University of Nevada, Reno

The Late Neolithic Portuguese Diet: an Assessment Through Stable Isotope Analysis of Dental Calculus

A thesis submitted in partial fulfillment of
the requirements for the degree of Master of Arts in
Anthropology
By

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May 2019

UNIVERSITY OF NEVADA
RENO

THE GRADUATE SCHOOL

We recommend that the
thesis prepared under
our supervision by

Amelia Eileen

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entitled

The Late Neolithic Portuguese Diet: an Assessment Through Stable Isotope Analysis of Dental Calculus

be accepted in partial fulfillment of the
requirements for the degree of

MASTER OF ARTS

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May 2019

Abstract:

The research presented involves stable isotope analysis of human dental calculus from Neolithic and Bronze Age remains from Portugal. For this thesis 80 dental calculus samples from Neolithic period remains and two calculus samples from Bronze Age remains were analyzed. This study looks to expand the understanding of the use of dental calculus for stable isotope analysis in comparison to traditional materials used to do stable isotope analysis, such as bone collagen, and the Neolithic diet.

Questions that are being addressed through this study are can dental calculus be used to understand the diet of a person through stable isotope analysis in comparison to using bone collagen for stable isotope analysis. Previous studies done in Portugal for the Neolithic time period are not as numerous as Mesolithic and the time periods after. During the Mesolithic, humans were still hunter/gatherers. In the Neolithic farming was introduced across Europe, changing the way that people obtained food. Stable isotope analysis can help in understanding that shift.

Dental calculus being used for stable isotope analysis is a new process that is still being tested for its accuracy. It can be used as a non-invasive way to run stable isotope analysis.

I would like to dedicate this thesis to my parents and brother who continuously push me to achieve my goals.

Table of Contents

List of Tables

List of Figures

Introduction

The research presented in this thesis involves stable isotope analysis of dental calculus from 80 Neolithic and two Bronze Age individuals found in Portugal. There have been several stable isotope studies to evaluate the paleodiet of human skeletons from the Neolithic period on the Iberian Peninsula. This study will add to the information available on the Neolithic diet of humans on the Iberian Peninsula, and further evaluate the value of dental calculus for stable isotope analysis.

The Importance of Stable Isotope Analysis

Stable isotope analysis was first used in an anthropological context in the 1970s to look at the paleodiet in the Americas, with particular attention to the spread of maize agriculture beyond its Mesoamerican homeland. An archaeologist and a geochemist collaborated on two studies to understand the carbon and nitrogen ratios in plants, animals, and humans (Vogel and van der Merwe, 1977; Vogel and van der Merwe, 1978). In subsequent years, stable isotope analysis has been used as a complementary means of understanding the paleodiet when used in concert with zooarchaeological and paleobotanical evidence.

Stable isotope analysis allows researchers to evaluate the last five to ten years of an individual's diet when examining traditional materials such as bone collagen or tooth dentin. Looking at an individual's diet allows researchers to track dietary change caused by resource availability, geographical migration, weaning age, and/or societal differentiation (Schoeninger and Moore, 1992; Schurr, 1998; Dupras et al., 2001; Privat and O'Connell, 2002; Richards et al., 2002; Ambrose and Krigbaum, 2003; Ambrose et al., 2003; Harrison and Katzenberg, 2003; Fraser et al., 2006; Fuller et al., 2006; Knudson et al., 2007, 2010). Stable isotope analysis allows researchers to track changes in the diet of the overall population, note the introduction of new food sources, and assess differences between sexes, age groups, and social classes, along with geographical and temporal differences within and between populations (Schoeninger and Moore, 1992; Harrison and Katzenberg, 2003; Kellner and Schoeninger, 2008; Kjelström et al., 2009; Schoeninger, 2010; Yoder, 2010; Webb et al., 2013).

This study contributes to the broad field of bioarchaeology in two ways. First, it expands our knowledge of the Neolithic diet on the Iberian Peninsula. Second, it is another test to determine if dental calculus is a suitable biomaterial to conduct non-invasive stable isotope analysis. That is, dental calculus may allow for research on populations when descendants choose not to allow destructive research on human skeletal and dental remains. As dental calculus is an add-on and not part of the skeleton, it may provide an alternative means of conducting stable isotope analysis without destroying bones or teeth.

Traditional Materials Used in Stable Isotope Analysis

Bone collagen is one of the most commonly used sample materials in isotopic analysis, primarily because it preserves well (Ambrose, 1990; Schoeninger and Moore, 1992). Bone collagen is made up of individual amino acids produced from the reactions of starting components of food or the breakdown of the animal's own tissue (Schoeninger and Moore, 1992; Schoeninger, 2010). The ratio of C13/C12 results from the differences in the mass of the isotopes and the reaction between products that have different isotopic compositions than the source material. The bonds that form C12 break and form more quickly than those of C13. Most biological material, bone included, has less C13 relative to C12 (van der Merwe, 1982; Schoeninger and Moore 1992). The nitrogen in bone collagen is ultimately a product of atmospheric nitrogen absorbed by plants in a food web or dissolved in the ocean. The change between the trophic levels is due to biological fractionation. This is the process whereby a lighter isotope is taken up by the body to conserve energy. This results in a change in the isotopic ratio which is passed along the food chain in a predictable pattern, between +2-4‰ (van der Merwe, 1982; Schoeninger and de Niro, 1984; Schoeninger, 1985; Ambrose, 1990; Schoeninger and Moore, 1992; Huray and Schutkowski, 2005; Hedges and Reynard, 2007).

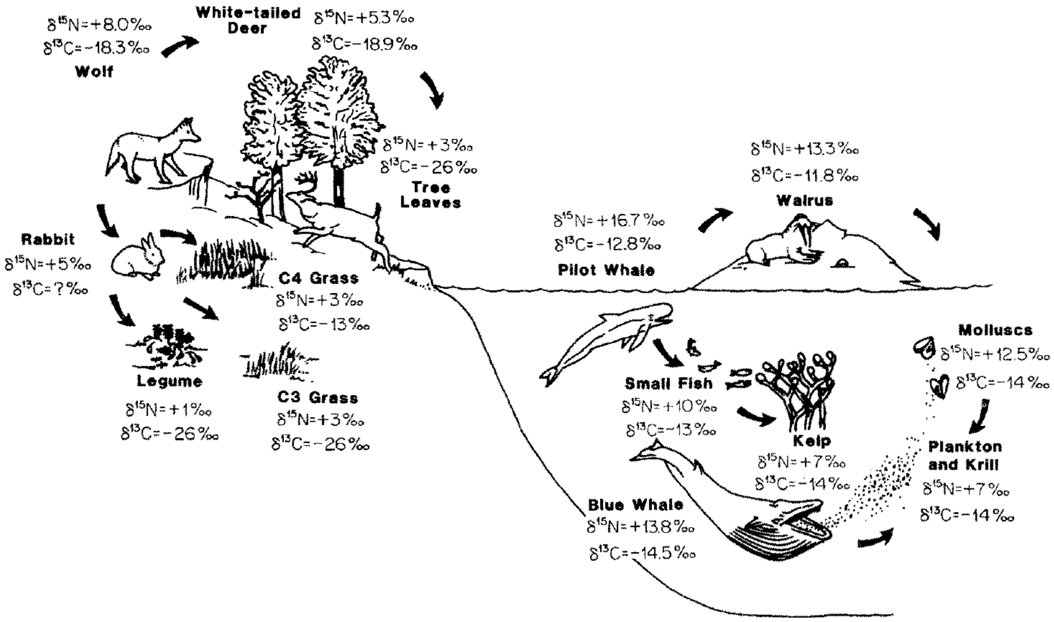


Fig 1.1. Drawing of the distribution of stable isotope ratios in the biosphere.

Schoeninger and Moore, 1992

$$\delta^{15}N = \left[\frac{(^{15}N/^{14}N)\ \text{sample}}{(^{15}N/^{14}N)\ \text{standard}} - 1 \right] \times 1000\ ‰$$

$$\delta^{13}C = \left[\frac{(^{13}C/^{12}C)\ \text{sample}}{(^{13}C/^{12}C)\ \text{standard}} - 1 \right] \times 1000\ ‰$$

Fig 1.2. Equations for calculating the δ for carbon and nitrogen. Schoeninger, 1985

Bone apatite is the inorganic material in bone. It is composed of calcium, phosphate, and hydroxide, which provide the rigidity of bone, and which provide carbon in the form of carbonate ions (Lee-Thorpe et al., 1989; Schoeninger and Moore, 1992; Harrison and Katzenberg, 2003; Hedges, 2003). Bone apatite is more susceptible to diagenetic changes, as the material is considered “sponge like," absorbing minerals and ions from the surrounding depositional environment (Lee-Thorpe et al., 1989). The C13 content from the bone apatite reflects the whole diet (Harrison and Katzenberg, 2003; Jim et al., 2004). The metabolic process in the body can affect the isotopic values of the apatite and the relationship between apatite (inorganic) and collagen (organic) (Lee-Thorpe et al., 1989; Hedges, 2003).

Keratin is a fibrous, sulfur-containing protein that is the primary component in hair and nails (O’Connell et al., 2001). Hair has a regular growth rate, averaging roughly 1 cm a month (Knudson et al., 2010). It is metabolically inactive once it grows out of the hair follicle. For this reason, it provides sequential archive information of diet. Hair follicles are active in the anagen phase, the growth phase of hair, which last 2-3 years before the hair is shed and replaced by new follicles. While hair is actively growing, it takes approximately 14 days from the time when the hair is forming to emergence from the scalp, leaving a 14-day gap of isotopic information before death (Webb et al. 2013, 2015). Hair provides parallel isotopic information to bone collagen on different temporal scales and provides isotopic information on protein intake of an individual (Knudson et al., 2010; Webb et al., 2013).

The active nature of hair allows researchers to look at recent dietary changes in an individual. The changes in carbon and nitrogen values can be detected within a few days in hair if a change in diet is maintained. Thus, changes in diet can be looked at incrementally along the hair shaft. During a dietary change, the body is in a state of equilibration, the act of creating equilibrium. Now, the isotopic values from the hair shaft will not match those of the bone collagen. It can take up to 5 months for nitrogen and 12 or more months for carbon to complete isotopic equilibration, of the body protein pool (Webb et al., 2013; Webb et al., 2015).

Nails also provide a good sample material to test carbon and nitrogen isotopes. The growth rate of nails is much slower than hair, but they do grow at a constant rate (Fraser et al., 2006; O'Connell et al., 2010). The rate is different for everyone and can be affected by stressors similar to those that influence hair. The growth of nails allows for the nitrogen values to be more variable than those of hair from the same individual. Basically, there is more time for biochemical processes to affect the isotopic content of nails compared to hair and collagen (Fraser et al., 2006).

The use of hair and nails for isotopic studies has been applied to both prehistoric and historic populations when there is good preservation (Aufderheide et al., 1994;

Knudson et al., 2010; O’Connell et al., 2010). These kinds of studies have also been applied to living populations. This allows researchers to evaluate longitudinal effects of diet and determine if isotopic analysis can be used in the identification of individuals (Fraser et al., 2006; Knudson et al., 2010; O’Connell et al., 2010).

The Use of Dental Calculus for Paleodiet

The use of dental calculus as a biomaterial for stable isotope analysis was pioneered within the past four years. It allows a researcher to conduct a dietary analysis without destroying parts of the skeleton. Traditional materials such as bone, teeth, hair, and nails may be prohibited for use from populations that do not allow destructive analysis (Scott and Poulson, 2012; Eerkens et al. 2014). Dental calculus may allow for more populations to be examined, provided that individuals who work in museums and descendant groups come to view it as a secondary biomaterial that allows for a non-invasive form of analysis (Scott and Poulson, 2012). This could greatly aid attempts to better understand paleodiet among diverse groups in a wide variety of geographic and temporal contexts.

Plaque, a biofilm that contains bacteria from the oral cavity, begins to harden after approximately 10 days (Hillson, 1979; Hillson, 1996; White, 1997; Lieverse, 1999; Scott and Poulson, 2012). Dental calculus is the hardened biofilm, also known as tarter. Within the calculus, organic and inorganic materials are incorporated. The inorganic materials that are part of dental calculus include hydroxyl apatite, octacalcium phosphate, brushite, and whitlockite. The organic material in calculus comes from the bacteria that obtain their nutrients from proteins, glycoproteins, amino acids, and peptides in the oral fluids and dietary materials. These comprise 15-20% of the dry weight of calculus (White, 1997; Lieverse, 1999; Scott and Poulson, 2012; Poulson et al., 2013; Eerkens et al., 2014). Silicates have been found within the calculus, likely when the teeth, and subsequently the dental calculus, comes in contact with ground water (Klepinger et al., 1977; Eerkens et al. 2014). The presence of the organic material allows for stable isotope analysis to be performed on the dental calculus.

European Paleodietary Changes

During the Mesolithic, populations relied on hunting and gathering for food resources. The diet consisted of terrestrial C3 plants, wild game, and aquatic resources from rivers, estuaries, and the coast. Aquatic resources were vital to survival in a period of climate change and decreasing herbivore biomass (Lubell et al., 1994; Richards and Hedges, 1999; Schulting and Richards, 2002; Milner et al., 2004; Garcia Guix et al., 2006; Fisher et al., 2007; Schulting et al., 2008; Lightfoot et al., 2010; Fernádez-López de Pablo et al., 2012; Fontanals-Coll et al., 2014).

The introduction of farming and domesticated animals to the European continent was a gradual process beginning around 7500 BCE. The practice of farming and domestication of animals was introduced to Europe through various routes, including along the Mediterranean coast and overland routes. With the introduction of domesticated animals, primarily sheep, Mesolithic populations shifted from hunting and gathering to agricultural and pastoral practices (Lubell et al., 1994' Zilhão, 2000; Milner et al., 2004; Bocherens et al., 2007; Fischer et al., 2007; Eriksson et al., 2008; Nehlich et al., 2009). With the introduction of agro-pastoral practices, the reliance on marine resources dwindled, as shown by decreasing shell middens. Though the populations did not always move away from the coast or other aquatic sources, they did rely less heavily on them. The change in diet decreased from around 50% marine resources to 5-12% (Lubell et al. 1994; Papathanasiou, 2003; Milner et al., 2004 Fischer et al., 2007; Eriksson et al., 2008 Lightfoot 2011; Fraser et al., 2013; Montgomery et al., 2013; Fontanals-Coll et al., 2015a).

The Neolithic period began reaching the Iberian Peninsula around 5500 BCE. There is an overlapping period with some populations that still practiced Mesolithic hunting and gathering. Over time, Mesolithic hunter-gatherer populations would have been integrated into the permanent agro-pastoral settlements of the Neolithic population (Fontanals-Coll et al., 2015a). This change from the hunter-gatherer practices to the Neolithic agro-pastoral practices has been called the Neolithic Revolution or Neolithic package (Zilhão, 2000; Eriksson et al., 2008). The Neolithic package includes the spread of ceramics, different burial practices, social stratification, and the domestication of plants and animals (Zilhão, 2000; Eriksson et al., 2008). These practices continued and mutated in post-Neolithic times.

Post-Neolithic populations continued to consume terrestrial C3 plants without much influence from marine protein (Pivat et al., 2002; Prowse et al., 2004; Honch et al., 2006; Jay and Richards, 2006; Müldner and Richards, 2007; Triantaphyllou et al., 2008; Tafuri et al., 2009; Jørkov et al., 2010; Knipper et al., 2013; Fontanals-Coll et al. 2015b). During the Neolithic, millet, a C4 plant, was introduced to Europe from Asia. It reached Eastern Europe between 6500 and 5000 BCE, eventually becoming part of the diet in post-Neolithic periods both as animal fodder and part of the human diet (Hunt et al., 2008; Fracetti et al., 2010; Motuzaite-Matuzeviciute et al., 2013; Svyatko et al., 2013; Valamoti, 2016). The use of millet was not readily accepted across all of Europe. There was more acceptance and reliance by inland populations compared to those living on the coast (Le Huray and Schutkoski, 2005; Honch et al., 2006; Tafuri et al., 2009; Knipper et al., 2013; Svyatko et al., 2013). During the post-Neolithic time periods, societies became more socially stratified with grave goods as indicators of this stratification. From the Copper Age through the 6th century CE, the difference in diet between social groups is barely detectable isotopically (Le Huray and Schutkowski, 2005; Honch et al., 2006; Jay and Richards, 2006; Triantaphyllou et al., 2008; Jørkov et al., 2010; Knipper et al., 2013). Diet changes during the post-Neolithic time periods were minimal to the diet of European populations. Populations continued to consume C3 plants, domesticated animals, minimal to no marine protein, the only difference being the introduction of millet, a C4 plant.

Site Information

Samples presented in this thesis come from 10 different sites in Southwestern Portugal. Three sites are coastal. The remaining 7 are further inland. All the sites have been dated to the late Neolithic through radiocarbon dating and burial practices (Silva et al., 2012; Bonaventure et al., 2014; Valera et al., 2014; Waterman et al., 2014; Emslie et al., 2015). The ten sites that are part of this study are as follows: Dolmende Ansião 2, Cavadas Lapas, Lugar do Canto, Tholos de Piamogo I/Tholos de Pia Mogo I, Cova de Moura, Cabeço do Arruda II, Hipogeu de São Pedro Do Estroil, Hipogeu de São Paulo II, Perdigões, Hipogeu de Monte Canelas. Various burial practices were used throughout

Neolithic Portugal. From various tombs (both constructed tombs and natural caves) to pit burials, and rarely cremation (Silval et al., 2012; Bonaventure et al., 2014; Silva et al., 2014; Valera et al., 2014; Waterman et al., 2014a; Waterman et al., 2014b; Emslie et al., 2015; Waterman et al., 2016). Many of the tombs contained high numbers of individuals. This is attributed to the accumulation of burials over time, not one deposit (Bonaventure et al., 2014).

One site, Perdigões, has been extensively studied. Perdigões is a Neolithic ditched enclosure in south-central Portugal that was used as a gathering place for around 1000 years. This site sits in a natural amphitheater that faces East, toward the valley of Riberia de Álamo where human occupation has been documented from the Neolithic onward (Silva et al., 2014; Verlera et al., 2014; Emslie et al., 2015). The site was used for ceremonial and burial practices. The kinds of burials found here are pit, vaulted monuments, ditches, and areas for cremated remains. Some of these different burial practices occurred at the same time (Silva et al., 2014).

Other sites are as follows: Tholos de Paimogo 1 is a vaulted tomb structure near the Atlantic coast. It was discovered in 1968 and excavated in 1971 (Waterman et al., 2014). Hipogeu de São Paulo II, Hipogeu de Monte Canelas, and Hipogeu de São Pedro Do Estroil are rock-cut tombs from southwest Portugal. These tombs consist of a chamber and a passage and are often found in clusters (Bonaventure et al., 2014). Tholos de Piamogo 1/Tholos de Pia Mogo1 is a coastal site that contains a manmade tomb containing over 400 individuals. Another tholos is Cabeço do Arruda II (Bonaventure et al., 2014). Cova de Moura, Cova das Lapas, and Lugar do Canto are natural caves used for deposition of human remains (Bonaventure et al., 2014). Dolmende Ansião 2 is a dolman in the mountains of southwestern Portugal (Bonaventure et al., 2014).

For this study, the sites will be divided into three groups: Perdigoes, Hipogeum, and tomb burials.

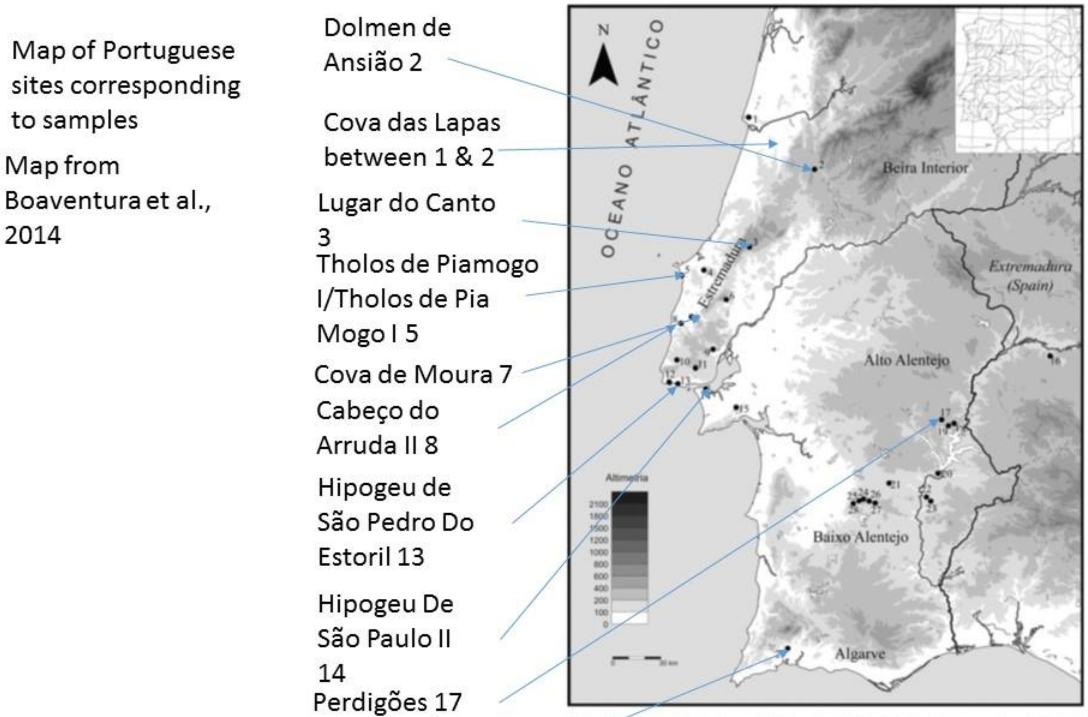


FIGURE 1. Sites mentioned in the text. 1, Cabeço dos Moinhos; 2, Ansião; 3, Lugar do Canto; 4, Serra da Roupa; 5, Paimogo 1; 6, Algar do Bom Santo; 7, Cova da Moura; 8, Cabeço da Arruda 2; 9, Carcavelos; 10, Pedras da Granja; 11, Carenque; 12, Poço Velho; 13, São Pedro do Estoril; 14, São Paulo 2; 15, Casal do Pardo; 16, La Pijotilla; 17, Perdigões; 18, Olival da Pega 2; 19, Santa Margarida 3; 20, Sobreira de Cima; 21, Monte das Covas 3; 22, Ribeiro de São Domingos 1; 23, Outeiro Alto 2; 24, Monte do Carrascal 2; 25, Horta do João da Moura; 26, Monte do Pombal 1; 27, Vale de Barrancas 1; 28, Monte do Cardim 6; 29, Monte Canelas. Map adapted from Carta Hispométrica de Portugal, 1:1.000.000.

Fig 1.3. Map of Neolithic Portuguese sites. Map provided from Boaventura et al., 2014.

Materials and Methods

Sample Information

The calculus samples for this thesis were collected by G. R. Scott during the summer of 2015 using techniques presented in Scott and Poulson (2012). Samples were collected from 80 Late Neolithic individuals that were part of three different burial areas: Pedgeões, Hipogeum, and tomb burials. Samples were taken from loose teeth and were a combination of subgingival and supragingival calculus taken from the buccal and lingual surfaces of both maxillary and/or mandibular teeth. Calculus was not removed in its entirety from any one individual. The calculus was removed from teeth using a dental pick to flake off samples that ranged from 0.5 mg to 147.3 mg. Care was taken to remove calculus without damaging the surfaces of the teeth. It was attempted to remove calculus samples from one tooth, but for individuals that did not have a large amount of calculus on one tooth, samples were taken from two or three teeth to obtain a sufficient quantity for testing.

Dental calculus was obtained from 79 individuals associated with three Late Neolithic burial groups, a single Late Neolithic individual not associated with a burial group, and two individuals dating to the Bronze Age. Perdigões is a site in the south of Portugal where the necropolis consisted of tomb and pit burials. The individuals (n =9) in this study came from three monuments. The Hipogeum burials (n =34) were in artificial caves built into the limestone. The last group was buried within tombs (n = 36). The Bronze Age burials (n = 2) are associated with the tomb burials of the Late Neolithic. EAII1107 (n = 1) is not associated with any of the burial groups.

Fig 2.1. Dental Calculus still attached to teeth. Photo provided by Dr. G. Richard Scott.

Methods for Processing Dental Calculus

Samples were processed at the Nevada State Isotope Lab at University of Nevada, Reno. Samples were weighed prior to cleaning. The weighing paper was zeroed out on a digital scale. The dental calculus was placed on the weighing paper and returned to the scale. All samples were weighed to the tenth of a milligram. Sample number and weight were recorded on paper before a sample was returned to a plastic vial for storage.

After the 82 samples were weighed and the weights recorded, the samples were washed twice. The first wash was done with tap water. The samples were left in the plastic vials and covered with enough tap water to submerge them. The vials were swirled in a circular motion to agitate the water and loosen dirt from the calculus. Water was then siphoned off, removing dirt from the samples. The second wash consisted of deionized water. Samples were left in the vials; enough deionized water was added to the vials to submerge the dental calculus. The vials were swirled again to remove any remaining dirt from the samples. Water was siphoned off without removing the calculus. All samples were then stored in vials to dry without the caps at least overnight if not longer until the samples were dry.

Once samples were dry, they were powdered with a metal mortar and pestle. The powdered samples were returned to the original vials and plastic caps were used to seal the vials. Between pulverizing each sample, the mortar and pestle were cleaned using ethanol to avoid cross contamination. All samples were sealed with a new plastic cap.

Larger samples of ca. 30mg were weighed out and run to test the nitrogen and carbon content. Samples were then divided into two groups, larger samples weighing 47.2mg to 6.9mg, and smaller samples, 0.5mg to 6.8mg. Larger samples were weighed in 6X10mm tin capsules with weights between 5.225 mg and 10.886 mg. The smaller samples were weighed in 4X6mm tin capsules with weights between 0.459 mg to 2.447 mg. With the smaller samples, the whole sample was used for testing.

All prepared control and calculus samples were processed through a mass spectrometer. In concurrence with Scott and Poulson (2012: 1389), “Stable isotope analysis and elemental concentrations (weight % C and weight % N) were performed using a Eurovector elemental analyzer (which releases all carbon and nitrogen as CO2 and N2, respectively) interfaced to an Iosprime stable isotope ratio mass spectrometer. Results are reported in the units of ‰ in the usual δ notation versus VPDB for carbon and versus air for nitrogen." The larger samples were run for both carbon and nitrogen isotopes. Smaller samples were analyzed only for carbon content. This was done in an attempt to obtain positive results. In all biological material there is a greater abundance of carbon in comparison to nitrogen. Nitrogen require a larger sample to obtain values.

Results

Late Neolithic Population

The bulk calculus samples for the Late Neolithic produced a carbon mean of -19.13 ± 2.025 (1σ) and a range of -25.0‰ to -13.6‰. The results suggest a mixed terrestrial diet, high in C3 plants with influence from C4 plants for certain individuals. The nitrogen values range from 0.2 to 13.2. The mean and standard deviation are +6.39 ± 3.910‰, suggesting a diet of primarily plant material with influence from animal and marine protein for some individuals. Complete Results are presented in Table 1.

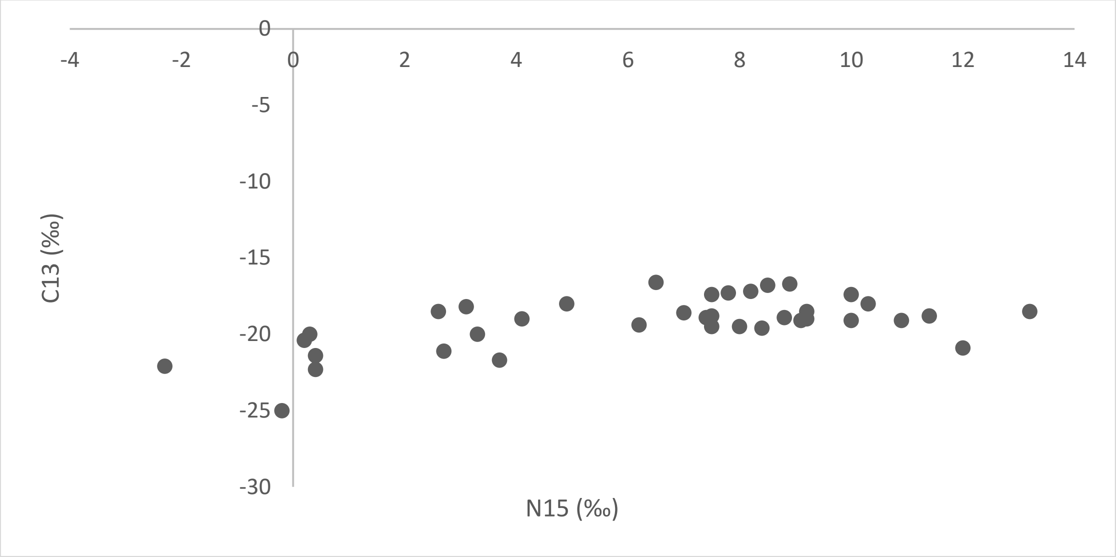
Table 3.1. Complete results of stable isotope compositions and elemental concentrations for the Late Neolithic population of study. Samples that were tested for both carbon and nitrogen are presented first.

Sample Names	δN15 (‰vs. air)	wt. % N	δC13 (‰ vs. VPDB)	wt. % C	C/N atomic raito
SP5217	7.0		-18.6		8.2
SP5127		0.2	-15.9	3.3	22.9
SP18923	7.5	0.4	-18.8	3.8	10.1
SP3759	3.3	0.6	-20.0	4.4	9.1

SP8009	-2.3	0.8	-22.1	5.6	8.1
SP14800	7.5	0.4	-19.5	3.9	11.7
SP15775	7.4	0.3	-18.9	4.3	15.7
SP3799	11.4	0.4	-18.8	3.9	11.5
SP10539	10.3	0.3	-18.0	3.4	12.8
SP15828	6.2	0.5	-19.4	4.1	10.2
SP17328	2.7	0.5	-21.1	3.6	8.6
SP18673	10	0.4	-17.4	3.8	10.1
SPE414		0.3	-18.3	3.4	16.0
SPE132		0.2	-18.0	3.2	16.1
SPE522	0.4	0.5	-21.4	5.1	11.0
SPE473		0.3	-18.3	3.7	14.9
MCI414-34		0.2	-15.8	3.9	30.4
MCI102-30	0.2	0.7	-20.4	5.4	9.6
MCI1-69		0.2	-17.7	2.8	17.9
MCI1-64		0.2	-18.1	3.3	16.6
MCI208-17	8.5	0.2	-16.8	3.6	18.1
MCI187-21	0.4	0.7	-22.3	5.7	9.0
MCI218-27	8.2	0.3	-17.2	3.3	13.7
MCI1-105	0.3	0.5	-20.0	4.7	11.6
MCI382-100		0.2	-17.3	2.5	14.7
EAII1107	6.5	0.2	-16.6	3.4	17.8
14842PM		0.2	-18.2	2.6	16.6
15244PM	4.9	0.4	-18.0	4.3	11.9
17112PM	3.1	0.2	-18.2	3.1	15.9
17210PM		0.2	-18.1	3.1	22.2
17204PM	9.1	0.4	-19.1	4.2	12.8
16283PM	2.6	0.2	-18.5	3.5	17.0
16642PM	7.8	0.2	-17.3	2.4	13.3
14603PM	4.1	0.4	-19.0	3.5	11.1
13289PM	-0.2	0.4	-25.0	10.2	27.0
CM44	10.9	0.4	-19.1	3.5	11.2
CM23	9.2	0.4	-19.0	3.1	8.7
CM547	8.4	0.4	-19.6	3.7	10.4
CM164	10.0	0.4	-19.1	3.5	11.3
CM625A	8.8	0.3	-18.9	3.2	11.7
CM528	13.2	0.4	-18.5	3.5	11.5
CM171		0.2	-19.5	2.2	14.2
CM95		0.3	-20.0	2.9	12.9

CM270	12.0	0.4	-20.9	3.3	9.5
CM513	3.7	0.7	-21.7	5.4	8.5
CLK968	8.0	0.5	-19.5	3.2	8.2
TV3806-39	8.9	0.2	-16.7	3.3	18.3
CL124-83	9.2	0.6	-18.5	3.5	7.2
LC1137-100		0.2	-13.6	8.5	58.1
TV3830-8		0.2	-17.8	3.3	21.2
UE76		0.2	-16.4	5.0	30.6
PDG283A		0.2	-15.7	3.2	23.5
PDG2621		0.2	-14.8	5.1	35.2
PDG2385	7.5	0.3	-17.4	3.1	12.9
PDG24716		0.2	-16.8	3.1	18.7
PDG187A		0.1	-20.0	1.4	12.3
PDG1710		0.2	-21.1	2.5	15.9
SP3415			-21.3	4.9	
SP1849			-22.7	4.3	
SPE318			-20.0	3.6	
SPE80			-23.4	7.5	
SPE112			-19.3	4.0	
SPE347			-19.3	4.7	
SPE418			-19.6	3.5	
MCI178-5			-19.8	3.9	
MCI178-10			-19.1	4.0	
8215PM			-17.6	3.5	
14886PM			-19.7	4.5	
15431PM			-19.3	2.5	
15974PM			-19.9	3.9	
10134PM			-20.4	4.2	
13360PM			-22.6	5.8	
CM652			-22.4	3.8	
CM390			-22.4	5.5	
CM426			-21.7	3.6	
DEA1575			-21.9	4.6	
DEA878			-18.1	7.1	
DEA1429			-20.3	4.0	
PDG2577			-20.3	2.8	
PDG2489			-16.9	2.9	

Fig 3.1: Stable carbon and nitrogen isotopic values based on analysis of dental calculus from the Late Neolithic Portugal.



Burial Groups (Perdigões, Hipogeum, Tombs)

Table 3.2. Late Neolithic Portugal descriptive statistics (Minimum value, maximum value, Mean±1σ) for carbon and nitrogen from dental calculus.

	Total Population	Pedigões	Hipogeum	Tomb burials
Number of samples	80	9	34	36
Carbon				
Mean±1σ	-19.13±2.025	-17.71±2.215	-19.25±1.846	-19.45±2.030
Minimum	-25.0	-21.1	-23.4	-25.0
Maximum	-13.6	-14.8	-15.8	-13.6
Nitrogen				
Mean±1σ	6.39±3.910	7.50	5.1±4.345	7.43±3.589
Minimum	-2.3		-2.3	-0.2
Maximum	13.3		11.4	13.20

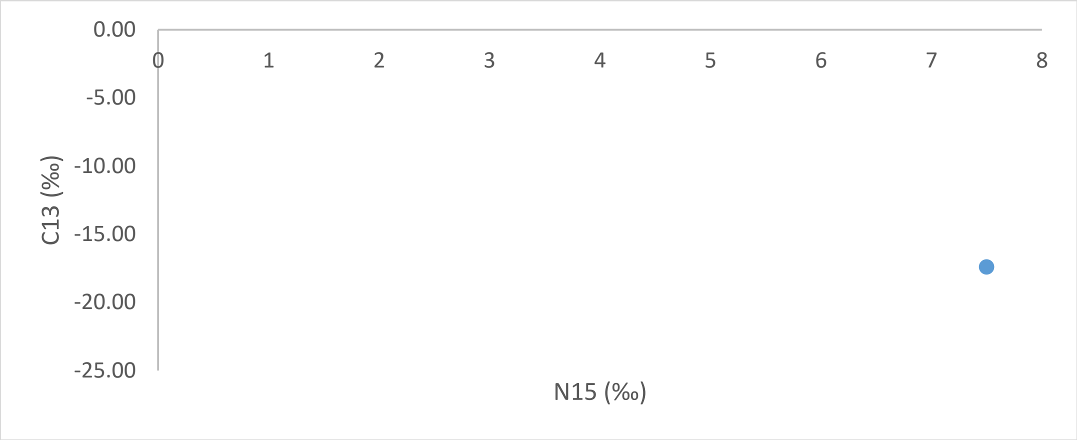
Perdigões

The samples from this group produced stable carbon values. However, most calculus samples from this group did not produce enough weight %N to test for nitrogen. The bulk calculus has a carbon mean and standard deviation of -17.71 ± 2.215‰ with a range of -21.1‰ to -14.8 ‰. This large carbon range suggests a diverse diet that centered on terrestrial, C3 plants with influence from C4 plants and animals that consumed them. The only sample to have enough weight %N was PDG2 385 and this yielded a nitrogen isotope value of 7.5‰, a value lower than omnivores but is more than the herbivorous animals of the Neolithic. This implies that this individual ate primarily plants with some input of animal protein. Full results are presented in Table 3.3 and Fig. 3.2.

Table 3.3. Stable isotope compositions and elemental concentrations of Pedigões burial group.

Sample #	d^{15}N (‰ vs. air)	wt.% N	d^{13}C (‰ vs. VPDB)	wt.% C	C/N atomic ratio
UE76		0.2	-16.40	5.0	30.6
PDG283A		0.2	-15.70	3.2	23.5
PDG2621		0.2	-14.80	5.1	35.2
PDG2385	7.50	0.3	-17.40	3.1	12.9
PDG24716		0.2	-16.80	3.1	18.7
PDG187A		0.1	-20.00	1.4	12.3
PDG1710		0.2	-21.10	2.5	15.9
PDG2577			-20.30	2.8	
PDG2489			-16.90	2.9	

Fig 3.2. Stable carbon and nitrogen isotope values based on analysis of dental calculus from Pedigões.



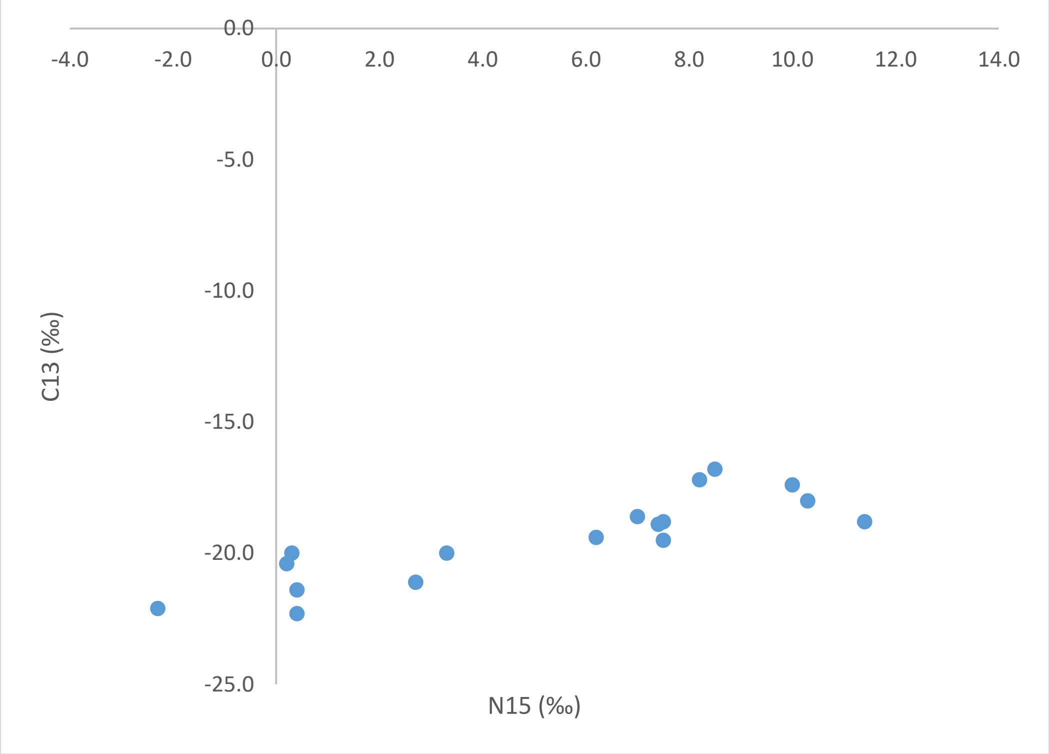
Hipogeum

This group produced enough carbon and nitrogen for 17 of 34 individuals sampled. The carbon mean and standard deviation is -19.25 ± 1.846‰ with a range of -15.9‰ to -23.4‰. These individuals show a wide range of carbon values that may indicate a wide variety of diets centered around a strongly terrestrial, C3 plant-based diet. The samples that were large enough to estimate nitrogen isotope compositions (N:17) produced a mean and standard deviation of 5.10 ± 4.345‰, with a range of 0.2 to 11.4‰. The low nitrogen mean falls within values found for herbivorous animals of the Neolithic. The extremely low nitrogen values skew the mean value for the population. The higher nitrogen values, on the other hand, fall within the published results for bone collagen nitrogen values. Results are presented in Table 3.4 and Fig. 3.3.

Table 3.4. Stable isotope compositions and elemental concentrations of Hipogeum burial group.

Sample #	d^{15}N (‰ vs. air)	wt.% N	d^{13}C (‰ vs. VPDB)	wt.% C	C/N atomic ratio
SP5217	7.0		-18.6		8.2
SP5127		0.2	-15.9	3.3	22.9
SP18923	7.5	0.4	-18.8	3.8	10.1
SP3759	3.3	0.6	-20.0	4.4	9.1
SP8009	-2.3	0.8	-22.1	5.6	8.1
SP14800	7.5	0.4	-19.5	3.9	11.7
SP15775	7.4	0.3	-18.9	4.3	15.7
SP3799	11.4	0.4	-18.8	3.9	11.5
SP10539	10.3	0.3	-18.0	3.4	12.8
SP15828	6.2	0.5	-19.4	4.1	10.2
SP17328	2.7	0.5	-21.1	3.6	8.6
SP18673	10.0	0.4	-17.4	3.8	10.1
SPE414		0.3	-18.3	3.4	16.0
SPE132		0.2	-18.0	3.2	16.1
SPE522	0.4	0.5	-21.4	5.1	11.0
SPE473		0.3	-18.3	3.7	14.9
MCI414-34		0.2	-15.8	3.9	30.4
MCI102-30	0.2	0.7	-20.4	5.4	9.6
MCI1-69		0.2	-17.7	2.8	17.9
MCI1-64		0.2	-18.1	3.3	16.6
MCI208-17	8.5	0.2	-16.8	3.6	18.1
MCI187-21	0.4	0.7	-22.3	5.7	9.0
MCI218-27	8.2	0.3	-17.2	3.3	13.7
MCI1-105	0.3	0.5	-20.0	4.7	11.6
MCI382-100		0.2	-17.3	2.5	14.7
SP3415			-21.3	4.9	
SP1849			-22.7	4.3	
SPE318			-20.0	3.6	
SPE80			-23.4	7.5	
SPE112			-19.3	4.0	
SPE347			-19.3	4.7	
SPE418			-19.6	3.5	
MCI178-5			-19.8	3.9	
MCI178-10			-19.1	4.0	

Fig. 3.3. Stable carbon and nitrogen isotope values based on analysis of dental calculus from Hipogeum Burial group.



Tomb Burials

These individuals, like those of the Hipogeum group, had calculus with adequate carbon for isotope estimates, but nitrogen was again a limiting factor. Only 18 of 36 individuals had sufficient nitrogen for isotope estimates. The carbon mean and standard deviation of this group is -19.45 ± 2.030‰ with a range of -25.0‰ to -13.6. The mean and range suggests a diet focused on terrestrial, C3 plants with some individuals consuming C4 plants. The samples with sufficient nitrogen for testing had a mean and standard deviation for δ^{15}N of 7.43 ±3.589‰, with a range from 3.1 to 13.2‰. Like the

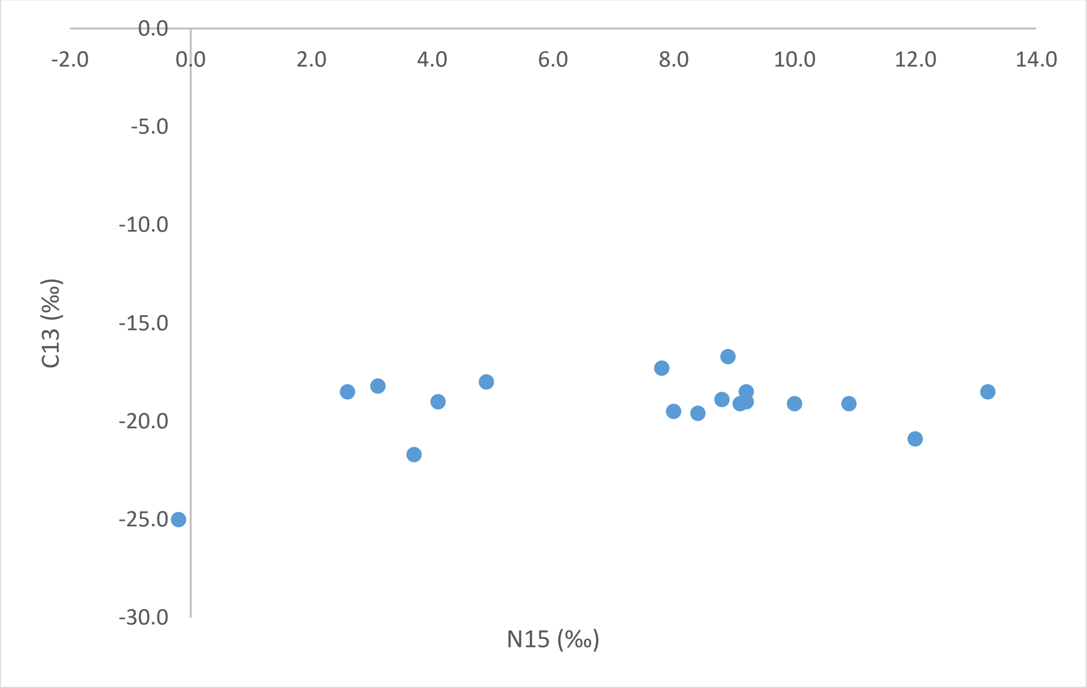
Hipogeum samples, the nitrogen mean is skewed by the inclusion of some extremely low values. Given those limitations, an omnivorous diet with a higher influence of plant material is implied. Complete results are presented in Table 3.5 and Fig. 3.4.

Table 3.5. Stable isotope compositions and elemental concentrations of the Tomb Burial group.

Sample #	d^{15}N (‰ vs. air)	wt.% N	d^{13}C (‰ vs. VPDB)	wt.% C	C/N atomic ratio
14842PM		0.2	-18.2	2.6	16.6
15244PM	4.9	0.4	-18.0	4.3	11.9
17112PM	3.1	0.2	-18.2	3.1	15.9
17210PM		0.2	-18.1	3.1	22.2
17204PM	9.1	0.4	-19.1	4.2	12.8
16283PM	2.6	0.2	-18.5	3.5	17.0
16642PM	7.8	0.2	-17.3	2.4	13.3
14603PM	4.1	0.4	-19.0	3.5	11.1
13289PM	-0.2	0.4	-25.0	10.2	27.0
CM44	10.9	0.4	-19.1	3.5	11.2
CM23	9.2	0.4	-19.0	3.1	8.7
CM547	8.4	0.4	-19.6	3.7	10.4
CM164	10.0	0.4	-19.1	3.5	11.3
CM625A	8.8	0.3	-18.9	3.2	11.7
CM528	13.2	0.4	-18.5	3.5	11.5
CM171		0.2	-19.5	2.2	14.2
CM95		0.3	-20.0	2.9	12.9
CM270	12.0	0.4	-20.9	3.3	9.5
CM513	3.7	0.7	-21.7	5.4	8.5
CLK968	8.0	0.5	-19.5	3.2	8.2
TV3806-39	8.9	0.2	-16.7	3.3	18.3
CL124-83	9.2	0.6	-18.5	3.5	7.2
LC1137-100		0.2	-13.6	8.5	58.1
TV3830-8		0.2	-17.8	3.3	21.2
8215PM			-17.6	3.5	
14886PM			-19.7	4.5	
15431PM			-19.3	2.5	
15974PM			-19.9	3.9	
10134PM			-20.4	4.2	
13360PM			-22.6	5.8	
CM652			-22.4	3.8	
CM390			-22.4	5.5	
CM426			-21.7	3.6	
DEA1575			-21.9	4.6	

DEA878	-18.1	7.1
DEA1429	-20.3	4.0

Fig 3.4: Stable carbon and nitrogen isotope values based on analysis of dental calculus from the Tomb Burial group.



Comparing the Three Burial Groups

All three groups show a consistent pattern of terrestrial, C3 plant based diet. When comparing the carbon values from Hipogeum and Tomb burials, there is no statistically significant difference (t = -1.488, df = 8, p-value = 0.175). Comparing Perdigões and the Tomb burials, there is also no statistically significant difference (t = 1.168, df = 8, p-value = 0.277). Comparing Perdigões and the Hipogeum burials showed no statistical significance between the carbon values (t = 1.488, df = 8, p-value = 0.175).

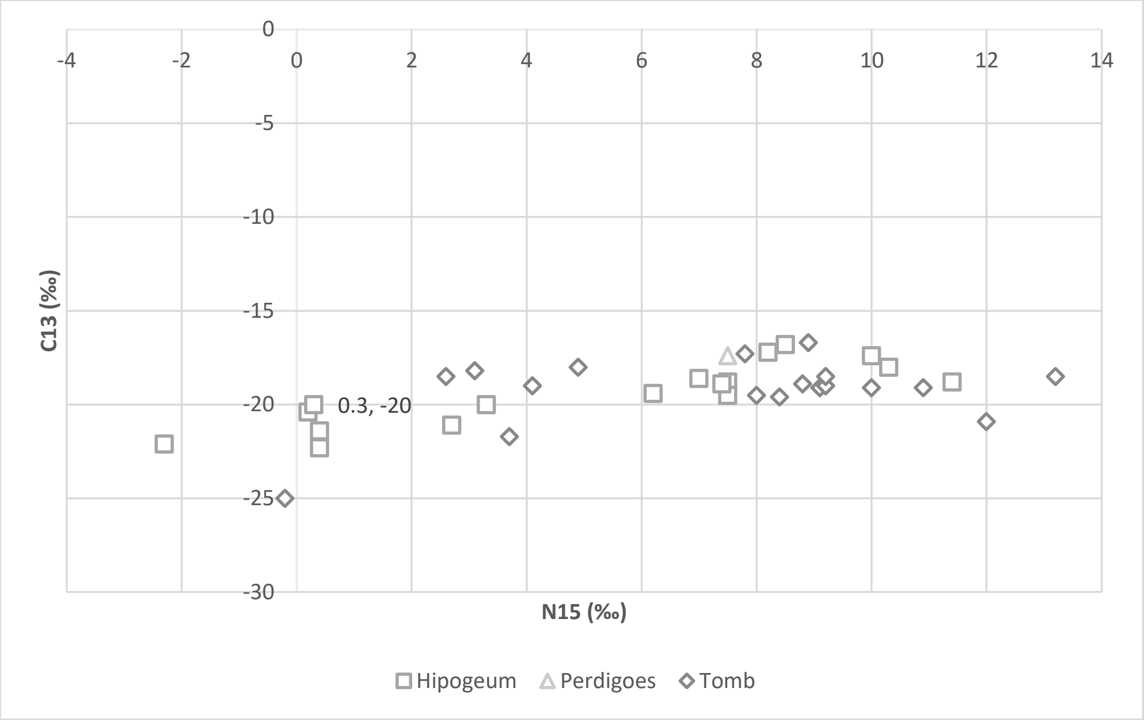
Conducting an ANOVA test for the stable carbon values of the three burial populations shows no statistical significance between the populations (F=2.126, df=2, p-value=0.127). With no statistically significant difference between the carbon values for the three three burial groups, a diet of C3 based food sources, with some influence from C4 plants, is indicated for the Neolithic populations in Portugal. Table 3.6 provides information for the ANOVA test. Fig 3.5 presents a graph of all burial groups for comparison.

Table 3.6. ANOVA statistical variance for the three burial groups: Perdigões, Hipogeum, and Tomb burials.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
-16.4	8	-143	-17.875	5.330714
-18.6	33	-636	-19.2727	3.502045
-18.2	35	-681.9	-19.4829	4.182639

Fig 3.5. Comparison of stable carbon and nitrogen isotopes of the three burial groups for the Late Neolithic.



Discussion

Neolithic Diet

Significant climate change occurred during the early Neolithic. The European continent experienced alternating dry and wet periods. Climate changes may be associated with advancing the agro-pastoral economy (Herrscher and La Bras-Goude 2010). Studies involving faunal samples show populations relied heavily on domestic animals, especially sheep, goats, cattle, and pigs, as the climate of Europe continued to dry (Bocherens et al. 2007; Fischer et al. 2007; Herrscher and Le Bras-Boude 2010; Lightfoot et al. 2010; Fraser et al. 2013; Fontanals-Coll et al. 2015a; Goude et al. 2015).

Paleobotanical studies have found wheat and barley seeds in various Neolithic habitation sites (Hunt et al., 2008; Fracetti et al., 2010; Fraser et al. 2013; Motuzaite-Matuzeviciute et al., 2013; Svyatko et al., 2013). Adopting an agro-pastoral economy decreased the diversity of the Neolithic diet compared to the Mesolithic. There is no apparent influence from C4 plants, such as millet, for most Neolithic populations. Papathanasiou (2003), Bocherens et al. (2007), Fischer et al. (2007), Eriksson et al. (2008), Nehlich et al. (2009), Herrscher and Le Bras-Goude (2010), Lightfoot et al. (2010), Oelze et al. (2010), Fraser et al. (2013), Fonvtanals-Coll et al. (2015a), and Goude et al. (2015) show Neolithic populations had a strong terrestrial-based diet with little to no influence from marine resources. Mollusk shells in Neolithic assemblages provide evidence for the utilization of shoreline resources, but these were not a large part of the diet. These food sources do not alter the stable isotopes of individuals or populations because mollusks are stationary marine organisms that rely on filtering out plankton for sustenance. Mollusks, at a lower trophic level than fish and marine mammals, would not alter human stable carbon and nitrogen isotope compositions (Schoeninger and DeNiro, 1984; Lubell et al., 1994; Milner et al., 2004).

Comparison of Stable Isotope Results with Collagen-based Values in Neolithic Populations of Europe

The calculus-based δ^{13}C values for Neolithic Portugal that averages -19.1 ± 2.0‰ are consistent with collagen-based carbon isotope values from other areas of Europe, including Portugal, Southwestern France, coastal Croatia, Denmark, southern Sweden, Greece, and central Germany (Lubell et al., 1994; Papathanasiou, 2003; Fischer et al., 2007; Eriksson et al., 2008; Herrscher and Le Bras-Goude, 2010; Lightfoot et al., 2010). The δ^{15}N mean of 6.8 ± 3.5, is low but a similar value was found in Northern Greece (7.4 ± 1.05), where the diet focused on plants with a little influence from animal protein (Papathanasiou, 2003). The whole of Europe varies for both the stable carbon and nitrogen isotope values. The variation is due in part to the contrasting environments where populations settled. This reflects varying practices associated with domestication and farming. The use of animal manure to fertilize crops added nitrogen into the soils. Deforestation changed the way plants grew, especially in coastal areas. The coast affects plants as salt water in the air changes the nitrogen intake of plants.

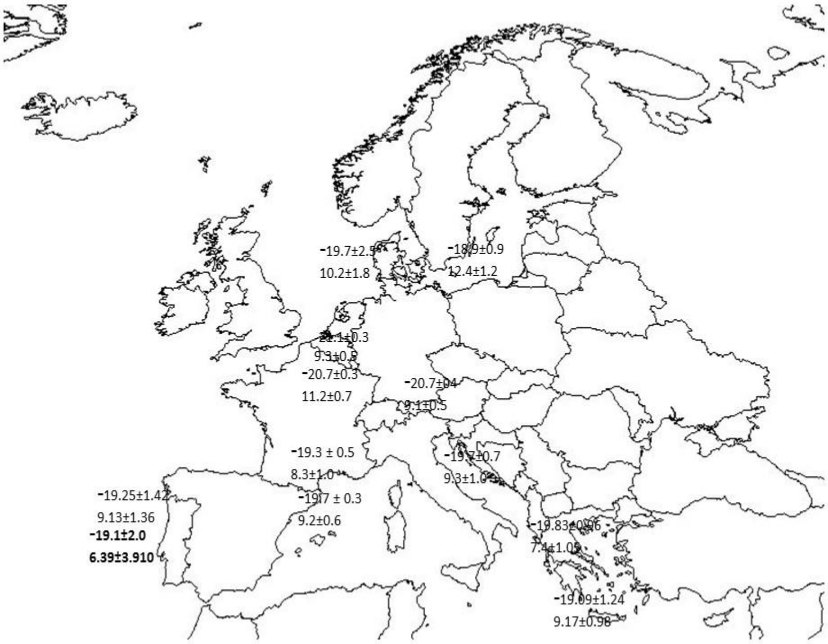


Fig 4.1. Map of Neolithic Europe with stable carbon and nitrogen values from published literature. Values from the bulk dental calculus in bold.

Carbon Stability vs. Nitrogen Variance

Variance between the nitrogen values can be explained through several means: environmental factors, preservation of material and diagenesis, and access to resources. Plants take up nitrogen through the soil. Constant planting without replenishing the nitrogen through human intervention depletes nitrogen for future crops. The influence of humans (e.g., the use of a fertilizer) and the lack of diversity in plants can change the nitrogen values in particular areas (Herrscher and Le Bras-Goude, 2010). Other reasons for variance within the nitrogen can be due to the preservation of the material and diagenesis. The preservation of material is affected by the environment after deposition and during curation. Lack of preservation of the biological material causes the breakdown of the stable isotopes, in turn creating variance in within the population.

The carbon found within the organic component of dental calculus has a greater abundance throughout the environment in comparison to nitrogen. Thus, it takes a much smaller amount of material to obtain a carbon value, as small as 0.5 mg for this study. The difference in the carbon available in plants is due to the difference in the photosynthetic pathway. C3 plants produce more negative carbon values, while C4 plants produce more positive carbon values. The combination of the different values and the changes between the trophic levels create the low level of variance in the carbon values within and between populations. The differences in this study are due to differential access to resources. The 19 individuals that produced more positive carbon values may have had access to C4 plants, such as millet.

Comparison of Dental Calculus Results to Collagen Values from Portugal

Carbon values for the bulk calculus samples yielded a mean of -19.13±2.025‰, which is consistent with studies done on bone collagen, -19.25±1.42‰ Lubell et al. (1994), -20.2±0.4‰ Waterman et al., (2014), -19.6±0.8‰ Guiry et al. (2016), and -20.1±0.5‰ Waterman et al., (2016). The nitrogen from the bulk calculus, with a mean of +6.39±3.910‰, falls outside two standard deviations of the previously stated articles: 9.13±1.36‰ Lubell et al. (1994), 8.2±0.5‰ Waterman et al., (2014), 8.7±1.0‰ Guiry et al. (2016), and 8.7±0.9‰ Waterman et al., (2016). The consistency of the carbon and the variability of the nitrogen values, when comparing results from dental calculus to bone collagen for the Neolithic populations, creates an uncertainty if dental calculus can be used for stable isotopic analysis of the whole diet for this particular population. The consistency of the carbon values between the dental calculus and the bone collagen allows for the wider interpretation of the plant part of a diet of a population. For the Neolithic Portuguese, a diet consistent with C3, terrestrial plants and animals can be inferred from both the results of the bone collagen studies and the bulk dental calculus. The variability of the nitrogen values, in particular the wide variation of the dental calculus in comparison to the bone collagen, presents new questions as to the preservation and amount of material within the calculus along with the possibility of social differentiation. The nitrogen values from Lubell et al. (1994), Waterman et al. (2014), Guiry et al. (2016), Waterman et al. (2016) all present as consuming terrestrial animals with little to no influence of marine resources. The mean of the dental calculus presents as the population consuming primarily plants with little meat or marine resources. This is not a normal type of diet found during the Neolithic.

Fig 4.2. Map of stable carbon and nitrogen values of the Iberian Peninsula from published literature and this study.

Inter-personal Variation

Inter-personal variation is present as the material within the dental calculus can and does produce different values than those of bone collagen or apatite. One of the samples from the dental calculus, UE 76, had stable isotope analysis conducted on both dental calculus and a sample of bone. The sample of bone was tested by Ana Maria Silva of the University of Coimbra, Portugal. The carbon value was -19.87 while the nitrogen isotope composition was 7.96, with a carbon-nitrogen (C:N) ratio of 3.3. The carbon value from dental calculus was -16.4 for carbon, but the sample was too small to test for nitrogen. The contrast in carbon values must reflect material the dental calculus contains that is not recorded in collagen.

The values obtained show two different diets. Values from the bone collagen show a diet of C3, terrestrial based plants. Values from the dental calculus are more consistent with a diet containing C4 plants. This discrepancy could reflect changes in the diet that are not yet present in the bone material. Another possible explanation for the discrepancy between bone collagen and the dental calculus values could be the amount of material processed. Small calculus samples may not allow for enough material to produce accurate values.

Quality Control

Looking at quality control in regard to dental calculus, the atomic C/N ratio can be a good indicator of "good" samples. For bone collagen, with its fixed chemical composition, the atomic C/N ratio is a good indicator of the quality of ancient bone (DeNiro, 1985; Eerkens et al, 2014). Due to the variation in the biomolecular composition of calculus, there is no acceptable range of C/N ratios to evaluate diagenesis (Eerkens et al., 2014). The ratios in this study range from 7.2 to 58.1, with a mean of 15.37 and a standard deviation of 8.31 (1 standard deviation). These values are far higher than any other calculus samples tested for isotope composition to date. Following Eerkens et al. (2014), I have separated the samples into 2 groups. One group consists of samples that fall at or below 12 C/N ratio, with another group above the C/N ratio of 12. Samples at or below 12 for the C/N ratio have a nitrogen mean of 6.28 and a standard deviation of 4.333. The carbon mean is -19.664 with a standard deviation of 1.289. Samples with C/N ratios above 12 have a nitrogen mean of 5.543 with a standard deviation of 3.662. The carbon mean is -18.111 with a standard deviation of 2.562.

When the two quality control groups are compared, there is a statistically significant difference between the carbon means for the samples below and above the C/N ratio of 12 (t = 2.365, df = 23, p = 0.027). For the nitrogen values, there is no statistically significant difference between the groups (t = 0.453, df = 11, p = 0.659). Quality control is an important consideration when using dental calculus as a biomaterial for isotope testing. Higher quality samples produce more consistent values, and both carbon and nitrogen isotope compositions can be estimated. In these circumstances (e.g., Scott and Poulson, 2012) calculus produces values consistent with those from other biomaterials. Samples with extremely high C/N ratios should be excluded from analysis in most instances.

Conclusion

Evaluating the role of dental calculus as a potential biomaterial for the analysis of stable carbon and nitrogen isotopes is a key part of this study. This study of the diet of Late Neolithic Portuguese groups is important to evaluate possible changes in diet across the geographic landscape, along with adding to the existing studies on dental calculus as a proxy for bone, hair, and/or nails in stable isotope analysis.

The δ^{13}C values in this study are consistent with that derived using bone collagen on other European populations. In particular, populations from Portugal, Southwestern France, coastal Croatia, Denmark, southern Sweden, Greece, and central Germany show similar carbon values to those derived from the dental calculus of Neolithic Portuguese (Lubell et al., 1994; Papathanasiou, 2003; Bocherens et al., 2007; Fischer et al., 2007; Eriksson et al., 2008; Nehlich et al., 2009; Herrscher and Le Bras-Goude, 2010; Lightfoot et al., 2010; Lightfoot et al., 2011; Oelze et al., 2011; Fraser et al., 2013; Fontanals-Coll et al., 2015a; Goude et al., 2015). The carbon isotope values show a wide range of variation, with a mean of -19.13‰, a standard deviation of ± 2.025‰, and a range of -13.6‰ to -25.0‰ The results indicate that Neolithic Portuguese populations relied heavily on C3 terrestrial plants and the animals that consumed them. There are individuals within the population that have more positive carbon values, consistent with consumption of C4 plants.

Nitrogen values are not congruent with collagen-based stable isotope studies of Neolithic European populations. A δ^{15}N mean of +6.8‰ ± 3.5‰ suggests a diet focused on plants (Lubell et al., 1994; Papathanasiou, 2003; Bocherens et al., 2007; Fischer et al., 2007; Eriksson et al., 2008; Nehlich et al., 2009; Herrscher and Le Bras-Goude, 2010; Lightfoot et al., 2010; Lightfoot et al., 2011; Oelze et al., 2011; Fraser et al., 2013; Fontanals-Coll et al., 2015a; Goude et al., 2015). Most Neolithic populations had nitrogen values consistent with the consumption of terrestrial herbivores, and for some populations, omnivores such as pigs: 9.8±0.4‰, 8.3±1.0‰, 9.1±0.5‰, 9.2±0.6‰ (Eriksson et al., 2008; Herrscher and Le Bras-Goude, 2010; Fraser et al., 2013; Fontanals-Coll et al., 2015a). The nitrogen results from Portugal may indicate a limitation of dental calculus as a biomaterial for stable isotopes, but this may reflect an issue with preservation. In all other calculus samples analyzed to date, the C/N ratios are consistently under 10.

Dividing the population into burial types and locations, Perdigões, Hipogeum, and the Tomb Burials allowed for some contrast between different populations in Portugal.

An ANOVA test shows there is no significant difference between the burial groupings (F = 2.126, df = 2, p-value = 0.127). All of the populations show some influence from C4 plants when the ranges are examined: Perdigões -21.1‰ to -14.8 ‰, Hipogeum -23.5‰ to -15.9‰, and the Tomb Burials -25.0 to -13.6‰. The ranges of these populations also imply a varied diet. The Hipogeum and tomb burials show values that are consistent with a diet containing terrestrial C3 plants and animals. There is no noteworthy input from marine resources in these populations.

Quality control of dental calculus is an aspect that has been addressed and will continue to be addressed through further studies. What has been discussed is that the C:N ratio of a good sample is 12.0 and under, making it closer to the C:N ratio of bone collagen, 3.0. Assessing the quality of a particular group of samples is an important part of understanding the results. Using the 12.0 C/N ratio as a guide, samples with ratio at or under 12.0 C/N ratio are considered a better quality since they are close to the C/N ratio of collagen. Those with a C/N ratio higher than 12.0 are often missing a nitrogen reading. The missing nitrogen does not mean that it is completely missing, just that it is not detectable by the machines testing the samples. The statistical difference between the groups, particularly with carbon values, indicates the importance of examining the samples both as a whole population and in quality control groups. Higher quality samples, as a group, produce values in both carbon and nitrogen closer to that of other biomaterials.

Limitations of Stable Isotope Analysis and Dental Calculus

Stable isotope analysis is one tool for understanding the diets of past populations. Traditional biomaterials, including collagen, dentine, apatite, hair, skin, and nails, can reflect in part early diets and foods consumed during the final years of an individual's life. The use of traditional materials limits what populations that can be analyzed. Limitations start with obtaining the material from not only institutions holding the material but also from descendent populations that may not approve of the invasive and destructive process. Even when material is available from museums and descendant groups for isotope analysis, lack of preservation limits the amount of organic or inorganic material available for testing.

The use of dental calculus as a proxy for stable isotope analysis is in its infancy. There are limitations associated with calculus and these are slowly being worked out. For example, it takes a fairly large sample to test for both carbon and nitrogen in dental calculus. Although all humans produce dental calculus, individuals produce differing amounts due to various factors (Hillson, 1979, 1996; White, 1997; Lieverse, 1999). The different amounts produced make it difficult to get a wider, generalized view of the diet of a population. Even with positive results from dental calculus, the results may not reflect material related directly to diet. The results are from what is consumed by the oral bacteria before it is trapped within the calculus, either food or non-food material.

Comparison to Published Studies on Dental Calculus and Stable Isotope Analysis

Dental calculus - used in the past to examine phytoliths, starch granules, and bacteria - has gained interest as a biomaterial for stable isotope analysis. Scott and

Poulson (2012) were the first to find dental calculus was a biomaterial that can be used for stable isotope analysis. The findings from a Basque sample in Spain were consistent with bone collagen-based $\delta^{13}C$ and $\delta^{15}N$ compositions. Additional studies have addressed intra- and inter-individual variation. Even within the same individual, stable isotope values can show differences (Poulson et al., 2013; Eerkens et al., 2014). The variation of stable isotopes, carbon and nitrogen, is due to the formation and composition of dental calculus. These variations do not always match up with the stable carbon and nitrogen values of bone collagen and should only be used when no other source of skeletal material is available and only to interpret general dietary patterns for a population (Salazar-Garcia et al., 2014). The carbon mean and range for this study, -19.13 ± 2.025‰ and -25.0‰ to -13.6‰, is consistent with the variation that is seen in other studies of dental calculus. The nitrogen mean and range for this study, 6.39 ± 3.910‰ and -2.3 to 13.6, has greater variation than previously reported (Scott and Poulson, 2012; Poulson et al., 2013; Eerkens et al., 2014; Salazar-Garcia et al.,2014). The large variation for the stable nitrogen values confirms there is a need to conduct more testing on dental calculus from various areas and time periods to obtain better insight in its role as an isotopic dietary marker. Means, standard deviation, and ranges for the bulk calculus for Scott and Poulson (2012), Poulson et al. (2013), Salazar-Garcia et al. (2014), and Eerkens et al. (2014) and this study are available in Table 12.

As more studies are conducted, the way in which dental calculus is collected and processed will become more standardized. With the wide variation within the bulk calculus, its use as a biomaterial for stable isotope analysis should be used to investigate the general diet of a population. When assessing diet on the individual level, the inconsistency in the amount of dental calculus available due to the various factors that compound the formation of calculus makes it difficult to interpret diet. Until more studies are conducted across various areas and time periods, and processing dental calculus is standardized, the use of dental calculus for stable isotope analysis should be conducted along with other means of examining diet, when they are available, to create a better understanding of the general diet of a population. Faunal and paleobotanical analysis, microscopic examination of organic material within the calculus, and using skeletal material along with dental calculus for stable isotope analysis should be conducted when these materials are available.

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