



# Stable isotope and multi-analytical investigation of Monte da Cegonha: A Late Antiquity population in southern Portugal

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## ABSTRACT

This study presents for the first time the diet of a Late Antiquity population in southern Portugal (Civitas of Pax Julia), from the Roman villa of Monte da Cegonha (predominantly 7th century CE). Stable isotope analysis ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ ,  $^{87}\text{Sr}/^{86}\text{Sr}$ ) of human and faunal bone collagen and apatite was conducted in order to understand the influence of Roman subsistence strategies on the way of life of rural inhabitants of the area of Pax Julia and to explore their diet (types of ingested plants, amount of animal resources, terrestrial versus marine resources). X-ray diffraction (XRD) and Fourier transform infra-red spectroscopy (FTIR) analyses were used to determine the degree of bone diagenesis and assess the reliability of the bone stable isotopic composition for palaeodietary reconstruction. Anthropological analysis revealed a cariogenic diet, rich in starchy food and carbohydrates, in at least in two individuals based on the frequency of dental caries. Collagen and apatite carbon isotopic analysis suggested that  $\text{C}_3$  plants were the basis of the population's diet, complemented with some terrestrial meat and its by-products as reflected by the observed bone collagen nitrogen isotopic composition. Moreover, whilst the fairly low apatite-collagen spacing recorded in some skeletons (at around 4‰) may have been due to freshwater organisms intake, the relatively low nitrogen values observed indicate that this consumption did not occur very often, unless in the form of fresh fish of low trophic level or fish sauces. There were no significant differences in isotopic values depending on gender or burial type.

Strontium and oxygen isotopic composition of bone apatite revealed a sedentary community, with the exception of a male individual who probably did not spend his childhood in Monte da Cegonha.

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## 1. Introduction

This study examines for the first time the way of life, and more specifically the diet, of a Late Antiquity population in southern Portugal (Civitas of Pax Julia), using the geochemical composition of human and faunal remains from the Roman villa of Monte da Cegonha.

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Stable isotope analysis is based on the principle that human and animal body tissues reflect the isotopic composition of the food and water ingested (Britton et al., 2008; Budd et al., 2013; Keenleyside et al., 2009; Müldner and Richards, 2007). Measuring the carbon ( $\delta^{13}\text{C}_{\text{co}}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopic ratios of bone collagen is a well-established technique for reconstruction of ancient diets (Bogaard et al., 2007; Craig et al., 2009; Keenleyside et al., 2009). Carbon isotope values can be used to distinguish plants using the  $\text{C}_3$  and  $\text{C}_4$  photosynthetic pathways and the individuals consuming these plants (Budd et al., 2013; Chenery et al., 2010). They can also be used to differentiate terrestrial and aquatic food sources (Chenery et al., 2010; Müldner et al., 2011). Carbon

isotopic ratios incorporated into bone collagen derive mainly from dietary proteins (Ambrose and Norr, 1993; Howland et al., 2003; Mays and Beavan, 2012). Bone apatite ( $\delta^{13}\text{C}_{\text{apa}}$ ) reflects the isotopic composition of the complete diet (proteins, carbohydrates and lipids) (Ambrose et al., 2003; Brown and Brown, 2011). Mays and Beavan (2012) suggested that the use of bone apatite in conjunction with bone collagen enables a more complete assessment of palaeodiet. Apatite-collagen spacing ( $\delta^{13}\text{C}_{\text{apa-col}}$  spacing) has been used to estimate the type and proportion of protein in the overall diet (Turner et al., 2012). Nitrogen isotope values are used to infer the position of an individual in the food chain (Keenleyside et al., 2009), based on increased  $\delta^{15}\text{N}$  values (2–6‰; Hedges and Reynard, 2007) with each trophic level, and are useful for distinguishing meat-rich diets from plant-rich diets, as well as the consumption of marine and freshwater resources (Bocherens and Drucker, 2003; Hedges and Reynard, 2007).

Human mobility can also be investigated using strontium and oxygen isotopes. Enamel  $^{87}\text{Sr}/^{86}\text{Sr}$  isotopic ratios reflect the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of the local geological region in which an individual grew up. After formation, there are no alterations of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in enamel during an individual's lifetime and, as such, a  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio different from that of the burial location suggests that the individual moved from their place of infancy (Bentley, 2006). Comparison between  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in the teeth and bones of an individual also suggest later mobility, because bones are continuously remodelled during a lifetime and their  $^{87}\text{Sr}/^{86}\text{Sr}$  values change if a person moves to a region with a different geological signature (Brown and Brown, 2011). Similar information can be obtained from oxygen isotopes. Oxygen isotopic ratios yielded by phosphates and carbonates of bone mineral (apatite) derive from dissolved oxygen that has been ingested with water and food (Longinelli, 1984) with an oxygen isotopic composition ( $\delta^{18}\text{O}$ ) that is dependent on geoenvironmental factors, i.e. latitude, altitude and distance to the coast (Dansgaard, 1964). Therefore,  $\delta^{18}\text{O}$  recorded in skeletons can be used to identify individuals who had access to a different water supply than rest of the local population. However, the suitability of  $\delta^{18}\text{O}$  to this application is still debated because for example, food preparation and drinking fermented beverages can alter isotopic ratio (Brettell et al., 2012).

Furthermore, bone isotopic composition might be altered during burial by diagenesis. Diagenesis is a complex process that involves biological and physico-chemical *post-mortem* alterations of skeletal material directly exposed to soil environments during burial (Beasley et al., 2014; Hollund et al., 2013; Lebon et al., 2010; Maurer et al., 2011, 2014). Such processes that affect bone preservation can be caused by microbial attack, temperature, humidity, hydrology, pH, and may lead to partial or complete degradation of organic matter (Beasley et al., 2014; Lebon et al., 2010). Diagenesis is also associated with uptake or exchange of ions (Wright and Schwarcz, 1996) and changes in the crystallinity of bone mineral (Berna et al., 2004). During recrystallization, bone mineral can incorporate or adsorb dissolved ionic species from groundwater into its crystal structure, but it is not inevitable that the original isotope composition is altered (Hollund et al., 2013; Lee-Thorp, 2008). Changes in crystallinity relevant to the evaluation of diagenesis can be measured by X-ray diffraction (XRD) and attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) (Beasley et al., 2014; Berna et al., 2004; Brown and Brown, 2011). FTIR also gives information about the composition and structure of the organic phase (Hollund et al., 2013; Lebon et al., 2010).

## 2. The archaeological site investigated

The Roman *villa* of Monte da Cegonha is located 13.5 km northeast of Beja (Alentejo region, southern Portugal) (Fig. 1). This *villa* was built in the first quarter of the 1st century CE and lasted, with successive modifications, until the 12th century CE. The soil and available water resources of this area facilitated agricultural and animal husbandry practices, and allowed the establishment of local populations through

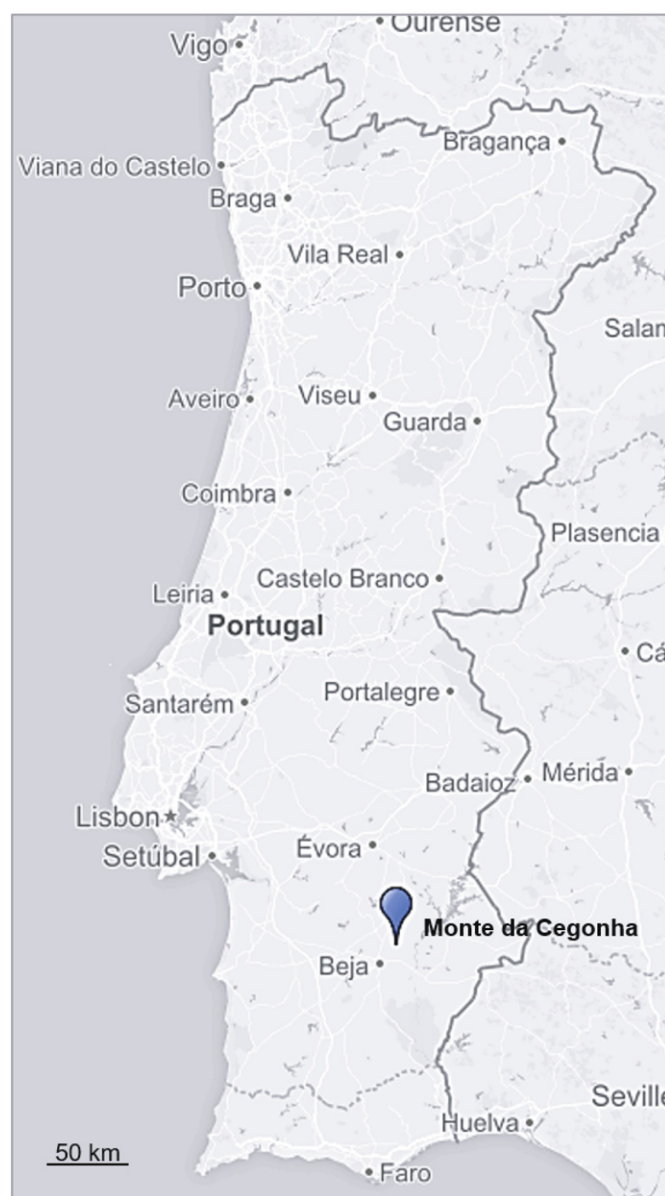
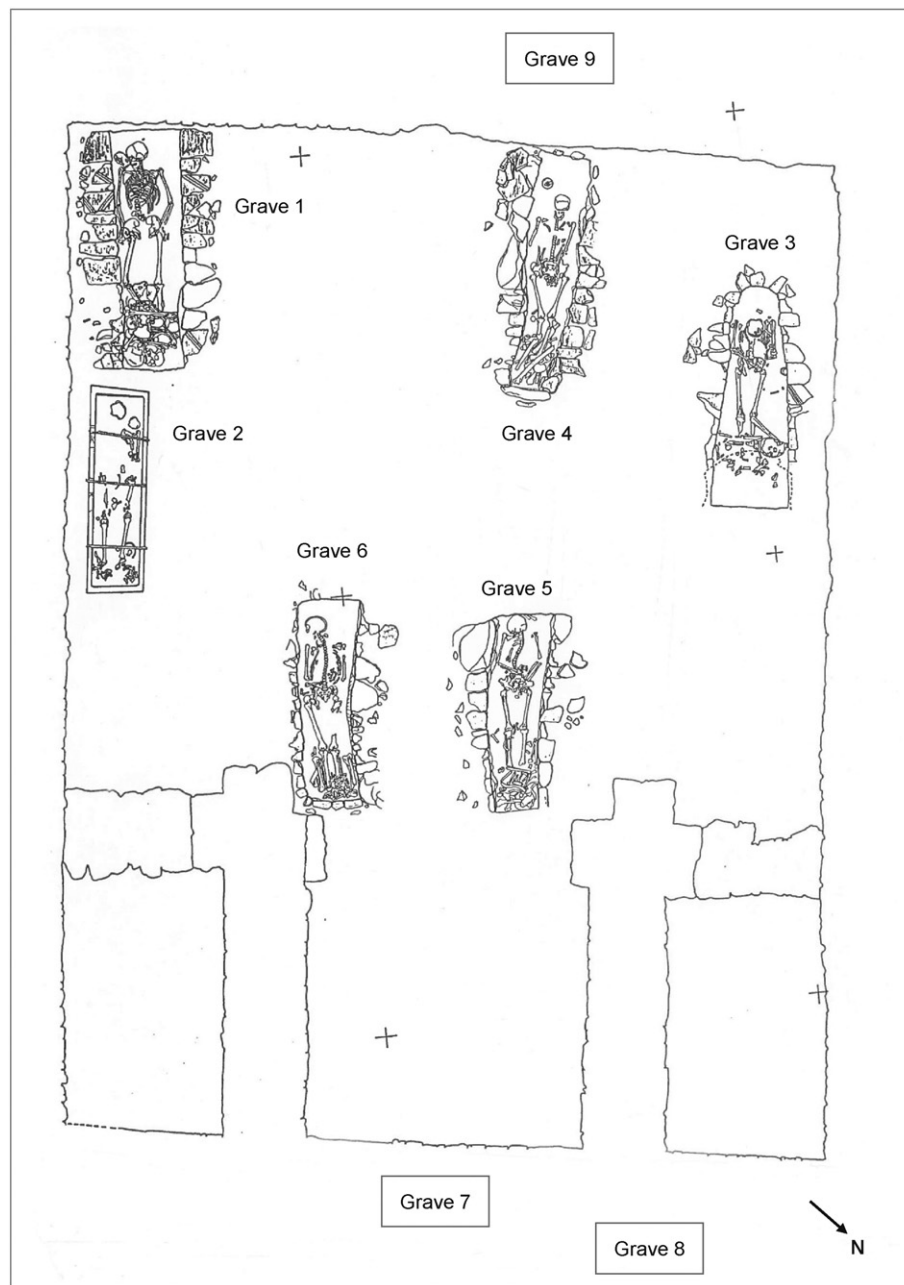


Fig. 1. Map showing the location of Monte da Cegonha.

time. The restructuring of the *villa* in the 4th century CE incorporated new buildings, including a Palaeochristian basilica. The basilica was used for interments until the end of the 6th century CE, although its funerary function was not the primary objective (Lopes and Alfenim, 1994). A total of 12 graves were uncovered, which were constructed with reused materials such as stone, brick and tiles, with the exception of one white marble sarcophagus (grave 2). All the graves were used and reused several times, as witnessed by the ossuaries accumulated at the foot of each tomb. The majority of primary burials found in articulation were inhumed in a *decubitus dorsalis* position with a West-East orientation. A minimum number of 25 individuals were retrieved for this study, although the total number of excavated individuals was higher (see Section 3.1 for more details). While nine graves were discovered inside the basilica, graves 7, 8 and 9 were buried outside the eastern and western church walls (Fig. 2). The council of Braga, which took place in 572 CE, where a ban on burials inside the temples was promulgated, provides *terminus antequem* for the use of this space as a necropolis and dates the new phase with establishment of a new scree, a new altar, a baptistery and a reliquary. This basilica was one of the oldest rural Palaeochristian churches in the Iberian Peninsula (Alfenim and



**Fig. 2.** Partial plan of the burials found within and around the Palaeochristian basilica in Monte da Cegonha from where osteological remains were collected. Graves 7, 8 and 9 are positioned approximately.

Lopes, 1992; Lopes and Alfenim, 1994). An unusual find was a reliquary found under the altar consisting of a gray marble box, possibly dated to the 6th century CE. Silver fragments were found inside the reliquary that may have belonged to an *ampulla*, two ceramic medallions and two ceramic medals. The reliquary artefacts display similarities with materials found in Palestine and are likely to have been transported by a pilgrim (Alarcão et al., 1992; Alfenim and Lopes, 1992; Lopes and Alfenim, 1994). The basilica inside the *villa* has been renewed more than once during its existence, from the 4th century onwards, and the graves and ossuaries have been introduced in a second phase, thought to be finished by the end of the 6th century CE (Alfenim and Lopes, 1992) based on archaeological evidence and artefacts discovered at Monte da Cegonha. However, recent radiocarbon dating of human bones undertaken during this project, from three graves and one ossuary, revealed that the burials dated were mainly interred during a

more recent period, by the mid of the 7th century CE, with a sample dating in the 8th–9th century CE (Table 1).

### 3. Materials

#### 3.1. Human samples

Unfortunately not all osteological material from Monte da Cegonha was available for sampling and analysis. The best possible skeletal representation was selected from available material. Long and flat bones from 25 individuals buried in Monte da Cegonha and excavated from individual graves comprising skeletons in articulation and ossuary bones were sampled. These 25 individuals correspond to 5 individual skeletons and 20 ossuary burials. The inhumations sampled were adult individuals (4 females and 1 male), while the 20 ossuary burials included 5



**Table 1**

Radiocarbon dates obtained for four human bones from Monte da Cegonha, performed at the Scottish Universities Environmental Research Centre, Glasgow, UK (SUERC). The calibrated age ranges were determined using the University of Oxford Radiocarbon Accelerator Unit calibration program (OxCal4).

Grave	Sample	Conventional radiocarbon years (BP)	Calibrated radiocarbon ages (1 $\sigma$ , cal CE) <sup>a</sup>	Laboratory code
2	HMC 6	1363 $\pm$ 28	647–672	SUERC-58539 (GU36586)
5	HMC 8	1228 $\pm$ 31	790–869	SUERC-58544 (GU36588)
	HMC 9 (O)	1373 $\pm$ 31	641–671	SUERC-58540 (GU36587)
7	HMC 13	1330 $\pm$ 31	654–690	SUERC-58545 (GU36589)

O – ossuary.

<sup>a</sup> Calibrated radiocarbon ages were obtained by using OxCal v4.2.4 and IntCal13.

children (<5 years old) and 15 adults (1 female, 5 males, 2 probable males and 7 undetermined individuals). For the skeletons found in articulation more than one anatomical element was analysed for examining potential intra-skeletal geochemical differences (Maurer et al., 2011, 2014). In total 40 human samples were submitted for analysis and are listed in Table 2.

### 3.2. Faunal samples

In total, 21 faunal samples were collected from a range of contemporaneous animal remains excavated outside the basilica at various locations at Monte da Cegonha. Specimens were deliberately collected from different individuals, using similar bone portions or remains collected in different areas. The zooarchaeological analysis, including species identification, age, biometry and pathology, was carried out according to current standard techniques (e.g. Bull and Payne, 1982; Grant, 1982; Payne, 1973; Silver, 1969; von den Driesch, 1976; Zeder, 2006; Zeder and Lapham, 2010; Zeder and Pilaar, 2010). Animal samples included two wild species (rabbit, *Oryctolagus cuniculus*, and red deer, *Cervus elaphus*), at least two domestic species (cattle, *Bos taurus*, and ovicaprids: goat, *Capra hircus*, and perhaps sheep, *Ovis aries*) and swine (pig or wild boar, *Sus* sp.). Further information is listed in Table 3.

## 4. Methods

### 4.1. Anthropological analysis

All individuals were aged and sexed (Table 2) according to standard anthropological techniques (Beck, 1995; Bruzek, 2002; Ferembach et al., 1980; Lovejoy et al., 1985; MacLaughlin, 1990; Scheuer and Black, 2004; Silva, 1995; Wasterlain, 2000).

Stature was estimated using humerus maximal length and physiological length of the femur according to the regression formulae proposed by Mendonça (2000). For the evaluation of dental caries size and location, Lukacs (1989) and Moore and Corbett (1971) were used, while dental calculus was reported following Brothwell (1981). The analysis of dental wear was based on the Smith (1984) scoring system. Indicators of physiological stress, namely porotic hyperostosis, cribra orbitalia and dental enamel hypoplasias were macroscopically surveyed following the recommendations proposed by Beck (1995).

### 4.2. Sampling

Approximately 1–2 g of human and faunal bone samples were collected with a saw and external surfaces cleaned with a DREMEL® drill to remove contaminating surface material and adhering soil.

### 4.3. To test bone preservation

#### 4.3.1. Infrared spectroscopy

A total of 20 human and 7 faunal bone samples were examined using ATR-FTIR (Table 5). Samples were ground using an agate mortar and pestle to a fine powder. Infrared spectra were collected on a Bruker Alpha spectrometer coupled with a single-reflection diamond ATR module (100 scans with a spectral resolution of 4 cm<sup>-1</sup>, from 4000 to 375 cm<sup>-1</sup>). All spectra were acquired in absorbance mode and were recorded and analysed using OPUS/Mentor software (version 6.5). Peak heights at wavenumber 565, 603, 1030, 1035, 1415 and 1640 cm<sup>-1</sup> were used for the calculation of the Infrared Splitting Factor (IRSF), relative carbonate content (C/P) and relative collagen content (Am/P). The sum of the absorbance values of the two phosphate peaks at wavenumbers 565 (ν<sub>4</sub> PO<sub>4</sub>) and 603 cm<sup>-1</sup> (ν<sub>4</sub> PO<sub>4</sub>) was then divided by the height of the valley between them to obtain the IRSF value, following the protocol described by Weiner and Bar-Yosef (1990). Relative carbonate content was measured by the peak heights at wavenumbers 1035 (ν<sub>3</sub> PO<sub>4</sub>) and 1415 cm<sup>-1</sup> (ν<sub>3</sub> CO<sub>3</sub>) (Wright and Schwarcz, 1996). Relative collagen content was measured by the intensity ratio of the amide I peak at 1640 cm<sup>-1</sup> versus the phosphate peak at 1030 cm<sup>-1</sup> (Trueman et al., 2008). All FTIR analyses were performed in the HERCULES Laboratory.

#### 4.3.2. X-ray diffraction analysis

A total of 20 human and 6 faunal bone samples were analysed using XRD (Table 5). Samples were ground using an agate mortar and pestle to a fine powder. X-ray diffraction data was obtained with Cu Kα radiation on a Bruker AXS D8 Advance with a DAVINCI design diffractometer, equipped with a Göbel mirror assembly and a LynxEye 1D detector (2θ angular range of 20.000°–40.184°, step size of 0.020° and a step time of 6 s). The peaks corresponding to reflections [211], [112], [300] and [202] were measured to calculate the crystallinity of the samples, Crystallinity Index (CI) (Person et al., 1995). All the X-ray diffractograms were checked for the presence of other minerals, such as quartz or calcite. All XRD analyses were performed in the HERCULES Laboratory.

### 4.4. Bone collagen extraction and analysis

Collagen was extracted using the method of Longin (1971) with some modification (Britton et al., 2008; Knipper et al., 2012; Salesse et al., 2014). Between 300 and 500 mg of bone sample was demineralized in 10 ml 0.5 M HCl at 4 °C for approximately 14 days, with regular vortex and an acid change after one week. After demineralization, the samples were rinsed to neutrality with ultrapure water and soaked in 0.125 M NaOH for 20 h at room temperature to oxidize fulvic and humic acids. The samples were rinsed again to neutrality with ultrapure water and gelatinized in 0.01 M HCl at 70 °C for 48 h. The liquid fraction containing the gelatinized protein was filtered using Eze-Filter™ separators (Elkay Laboratory Products) to remove impurities. The solubilized collagen was frozen, lyophilized for 48 h and analysed.

Extracted and freeze-dried collagen samples were weighed into tin capsules (between 0.7 and 1.2 mg) and combusted into CO<sub>2</sub> and N<sub>2</sub> in an elemental analyser (EA) with oxygen (Flash 2000 HT, Thermo Fisher Scientific, Bremen, Germany) using pure helium as carrier gas. Isotopic ratios of carbon and nitrogen were obtained on a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) coupled to the EA via ConFlo IV interface (Thermo Fisher Scientific, Bremen, Germany).

The raw data were normalized by two-point calibrations using international reference materials, such as IAEA-CH-6 (sucrose, −10.449‰) and IAEA-CH-7 (polyethylene, −32.151‰) for carbon and IAEA-N-1 (ammonium sulphate, +0.4‰) and IAEA-N-2 (ammonium sulphate, +20.3‰) for nitrogen isotopic composition. Measurement errors were less than ±0.1‰ for carbon and ±0.2‰ for nitrogen. The δ<sup>13</sup>C and δ<sup>15</sup>N values are expressed in per mill and relative to VPDB and

**Table 2**  
Information pertaining to individual archaeological contexts, estimated age-at-death, sex, skeletal element analysed and pathologies of studied individuals from Monte da Cegonha.

Grave	Age	Sex	Sample	Skeletal element	Pathologies
1	Adult	Male	HMC 1 (O)	Femur (R)	–
	Young adult	Female	HMC 2 (O)	Femur (R)	–
	Non adult (~5 years)	Undetermined	HMC 3 (O)	Femur (R)	–
	Non adult (~3 years)	Undetermined	HMC 4 (O)	Femur (R)	–
	Non adult (~6 months)	Undetermined	HMC 5 (O)	Femur (R)	–
2	Young adult (<27 years)	Female	HMC 6	Femur (L)	–
			HMC 26	Sacrum	–
			HMC 27	Rib (L)	–
			HMC 28	Scapula	–
4	Young adult (<27 years)	Female	HMC 7	Femur (R)	Cariogenic lesions (grade 1), dental calculus (grades 1–3), dental wear (grades 1–2) and dental enamel hypoplasia
			HMC 29	Humerus (R)	
			HMC 30	Ulna	
			HMC 31	Tibia (L)	
			HMC 8	Humerus (L)	
5	Adult (~40 years)	Female	HMC 32	Cranial	Cariogenic lesions (grades 1–3), dental calculus (grades 1–2), dental wear (grades 3–4) and dental enamel hypoplasia
			HMC 33	Humerus (L)	
			HMC 34	Ulna	
			HMC 9 (O)	Femur (R)	
			HMC 10 (O)	Femur (R)	
6	Adult	Male	HMC 11	Femur (L)	Dental calculus (grades 1–2), dental wear (grades 4–7) and dental enamel hypoplasia
			HMC 35	Rib (L)	
			HMC 36	Scapula	
			HMC 37	Humerus (R)	
			HMC 12 (O)	Femur (L)	
7	Young adult (<23 years)	Female	HMC 13	Femur (L)	Dental calculus (grades 1–2), dental wear (grades 1–2) and dental enamel hypoplasia
			HMC 38	Cranial	
			HMC 39	Rib (R)	
			HMC 40	Radius (R)	
	Young adult	Male	HMC 14 (O)	Femur (R)	–
	Adult	Male?	HMC 15 (O)	Femur (R)	–
			HMC 16 (O)	Humerus (R)	–
	Adult	Undetermined	HMC 17 (O)	Humerus (L)	–
8	Non adult (38 weeks in uterus–1.5 months)	Undetermined	HMC 18 (O)	Femur (L)	–
	Non adult (6 months–1 year)	Undetermined	HMC 19 (O)	Femur (L)	–
	Adult	Male?	HMC 20 (O)	Femur	–
	Adult	Undetermined	HMC 21 (O)	Femur (L)	–
	Undetermined	Undetermined	HMC 22 (O)	Femur (L)	–
	Young adult	Male	HMC 23 (O)	Femur (L)	–
	Adult	Male	HMC 24 (O)	Femur (L)	–
	Adult	Undetermined	HMC 25 (O)	Femur (L)	–

L – left; R – right; O – ossuary.

AIR, with analytical precision of 0.1‰ and 0.2‰, respectively. A calibrated in-house standard (L-alanine,  $\delta^{13}\text{C} = -19.17\text{‰}$ ;  $\delta^{15}\text{N} = +4.36\text{‰}$ ) was measured at regular intervals throughout analytical sequences (every eight analyses) to check and correct for instrumental drift.

Samples were run at least in duplicates. The elemental (C%, N%) and isotopic composition of analysed collagen samples is reported in Table 6. All EA-IRMS analyses were performed in the HERCULES Laboratory.

**Table 3**

Information on taxonomy, skeletal element analysed and age of faunal species studied from Monte da Cegonha. N.B. when possible the distinction between *Ovis aries* (sheep) and *Capra hircus* (goat) was made using the criteria of Zeder and Lapham (2010) and Zeder and Pilaar (2010).

Taxonomy	Skeletal element	Age	Sample	Comments
<i>Cervus elaphus</i>	Tibia	Adult	FMC 2	–
<i>Oryctolagus cuniculus</i>	Mandible	Adult	FMC 21	–
<i>Capra hircus</i>	Mandible	Adult	FMC 6	–
<i>Capra hircus</i>	Mandible	Adult	FMC 7	–
<i>Capra hircus</i>	Mandible	Young adult	FMC 9	–
<i>Capra hircus</i>	Mandible	Adult	FMC 14	–
<i>Capra hircus</i>	Mandible	Adult	FMC 15	–
<i>Capra hircus</i>	Mandible	Adult	FMC 20	–
<i>Ovis aries</i> / <i>Capra hircus</i>	Humerus	Adult	FMC 4	Probably <i>Ovis aries</i>
<i>Ovis aries</i> / <i>Capra hircus</i>	Mandible	Adult	FMC 8	–
<i>Ovis aries</i> / <i>Capra hircus</i>	Mandible	Young adult	FMC 10	–
<i>Ovis aries</i> / <i>Capra hircus</i>	Tibia	Adult	FMC 11	Probably <i>Ovis aries</i>
<i>Ovis aries</i> / <i>Capra hircus</i>	Pelvis	Adult	FMC 17	–
<i>Bos taurus</i>	Proximal phalanx	Adult	FMC 1	–
<i>Bos taurus</i>	Metacarpal	Adult	FMC 3	–
<i>Bos taurus</i>	Radius	Adult	FMC 5	–
<i>Bos taurus</i>	Astragalus	Adult	FMC 12	–
<i>Bos taurus</i>	Mandible	Adult	FMC 13	–
<i>Bos taurus</i>	Maxillary	Adult	FMC 16	–
<i>Bos taurus</i>	Astragalus	Juvenile	FMC 19	–
<i>Sus</i> sp.	Metacarpal IV	Young adult	FMC 18	–

#### 4.5. Bone apatite extraction and analysis

Apatite was extracted from the bone samples following the method of Koch et al. (1997) with modifications (Rand, 2011; Salesse et al., 2013). Bone samples were ground using an agate mortar and pestle and approximately 30 mg of bone powder was soaked in 1.2 ml 2.5% NaOCl solution at room temperature for 24 h to remove any organic matter. The samples were rinsed with ultrapure water and centrifuged five times to remove the NaOCl. The remaining fraction was treated with 1.2 ml 1 M CH<sub>3</sub>COOH buffered with CH<sub>3</sub>COOLi at room temperature for 24 h to eliminate exogenous and adsorbed carbonate. The samples were rinsed with ultrapure water and centrifuged five times for 5 min to remove the buffered acetic acid. The samples were then oven-dried at 40 °C for 24 h before being analysed at the Isotope Mass Spectrometry Service of the National Museum of Natural History (MNHN, Paris). Around 600 µg of apatite powder were reacted with 100% H<sub>3</sub>PO<sub>4</sub> at 70 °C in a Kiel IV device connected to a Delta V Advantage (Thermo Scientific) isotope ratio mass spectrometer for measuring  $\delta^{13}\text{C}_{\text{apa}}$  and  $\delta^{18}\text{O}_{\text{carb}}$  values.

The internal carbonate standard (Marble LM), normalized to the international standard NBS 19, was used for checking the accuracy of the measurements (theoretical  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of 2.13‰ and –1.83‰, respectively) and to apply a correction to the samples varying from –0.01 to 0.01‰ for  $\delta^{13}\text{C}_{\text{apa}}$  and –0.16 to –0.30‰ for  $\delta^{18}\text{O}_{\text{carb}}$ .

Between 14 and 18 analyses of the standard Marble LM provided an analytical precision within each run, of 0.01 to 0.04‰ for  $\delta^{13}\text{C}$  and of 0.01 to 0.12‰ for  $\delta^{18}\text{O}$ .

#### 4.6. Strontium isotope: sample preparation and analysis

Strontium isotopic analyses were performed on human tooth enamel of four individuals (graves 4, 5, 6 and 7) and on two human bones (graves 5 and 6). Around 20 mg of powder samples were collected using a drill diamond bit. All skeletal tissues were pre-treated with 1 M Li acetate-acetic acid. Bones were then ashed at 550°/12 h in acid-cleaned ceramic crucibles.

In order to determine the local bioavailable  $^{87}\text{Sr}/^{86}\text{Sr}$  signature of Monte da Cegonha site, two modern water and eight vegetation samples were collected from variable geological outcrops (Maurer et al., 2012), in the vicinity of Monte da Cegonha (Table 4), characterized during the field work that took place in October 2014. After collection, the plant leaves were cleaned with ultrapure water, dried in an oven at 50 °C and then ashed in a muffle furnace at 550°/12 h in acid-cleaned ceramic crucibles. Water samples were acidified with Suprapur® HNO<sub>3</sub> (to get a final solution of 0.2% HNO<sub>3</sub>) and filtered with 0.45 µm Nylon membrane (VWR). Environmental samples and skeletal tissues were digested, Sr extracted and analysed at the Isotope Geology Laboratory of the University of Aveiro. All samples were digested using Suprapur® nitric acid (with the addition of H<sub>2</sub>O<sub>2</sub> for plant samples) and evaporated to dryness. AG8 50W Bio-Rad resin was used for separating Sr from isobaric interferences, further loaded onto Ta filaments with H<sub>3</sub>PO<sub>4</sub> and measured with a VG Sector 54 TIMS in dynamic mode. The standard reference material NIST SRM 987 was concomitantly measured and provided a value of  $0.710278 \pm 14$  (conf. limit 95%, N = 13). Strontium procedural blanks were <500 pg Sr.

#### 4.7. Statistical analyses

Statistical analyses were performed using ANDAD (developed by Jorge de Sousa, Technical University of Lisbon, <http://biomonitor.ist.utl.pt/~ajsousa/Andad.html> [accessed 20 November 2015]) and PAST 3.10 for Windows. Results were investigated using principal component analysis (PCA) on ANDAD and non-parametric Mann-Whitney *U* tests on PAST.

### 5. Results

#### 5.1. Anthropological analysis

From the 25 individuals analysed, stature was estimated on eight individuals, four of them represented by skeletons in articulation and the remaining four only by femurs. The male average,  $164.60 \pm 6.90$  cm (*n* = 3) was obtained using the physiological length of the femur. For females using the physiological length of the femur, an average of  $154.90 \pm 5.92$  cm (*n* = 4) was achieved. The humerus maximal length of another female gave a stature of  $154.70 \pm 7.70$  cm. These values are consistent with expected values for both sexes and they are compatible with those referred by Cardoso and Gomes (2009) for Portuguese populations of the same timeline.

**Table 4**

Geological substrate and or field observation at each sampling point for water and plant collected in the vicinity of Monte da Cegonha site.

Vegetation sample	Geological substrate/observation
1 plant	Metapelite
2 plant	Metapelite
3 plant	Weathered diorite
4 plant	Dioritic, variation of granites, no schists
5 plant	Amphibolite
6 water	Local stream
7 water	Ribeira do Freixo
7 plant	Adjacent to Ribeira do Freixo
8 plant	Metabasic outcrop

Only four individuals could be considered for the study of oral pathology, since the remaining one from grave 2 had no associated cranial or dental remains. From these, only two showed the presence of cariogenic lesions, inhumations in graves 4 and 5. Dental calculus was observed on all four individuals analysed (graves 4, 5, 6 and 7) and all of them displayed dental wear. The indicators of physiological stress were applied only to four individuals. Porotic hyperostosis and cribra orbitalia were not recorded. However, dental enamel hypoplasias (DEH) were detected in four analysed individuals. All of the oral pathologies and physiological stress indicators are presented in Table 2.

**Table 5**

Results of FTIR (IRSF, C/P and Am/P) and X-ray diffraction (CI and secondary minerals present in bone porosity) analyses of 20 human bone samples (5 individual skeletons from graves 2–7 and 9 individuals from ossuaries) and 7 faunal bone samples.

	Grave	Sample	ATR-FTIR			XRD	
			IRSF	C/P	Am/P	CI	Minerals
Skeletons	2	HMC 6	3.34	0.45	0.28	0.04	Calcite
		HMC 27	4.10	0.22	0.09	0.10	–
	4	HMC 7	3.78	0.27	0.07	0.11	Calcite
		HMC 31	5.92	0.11	0.03	0.24	Calcite
	5	HMC 8	4.05	0.21	0.08	0.14	Calcite
		HMC 32	5.33	0.10	0.02	0.32	Calcite
	6	HMC 11	5.20	0.10	0.02	0.30	Calcite
		HMC 36	4.31	0.21	0.08	0.27	Quartz
	7	HMC 13	4.09	0.21	0.10	0.19	–
		HMC 39	4.04	0.16	0.06	0.28	–
		HMC 40	4.19	0.22	0.10	0.19	–
	Average		4.40 ± 0.8	0.21 ± 0.1	0.08 ± 0.1	0.20 ± 0.1	–
Ossuaries	1	HMC 2	4.69	0.12	0.02	0.41	–
		HMC 3	4.17	0.19	0.02	0.31	Calcite
	5	HMC 9	4.24	0.19	0.09	0.10	Calcite
		HMC 10	5.67	0.09	0.02	0.32	Calcite
	7	HMC 14	4.14	0.22	0.10	0.18	–
	8	HMC 18	4.05	0.18	0.04	0.35	–
		HMC 20	4.37	0.14	0.08	0.31	–
	9	HMC 24	4.56	0.13	0.02	0.33	–
		HMC 25	4.30	0.17	0.16	0.23	Calcite
	Average		4.47 ± 0.5	0.16 ± 0.0	0.06 ± 0.0	0.28 ± 0.1	–
Fauna	–	FMC 1	3.44	0.28	0.30	0.05	Calcite, quartz
	–	FMC 2	3.75	0.21	0.07	0.24	–
	–	FMC 3	3.50	0.28	0.08	0.15	–
	–	FMC 13	3.76	0.21	0.06	0.19	–
	–	FMC 14	3.70	0.24	0.05	0.21	Calcite
	–	FMC 16	3.22	0.47	0.18	–	–
	–	FMC 19	3.58	0.41	0.08	0.12	Calcite, quartz
	Average		3.56 ± 0.2	0.30 ± 0.1	0.12 ± 0.1	0.16 ± 0.1	–

## 5.2. Bone preservation

### 5.2.1. Infrared spectroscopy

All of the ATR-FTIR data are presented in Table 5. Statistical analysis revealed that there were no significant differences in IRSF, C/P and Am/P values (Mann-Whitney  $p > 0.05$ ) between individual inhumations and ossuary bones. Statistical analysis also revealed that there were significant differences in IRSF and C/P values (Mann-Whitney  $p < 0.05$ ) between human and faunal bones, as the latter have lower IRSF and C/P values. However, they show no significant differences in Am/P values (Mann-Whitney  $p > 0.05$ ).

Samples collected from various skeletal elements of the same individual skeletons (Table 5) from graves 2, 4, 5, 6 and 7 confirmed intra-skeletal differences based on IRSF, C/P and Am/P values.

### 5.2.2. X-ray diffraction

Full details of XRD analyses are listed in Table 5. Statistical analysis showed that there were no significant differences in CI values (Mann-Whitney  $p > 0.05$ ) between individual inhumations and ossuary bones. Statistical analysis also revealed that there were significant differences in CI values (Mann-Whitney  $p < 0.05$ ) between human and faunal bones, which have lower CI values than human bones. XRD analyses revealed the presence of calcite in the majority of samples (humans and faunal samples). The analysis of two faunal samples (FMC 1 and 19) also revealed the presence of quartz.

Similar to ATR-FTIR analyses, some samples analysed by XRD were collected from different skeletal elements of the same human individual (Table 5) from graves 2, 4, 5, 6 and 7. The results further corroborate the intra-skeletal differences in crystallinity.

### 5.2.3. Collagen quality

Collagen extraction was successful for most of the human and faunal bone samples, based on the criteria for well-preserved collagen (i.e. C/N ratios between 2.9 and 3.6 (DeNiro, 1985), carbon content between 15.3 and 47.0% (Ambrose, 1990), nitrogen content between 5.5 and 17.3% (Ambrose, 1990) and collagen yields  $> 1\%$  (van Klinken, 1999)). The bone collagen atomic C/N ratios for all samples showed ranges between 3.2 and 3.5. The carbon and nitrogen amount in all bone collagen samples ranged from 40.4 to 44.8% and from 14.2 to 16.1%, respectively. The bone collagen yields showed a large variability, ranging from 0.5 to 12.5%. All the data is listed in Table 6.

## 5.3. Dietary patterns

All isotopic measurements ( $\delta^{13}\text{C}_{\text{co}}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{13}\text{C}_{\text{apa}}$  and  $\delta^{18}\text{O}_{\text{carb}}$ ) of human and faunal samples are presented in Table 6. Stable isotope analysis of the human samples from Monte da Cegonha yielded  $\delta^{13}\text{C}_{\text{co}}$  values ranging from  $-17.40$  to  $-18.80\%$ .  $\delta^{15}\text{N}$  values ranged from  $9.40$  to  $13.20\%$ . Statistical analysis revealed that there were no significant differences in  $\delta^{13}\text{C}_{\text{co}}$  and  $\delta^{15}\text{N}$  values (Mann-Whitney  $p > 0.05$ ) between individual inhumations and ossuary bones, and between female and male individuals (Mann-Whitney  $p > 0.05$ ). There were no significant differences in  $\delta^{13}\text{C}_{\text{co}}$  values (Mann-Whitney  $p > 0.05$ ) between bone samples excavated from graves inside and outside the basilica. However, statistical analysis also revealed that there were significant differences in  $\delta^{15}\text{N}$  values (Mann-Whitney  $p < 0.05$ ) between bone samples excavated from graves inside and outside the basilica. Stable isotope analysis of the faunal samples yielded  $\delta^{13}\text{C}_{\text{co}}$  values ranging from  $-18.78$  to  $-21.38\%$ , and  $\delta^{15}\text{N}$  values ranging from  $4.80$  to  $7.95\%$ .

Stable isotope analysis of the human apatite samples yielded  $\delta^{13}\text{C}_{\text{apa}}$  values ranging from  $-11.57$  to  $-14.91\%$ . Stable isotope analysis of the faunal samples yielded  $\delta^{13}\text{C}_{\text{apa}}$  values ranging from  $-10.80$  to  $-12.67\%$ .

A selection of samples from various skeletal elements of individual skeletons from graves 2, 4, 5, 6 and 7, prepared for  $\delta^{13}\text{C}_{\text{apa}}$ , were analysed with and without acetic acid treatment. The analysis was done in order to monitor the potential impact of diagenesis on  $\delta^{13}\text{C}_{\text{apa}}$ . Results show that



**Table 6**Carbon, nitrogen and oxygen stable isotope results and collagen quality indicators for human and faunal samples with  $^{87}\text{Sr}/^{86}\text{Sr}$  values from four human individuals (bones and teeth).

	Grave	Sample	Collagen yield (%)	%C	%N	C:N	$\delta^{13}\text{C}_{\text{col}}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}_{\text{apa}}$ (‰)	$\delta^{13}\text{C}_{\text{apa-col}}$ (‰)	$\delta^{18}\text{O}$ (‰)	$^{87}\text{Sr}/^{86}\text{Sr}$	
												Bone	Tooth
Humans – skeletons	2	HMC 6	7.6	43.48	15.77	3.22	−18.10	10.90	−14.19	3.91	1.00	–	–
		HMC 26	–	–	–	–	–	–	−15.04	–	1.52	–	–
		HMC 27	–	–	–	–	–	–	−15.26	–	1.24	–	–
		HMC 28	–	–	–	–	–	–	−14.91	–	2.00	–	–
	4	HMC 7	8.6	44.83	16.14	3.24	−18.10	11.00	−14.91	3.19	−0.58	–	0.7111
		HMC 29	–	–	–	–	–	–	−14.09	–	−0.29	–	–
		HMC 30	–	–	–	–	–	–	−13.99	–	0.15	–	–
		HMC 31	–	–	–	–	–	–	−14.45	–	0.07	–	–
	5	HMC 8	5.8	43.90	15.67	3.27	−18.40	10.00	−14.21	4.19	−0.10	0.7108	0.7108
		HMC 32	–	–	–	–	–	–	−14.80	–	−0.15	–	–
		HMC 33	–	–	–	–	–	–	−14.11	–	0.16	–	–
		HMC 34	–	–	–	–	–	–	−14.17	–	0.74	–	–
	6	HMC 11	–	–	–	–	–	–	−13.76	–	−1.18	0.7103	0.7097
		HMC 35	–	–	–	–	–	–	−14.11	–	0.10	–	–
		HMC 36	–	–	–	–	–	–	−14.29	–	−0.19	–	–
		HMC 37	–	–	–	–	–	–	−14.08	–	−1.01	–	–
	7	HMC 13	6.6	44.86	15.59	3.36	−18.30	9.90	−11.90	6.40	−1.45	–	0.7109
		HMC 38	–	–	–	–	–	–	−13.90	–	−1.71	–	–
		HMC 39	–	–	–	–	–	–	−11.67	–	−1.50	–	–
		HMC 40	–	–	–	–	–	–	−14.67	–	−0.15	–	–
Humans – ossuaries	1	HMC 1	5.5	43.84	15.62	3.27	−18.00	10.80	−11.57	6.43	−0.58	–	–
		HMC 2	0.5	–	–	–	−18.70	11.20	−13.01	5.69	0.50	–	–
		HMC 3	6.2	43.62	15.88	3.20	−18.70	10.00	−13.14	5.56	−1.58	–	–
		HMC 4	3.2	43.62	15.91	3.20	−18.50	10.40	−12.40	6.10	−0.86	–	–
	5	HMC 5	2.0	41.71	14.82	3.28	−17.40	13.20	−12.70	4.70	−1.28	–	–
		HMC 9	4.4	42.52	15.42	3.22	−18.00	9.80	−13.54	4.46	−0.46	–	–
		HMC 10	–	–	–	–	–	–	−14.19	–	−0.95	–	–
		HMC 12	0.6	–	–	–	−18.20	11.60	−14.23	3.97	−1.02	–	–
	7	HMC 14	6.3	44.37	15.76	3.29	−18.80	10.50	–	–	–	–	–
		HMC 15	5.8	43.58	15.80	3.22	−18.00	9.60	–	–	–	–	–
		HMC 16	12.5	41.31	14.27	3.38	−17.90	11.20	−13.54	4.36	0.74	–	–
		HMC 17	7.5	44.33	15.84	3.27	−18.70	10.10	−13.42	5.28	0.66	–	–
	8	HMC 18	2.3	40.38	14.25	3.31	−18.40	9.60	−11.70	6.70	−1.21	–	–
		HMC 19	5.2	44.15	15.42	3.34	−18.30	10.10	−12.84	5.46	−1.24	–	–
		HMC 20	3.9	43.68	15.14	3.37	−18.40	10.80	−14.01	4.39	−1.76	–	–
		HMC 21	6.7	44.12	15.77	3.26	−18.60	9.70	−12.67	5.93	−0.72	–	–
	9	HMC 22	3.9	43.24	15.65	3.22	−18.70	10.20	−13.55	5.15	−1.21	–	–
		HMC 23	7.7	44.01	15.90	3.23	−18.40	9.40	−13.71	4.69	−0.31	–	–
		HMC 24	4.7	43.41	15.68	3.23	−18.40	10.40	−13.81	4.59	−1.57	–	–
		HMC 25	3.8	42.77	15.47	3.23	−18.60	9.60	−13.58	5.02	−0.94	–	–
Fauna	–	FMC 1	0.96	41.95	14.38	3.40	−21.27	7.57	−11.96	9.31	0.00	–	–
	–	FMC 2	2.0	43.48	15.65	3.24	−20.49	4.80	−11.75	8.74	−1.25	–	–
	–	FMC 3	3.8	43.07	15.42	3.26	−20.16	6.05	−11.38	8.78	0.33	–	–
	–	FMC 4	1.2	–	–	–	–	–	−12.29	–	−1.06	–	–
	–	FMC 5	0.7	42.84	14.22	3.51	−20.27	6.64	−11.04	9.23	−1.34	–	–
	–	FMC 6	8.7	44.14	15.26	3.37	−19.87	7.61	−12.55	7.32	0.30	–	–
	–	FMC 7	1.6	–	–	–	–	–	−13.36	–	0.15	–	–
	–	FMC 8	0.7	–	–	–	–	–	−13.89	–	−0.51	–	–
	–	FMC 9	0.8	43.16	15.47	3.25	−19.18	7.58	−12.48	6.70	1.15	–	–
	–	FMC 10	5.6	–	–	–	–	–	−10.45	–	−0.04	–	–
	–	FMC 11	3.9	42.54	15.02	3.30	−18.78	6.95	−12.44	6.34	1.00	–	–
	–	FMC 12	1.0	–	–	–	−20.86	6.57	−10.80	10.06	−0.69	–	–
	–	FMC 13	2.1	42.13	15.20	3.23	−20.99	6.45	−11.58	9.41	−0.99	–	–
	–	FMC 14	0.5	–	–	–	−19.24	5.51	−11.76	7.48	−0.05	–	–
	–	FMC 15	8.0	41.99	15.33	3.20	−18.87	7.17	−12.57	6.30	0.07	–	–
	–	FMC 16	6.0	43.09	15.42	3.26	−19.46	7.76	−11.04	8.42	0.67	–	–
	–	FMC 17	4.9	42.03	15.15	3.24	−19.99	6.73	−11.31	8.68	0.28	–	–
	–	FMC 18	7.7	42.66	15.31	3.25	−19.88	7.95	−12.56	7.32	−1.72	–	–
	–	FMC 19	4.1	42.72	15.16	3.29	−19.86	7.78	−11.22	8.64	−0.48	–	–
	–	FMC 20	8.8	43.12	15.39	3.27	−19.00	6.16	−12.67	6.33	2.67	–	–
	–	FMC 21	4.6	43.14	14.97	3.36	−21.38	5.25	–	–	–	–	–

samples without acetic acid treatment present enriched  $\delta^{13}\text{C}_{\text{apa}}$  values. However, two samples (HMC 13 and 39) from grave 7 also show enriched  $\delta^{13}\text{C}_{\text{apa}}$  values, although they were treated with acetic acid.

#### 5.4. Mobility

Monte da Cegonha's  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios of modern water samples were 0.7124 (Fig. 7) while the values for modern vegetation samples ranged

from 0.7083 to 0.7125. Tooth enamel  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio values of four individuals (graves 4, 5, 6 and 7) ranged from 0.7096 to 0.7111. The bone apatite  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from two different human individuals (graves 5 and 6) varied between 0.7103 and 0.7108.

Regarding  $\delta^{18}\text{O}_{\text{carb}}$ , obtained human values range from −1.76‰ to 1.00‰ while faunal sample analysis yielded values ranging from −1.72‰ to 2.67‰. Similar to  $\delta^{13}\text{C}_{\text{apa}}$  we observed differences in  $\delta^{18}\text{O}_{\text{carb}}$  values between samples treated and not treated with acetic



acid, which depleted values, although two samples (HMC 13 and HMC 39) from grave 7 treated with acetic acid behave similarly to untreated samples.

## 6. Discussion

### 6.1. Anthropological analysis

The anthropological data does not allow any conclusions to be made on a population level, due to the small sample size (only five skeletons in articulation), as well as their poor preservation state.

The frequency of dental caries, with two out of four individuals affected, cannot be used to infer diet on a population level but the high number of caries affecting each individual indicates that at least these two had a cariogenic diet, comprising rich starchy food and/or carbohydrates, and negligent dental hygiene. Dental calculus and wear are not significant.

Hypoplastic defects observed on all analysed teeth can be interpreted as the result of physiological stress suffered by these individuals during their early growth, between 1.5 and 3.5 years of age, which corresponds (according to AlQahtani et al. (2010)) to the chronological range of the crown formation of incisors and canines.

### 6.2. Bone preservation

Diagenetic alterations of archaeological bones present a potential problem for the interpretation of isotopic ratios as a source of information for dietary and mobility reconstructions (Hollund et al., 2013; Lebon et al., 2010). Through FTIR and XRD analyses of bone samples it is possible to screen for diagenetic alterations (differences in bone carbonate and collagen content, and in bone crystallinity) in order to understand the reliability of the isotopic data used for dietary and mobility studies.

Principal component analysis involving XRD and FTIR data acquired from human and faunal bones was performed. The correlation circle (Fig. 3) shows a projection of the initial variables (CI, IRSF, C/P and Am/P) in the factors' space. F1 axis (78.2% of the variation) correlates strongly with the decreasing values for C/P and Am/P ratios, while the F2 axis (11.4% of the variation) correlates positively with Am/P ratio and IRSF, but negatively with CI. The positions of variables CI and Am/P suggest they are negatively correlated, i.e., high crystallinity is strongly correlated to lower relative collagen content, which is commonly observed in temperate climates. As the same applies to the variables IRSF and C/P, an increase in crystallinity is also correlated with a diagenetic loss of carbonate, which is linked to the loss of bone organic matter (Nielsen-Marsh and Hedges, 2000; Person et al., 1995; Salesse et al., 2014). XRD analysis revealed the presence of calcite in 13 samples and the presence of quartz in three samples. The increase of bone porosity, due to the loss of organic matter, subsequently allows interactions between bone components and groundwater, as well as promoting the precipitation of secondary minerals (Lebon et al., 2010), such as calcite. Therefore, FTIR and XRD analysis indicate that the samples have been diagenetically transformed in comparison to fresh bones (IRSF > 3.4 (Beasley et al., 2014; Berna et al., 2004; Salesse et al., 2014), C/P values out of the range 0.23 to 0.34 (Beasley et al., 2014), Am/P values below 0.9 (Salesse et al., 2014) and CI > 0 (Person et al., 1996)).

FTIR and XRD also indicate that there are no differences in preservation between overall individual inhumations and ossuary bones. On the other hand, faunal bone samples were less altered compared to human bone samples. Such alteration can be due to a different burial context. Faunal remains were excavated outside the basilica at various locations, suggesting that they could be domestic refuse.

Bone samples from graves 2, 4, 5, 6 and 7, analysed by FTIR and XRD, were collected from different skeletal elements of the same individual to check for intra-skeletal differences. Results show that there are differences in crystallinity at the intra-skeletal scale, as well as at the grave

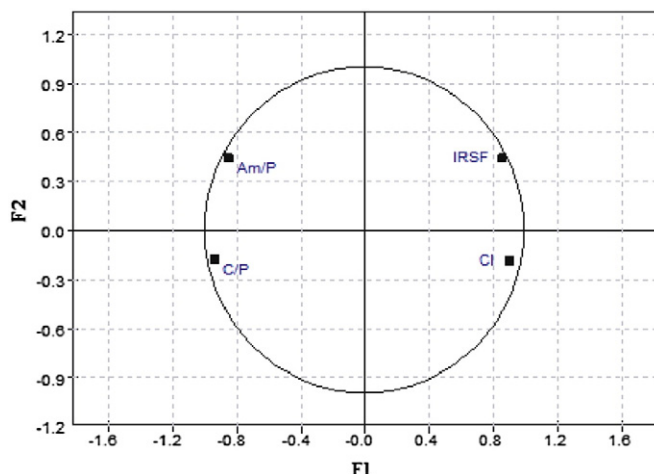


Fig. 3. Correlation circle for principal components 1 and 2 after principal component analysis of FTIR and XRD data.

level, between primary inhumations and ossuaries buried in the same grave (Table 5). These intra-skeletal differences may be due to taphonomic processes and differential kinetic diffusion parameters depending on the part of the skeleton and the individuals analysed (Maurer et al., 2011).

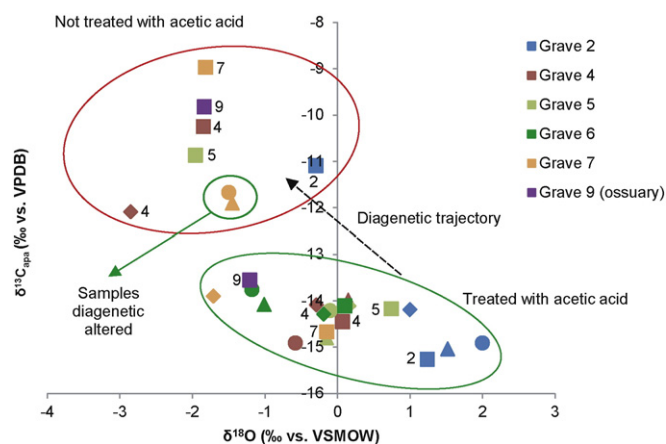
Additionally, six samples were analysed with and without acetic acid treatment to better understand the diagenetic trajectory of the  $\delta^{13}\text{C}_{\text{apa}}$  and  $\delta^{18}\text{O}_{\text{carb}}$  isotopic ratios. Although, acetic acid can have an impact on sample stable isotope values, with increasing  $\delta^{18}\text{O}$  values (Garvie-Lok et al., 2004), acetic acid is usually used to remove calcite precipitated in bone porosity, before analysing bone apatite  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ . Results show that samples without acetic acid treatment present enriched  $\delta^{13}\text{C}_{\text{apa}}$  and depleted  $\delta^{18}\text{O}_{\text{carb}}$  values (Fig. 4). Moreover, as can be seen from Fig. 4, two samples from grave 7 (HMC 13 and 39) although treated with acetic acid show relatively enriched  $\delta^{13}\text{C}_{\text{apa}}$  values, which may indicate that these two samples are “on the diagenetic trajectory” and should therefore be excluded from palaeodietary reconstruction. Skeletons, 2, 4, 5, 6, do not show important isotopic intra-skeletal differences ( $\delta^{13}\text{C}_{\text{apa}} < 0.5\text{‰}$  and  $\delta^{18}\text{O}_{\text{carb}} < 0.6\text{‰}$  at the intra-skeletal scale) nor any values “on the diagenetic trajectory” indicating the preservation of their isotopic composition.

In summary, although modified *post-mortem*, according to collagen parameters (C/N ratio, %C, %N, collagen yield) and results issued from FTIR and XRD analysis, the isotopic information obtained is, overall, reliable for reconstructing diet and mobility of this Late Antiquity population.

### 6.3. Dietary patterns

In order to accurately determine isotopic signatures ( $\delta^{13}\text{C}_{\text{co}}$ ,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}_{\text{apa}}$ ) of food sources potentially consumed by the human population, it is essential to determine the isotopic values of contemporaneous animal remains (Jørkov et al., 2010; Stevens et al., 2012).

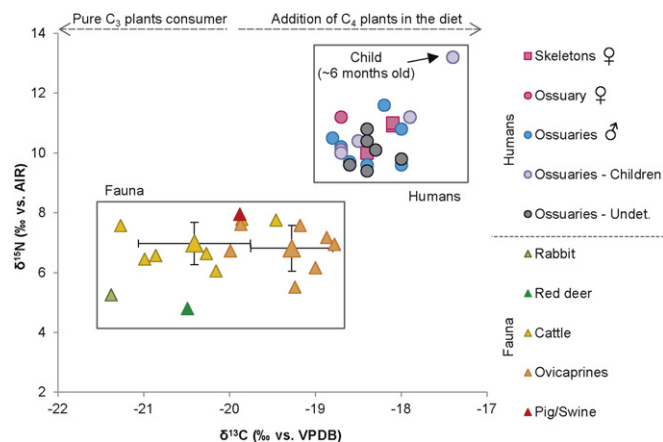
The wild animals analysed (red deer and rabbit), show carbon isotopic ratios typical for terrestrial wild herbivores that feed on  $\text{C}_3$  plants ( $\delta^{13}\text{C}_{\text{co}}$  median =  $-20.94\text{‰}$ ; 25th percentile:  $-24.16\text{‰}$ , 50th percentile:  $-20.94\text{‰}$ ; 75th percentile:  $-20.71\text{‰}$ ; Fig. 5 and Table 6; Schoeninger and DeNiro, 1984; Schoeninger and Moore, 1992). Domestic fauna, cattle and ovicaprids, show relatively similar  $\delta^{13}\text{C}_{\text{co}}$  (average =  $-20.41 \pm 0.70\text{‰}$ ) or enriched  $\delta^{13}\text{C}_{\text{co}}$  (average =  $-19.30 \pm 0.50\text{‰}$ ) values, respectively, in comparison to wild animals (Fig. 5 and Table 6). The enrichment observed for ovicaprids may be due to the consumption of  $\text{C}_4$  plants. Occasional finds of millet seeds provide some evidence for its introduction to Northern Italy by the Early Bronze Age (Tafuri et al., 2009). Written historical sources reveal that millet



**Fig. 4.** Oxygen and carbon isotope composition from human bone apatite samples from graves 2, 4, 5, 6, 7 (primary inhumations) and 9 (ossuary). Data are provided for samples pre-treated with acetic acid (highlighted with a green circle) as well as for samples with no acetic acid treatment (highlighted in red). Samples submitted to both treatments are accompanied with their grave number, and represented with the same symbol. When other skeletal elements were analysed from the same individual, they are represented with different symbols but with the same color.

was grown for human consumption as well as animal fodder in Italy, during the Roman and Medieval periods (Tafari et al., 2009). In the Iberian Peninsula, the earliest evidence for millet is dated to the 3rd century CE in the Portuguese site of Castro de Palheiros (Moreno-Larrazabal et al., 2015). According to Tereso (2007), one of the most cultivated species in the site of Terronha de Pinheiro (Northeast Portugal) during the 4th and 5th centuries CE was millet. However, there is no archaeobotanical data confirming the cultivation of millet at Monte da Cegonha. Moreover, the slightly enriched  $\delta^{13}\text{C}_{\text{co}}$  values for ovicaprine could also arise from the consumption of different  $\text{C}_3$  plants; or plant tissue with enriched  $\delta^{13}\text{C}_{\text{co}}$  values (Dungait et al., 2011); or plants growing in fields subjected to different irrigation practices (Stokes et al., 2011).

Concerning the  $\delta^{15}\text{N}$  values (Fig. 5), cattle and ovicaprine animals display enriched  $\delta^{15}\text{N}$  ratios (average =  $6.90 \pm 0.70\text{‰}$ ) in comparison to wild herbivores, i.e., red deer and rabbit (median =  $5.03\text{‰}$ ; 25th percentile:  $4.91\text{‰}$ ; 50th percentile:  $5.03\text{‰}$ ; 75th percentile:  $5.14\text{‰}$ ). This  $\delta^{15}\text{N}$  enrichment may be the result of field manuring where domesticated animals were kept (Bogaard et al., 2007). The application of animal dung results in artificially raised  $\delta^{15}\text{N}$  values in soil and plants, causes a significant enrichment in bone  $\delta^{15}\text{N}$  values of animals and human consumers (Bogaard et al., 2007; van Klinken et al., 2000). Furthermore,



**Fig. 5.** Bone collagen carbon and nitrogen isotope data from human and faunal remains recovered at Monte da Cegonha. Cattle and ovicaprine  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ratios with average  $\pm 1\sigma$  are indicated.

$\delta^{15}\text{N}$  of plants may be affected by differences in temperature, precipitation and nitrogen content in soil, even in the same locality (Amundson et al., 2003). Swine  $\delta^{13}\text{C}_{\text{co}}$  and  $\delta^{15}\text{N}$  values ( $-19.88\text{‰}$  and  $7.95\text{‰}$ , respectively; Fig. 5 and Table 6) are clustered with cattle and ovicaprine values, which suggests a similar herbivorous diet (Craig et al., 2009; Knipper et al., 2012; Prowse et al., 2004; Vika, 2011). Zooarchaeological analyses of the swine specimen were not able to distinguish between domestic pig and wild boar (Albarella et al., 2005; Valente and Carvalho, 2014) but, given the residential/farming status of the site, most of the swine remains can be attributed to domestic animals.

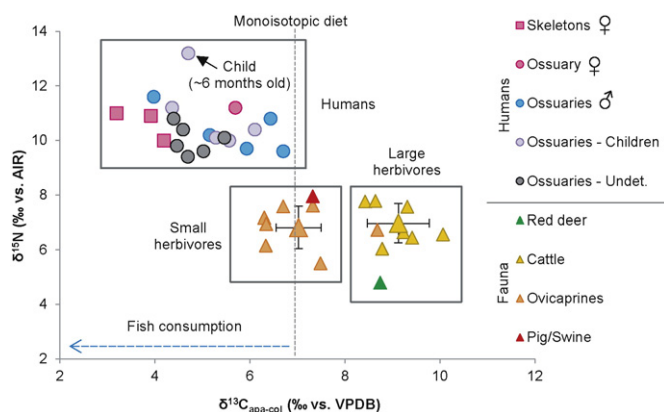
As can be seen from Fig. 5 and Table 6, most of the human collagen  $\delta^{13}\text{C}_{\text{co}}$  and  $\delta^{15}\text{N}$  values are closely clustered, with a single notable outlier (HMC 5) associated with an enriched  $\delta^{15}\text{N}$  value. This individual was an infant (~6 months), whose  $\delta^{15}\text{N}$  value of  $13.20\text{‰}$ , is around  $3\text{‰}$  above the average  $\delta^{15}\text{N}$  value for the human population of Monte da Cegonha. This  $\delta^{15}\text{N}$  enrichment seems to indicate a nursing signal. Some authors have addressed the issue of breastfeeding and weaning practices by analysing teeth (Richards et al., 2002; Beaumont et al., 2013, 2015) while others have investigated bone samples (Dupras et al., 2001; Fuller et al., 2006; Jørkov et al., 2010). In this study, breastfeeding and weaning was investigated using bone samples as teeth were not available for infant. When infants are breastfed, all protein is obtained from their mother's milk (Dupras et al., 2001), and thus  $\delta^{15}\text{N}$  values of the infant's collagen reflect a trophic shift of 2 to  $3\text{‰}$  compared to the mother (Dupras et al., 2001; Fuller et al., 2006). The  $\delta^{15}\text{N}$  collagen ratios of four other infants analysed (HMC 3, 4, 16 and 17; aged 38 weeks in uterus–1.5 months, 6 months–1 year, three and five years old) plot closely to the adult isotope ratios, suggesting that these individuals were either not breastfed or had already been weaned (Budd et al., 2013). Weaning practices of the Roman era were described by Soranus and Galen, medical scholars of the Roman world (2nd century AD) (Dupras et al., 2001; Fuller et al., 2006). They reported that supplementary foods (boiled honey or a mixture of honey and goat's milk) were introduced gradually at 6 months of age, with complete termination of breastfeeding by 3 years of age. During the weaning process, the introduction of supplementary foods results in a gradual depletion of infant  $\delta^{15}\text{N}$ , reaching adult values when the child is fully weaned (Dupras et al., 2001; Fuller et al., 2006). Average enrichment in bone  $\delta^{13}\text{C}_{\text{co}}$  and  $\delta^{15}\text{N}$  values of the humans analysed (at approximately  $1.5\text{‰}$  and  $3.8\text{‰}$ , respectively), is consistent with a trophic level enrichment between herbivores and carnivores. Both carbon and nitrogen isotopic values therefore suggest that animal meat constituted an important dietary protein resource for the human population of Monte da Cegonha. Therefore, the human  $\delta^{13}\text{C}_{\text{co}}$  and  $\delta^{15}\text{N}$  collagen signatures observed suggest that  $\text{C}_3$  plants were the basis of the Monte da Cegonha population diet, complemented with meat from terrestrial herbivores alongside their by-products with perhaps some input of  $\text{C}_4$  plants. This  $\text{C}_4$  input could be due to the consumption of domestic animals, which were fed  $\text{C}_4$  with plants.

The results from Monte da Cegonha are consistent with the Roman diet, as reported in literature sources, which focused on a "Mediterranean triad" of cereals, wine and olive oil, together with dry legumes (Keenleyside et al., 2009; Prowse et al., 2005). Prowse et al. (2004) suggested that cereals made up around 70–75% of the dietary intake of the Roman diet, while meat and other animal products were not major dietary components. Pork was the most popular meat consumed by the Romans, followed by sheep and goats' meat (approximately 25 to 50%). However, consumption varied by region and chronology (King, 1999). Zooarchaeological studies concur that the predominant domesticates in Southern Portugal during the Roman period and Late Antiquity were ovicaprine (sheep and goats) and pigs, followed by cattle (for the Alentejo region see: MacKinnon, 1999–2000; Cardoso and Detry, 2005; similar results for Horta da Torre (personal communication by M. J. Valente)). Ovicaprines were kept primarily for secondary products, such as wool, milk and cheese, while beef represented only a small component of the Roman diet because cattle were used primarily as draft

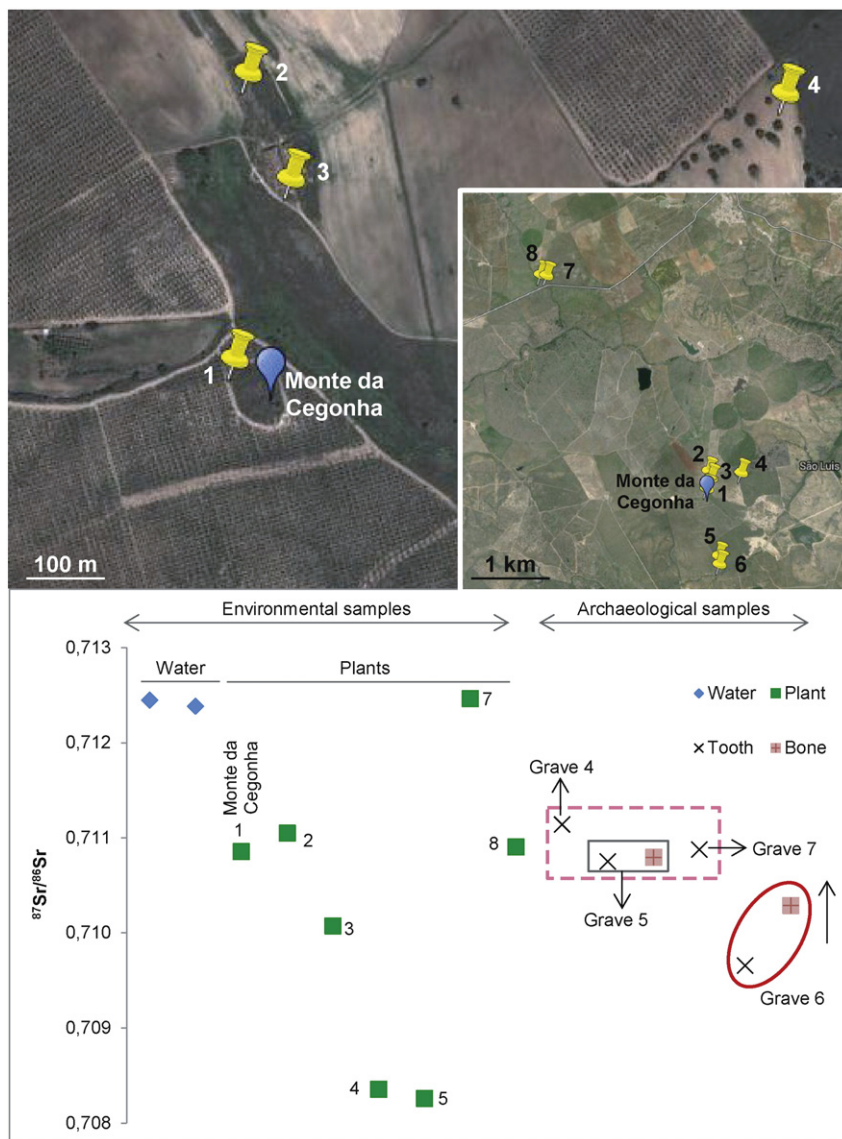
animals. This dietary picture is consistent with the results obtained from human and animal bone collagen stable isotopic composition analysis. The human  $\delta^{13}\text{C}_{\text{co}}$  and  $\delta^{15}\text{N}$  isotopic collagen signatures also revealed that there were no dietary differences related to gender and burial type (primary inhumations versus ossuary bones, sarcophagus versus other graves). However, human  $\delta^{15}\text{N}$  isotopic collagen signatures revealed dietary differences related to grave location (graves inside versus outside the basilica), potentially due to social stratification in dietary habits.

Comparison with other roughly contemporaneous European sites revealed similar diets as well as dietary practices which rarely show sex and age related patterns (Chenery et al., 2010; Killgrove and Tykot, 2013; Müldner et al., 2011; Pollard et al., 2011; Prowse et al., 2004, 2005; Rutgers et al., 2009; Stevens et al., 2012). Overall, these studies (including the results discussed herein) show that the diet of these populations was more dependent on the environment and local availability of food sources rather than on cultural habits.

Although currently under debate, the application of  $\delta^{13}\text{C}_{\text{apa-col}}$  spacing allows discrimination of trophic levels (Sponheimer and Cerling, 2014), as  $\delta^{13}\text{C}_{\text{apa-col}}$  increases with decreasing trophic level



**Fig. 6.**  $\delta^{13}\text{C}_{\text{apatite-collagen}}$  spacing and  $\delta^{15}\text{N}$  values for human and faunal samples. The gray dashed line corresponds to values reported for monoisotopic diet (Salesse et al., 2013). The blue dashed line corresponds to a theoretical dietary increase of fish consumption. Cattle and ovicaprids  $\delta^{13}\text{C}_{\text{apatite-collagen}}$  and  $\delta^{15}\text{N}$  ratios with average  $\pm 1\sigma$  are indicated.



**Fig. 7.** The map represents the GPS locations of collected vegetation and water samples in the area of Monte da Cegonha site and the graph represents the  $^{87}\text{Sr}/^{86}\text{Sr}$  values of water, vegetation, bone and tooth samples. The pink rectangle corresponds to the local  $^{87}\text{Sr}/^{86}\text{Sr}$  range of Monte da Cegonha including the majority of teeth analysed and one human bone. Plant numbers indicate samples on the map. The red circle represents a potentially non-local  $^{87}\text{Sr}/^{86}\text{Sr}$  signal from the individual in grave 6.



(Keenleyside et al., 2009). Herbivores have  $\delta^{13}\text{C}_{\text{apa-col}}$  values of 7‰ (Ambrose et al., 2003) and carnivores have values of 3–4‰ (Ambrose et al., 2003; Hedges, 2003). The  $\delta^{13}\text{C}_{\text{apa-col}}$  values obtained for the human population and animal species are reported in Fig. 6 and Table 6. The  $\delta^{13}\text{C}_{\text{apa-col}}$  values for herbivores fall within the previously reported range (7‰; Ambrose et al., 2003) however, there are  $\delta^{13}\text{C}_{\text{apa-col}}$  differences between large (cattle and red deer) and small herbivores (ovicaprines and swine). Cattle exhibit enriched  $\delta^{13}\text{C}_{\text{apa-col}}$  values, probably due to methanogenesis (Hedges, 2003). According to Ambrose and Krigbaum (2003), ruminant herbivores have symbiotic digestive microbes that produce large amounts of methane. As such, apatite is enriched relative to diet but collagen enrichment is unchanged, resulting in higher  $\delta^{13}\text{C}_{\text{apa-col}}$  values in ruminant herbivores. As physiological differences appear to be preserved between large and small herbivores in this study, the isotopic dataset has not been altered by diagenetic modifications.

As can be seen from Fig. 6 and Table 6 the relatively low  $\delta^{13}\text{C}_{\text{apa-col}}$  spacing (<7‰) exhibited by the humans from Monte da Cegonha indicates that the protein component of their diet is  $^{13}\text{C}$ -enriched compare to the whole diet (Salesse et al., 2013) and that the presence of lipids in the diet reduced the carbon isotope spacing values (München, 2007). Fig. 6 further suggests that four of the five children (HMC 3, 4, 16 and 17) were consuming the same food as the adults in the population and that these individuals were weaned. Freshwater intake may have been reflected in some skeletons with fairly low  $\delta^{13}\text{C}_{\text{apa-col}}$  spacing (around 4‰; Fig. 6), but the relatively depleted  $\delta^{15}\text{N}$  values observed suggest that this consumption was minimal, perhaps in the form of low trophic fish or fish sauces produced from such species (Prowse et al., 2004). According to Prowse et al. (2004), fish were considered an expensive food item in the Roman diet, suggesting that regular fish consumption may have been restricted to elite members of society, especially in regions located far from the coast. Fish were also consumed salted (*salsamenta*) and in the form of various fish sauces (e.g., *liquamen*, *garum*) (Prowse et al., 2004).

#### 6.4. Mobility

As can be seen from Fig. 7 and Table 6, it is apparent that the individuals from graves 4, 5 and 7 have  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios that are comparable to vegetation samples collected around the Roman villa. A tooth from the individual buried in grave 6 presented lower  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios in comparison to  $^{87}\text{Sr}/^{86}\text{Sr}$  recorded in this individual's femur which is closer to the local bioavailable  $^{87}\text{Sr}/^{86}\text{Sr}$  signal. This suggests that this male individual could be local to the geographical area but non-local to the site of Monte da Cegonha. This shift in the  $^{87}\text{Sr}/^{86}\text{Sr}$  signature may have occurred in later life or *post-mortem*. Considering that the observed  $\delta^{18}\text{O}_{\text{carb}}$  value does not appear to be diagenetically impacted (cf. 6.2.), individual 6 appears to have resided at Monte da Cegonha long enough for the  $\delta^{18}\text{O}_{\text{carb}}$  to be the same as individuals 4, 5 and 7, as well as the small and large herbivores studied (see Fig. 8). Interestingly, the individual from grave 2 had the most enriched  $\delta^{18}\text{O}_{\text{carb}}$  value compared to other graves, ossuaries and faunal remains (small and large herbivores) found in Monte da Cegonha. Additionally, this individual presented one of the lowest  $\delta^{13}\text{C}_{\text{apa-col}}$  spacings (<4‰) probably reflecting fish consumption (Fig. 6). As this individual was interred inside a white marble sarcophagus (see Fig. 2), access to different water resources and a higher fish consumption may reflect a different social status or provenance. Teeth from this individual were not available sampling.

## 7. Conclusions

The stable isotope analysis of human and animal remains from the site of Monte da Cegonha provides the first dietary evidence of a Late Antiquity population in southern Portugal.

Anthropological analysis revealed no dietary-driven gender differences. However, the frequency of dental caries indicated a cariogenic

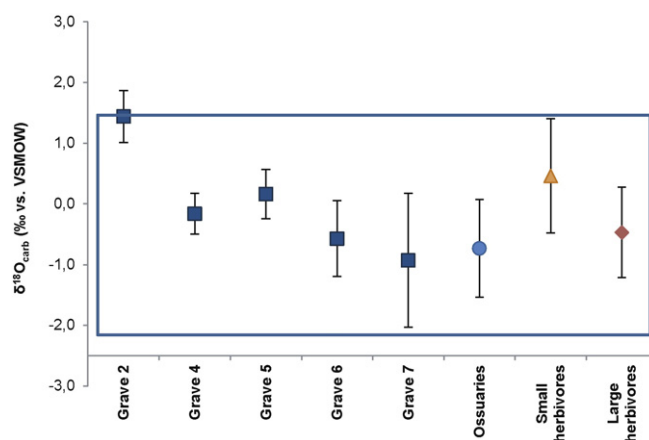


Fig. 8. Oxygen isotope values of bone apatite samples from inhumations 2, 4, 5, 6 and 7, ossuaries as well as small and large herbivores displayed as average values  $\pm 1\sigma$ . The blue rectangle includes all human individuals (primary inhumations and ossuaries) and the small and large herbivores.  $\delta^{18}\text{O}_{\text{carb}}$  values obtained from grave 2 stand out of this rectangle.

diet comprising rich starchy food and carbohydrates for at least two individuals alongside poor dental hygiene. Mineralogical and geochemical investigation of bone preservation affirmed the reliability of isotopic results for dietary and mobility studies, despite slight diagenetical transformation. Isotopic data confirmed the results of anthropological analyses and indicated that people from Monte da Cegonha consumed a diet consisting of  $\text{C}_3$  plants, complemented by some meat from terrestrial herbivores and their secondary products. Low nitrogen isotopic ratios suggest minimal freshwater fish intake, or perhaps low trophic level fish or fish sauce consumption, as reflected by the low  $\delta^{13}\text{C}_{\text{apa-col}}$  spacings obtained.

Dietary differences were not observed for gender or burial type. In contrast, isotopic data implied dietary differences between adults and one very young infant that exhibited a nursing signal. Four other children of varying age plotted closely to the adult isotope ratios and were likely already weaned.

Finally, strontium and oxygen isotopic ratios revealed a sedentary community, with the exception of a male individual who may have been a geographical local but non-local to the site of Monte da Cegonha. A single female individual buried in a marble sarcophagus, suggesting a different social status, was characterized by an enriched potentially non-local  $\delta^{18}\text{O}_{\text{carb}}$  signature as well as a relatively low  $\delta^{13}\text{C}_{\text{apa-col}}$  spacing likely suggesting more frequent access to fish products.

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