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Dieta e saúde oral em Flõr Da Rosa medieval, Portugal

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Diet and oral health at medieval Flôr da Rosa, Portugal

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Abstract

The analysis of oral health and stable isotope ratios in human skeletal remains are important tools for the reconstruction of past human health and diet. However, there is a lack of studies combining these two methods in the context of medieval Portugal. This master thesis aims to investigate if there is a correlation between oral health and diet at medieval Flôr da Rosa, Portugal.

Osteological samples were collected from 26 human individuals and 12 faunal remains from Flôr da Rosa monastery (13^{th} to 18^{th} century). Human samples were divided by sex and age at death. The species of the faunal remains were identified when possible. The frequencies of dental caries, calculus and ante mortem tooth loss (AMTL) were recorded for each individual if it was possible. Human and faunal bone collagen $\delta^{13}C$ and $\delta^{15}N$ were analyzed using EA-IRMS. No statistically significant difference was observed between sex and age groups. However, the frequency of dental caries is slightly higher among females than males and calculus is more frequent among males. Based on the findings of stable isotope analysis, the diet at Flôr da Rosa relied on C_3-terrestrial animal intake. There are no significant differences in $\delta^{13}C$ and $\delta^{15}N$ between sex ($\delta^{13}C$: p=0.36; $\delta^{15}N$: p=0.97) and age groups ($\delta^{13}C$: p=0.14; $\delta^{15}N$: p=0.45). This suggests that the observed differences in oral health between sexes might be behavioral, biological and physiological differences rather than dietary differences. A small correlation was found between dental caries and $\delta^{13}C$ and between AMTL and age groups. A continuation of this study in a larger sample would improve our knowledge about the relationship between diet and oral health in past populations.

Keywords: isotopes, diet, Flôr da Rosa**,** oral health

Resumo

A análise da saúde oral e das proporções de isótopos estáveis em restos de esqueletos humanos são ferramentas importantes para a reconstrução da saúde e da dieta humana no passado. No entanto, faltam estudos que combinem estes dois métodos no contexto do Portugal medieval. Esta dissertação de mestrado tem como objetivo investigar se existe uma correlação entre saúde oral e alimentação em Flôr da Rosa medieval, Portugal.

Foram amostrados ossos de 26 indivíduos humanos e 12 vestígios faunísticos do mosteiro de Flôr da Rosa (séculos XIII a XVIII). As amostras humanas foram divididas por grupos etários e por sexo. As espécies dos restos faunísticos foram identificadas sempre que possível. As frequências de cárie dentária, cálculo dentário e perda dentária ante mortem (AMTL) foram registadas para cada indivíduo. Os valores de $\delta^{13}C$ e $\delta^{15}N$ do colagénio ósseo humano e faunístico foram analisados usando um EA-IRMS. Não foram observadas diferenças estatisticamente significativas entre sexos e grupos etários. No entanto, a frequência de cárie dentária é ligeiramente maior no sexo feminine do que no masculino e o cálculo é mais frequente entre o sexo masculino. Com base nos resultados da análise de isótopos estáveis, a dieta do Flôr da Rosa era composta maioritariamente de uma dieta terrestre. Não há diferenças significativas em $\delta^{13}C$ e $\delta^{15}N$ entre sexos ($\delta^{13}C$: p=0.36; $\delta^{15}N$: p=0.97) e grupos (($\delta^{13}C$: p=0.14; $\delta^{15}N$: p=0.45) etários, sugerindo que as diferenças observadas na saúde oral entre os sexos podem seatr mais relacionadas com comportamentos, biologia e fisiologia do que apenas com a dieta. Foi encontrada uma correlação fraca entre cárie dentária e $\delta^{13}C$ e entre AMTL e grupos etários. A continuação deste estudo em uma amostra maior melhoraria nosso conhecimento sobre a relação entre dieta e saúde oral em populações do passado.

Palavras-chave: isótopos, dieta, Flôr da Rosa, saúde oral

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This project is a part of HD-TEC: The impact of childhood Diet on adult Health, comparing three Portuguese archaeological collections: Tomar, Estremoz and Crato.

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Introduction

Comparative studies of human health and dietary patterns are frequently conducted over different time periods characterized by distinct social structures or material cultures (Ventresca-Miller et al., 2014). Diet is vital for health (prevention), and a poor diet may cause illness in the long run (Turrini, 2022). Understanding public health requires an appreciation of the interplay between nutrition, infection, and immunity. This field holds evolutionary significance as it allows for the observation of the impact of an individual's diet on their susceptibility to pathogens. Nutritious diets may boost survival and capacity to recover from infectious illnesses, but poorer diets may increase susceptibility to pathogens leading more often to widespread infections (Bogin et al., 2007; Calder, 2013). Hence, an investigation will be conducted to analyze the relationship between oral health and nutritional intake, with the aim of gaining insights into the oral health and diet of the medieval population. In Flôr da Rosa during the 13th to 18th century, it is expected that agriculture relied on cultivating cereal crops and consumption of meat from livestock such as cattle, sheep, goats, domestic and wild pigs, birds. It is anticipated that there will be elevated levels of animal protein from terrestrial sources due to the inland location of the monastery. The monastery of Flôr de Rosa is located far from aquatic sources but the population might get it through trade/exchange. The consumption of fish is often associated with elevated socio-economic status in Medieval Iberia (Lubritto et al., 2017; Veiga-Rilo & López-Costas, 2023).

This master thesis studies the potential correlation between diet and oral health, as evaluated through the utilization of δ^{13}C and $\delta^{15}N$ ratios. Utilization of bone collagen δ^{13}C and $\delta^{15}N$ isotopes can give us direct information about the past food intake of individuals derived from protein part of the diet. Meanwhile, examination of dental diseases can indirectly inform us about diet. There is a strong correlation between high frequency of dental caries and consumption of food rich in carbohydrates and between the formation of dental calculus and meat consumption. Poor dietary habits are associated with negative health outcomes. It is possible that the individual's diet may affect their health status. Elevated $\delta^{15}N$ levels are typically indicative of diets that are rich in protein. The intake of $\delta^{15}N$ may be impacted by dental attrition, resulting in reduced values among elderly individuals. There is no distinction between burial types or locations relative to church in Flôr da Rosa. Therefore, diet and health cannot be compared to social status. Furthermore, skeletal lesions may indicate a favorable physiological condition. Periostitis may indicate the presence of physiological stress and inflammation, as well as the process of recovery. The bone collagen isotopic ratios of $\delta^{15}N$ and δ^{13}C were analyzed. The investigation will focus on examining the potential influence of diet on an individual's susceptibility to generalized infections.

Since archaeological collections are a good model to study diet and health without the confounding factor of modern medicine, the osteological collection from the Monastery of Flôr da Rosa humans will be used to address the main objectives of this study: 1) Estimate the adult diet at Medieval Flôr da Rosa using stable isotopes of carbon and nitrogen; 2) Compare the diet between sexes and ages; 3) Investigate the frequencies of dental caries, calculus and ante mortem tooth loss (AMTL) in Flôr da Rosa; 4) Investigate if there is a correlation between dental pathologies and values of $\delta^{13}C$ and $\delta^{15}N$. The diet of 26 adults will be estimated using bone collagen $\delta^{13}C$ and $\delta^{15}N$ extracted from ribs. 12 faunal remains will be studies to reconstruct the local baseline diet. The analysis of carbon and nitrogen stable isotopes will be done using mass spectrometry (EA-IRMS). This study will highlight the importance of archaeological sciences to understand the evolutionary relationship between diet and disease at a time when the supply of effective antibiotics is under threat.

2. Context of study

2.1. Monastery

The Monastery of Santa Maria de Flôr da Rosa is situated in the village of Flôr da Rosa, which is situated approximately 2 kilometers from the town of Crato, in the district of Portalegre, Portugal. The earliest reference to Flôr da Rosa dates back to 1351 when it was referred to as Flôr de Rosa and was linked to the Hospitaller Order (Fig. 1) (Rodrigues & Pereira, 2009). The name of the monastery of Flôr da Rosa could be derived from the Greek words "Rhodon" or "rosa," which relate to the island of Rhodes (Fig. 2) (Costa, 2014). In the Alentejo region of southern Portugal, the Order intended to construct a second Rhodes (Costa, 2014).

During the fourteenth to sixteenth centuries, the Portuguese Hospitallers faced challenges from the late Crusade and reshaped their identity (Costa, 2014). They focused on military capabilities and ventured into uncharted territories, establishing new centers within the Portuguese kingdom. They initially shifted their focus to the northern region, focusing on pilgrimage and the expansion of the diocesan Church's authority. They later shifted their focus to the southern region, specifically the Alentejo region, where they participated in the new crusade, particularly the pivotal battle of Salado in 1340. The closeness to royal power played a crucial role in shaping the evolution of the Order. The Livro de Linhagens, a manuscript praising the Order and highlighting the brothers' involvement in the battle (Costa, 2014).

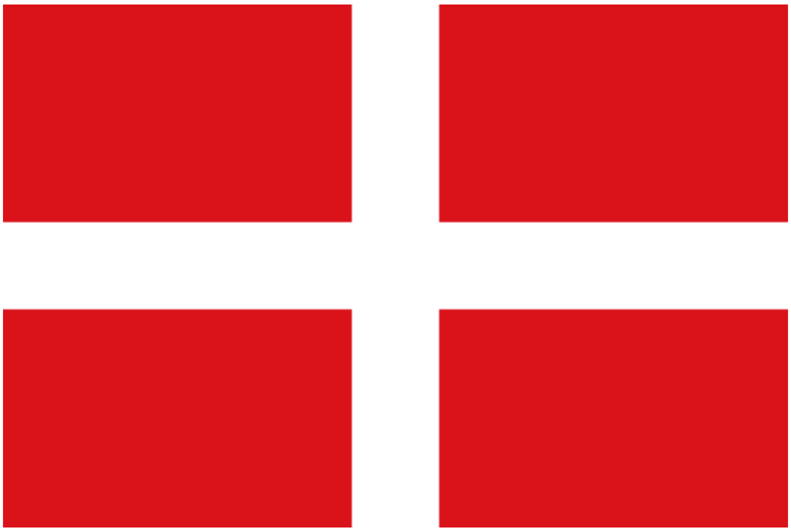

Figure 1 Flag of Sovereign Military Hospitaller Order of Saint John of Jerusalem, of Rhodes and of Malta by Zscout370 from https://commons.wikimedia.org/wiki/File:Flag_of_the_Order_of_St._John_(various).svg#/media/File:Flag_of_the_Order_of_St._John_(various).svg

Figure 2 Hospitallers Knights in 13th century by Ralph Hammann from https://commons.wikimedia.org/wiki/File:Soultz_Commanderie_09.JPG#/media/File:Soultz_Commanderie_09.JPG

The establishment of the monastery dates back to the year 1356, although a pre-existing structure existed at the same location, with a quadrilateral shape (Rodrigues & Pereira, 2009). It is speculated that this structure may have been affiliated with the Religious Military Order prior to the construction of the monastery. The monastery served as a stronghold for the spiritual and military influence of the Order in the southern region of Portugal. The building in question served as a convent residence and a fortified structure with the ability to impede the advancement of hostile forces, specifically the Islamic, into Portuguese territory (Rodrigues & Pereira, 2009). According to Herculano (1916), the territory of Ucrate (Crato) was granted to the order by D. Sancho II in 1232.

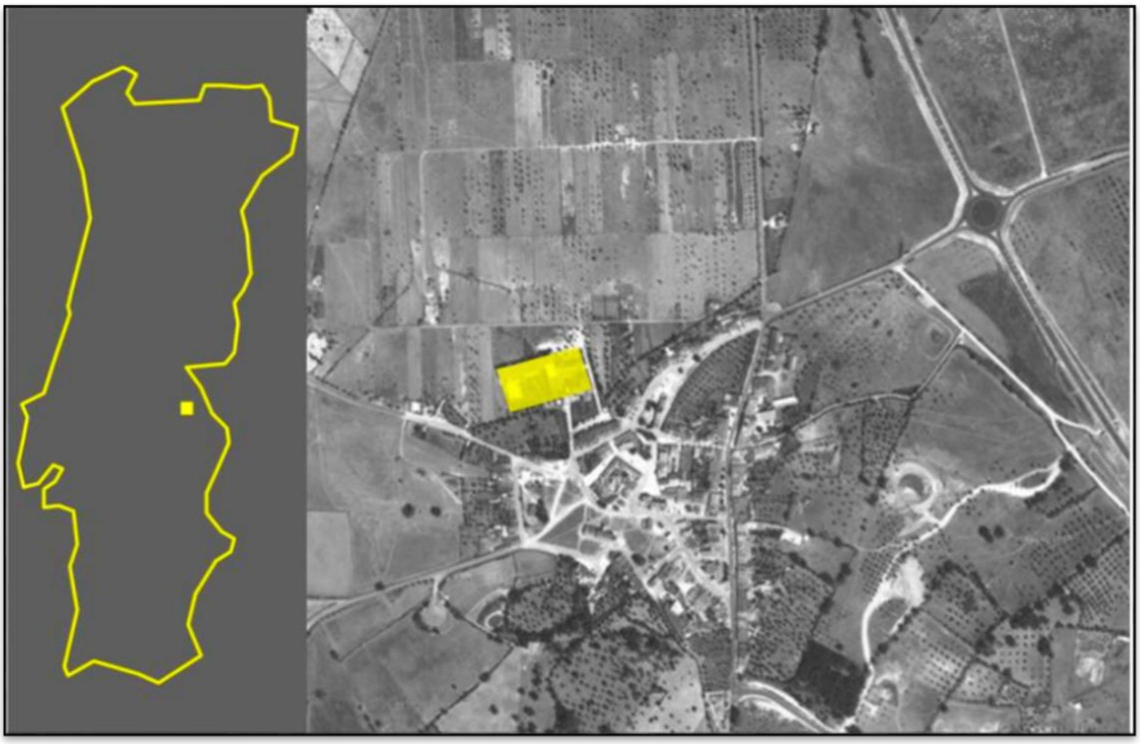

Figure 3 Orthophotmap of Crato (Portalegre, Portugal) – adopted from Google maps

The arrival of the Order of Malta in Portugal occurred sometime between 1114 and 1132 (Gordalina & Bucho, 1998). Upon their arrival, the order settled in the region of Leça De Balio or Belver, where they established their presence and continued to be known as the Order of Malta. Due to its role in territorial defense and its status as a military institution, the order acquires additional attributes provided by affluent monarchs as its domain expands, thereby augmenting its military power (Gordalina & Bucho, 1998).

During the period from 1340 to 1350, a significant relocation of the Hospitaller order's headquarters occurred, as it was transferred from Leça De Balio to the municipality of Crato (Fig. 3)(Rodrigues & Pereira, 2009). The move was planned by D. Alvaro Goncalves Pereira, who was the prior of the Hospitallers order. (Gordalina & Bucho, 1998; Rodrigues & Pereira, 2009). As part of this transition, D. Álvaro Gonçalves Pereira commissioned the construction of the Monastery of Flôr da Rosa, which served as both a religious institution and his personal residence (Gordalina & Bucho, 1998). Due to its geographical placement in a border region and its distinctive architectural design, the Monastery of Flôr da Rosa bears a resemblance to a fortified structure (Fig. 4) rather than a conventional religious building (Gordalina & Bucho, 1998).

Figure 4 Exterior view of the Flôr da Rosa monastery near Crato, Portugal / By Roundtheworld - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=116257790

The main towers were situated on the southern side of the monastery and were designed to possess a robust architectural style reminiscent of palaces. The Palace with castellated architecture (Fig. 4), which served as the residence for the prior of Crato, is accompanied by a church that occupies a significant portion of the early section of the monastery and encompasses the Conventual dependencies. The collection of towers found within the palace and church exhibits a significant sense of strength, attributed to the substantial nature of the stone walls (Gordalina & Bucho, 1998).

During the initial decades of the monastery's construction, significant emphasis was placed on incorporating elements that allude to various architectural styles, namely Manueline, Mudejar, and Renaissance (Gordalina & Bucho, 1998). The convergence of various architectural styles is represented by the cloister, which is formed by the intersection of these traces in both the palace and the church, spanning across different eras. During the Manueline period, specifically in the 15th and early 16th centuries, significant architectural modifications were made to the late façade and cloister of a certain establishment. These alterations were commissioned by Prior D. Diogo De Almeida, who oversaw the addition of new structures to the preexisting spaces (Gordalina & Bucho, 1998).

The church (Fig. 5) holds significant prominence within the monastery, serving as the primary architectural feature and positioned adjacent to the main facade. Entry is via a portico, traversing a corridor that leads to a door in the Renaissance architectural style, which provides ingress to the cloister. The nave of the church can be accessed to the north, while the interior is located to the west. Located within the nave of the structure is the Tomb of D. Friar Álvaro Gonçalves Pereira, responsible for its establishment.

The ceiling of the nave's interior is composed of a fragmented cradle vault structure (Gordalina; Bucho, 1998), featuring a prominent right foot and constructed using intricately cut stone materials (Gordalina; Bucho, 1998).

Figure 5 Interior view of the church By LuizaSerpaLopes - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=116257883

The cloister is supported by a series of eight pillars located at its base, which serve as the foundation for the toral arches and formers. The architectural elements known as "Arcos formeiros" (Gordalina; Bucho, 1998) made of granite, which are in the garden, are upheld by marble columns. These elements consist of a "smooth shaft" (Gordalina; Bucho, 1998) with a decorated base and capital in the Mudejar style. On the western side, there is a chamber that provides access to the garden through two windows characterized by their round-arched design (Gordalina; Bucho, 1998). In the southern direction, there is an area that establishes a connection with the central tower of the monastery.

Located in the western section of the premises, there is a compact chamber that serves as a conduit to the dining area, providing a means of ingress to the upper level via a staircase. Located in the northern direction, there exists a door designed in the Manueline style, which serves as an entrance to the historical monastery kitchen (Gordalina & Bucho, 1998).

The monastery underwent substantial destruction in the year 1615, while a notable portion of the church was subjected to damage in 1897. The monastery was abandoned in 1834, after the abolition of religious organizations in Portugal, hastening its decline and collapse. In the year 1940, a portion of the church experienced structural failure, resulting in its subsequent restoration and reconstruction (Gordalina & Bucho, 1998).

The designation of a national monument was conferred upon it in the year 1910. The monument underwent a comprehensive restoration process during the 1940s and 1960s of the twentieth century, carried out by the Directorate-General for National Heritage and Monuments. The project for the adaptation of the monastery into a Pousada for hotel was initiated in the 1990s under the direction of architect João Luís Carrilho da Graça (Melo & Toussaint, 2018).

The Monastery of Flôr da Rosa can be described as a place that exhibits a wide range of civil, military, and religious influence on the region (Gordalina & Bucho,1998). The structure is widely regarded as one of the most significant architectural landmarks in Portugal (Gordalina and Bucho, 1998).

2.2. Archaeological Context

The excavation of the monastery of Flor da Rosa near Crato (Portugal) was conducted during the period of 1989-92 (Rodrigues, 2009). The church, cloister, and building exterior were utilized as a necropolis. Based on the numismatic evidence, it appears that interments were conducted outside the edifice during the 16th century. The interment of human remains within the edifice during the period spanning from the 14th to the 19th centuries, as determined by the dating of coins (Rodrigues,2009).

Figure 6 Main façade after excavation (Graça, 1995b, p.67)

The graves were excavated directly into the soil (Fig.6) and exhibited evidence of both shrouds and clothing (Rodrigues, 2009). Two graves were found to contain coffins made of wood, secured with nails, and containing the soles of shoes. Most of the skeletons were found in a supine position with flexed elbows and arms crossed over the chest or abdomen, while their legs were either extended parallel to each other or crossed at the ankles. A few skeletons were discovered in a lateral decubitus position. The predominant orientation is from the West to the East, while a minority of orientations are from the North to the South. A total of 754 coins were recovered, including coins from D. Manuel I and D. Maria I (Rodrigues & Pereira, 2009).

2.3. Medieval Diet in Portugal

There is a lack of information related to the dietary habits of the medieval era within the geographical boundaries of contemporary Portugal. In the context of the Iberian Peninsula, it is relevant to acknowledge the emergence of distinct culinary variations that have arisen because of the assimilation of earlier gastronomic traditions, specifically those introduced by Phoenician, Greek, Roman, Islamic, and Christian cultures (Adamson 2004). During the late medieval period, the southern region of Portugal, where Flôr da Rosa is located, exhibited limited growth of wheat and cereals, necessitating the importation of such agricultural products from other regions within Portugal, including Entre-Douro-e-Minho, Beiras, Ribatejo, and Estremadura. (Gonçalves, 1984; Constable, 1996). According to Vicente's (2013) research, the fundamental components of the medieval diet in Portugal consisted of bread, wine, and olives in any form. A considerable proportion of the agricultural sector was centered on the cultivation of cereals, which are considered essential to producing bread, a staple component of the dietary intake in medieval Portugal. A significant proportion of the crop yield was likely allocated toward fulfilling obligations to the feudal lords and religious institutions (Vicente, 2013). Chestnuts and sweet acorns might replace bread and legumes could have been ground into flour if grains were limited (Vicente 2013; Gonçalves 2004).

According to Estaca-Gomez et al. (2018), during the Middle Ages in the Iberian Peninsula, livestock farming constituted a significant economic activity. Several zooarchaeological studies indicate that Christian sites exhibit the use of cattle, sheep/goats, and pigs as the most prevalent species (Grau-Sologestoa, 2017). Secondary animal products, including cheese, milk, and eggs, were significant sources of diet for the lower classes. However, distinguishing these products from meat based on their isotopic values is not possible.

Salmon, red bream, flounder, hake, shad, lamprey, and eel were considered expensive food items, whereas sardines, mollusks, and crustaceans were comparatively more abundant (Gonçalves, 2004). During religious fasting in medieval times, fish played a crucial role (Müldner et al. 2009; Vicente, 2013). The access of individuals of low socio-economic status to fish in rivers was limited (Vicente et al., 2013). During medieval Portugal, high socio-economical classes such as lords and military orders held dominion over the privileges of hunting and angling according to written texts (Vicente 2013).

3. Scientific Background

3.1. Bone Structure

The bone is a living tissue that undergoes continuous remodeling during its lifespan (White and Folkens, 2005). Osteoblasts and osteoclasts are cells accountable for the formation and breakdown of bone tissue (Bab and Sela, 2012). Osteoblasts are responsible for synthesizing various proteins, such as collagen, in addition to facilitating the formation of the inorganic component of bone through the combination of calcium, magnesium, and phosphate ions (Britannica, 2018). The chemical composition of hydroxyapatite is represented by the formula:

$$Ca_{10}(PO_4)_6(OH)_2$$

Bioapatite is the mineralized component of bone tissue, referred to as a crystalline hydroxyapatite carbonate in scientific nomenclature (Krueger and Sullivan 1984; Merwe 1991, Lee-Thorp et al. 1989). This inorganic component of bone serves as a barrier protecting bone collagen from enzymatic degradation (Child, 1995).

Approximately 20% of the dry weight of bone is composed of the organic component, which is primarily comprised of collagen (Pate 1994, Lightfoot 2009; Shoeninger & Moore, 1992). This collagen is arranged in triple helical structures known as tropocollagen, which subsequently combine to form larger collagen fibrils (Shoeninger & Moore, 1992). The microstructure of bone is characterized by the organization of collagen fibrils into three distinct groups, namely woven bone, lamellar bone, and parallel-fibered bone (Brown, 2011).

Two distinct types of bone are identified at the macroscopic level, namely compact and cortical bone (Morgan, 2018).

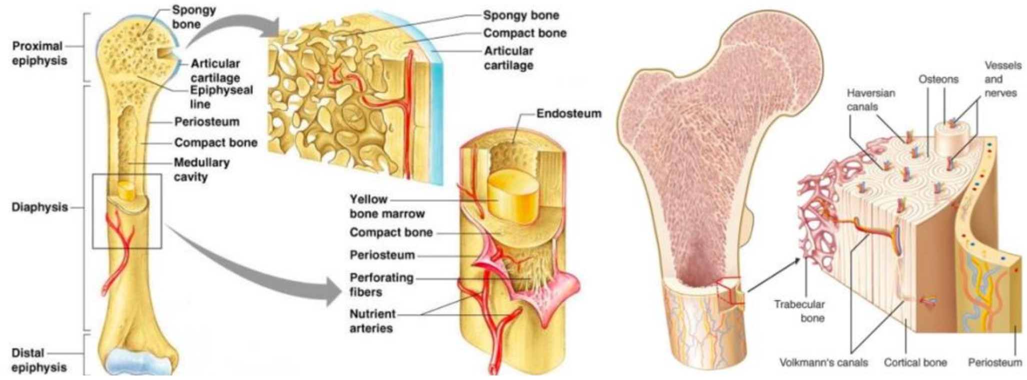

Figure 7 Anatomy and structure of the bone tissue. Adopted from Bartl & Bartl, 2019

Compact bone is composed of parallel osteons binders and exhibits remarkable strength. The cortical bone forms the diaphysis of long bones as well as the outer regions of all of the other bones (White and Folkens, 2005). Trabecular bone, characterized by its porous nature and the presence of interconnected channels, is predominantly located in articular zones of long bones, the vertebrae, ribs and tarsus, where it serves as the site for hematopoiesis, i.e., the production of blood cells (Child, 1995).

3.1.1. Bone Turnover Rate (BTR)

The process of bone remodeling is characterised by the resorption of old tissue by osteoclasts and the subsequent production of new tissue by osteoblasts (Robling et al., 2008). The isotopic makeup of an individual's food is incorporated into the formation of new bone (Fry & Arnold 1982). Bone turnover is a factor that has the potential to impact stable isotope analyses, yet remains incompletely comprehended. Remodeling occurs throughout an individual's lifespan (Sealy et al. 1995; Cox & Sealy, 1997). Various skeletal components develop at various stages of life and, remodel at various speeds (Manolagas and Jilka 1995). The turnover rate plays a crucial role as it establishes the duration of the dietary intake that may be seen by isotopic studies (Sealy et al., 1995).

The important aspect of the turnover rate lies in its ability to provide insights into dietary intake during isotope analysis (Fry & Arnold 1982). The turnover rate of human bones was determined based on the inorganic constituents, namely calcium, and strontium. The results indicate a annual turnover rate of 100-200% for individuals aged with one year old, 10% for those aged 3-7 years old, and 1% for those aged 8 years old and above (Cox and Sealy, 1997). The annual turnover rate for adults falls within the range of 0.3% to 3% (Cox and Sealy, 1997). Additionally, the rate of turnover varies depending on the bone (Libby et al.,1964; Mays, 1998). For instance, compared to other bones , the annual turnover rate of the vertebrae is higher at one year of age (72%), 5-6 years (20%), 10 years (30%), 20 years (24%), and adults (8%) (Cox and Sealy, 1997). The annual rate of bone turnover in cortical bone is comparatively lower, ranging from three to ten times slower than that of trabecular bone (Tieszen et al., 1983).

The relationship between isotopic variation and human bone remodeling is a topic of interest due to the correlation between isotopic ratios and bone turnover rates. The application of isotope analysis on the rib bones, for example, allows the reconstruction of a shorter time of dietary consumption prior to the occurrence of death than … long bonesfemoral bone which reflect dietary signal up to 10 years (Hedges et al., 2007). However, the study conducted by Fahy et al. (2017) suggests that the femur does not exhibits a slower turnover rate in comparison to the rib. The osteon population density (OPD) of the femur and rib did not exhibit a significant variation (Fahy et al., 2017). However, the mean OPD of the occipital bone was found to be considerably different from that of the rib (Fahy et al.,2017). Similarly, Fahy et al. (2017) found intraskeletal isotopic variations up to -1,6‰ in $\delta^{13}C$ and up to 3,1‰ in $\delta^{15}N$. The study conducted by Jørkov et al. (2008) revealed that both nitrogen and carbon stable isotope signals exhibit similarity between the femur and rib, despite variations in their turnover rates and load-bearing characteristics. However, Sykut et al., (2020) found a mean variation of 0.88‰±0.24 for $\delta^{15}N$ and 0.46‰±0.07 for $\delta^{13}C$ between samples taken from different bones of red deer (*Cervus elaphus*) within the same individual. It is important to note that there is a lack of research analyzing isotopic variability between faunal bones from the same individual. The study conducted by Jørkov et al. (2008) revealed that both nitrogen and carbon stable isotope signals exhibit similarity between the femur and rib, despite variations in their turnover rates and load-bearing characteristics. Nevertheless, the isotopic signature of a petrous bone exhibits a combination of nutritional signals, with a notable emphasis on early eating patterns (Jørkov et al., 2008). This trend is also observed in dentine, albeit to a greater extent (Gage, 1989; Hillson, 1996). The ribs, humeri, and metacarpals exhibit the highest bone-tissue ratio (MacKinnon et al., 2015; Fahy et al., 2017). The practice of sampling various bone parts for the purpose of dietary reconstruction sheds light on distinct phases of human dietary patterns over time (Lamb et al., 2014). The femoral bone provides a more extended dietary record of up to 10 years, as opposed to the ribs which offer a short-term dietary record before death, from an isotopic perspective (Hedges & Reinard, 2007).

3.2. Teeth

3.2.1. The Structure and Composition of Teeth

The morphologies of teeth in vertebrates exhibit a wide range of variations adapted to their diet and have distinct roles in the processing of food (Weiner, 2010; Hillson, 2018). The dental crown and the root make up the two primary components of a tooth. The dental structure of each tooth consists of a centrally located pulp cavity that is encircled by dentine (Weiner, 2010; Hillson, 2018). Enamel is made of nearly all inorganic material, including calcium phosphate, fluoride, magnesium, and strontium (96%), and is thus the most mineralized tissue in the body, the rest is comprised of organic matter (Kohn, et al., 1999; Wang et al., 1994). Enamel lacks collagen and does not undergo remodeling post-mineralization, thereby maintaining isotopic signatures over an individual's lifespan (Lee-Thorp et al., 1989; Cox & Sealy, 1997).

Most of the tooth is made up of dentin, which is distinguished by its mostly calcium phosphate ion composition. Hydroxyapatite crystals are formed within the dentin matrix as a result of the molecular arrangement of these ions. Mineralized collagen fibrils make up most of the structural elements of the dentin and the cementum. Pre-dentine, which is formed under the cusp's core and is followed by the development of immature enamel, and subsequent addition of apatite crystals, take place after the creation of the root (Turner-Walker, 2007). Cementum protects the root surface by encasing it and giving teeth stability (Pate, 2008). Cementum primarily consists of type I collagen, characterized by the presence of thick collagen fibrils.

Permanent tooth crown development entails three stages (Hillson 2018). The first stage of permanent crown development involves incisors, canines, and first molars, which are completed between 3 and 7 years. The formation of premolars and second molars typically commences during the age range of 2 to 8 years, while the development of third molars, occurs between 7 and 12 years. Because teeth are formed during childhood and do not go through remodeling, they serve as a primary source of dietary information during childhood, whereas compact bone serves as an indicator of an individual's long-term dietary patterns (Cox and Sealy,1997, Hillson, 2018).

3.2.2. Oral Health

Diet plays a crucial role in developing diseases associated with plaque formation (Tomczyk, J. et.al, 2013), such as dental calculus and dental caries (Hillson 2018). Dental plaques a compact aggregation of microorganisms that forms on the surface of teeth and has been associated with tooth loss among humans for an extensive period of time (Hillson, 2018). The composition of plaque fluid undergoes alterations characterized by reduced oxygen levels, decreased pH, diminished nutrient concentrations, and elevated levels of waste products (Hillson, 2018).

Dental calculus is the mineralization of plaque (Lieverse, 1999). The formation of calculus initiates with the development of a pellicle on the enamel surface, which occurs as a result of the adsorption of salivary glycoproteins with high specificity. This process serves as a protective mechanism against the detrimental effects of bacterial metabolic acids. Additionally, the accumulation of human saliva minerals, particularly calcium phosphate, contributes to the formation of calculus (Samaranayake, 2006). Subsequently, a plaque is formed consisting of many bacterial species, such as streptococci, staphylococci, lactobacilli, and corynebacteria (Radini et al., 2017). According to Marcotte and Lavoie (1998), the bacteria's metabolic process results in the creation of substances like ammonia, which raises the local pH of the mouth. The phenomenon of plaque formation results in an additional elevation of the surrounding pH, which subsequently facilitates the mineralization process and ultimately leads to the development of dental calculus. Calculus forms more frequently on the lingual surfaces of front teeth and the buccal surfaces of back molars, because these areas are closest to the salivary gland ducts, from whence the mineral is deposited (Driessens & Verbeeck, 1989). The classification of dental calculus comprises two distinct categories: supragingival and subgingival (Hillson, 2018). Supragingival dental calculus refers to the occasional accumulation of mineralized deposits on the gingival tissue. The formation of subgingival calculus is closely associated with the occurrence of periodontal disease (Hillson, 2018). The starting point of mineralization is associated with the degree of plaque formation, and consequently, with factors that contribute to heightened plaque accumulation, such as poor oral hygiene or excessive carbohydrate intake. According to Beiswanger et al. (1989), there is a notable disparity in the prevalence and severity of supra-gingival calculus deposits between males and females within living populations. Furthermore, it has been observed that the frequency and magnitude of these deposits become greater with older age.

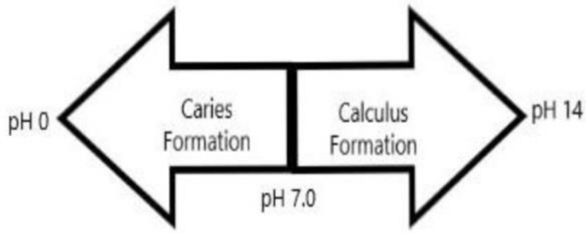

Figure 8 Illustration depicting the relationship between oral pH and associated pathology. Adopted from Roshan, 2018

Caries develop as the pH is reduced and the produced plaque does not mineralize. Dental caries result from a process of demineralization that occurs because of bacterial activity on the surface of the teeth (Tomczyk, J. et al., 2013). Caries can arise from a multitude of etiological factors, encompassing both environmental and internal influences. Environmental factors encompass trace elements found in food and water, while external factors involve oral health and dietary habits (Powell, 1997). Internal factors encompass teeth' inherent structure and morphology (Powell, 1997). Hillson (2018) noted that there is a lack of observable disparity in carcinogenic potential among sucrose, fructose, glucose, and lactose. The development of dental caries is correlated with a high intake of carbohydrates (Larsen, 2002). The increased occurrence of caries is frequently associated with the development of agriculture (Larsen 1997; Hillson 2007). The Inuit, a hunter-gatherer population, exhibits a notable absence of dental caries (Pedersen,1966). Furthermore, starch possesses the ability to cause dental caries,while milk has a protective effect against them (Guggenheim, et al.,1999). The presence of severe gross caries can result in teeth loss (Hillson, 2018). Dental calculus and dental caries are chronic conditions that persist throughout an individual's lifespan (Bonsall & Pickard, 2015). Dietary habits have an impact on oral health since they directly affect the teeth. Consequently, dental pathology data can be employed to infer dietary patterns in the past (Hillson, 1996). An illustrative case is the investigation carried out by Larsen in 1997 on the Archaic Native Americans. This study reveals that the examined group had significant dental wear while presenting little indications of dental caries, substantiating their classification as hunter-gatherers. Ventresca Miller et al. (2014) conducted a study that examined the oral health of individuals from the Bronze Age in northern Kazakhstan. The study findings suggest that dental diseases observed are associated with a noncariogenic diet characterised by a high proportion of protein and a deficiency of carbohydrate intake. Researchers linked these findings to typical trends observed in pastoral cultures. Moreover, Ventresca Miller et al. (2014) aligned dental pathologies with the stable carbon and nitrogen isotopic data, demonstrating that the diet of Bronze Age population in northern Kazakhstan was protein-rich and therefore non-cariogenic. In archaeology, diet and food preparation are utilized to investigate changes in subsistence practices since they are frequently linked to the frequency of carious lesions (Schats et al., 2021; Tomczyk, J. et.al, 2013; Bonsall & Pickard, 2015). Several studies have indicated that women have a somewhat higher susceptibility to the condition compared to males, however the extent of this disparity varies and is quite modest when compared to other factors such as socioeconomic status (Hillson, 2018). According to Hillson (2000), individuals often have caries and calculus in their teeth, despite their associations with contrasting pH environments.

The primary factors contributing to tooth loss are believed to be dental caries, periodontal disease, and periapical lesions (Hillson, 2008; Lukacs, 1995). Additional factors identified as potential causes of AMTL in historical contexts include trauma, the extraction process, diseases such as leprosy, severe attrition, and ongoing eruption (Waldron, 2009). The occurrence of tooth loss is improbable to be primarily attributed to bone loss specifically near the apex (Hillson 2018). The significance of dental caries in tooth loss is limited unless teeth are deliberately taken for the purpose of managing acute pulpitis, or if bone loss resulting from periodontal disease coincides with loss from the alveolar process (Hillson 2018; Lukacs, 1995). Granulomas can develop in proximity to the alveolar crest, namely around lateral canals within the root, which may have implications for the eventual loss of the affected tooth (Hillson, 1996; Hillson 2018).

Overall, the dental pathologies observed in archaeological populations are indicative of dietary patterns, oral hygiene practices, and subsistence economy (Roberts et al.,1995).

3.3 Stable Isotopes Analysis (SIA)

An isotope is an element characterized by an identical number of protons and electrons, but a distinct number of neutrons. Therefore, they exhibit variance in their atomic masses (Smith, 1972). Atoms contain different numbers of protons, electrons, and neutrons. An atom is composed of three components: electrons which orbit the nucleus, protons, and neutrons which exist inside the nucleus.

The process of isotope fractionation results in changes in the relative abundance of isotopes in the reaction products as compared to their abundance in their initial substrates (Fry 2006; Hoefs 2015). There are two main types of isotopic fractionation. The first process is equilibrium fractionation, which finds its application in paleoclimate investigations but holds a relatively lower significance in the field of biomolecular archaeology (Brown, 2011). The second process is kinetic fractionation, which happens as a result of one-way physical and chemical processes involving lighter and heavier isotopes (Fry, 2006). There exist two principles that must be considered. Light isotopes exhibit higher reactivity in kinetic processes. In exchange reactions, isotopes with greater mass tend to accumulate in regions where the bonding is strong. (Fry, 2006). The difference in mass impacts the amount of energy required to use them (Craig, 1957). Nitrogen 15 (^{15}N) and carbon 13 (^{13}C) are heavier isotopes that move and react more slowly and have greater energy needs (Fry, 2006; Hoefs, 2015). In general, lighter isotopes tend to be more dominant compared to heavier ones (Lightfoot 2009).

Table 1 Naturally occurring isotopes of element relevant to biomolecular archaeology / Biomolecular archaeology, Brown, 2011

Element	*Isotope mass numbers*	*Proportion in nature*	*Stability*
Carbon	12	98.93%	Stable
	13	1.07%	Stable
	14	one part per trillion	Half-life of 5730 ± 40 years
Nitrogen	14	99.64%	Stable
	15	0.36%	Stable
Oxygen	16	99.76%	Stable
	17	0.04%	Stable
	18	0.20%	Stable
Strontium	84	0.56%	Stable
	86	9.86%	Stable
	87	7.00%	Stable
	88	82.58%	Stable

Isotopes can be found in a stable state, characterized by a consistent number of protons and neutrons, or in a radioactive state, where they undergo decay as time progresses. Stable isotopes are utilized to reconstruct the dietary patterns of past populations (De Niro, Epstein 1978; Ambrose,1993; Shoeninger, Moore 1992). The process of dietary reconstruction using stable isotope analysis is based on the concept that the chemical compositions of consumed food and water are retained in the bodily tissues of humans and remain relatively constant even after death (Schoeninger and Moore 1992, R. Tykot 2006, Vaiglova et al. 2014, Ambrose, 1993; Cox, Sealy, 1997). The relative proportions of an element's stable isotopes can vary due to isotope fractionation (J.C. Vogel,1980). A trophic level is a stepped enrichment that follows the food chain, from the primary producers to the highest consumers (Ambrose, 1991). Isotopes contain within biomolecules, and their analysis is conducted with bulk protein or lipid preparations from archaeological samples (Brown, 2011). The principle behind this method is "you are what you eat (plus a few)" (Tykot, 2006). The dietary habits of a consumer can be inferred from the isotopic composition of their tissues (DeNiro, Epstein 1978).

The symbol delta, denoted by the Greek letter δ, is utilized to represent values in parts per thousand and is conventionally expressed as ‰, which is read as "per mil." (Shoeninger, Moore 1992; Katzenberg, 2008). PeeDee Belemnite (PDB) is the commonly accepted standard for carbon isotope measurements (Craig, 1957, Cai, Qui 1984, Fry 2006). Atmospheric nitrogen - AIR (Atmospheric Inhalable Reservoir) is the standard for nitrogen isotope measurements because the N_2 isotope ratio is constant throughout the globe. (Fry 2006).

There are only a few parts per thousand differences between the stable isotopes of the biological and environmental components (Fry 2006, Brown 2011, Hoefs, 2015). The notation used to describe and calculate:

$$\delta^{13}C = (\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{standard}} - 1) \times 1000‰$$

Equation 1 Calculation of $\boldsymbol{\delta^{13}C}$ values from $\boldsymbol{^{13}C}/\boldsymbol{^{12}C}$/ ratios

$$\delta^{15}N = (\frac{(^{15}N/^{14}N)_{sample}}{(^{15}N/^{14}n)_{AIR\ standard}} - 1) \times 1000‰$$

Equation 2 Calculation of $\boldsymbol{\delta^{15}N}$ values from $\boldsymbol{^{14}N}/\boldsymbol{^{15}N}$ ratios

Stable isotope ratios are calculated through the comparison of a given sample against an established standard. A positive value of heavier isotope (e.g. ^{13}C) indicates that the sample has a higher concentration of heavier isotope (e.g. ^{13}C) than the standard, while a negative value indicates that the sample has a lower concentration of heavy isotope (e.g. ^{13}C) than the standard one (Craig, 1957). Both lighter (e.g. ^{12}C) and heavier isotopes (e.g. ^{13}C) exhibit similar behavior in biological processes and chemical reactions; however, they differ in their physical mass or weight (Fry 2006; Katzenberg, 2018).

The carbon present in bone collagen is utilized for the differentiation of plant types, namely C_3 and C_4, as well as for distinguishing between aquatic and terrestrial diets. The estimation of trophic levels and protein consumption is accomplished through the utilization of nitrogen isotopes (Minagawa & Wada, 1984, Katzenberg 2008).

Stable isotope analysis has the capacity to provide insights into inquiries pertaining to human biology, specifically regarding food, mobility, and nutritional strain. Initially, stable isotope research primarily served the purpose of elucidating dietary patterns among individuals. Nevertheless, subsequent applications of this methodology have encompassed questions pertaining to the duration of breastfeeding, the transmission of diseases, and patterns of human mobility (Schoeninger et al. 1983; Schoeninger and DeNiro 1984).

A notable elevation of $\delta^{15}N$ values, ranging from 3‰ to 5‰, is observed across trophic levels in relation to the dietary composition of the consumers (Minagawa and Wada 1984). Fractionation allows for the utilization of $\delta^{15}N$ values as a means to deduce trophic level (Sponheimer et al. 2003), with elevated $\delta^{15}N$ values typically indicating consumption of diets rich in protein, as observed in bone collagen (Bocherens and Drucker 2003).

The resistance of bone collagen to deterioration and contamination makes it suitable for paleo-dietary studies (DeNiro, 1985). The alteration in the carbon-to-nitrogen ratio of bone occurs only when a significant depletion of collagen, amounting to approximately 97%, takes place (Child, 1995). The collagen component reflects protein consumption, in contrast to bioapatite which represents the entire diet (Schoeninger & Moore 1992).

3.3.1 Carbon Isotope Analysis

Three naturally occurring isotopes of carbon exist, each with six protons but different numbers of neutrons (carbon-12 (^{12}C), carbon-13 (^{13}C), and carbon-14 (^{14}C)) (O'Leary, 1988). The number of protons and neutrons in a nucleus determines their mass numbers, which are different. The natural abundance of the carbon isotope ^{12}C is 98.89%, while the quantity of ^{13}C is just 1.1% (Craig, 1957).

Carbon isotope fractionation mechanisms take place during the process of photosynthesis (De Niro, Epstein 1978). This results in the enrichment or depletion of the ^{12}C isotope (Sharp, 2007).

The classification of C_3(Calvin-Benson) and C_4(Hatch-Slack) plants are attributed to the distinct enzymatic pathways employed in carbon dioxide fixation, leading to the production of molecules containing three or four carbon atoms during the primary phase of photosynthesis (Craig 1957, Vogel, 1980). C_3 plants, which include a variety of vegetation such as legumes, trees, herbivorous plants, grasses, wheat, rice, and shrubs, make up approximately 90% of the world's vegetation with more than 150 species (J.C. Vogel & N.J. Van der Merwe 1977.). The C_3 is characteristic of the European ecosystem, whereas C_4 plants, including sugar cane, maize, millet, and sorghum, have their origins in tropical and savannah regions (Chisholm,1982). Only millet can be relevant C_4 plant in this European context (Spengler 2017).

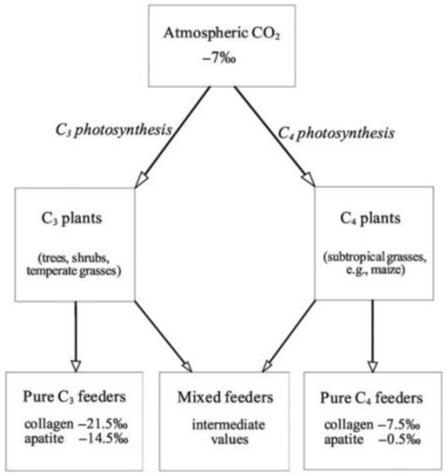

Figure 9 $\boldsymbol{C_3}$ and $\boldsymbol{C_4}$ pathways (Tykot, 2004)

C_3 or C_4 pathways determine the amount of carbon absorbed during photosynthesis (Schwarz, Shoeninger 1991, Hoefs, 2015). There is an initial enrichment of ambient CO2 entry into the plant because lighter isotopes diffuse more quickly in small pores in the outer layer of the plant (O'Leavy 1981). During the photosynthetic process itself, the ribulose-1,5-bisphosphate carboxylase (Rubisco) converts CO2 into glucose (Park, Epstein 1960). The ^{13}C value is depleted relative to the atmospheric one, which is 8‰, but within C_3 plants is -26,5, ranging from -24 to -36‰ (Vogel, Van der Merwe 1977). This is due to two ^{12}C enrichments. Humidity, temperature, and light are a few environmental factors that affect this value (Yakir, Israeli 1995). C_3 plants are widely spread in the temperate regions of North America, Europe, and Asia.

An alternative photosynthetic pathway is employed by a distinct group of plants, that exhibits a less selectivity against the heavier isotope. Maize, millet, and sugarcane are C_4 plants that thrive in hot, dry climates (Chisholm,1982; J.C. Vogel and N.J. Van der

Merwe 1977). Thus, $^{12}C/_{^{13}C}$ range from -9 to -14 ‰ (Vogel, Van der Merwe 1977, Shoeninger, Moore 1992).

Succulents including cactus, agave, orchids, and bromeliads use the third photosynthetic pathway, CAM, to switch between C_3 and C_4 depending on environmental conditions (Shoeninger, Moore 1992, Katzenberg, 2008). CAM plants are crassulacean acid metabolism plants. CAM succulents like Opuntia use C_4 pathway continually (Shoeninger, Moore 1992).

The stable isotope of carbon indicates the trophic level distinction through shifts of approximately 1‰. According to De Niro and Epstein (1978), there is a trend of decreasing negativity in carbon values as they progress from plants to herbivorous animals, then to carnivores, and finally to marine organisms. This trend is associated with an enrichment in the heavier isotope ^{13}C. The primary reason for the observed (1‰) $\delta^{13}C$ elevation across trophic levels is due to the phenomenon of preferential uptake by tissues. For example, various animal tissues exhibit slight variations in their carbon ranges (DeNiro et al., 1978; Ambrose et al., 1991). The presence of variability in the trophic level impacts various tissues within a single organism as well as among different taxonomic groups. Therefore, due to the rise in tissue variation in biochemical composition, the trophic level impact would be more evident in carnivores than in herbivores. The composition of plant parts, such as stems and leaves, exhibits variation. However, it has been observed that botanical components tend to exhibit an average $\delta^{13}C$ content of carbohydrates. A comprehensive understanding of these variations, including their impact on studies pertaining to dietary patterns, remains incomplete. Although the relationship between secondary isotopic fraction patterns and metabolism is not well understood (Ambrose and DeNiro 1986, Deschner et al. 2012, D'Ortenzio et al. 2015, Gaye-Siesseger et al. 2004).

3.3.2 Nitrogen Isotope Analysis

There are two stable isotopes of nitrogen: ^{14}N and ^{15}N. 99,64% of all nitrogen is made up of ^{14}N (Ambrose 1993, Hoefs 2015). The balance between microbial nitrogen fixation from the atmosphere and the subsequent denitrification process leads to nitrogen isotope fractionation (Sharp 2007, Malainey 2010).

Sincet that a significant proportion of denitrification takes place in the ocean, the $\delta^{15}N$ values of marine organisms exhibit slightly higher positive values (Wada, 1980). $\delta^{15}N$

values in the coastal marine region range from 5-6‰, whereas the terrestrial region exhibits values ranging from 1-4‰ (Shoeninger and DeNiro, 1984).

In contrast to non-leguminous plants, which must obtain nitrogen from degraded organic matter such as ammonia (NH_3) and nitrates (NO_3), legumes have nitrogen isotope ratios near the atmospheric one (Shearer and Kohl, 1989). As a result of their symbiotic relationship with the bacteria Rhizobium, legumes fix nitrogen through their roots (Katzenberg, 2008; Malainey, 2010; Mora, 2022).

The nitrogen isotope ratios in terrestrial ecosystems are dependent on the trophic level, rather than being influenced by the type of plant (Shoeninger et al. 1983; Minagawa & Wada, 1984). The herbivorous diet exhibits a 3‰ increase in $\delta^{15}N$ when compared to their respective dietary intake (Wada et al 1975; Shoeiniger and DeNiro 1984; Minagawa & Wada 1984). $\delta^{15}N$ values in carnivorous tissue exhibit an enrichment of approximately 3‰ (Ambrose 1993). The isotopic composition of prey and predator exhibits a distinct variation ranging from +2.4 to +4.8‰ (Bocheres and Drucker 2003; Wada et al 1975). Most of the human population can be classified as omnivorous, occupying a dietary niche that lies between herbivorous and carnivorous feeding strategies (Ambrose & Norr, 1993; Katzenberg & Saunders, 2008). Vegans exhibit a significantly lower stable nitrogen isotope ratio in comparison to individuals who consume meat (Fuller et al., 2005). Therefore, the stable nitrogen isotope levels of an individual can serve as an indicator of the proportion of meat and plant consumption (Sponheimer et al., 2003).

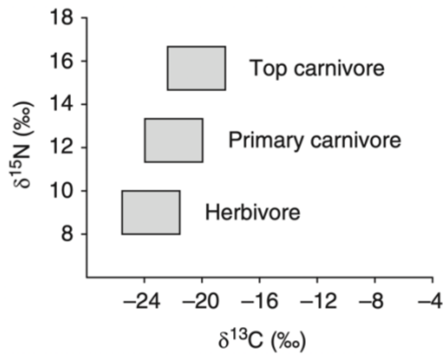

Figure 10 A plot demonstrating isotope shifts with $\boldsymbol{\delta^{13}C}$ and $\boldsymbol{\delta^{15}N}$. From Brown, 2011

$\delta^{15}N$ values of terrestrial plants from the same habitat are similar (Smith and Epstein 1971). Therefore, nitrogen measurements are unable to differentiate between $C_3$from C_4 plants (Shoeninger & DeNiro, 1984). Alternatively, marine plants have a distinct nitrogen signature (DeNiro & Epstein, 1981). Marine Flôra exhibits $\delta^{15}N$ values that are roughly 4‰ greater than those of terrestrial vegetation (Ambrose et al., 1997). $\delta^{13}C/\delta^{15}N$ may be used to establish whether the fish came from an ocean or a river (Shoeninger et al., 1983; Richards & Hedges, 1999). Freshwater photosynthesis requires air carbon dioxide, while marine photosynthesis uses dissolved bicarbonate (Fry, 2006, Hoefs, 2015). As a result, freshwater values for $\delta^{15}N$ are more like terrestrial values than to marine ones (Brown, 2011). Freshwater fish exhibits a trophic level impact, which is reflected in greater $\delta^{15}N$ levels and slightly increased $\delta^{13}C$ values in carnivorous fish (Katzenberg 1989, Minagawa & Wada 1984, Schoeninger & DeNiro 1984).

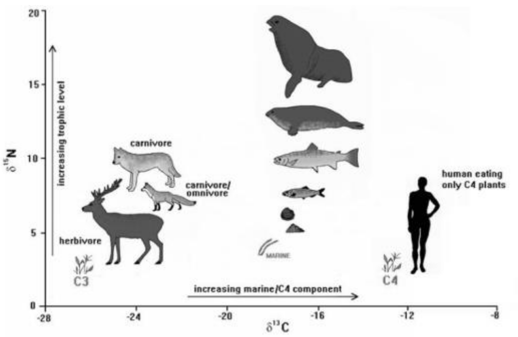

Figure 11 Nitrogen and Carbon Different organisms' isotopic compositions, from http://chrono.qub.ac.uk (derived from Schulting 1998)

3.3.3 Other Factors that Affect Stable Isotopes

Other factors that may elevate $\delta^{15}N$ values include water stress, physiological stress, and protein stress (Heaton & Sealy, 1993). Elevated $\delta^{15}N$ values can be misinterpreted as marine resource consumption, but they may instead be due to the population eating water-stressed terrestrial species (Heaton, 1986). Nitrogen loss in arid environments is connected to experiencing water stress, and urea excretion is increased relative to the overall urine volume (Ambrose and De Niro 1986). This leads to the excretion of lighter $\delta^{15}N$, causing a subsequent increase in $\delta^{15}N$during prolonged periods of water stress (Cox & Sealy,1997).

Factors that Affect Values of $\delta^{13}C$

Firstly, it is important to note that the "canopy effect" has been observed to result in carbon isotope ratios that are lower than the average values of C_3 and C_4 plants. The incomplete atmospheric mixing observed in this region can be attributed to the presence of dense forest cover and reduced light intensity. The recycling of fractionated respired carbon dioxide (CO2) by plants, as well as the decomposition of plants on the forest floor, contribute to an increase in the $\delta^{13}C$ values of atmospheric CO2. This increase typically falls within the range of -21 to -26 per mil, as reported by Ambrose et al. (2001) and Keegan et al. (1989).

According to previous studies conducted by Tykot et al. (2004) and Heaton et al. (1999), it has been observed that there is a general decrease in $\delta^{13}C$ values near the ground, typically ranging from 3 to 4‰, resulting in a reduction of $\delta^{13}C$. In this scenario, it is imperative to consider various species originating from diverse ecosystems, such as a deer dwelling within a forested habitat as opposed to a cattle inhabiting an open field, in conjunction with the isotopic values pertaining to local food webs. Indeed, it has been observed that animals that consume food sources from the lower levels of the canopy exhibit $\delta^{13}C$ values that are up to 5 per mil lower compared to animals that have access to the upper canopy or consume food from other locations (Ambrose & DeNiro et al., 1986).

The $\delta^{13}C$ values exhibit variations in response to stomatal conductance in plants regarding climate. Plants can regulate the opening and closing of their stomata in response to environmental conditions, which in turn affects the isotopic composition of their tissues. During periods of high temperature and low humidity, plants tend to close their stomata to minimize water loss. Conversely, when stomata are open or have a high conductance, water use efficiency decreases, leading to reduced photosynthetic rates and more negative isotopic values (Ambrose, 2001).

Factors that Affect Values of δ^{15}N

The significance of nitrogen values is also noteworthy. The study conducted by Ambrose (1991) demonstrates that $\delta^{15}N$ values exhibit sensitivity to climate and tend to be higher in arid regions. In general, it can be observed that temperate forest soils exhibit relatively lower $\delta^{15}N$ values in comparison to desert or tropical environments, where higher $\delta^{15}N$ values are typically observed. This pertains to the relationship between aridity and the nitrogen cycle. It is widely recognized that nitrogen fractionation processes, including nitrification, mineralization (the conversion of organic nitrogen into ammonium by bacteria), and denitrification, significantly influence the loss of nitrogen, resulting in $\delta^{15}N$ enrichment in both soil and plants. In addition, it has been observed that arid and hot ecosystems are susceptible to nitrogen loss, resulting in an increase in $\delta^{15}N$ values. Conversely, wet, and cold environments exhibit a higher degree of nitrogen conservation and recycling through the nitrogen pools (Szpak et al., 2014; Ambrose et al., 1993; Martinelli et al., 1999).

According to Ambrose et al. (1991), there is evidence to suggest that $\delta^{15}N$ values in plants from arid regions may exhibit variability, reaching values as high as +13‰. This variability is likely attributed to the distribution of nitrate within the soil. In addition to these factors, the salinity of soils and the presence of organic materials can also contribute to higher $\delta^{15}N$ values. For instance, areas with a history of evaporation or high levels of animal residue, such as manure, have been found to exhibit elevated $\delta^{15}N$ values (Larsen et al., 2016). Saline environments have been found to exhibit elevated $\delta^{15}N$ values, which can be attributed to the increased presence of soil nitrate and ammonium (Pate et al., 1994).

In contrast to the aridity and soil conditions, precipitation also exerts an influence on the alteration of nitrogen levels. According to Heaton et al. (1986), there exists a negative correlation between receiving an annual rainfall of less than 400mm and the increase in $\delta^{15}N$ values. This correlation assumes of water availability and aridity, with $\delta^{15}N$ values ranging from 10 to 13 per mil. Finally, it has been observed that altitude is associated with lower $\delta^{15}N$ values, with a decrease of a few percentage points as altitude increases. This observation assumes that the changes in altitude lead to these variations.

Protein stress also raises bodily tissue $\delta^{15}N$ levels (Fuller et al. 2005, Lightfoot, 2009). Paleopathology is an evolving field that uses stable isotopes to research disease processes and metabolism (Long et al. 1981). The breakdown and reutilization of existing $\delta^{15}N$ are linked to protein stress and higher $\delta^{14}N$due to the preferred excretion of $\delta^{14}N$ (Reitsema et al., 2016). Humans are frequently shown to synthesize new proteins from the byproducts of current proteins being catabolized as a result of nutritional stress (Hobson & Clark, 1992; Hobson et al., 1993). Diseases like AID can cause osteomyelitis and new bone deposition with increased $\delta^{15}N$ (Olsen et al., 2014).

3.4. IRMS

The quantification of stable isotopes of carbon and nitrogen in bulk materials, such as bone collagen, is achieved through the utilization of an isotopic ratio mass spectrometer (IRMS). Mass spectrometry is the most efficient technique for determining the relative abundance of isotopes (Muccio, et al., 2009). Because of their distinct mass-to-charge ratios, ionized forms of 12C and ^{13}C may be separated in the mass spectrometer (Brown, 2011).

The system can be technically organized into four main components: an inlet mechanism, an ionization source, a mass analyzer, and an ion detector (Muccio & Jackson 2008). Initially, ionized isotopes are transported via helium gas. The conversion of samples to carbon dioxide and nitrogen is achieved through combustion at a high temperature of 1020 °C (Schoeninger & Moore, 1992; Brown, 2011). This process takes place in a chamber that is equipped with wires made of copper, nickel, and platinum. Subjecting the second set of copper wires to a temperature of 600° C facilitates the reduction of residual nitrogen to nitrogen (Katzenberg et al. 2008; Sharp et al. 2007). Before entering the mass spectrometer, electron ionization turns nitrogen and carbon dioxide into ions (Muccio & Jackson, 2009). The Faraday collector is utilized to capture ions of distinct isotopes that undergo varying degrees of deflection within the magnetic sector. The Faraday collector produces an electric current that is utilized to quantify each isotope present in the initial sample (Muccio, et al., 2009).

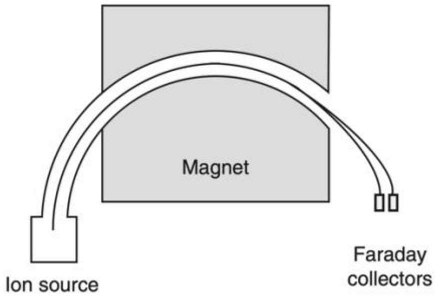

Figure 12 Scheme of IRMS. Adopted from Brown, 2011

The preference for compact bone over spongy bone is attributed to the latter's greater susceptibility to taphonomic and diagenetic processes.

3.5. Stable Isotope Analysis (SIA) in Medieval Europe

This chapter will provide a comprehensive overview of prior paleodietary investigations conducted in the Iberian Peninsula (Fig. 13 and Table 2), as well as selected European sites (Holland, Poland) during the medieval period. The objective of this chapter is to examine the correlation between diet changes across Iberia, with the aim of identifying the potential contributions that can be made by research of Flôr da Rosa in relation to neighboring communities.

The Iberian Peninsula is considered a subcontinent due to its geographical location in the southwestern region of Europe and its possession of unique cultural, geological, and climatic characteristics that differentiate it from the rest of the European continent (Britannica, 2023). The aforementioned qualities exhibit significant variations based on geographical factors such as coastal and inland locations, north and south regions of the Iberian peninsula, and urban and rural settings (Britannica, 2023). Over the course of Mediaeval Iberian history, a distinct and amalgamated culture emerged, characterized by the assimilation of a wide range of traditions, practices, and languages from other civilizations including the Romans, Visigoths, Muslims, Jewish, and Christian communities (Peña-Chocarro et al., 2019). For example, the implementation of technological advancements, such as the green revolution, by the Islamic populace has had a substantial impact on the development of agricultural practices in the region (Kirchner et al., 2019). Hence, the examination of the Mediaeval Iberian paleodiet holds significant value in the context of archaeological investigations pertaining to diverse human lifestyles prevalent in that particular historical era (Veiga-Rilo & Lopez-Costas, 2023; Mackinnon et al. 2015).

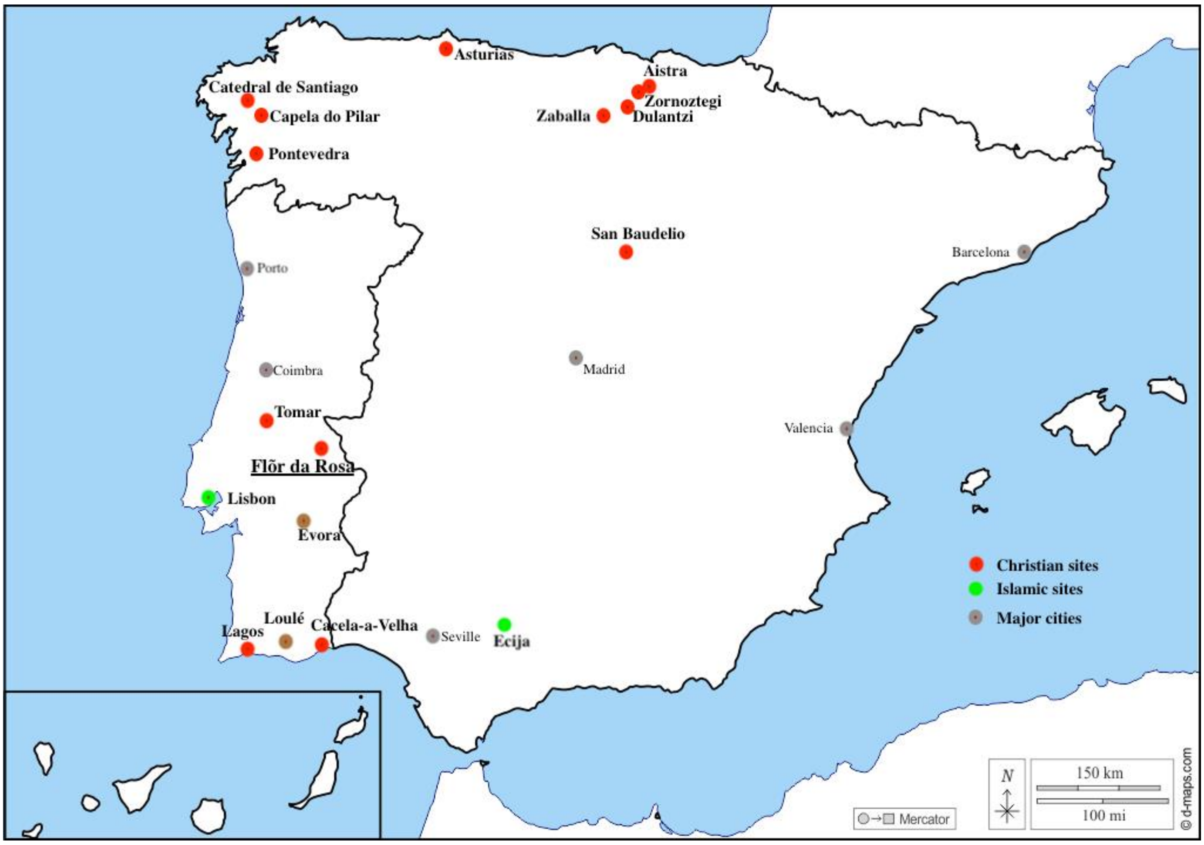

Figure 13 Iberian map that depicts the location of Flôr da Rosa together with comparative sites (represented by different colors: red – Christian sites, green - Islamic sites; gray – Major cities; brown – Mixed Christian and Islamic), adapted from d-maps.com

A positive correlation between protein consumption and social position has frequently been seen(e.g. Quirós Castillo et al. 2013.) Basque country, in the northern Iberian Peninsula, exhibits discernible isotopic variations that correspond to social stratification (Lubritto et al., 2017). The isotopic results suggest that individuals residing in the prominent locations of Zaballa and Treviño (Mean and SD = $\delta^{15}N$ 9.6 ± 1.1 ‰, and $\delta^{13}C - 19.5$ ± 0.7 ‰) mostly consumed a diet consisting of C_3 plants, with a notable inclusion of animal protein (Lubritto et al., 2017). Contrarily, it can be observed that C_4 plants held significant prominence in the context of Aistra (Mean and SD = $\delta^{15}N$ 8.0 ± 1.1 ‰, and $\delta^{13}C - 18.9$ ± 1.0 ‰) and lower-status Zornoztegi (see Tab.1), whereas the consumption of animal protein was found to be somewhat restricted (Lubritto et al., 2017). Dulantzi (Mean and SD = $\delta^{15}N$ 9.1 ± 1.2 ‰, and $\delta^{13}C - 18.8$ ± 1.4 ‰), a community of considerable social standing, was situated between the aforementioned groupings throughout the Early Middle Ages (Quiro ́s Castillo et al., 2013). However, it has been observed that in rural locations in Asturias, individuals of high social standing who were interred within churches did not consistently exhibit increased $\delta^{15}N$ values

(MacKinnon et al., 2019). Although, Toso et al. (2019) provide evidence indicating that the high-status male group in Lisbon (Saõ Jorge Castle) displays greater δN15 values in comparison to the low-status male group. Curto et al. (2018) suggest that the medieval population from the Tomar collection may have been influenced by the intake of freshwater fish, however, Curto et al. (2018) notes that the extent of this influence remains questionable. One potential factor contributing to the intake of freshwater fish, as suggested by Curto et al. (2018), is its association with religious dietary practices. Similarly, MacKinnon et al. (2019) established a connection between the heightened $\delta^{15}N$ value and comparatively low $\delta^{13}C$ value from San Miguel de Lillo in Oviedo (Mean and SD = $\delta^{15}N$ 12.0 ± 1.2 ‰, and $\delta^{13}C -$ 18.6 ± 0.2 ‰), attributing it to periods of fasting and the intake of freshwater fish. However, the exact nature of this relationship remains uncertain (Curto et al. 2018; MacKinnon et al. (2019).

In general, Medieval Iberian sites (Table 1 and Figure 1) exhibit $\delta^{13}C$ mean values within the range of -20.6‰ to -16.40‰. If we eliminate Christian Pontevedra and Islamic Ecija sites, the other sites exhibit $\delta^{13}C$ values ranging from -20.6‰ to -17.0 ‰. These $\delta^{13}C$ values indicate that the individuals at most Medieval Iberian sites consumed a diet primarily composed of C_3-rich plants or domestic herbivorous animals that fed on C_3 plants. When examining the $\delta^{15}N$ values, they exhibit a range spanning from 8‰ to 13.4‰, with an average value of 5.40‰. If the North Iberian Christian sites (Aistra, Zornoztegi, Dulantzi) are excluded, the mean $\delta^{15}N$ values of the remaining Iberian sites would fall within a range of 9.4‰ to 13.4‰, with a mean value of 8.4 ± 0.6‰. This suggests that the consumption of animal protein is primarily derived from terrestrial sources, potentially with limited contributions from aquatic animal protein. It is important to note that Christian Capela do Pilar in North-West Iberia demonstrate elevated nitrogen isotope levels compared to other locations (López-Costas et al., 2021). Some Medieval Iberian sites are situated in close proximity to the coastline, so benefiting from access to marine resources (e.g. Islamic site of São Jorge Castle). Nevertheless, the stable isotopic analysis indicates that the individuals inhabiting Medieval Lisbon did not primarily depend on marine protein supplies (Toso et al., 2018). It is possible that the population may have been fed marine fish from lower trophic levels, such as sardines (Veiga-Rilo & Lopez-Costas, 2023). Consequently, the $\delta^{15}N$ value would likely reflect this dietary behavior, resulting in an isotopic signature comparable to that of terrestrial animals (Lopez-Costas et al., 2019). Only Christian Pontevedra and Asturia show elevated $\delta^{13}C$ and $\delta^{15}N$ associated with marine and millet intake in Medieval Iberia. The isotopic results presented by Lopez-Costas and Müldner (2018) indicate the presence of marine resources in medieval Pontevedra in NW Iberia. Additionally, based on $\delta^{13}C$ values, Pontevedra demonstrates the variable input of C_4 plants (such as millets or later maize) (Lopez-Costas & Müldner, 2018). However, it should be noted that the intake of crops in medieval Pontevedra was predominantly C_3-based (López-Costas and Müldner, 2019). According to Alonso (1999), millets were commonly utilized in the Iberian Peninsula starting from the Iron Age. According to Lopez-Costas and Müldner (2018), regions such as northern Portugal and Galicia had a higher degree of dependence on C_4 plants. In particular, the Christian town of Pontevedra, specifically those belonging to the middle-low socioeconomic class, ingested millet (C_4 plant) in significant quantities (López-Costas and Müldner, 2018). Similarly, the utilization of C_4 plants, specifically millet, has been recorded isotopically in medieval Northern Spain (Asturias) (MacKinnon et al., 2019). However, millet (C_4 plant) was not a dietary staple for the high-status Islamic individuals interred at São Jorge Castle in Lisbon, as well as the local fauna investigated in the study conducted by Toso et al. (2019). Nevertheless, it is worth noting that C_4 plants, which were formerly considered essential and extensively utilized during the period of Islamic control, experienced a decline in significance following the Christian conquest (Toso et al. 2021). It is plausible that they became more closely linked to lower socioeconomic classes and rural communities (Alexander et al., 2019). Identifying the consumption of C_4 plants based on δC13 and δN15, both directly and indirectly through C_4 feeders, poses a challenge (Curto et al., 2018).

Only two Medieval Iberian sites (Islamic Lisbon and Christian San Baudelio) found isotopic differences between sexes (Toso et al., 2019; Jimenez-Probeil et al., 2020). Toso et al. (2019) correlate isotopic gender differences with the Islamic cultural context. Based on isotopic values of $\delta^{15}N$ and $\delta^{13}C$ (Mann-Whitney U test, p = 0.02 and p = 0.89, respectively), they noted that in Islamic societies, men often occupy positions of higher social standing and hence have greater access to protein-rich dietary sources (Toso et al., 2019). Similarly, another study on the Christian site San Baudelio revealed that male individuals exhibited elevated $\delta^{15}N$ land $\delta^{13}C$ values (Jimenez-Brobeil et al., 2020). Researchers connected it with a pattern of males having a greater likelihood of accessing resources that are highly esteemed within the community (Jimenez-Brobeil et al., 2020). Only these two medieval Iberian sites found isotopic differences between sexes, other coetaneous sites in the Iberian Peninsula demonstrate no statistically significant differences between male and female individuals (Table1) based on their isotopic values (Tawanda, 2020; Curto et al. 2018; Aceves, 2019; Gonzalez, 2019; MacKinon et al. 2019, Perez-Ramalho et al. 2022, Lubritto et al. 2017). It is important to mention that the isotopic variations seen between sexes can potentially be connected to additional factors, such as the geographical origin of the individuals (Jimenez-Brobeil et al. 2020; Lubritto et al., 2017; Guede et al., 2017).

Table 2 Carbon and nitrogen isotope values of $\boldsymbol{\delta^{13}C}$ and $\boldsymbol{\delta^{15}N}$ among coetaneous sites

Population	N	$\delta^{13}C$ mean	SD ±	$\delta^{15}N$ mean	SD ±	Sample	Reference	Chronology	Culture	Geography
Écija	38	-16.4	0.80	10.40	1.40	Tooth	Inskip et al., 2019	8– 12	Islamic	Andalusia, Spain
Évora	11	-17.8	0.70	11.90	1.40	Tooth	MacRoberts et al., 2020	8-13	Mixed (Christian and Islamic)	Alentejo, Portugal
Capela do Pilar	6	-18.6	0.40	13.40	1.20	Bone	López-Costas et al., 2021	12-14	Christian	NW Spain
Catedral de Santiago	40	-17.8	0.80	10.90	0.80	Bone	Pérez-Ramallo et al., 2022	9–13	Christian	Northern Iberia
Medieval Pontevedra	63	-16.4	0.80	12.60	0.90	Tooth	Veigo-Rillo & López-Costas, 2023	13–18	Christian	NW Spain
San Baudelio de Berlanga	57	−18.2	0.40	10.03	0.50	Bone	Sylvia A. Jiménez-Brobeil et al., 2020	11-13	Christian	N Spain, Soria
Tomar	33	−18.6	0.50	10.08	0.80	Bone	Curto et al., 2018	11-17	Christian	Portugal
Lisbon	27	−18.9	0.30	9.09	0.80	Bone	Toso et al., 2018	12–13	Islamic	Portugal
Asturia	56	-18.30	1.80	10.30	1.02	Bone	MacKinnon et al. 2019	6–18	Christian	Northern Iberia
Basque Country	147	-19.00	1.20	8.80	1.30	Bone	Lubritto et al., 2017	5-15	Christian	N Spain, Basque
Dulantzi	61	-18.80	1.40	9.10	1.20	Bone	Lubritto et al., 2017	5-15	Christian	N Spain, Basque
Aistra	44	-18.90	1.00	8.00	1.00	Bone	Lubritto et al., 2017	5-15	Christian	N Spain, Basque
Zaballa	14	-19.90	0.90	9.40	1.30	Bone	Lubritto et al., 2017	5-15	Christian	N Spain, Basque
Zornoztegi	9	-18.20	0.80	8.00	0.60	Bone	Lubritto et al., 2017	5-15	Christian	N Spain, Basque

Radom	55	−19.7	0.3	10,08	1,03	Bone	Tomczyk et al., 2020	11-19	Christian	Poland
Blokhuizen	50	-20.6	0.36	11,08	0,88	Bone	Schats et al., 2021	8–12	Christian	Holland
Total	819	-18,19	0,38	10,35	1,03					

When we consider isotopic differences between age groups in the Medieval Iberian Peninsula only the Christian sites of Tomar, Boadilla and Sao Baudelio show statistically significant differences. Curto et al. (2018) found statistically significant differences between young adult individuals and mature adults in the medieval Christian site of Tomar, Portugal. In another study by García-Collado et al. (2018), the Early Mediaeval population of Boadilla shows isotopic differences between juveniles and adults. Juveniles displayed lower $\delta^{15}N$ levels in comparison to adults (García-Collado et al., 2018). Researchers suggested that juveniles have had a reduced consumption of animal protein when compared to the adult population (García-Collado et al. 2018). In the Northern Iberian site of San Baudelio (Mean and SD = $\delta^{15}N$ 10.3 ± 0.5 ‰, and $\delta^{13}C - 18.2$ ± 0.4 ‰), Jimenez-Brobeil et al. (2020) discovered a statistically significant difference (p-value=0.031) when comparing age groups based on sex. Thus, the young adult male group demonstrated higher ^{15}N values than the young adult female group. No statistically significant difference in $\delta^{13}C$ and $\delta^{15}N$ was observed (Jimenez- Brobeil, 2020). Sarkic et al. (2018) found a statistically significant isotopic difference in one female individual within a post-medieval nun community in Belmonte. Sarkic et al. (2018) propose that the migration of outliers to this convent during the latter years of their lives is the cause. The remaining Medieval Iberian found no statistically significant isotopic difference between age groups (Lopez-Costas et al., 2021, Aceves, 2019). The exploration of dietary patterns across different age groups is often limited, primarily because it requires a substantial sample size that includes well-defined age categories (Lopez-Costas et. al., 2019).

Isotopic studies have become an important tool in understanding the dietary patterns and social dynamics of past populations (Alexander et al., 2019; Curto et al., 2018; García-Collado et al., 2018). Pérez-Ramalho et al. (2022) conducted a study on the isotopic difference in North-Eastern Christian Iberian sites (9th-13th Centuries), specifically examining the distinction between urban and rural populations. The Catedral de Santiago site exhibits notable variations in $\delta^{15}N$ values but no discernible distinctions in $\delta^{13}C$ (Pérez-Ramalho et al., 2022). It was suggested that urban people exhibited higher average $\delta^{15}N$ values (mean = 10.5 ± 1.0 ‰) compared to individuals residing in rural areas (Pérez-Ramalho et al., 2022). This finding implies a potential correlation between urban living and enhanced availability of animal and/or freshwater protein sources (Pérez-Ramalho et al., 2022). Additionally, physiological stress has been observed to have an impact on increased $\delta^{15}N$ levels (Reitsema, 2013; Nicholls et al., 2020).

Stable isotope analysis has the capacity to yield precise insights into dietary patterns and dental pathologies, given that all substances come into direct contact with the teeth. The correlation between dietary patterns and oral health in historical populations continues to be a subject of scholarly discourse, primarily due to the presence of genetic diversity and the impact of non-dietary factors such as environmental and social influences (Moles, 2012). Nevertheless, it is widely acknowledged that diet plays a significant role and can be utilised to corroborate stable isotope analysis findings when interpreting the dietary patterns of Flôr da Rosa. Dental pathologies such as calculus and caries serve as valuable indicators of dietary patterns, providing insights into the consumption of proteins and carbohydrates (Hilson, 1996).

There are few studies available regarding the association between food and oral health in the archaeological context of Medieval Portugal (Veiga-Rilo & Lopez-Costas, 2023). A sample from Medieval Lisbon (Toso et al. 2019) shows a small prevalence of dental caries with only 4% of the sample exhibiting dental caries. This observation is noteworthy, particularly when considering other contemporary studies. In Leiria, for example, dental caries were recorded in 56% of the individuals studied (Garcia, 2007). São Martinho (84%; Garcia, 2007), Pontevedra (64%; Lopez-Costas in 2023) and the Xarea sites exhibited even higher frequencies of dental caries (73%; Robledo and Trancho, 2003).

4. Material and Methods

4.1. Samples

This chapter will provide a comprehensive description of the materials and methods that were analyzed. The total number of samples is 38: 26 human skeletons and 12 animal bones. Sex was determined through the assessment of pelvic and cranial characteristics (Phenice, 1969; Buikstra and Ubelaker, 1004). The determination of age at death was established by examining the degeneration of the pubic symphysis (Brooks and Suchey, 1990; Buikstra and Ubelaker, 1994) and the degeneration of the auricular surface (Lovejoy et al., 1985). The samples were gathered from regions of the bone that exhibited no discernible indications of lesions.

4.1.1. Human Samples

All human skeletons under study were identified as adults. 10 individuals were identified as male; 12 individuals were identified as female; for 4 individuals it was not possible to estimate age (See Tab). Osteological analysis of the Flôr da Rosa collection was conducted by Dr. Ana Curto.

The human skeletons under study were divided into four age groups: young (18-24 years), mature (25-44), elderly (>44), and an undetermined age group (See Appendix: Tab.15):

- 18-24 years, which represents 15,38% (4 individuals).
- 25-44 years, which represents 26,9% (7 individuals).
- >44 years, which represents 26,9% (7 individuals).
- Undetermined, which represents 30,7% (8 individuals).

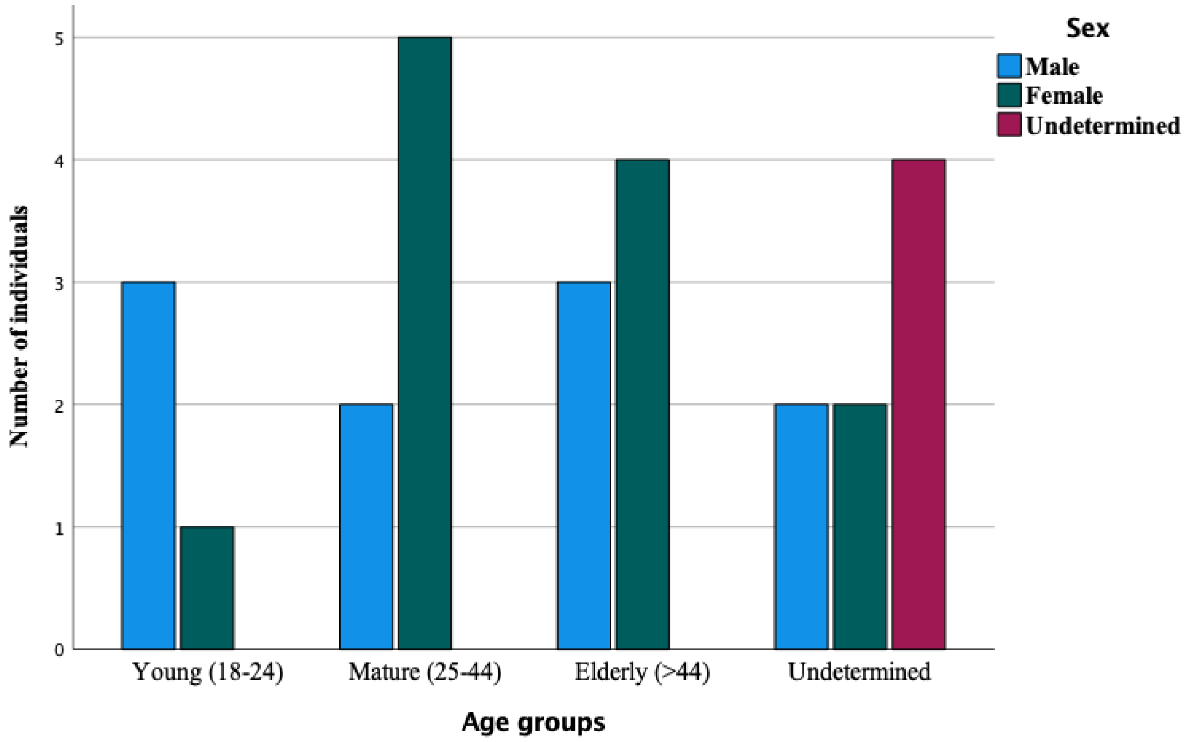

Figure 14 Bar chart of selected samples of Flôr da Rosa site for SIA divided by age groups and sex

All 26 human samples consist solely of rib bones. So far three palaeopathological cases have been described in Flôr da Rosa’s osteological collection. One of them is a potential case of diffuse idiopathic skeletal Hyperostosis (DISH) in a mature female skeleton (FR 885) (Candido et al. 2018) This pathology is a rheumatologic disorder characterized by excessive formation of bone resembling melted candle wax in the anterior common vertebral ligament, especially if affecting at least three consecutive vertebrae(Ortner, 2003). Apart from the vertebral lesions, FR 885 also displays ossification of entheses and ligaments throughout the rest of the skeleton (Candido et al., 2018). This condition tends to rise with advancing age and higher body weight (Ortner, 2003).

Another case is the identification of two pleural plaques associated with tuberculosis in female FR 857 (Fernandes et al., 2014). Pleural plaques were a rare occurrence in the medieval era (Fernandes et al., 2014). Multiple lytic lesions were observed in the thoracic vertebral bodies (Fernandes et al., 2014). The presence of apatite remains indicates that infection was still present at the time of death (Fernandes et al, 2014).

The third case is a potential case of poliomyelitis FR 885 in a male adult who died at the age of 50–60 years (Santos et al., 2002). Poliomyelitis is a viral infection affecting the central nervous system, characterized by the clinical manifestation of paralysis in one or multiple muscle groups (Meyer et al., 1960). According to S. Santos, the observed entesopathies are likely a consequence of excessive use of the unaffected limb. The occurrence of limb paralysis during the early stages of development may lead to muscular atrophy and a potential impairment of bone growth in the affected limb(s) (Santos et al.,).

The recent study by A.Curto et al. (2023) examines a potential case of anemia in a non-adult individual FR 1299. The FR 1299's medical records indicate the presence of cranial diploic hyperplasia, long bone porosity, hair-on-end appearance, rib porosity, and thickening (Curto et al., 2023). Additionally, there were observations of non-specific skeletal lesions such as endocranial bone growth and enlarged foramina of the hand's phalanges (Curto et al., 2023). Based on the exuberant lesions, premature mortality, and patterns of maturation and development, Curto et al. (2023) suggested the potential indication of β-Thalassemia major, a condition that necessitates regular blood transfusions for sustained survival. β-thalassemia is an epidemiological condition that exhibits a correlation with malaria, historical slavery, and cultural practices such as intermarriage or consanguineous marriages (Curto et. al, 2023).

4.1.2. Faunal Samples

The present study examines a collection of 12 faunal remains originating from the historical monastery of Flôr da Rosa, dating back to the 14th to 18th centuries. These remains consist of specimens from three distinct genera, namely two from the *Ovis*, one from the *Capra*, three from the *Ovis/Capra* genus, four from the *Bos* genus, and two from the *Aves* genus. The study examined several bones, including the humerus (n=3), tibia (n=3), pelvis (n=3), metacarpal (n=1), vertebra (n=1), and phalange (n=1). The faunal bones were classified by Dr. Vanessa Navarrete. The stable isotopic analysis of fauna was conducted to establish a baseline diet.

Table 3 Faunal samples from Flôr da Rosa

Individual	Bone	Species
FR 642 bird	Metacarpal	Aves
FR 1767	Phalange	Aves
FR 955	Pelvis	Bos
FR 2222 F	Vertebra	Bos
FR 2222 D	Tibia	Bos
FR 2222 E	Tibia	Bos
FR 013	Humerus	Ovis/Capra
FR 642 ovis	Pelvis	Ovis

FR 698	Humerus	Ovis
FR 2222 B	Humerus	Capra
FR 2222 C	Pelvis	Ovis/Capra
FR 2222 A	Tibia	Ovis/Capra

4.2. Collagen Extraction

38 samples were prepared: 26 human bones and 12 faunal bones. The samples were prepared and cleaned at the Laboratory of Biological Anthropology located at Mitra Pole of the University of Évora. Approximately 5 grams of compact bone were extracted by cutting bones into small pieces and subjected to surface cleaning using a Dremel. As part of the procedure to prepare the sample, the ribs were first divided longitudinally into two independent parts. This made it possible to completely remove all of the trabecular bone that was included within the ribs. After being weighed, the bones were placed in glass tubes that had been labeled.

The collagen extraction protocol employed in this study was based on the method originally developed by Longin (1971) and subsequently modified by DeNiro and Epstein in 1981. The collagen extraction was conducted at the HERCULES Laboratory, Universidade de Évora, Portugal. The bone fragments were placed inside identified tubes that were then filled with approximately 10 milliliters of hydrochloric acid (HCl) solution with a concentration of 0.5 moles per liter to demineralize the bones. The samples were vortexed daily for a week. After one week the acid was replaced and the solution vortex daily for another week until the bone had a soft texture. The samples were to remain at room temperature during the day and refrigerated overnight. After the elimination of the mineral component, the samples were washed to eliminate any remaining HCl, for this the tubes were filled with milli-Q water and centrifuged (at 5.0 RPM x 1000 for 5 minutes) this process was repeated about five times until a neutral pH (pH=7)was achieved.

To eliminate any remaining acid and organic residues, the milli-Q water was replaced with 10 ml of a 0.125M sodium hydroxide (NaOH) solution. The tubes then remained at room temperature for twenty hours. The samples were then subjected to centrifugation five times using milli-Q water at a speed of 5000 revolutions per minute for 5 minutes. This process was carried out to remove the NaOH until a pH level of 7 was reached.

In the gelatinization phase, to achieve a lower pH level to inhibit microorganism growth, 10 milliliters of a 0.01 molar hydrochloric acid solution (HCl 0.1M) replaced the solution in the tubes. These tubes were then placed in an oven at 70°C for 48 hours, during which they were regularly vortexed to stimulate gelatinization. When the dissolution of the samples from the initial batch was incomplete, 15 milliliters of hydrochloric acid with a concentration of 0.5 molars was added.

The remaining insoluble residues were filtered out using 5µm EZEE©. The filter was replaced for every sample. A total of 38 plastic containers were identified, weighed, and labeled. The extracted solution was introduced into the containers and freezed.

The collagen containers were freezed in LN2 (liquid nitrogen). The frozen containers underwent a 48-hour freeze-drying (lyophilization) process.

Collagen yields were determined by weighing the samples. The collagen yield is calculated by dividing the sample's collagen weight by its bone weight and multiplying the result by 100.

4.3. Sample Analysis by EA-IRMS

A total of 38 samples were subjected to EA-IRMS analysis, comprising 26 human samples and 12 faunal samples. The specimens were inserted into tin capsules and subjected to combustion.

The samples were analysed at the University of Montpellier (France) – platform AETE-ISO of OSU OREME, using a ThermoFischer Delta Vplus coupled to a Flash Elemental Analyzer Isolink NCS.

Carbon and nitrogen contents (%) were obtained using a calibration based on acetanilide (C : 71.1 %; N: 10.36 %) and alanine (C : 40.4 %; N : 15.7 %). Carbon and nitrogen isotopes $\delta^{13}C$ and $\delta^{15}N$ were calibrated relative to VPDB and AIR, respectively, and analysed using a three-points calibration with USGS61 ($\delta^{13}C$: -35.05 ‰; $\delta^{15}N$: -2.87‰), USGS62 ($\delta^{13}C$: -14.79 ‰; $\delta^{15}N$: 20.17 ‰) and USGS63 ($\delta^{13}C$: -1.17 ‰; $\delta^{15}N$: 37.83 ‰). NIST 1577C was used to check %C (48.8%) and %N (10.3%) contents, as well as isotopic measurement error ($\delta^{13}C$: -17.52 ‰; $\delta^{15}N$: 8.21‰, Guiry and Szpak, 2017). Precision was 0.19 ‰ for $\delta^{13}C$ and 0.37 ‰ for $\delta^{15}N$, while accuracy was 0.24 ‰ for $\delta^{13}C$ and 0.55 ‰ for $\delta^{15}N$. The total analytical uncertainty was determined to be 0.30 ‰ for $\delta^{13}C$ and 0.66 ‰ for $\delta^{15}N$. Precision, accuracy and analytical uncertainty were calculated according to Szpak et al. (2017).

4.4. Dental examination

Oral health was analyzed for the 26 adult human skeletons selected for this study. Dr. Ana Curto conducted a dental examination. The limited sample size was attributed to constraints related to preservation, resulting in the absence of numerous teeth. The samples in this study were previously assessed for the presence or absence of caries, dental calculus, and tooth loss, as indicated in Table 5. The frequency of these lesions was calculated for each individual. Additionally, the frequency of affected teeth by pathologies was recorded and presented in Table 6.

4.5. Statistical analysis

The statistical analysis was performed utilizing the SPSS programme version 24.0 for Mac, as well as employing a manual calculator for supplementary calculations. The Mann-Whitney and Kruskal-Wallis tests were utilized to assess the differences in $\delta^{13}C$ and $\delta^{15}N$ between sexes and age groups.

The Pearson correlation coefficient was utilized to examine the relationships between variables.

5. Results

5.1. Dental Pathology

The human skeletons of Flôr da Rosa under study display dental caries, dental calculus, and antemortem tooth loss (Tables 5,6).

A total of 57.6% (Table 4) of the human skeletons exhibited dental caries. Caries is more frequent in males (70%) than females (58.3%) (Table 4), but females exhibit a higher mean frequency of caries (42%) per individual than males (25%) (Table 3). Neither difference is statistically significant ($p>0.5$). The mean frequency of dental caries of 25% for young adults, 47% for mature adults and 50% for elderly adults. There was no statistically significant difference in the frequency of dental caries between the age groups, $\chi2(2) = 1.94$, $p = 0.37$. There were no statistically significant differences observed between the young and mature adult age groups (U-test = 6.50, $p = 0.24$), as well as between the mature and elderly adult age groups (U-test = 13.50, $p = 0.78$). There is a positive link between age and the frequency of caries. However, it is important to note that the sample size of the study is inadequate to provide statistically significant findings. The individuals FR 956 and FR 2485, both females, had the highest number of teeth affected by dental caries (Table 7).

Table 4 Number of individuals with dental pathologies in Flôr da Rosa by age group and sex. Note that an undetermined group was included in the total. AMTL*= ante mortem tooth loss.

	Age groups	Males N	%	Females N	%	Total	%
Number of dental caries per individual	Young (18-24)	3	66.00	1	100.00	4	75.00
	Mature (25-44)	2	100.00	5	60.00	7	71.40
	Elderly	3	66.00	4	75.00	7	71.40
	Undetermined	2	50.00	2	0	8	25.00
	Total	10	70.00	12	58.30	26	57.60
Number of teeth with calculus per individual	Young (18-24)	3	100.00	1	100.00	4	100.00
	Mature (25-44)	2	50.00	5	100.00	7	85.70
	Elderly	3	66.00	4	75.00	7	71.40
	Undetermined	2	50.00	2	0	8	25.00
	Total	10	70.00	12	75.00	26	65.30
Number of	Young (18-24)	3	100.00	1	100.00	4	100.00

	Mature (25-44)	2	100.00	5	80.00	7	85.70
	Elderly	3	66.00	4	75.00	7	85.70
	Undetermined	2	100.00	2	0	8	37.50
	Total	10	90.00	12	75.00	26	73.00

Table 5 Mean frequency of teeth affected by dental caries, calculus and AMTL*=ante mortem tooth loss in Flôr da Rosa

		Age groups				Sex		
		18-24	25-44	>44	Total	Female	Male	Total
Mean frequency of caries	n	3	5	5	13	7	7	14
	Mean	0.25	0.47	0.50	0.38	0.42	0.25	0.42
	sd	0.28	0.26	0.44	0.29	0.40	0.21	0.33
	Minimum	0.13	0.25	0.25	0.13	0.15	0.10	0.10
	Maximum	0.47	0.88	1.00	1	1	0.59	1
	Test	Kruskal-Wallis H		χ2=1.94	p-value=0.37	Mann-Whitney U	W=26	p-value=0.56
Mean frequency of calculus	n	4	6	5	15	9	7	16
	Mean	0.66	0.59	0.58	0.65	0.55	0.74	0.64
	sd	0.39	0.39	0.31	0.28	0.23	0.26	0.24
	minimum	0.10	0.71	0.25	0.25	0.10	0.29	0.10
	maximum	1.00	0.29	1.00	1.00	0.75	1.00	1.00
	test	Kruskal-Wallis H		χ2=1.11	p-value=0.57	Mann-Whitney U	W=17	p-value=0.12
Mean frequency of AMTL*	n	4	6	6	16	9	9	18
	Mean	0.08	0.23	0.80	0.35	0.21	0.37	0.26
	Sd	0.05	0.30	0.20	0.30	0.29	0.43	0.57
	Minimum	0.04	0.05	0.61	0.04	0.00	0.00	0.04
	Maximum	0.13	0.59	1.00	1.00	0.67	0.92	1.00
	test	Kruskal-Wallis H		χ2=16	p-value=0.04	Mann-Whitney U	W=36.5	p-value=0.72

Dental calculus has been observed in 65% of the human samples. Dental calculus was more common in females (75%) than males (70%) (Table 4), however, males exhibited a higher mean frequency of calculus (74%) than females (55%) per individual (Table 5). There was no statistically significant difference between the sexes ($p>0.5$) or age groups ($\chi2(2)=1.11$, $p=0.57$). It is important to underline the small sample size. Individuals FR1431 and FR1306, both males, have the highest frequency of dental calculus (Table 14).

A total of 73% of the individuals within the study samples had tooth loss prior to death. Tooth loss was more common in males (90%) than in females (75%) (Table 4), similarly, males displayed a higher mean frequency of antemortem tooth loss (36%) than females (21%) (Table 5). The mean frequencies of AMTL were found to be 0.8% for young people, 23% for mature adults, and 80% for the elderly group. There were no statistically significant differences between sexes ($p>0.5$). The results indicated a statistically significant difference in the frequency of AMTL across different age categories, $\chi 2(2) = 10.837$, $p = 0.004$. The individuals FR 957 and FR 2485, both females, had the highest number of teeth affected by AMTL (Table 7). Among individuals who had AMTL, a significant proportion of 63% exhibited the coexistence of carious lesions.

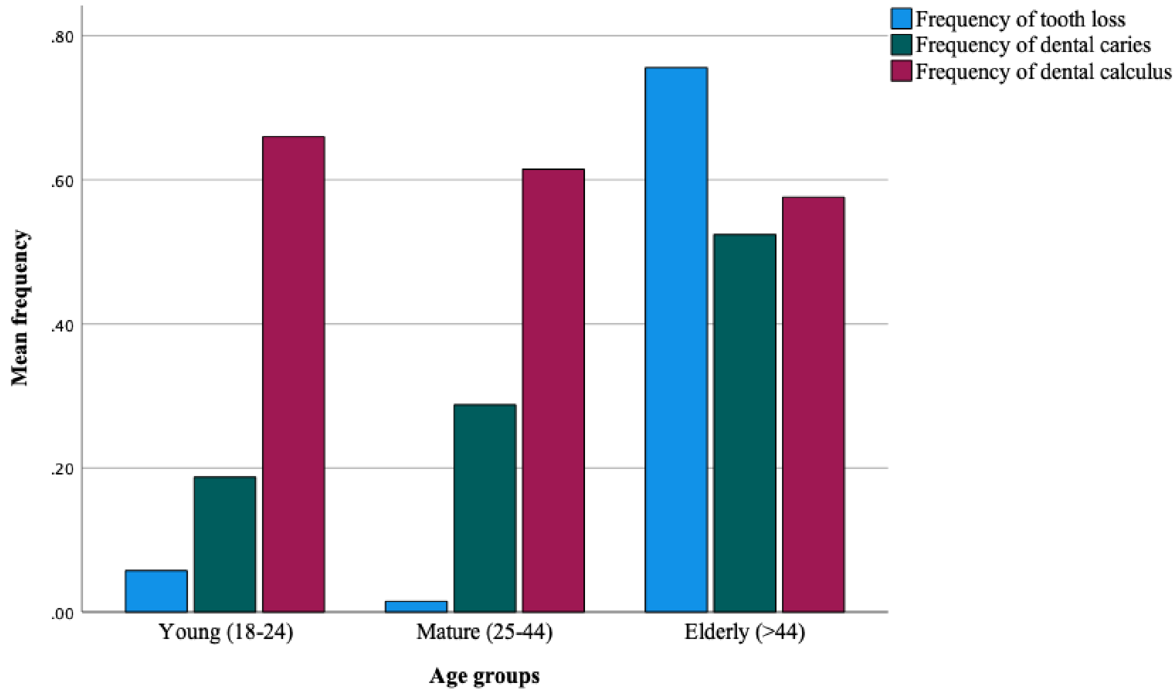

Figure 15 Graphic representation of the mean frequencies of dental pathologies by age group

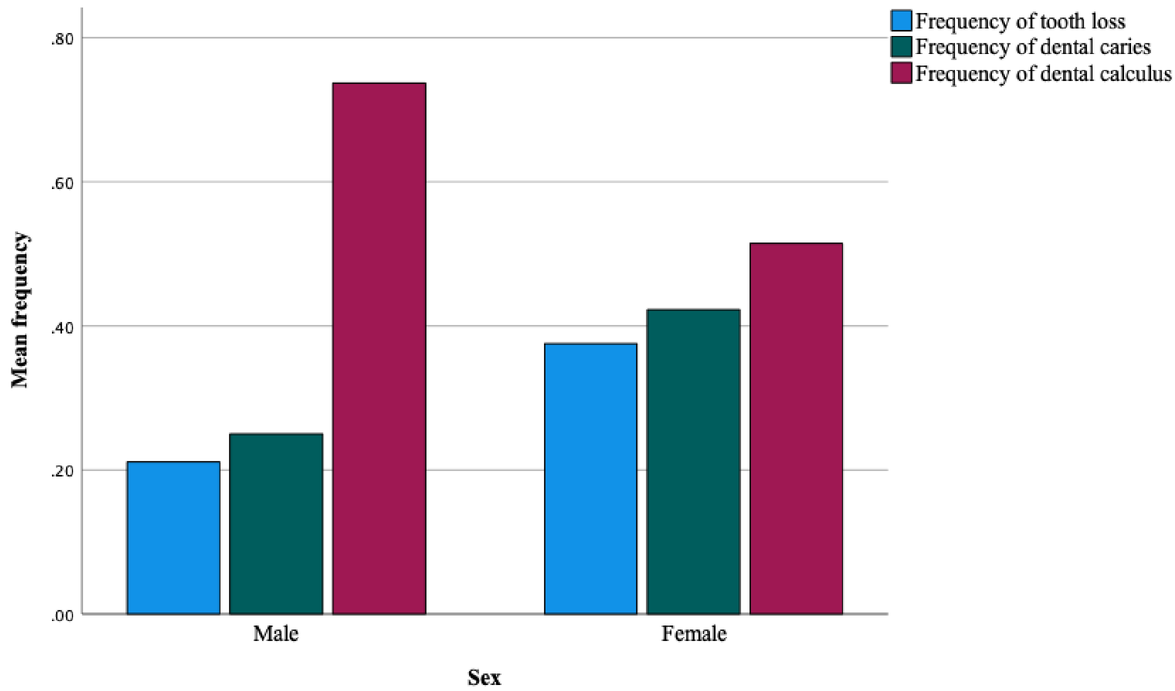

Figure 16 Graphic representation of the mean frequencies of dental pathologies by sex

Table 6. Pearson correlation analysis of dental diseases

		Frequency of dental caries	**Frequency of dental calculus**	**Frequency of dental tooth loss**
Frequency of dental caries	Pearson correlation	1		
	p-value			
Frequency of dental calculus	Pearson correlation	-0.42	1	
	p-value	0.11		
Frequency of dental tooth loss	Pearson correlation	0.45	-0.23	1
	p-value	0.07	0.38	

A Pearson correlation coefficient was tested to assess the relationship between the frequencies of dental caries, calculus and tooth loss.

There was a moderate positive correlation between the frequency of dental caries and tooth loss (r=0.45, N=16). Between the frequency of dental caries and calculus there was a moderate negative correlation (r=-0.42, N=16). The correlation between the frequency of dental tooth loss and calculus was a weak negative (r=-0.42, N=16). Overall, the relationship between frequencies of dental diseases was not statistically significant (p>0.5).

5.2. Isotopic composition

5.2.1. Faunal bone collagen samples

Animal/human sample ratio in this study (Animal:Human = 0.46) was within recommended range (0.3 to 0.5) as advised by Lopez-Costas et al. (2019).

The faunal bone collagen samples (Table 7) from Flôr da Rosa exhibited acceptable C/N ratios between 3.2 and 3.3 (De Niro et al., 1985), %C and %N values are within the quality indices proposed by DeNiro (1985), Ambrose (1990) and Van Klinken (1999) for preserved collagen. Stable isotope analysis of faunal remains from Flôr da Rosa resulted in a mean $\delta^{13}C$ value of -20.40 ± 2.01‰ (ranging from -21.86‰ to -15.60‰) and $\delta^{15}N$ of 7.27 ±1.26‰ (ranging between 5.72‰ to 9.76‰) (Table 8). The faunal samples (without Aves) bone collagen resulted in a mean $\delta^{13}C$ value of -21.19 ± 0.82‰ (ranging from -21.86‰ to -19.49‰) and $\delta^{15}N$ value of 6.83‰±0.80 (ranging from 5.72‰ to 8.45‰) (Table 8).

Table 7 . $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen of human and faunal samples from Flôr da Rosa

	ID	Genus		$\delta^{13}C$	%C	$\delta^{15}N$	%N	C/N
Fauna	FR698	*Ovis aries*	Humerus	-21.72	39.88	5.81	14.38	3.2
	FR013	*Ovis aries / Capra hircus*	Humerus	-21.86	39.06	5.72	14.27	3.2
	FR2222B	*Capra hircus*	Humerus	-21.50	40.05	6.52	14.59	3.2
	FR2222F	*Bos taurus*	Vertebra	-21.78	39.23	6.85	14.18	3.2
	FR2222A	*Ovis aries / Capra hircus*	Tibia	-21.48	39.92	7.51	14.56	3.2
	FR2222C	*Ovis aries / Capra hircus*	Pelvis	-19.49	40.48	7.11	14.64	3.2
	FR642Ovi	*Ovis aries*	Pelvis	-20.10	33.76	8.45	12.31	3.2
	FR2222F	*Bos taurus*	Vertebra	-21.78	39.23	6.85	14.18	3.2
	FR2222E	*Bos taurus*	Tibia	-21.78	39.38	6.37	14.42	3.2
	FR955	*Bos taurus*	Pelvis	-21.55	40.55	6.86	14.55	3.3
	FR2222D	*Bos taurus*	Tibia	-20.68	40.08	7.12	14.68	3.2
	FR642Ave	Aves	Metacarpal	-17.33	37.75	9.19	13.43	3.3
	FR1767	Aves	Phalange	-15.60	40.33	9.76	14.60	3.2

Humans	FR 500	Homo	Rib	-18.28	39.89	9.73	14.62	3.2
	FR 1138			-18.58	39.00	11.73	14.03	3.2
	FR 1301			-18.73	40.25	10.64	14.53	3.2
	FR 1304			-18.44	40.77	10.77	15.08	3.2
	FR 1305			-18.94	40.63	11.06	14.68	3.2
	FR 1306			-18.72	39.55	11.30	14.27	3.2
	FR 1307			-19.08	40.69	11.05	15.02	3.2
	FR 1309			-18.87	39.85	11.03	14.87	3.1
	FR 1414			-18.56	39.19	11.77	14.16	3.2
	FR 1431			-18.57	37.94	12.29	13.66	3.2
	FR 1432A			-18.50	39.70	11.21	14.38	3.2
	FR 1432B			-18.44	38.48	11.33	14.14	3.2
	FR 1435			-19.13	39.93	10.98	14.58	3.2
	FR 1436			-18.59	38.77	10.78	14.35	3.2
	FR 1448			-18.76	40.94	10.76	14.44	3.3
	FR 1499			-18.64	37.92	10.46	13.78	3.2
	FR 1614			-18.70	40.84	10.53	14.64	3.3
	FR 1986			-19.24	42.13	11.84	14.31	3.2
	FR 2485			-18.80	40.41	11.54	14.67	3.2
	FR 642			-18.43	35.10	11.17	12.90	3.2
	FR 885			-17.80	39.96	13.05	14.61	3.2
	FR 953 A			-18.62	40.93	10.98	15.03	3.2
	FR 953 B			-18.59	40.08	10.91	14.68	3.2
	FR 956			-18.53	41.63	11.00	15.40	3.2
	FR 957			-18.98	39.33	10.27	14.36	3.2
	FR 1449			-21.45	31.08	8.12	12.78	2.8

Table 8 Comparative depiction of $\delta^{13}C$ and $\delta^{15}N$ mean values of faunal samples from Flôr da Rosa

Species	N	$\delta^{13}C$ (Mean)	$\delta^{13}C$ (SD)	$\delta^{13}C$ (Min)	$\delta^{13}C$ (Max)	$\delta^{15}N$ (Mean)	$\delta^{15}N$ (SD)	$\delta^{15}N$ (Min)	$\delta^{15}N$ (Max)
Ovis/Capra	3	-20.94	1.27	-21.86	-19.49	6.78	0.93	5.72	7.51
Ovis	2	-20.91	-	-21.72	-20.10	7.13	-	5.81	8.45
Capra	1	-	-	-	-	-	-	-	-
Bos	4	-21.44	0.52	-21.78	-20.68	6.80	0.31	6.37	7.12
Aves	2	-16.46	1.22	-17.33	-15.60	9.47	0.40	9.19	9.76
Total	12	-20.40	2.01	-21.86	-15.60	7.27	1.26	5.72	9.76

The *Aves* showed the highest values of $\delta^{13}C$ (-17.33‰ to -15.60‰) and bone collagen $\delta^{15}N$ (9.19‰ to 9.76‰) among the faunal samples from Flôr da Rosa (Table 8). $\delta^{13}C$ values (-21.86‰ to -19.49‰) and $\delta^{15}N$ (5.72‰ to 8.45‰) of *Ovis aries, Capra hircus* and *Ovis* *aries/Capra hircus* demonstrated higher range compared to their $\delta^{13}C$ values (-21.78‰ to -20.68‰) and $\delta^{15}N$ values (6.37‰ to 7.12‰) of *Bos taurus* (Table 8).

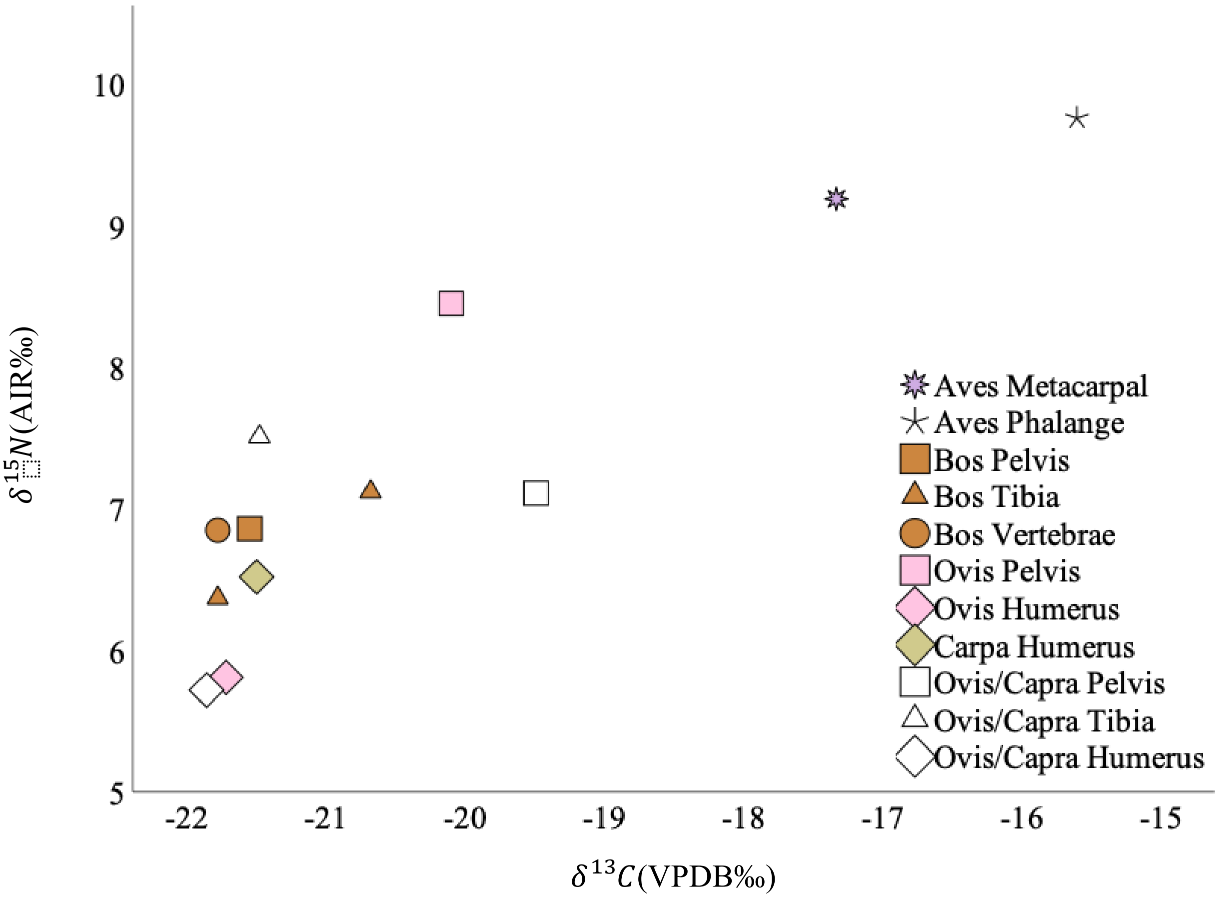

Figure 17 $\delta^{13}C$ and $\delta^{15}N$ values of faunal samples and sampled bone

Table 9 Mean $\delta^{13}C$ and $\delta^{15}N$ values of domestic herbivores (*Ovis aries, Capra hircus and Bos taurus*) in Medieval Iberian Peninsula (12-18th century)

Site	$\delta^{13}C$	$\delta^{15}N$	Reference
Flôr da Rosa	-21.2 ±0.82	6.8 ± 0.80	This study
Tomar	-21.1	6.2	Curto et al., 2018
Evora	-20.2	7.5	MacRoberts et al., 2020
Lisbon	-20.9	6.4	Toso et al., 2019
Capelo do Pilar	-21.7	9.5	Lopez-Costas, 2012
Pontevedra	-21.1	5.7	Lopez-Costas, 2012
Zaballa	-20.6	5.7	Lubritto et al., 2017

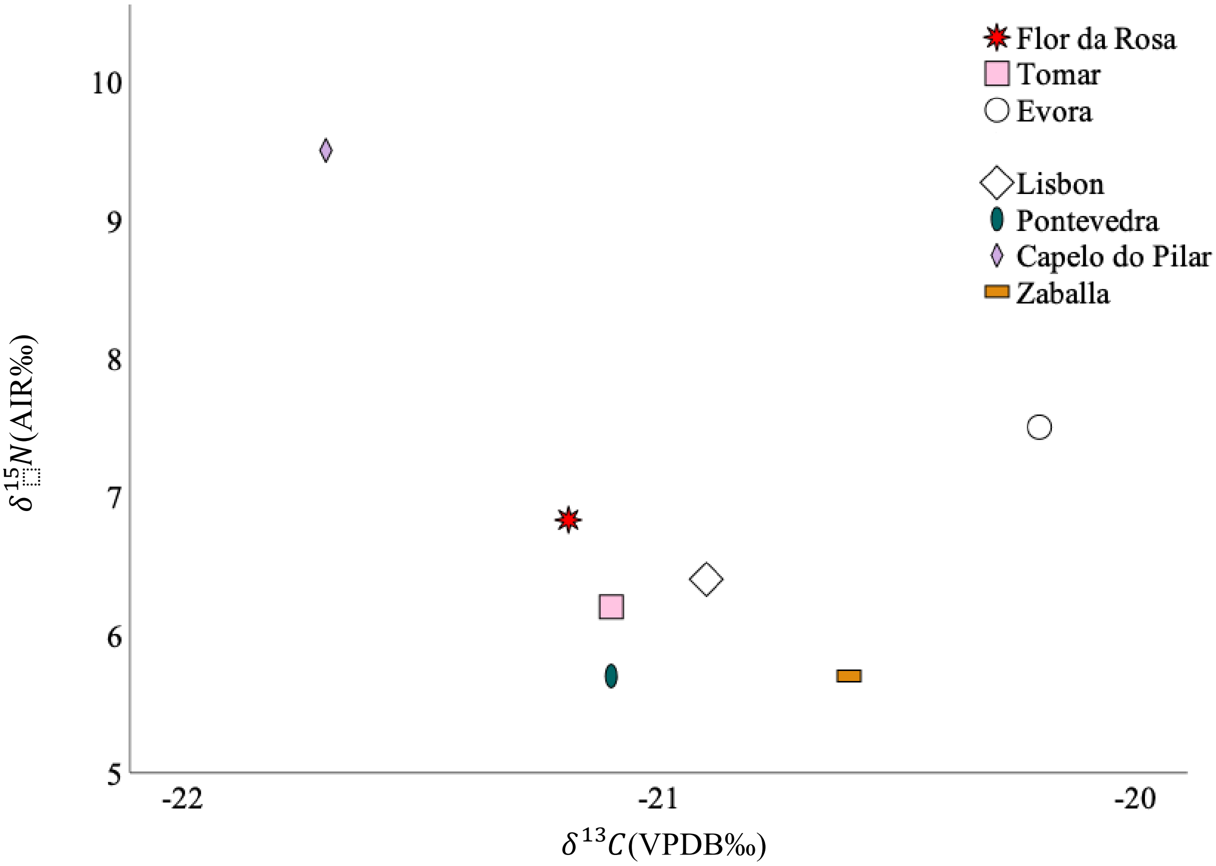

Figure 18 Scatterplot with mean herbivores $\boldsymbol{\delta^{13}C}$ and $\boldsymbol{\delta^{15}N}$ values from Medieval Iberia

5.2.2. Human bone collagen samples

Apart from FR1449, all human bone collagen samples from Flôr da Rosa exhibited acceptable C/N ratios between 3.1 and 3.3 (De Niro et al., 1985). %C and %N values are within the quality indices proposed by DeNiro (1985), Ambrose (1990) and Van Klinken (1999) for preserved collagen.

The analysis of $\delta^{13}C$ and $\delta^{15}N$ of human samples resulted in a mean $\delta^{13}C$ value of -18.66‰ $\pm$0.29 (ranging from -19.24‰ to -17.80‰) and a mean $\delta^{15}N$ value of 11.12‰$\pm$0.67 (ranging from 9.73‰ to 13.05‰) (Table 10). Amongst the human individuals studied, there is one outlier, elderly (>44) male (FR885). His bone collagen $\delta^{13}C$ (-17.8‰) and bone collagen $\delta^{15}N$ (13.05‰) are the highest compared to the Flôr da Rosa of the population.

Human $\delta^{13}C$ of bone collagen in Flôr da Rosa exhibited a range between -19.3‰ to 18.28‰ $\delta^{15}N$ of bone collagen was from 9.73‰ to 12.29‰ (Table 10). Male bone collagen $\delta^{13}C$ showed a range between -19.3‰ and -18.28‰, whereas female bone collagen $\delta^{13}C$ showed a range between -18.98‰ and -18.50‰. Male bone collagen ^{15}N ranged from 9.73‰ to 12.29‰, whereas female bone collagen ^{15}N ranged from 10.27‰ to 11.77‰ (Table 10).

Table 10 Descriptive statistics for the stable isotope ratios analyzed without mature female FR1449 and elderly male FR885

	Female		Male		Female and male	
	Isotope					
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Mean	-18.72	10.96	-18.65	10.97	-18.69	10.92
SD	0.16	0.43	0.28	0.68	0.22	0.54
Variance	0.02	0.19	0.08	0.46	0.05	0.29
Max	-18.50	11.77	-18.28	12.29	-18.28	12.29
Min	-18.98	10.27	-19.13	9.73	-19.13	9.73
N	11	11	9	9	20	20
Non-parametric statistics tests	Mann-Whitney U				37.50	49
	p value				0.36	0.97

Overall, bone collagen $\delta^{15}N$ registered in humans is more variable than bone collagen $\delta^{13}C$ (Table 7 and Fig. 19). Males displayed more variance in bone collagen's $\delta^{13}C$ and $\delta^{15}N$ than females (Table 10). There is no statistically significant difference in bone collagen $\delta^{13}C$ and $\delta^{15}N$ between sexes (p>0.05) (Table 10).

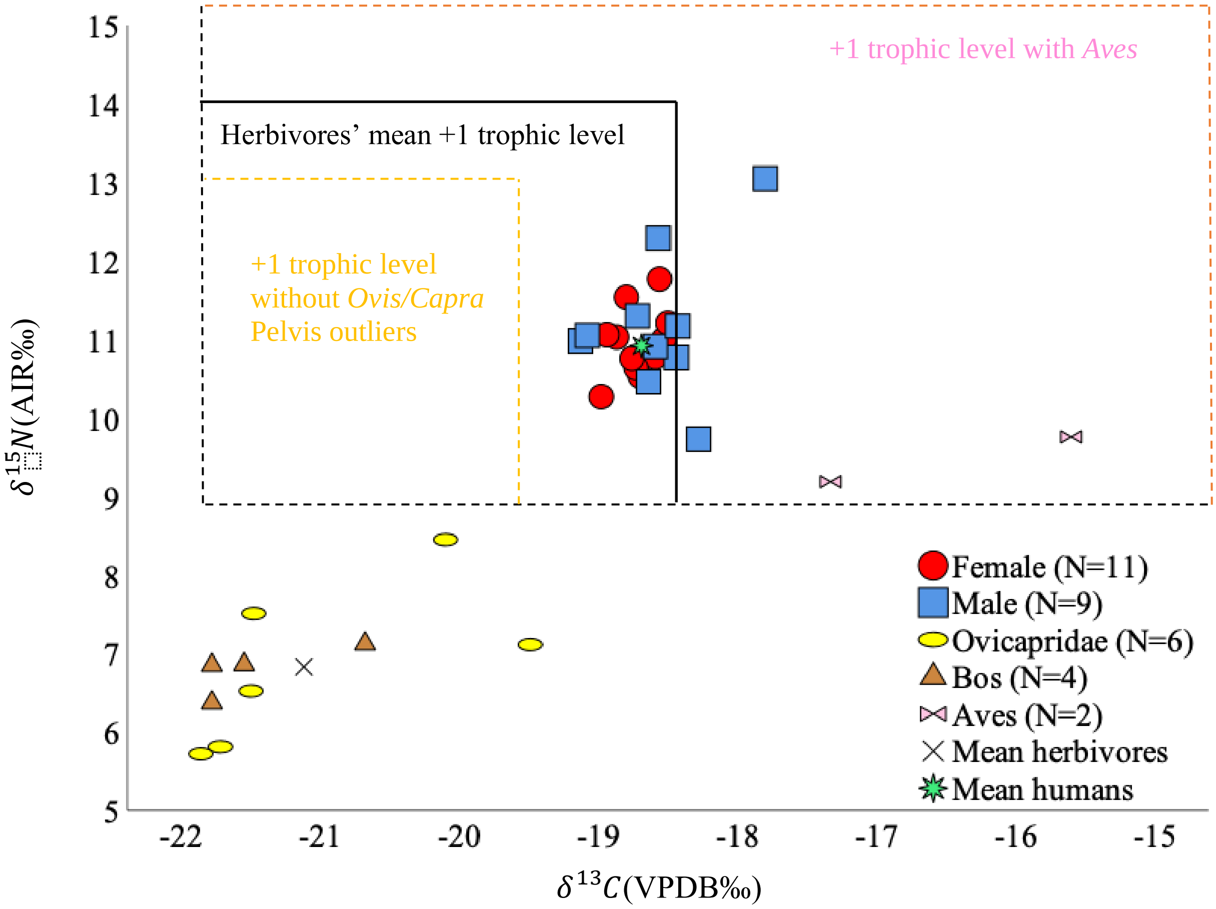

Figure 19. Scatter plot of distribution $\delta^{13}C$ and $\delta^{15}N$ values for males and females together with fauna from Flôr da Rosa. Expected + 1 trophic level shift range is given for Herbivores (*Bos taurus and Ovis aries, Capra hircus and Ovis aries/Capra hircus*) mean values.

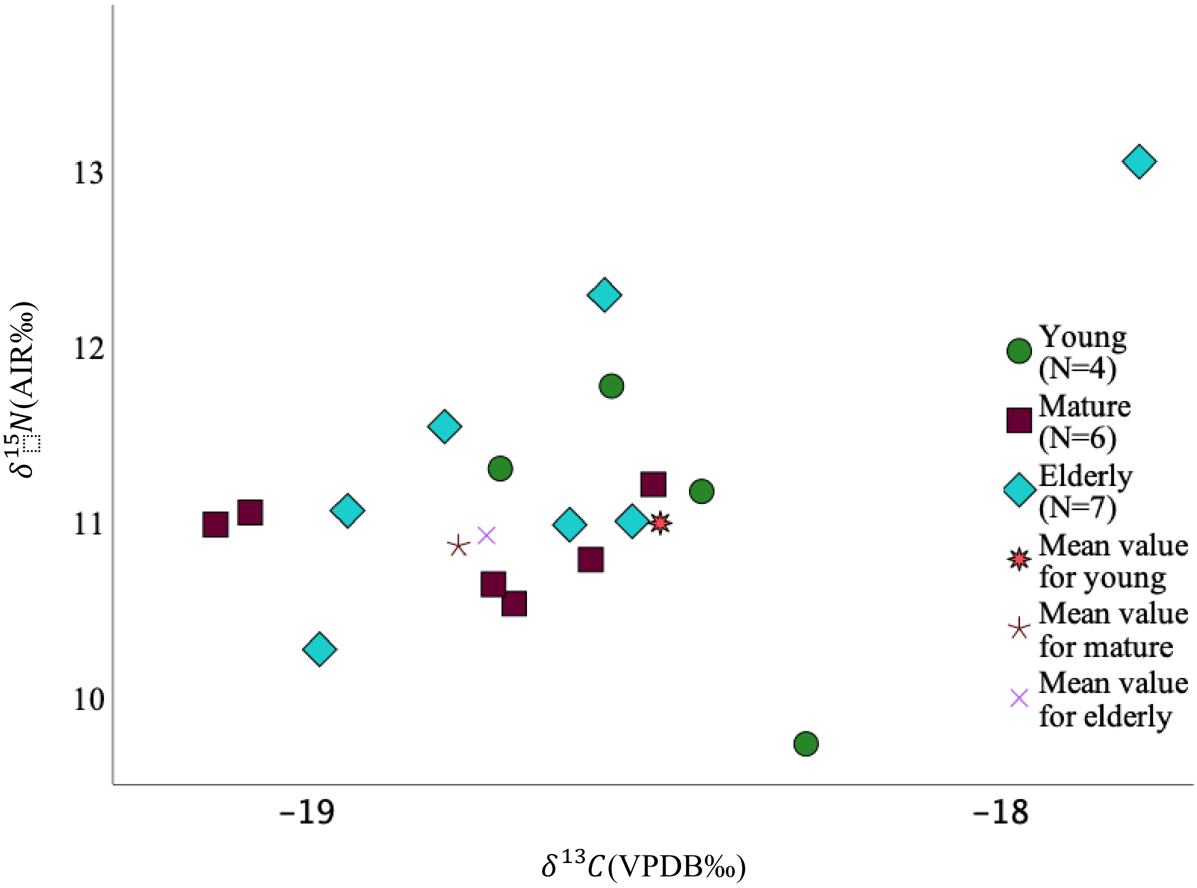

Figure 20 Scatter plot of distribution $\delta^{13}C$ and $\delta^{15}N$ values for young, mature adults and elderly. Stable isotope values of individuals with estimated age. Mean values for all the samples except the outlier ($\delta^{13}C$ -17.8‰ and $\delta^{15}N$ 13.05‰).

The mean $\delta^{13}C$ values of young (-18.49 ± 0.18‰), mature ($\delta^{13}C$ -18.78 ± 0.25‰) and elderly (-18.74 ± 0.19‰) groups were comparable (Fig. 20). Additionally, the mean $\delta^{15}N$ values of young (10.99 ± 0.88‰), mature (10.86 ± 0.25‰) and elderly (10.92 ± 0.67‰) groups showed similar values (Fig. 20).

^{13}C of young adults exhibited a range between -18.72‰ and -18.28‰ (Table 11). Mature bone collagen ^{13}C ranged between -19.13‰ and -18.50‰, whereas elderly bone collagen ^{13}C ranged between -18.98‰ and -18.53‰. The range for young bone collagen ^{15}N was 9.73‰ to 11.77‰; for mature bone collagen ^{15}N, it was 10.53‰ to 11.21‰; and for old bone collagen ^{15}N, it was 10.27‰ to 12.29‰ (Table 11).

Bone collagen $\delta^{15}N$ of the young and elderly individuals showed more variance than bone collagen $\delta^{15}N$ of the mature (Table 11). There were no statistically significant differences in bone collagen $\delta^{13}C$ and $\delta^{15}N$ between age groups, p=0.14 and p=0.45 respectively (Table 11).

Table 11 Descriptive statistics of stable isotope values by age groups

	Young (18-24)		Mature (25-44)		Elderly (>44)		All age groups	
	Isotope							
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Mean	-18.49	10.99	-18.78	10.86	-18.74	10.92	-18.69	11.01
SD	0.18	0.88	0.25	0.25	0.19	0.67	0.23	0.59
Variance	0.03	0.77	0.06	0.06	0.03	0.45	0.05	0.35
Max	-18.28	11.77	-18.50	11.21	-18.53	12.29	-18.28	12.29
Min	-18.72	9.73	-19.13	10.53	-18.98	10.27	-19.13	9.73
N	4	4	6	6	6	6	16	16
Kruskal-Wallis H							3.84	1.59
p value							0.14	0.45

A Pearson correlation coefficient was tested to assess the relationship between the bone collagen $\delta^{13}C$, $\delta^{15}N$ and dental diseases (Table 12).

Bone collagen $\delta^{13}C$ showed a weak correlation with frequencies of dental caries (r=-0.38), dental calculus (r=0,11), and tooth loss (r=-0.20) (Table 12). Similarly, bone collagen $\delta^{15}N$ demonstrated a weak correlation with frequencies of dental caries (r=0.11), calculus (-0.07) and tooth loss (r=0.13). Overall, there was no statistically significant correlation between stable isotopes (C, N) and dental diseases (p>0.5) (Table 12).

Table 12. Pearson correlation analysis of bone collagen $\delta^{13}C$, $\delta^{15}N$ to frequencies of dental caries, calculus and tooth loss

		Frequency of dental caries	**Frequency of dental calculus**	**Frequency of tooth loss**
$\boldsymbol{\delta^{13}C}$	Pearson correlation	-0.38	0.11	-0.20
	p-value	0.14	0.67	0.40
$\boldsymbol{\delta^{15}N}$	Pearson correlation	0.11	-0.07	0.13
	p-value	0.19	0.98	0.59

Table 13. Pearson correlation analysis of dental diseases

		Frequency of dental caries	**Frequency of dental calculus**	**Frequency of dental tooth loss**
Frequency of dental caries	Pearson correlation	1		
	p-value			
Frequency of dental calculus	Pearson correlation	-0.42	1	
	p-value	0.11		
Frequency of dental tooth loss	Pearson correlation	0.45	-0.23	1
	p-value	0.07	0.38	

A Pearson correlation coefficient was tested to assess the relationship between the frequencies of dental caries, calculus and tooth loss (Table 13).

There was a moderate positive correlation between the frequency of dental caries and tooth loss, r=0.45 (Table 13). Between the frequency of dental caries and calculus there was a moderate negative correlation, r=-0.42 (Table 13). The correlation between the frequency of dental tooth loss and calculus was a weak negative, r=-0.42 (Table 13). Overall, the relationship between dental diseases was not statistically significant (p>0.5) (Table 13).

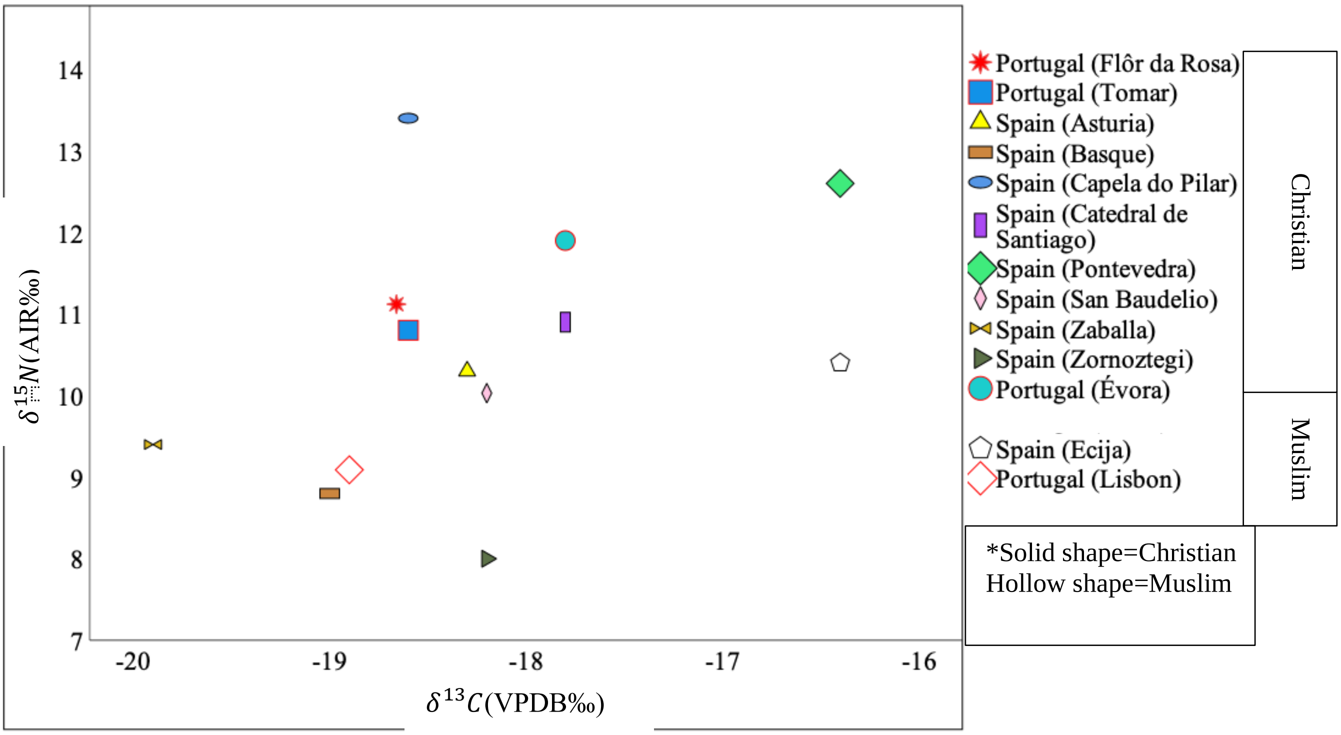

Figure 21. The mean $\delta^{13}C$ and $\delta^{15}N$ values of human samples from different medieval sites in Iberia

When human bone collagen $\delta^{13}C$ and $\delta^{15}N$ of Flôr da Rosa collection was compared with other medieval Iberian sites, $\delta^{13}C$ and $\delta^{15}N$ of Tomar from Portugal was the closest (Fig. 21).

6. Discussion

In this study, we aimed to investigate the possible relationship between diet and oral health at medieval Flôr da Rosa Portugal, by utilizing stable isotope analysis of bone collagen $\delta^{13}C$ and $\delta^{15}N$ values and correlating it with the frequencies of dental caries, calculus, and ante-mortem tooth loss.

Oral health at medieval Flôr da Rosa

Table 14 shows that the frequency of dental caries in Flôr da Rosa (57,6%) is above the average if we compare it with other medieval sites (46%) in the Iberian peninsula (Veiga-Rilo & Lopez-Costas, 2023) while dental calculus in FR (65%) is below the average (75.2%) (Toso et al., 2019; Garcia, 2007; Jordana Comin, 2007; Marques, 2007; López-Morago, 2020).

Table 14 Dental caries and calculus across medieval sites across Iberia, adopted from Veiga-Rilo, C., & López-Costas, O.,2023.

Population	Dental caries (%)	Dental calculus (%)	Location	Reference
Flôr da Rosa	57.6	65	Portugal	This study
Medieval Pontevedra	64	72	Portugal	Veiga-Rilo, C., & López-Costas, O. ,2023
Lisbon	4	-	Portugal	Toso et al., 2018
Sao Martinho	84	-	Portugal	Garcia, 2007
Xarea	73	-	Portugal	Sanz & Gayo, 2003
Sant Pere de Terrassa	10	59	Spain	Jordana Comin, 2007
Capela da Nossa Senhora da Vitória	26	37	Spain	Marques, 2007
Poblaciones históricas de Castilla y Leon	4	77	Spain	López Martinez, 2000
San Baudelio de Berlanga	20	80	Spain	López-Morago, 2020
El Castillo	40	90	Spain	López-Morago, 2020
Capela do Espírito Santo	80	-	Portugal	Ferreira, 2015
Igreja da N.Sra. Anunciada	92	-	Portugal	Ferreira, 2015
Monasterio de Suso	43	100	Spain	López-Morago, 2020
Sahl ben Malik	39	65	Spain	López-Morago, 2020
Villanueva de Soportilla	40	90	Spain	López-Morago, 2020
San Lorenzo y San Nicolás	61	82	Spain	López-Morago, 2020
Average	46	75.2		

There is a correlation between the consumption of a carbohydrate-rich diet and the elevated occurrence of dental caries (Temple ö Larsen, 2007; Tomczyk et al., 2020; Schats et al., 2021; Veiga-Rilo & Lopez-Costas, 2023). Diets rich in fermentable carbohydrates are known as cariogenic because they lower oral pH levels, which erode enamel and promote cariogenesis (Schmidt, 1998; Walter, DeWitte, & Redfern, 2016). Malnutrition in childhood and decreased salivary flow rates associated with religious fasting are other factors can also increase the development of dental caries (Lingström and Moynihan, 2003; Psoter, Reid, & Katz, 2005). Additionally, the increased frequency of dental caries has been related to a high consumption of shellfish and maize (Veiga-Rilo & Lopez-Costas, 2023; López-Costas and Müldner, 2019). The aforementioned information suggests that the high consumption of carbohydrates mostly processed cereal grains, shellfish intake, malnutrition and/or religious fasting could be the reasons behind higher frequencies of dental caries in Flôr da Rosa. The frequency of dental calculus (65%) is higher than dental caries (57.6%) in Flôr da Rosa. The formation of dental calculus is associated with the ingestion of food rich in animal protein, and its by products, and insufficient in carbohydrates intake (Hillson, 1979). The high frequency of dental calculus (65) in Flôr da Rosa (Table 14) suggests that animal-derived food had a significant role in the dietary practices of this population. Figure 16 shows that females exhibited a higher mean frequency of caries (42%) per individual than males (25%) but it was not statistically significant (p=0.56). The higher frequency of dental caries among females has been observed frequently since the beginning of agriculture including the medieval period (Larsen, 1995; Lukacs, 2008; Walter, DeWitte, & Redfern, 2016). Behavioral differences in oral hygiene (Walter, DeWitte, & Redfern, 2016), dietary differences (Lukacs, 2008), biological factors such as pregnancy (Lukacs & Largaespada, 2006), and physiological differences in estrogen level, saliva chemical composition and flow rate (Lukacs, 2011b; Lukacs & Largaespada, 2006) could be the potential reasons behind the higher frequency of dental caries among females in Flôr da Rosa. On the contrary, the increased prevalence of dental calculus among men in Flôr da Rosa (Figure 16) may be attributed to the sexual division of labor and gender-specific dietary preferences, in which males have greater access to consuming meat and animal-based goods (Lukacs, 2008).

There is a lack of studies available regarding the association between dietary intake and oral health in the archaeological context of Medieval Portugal. Coastal Medieval Lisbon shows a small prevalence of dental caries with only 4% of the sample exhibiting dental caries (Toso et al., 2019). Yanko et al. (2021) conducted a study that revealed a positive relationship between marine diets and a reduced occurrence of dental caries, accompanied by an increased incidence of dental calculus. Furthermore, the correlation between increased levels of fluoride and silica in coastal environments has been recognized as an important factor in the decreased prevalence of dental caries and the heightened occurrence of dental calculus (Larsen, 1999). However, Toso et al. (2019) based on isotopic results, concluded that the low prevalence of dental caries in Lisbon relates to the reduced intake of carbohydrates and consistent intake of animal-derived proteins and dietary fats, within the Islamic setting of Lisbon. In Christian Medieval Leiria (Portugal), dental caries was recorded in 56% of the individuals studied (Garcia, 2007) which is close to Flôr da Rosa (Table 13). This can be explained by the rural and inland locations of both Leiria and Flôr da Rosa. Table 14 shows that São Martinho (84%; Garcia, 2007), Pontevedra (64%; Veiga-Rilo & Lopez-Costas, 2023) and the Xarea sites exhibited even higher frequencies of dental caries (73%; Sanz & Gayo, 2003) than Flôr da Rosa. If we compare the oral pathologies of the Flôr da Rosa population with other medieval sites from the Iberian peninsula, Flôr da Rosa samples exhibit in general worse oral health in terms of caries but better in calculus (Table 14). A Pearson correlation analysis did not find strong correlations between dental caries, calculus and tooth loss in Flôr da Rosa (Table 13). However, it should be noted that the correlation between the frequency of dental caries and tooth loss is the highest (r=0.45) among all correlations and is very close to being statistically significant (p=0.07), even in a small sample size (Table 13). It is an expected phenomenon because the primary cause of ante mortem tooth loss is dental caries (Nelson, Lukacs, & Yule, 1999; Lukacs, 2008; Walter, DeWitte, & Redfern, 2016). Figure 15 shows that the frequency of dental caries and tooth loss increased with advancing age, which is expected. There was a statistically significant difference in the frequency of AMTL across different age categories($\chi2(2) = 10.837$, $p = 0.004$; Table 5). The frequency of tooth loss was found to be substantially higher among older persons compared to younger ones (Table 5). As expected, the females exhibited higher average AMTL values than males in Flôr da Rosa (Fig. 16). However, the difference in AMTL between sexes is not statistically significant (p=0.72) (Table 5). In general, it is expected that there is a correlation between advancing age and a greater number of oral health problems. The lower p-values and correlations may also relate to the small sample size of this study (Table 5).

Stable isotope analysis of bone collagen δ^{13}C and δ^{15}N

The mean faunal bone collagen $\delta^{13}C$ of -20.40‰±2.01 and $\delta^{15}N$. 7.27±1.26 values suggest their diet mainly relied on C_3 -based plants (Table 8, Figure 19). The mean faunal bone collagen $\delta^{13}C$ value from Flôr da Rosa is comparable with the mean faunal bone collagen $\delta^{13}C$ values of -20.0‰1±0.7 from the Iberian Peninsula (López-Costas & Alexander, 2019). The herbivores bone collagen $\delta^{13}C$ (mean $\delta^{13}C$ value of -21.19‰±0.82, $\delta^{15}N$ 6.83‰ $\pm$ 0.80) (Table 9) is suggesting mainly C_3 plant consumption (Fig.19). C_4 plants such as broomcorn millet (*Panicum miliaceum*) and foxtail millet (*Setaria italica*) might be used as animal fodder for birds (*Aves*: mean $\delta^{13}C$ value of -16.46‰±1.22) (Table 8). Also, the difference in isotopic values of birds might be connected that birds in Flôr da Rosa collection were wild birds. Millets made their way to the Iberian Peninsula in the Late Bronze Age (Lopez-Costas et al., 2015), and were the only C_4 plants until the eighth century AD when sugarcane and sorghum were later introduced with invasion of Arabs (Peña-Chocarro et al. 2019). The chronology of the Monastery of Flôr da Rosa is long from the 13th to 18th century and it is possible that human individuals used new C_4 plant from the New World such as maize (*Zea mays*). However, there is no historical and archaeobotanical information regarding maize consumption in Flôr da Rosa.

Herbivores bone collagen $\delta^{15}N$ is more variable than $\delta^{13}C$ in Flôr da Rosa (Table 8). In Flôr da Rosa, the *Ovis/Capra* have comparable $\delta^{15}N$ values as *Bos* (Table 8). Other medieval Iberian sites shows a trend of elevated ^{15}N in *Ovicapridae* than *Bos* is seen in medieval Christian Tomar (Curto et al., 2018), Islamic Lisbon (Toso et al., 2019), and Christian Asturias (MacKinnon et al. 2019). In Asturias, the range of sheep/goats $\delta^{13}C$ is 9‰ while in Flôr da Rosa it is 2.37‰ which suggests comparatively a more homogenous diet for *Ovis/Capra* in Flôr da Rosa than Asturias. The variability in herbivores' bone collagen $\delta^{15}N$ also can be associated with the manuring of soils and land management practices (Boggard et al., 2013). Additionally, the variability between $\delta^{13}C$ and $\delta^{15}N$ values of herbivores could be attributed to the difference in bone turnover rates of various bones within the same species (Fig.17). In Fig. 17, the $\delta^{13}C$ and $\delta^{15}N$ of the humerus bones of *Ovicapridae* showed a difference from the pelvis bones of *Ovicapridae*. Sykut et al., (2020) found a mean variation of 0.88‰±0.24 for $\delta^{15}N$ and 0.46‰±0.07 for $\delta^{13}C$ between samples taken from different bones of red deer (*Cervus elaphus*) within the same individual. However, there is a lack of research analyzing isotopic variability between faunal bones from the same individual, especially for sheep/goats (*Ovis/Capra*). Another potential distinction between *Bos* and *Ovis/Capra* lies in their respective dietary preferences, with sheep and cattle mostly consuming grass and hay, whereas goats choose to feed on trees and shrubs (Curto et al., 2018).

Based on the herbivores isotope values the expected trophic level increase would be between 8.72 and 14.15‰ for $\delta^{15}N$ and from -21.86 to -18.49‰ for $\delta^{13}C$ (Fig. 19) which is within the expected trophic level increase of 0-2‰ for $\delta^{13}C$ and 3-5‰ for $\delta^{15}N$ (Lee-Thorp et al., 1989; DeNiro and Epstein, 1978; Hedges and Reynard, 2007). The mean increase from faunal to human bone collagen is 1.62‰ for $\delta^{13}C$ and 3.81‰ for $\delta^{15}N$ (Tables 7 and 8).

The human bone collagen $\delta^{13}C$ of Flôr da Rosa (-18.66‰) is consistent with other Medieval sites in Portugal (Tomar, Lisbon) where C_3 -based plant intake is dominated (Table 2, Fig. 21). However, $\delta^{13}C$ of Flôr da Rosa differs from $\delta^{13}C$ of north Iberian sites of Pontevedra (-16.4‰) and Asturias where C_4 -plants such as millet (later maize) played an important role in the diet of middle-low social classes (López-Costas and Müldner, 2019; MacKinnon et al. 2019). Most of the human samples were within the expected trophic level increase except for one male outlier (FR885) (Fig. 21). The different $\delta^{13}C$ and $\delta^{15}N$ values of elderly male FR885 could be explained by the FR885's health problems (See chapter 4.1.1. Human samples). FR885 exhibited signs of DISH (diffuse-idiopathic skeletal hyperostosis) and poliomyelitis (Santos, Candido et al. 2018). DISH tends to rise with advancing age and higher body weight, and is associated with high meat consumption (Ortner, 2003).

Different protein sources could be responsible for the variation in the human bone collagen $\delta^{15}N$ of 3.32‰ (Table 10; Table 11). It could be connected to ingesting modest amounts of fish. However, Flôr da Rosa is in 175 kilometers from the Atlantic Ocean and 35 km from the Tagus River (according to Google Maps). It is important to note that the population Flôr da Rosa could have bought fish from the coastal area but there is no historical and archaeological record of it. Therefore, it is improbable that human individuals consume proteins derived from fish. Birds (*Aves*) might consume C_4 plants such as millets as fodder (Table 8). Male outliers FR885 and FR500 might consumed birds which might affected their $\delta^{13}C$ values (Table 7; Fig. 21). Additionally, small amounts of millet might be ingested by young male FR500 ($\delta^{13}C$: -18.28‰) and elderly male FR885 (-17.8‰). To wrap up, the human bone collagen $\delta^{13}C$ from Flôr da Rosa indicates general consumption of C_3 plants. The diet at Flôr da Rosa likely utilized C_3 as a staple with minimal contributions of C_4 plants based on stable isotope values of bone collagen $\delta^{13}C$. The bone collagen $\delta^{15}N$ of human samples from Flôr da Rosa is indicative of terrestrial food intake. The $\delta^{15}N$dietary intake of Flôr da Rosa mostly consisted of domestic herbivores (sheep/goats, cattle). Compared to different Medieval Iberian sites (Table 2; Fig. 21), human bone collagen values of $\delta^{13}C$ and $\delta^{15}N$ from Flôr da

Rosa are close to the values of Tomar (Curto et al., 2018). Nevertheless, the human samples collected in Tomar did not align with the anticipated trophic level increase observed from animals to humans (Curto et al., 2018). However, it was observed that most of the human samples collected in Flôr da Rosa exhibited the expected increase in trophic levels from fauna to humans (Fig. 19). The difference in baseline dietary patterns between Tomar and Flôr da Rosa implies that the latter community predominantly relied on terrestrial protein sources derived from C_3 plants, rather than aquatic resources. Based on the findings shown in Figure 21, it can be inferred that the dietary patterns seen in the Iberian Peninsula exhibited a significant degree of diversity. The factor influencing this phenomenon was mostly geographical and locational, rather than being associated with religious beliefs or societal factors (López-Costas& Alexander, 2019).

Bone collagen $\delta^{13}C$ and $\delta^{15}N$ for both sexes is similar (Table 10; Fig. 19). There is a strong relationship between dietary intake and dental caries (Hillson, 1996; Ortner, 2003). Based on a bone collagen $\delta^{13}C$ from Flôr da Rosa (Table 10) we can suggest that the carbohydrate-diet between sexes did not differ significantly. Hence, a slightly elevated frequency of dental caries among females (Table 5) can be connected to behavioral differences in oral hygiene (Walter, DeWitte, & Redfern, 2016), dietary differences (Lukacs, 2008), biological factors such as pregnancy (Lukacs & Largaespada, 2006), and physiological differences in estrogen level, saliva flow rate (Lukacs, 2011b; Lukacs & Largaespada, 2006). The elevated mean frequency of dental calculus for males of Flôr da Rosa (Table 5) suggests that males could have a greater access to meat (Ventresca-Miller et al., 2014). Individuals FR1431 and FR1306, both males, have the highest frequency of dental calculus (Appendix: Table 15). However, the results of $\delta^{15}N$ of males and females indicate that the protein intake for both sexes was similar (Table 10). Furthermore, there are no sex-related statistically significant differences ($\delta^{13}C$: p=0.36; $\delta^{15}N$: p=0.97). Also, it can be because of small sample size.

Overall, results of dental pathologies and stable isotope analysis suggest that there no statistically significant difference in carbohydrate consumption and no sex and age difference in protein intake in Flôr da Rosa population. In Medieval Iberian context, only Islamic Lisbon and Christian San Baudelio showed isotopic differences between the sexes (Toso et al., 2019; Jimenez-Brobeil et al., 2020). The isotopic difference between males and females in Lisbon and San Baudelio was linked to cultural context. Toso et al. (2019) suggest that in Sao Jorge castle (Lisbon) men occupied higher social positions than women which researchers linked to Islamic traditions.

Due to one outlier (young male FR500: $\delta^{13}C$ of -18.28‰) (Appendix: Table 15), bone collagen $\delta^{13}C$ of young individuals diverged from mature and old individuals (Fig. 20). If we exclude individual FR500, mean values of $\delta^{13}C$ and $\delta^{15}N$ for young, mature and elderly are comparable (Table 11). There is no age-related ($\delta^{13}C$: p=0.14; $\delta^{15}N$: p=0.45) statistically significant differences (Table 11). Again, it can be related to the small sample size. Only the Christian sites of Tomar, Boadilla and Sao Baudelio show statistically significant isotopic differences between age groups. Curto et al. (2018) found statistically significant differences between young adult individuals and mature adults in the medieval Christian site of Tomar, Portugal. In another study by García-Collado et al. (2018), the Early Mediaeval population of Boadilla shows isotopic differences between juveniles and adults. Juveniles displayed lower $\delta^{15}N$ levels in comparison to adults (García-Collado et al., 2018). Researchers suggested that juveniles have had a reduced consumption of animal protein when compared to the adult population (García-Collado et al. 2018). In the Northern Iberian site of San Baudelio, Jimenez-Brobeil et al. (2020) discovered a statistically significant difference (p-value=0.031) in $\delta^{15}N$ between young adult males and young adult females. The young adult male group demonstrated higher $\delta^{15}N$ values than the young adult female group. Sarkic et al. (2019) found a statistically significant isotopic difference in one female individual within a post-medieval nun community in Belmonte. Sarkic et al. (2019) propose that the migration of outliers to this convent during the latter years of their lives can be the cause.

There are no strong correlations between stable isotopes and oral health for the studied individuals. Still, the correlation between the frequency of dental caries and $\delta^{13}C$ is the highest among all correlations (r=-0.38; p=0.14) in Flôr da Rosa. The high frequencies of dental caries are frequently connected to elevated $\delta^{13}C$ levels which are associated with the high consumption of cariogenic food (e.g. cereals) (Tomczyk et al., 2020; Bonsall & Pickard, 2015; Schats et al., 2021).

7. Conclusion

This study improved our understanding of the health and diet of the Medieval population at Flôr da Rosa, Portugal. This work highlights the complex relationship between health and diet even in oral health, which is highly releated to the ingested foods.

The frequencies of dental caries and tooth loss are slightly higher in females. Dental calculus is more frequent in males. However, these differences are not statistically significant. Higher frequencies of dental caries in medieval settings are commonly connected to a diet rich in carbohydrates, conversely, a high frequency of dental calculus is usually linked to a diet rich in animal protein. In Flôr da Rosa population, we can see an overall decline in oral health with advancing age. There are statistically significant differences between the frequency of AMTL and age groups, despite the elderly having poor oral health. On a broader scale, the Flôr da Rosa population showed poorer oral health than other Medieval Iberian sites.

The adult diet at medieval Flôr da Rosa is mainly terrestrial. The human samples are within the expected trophic level increase from local fauna to humans. $\delta^{13}C$ suggests that both humans and herbivores relied on the C_3 plant as a main staple. Variability in $\delta^{15}N$ of faunal samples might be associated with husbandry and land management practices. Consumption of C_4 plants such as millet by birds as animal fodder might have affected the values of outliers FR500 and FR885. Health problems such as DISH (diffuse idiopathic skeletal homeostasis) and poliomyelitis might have influenced the $\delta^{13}C$ and $\delta^{15}N$ values of the male outlier FR885. The $\delta^{15}N$ of human individuals suggests that their diet is based on terrestrial animal intake with some minor contributions of birds, C_4 plants or aquatic protein. The results of stable isotope analysis do not reveal statistically significant differences in $\delta^{13}C$ and $\delta^{15}N$ between sexes and ages. This suggested that there is no dietary difference between age and sex groups in Flôr da Rosa. The disparity in dental pathologies between sexes might be mainly related to biological (e.g. pregnancy), physiological (e.g. estrogen level, saliva flow rate and composition) and behavioral factors. There is a small correlation between dental caries and $\delta^{13}C$ but it is not statistically significant. The main limitations of this study are the small sample size, lack of historical records, and lack of information from archaeological contexts regarding socio-economic and cultural differences between individuals. Further research combining stable isotopic data with oral health can give a better understanding of how diet related to oral health in past populations.

Bibliography

Ambrose, S. H. (1991). Effects of diet, climate and physiology on nitrogen isotope abundances in terrestrial foodwebs. Journal of Archaeological Science, 18(3), 293-317.

Ambrose, S. H., & Norr, L. (1993). Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. Prehistoric human bone: archaeology at the molecular level, 1-37.

Bocherens, H., & Drucker, D. (2003). Trophic level isotopic enrichment of carbon and nitrogen in bone collagen: case studies from recent and ancient terrestrial ecosystems. International Journal of osteoarchaeology, 13(1-2), 46-53.

Bogaard, A., Fraser, R., Heaton, T. H., Wallace, M., Vaiglova, P., Charles, M., ... & Stephan, E. (2013). Crop manuring and intensive land management by Europe's first farmers. Proceedings of the National Academy of Sciences, 110(31), 12589-12594.

Bonsall, L. A., & Pickard, C. (2015). Stable isotope and dental pathology evidence for diet in late Roman Winchester, England. Journal of Archaeological Science: Reports, 2, 128-140.

Britannica, T. Editors of Encyclopaedia (2023, August 10). Iberian Peninsula. Encyclopedia Britannica. https://www.britannica.com/place/Iberian-Peninsula

Brown, T. A., & Brown, K. (2011). Biomolecular archaeology: an introduction. John Wiley & Sons

Cai, L. Z., & Qiu, S. (1984). Carbon-13 evidence for ancient diets in China. Kǎogǔ, (10), 949-955.

Cândido, C., Relvado, C., Fernandes, T., & Lopes, C. Possível caso de HID num esqueleto feminino proveniente da necrópole do Mosteiro da Flor da Rosa (séc. XIII-XVIII), Crato, Portugal.

Chisholm, B. S., Nelson, D. E., & Schwarcz, H. P. (1982). Stable-carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science, 216(4550), 1131-1132.

Craig, H. (1957). Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochimica et cosmochimica acta, 12(1-2), 133-149.

Curto, A. (2023). *Possible β-Thalassemia major in an infant from 17th century Portuguese countryside (Flôr da Rosa, Crato)* (p. 18). INTERNATIONAL MEETING ON

POROUS SKELETAL LESIONS: Achievements and Future Directions. https://www.uc.pt/events/pslmeet2023/news/program-and-abstract-book/

DeNiro, M. J. (1985). Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. nature, 317(6040), 806-809.

DeNiro, M. J., & Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. Geochimica et cosmochimica acta, 42(5), 495-506.

DeNiro, M. J., & Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et cosmochimica acta, 45(3), 341-351.

ENATUR, Empresa Nacional de Turismo, S.A. Pousada Flôr da Rosa. Portugal: SOCTIP - Sociedade Tipográfica, SA, 2001.

Eric Guiry and Paul Szpak. 2017. Isotopic analyses reveal geographical and socioeconomic patterns in historical domestic animal trade between predominantly wheat- and maize-growing agricultural regions in eastern north America. American Antiquity, Vol. 82, No. 2 (April 2017), pp. 341-352.

Ferreira, N. A. (2015). Antropologia Funerária e Paleobiologia das Populações Pós-Medievais Portuguesas: Os Casos de Nossa Senhora da Anunciada e Espírito Santo.

Fry, B. (2006). Stable isotope ecology (Vol. 521, p. 318). New York: Springer.

Fuller, B. T., Fuller, J. L., Sage, N. E., Harris, D. A., O'Connell, T. C., & Hedges, R. E. (2005). Nitrogen balance and $\delta^{15}N$: why you're not what you eat during nutritional stress. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry, 19(18), 2497-2506.

Garcia MSJ (2007) Maleitas do corpo em tempos medievais: indicadores paleodemográficos, de stresse e paleopatológicos numa série osteológica urbana de Leiria. PhD Dissertation. University of Coimbra

García-Collado, M. I., Ricci, P., Catalán Ramos, R., Altieri, S., Lubritto, C., & Quirós Castillo, J. A. (2019). Palaeodietary reconstruction as an alternative approach to poorly preserved early medieval human bone assemblages: the case of Boadilla (Toledo, Spain). Archaeological and Anthropological Sciences, 11, 3765-3782.

Gonçalves, A. 1989/1992. Relatório Final. Intervenção Arqueológica ao Mosteiro da Flôr da Rosa.

Guggenheim, B., Schmid, R., Aeschlimann, J. M., Berrocal, R., & Neeser, J. R. (1999). Powdered Milk Micellar Casein Prevents Oral Colonization by Streptococcus sobrinus and Dental Caries in Rats: A Basis for the Caries–Protective Effect of Dairy Products. Caries research, 33(6), 446-454.

Hillson, S. (2018). Dental pathology. Biological anthropology of the human skeleton, 293-333.

Hobson, K. A., Alisauskas, R. T., & Clark, R. G. (1993). Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. The Condor, 95(2), 388-394

Hobson, K. A., Alisauskas, R. T., & Clark, R. G. (1993). Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. The Condor, 95(2), 388-394.

Hoefs, J., & Hoefs, J. (1997). Stable isotope geochemistry (Vol. 201). Berlin: Springer.

Jiménez-Brobeil, S. A., Maroto, R. M., Laffranchi, Z., Roca, M. G., Torres, A. G., & Huertas, A. D. (2020). Exploring diet in an isolated medieval rural community of Northern Iberia: The case study of San Baudelio de Berlanga (Soria, Spain). Journal of Archaeological Science: Reports, 30, 102218.

Jordana Comin, X. (2007). Caracterització i evolució d'una comunitat medieval catalana: estudi bioantropològic de les inhumacions de les esglèsies de Sant Pere. Universitat Autònoma de Barcelona,.

Katzenberg, M. A., & Waters-Rist, A. L. (2018). Stable isotope analysis: a tool for studying past diet, demography, and life history. Biological anthropology of the human skeleton, 467-504.

Kirchner, H., García-Contreras, G., Fenwick, C., & Pluskowski, A. (2023). Re-thinking the 'Green Revolution'in the Mediterranean world. Antiquity, 1-11.

Larsen, B., & Aone, C. (1999, August). Fast and effective text mining using linear-time document clustering. In Proceedings of the fifth ACM SIGKDD international conference on Knowledge discovery and data mining (pp. 16-22).

Larsen, C. S. (1995). Biological changes in human populations with agriculture. Annual Review of Anthropology, 24(1), 185-213.

Lee, H., Hong, J. H., Hong, Y., Shin, D. H., & Slepchenko, S. (2019). Caries, antemortem tooth loss and tooth wear observed in indigenous peoples and Russian settlers of 16th to 19th century West Siberia. Archives of oral biology, 98, 176-181.

Lightfoot, E., O'CONNELL, T. C., Stevens, R. E., Hamilton, J., Hey, G., & Hedges, R. E. (2009). An investigation into diet at the site of Yarnton, Oxfordshire, using stable carbon and nitrogen isotopes. Oxford Journal of Archaeology, 28(3), 301-322.

Long, C. L., Birkhahn, R. H., Geiger, J. W., & Blakemore, W. (1981). Contribution of skeletal muscle protein in elevated rates of whole body protein catabolism in trauma patients. The American Journal of Clinical Nutrition, 34(6), 1087-1093.

Longin, R. (1971). New method of collagen extraction for radiocarbon dating. Nature, 230(5291), 241-242.

López-Costas, O., & Alexander, M. (2019). Paleodiet in the Iberian Peninsula: exploring the connections between diet, culture, disease and environment using isotopic and osteoarchaeological evidence. Archaeological and Anthropological Sciences, 11, 3653-3664.

López-Costas, O., & Müldner, G. (2019). Boom and bust at a medieval fishing port: dietary preferences of fishers and artisan families from Pontevedra (Galicia, NW Spain) during the Late Medieval and Early Modern Period. Archaeological and Anthropological Sciences, 11, 3717-3731.

López-Costas, O., Müldner, G., & Cortizas, A. M. (2015). Diet and lifestyle in Bronze Age Northwest Spain: the collective burial of Cova do Santo. Journal of Archaeological Science, 55, 209-218.

LÓPEZ-MORAGO, C. L. A. U. D. I. A., ESTÉVEZ, E. J., ALEMÁN, I., & BOTELLA, M. (2020). Dental health and diet in a medieval Muslim population from southern Spain. Anthropologie (1962-), 58(1), 3-16.

Lukacs, J. R. (2008). Fertility and agriculture accentuate sex differences in dental caries rates. Current Anthropology, 49(5), 901-914.

Lukacs, J. R. (2011). Sex differences in dental caries experience: clinical evidence, complex etiology. Clinical oral investigations, 15, 649-656

Lukacs, J. R., & Largaespada, L. L. (2006). Explaining sex differences in dental caries prevalence: Saliva, hormones, and “life‐history” etiologies. American Journal of Human Biology: The Official Journal of the Human Biology Association, 18(4), 540-555.

MacKinnon, A. T., Passalacqua, N. V., & Bartelink, E. J. (2019). Exploring diet and status in the Medieval and Modern periods of Asturias, Spain, using stable isotopes from bone collagen. Archaeological and Anthropological Sciences, 11, 3837-3855.

Malainey, M. E., & Malainey, M. E. (2011). Stable isotopes. A Consumer's Guide to Archaeological Science: Analytical Techniques, 35-44.

Marques, R. A. B. D. C. (2007). Capela de Nossa Senhora da Vitória. Paleobiologia de uma série osteológica humana proveniente de Porto do Mós, Batalha (Master's thesis).

Miller, A. V., Usmanova, E., Logvin, V., Kalieva, S., Shevnina, I., Logvin, A., ... & Suslov, A. (2014). Dental health, diet, and social transformations in the Bronze Age: Comparative analysis of pastoral populations in northern Kazakhstan. Quaternary International, 348, 130-146.

Minagawa, M., & Wada, E. (1984). Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et cosmochimica acta, 48(5), 1135-1140.

Mora, A. (2022). Stable carbon and nitrogen isotope analysis of archaeological human hair: Reconstructing diet and health of ancient individuals. Journal of Archaeological Science: Reports, 43, 103439.

Muccio, Z., & Jackson, G. P. (2009). Isotope ratio mass spectrometry. Analyst, 134(2), 213-222.

Nelson, G. C., Lukacs, J. R., & Yule, P. (1999). Dates, caries, and early tooth loss during the Iron Age of Oman. American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists, 108(3), 333-343.

O'Leary, M. H. (1988). Carbon isotopes in photosynthesis. Bioscience, 38(5), 328-336.

Ortner, D. J. (2011). Human skeletal paleopathology. International Journal of Paleopathology, 1(1), 4-11.

Park, R., & Epstein, S. (1960). Carbon isotope fractionation during photosynthesis. Geochim. et cosmochim. acta, 21.

Peña-Chocarro, L., Pérez-Jordà, G., Alonso, N., Antolín, F., Teira-Brión, A., Tereso, J. P., ... & Reyes, D. L. (2019). Roman and medieval crops in the Iberian Peninsula: A first overview of seeds and fruits from archaeological sites. Quaternary International, 499, 49-66.

Preus, H. R., Marvik, O. J., Selvig, K. A., & Bennike, P. (2011). Ancient bacterial DNA (aDNA) in dental calculus from archaeological human remains. Journal of Archaeological Science, 38(8), 1827-1831.

Reitsema, L. J., Vercellotti, G., & Boano, R. (2016). Subadult dietary variation at Trino Vercellese, Italy, and its relationship to adult diet and mortality. American journal of physical anthropology, 160(4), 653-664.

Richards, M. P., & Hedges, R. E. (1999). Stable isotope evidence for similarities in the types of marine foods used by Late Mesolithic humans at sites along the Atlantic coast of Europe. Journal of Archaeological Science, 26(6), 717-722.

Rodrigues, J. (2009). O mosteiro de Flôr da Rosa. IGESPAR.

Santos, S. A.; Costa, C. & Almeida, P. (2002). Study of a probable case of poliomyelitis (Flor da Rosa).

Sanz, B. R., & Gayo, G. J. T. (2003). Patología oral en la población Hispano-Musulmana de Xarea (Almería). In ¿ Dónde Estamos?: Pasado, presente y futuro de la Paleopatología: actas del VI Congreso Nacional de Paleopatología (Madrid, 13 al 16 de septiembre de 2001) (pp. 375-385). Universidad Autónoma de Madrid.

Sarkic, N., López, J. H., López-Costas, O., & Grandal-d'Anglade, A. (2019). Eating in silence: isotopic approaches to nuns' diet at the convent of Santa Catalina de Siena (Belmonte, Spain) from the sixteenth to the twentieth century. Archaeological and Anthropological Sciences, 11, 3895-3911.

Schats, R., van Hattum, I., Kootker, L. M., Hoogland, M. L., & Waters ‐ Rist, A. L. (2022). Diet and urbanisation in medieval Holland. Studying dietary change through carious lesions and stable isotope analysis. International Journal of Osteoarchaeology, 32(1), 142-155.

Schmidt, C. W. (1998). Dietary reconstruction in prehistoric humans from Indiana: an analysis of dental macrowear, dental pathology, and dental microwear. Purdue University.

Schoeninger, M. J., & DeNiro, M. J. (1984). Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochimica et Cosmochimica acta, 48(4), 625-639.

Schoeninger, M. J., & Moore, K. (1992). Bone stable isotope studies in archaeology. Journal of World Prehistory, 6, 247-296.

Schoeninger, M. J., DeNiro, M. J., & Tauber, H. (1983). Stable nitrogen isotope ratios of bone collagen reflect marine and terrestrial components of prehistoric human diet. Science, 220(4604), 1381-1383.

Schwarcz, H. P. (1991). Some theoretical aspects of isotope paleodiet studies. Journal of archaeological science, 18(3), 261-275.

Schwarcz, H. P., & Schoeninger, M. J. (1991). Stable isotope analyses in human nutritional ecology. American Journal of Physical Anthropology, 34(S13), 283-321.

Sharp, Z. (2017). Principles of stable isotope geochemistry.

Shearer, G., & Kohl, D. H. (1989). Estimates of N 2 fixation in ecosystems: the need for and basis of the 15 N natural abundance method. In Stable isotopes in ecological research (pp. 342-374). Springer New York.

Smith, B. N. (1972). Natural abundance of the stable isotopes of carbon in biological systems. BioScience, 22(4), 226-231.

Sponheimer, M., Robinson, T., Ayliffe, L., Passey, B., Roeder, B., Shipley, L., ... & Ehleringer, J. (2003a). An experimental study of carbon-isotope fractionation between diet, hair, and feces of mammalian herbivores. Canadian Journal of Zoology, 81(5), 871-876.

Sponheimer, M., Robinson, T., Ayliffe, L., Roeder, B., Hammer, J., Passey, B., ... & Ehleringer, J. (2003b). Nitrogen isotopes in mammalian herbivores: hair $\delta^{15}N$ values from a controlled feeding study. International Journal of Osteoarchaeology, 13(1-2), 80-87.

Staller, J., Tykot, R., & Benz, B. (Eds.). (2006). Histories of maize: multidisciplinary approaches to the prehistory, linguistics, biogeography, domestication, and evolution of maize. Left Coast Press.

Szpak, P., J. Z. Metcalfe, and R. A. Macdonald. 2017. "Best Practices for Calibrating and Reporting Stable Isotope Measurements in Archaeology." Journal of Archaeological Science: Reports 13: 609–616.

Temple, D. H., & Larsen, C. S. (2007). Dental caries prevalence as evidence for agriculture and subsistence variation during the Yayoi period in prehistoric Japan: biocultural interpretations of an economy in transition. American Journal of Physical Anthropology, 134(4), 501-512.

Tieszen L, Boutton L, Tesdahl K, Slade N. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: implications for δ 13C analysis of diet. Oecologia 57: 32–37.

Tomczyk, J., Regulski, P., Lisowska-Gaczorek, A., & Szostek, K. (2020). Dental caries and stable isotopes analyses in the reconstruction of diet in Mesolithic (6815–5900 BC) individuals from Northeastern Poland. Journal of Archaeological Science: Reports, 29, 102141.

Toso, A., Gaspar, S., Banha da Silva, R., Garcia, S. J., & Alexander, M. (2019). High status diet and health in Medieval Lisbon: a combined isotopic and osteological analysis of the Islamic population from São Jorge Castle, Portugal. Archaeological and Anthropological Sciences, 11, 3699-3716.

Turrini A. (2022). Perspectives of Dietary Assessment in Human Health and Disease. Nutrients, 14(4), 830. https://doi.org/10.3390/nu14040830

Vaiglova, P., Bogaard, A., Collins, M., Cavanagh, W., Mee, C., Renard, J., ... & Fraser, R. (2014). An integrated stable isotope study of plants and animals from Kouphovouno, southern Greece: a new look at Neolithic farming. Journal of Archaeological Science, 42, 201-215.

Veiga-Rilo, C., & López-Costas, O. (2023). Biting into the Truth: Connecting oral pathology and stable isotopes through the paradigmatic example of a hyper-specialized marine diet in Medieval Pontevedra (NW Iberia).

Vogel, J. C. (2012). Fractionation of the Carbon Isotopes During Photosynthesis: Submitted to the Session of 19 April, 1980. Springer Science & Business Media.

Vogel, J. C., & Van Der Merwe, N. J. (1977). Isotopic evidence for early maize cultivation in New York State. American Antiquity, 42(2), 238-242.

Wada, E., Kadonaga, T., & Matsuo, S. (1975). ^{15}N abundance in nitrogen of naturally occurring substances and global assessment of denitrification from isotopic viewpoint. Geochemical Journal, 9(3), 139-148.

Walter, B. S., DeWitte, S. N., & Redfern, R. C. (2016). Sex differentials in caries frequencies in Medieval London. Archives of Oral Biology, 63, 32-39.

Yakir, D., & Israeli, Y. (1995). Reduced solar irradiance effects on net primary productivity (NPP) and the $\delta^{13}C$ and δ18O values in plantations of Musa sp., Musaceae. Geochimica et Cosmochimica Acta, 59(10), 2149-2151.

Yanko, N. V., Artemyev, A. V., & Kaskova, L. F. (2021). Dental health indicators of the Chernyakhov population from Shyshaki (Ukraine). Anthropological Review, 84(1), 17-28.

Appendix

Table 15 Human samples from Flôr da Rosa with age, sex and oral health

Individual	Bone	Sex	Age	Frequency of tooth loss	Frequency of dental caries	Frequency of dental calculus
FR 500	Rib	M	18-24	0	0	0.68
FR 642	Rib	M	18-24	0.06	0.47	0.86
FR 885	Rib	M	>44			
FR 953 A	Rib	M	>44	0.61	0.27	0.55
FR 953 B	Rib					
FR 956	Rib	F	>44	0.71	1	0.25
FR 957	Rib	F	>44	1		
FR 1138	Rib					
FR 1301	Rib	F	35-44	0.05		0.71
FR 1304	Rib	M		0.75		
FR 1305	Rib	F	>44	0.87	0.25	0.58
FR 1306	Rib	M	18-24	0.04	0.13	1
FR 1307	Rib	M	35-44	0.06	0.59	0.29
FR 1309	Rib	F				
FR 1414	Rib	F	18-24	0.13	0.15	0.1
FR 1431	Rib	M	>44	0.67	0.1	1
FR 1432 B	Rib					
FR 1432 A	Rib	F	35-44	0	0	0.7
FR 1435	Rib	M	35-44	0.59	0.88	
FR 1436	Rib	F	25-34	0	0.25	0.75
FR 1448	Rib	F				
FR 1449	Rib	F	35-44		0.33	0.67
FR 1499	Rib	M		0.04	0.19	0.78

FR 1614	Rib	F	35-44	0	0.31	0.72
FR 1986	Rib			0.1	0.44	0.74
FR 2485	Rib	F	>44	0.92	1	0.5

Table 16 Collagen weight of samples from Flôr da Rosa

Sample ID	Archaeological site	Genus	Bone sampled	Bone sample weight	Container weight	Container with collagen weight	Collagen weight
FR 642 bird	Flôr da Rosa	Aves	Metacarpal	0,5818	6,1569	6,172	0,0151
FR 642 ovis	Flôr da Rosa	Ovicap	Pelvis	0,479	6,1809	6,2012	0,0203
FR 1767 bird	Flôr da Rosa	Aves	Phalange	0,1875	6,1931	6,2324	0,0393
FR 013	Flôr da Rosa	Ovicap	Rib	0,4156	6,2141	6,2346	0,0205
FR 698	Flôr da Rosa	Ovicap	Humerus	0,5421	6,1597	6,1819	0,0222
FR 2222 B	Flôr da Rosa	Ovicap	Humerus	0,589	6,1851	6,2108	0,0257
FR 2222 A	Flôr da Rosa	Ovicap	Tibia	0,555	6,19165	6,2295	0,03785
FR 1436	Flôr da Rosa	Homo	Rib	0,648	6,1636	6,2695	0,1059
FR 1309	Flôr da Rosa	Homo	Rib	0,5901	6,1973	6,23	0,0327
FR 1448	Flôr da Rosa	Homo	Rib	0,3802	6,166	6,1976	0,0316
FR 1305	Flôr da Rosa	Homo	Rib	0,5203	6,1113	6,1443	0,033
FR 1432 B	Flôr da Rosa	Homo	Rib	0,6202	6,14459	6,1794	0,03481
FR 1435	Flôr da Rosa	Homo	Rib	0,6301	6,1456	6,1767	0,0311
FR 1449	Flôr da Rosa	Homo	Rib	0,6235	6,1868	6,1964	0,0096
FR 642	Flôr da Rosa	Homo	Rib	0,6012	6,1913	6,2205	0,0292
FR 1307	Flôr da Rosa	Homo	Rib	0,602	6,1501	6,1971	0,047
FR 1304	Flôr da Rosa	Homo	Rib	0,683	6,1851	6,2428	0,0577
FR 1301	Flôr da Rosa	Homo	Rib	0,5421	6,1892	6,2306	0,0414
FR1414	Flôr da Rosa	Homo	Rib	0,5452	6,166	6,2058	0,0398
FR1138	Flôr da Rosa	Homo	Rib	0.5092	6,1932	6,2253	0,0321
FR1499	Flôr da Rosa	Homo	Rib	0,5004	6,1122	6,1437	0,0315

FR500	Flôr da Rosa	Homo	Rib	0,5014	6,1861	6,2309	0,0448
FR1986	Flôr da Rosa	Homo	Rib	0,5677	6,1943	6,2309	0,0366
FR953 B	Flôr da Rosa	Homo	Rib	0,3305	6,1869	6,2224	0,0355
FR 1432 A	Flôr da Rosa	Homo	Rib	0,5071	6,2069	6,238	0,0311
FR 957	Flôr da Rosa	Homo	Rib	0,504	6,2089	6,2468	0,0379
FR 955 fauna	Flôr da Rosa	Bos		0,5169	6,1569	6,211	0,0541
FR 885	Flôr da Rosa	Homo	Rib	0,4544	6,1941	6,282	0,0879
FR 953 A	Flôr da Rosa	Homo	Rib	0,5437	6,1864	6,243	0,0566
FR 1431	Flôr da Rosa	Homo	Rib	0,5201	6,2058	6,2411	0,0353
FR 1306	Flôr da Rosa	Homo	Rib	0,5188	6,2096	6,2376	0,028
FR 956	Flôr da Rosa	Homo	Rib	0,5125	6,198	6,2392	0,0412
FR 2485	Flôr da Rosa	Homo	Rib	0,5379	6,1903	6,2931	0,1028
FR 2222, E	Flôr da Rosa	Bos	Tibia	0,5353	6,1773	6,2004	0,0231
FR 2222, F	Flôr da Rosa	Bos	Vertebra	0,5138	6,151	6,2002	0,0492
FR 2222, C	Flôr da Rosa	Ovicap	Pelvis	0,4893	6,2267	6,2891	0,0624
FR 1614	Flôr da Rosa	Homo	Rib	0,5376	6,1785	6,2544	0,0759
FR 2222, D	Flôr da Rosa	Bos	Tibia	0,5213	6,181	6,2392	0,0582

Figure 22 Individual FR 956’ mandibular tooth with dental caries

Figure 23 Individual FR 1431 with dental calculus

Figure 24 Individual FR 957 with ante mortem tooth loss

Figure 25 Rib bone of individual FR1436

Figure 26 Faunal remains of *Ovis/Capra* from Flôr da Rosa sampled for stable isotope analysis

Figure 27 Faunal remains of *Bos* from Flôr da Rosa used for stable isotope analysis