

GENDER-BASED DIETARY DIFFERENCES AT MERSIN CHAL CEMETERY DURING THE SECOND HALF OF THE FIRST MILLENNIUM

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Abstract: The Mersin Chal cemetery is situated in the village of Telajim, within Mehdi Shahr city in Semnan Province. During the third season of archaeological excavations at the Mersin Chal cemetery in 2021, a total of 49 burials were identified, dating back to the second half of the first millennium BC. One of the main focuses in bioarchaeological research involves examining how physiological differences between sexes influence dietary patterns, often analyzed through the stable isotope $\delta^{15}\text{N}$. This research aimed to investigate the biological differences in the diets of individuals from the Mersin Chal cemetery through bioarchaeological analysis. Aligned with this goal, a question emerged regarding whether the gender differences between males and females result in variations in their nutritional mechanisms. This research addresses the question by analyzing human skeletal remains from 12 of the 49 excavated graves in the Mersin Chal cemetery. This research employs $\delta^{15}\text{N}$ stable isotope analysis on dental collagen samples, combined with SPSS statistical methods, to interpret the findings. The analysis of $\delta^{15}\text{N}$ stable isotope ratios from adult male and female skeletons in the Mersin Chal cemetery reveals that males exhibit slightly higher $\delta^{15}\text{N}$ values compared to females. This suggests greater variation in the amount of protein consumed by males. This difference likely reflects greater protein intake in males, consistent with their higher proportional protein requirements relative to body mass.

Keywords: Stable Isotope Analysis, Human Tooth Collagen, Diet, Gender, $\delta^{15}\text{N}$.

چکیده: گورستان مرسین چل در روستای تلایجیم، در محدوده شهر مهدی شهر استان سمنان واقع شده است. در فصل سوم کاوش‌های باستان‌شناسی در گورستان مرسین چل در سال ۱۴۰۰، مجموعاً ۴۹ تدفین شناسایی شد که مربوط به نیمه دوم هزاره اول پیش از میلاد هستند. یکی از تمرکزهای اصلی در پژوهش‌های باستان‌زیست‌شناسی، بررسی چگونگی تأثیر تفاوت‌های فیزیولوژیکی بین جنسیت‌ها بر الگوهای غذایی است که اغلب از طریق ایزوتوپ پایدار $\delta^{15}\text{N}$ تحلیل می‌شود. این پژوهش با هدف بررسی تفاوت‌های زیستی در رژیم غذایی افراد گورستان مرسین چل از طریق تجزیه و تحلیل باستان‌زیست‌شناسی انجام شد. در راستای این هدف، این سوال مطرح شد که آیا تفاوت‌های جنسیتی بین مردان و زنان منجر به تغییراتی در مکانیسم‌های تغذیه‌ای آن‌ها می‌شود؟ این پژوهش با تجزیه و تحلیل بقایای اسکلت انسانی ۱۲ قبر از ۴۹ قبر کاوش شده در گورستان مرسین چل به این سوال پاسخ می‌دهد. این تحقیق از تحلیل ایزوتوپ پایدار $\delta^{15}\text{N}$ بر روی نمونه‌های کلژن دندان، همراه با روش‌های آماری SPSS، برای تفسیر یافته‌ها استفاده می‌کند. تجزیه و تحلیل نسبت‌های ایزوتوپ پایدار $\delta^{15}\text{N}$ از اسکلت‌های مردان و زنان بالغ در گورستان مرسین چل نشان می‌دهد که مردان مقادیر کمی بالاتر $\delta^{15}\text{N}$ نسبت به زنان دارند. این نشان‌دهنده تنوع بیشتر در میزان پروتئین مصرفی توسط مردان است. این تفاوت احتمالاً نشان‌دهنده مصرف پروتئین بیشتر در مردان است که با نیازهای پروتئینی متناسب با توده بدنی بالاتر آن‌ها مطابقت دارد.

کلیدواژه: تحلیل ایزوتوپ پایدار، کلژن دندان انسان، رژیم غذایی، جنسیت، $\delta^{15}\text{N}$.

I. Introduction

Bioarchaeology studies the biological remains of ancient populations within the context of their cultural environment. Bioarchaeology research primarily relies on the chemical analysis of skeletal remains as its foundational approach (Lewis et al., 2017:45; Basu et al., 2015; Bogaard and Outram, 2013:333). "Stable isotope research is providing new perspectives into the human past, uncovering ecological and cultural dimensions that hold significant relevance" (Somerville et al., 2015:410). Stable isotope analysis studies are interpreted within archaeological theoretical frameworks to investigate various topics. These include palaeodiet, livelihood strategies, approaches to utilizing and managing biological resources, food preparation and consumption practices, changes in human dietary patterns, and the social dynamics of historical communities. Additionally,

these studies shed light on levels of societal complexity, the distinct mechanisms tied to plant and animal management as well as domestication processes, patterns of human and livestock migration, and aspects of social identity and gender distinctions, among other subjects (Somerville et al., 2015:410; Makarewicz and Sealy, 2015:146-147).

Accurate dietary reconstruction necessitates a detailed understanding of the isotopic composition of local food sources, as well as a comprehensive grasp of how nutrition, environmental factors, and physiological processes influence the functioning of dietary systems (Ambrose and Lynette, 1993:1). "Food holds a significant place in human social life, as its acquisition, preparation, distribution, and consumption are deeply embedded within the structure of social relationships. The reconstruction of historical diets and eating habits

plays a crucial role in analyzing gender roles within archaeological research. This is because food preparation and consumption practices are among the most significant ways individuals fulfill their social roles" (Somerville et al., 2015:410). "One key area of research in bioarchaeology focuses on investigating gender differences in physical activity, health, and access to food resources. It also examines potential connections between these differences and social inequalities or distinctions linked to gender roles. Variables used to evaluate potential gender differences in physical activity, health, and diet encompass skeletal and dental features linked to biomechanical stress and certain pathological conditions, as well as stable isotope ratios (especially $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) that facilitate the reconstruction of past dietary habits.

This approach further facilitates the investigation of how biological (physiological) and cultural factors influence the varying responses of males and females to their natural and social environments" (Jiménez-Brobeil et al., 2021:2). To examine the biological distinctions between the two sexes, one of the key and most significant factors is the hormonal variance, which drives differing bodily functions in males and females (Sheikhshoae and Niknami, 2016:80). One key hormonal difference is the presence of testosterone in males. This hormone plays a crucial role as the primary male hormone, significantly influencing the development and maintenance of male secondary sexual characteristics. It also promotes anabolic effects such as increased muscle growth and bone density. Testosterone enhances protein synthesis and promotes muscle growth, leading to male muscle mass being, on average, approximately 50% greater than female muscle mass. Additionally, it contributes to increased protein levels in non-muscular parts of the body as well.

The development and upkeep of muscle mass (approximately 50% greater than that of females), muscle strength, bone density, elevated basal metabolic rate (by 10-15%), overall physical strength, and increased appetite (reflecting higher nutritional requirements compared to women) are heavily influenced by testosterone. This hormone plays a multifaceted and vital role in male physiology (Rojas-Zambrano et al., 2025). It is reasonable to consider that the levels of the stable isotope $\delta^{15}\text{N}$ found in skeletal remains, which are related to the body's protein consumption, might vary between males and females (Sheikhshoae and Niknami, 2016:80). The present study aims to investigate the livelihood strategies and dietary patterns of both sexes within this cemetery by analyzing stable nitrogen isotopes ($\delta^{15}\text{N}$). This analysis is conducted on 12 dental collagen samples extracted from human remains found at the Mersin Chal cemetery.

II. Location of the Research Site

The Mersin Chal cemetery is situated at geographical coordinates X:719131.920 and Y:3991489.190 within the watershed of the Finsk Dam. It lies to the east of Telajim village, on the southern bank of the Esperu River, the Poshtkouh district of Mehdi Shahr city, in Semnan Province (Fig 1). This site was initially surveyed in 2010 by Mortezaei and Maleki (Mortezaei and Maleki, 2010). The first and second archaeological excavation seasons were conducted in 2014 and 2015, led by Mehrdad Malekzadeh (Malekzadeh et al., 2023). The third season of excavation was done in 2021 by two expeditions under the supervision of Mohammad Reza Nemati and Ata Hassanpour. In the third season of archaeological excavations at the Mersin Chal cemetery in 2021, under the supervision of Mohammad Reza Nemati, three trenches, C11, D11, and E11, were opened in the northwestern section of this site (Fig2). These excavations uncovered 49 graves, which were determined to date back to the second half of the first millennium BC (Nemati, 2021).

III. Nitrogen Isotope Studies

Bioarchaeological research utilizes nitrogen and carbon isotopes found in bone or dental collagen to analyze and interpret ancient human diets, offering insights into the consumption of marine resources, terrestrial food sources, or nitrogen-fixing plants (Kelly, 2000: 14; Richards, 2019: 137-138). Nitrogen exists in two naturally occurring stable isotopes, ^{14}N and ^{15}N , whose ratio is typically expressed as $\delta^{15}\text{N}$ (Price, 2015: 73). The nitrogen isotope ratios in plants are largely influenced by the method through which they acquire nitrogen. Nitrogen transfer to the biological domain primarily occurs through two key processes: the activity of nitrogen-fixing organisms and the bacterial decomposition of complex nitrogen-containing compounds in organic matter after an organism's death. Non-nitrogen-fixing plants tend to display higher $\delta^{15}\text{N}$ values compared to nitrogen-fixing plants, which acquire part of their nitrogen directly from atmospheric sources (Agarwal and Glencross, 2011: 414-415; Katzenberg, 2008: 425-426, Price, 2015: 75; Schoeninger and Moore, 1992: 256). Stable nitrogen isotope ratios ($\delta^{15}\text{N}$) serve as a tool for identifying an organism's trophic level within a food web. These ratios are closely linked to protein consumption and typically exhibit $\delta^{15}\text{N}$ enrichments ranging from approximately 3–5‰ (Budd et al., 2017: 5). Carnivores typically exhibit $\delta^{15}\text{N}$ values that are about 3‰ higher than those of herbivores (Richards, 2019: 135–136). This difference is tied to the trophic level effect, where $\delta^{15}\text{N}$ levels in the tissues of carnivores are approximately 3–5‰ higher than those found in herbivorous diets with each successive step along the food chain (Agarwal and Glencross, 2011: 414–415). The $\delta^{15}\text{N}$ values in collagen

typically range between 6–10‰ for human consumers of terrestrial animals and plants. In contrast, individuals who consume freshwater or marine fish, as well as seals and sea lions, often exhibit $\delta^{15}\text{N}$ values of approximately 15–20‰. Higher $\delta^{15}\text{N}$ values are typically

associated with the utilization of marine resources, as more positive nitrogen isotope ratios often reflect a higher trophic level within the food chain (Price, 2015: 75; Richards, 2019: 136).



Figure 1: The location of the Mersinchal cemetery in the aerial image (Google Earth, 2024).

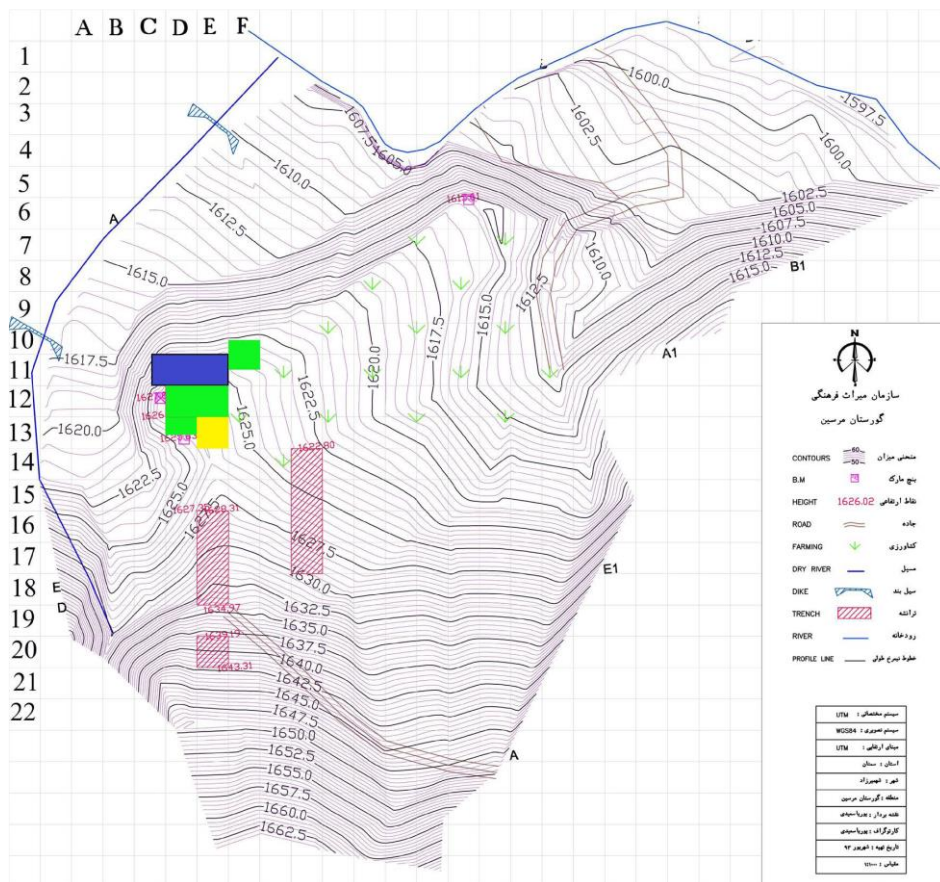


Figure 2: Topographic map of the Mersin Chal cemetery and the location of the excavated trenches in the third and fourth Seasons (Nemati, 2021).

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The ratio of stable nitrogen isotopes in organisms is influenced by their diet and habitat (Katzenberg, 2008: 430-431). $\delta^{15}\text{N}$ values, on the other hand, are determined by factors such as temperature and environmental conditions (Richards, 2019: 136-137). Nitrogen isotope ratios often show significant variability across plant species within the same area. In arid regions, $\delta^{15}\text{N}$ values are typically higher in food webs (Price, 2015: 75). The $\delta^{15}\text{N}$ values can also rise in cases where the diet lacks sufficient protein or when agricultural fertilization is heavily utilized. Furthermore, $\delta^{15}\text{N}$ levels tend to rise during periods of water stress or protein deficiency caused by inadequate protein intake

(Katzenberg, 2008: 428). The analysis of nitrogen isotope ratios serves as a valuable tool for differentiating diets that rely on marine, terrestrial, or nitrogen-fixing plants. Additionally, fluctuations in $\delta^{15}\text{N}$ values may arise as a consequence of food and water crises, highlighting their potential impact (Kelly, 2000: 14). In addition to providing insights into nutritional levels, nitrogen isotope values ($\delta^{15}\text{N}$) are instrumental in identifying environmental factors such as drought resulting from water stress or human activities. They also offer a means of understanding animal management practices, particularly those involving livestock manure (Soltysiak and Schutkowski, 2018: 861). Drier climates or less rainfall have been associated with higher $\delta^{15}\text{N}$ levels in plants (Soltysiak, 2020: 117). Furthermore, agricultural practices involving natural fertilizers, especially animal manure, are known to increase $\delta^{15}\text{N}$ levels (Soltysiak, 2020: 117, Soltysiak and Fernandes, 2021: 1156, Soltysiak and Schutkowski, 2018: 869). Moreover, diets rich in animal-based products also show an association with higher $\delta^{15}\text{N}$ levels (Soltysiak and Schutkowski, 2018: 869). Pregnancy can also affect $\delta^{15}\text{N}$ values (Cooper et al., 2019: 3), highlighting the significance of nitrogen isotopes for studying the timing and patterns of weaning in ancient human populations. Breastfed infants generally exhibit $\delta^{15}\text{N}$ levels that are 2–3‰ higher than their mothers, as their primary nutrition comes directly from maternal tissue through breast milk. During the transitional feeding stage, when breast milk is gradually supplemented with other complementary foods, $\delta^{15}\text{N}$ levels start to decrease as the reliance on breast milk lessens (Agarwal and Glencross, 2011: 415; Richards, 2019: 136). Once weaning is complete, the diet of the child stabilizes, reflecting their typical long-term nutritional intake. Nondietary factors, including nutritional stress, disease, and environmental conditions like extreme drought, can influence $\delta^{15}\text{N}$ levels in humans. Archaeological population studies reveal a notable dietary distinction between males and females (Schoeninger and Moore, 1992: 277). Consequently, any observed gender-based variation in isotopic values could point to a shift in dietary patterns (Agarwal and Glencross, 2011: 415). Physiological factors and differences in protein consumption are generally responsible for variations in stable isotope $\delta^{15}\text{N}$ levels between males and females (Sheikhshoae and Niknami, 2016:80). This research focuses on exploring possible gender-related differences in dietary habits by analysing $\delta^{15}\text{N}$ stable isotope.

IV. Methods and Materials

Twelve human skeletal remains from the Mersinchal cemetery were selected for nitrogen isotopic analysis studies (Fig 3). The skeletal remains studied in the

current research are preserved and relatively articulated. So, the sex estimation of the skeletal remains was done based on the pelvis bone (pubic arch, sciatic notch) and skull bone (glabella, mastoid process, frontal prominence, parietal prominence, occipital prominence, supraorbital margin, mental eminence) (Buikstra, and Ubelaker, 1994; Hager, 1996; Mays, 1998; Tague, 1995; White and Folkens, 2005). The age estimation of the skeletal remains was done based on the anatomical structures of the skeletal remains through the closure of the cranial sutures, the epiphyseal fusion, alterations in the pubic symphysis, stages of degeneration of sternal

rib ends, changes in the auricular surface of the sacroiliac joint, vertebral osteophytes, ossification of the thyroid cartilage, fusion of the hyoid bone and tooth attrition (Buikstra and Ubelaker, 1994; Işcan et al., 1984; Lovejoy et al., 1985; Meindl and Lovejoy, 1985; Oliveira et al., 2006; Todd, 1920; White and Folkens, 2005). Based on age and sex estimation studies, the samples selected for this research all belong to adult individuals, and the samples were a mix of feminine and masculine skeletons. Comprehensive details regarding the samples are provided in Table 1.

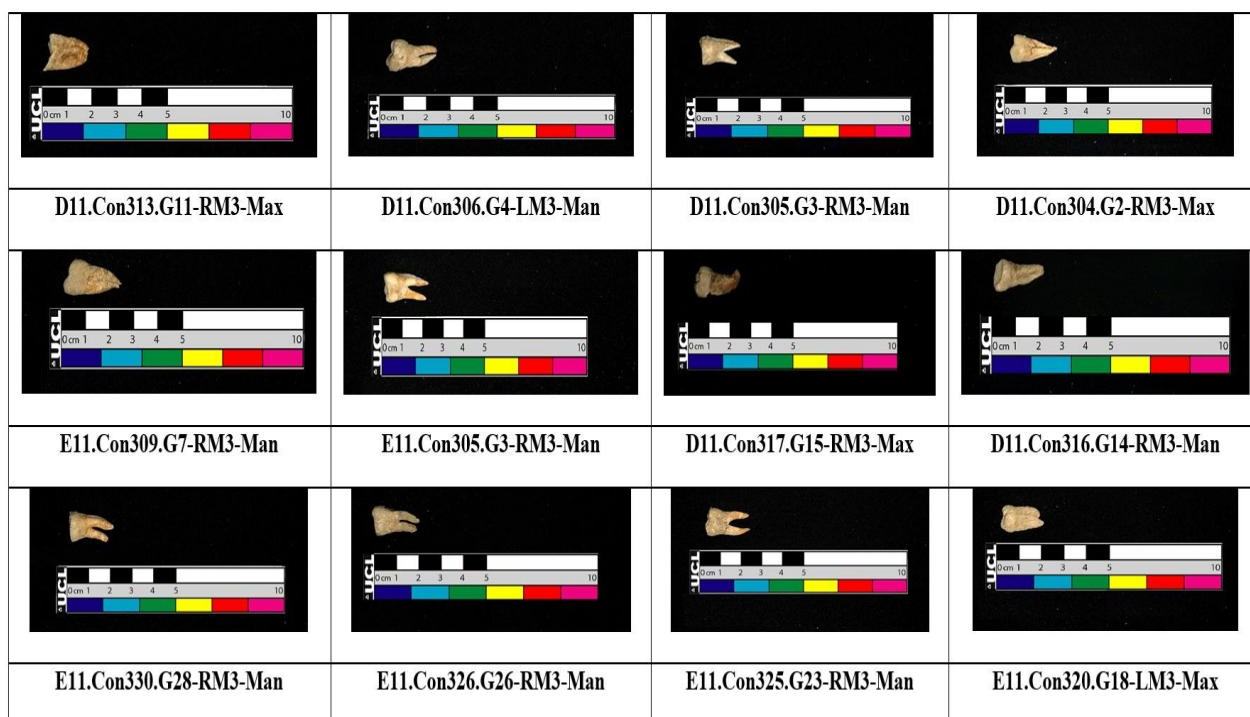


Figure 3: The tooth samples from the Mersin Chal cemetery (Authors, 2021).

Collagen extraction followed a modified Longin procedure as described by Fetner (2015). “First, a dentin sample (c. 100-200 mg) was cleaned in an air blaster with particles of aluminum oxide and then powdered in a hand mortar. Next, the sample was put in a weak (0.5 M) water solution of hydrochloric acid (HCl) for demineralization. The process of demineralization proceeded for a few days at a temperature of about 4°C. Then the sample was washed three times with MilliQ water, and the solution of the sample in the pH 3 MilliQ water was heated to 65–70°C for 48 hours to gelatinize the organic fraction of the bone. After two days, the gelatinized sample was filtered with an Ezee Filter separator. Then, the sample was frozen in liquid nitrogen and freeze-dried.

The $\delta^{15}\text{N}$ measurements were completed in the Center for Physical Sciences and Technology, Vilnius, Lithuania, using an isotope ratio mass spectrometer (Thermo Delta V Advantage) connected with an input

device, an elemental analyzer (Thermo Flash EA 1112), via the ConFlo III interface. The isotopic ratios for nitrogen ($^{15}\text{N}/^{14}\text{N}$) are expressed in permille relative to the international standards (AIR for $\delta^{15}\text{N}$). Caffeine IAEA- 600 ($\delta^{15}\text{N} = +1\text{‰}$) was used as secondary reference material” (Bakhtiari et al., under review).

Human remains that contained well-preserved "collagen" had nitrogen concentrations (above 4.8%), carbon concentrations (above 13%) (Ambrose, 1990: 447), and C/N ratios (2.9-3.6) (Ambrose, 1990: 443, DeNiro, 1985:808). The yield of collagen is suitable (>0.5%) because if the "collagen" content drops below 0.5% (low "collagen" bone), it becomes difficult to remove contamination, and the bones with low collagen are unsuitable for analysis (Van Klinken, 1999: 689).

To examine the differences in $\delta^{15}\text{N}$ value distributions among groups, the results of the stable nitrogen isotope analysis ($\delta^{15}\text{N}$) were evaluated using Standard Deviation (SD) in statistical software (SPSS).

V. Discussion

Table 1 presents data from the nitrogen isotope analysis ($\delta^{15}\text{N}$) conducted on 12 dental collagen samples

obtained from human remains at the Mersin Chal cemetery. The results reveal that the $\delta^{15}\text{N}$ values for these samples range between 8.61‰ and 11.12‰.

Table 1: Nitrogen isotope analysis results ($\delta^{15}\text{N}$) of dental collagen from human remains at the Mersin Chal cemetery (Authors, 2022).

Mersin Chal Cemetery (2021)											
No	Lab Code	T.T.R	Context	Grave	Sex	Age	Tooth	$\delta^{15}\text{N}$ ‰	N %	C/N atom	Collagen yield, %
1	FTMC-SN54-1	D11	304	2	Male	>45	RM3-Man	10/35	13/98	3/18	15/86
2	FTMC-SN54-2	D11	305	3	Male	40-45	RM3-Man	10/36	13/28	3/19	13/34
3	FTMC-SN54-3	D11	306	4	Female	40-45	LM3-Man	10/85	13/75	3/21	14/03
4	FTMC-SN54-4	D11	313	11	Male	>45	RM3-Max	8/61	13/31	3/23	14/53
5	FTMC-SN54-5	D11	316	14	Female	>35	RM3-Man	10/05	13/52	3/21	15/19
6	FTMC-SN54-6	D11	317	15	Female	35-40	RM3-Man	9/46	13/46	3/22	15/72
7	FTMC-SN54-7	E11	305	3	Male	Adult?	RM3-Man	11/12	13/67	3/20	16/12
8	FTMC-SN54-8	E11	309	7	Male	45-55	RM3-Man	10/51	14/39	3/21	15/5
9	FTMC-SN54-9	E11	320	18	Female	Adult?	LM3-Max	9/46	13/98	3/20	10/17
10	FTMC-SN54-10	E11	325	23	Female	Adult?	RM3-Man	9/6	14/49	3/23	10/36
11	FTMC-SN54-11	E11	326	26	Female	Adult?	RM3-Man	9/28	13/61	3/25	7/67
12	FTMC-SN54-12	E11	330	28	Male	>50	RM3-Man	10/78	14/60	3/20	15/27

The average nitrogen isotope $\delta^{15}\text{N}$ levels in the dental samples from the Mersin Chal cemetery reveal a mean value of 10.0358 with a standard deviation of 0.75548, as determined through statistical analysis. Table 3 highlights that six male-identified skeletons exhibit $\delta^{15}\text{N}$ levels ranging from 8.61 to 11.12, with a mean of 10.2883. Meanwhile, six female-identified skeletons display $\delta^{15}\text{N}$ levels ranging from 9.28 to 10.85,

with a mean of 9.7833. Based on isotopic data from the 12 analyzed samples, males show a $\delta^{15}\text{N}$ value approximately 0.505 units higher than females. This difference in nitrogen isotope levels suggests variations in dietary habits between males and females, which is further supported by the statistical analysis of the standard deviation results.

Table 2: Statistical results presenting the standard deviation of nitrogen $\delta^{15}\text{N}$ values for males and females, derived from dental collagen analysis of humans (Authors, 2022)

Descriptives									
$\delta^{15}\text{N}$	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	
					Lower Bound	Upper Bound			
Male	6	10.2883	.87273	.35629	9.3725	11.2042	8.61	11.12	
Female	6	9.7833	.58394	.23839	9.1705	10.3961	9.28	10.85	
Total	12	10.0358	.75548	.21809	9.5558	10.5158	8.61	11.12	

The $\delta^{15}\text{N}$ stable isotope analysis and SPSS statistical studies revealed that $\delta^{15}\text{N}$ levels were slightly higher in males compared to females, with a difference of approximately <0.5. This likely reflects variations in dietary habits between males and females, with females consuming a more homogeneous and closely related diet, while males likely showed greater variation in the amount of protein consumed. This is primarily due to the physiological differences between males and females. Testosterone, the key male hormone, plays a significant role in muscle growth, development, bone density, and tissue formation. It also boosts protein synthesis. Enhanced muscle activity contributes to an increase in both their length and volume. Proteins play a crucial role in this process by forming the protein fibers within muscles that serve as essential contractile units. Males typically engage in more physically demanding activities compared to females, which explains why they generally have greater muscle mass. As a result, males require higher protein intake to support muscle development. This increased protein

consumption leads to higher nitrogen isotope levels ($\delta^{15}\text{N}$) in males compared to females.

VI. Conclusion

Mersin Chal Cemetery, located in Telajim Village within Semnan Province, serves as the focus area for this research. 12 dental collagen samples from human remains excavated at the Mersin Chal cemetery were analyzed to investigate the connection between the stable nitrogen isotope $\delta^{15}\text{N}$ and gender (male and female). The results of the nitrogen isotope analysis on the skeletal remains of males and females from Mersin Chal revealed slight differences in $\delta^{15}\text{N}$ levels, probably suggesting variations in dietary habits between genders. The nitrogen isotope level ($\delta^{15}\text{N}$) in males is approximately 0.505 higher than in women. This variation in $\delta^{15}\text{N}$ levels stems from the distinct biological traits of males and females, which in turn influence the differences in their dietary patterns. The physiological differences between males and females, such as males generally having 1.5 times more muscle

mass on average, a higher metabolic rate, and differing nutritional demands, result in males requiring greater protein intake. This is essential for building muscle and carrying out more physically demanding activities relative to their body mass. The protein intake of the body is linked to the concentration of stable nitrogen isotopes ($\delta^{15}\text{N}$) found in skeletal remains. In the Mersin Chal cemetery, the slightly higher nitrogen isotope levels ($\delta^{15}\text{N}$) observed in males compared to females (approximately 0.505 greater) could correspond to the physiological differences between genders.

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