture during this period can be directly traced through isotope analysis. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses conducted in Europe demonstrate that hunter-gatherer societies maintained a diet comprising marine and hunter-gatherer food sources with elevated $\delta^{15}N$ values [61]. In Neolithic agricultural societies, an analysis of δ13C values indicates a transition to a diet comprising C3 plants, accompanied by a significant decrease in δ15N values. This change is indicative not only of the transformation of the economic system, but also of the diversification of food sources and the contribution of animal husbandry to social organisation.

The vast geography and multicultural structure of the Roman Empire renders it a significant case study for research into mobility. Strontium ($^{87}Sr/^{86}Sr$) and oxygen ($\delta^{18}O$) isotope analyses conducted on cemeteries from the Roman period have enabled the biochemical identification of individuals who migrated to the empire's major cities [17]. For instance, analyses conducted at the Casal Bertone cemetery in Rome revealed that some individuals came from outside Italy, providing strong evidence of the city's cosmopolitan structure [47]. The findings of this study illuminate not only the phenomenon of population movements but also the social dynamics associated with labour, military campaigns, and trade within the empire.

Isotope analysis of medieval European remains has provided biochemical evidence indicative of social differences. A comparative analysis of monastic and lay cemeteries in England revealed distinct differences in the isotope values of carbon and nitrogen. As Müldner and Richards [49] observed, low $\delta^{15}N$ values were detected in monastic communities due to limited meat consumption, while higher protein consumption was observed among the lay population. This discrepancy is indicative of the influence of religious doctrine on day-to-day life and the manner in which dietary practices have contributed to the formation of social structures.

Carbon and nitrogen isotope analyses conducted in Maya societies in Mesoamerica reveal how social stratification was reflected through diet. The δ13C values of the elite classes indicate a high consumption of maize (a C4 plant) and a greater inclusion of marine products in their diet [80]. Conversely, the general populace adhered to a dietary regime comprising primarily C3 plants and land animals. This discrepancy suggests that economic power was not the sole factor influencing the dietary choices of the elite; religious rituals and ideological systems also played a significant role.

Türkiye boasts a rich archaeological heritage, spanning from the prehistoric era to the Byzantine period. Isotope analysis is a pivotal tool in understanding dietary habits, mobility patterns, and social structures during this historical era. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses conducted on Neolithic settlements, particularly at centres such as Çatalhöyük and Aşıklı Höyük, demonstrate that the diets of these societies were predominantly based on C3 plants (e.g. wheat, barley, legumes) and that animal protein consumption was limited [56, 2]. The data presented herein provide direct evidence of the economic and social dimensions of the transition to Neolithic agriculture.

Table 2. Isotope analysis studies conducted on Anatolian archaeological societies

Archaeological Period	Site / Region	Main Isotopes Analysed	Key Findings (Diet, Mobility, Weaning)	References
Neolithic	Çatalhöyük (Central Anatolia)	$\delta^{13}C$, $\delta^{15}N$	C_3-based terrestrial diets; moderate animal protein; infant $\delta^{15}N$ elevation indicating breastfeeding; weaning generally completed around 2.5-3 years.	Richards et al. 2003; Pearson et al. 2015
Neolithic	Aşıklı Höyük & Çayönü	$\delta^{13}C$, $\delta^{15}N$	Breastfeeding detectable through $\delta^{15}N$ enrichment; weaning estimated at ~1–2 years for Aşıklı and ~2–3.5 years for Çayönü.	Pearson et al. 2015
Neolithic–Chalcolithic	Tepecik-Çiftlik	$\delta^{13}C$, $\delta^{15}N$	Very early weaning (0.2–1.3 years); C_3-based plant diets; limited high-trophic protein.	Özdemir et al. 2024
Late Neolithic–Chalcolithic	Aktopraklık (NW Anatolia)	$\delta^{13}C$, $\delta^{15}N$	Late weaning (>3–4 years in some contexts); evidence for subsistence diversification.	Budd et al. 2013
Late Chalcolithic – Early Bronze Age	İkiztepe (Black Sea region)	$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	Weaning beginning at 1–1.5 years and ending ~2.25 years; mainly C_3 terrestrial diet; limited marine protein despite coastal location.	Özdemir et al. 2019; Irvine & Erdal 2020
Early–Middle Bronze Age	Kültepe (Central Anatolia)	$\delta^{13}C$, $\delta^{15}N$	Weaning begins at ~1–1.5 years, ends ~3 years; exclusively C_3 terrestrial foods; isotopic data match textual evidence on childcare and wet-nursing.	Özdemir et al. 2025
Middle–Late Bronze Age	Alalakh (Hatay)	$^{87}Sr/^{86}Sr$, $\delta^{18}O$	Clear local vs non-local differentiation; substantial human mobility consistent with trade networks of the Levant–Anatolia corridor.	Ingman et al. 2021

Roman	Ephesus (Gladiator Cemetery)	$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$	Distinct diet groups; mostly C_3 terrestrial diet; sulfur values show limited marine input; social stratification detectable isotopically.	Lösch et al. 2014
Roman–Byzantine	Hierapolis (SW Anatolia)	$^{87}Sr/^{86}Sr$, $\delta^{18}O$	Mostly local individuals; a subset of immigrants, especially in Byzantine layers—consistent with pilgrimage and regional mobility.	Wong et al. 2018
Byzantine	Kovuklukaya (Sinop)	$\delta^{13}C$, $\delta^{15}N$ (+ AA-specific data)	C_3 diets with modest C_4 contribution (millet); $\delta^{15}N$ shows limited marine input despite Black Sea proximity.	Özdemir et al. 2025

Research on stable isotopes in Anatolia and the Near East has documented a long-term stability of C_3-dominated terrestrial diets, versatile mobility, and culturally based weaning in the region over thousands of years. Neolithic settlements including Çatalhöyük, Aşıklı Höyük, and Çayönü provide clear $\delta^{15}N$-based evidence of breastfeeding, and on average weaning occurred between 2 and 3.5 years of age [52, 56, 62]. In contrast, the site of Tepecik-Çiftlik noted remarkably early weaning (0.2–1.3 years) which likely reflects ecological or cultural circumstances [52]. Late Chalcolithic and Early Bronze Ages settlements, such as Aktopraklık and İkiztepe, display an overall pattern of heterogeneous weaning, from early weaning (~1.5 years) to later weaning (>4 years) [8, 53]. Within the general pattern of changing weaning, Kültepe provides one of the best datasets for isotopic evidence of weaning practices, where $\delta^{13}C$ and $\delta^{15}N$ values demonstrate a regular process of weaning beginning c. 1–1.5 years, ending around 3 years of age, and correlating closely with textual evidence pertaining to wet nurses, child care, and household labor [54]. The mobility indicators in the Bronze and Iron Ages (Alalakh to Van and the later Roman-Byzantine Hierapolis) indicate that Anatolia was a pathway for constant migration. Dietary reconstructions of infants from Ephesus and Sinop further represent potential social and geographical variation in protein source of Terrestrial diets with C_3 [29, 30, 44, 55, 82]. Overall, these datasets support that as infant feeding strategies, diet compositions, and mobility were happening concurrently and in relation to an evolving economic structure and urbanization, as well as interregional interactions, Kültepe remains important for assessing childhood, diet, and cultural practice in early complex societies of Anatolia.

Conclusion

Isotope analysis of archaeological human skeletons provides comprehensive information about the lifestyles of past communities. Carbon and nitrogen isotopes have been shown to reveal the basic character of the diet, while strontium and oxygen isotopes have been demonstrated to provide direct evidence about individuals' lifelong mobility and migration movements. Isotopes such as sulfur and lead provide additional information, particularly about protein sources and environmental interactions, illuminating the ecological context of diets and lifestyles.

The isotopic studies conducted across Anatolia on Neolithic farming villages, complex Bronze Age trade centers, and later Byzantine urban contexts reveal that diet and mobility were assimilated into the economic and social strategies of each time period. Variation in $\delta^{13}C$, $\delta^{15}N$ and strontium–oxygen signatures illustrates how access to food resources, exposure to different ecological zones, and participation in regional networks were influenced by the social status of the individual, household labor, and broader political arrangements. High and low-status groups frequently consumed different types of protein, or resources drawn from different locations, and individuals with non-local isotopic signatures indicate long-distance movement, interregional exchange, and cultural integration. The patterns identified in this study therefore extend beyond the biochemical signatures of individuals; rather, they reflect how communities organized labor, created and maintained social hierarchies, formed external connections, and practiced adaptability to changing environments. In this regard, isotopic evidence is a powerful lens in which to view the more holistic economic, cultural, and demographic processes of ancient Anatolian societies.

Methodologically, the combined use of different tissues (bone collagen, dental dentin, dental enamel, carbonate in apatite) allows for detailed tracking of an individual's lifetime diet and migration movements. However, limitations such as post-mortem changes, tooth wear, and environmental effects must be considered. The reliability of the results is increased by the interpretation of isotope analyses in conjunction with archaeological context and other paleoecological data.

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