#### **ORIGINAL PAPER**



# Stable isotope ratio analysis of bone collagen as indicator of different dietary habits and environmental conditions in northeastern Iberia during the 4th and 3rd millennium cal B.C.

Vanessa Villalba-Mouco <sup>1</sup> • Izaskun Sarasketa-Gartzia <sup>2</sup> • Pilar Utrilla <sup>1</sup> • F. Xavier Oms <sup>3</sup> • Carlos Mazo <sup>1</sup> • Susana Mendiela <sup>4</sup> • Artur Cebrià <sup>3</sup> • Domingo C. Salazar-García <sup>5,6</sup>

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#### Abstract

The Late Neolithic and Chalcolithic periods are poorly understood in northeastern Iberia. Most of the information comes from the sepulchral structures rather than habitat settlements. The high number of individuals usually recovered from this types of collective burial spaces, together with the low number of direct radiocarbon dates available on them, forces us to be cautious and consider all the studied assemblages as belonging to the so-called Late Neolithic-Chalcolithic time period. To evaluate human dietary patterns of the Late Neolithic-Chalcolithic populations from the northeast of Iberia, stable carbon and nitrogen isotope analysis was carried out on 78 humans and 32 faunal bones from Cova de la Guineu (Font-rubí, Barcelona) and Cueva de Abauntz (Arraitz, Navarra), both of them sepulchral sites. Results show a common dietary pattern in both sites, indicating an homogeneous protein diet based on  $C_3$  terrestrial resources and no isotopic evidence of the consumption of  $C_4$  plants. Only one individual from Cueva de Abauntz, who directly dates to the first moments of the use of the cave as a burial place, suggests a different protein intake. The inter-population analysis shows a significant difference between both human and faunal  $\delta^{13}C$  values, suggesting an environmental influence on the isotope values depending on the geographic location. This effect should not be discarded and always assessed with baseline isotopic values in future studies at each area of Iberia and for different chronological moments.

Keywords Sepulchral caves · Late Neolithic-Chalcolithic · Carbon and nitrogen isotopes · Cova de la Guineu · Cueva de Abauntz

- ✓ Vanessa Villalba-Mouco vvmouco@unizar.es
- Domingo C. Salazar-García domingocarlos@gmail.com
- Departamento de Ciencias de la Antigüedad, Grupo Primeros Pobladores del Valle del Ebro (PPVE), Instituto de Investigación en Ciencias Ambientales (IUCA), Universidad de Zaragoza, Pedro Cerbuna 12, 50009 Zaragoza, Spain
- Departamento de Geografía, Prehistoria y Arqueología, Universidad del País Vasco-Euskal Herriko Unibertsitatea, C/Francisco Tomás y Valiente s/n., 01006 Vitoria-Gasteiz, Spain
- <sup>3</sup> Seminari d'Estudis i Recerques Prehistòriques (SERP), Secció de Prehistòria i Arqueologia, Universitat de Barcelona, C/Montalegre 6-8, 08001 Barcelona, Spain
- Departamento de Prehistoria, Arqueología, Historia Antigua, Historia Medieval y Ciencias y Técnicas Historiográficas, Universidad de Murcia, C/Santo Cristo 1, 30001 Murcia, Spain
- <sup>5</sup> Grupo de Investigación en Prehistoria IT-622-13 (UPV-EHU)/ IKERBASQUE-Basque Foundation for Science, Vitoria, Spain
- Department of Geology, University of Cape Town, Cape Town, South Africa

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# Introduction

The Chalcolithic period is traditionally defined by the emergence of copper elements and associated to the beginning of defensive-style architecture (Esquivel and Navas 2007). This last characteristic only seems to appear clearly in the southeast of the Iberian Peninsula, with the denominated Millares Culture (e.g. García Sanjuán 2013; Valera et al. 2014). In the rest of the Iberian Peninsula, the Neolithic-Chalcolithic transition is scarcely defined. In fact, it is possible that this transition does not even strictly exist and rather results from the evolution of villages present in the most advanced phases of the Neolithic (e.g. Blasco et al. 2007). This continuity is also perceptible in most of the sepulchral caves over time, where radiocarbon dates show a continued use from the 4th to the 3rd millennium cal B.C. (Fernández-Crespo 2016; Utrilla et al. 2015; Villalba-Mouco et al. 2017). Moreover, it is possible to find some copper materials normally associated with burial contexts as prestigious grave goods (Blasco and Ríos 2010), but not as evidence of a massive replacement of commonly



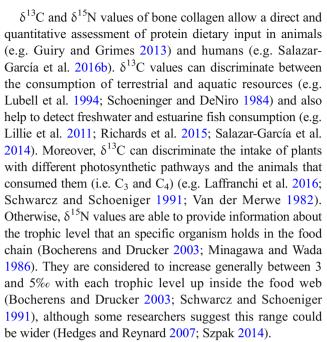
used tools such as flint blades, bone industry, polished stones or pottery without singular characteristics from a unique period (Pérez-Romero et al. 2017). In this context, except for the copper elements which are not always present, the Late Neolithic-Chalcolithic transition cannot be clearly delimited. Only Bell-Beaker ware, always associated to burial contexts, could be consistently identified as a new component, although it seems to appear when the villages defined as Chalcolithic had already been settled (Blasco et al. 2007). In this sense, genomic studies pointed out that Bell-Beaker-related individuals showed different genetic ancestries along Europe, suggesting a cultural diffusion rather than long-distance movement of people (Olalde et al. 2018).

Finally, the archaeological evidence of Late Neolithic-Chalcolithic settlements from the upper half part of the Iberian Peninsula is very scarce. There are only examples of Chalcolithic villages in the north central plateau (e.g. Delibes et al. 1995; Díaz-Andreu et al. 1992). The causes for this absence are probably erosive episodes and modern agrarian works that affected the conservation of the sites (Montes and Domingo 2014). As a result, the knowledge about this Late Neolithic-Chalcolithic period on great parts of Iberia is basically based on burial structures (e.g. Andrés 1998), anthropological studies (e.g. García-Guixé 2011; Gimeno 2009), radiocarbon dates (e.g. Salazar-García et al. 2016a), isotope studies (Fernández-Crespo et al. 2016; García-Borja et al. 2013; McClure et al. 2011; Salazar-García 2011, 2014; Sarasketa-Gartzia et al. 2017; Villalba-Mouco et al. 2017) and DNA analysis (Alt et al. 2016; Olalde et al. 2018; Szécsényi-Nagy et al. 2017).

In this context, the aim of this work is to use an isotopic approach to study the subsistence patterns and the environmental influence from two different Late Neolithic-Chalcolithic communities from the northeast of the Iberian Peninsula which are located in different geographical areas. These new isotopic data are provided to contribute increasing the scarce knowledge existing from the Late Neolithic-Chalcolithic period in most of Iberia. Moreover, we aim to discuss if it is possible to directly compare contemporary human isotopic data from different locations in Iberia due to environmental influences on the isotopic values of both fauna and humans.

# $\delta^{13} \text{C}$ and $\delta^{15} \text{N}$ stable isotope from collagen bone: diet and environment

Stable isotope ratio analysis of carbon and nitrogen has many uses in ecology (e.g. Ambrose and DeNiro 1986; Vogel 1978), plant physiology (e.g. Smith and Epstein 1971; Tieszen 1991) and geochemistry (Craig 1953). All this knowledge has been used in archaeology to know more about past human subsistence strategies (e.g. Lee-Thorp 2008) and environmental conditions (e.g. Drucker et al. 2003; Goude and Fontugne 2016).



Diet studies based on stable isotope analysis of bone collagen reflect an average diet of the last 10–15 years before the individual died, depending on the collagen turnover rate of the studied bones(Hedges et al. 2007). In addition, stable isotope ratios mainly reflect protein sources, resulting in having plant intake masked by animal protein consumption due to the higher density of proteins in animal lean meat than in plant foods (Ambrose and Norr 1993). In that case, the study of plant microremains trapped inside dental calculus by microscopy (e.g. Power et al. 2014; Salazar-García et al. 2013a), or metagenomics analysis (e.g. Weyrich et al. 2017) can be very useful to detect plant consumption and complement isotopic dietary information in this regard.

From an environmental point of view,  $\delta^{13}$ C could reflect many features about plant physiology (Seibt et al. 2008). The range of  $\delta^{13}$ C values in plants is mainly related to the CO<sub>2</sub> catchment efficiency from the atmosphere, which depends on many environmental factors such as luminosity, water availability and temperature (O'Leary 1981; Tieszen 1991). This  $\delta^{13}$ C variability is not only recorded in plants, as it is introduced also in higher trophic levels through their consumption, and plays an important role in the study of shepherding and livestock management (Tornero et al. 2016a). Plants also take organic nitrogen from the soil, resulting in their  $\delta^{15}$ N values showing variations depending on natural or anthropic factors which affect the soil-plant system (Szpak 2014). Among the natural factors, the presence of organic material in the soil, temperature and water availability could be the most significant ones (Ambrose 1991; Handley et al. 1999). Otherwise, human ecosystem modifications like the use of fertilisers may also modify the expected  $\delta^{15}N$  along the food web (Bogaard et al. 2007). All these features can be tested in bone collagen values (Drucker et al. 2003; Goude and Fontugne 2016;



Heaton et al. 1986) and help us know more about past environmental conditions.

# Archaeological context from the studied sites

# Cova de la Guineu

Cova de la Guineu is a sepulchral cave located in the village of Font-rubí (Alt Penedès, Barcelona) (X. 377972; Y. 4664374, UTM31N, WGS84) at 738 m above sea level (m.a.s.l.) (Fig. 1). The site was discovered by the Asociació d'Estudis Cientifics i Culturals de Mediona (AECCM) in the seventies, but the first archaeological intervention was conducted by Josep Mestres in 1983. Nowadays, the cave continues being excavated by Xavier Oms and Artur Cebriá (Oms et al. 2016a; Oms et al. 2016b). The excavation process verified the use of the cavity since the Epipalaeolithic and up to the Bronze Age (11–4 ka BP). The burial use of the cave took place during the 4th and second half of the 3rd millennium cal B.C. (Fig. 2, Table 1), working as a paradolmenic structure (Oms et al. 2016a).

The archaeological materials and grave goods recovered from the sepulchral levels help to prove a continuous funerary use during time: (1) the oldest phase dates from the Late Neolithic, represented by discoidal bone and seashell beads, and V-perforated buttons typical from the end of the 4th millennium cal. BC (Alday 1995); (2) a second phase is represented with the presence of a specific and regional Bell-Beaker pottery called Salomó (Harrison 1974) and a large set of lithic industry based on long flint blades and arrows, corresponding with the Chalcolithic period at the first half of the 3rd millennium; and (3) a third phase with some fragments of carinated pottery, typical from the beginning of the Bronze Age (Oms et al. 2016a). Only one metallic tool was found outside the cave (Oms et al. 2016a). Most of the human remains were found commingled and disarticulated as a result of accumulative primary and secondary burials and clandestine

activities. The first anthropological study gave an MNI (Minimal Number of Individuals) of 30 (Mercadal and Campillo 1995), but human remains recovered from the cave continue being studied by S. Mendiela. The faunal remains are largely present in the sepulchral level and many of these are burned and show cut marks. They were interpreted as food-offerings (Oms et al. 2016a).

# Cueva de Abauntz

Cueva de Abauntz is another sepulchral cave located in the village of Arraitz (Navarra) (X. 610735; Y. 4763270, UTM30N, WGS84) at 610 m.a.s.l. (Fig. 3). The cave was excavated by Pilar Utrilla and Carlos Mazo over ten campaigns (Utrilla et al. 2015). In this case, the use of the cave dates back to the Middle Palaeolithic, with the presence of Mousterian tools and a large amount of macrofauna remains (Altuna et al. 2002), and arrives up to the Late Roman Period when the cavity was used as a hiding place for coins and metallic tools (Utrilla et al. 2014a). During the Late Neolithic-Chalcolithic, the cavity was used as a burial space where the human corpses were accumulated inside individually. The MNI of the three later campaigns was of 108, calculated with dental elements (Utrilla et al. 2014b). The preference type of burial was the simple inhumation, but there were also pit and cist burials inside the cave (Utrilla et al. 2007). Some of the human remains were burned, the same as happens in other nearby burial cave sites (Fernández-Crespo 2016) probably as corpse treatment (Utrilla et al. 2015). The high number of radiocarbon dates performed shows the use of the cavity as a burial enclosure during one millennia, from the 4th to the 3rd millennium cal B.C. (Fig. 2, Table 1). All the archaeological materials recovered at the site from this period are basically grave goods, with a predominance of bone industry composed by awls and spatulas (more present in pit burials), and pedunculated and leaf-shaped points, necklace beads and pendants made from the teeth of wild boar.

Fig. 1 Location map. a Location of the Iberian Peninsula inside Western Europe. b Map of the northeast of the Iberian Peninsula, the star shows the location of Cova de la Guineu. c Topography of Cova de la Guineu with plan and profile views

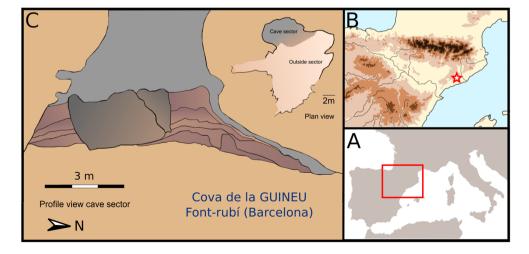
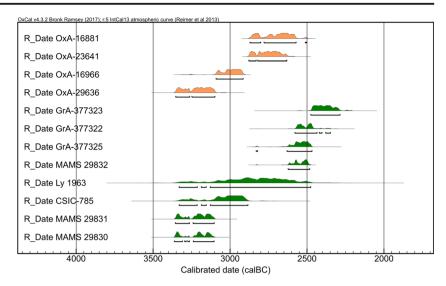




Fig. 2 AMS radiocarbon dates from Cova de la Guineu (in orange) and Cueva de Abauntz (in green) sepulchral levels. All dates have been calibrated with OxCal v4.2.3 and using the IntCal13 calibration curve (Bronk Ramsey 2009; Reimer et al. 2013)



Metallic tools are not very common, but they were present (Utrilla et al. 2007).

radiocarbon date all the individuals. However, the dates obtained give an approximated timeframe for the burials.

# Materials and methods

#### **Human and faunal remains**

The sampling strategy was different for each archaeological site. It was adapted to the characteristics of the site and the availability of sampling material, with the aim to obtain the maximum information from the remains. The possibility of carrying out one diachronic study in both sites was impossible due to all the human remains appeared commingled and disarticulated, not having enough economic resources to

#### Cova de la Guineu

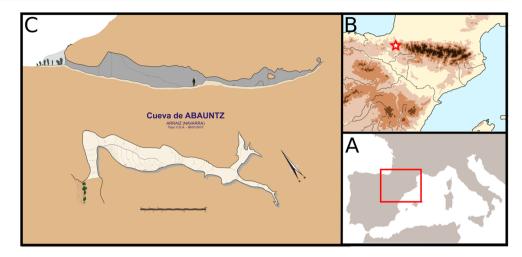
The anthropological report of Cova de la Guineu indicated that the talus was the most abundant bone among the human remains. However, this element was not chosen for the study because of its large spongy portion that could reflect a short-time diet closer to life's end (Cox and Sealy 1997). The next skeletal element with a higher frequency among the available remains was the humerus, which has an overall good preservation and helps to distinguish age groups. Therefore this skeletal element was chosen one for sampling. Specifically, the part of the humerus that showed more repetitions was the left olecranean fossa, with a completed or fragmented distal

**Table 1** AMS radiocarbon dates from the Sepulchral Late Neolithic-Chalcolithic levels of Cova de la Guineu and Cueva de Abauntz, calibrated with OxCal v4.2.3 using the IntCal13 calibration curve (Bronk Ramsey 2009; Reimer et al. 2013)

Lab code	Sample	<sup>14</sup> C age	2 $\sigma$ Cal BC	Site	Reference
OxA-29636	Human bone (phalanx)	4513 ± 30	3353–3099	Cova de la Guineu	This study
OxA-16966	Human bone (ulna)	$4385\pm32$	3091-2916	Cova de la Guineu	Oms et al. 2016a
OxA-23641	Charcoal (Quercus sp.)	$4156\pm28$	2878-2632	Cova de la Guineu	Oms et al. 2016a
OxA-16881	Human bone (patella)	$4110\pm38$	2871-2505	Cova de la Guineu	Oms et al. 2016a
MAMS 29830	Human bone (mandible)	$4534 \pm 24$	3362-3105	Cueva de Abauntz	This study
MAMS 29831	Human bone (mandible)	$4523\pm24$	3356-3104	Cueva de Abauntz	This study
CSIC-785	Human bone (no specification)	$4370\pm70$	3331-2885	Cueva de Abauntz	Utrilla et al. 2007
Ly-1963	Charcoal	$4240\pm140$	3332-2476	Cueva de Abauntz	Utrilla et al. 2007
MAMS 29832	Human bone (mandible)	$4040\pm23$	2622-2484	Cueva de Abauntz	This study
GrA-377325	Human bone (no specification)	$4025\pm35$	2831-2468	Cueva de Abauntz	Utrilla et al. 2007
GrA-377322	Human bone (no specification)	$3975\pm35$	2579-2349	Cueva de Abauntz	Utrilla et al. 2007
GrA-377323	Human bone (no specification)	$3900\pm35$	2476–2286	Cueva de Abauntz	Utrilla et al. 2007



Fig. 3 Location map. a Location of the Iberian Peninsula inside Western Europe. b Map of the northeast of the Iberian Peninsula, the star shows the location of Cueva de Abauntz. c Topography of Cueva de Abauntz with plan and profile views



epiphysis. Thirty-nine human left olecranean fossas were sampled: 18 classified as *adult* (when the epiphysis is fused), nine as *possible adult* (when only the fossa is present but the dimensions are the same as samples defined as adult), nine as *subadult* (when the epiphysis is unfused) and three as *possible subadult* (when the dimensions are equivalent to subadult samples but the epiphysis is absent). One phalanx was sampled in only one individual found in primary position to avoid the destruction of other skeletal elements (Table 2). The impossibility of associating humerus with other skeletal elements of a same individual makes impossible the sex-determination nor accurate age estimation of the individuals.

Cova de la Guineu faunal samples were taken from all species present: five rabbits (*Oryctolagus cuniculus*), two deer (*Cervus elaphus*), six domestic ovicaprids (*Ovis aries/Capra hircus*), two suids (*Sus domesticus*), one horse (*Equus ferus*) and one mustelid (genus unknown). Animal samples came from the funerary archaeological level but were not able to be selected based on a minimum number of individuals (MNI). Even so, we tried to select the same skeletal elements with a farther location to each other inside the cave whenever was possible. All the animal samples were adults, except for one pig sample (S-UCT 19023) (Table 2).

# Cueva de Abauntz

Cueva de Abauntz presents many human remains burned. The bones with clear signs of thermic alteration were discarded for sampling. Besides this limitation, we must also acknowledge that long bones were highly fragmented and made it difficult to get an accurate MNI with them. Of the remaining available skeletal material, mandibles were the most appropriate element to be assured that no the same individual was sampled twice; and they also presented a good preservation. The number of mandibles was large enough to have a representative sample of the individuals buried at the site. Moreover, they are a suitable skeletal element for retrieving information about

age, which is very important in isotopic studies where  $\delta^{13}$ C and  $\delta^{15}$ N are physiologically and age related (Beaumont et al. 2013). In this case, adults and subadults were distinguished more accurately according to bone dimensions and the presence of deciduous dentition (Ubelaker 1989). Finally, human samples consist of 40 mandibles divided in three biological age groups according to Buikstra and Ubelaker (1994): eight *child* (second permanent molar erupted;  $\approx$  up to 12 years), four *adolescent* (third permanent molar in the process of eruption;  $\approx$  up to 18 years) and 28 *adult* (full permanent dentition; > 18 years) (Table 3). The impossibility of associating mandibles with other skeletal elements more useful for sex determination (Buikstra and Ubelaker 1994) has lead us to be cautious and avoid using sex-categories to discuss the isotopic data.

Cueva de Abauntz faunal samples consist of two cattle (Bos taurus), six ovicaprids (Ovis aries/Capra hircus), four suids (Sus domesticus), two felids (Felis sp.) and one horse (Equus ferus). Animal samples were not selected based on MNI because of the scarcity of remains. Even so, we tried to select animal samples that were located far away from each other inside the cave. These faunal remains appeared commingled with the human remains, assuming a common stratigraphical level. Some faunal remains showed thermic alterations, which suggest they are from the same time period as the human remains. All the faunal samples were adults, except for one pig (S-UCT 19090), one ovicaprid (S-UCT 19083) and the horse (S-UCT 19094) (Table 3).

# Collagen extraction and $\delta^{13}$ C and $\delta^{15}$ N analysis

Sample preparation and analysis were carried out at the isotope dedicated facilities of the University of Cape Town (South Africa), as described below.

Before the analysis, visible contaminants were removed by abrasion using a Dremmel 3500 drill with a diamond grinder bit attached. Collagen extraction for carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) isotope analysis was extracted following the



**Table 2** Cova de la Guineu stable isotope ratio results, including S-UCT code, species, sampled bone, biological age group [P. adult: possible adult; P. subadult: possible subadult], %Collagen (> 30 kDa fraction),  $\delta^{13}$ C,  $\delta^{15}$ N and collagen quality indicator (%C, %N, C:N elemental) average values

S-UCT code	Archaeological ID	Species	Sampled bone	Age Group	Collagen (%) (> 30 kDa fraction)	δ <sup>13</sup> C (‰)	δ <sup>15</sup> N (‰)	% C	% N	C:N (elemental)
18979	Gn.RX.293	Human	Humerus	Subadult	4.73	- 19.0	7.8	41.5	15.4	3.1
18981	Gn.M.323	Human	Humerus	Subadult	8.70	- 19.4	6.4	43.1	15.5	3.2
19027	Gn'1331.VII.Ia(b) C11.366F	Human	Phalanx	Subadult	8.43	-18.6	9.5	40.7	14.8	3.2
18977	Gn.M.327	Human	Humerus	Subadult	5.27	-18.9	8.8	41.5	15.5	3.1
18980	Gn.M.321	Human	Humerus	Subadult	4.48	-18.9	7.4	41.6	15.3	3.2
18990	Gn.Rx.396	Human	Humerus	Subadult	0.92	- 19.5	8.1	41.5	14.5	3.3
18999	Gn.Rx.12157	Human	Humerus	Subadult	1.64	- 19.4	8.3	41.7	15.1	3.2
19000	Gn.Rx.13291	Human	Humerus	Subadult	2.82	- 19.3	8.6	33.2	11.9	3.2
19005	Gn. Rx. 17370	Human	Humerus	Subadult	1.68	- 19.1	8.3	28.3	10.0	3.3
18988	Gn.M.335	Human	Humerus	P. subadult	3.05	-19.2	9.0	37.7	13.5	3.3
19003	Gn.Rx. 13311	Human	Humerus	P. subadult	0.83	- 19.9	8.4	41.2	14.5	3.3
19006	Gn.Rx.17371	Human	Humerus	P. subadult	1.50	- 19.5	7.4	36.2	12.4	3.4
18972	E5.11	Human	Humerus	P. adult	4.01	- 19.6	8.7	42.5	15.4	3.2
18974	Gn.Rx.99	Human	Humerus	P. adult	0.67	-19.8	9.4	33.2	10.8	3.6
18975	Gn.M.324	Human	Humerus	P. adult	3.99	- 19.4	8.5	41.7	14.7	3.3
18986	Gn.M.333	Human	Humerus	P. adult	1.40	-19.2	9.4	41.9	14.4	3.4
18989	Gn.M.338	Human	Humerus	P. adult	6.38	- 19.5	8.6	38.9	14.0	3.2
18991	Gn.Rx.401	Human	Humerus	P. adult	1.44	- 19.7	8.8	30.9	10.8	3.4
18993	Gn.Rx.7838	Human	Humerus	P. adult	1.47	- 19.2	9.5	35.8	12.7	3.3
18995	Gn.RX.7847	Human	Humerus	P. adult	2.44	-19.3	10.1	38.4	13.7	3.3
18996	Gn.RX.7849	Human	Humerus	P. adult	4.83	- 19.1	8.2	42.0	15.6	3.1
18973	Gn.Rx.10	Human	Humerus	Adult	0.86	- 19.8	9.5	38.3	12.8	3.5
18976	Gn.M.326	Human	Humerus	Adult	2.12	- 19.3	9.0	36.9	13.0	3.3
18978	Gn.M.328	Human	Humerus	Adult	1.24	-19.2	9.2	43.4	15.3	3.3
18982	Gn.M.329	Human	Humerus	Adult	4.66	- 19.4	9.2	42.9	15.4	3.2
18983	Gn.Rx.330	Human	Humerus	Adult	2.03	- 19.4	8.4	36.2	12.7	3.3
18984	Gn.M.331	Human	Humerus	Adult	5.76	-18.8	9.0	40.2	14.3	3.3
18985	Gn.M.332	Human	Humerus	Adult	2.67	-19.3	8.7	39.5	13.8	3.3
18987	Gn.M.334	Human	Humerus	Adult	0.39	-19.5	9.1	28.0	9.4	3.5
18992	Gn.Rx.1286	Human	Humerus	Adult	1.20	-19.4	8.5	37.3	13.0	3.4
18994	Gn.RX.7841	Human	Humerus	Adult	1.98	-19.1	9.3	39.1	14.1	3.2
18997	Gn.RX.7853	Human	Humerus	Adult	1.70	-19.3	8.2	39.3	14.2	3.2
18998	Gn.RX.12156	Human	Humerus	Adult	0.70	-19.3	8.8	25.7	9.2	3.3
19001	Gn.Rx. 13294	Human	Humerus	Adult	3.27	- 19.3	8.7	39.9	14.4	3.2
19002	Gn.RX.13309	Human	Humerus	Adult	3.01	- 19.4	9.9	39.9	14.3	3.3
19004	Gn.RX.17368	Human	Humerus	Adult	1.21	-19.4	8.7	39.4	13.3	3.4
19007	Gn.Rx.17836	Human	Humerus	Adult	2.84	-19.3	8.6	41.9	14.8	3.3
19008	Gn.RX.7844	Human	Humerus	Adult	5.01	- 19.5	9.9	41.7	14.9	3.3
19009	17842	Human	Humerus	Adult	0.79	-19.2	9.0	37.7	13.0	3.4
19010	Gn/E3/1047	Oryctolagus cuniculus	Diaphysis	Adult	2.79	-21.1	2.3	41.8	15.0	3.3
19011	Gn/E3/1046	Oryctolagus cuniculus	Vertebra	Adult	2.50	-20.5	3.7	41.1	14.9	3.2
19012	Gn/E3/589	Oryctolagus cuniculus	Maxilla	Adult	1.73	-20.9	4.9	40.3	13.5	3.5
19013	Gn/E3/1039	Oryctolagus cuniculus	Maxilla	Adult	2.66	-20.3	2.6	42.5	14.8	3.3
19014	Gn/E3/795	Oryctolagus cuniculus	Mandible	Adult	0.83	-20.8	2.6	33.1	11.8	3.3
19015	Gn/D3/47	Ovis aries/Capra hircus	Tibia	Adult	1.02	-20.4	4.4	38.6	13.7	3.3
19016	GN/E2/175B	Ovis aries/Capra hircus	Scapula	Adult	0.12	- 19.4	4.3	39.7	14.0	3.3



Table 2 (continued)

S-UCT code	Archaeological ID	Species	Sampled bone	Age Group	Collagen (%) (> 30 kDa fraction)	δ <sup>13</sup> C (‰)	δ <sup>15</sup> N (‰)	% C	% N	C:N (elemental)
19017	Gn/D1/272	Ovis aries/Capra hircus	Humerus	Adult	3.74	-20.1	4.2	41.5	14.7	3.3
19018	Gn/D1/862	Ovis aries/Capra hircus	Cranial fragment	Adult	8.93	-19.7	4.1	42.1	15.3	3.2
19019	Gn/D1/461a	Ovis aries/Capra hircus	Vertebra	Adult	4.46	-19.7	6.4	42.6	15.4	3.2
19020	Gn/D1/906	Ovis aries/Capra hircus	Phalanx	Adult	5.89	-20.4	4.3	43.3	15.6	3.2
19021	Gn/D1/532	Cervus elaphus	Metapodia	Adult	2.30	-19.7	3.7	41.7	15.1	3.2
19022	Gn/D1/842	Cervus elaphus	Maxilla	Adult	2.27	- 19.9	3.8	41.3	14.9	3.2
19023	Gn/D3/832	Sus domesticus	Mandible	Subadult	1.64	- 19.5	5.8	37.4	13.0	3.4
19024	Gn/D1/859	Sus domesticus	Mandible	Adult	3.98	-19.3	5.5	43.2	15.5	3.2
19025	Gn/D3/845	Equus ferus	Phalanx	Adult	1.20	-20.8	2.6	33.6	11.8	3.3
19026	Gn/D1/568	Mustelidae	Mandible	Adult	3.96	-18.5	8.2	42.3	14.8	3.3

Longin (1971) method with the addition of an ultrafiltration step (Brown et al. 1988). Approximately 300 mg of chunk bone samples from each specimen were demineralised in 0.5 M HCl solution at 5 °C until fully demineralisation (over the course of a week in most cases). When demineralisation finished, samples were rinsed three times with deionised water until pH became neutral and then started the gelatinisation at 70 °C during 48 h using a heater block (FMH instruments, South Africa). The solutions were filtered with a 5-mm EZEEfilter (Elkay, United Kingdom) and ultrafiltered with 30 kDa ultrafilters previously cleaned with 0.5 M NaOH (Amicon, Germany) using a centrifuge (Thermo Fisher Scientifics Megafuge 16, USA) at 2500 rpm during variable times depending on the filtering speed of each sample. After that, solutions were frozen and lyophilised. Finally, duplicate ca. 0.5 mg of collagen per sample was microweighed into tin capsules and loaded into the mass spectrometers.

The carbon and nitrogen isotope ratio measurements were performed using a Finnigan Delta plus XP continuous-flow isotope ratio mass spectrometer (Thermo Fisher Scientific, USA) after being combusted in an elemental analyser Flash EA 1112 interfaced with it (Thermo Fisher Scientific, USA). Stable carbon isotope values were calibrated and expressed relative to the reference V-PDB scale (Vienna PeeDee Belemnite), and stable nitrogen isotope values were calibrated and expressed relative to the reference AIR scale (atmospheric  $N_2$ ), using the delta notation ( $\delta$ ) in parts per thousand (%0). The accuracy of measurements was monitored using international and in-house standards with well-known isotopic composition (MG:  $\delta^{13}C$  21.3  $\pm\,0.3,~\delta^{15}N$  7.3  $\pm\,0.1\%{\it o};$  seal:  $\delta^{13}C$  $12.7 \pm 0.1\%$ ;  $\delta^{15}$ N  $15.6 \pm 0.1\%$ ; valine:  $\delta^{13}$ C  $27.7 \pm 0.1\%$ ;  $\delta^{15}$ N 15.6 ± 0.1%.). Precision was determined with the repeated measurements of standards and sample replicates, determined an average analytical error below than 0.1% (1 $\sigma$ ) for  $\delta^{13}$ C and  $\delta^{15}$ N in standards and in sample replicates. All the samples were measured in duplicate. Samples which showed bad quality of collagen according to Ambrose (1993), DeNiro (1985) and Van Klinken (1999) parameters were not considered for interpretation of the results.

# Results

The results from both sepulchral caves are presented in Figs. 4 and 5, as well as in Tables 2 and 3. The results of the different sites are shown in different sections but are compared together at the end.

# Cova de la Guineu $\delta^{13}$ C and $\delta^{15}$ N results

Bone samples from 39 humans and 17 fauna were analysed from Cova de la Guineu. The  $\delta^{13}C$  and  $\delta^{15}N$  results are presented in Table 2 and plotted in Fig. 4. Only one sample from Cova de la Guineu (S-UCT 19016, ovicaprid) did not yield enough collagen at the > 30 kDa fraction to run the analysis in duplicate. However, for all samples (including the latter mentioned) the %C, %N and C:N elemental ratio values indicate a good collagen quality according to Ambrose (1993), DeNiro (1985) and Van Klinken (1999). As we use ultrafiltration, we have not considered the 1% of collagen as a quality control when all other indicators were acceptable.

Herbivore  $\delta^{13}$ C values range between -21.1% and -19.4%, with a mean value of  $-20.3 \pm 0.5$  [ $1\sigma$ ]% (n = 14). These  $\delta^{13}$ C values are consistent with typical values for a terrestrial C<sub>3</sub> European ecosystem (DeNiro and Epstein 1978; Schwarcz and Schoeniger 1991). Leporids, together with the horse value, show  $^{13}$ C-depleted values the same as happen in other studies (Villalba-Mouco et al. 2017), and wild herbivores (*Cervus elaphus*) ( $\delta^{13}$ C = 19.8  $\pm$  0.2%) are enclosed inside the  $\delta^{13}$ C domestic herbivore range (*Ovis aries/Capra hircus*) ( $\delta^{13}$ C = 19.9  $\pm$  0.4%), showing no difference between them. Herbivore  $\delta^{15}$ N values range between



Table 3 Cueva de Abauntz stable isotope ratio results, including S-UCT code, species, sampled bone, biological age group, %Collagen (> 30 kDa fraction),  $\delta^{13}$ C and  $\delta^{15}$ N average values, collagen control indicators (%C, %N, C:N elemental) and radiocarbon dates

S-UCT code	Archaeological ID	Species	Sampled bone	Age group	Collagen (%) (> 30 kDa fraction)	δ <sup>13</sup> C (‰)	δ <sup>15</sup> N (‰)	%C	%N	C: N (elemental)
19064	Ab.4F.139.1	Human	Mandible	Child	3.45	-20.2	8.9	39.8	14.4	3.2
19063	Ab.27D.342.155	Human	Mandible	Child	5.71	-20.5	9.0	44.1	15.6	3.3
19058	Ab.2F.95.2	Human	Mandible	Child	7.22	-20.5	9.0	44.1	15.6	3.3
19056	Ab.3D.142.2.68	Human	Mandible	Child	2.76	-20.0	8.3	43.7	15.3	3.3
19067	Ab.5C.b1b2.159	Human	Mandible	Child	3.72	-20.3	9.1	43.0	15.6	3.2
19059	Ab.1D.146.11	Human	Mandible	Child	2.05	-19.3	9.8	40.4	14.7	3.2
19060	Ab.3D.142.2.67	Human	Mandible	Child	4.81	-20.0	9.7	41.6	15.1	3.2
19062	Ab.27-33.100?	Human	Mandible	Child	4.46	-20.0	8.5	42.9	15.7	3.2
19057	Ab.35E.401.6	Human	Mandible	Adolescent	2.57	-20.7	7.9	39.5	14.2	3.3
19061	Ab.9C.230.860	Human	Mandible	Adolescent	4.78	-20.6	9.3	43.4	15.0	3.4
19065	Ab.3C.161.19.134	Human	Mandible	Adolescent	2.40	-20.8	8.9	41.3	14.7	3.4
19066	Ab.7A.146.295	Human	Mandible	Adolescent	2.80	-20.4	8.3	40.8	14.5	3.2
19033	Ab.29F.rev.2	Human	Mandible	Adult	3.17	-20.3	8.8	39.8	14.6	3.2
19038	Ab.25D.371.746	Human	Mandible	Adult	1.74	-20.5	9.5	37.6	13.5	3.2
19039	Ab.23D.421.221	Human	Mandible	Adult	4.26	-19.8	9.0	40.8	15.3	3.1
19041	Ab. 23D/F.rev.	Human	Mandible	Adult	5.68	-20.2	8.2	42.8	15.7	3.2
19042	Ab.2C.64.42	Human	Mandible	Adult	2.79	-20.1	9.1	39.9	14.6	3.2
19045	Ab.rev(35)439	Human	Mandible	Adult	2.83	-20.2	8.8	40.8	14.7	3.2
19047	Ab.4B.100.638	Human	Mandible	Adult	2.96	-20.5	9.1	41.1	14.7	3.3
19049	Ab.29F.b1.2	Human	Mandible	Adult	5.28	-20.1	8.8	42.9	15.6	3.2
19051	Ab.25D.nivel I. X:375	Human	Mandible	Adult	4.40	-20.3	9.5	43.5	15.8	3.2
19055	Ab.25D.nivel I.430-431	Human	Mandible	Adult	4.96	-20.1	8.4	43.1	15.5	3.2
19028	Ab.25D.371.768	Human	Mandible	Adult	2.37	-20.1	9.0	38.1	13.7	3.2
19029	Ab.21D.360.7	Human	Mandible	Adult	4.75	-19.3	11.3	41.7	15.1	3.2
19034	Ab.rev(35).146	Human	Mandible	Adult	2.30	-19.6	9.2	41.0	15.3	3.1
19035	Ab.rev(35).672	Human	Mandible	Adult	2.89	-20.1	9.1	39.7	14.7	3.1
19036	Ab.rev(35).619	Human	Mandible	Adult	5.55	-20.1	9.0	42.2	15.7	3.1
19037	Ab.27-33.109	Human	Mandible	Adult	3.08	-20.2	8.2	41.7	15.5	3.1
19053	Ab.19E.364.2	Human	Mandible	Adult	2.38	- 19.9	8.8	38.6	13.7	3.3
19030	Ab.1A.169.169	Human	Mandible	Adult	6.32	-20.8	9.3	42.5	15.2	3.3
19040	Ab.27-33.108	Human	Mandible	Adult	4.52	- 19.9	9.0	41.2	15.3	3.1
19044	Ab.25C.nivel I.x: 375 (17)	Human	Mandible	Adult	2.80	-20.2	8.6	41.0	14.8	3.2
19048	Ab.21D.360.8	Human	Mandible	Adult	4.09	-20.4	9.7	41.2	14.8	3.3
19046	Ab.rev(35).126	Human	Mandible	Adult	2.36	-20.3	9.0	38.8	14.0	3.2
19031	Ab.27C.r.50	Human	Mandible	Adult	5.06	-20.3	8.8	41.2	14.8	3.3
19043	Ab.25D.r.845	Human	Mandible	Adult	3.05	-20.4	9.5	38.0	14.0	3.2
19054	Ab.23D/E.rev.181	Human	Mandible	Adult	2.96	- 19.9	9.4	40.5	14.5	3.3
19032	Ab.23r.421.219	Human	Mandible	Adult	5.72	-20.3	9.2	42.8	15.6	3.2
19050	Ab.35E.430.138	Human	Mandible	Adult	2.61	-20.0	9.2	41.2	14.8	3.2
19052	Ab.25E.rev.51	Human	Mandible	Adult	3.69	-19.7	9.6	41.4	19.7	3.3
19074	Ab.27-33E/F.389	Bos taurus	Tibia	Subadult	4.86	-22.1	4.6	40.4	14.7	3.2
19075	Ab.35E.430.173	Bos taurus	Talus	Adult	6.29		4.3		15.7	
19084	Ab.27-33EF.203-215	Ovis aries/Capra hircus	Phalanx	Adult	1.30	-22.5	3.8		13.2	
19079	Ab.11D.rev.144	Ovis aries/Capra hircus	Talus	Adult	3.56	-20.9			15.6	
10000	Ab.3C.rev.43	Ovis aries/Capra hircus		Adult	1.69	-21.2			15.2	
19080	110.5 0.101.15	Ovis aries, capita micus	Tarus	raun	1.07	21.2	7.0	71.0	10.2	5.2



Table 3	(continued)
Table 3	ccommunica

S-UCT code	Archaeological ID	Species	Sampled bone	Age group	Collagen (%) (> 30 kDa fraction)	δ <sup>13</sup> C (‰)	δ <sup>15</sup> N (‰)	%C	%N	C: N (elemental)
19083	Ab.3C.rev.31	Ovis aries/Capra hircus	Tibia	Subadult	4.32	-21.0	5.1	41.8	15.6	3.1
19092	Ab.25C.rev.127	Ovis aries/Capra hircus	Femur	Adult	1.75	-21.4	4.4	35.8	12.7	3.3
19086	Ab.4F.139.7	Sus domesticus	Ulna	Adult	1.21	-20.7	4.1	42.0	14.4	3.4
19087	Ab.7BCD.rev.68	Sus domesticus	Maxilla	Adult	3.23	-21.1	5.8	39.7	13.8	3.3
19089	Ab.5D.196.13a78	Sus domesticus	Phalanx	Adult	4.57	-20.1	9.4	42.3	15.5	3.2
19090	Ab.5D.196.13a78	Sus domesticus	Maxilla	Subadult	7.72	-20.6	6.3	40.7	14.5	3.3
19094	Ab.5C.300.38	Equus ferus	Metapodia	Subadult	7.82	-21.5	4.9	42.3	15.3	3.2
19076	Ab.3C5C.rev.34	Felis sp.	Radius	Adult	4.63	-19.0	7.8	42.2	15.4	3.2
19091	Ab.3C.rev.32	Felis sp.	Vertebra	Adult	5.77	-18.8	6.6	42.3	15.4	3.2

2.3% and 6.4%, with a mean value of  $3.9 \pm 1.1 [1\sigma]$ %, defining the trophic baseline of the ecosystem food web for the region at the time. The lowest  $\delta^{15}$ N value belongs to a horse and the highest to an ovicaprid. Most of the  $\delta^{15}$ N domestic herbivore values (*Ovis aries/Capra hircus*) show similar  $\delta^{15}$ N values to the wild ones (*Cervus elaphus*) except for one sample whose  $\delta^{15}$ N value is higher (S-UCT 19019,  $\delta^{15}$ N = 6.4).

There are only two omnivores, whose  $\delta^{13}$ C values are -19.5%0 and -19.3%0, with a mean value of  $-19.4\pm0.2$  [ $1\sigma$ ]%0 (n=2). Their  $\delta^{15}$ N values are 5.8%0 and 5.5%0 with a mean value of  $5.7\pm0.2$  [ $1\sigma$ ]%0. The difference between  $\delta^{15}$ N herbivores and  $\delta^{15}$ N omnivores values is not as high as a complete trophic level change (1.8%0) (Bocherens and Drucker 2003). The singular carnivore datum from a mustellidae shows a  $\delta^{13}$ C value of -18.5%0 and a  $\delta^{15}$ N value

of 8.2%, showing also a  $C_3$  terrestrial environment and placing it in a higher trophic level (4.3% higher than herbivores and 3.2% higher than omnivores).

The human  $\delta^{13}$ C values range between -19.9% and -18.6%, with a mean value of  $-19.3\pm0.3$  [ $1\sigma$ ]% (n=39). Human  $\delta^{15}$ N values range between 7.4% and 9.9%, with a mean value of  $8.8\pm0.7$  [ $1\sigma$ ]%. Humans are in a higher trophic level than herbivores ( $\delta^{15}$ N 4.9% higher) and even omnivores ( $\delta^{15}$ N 3.1% higher). The single carnivore value is in the same trophic level as humans (Fig. 4). A non-parametric statistical test (Mann-Whitney) reveals that isotopic  $\delta^{13}$ C values do not differ between age groups (adult and subadult,  $\delta^{13}$ C p=0.142). However, the same test shows a difference in  $\delta^{15}$ N between these two groups that is statistically significant ( $\delta^{15}$ N p=0.007), showing lower  $\delta^{15}$ N values in the subadult

Fig. 4 Scatter plot of human and fauna bone collagen  $\delta^{13}C$  and  $\delta^{15}N$  values from Cova de la Guineu

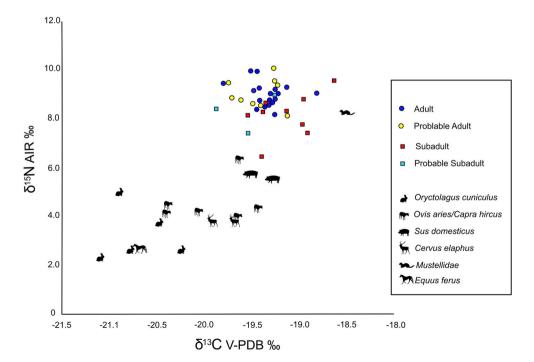
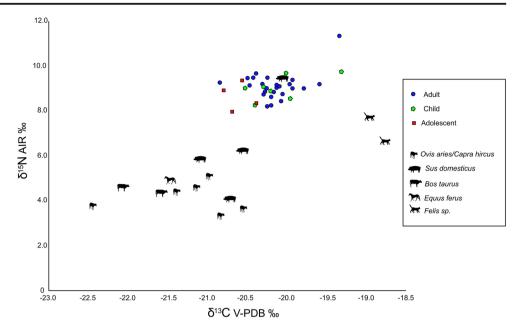




Fig. 5 Scatter plot of human and fauna bone collagen  $\delta^{13}C$  and  $\delta^{15}N$  values from Cova de Abauntz

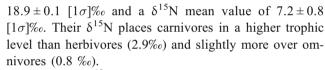


group. *Possible adult* and *possible subadult* categories were ruled out of this statistical test.

# Cueva de Abauntz $\delta^{13}$ C and $\delta^{15}$ N results

Bone samples from 40 humans and 15 animals were analysed from Cueva de Abauntz. The  $\delta^{13}C$  and  $\delta^{15}N$ results are presented in Table 3 and plotted in Fig. 5. All the samples yielded enough collagen at the > 30-kDa fraction for analysis in duplicate, but three duplicates were ruled out because they did not show a good collagen C:N elemental ratio (DeNiro 1985). All the other runs show good collagen quality parameters (%C, %N and C:N elemental ratio) according to Ambrose (1993), DeNiro (1985) and Van Klinken (1999). Herbivore  $\delta^{13}$ C values range between -22.5 and -20.6% with a mean value of  $-21.4 \pm 0.6$  [ $1\sigma$ ]% (n = 9). These  $\delta^{13}$ C values are consistent with typical values for a terrestrial C<sub>3</sub> European ecosystem (DeNiro and Epstein 1978; Schwarcz and Schoeniger 1991). Herbivore  $\delta^{15}N$  values range between 3.3 and 5.1% with a mean value of  $4.3 \pm$  $0.6 [1\sigma]\%$ , defining the trophic baseline of the ecosystem food web. The higher and lower values belong to different ovicaprids. In this case, no wild herbivore was analysed, as none was found in the available faunal assemblage of

There are four omnivores, whose  $\delta^{13}$ C values range between -21.1%0 and -20.1%0, with a mean value of  $-20.6 \pm 0.4$  [ $1\sigma$ ]%0. Their  $\delta^{15}$ N values range between 4.1%0 and 9.4%0, with a mean value of  $6.4 \pm 2.2$  [ $1\sigma$ ]%0, although the variability is high enough for not grouping of all of them in a single category. Carnivores are only represented by two felids with a  $\delta^{13}$ C mean value of -



The human  $\delta^{13}$ C values range between -19.3% and -20.5%, with a mean value of  $-20.2 \pm 0.3$  [ $1\sigma$ ]% (n = 40). Their  $\delta^{15}$ N values range between 8.2% and 11.3%, with a mean value of  $9.0 \pm 0.6$  [ $1\sigma$ ]%o. Humans are clearly in a higher trophic level than herbivores ( $\delta^{15}$ N 4.7% higher) and also in a higher step than omnivores ( $\delta^{15}$ N 2.6%) higher) and carnivores ( $\delta^{15}$ N 1.8% higher). There is an adult human (S-UCT 19029) whose  $\delta^{15}N$  values are enriched in 2.3% compared to the human mean. The direct radiocarbon date for this human is  $4534 \pm 24$  BP (MAMS-29830), contemporary to sample S-UCT 19048 (MAMS-29831:  $4523 \pm 24$  BP) whose  $\delta^{15}$ N values are enclosed in the human mean (Table 1, Fig. 5). A nonparametric statistical test (Mann-Whitney) reveals no significant differences between age groups (adult and subadult),  $\delta^{13}$ C data (p = 0.063) and  $\delta^{15}$ N values (p = 0.373).

# Inter-population $\delta^{13}$ C and $\delta^{15}$ N analysis

A non-parametric statistical test (Mann-Whitney) reveals that isotopic results from each site differ significantly in their  $\delta^{13}$ C values ( $p = 1.05 \times 10^{-12}$ ) but not in their  $\delta^{15}$ N values (p = 0.062). Inter-population analysis has been also applied to the herbivores from both sites to assess if the difference could be associated to different environments rather than dietary patterns via the same statistical test. It reveals also significantly differences only in their  $\delta^{13}$ C values (p = 0.0004), not in their  $\delta^{15}$ N values (p = 0.1644) (Fig. 6).



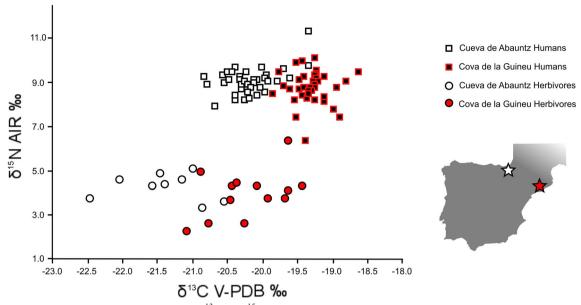


Fig. 6 Scatter plot of human and fauna bone collagen  $\delta^{13}C$  and  $\delta^{15}N$  values from Cova de la Guineu and Cueva de Abauntz according to their location inside Iberia

# Discussion

Collective burials and, specially, sepulchral caves, usually present an heterogeneous archaeological record that limits an in-depth discussion. The first limitation can be found in the chronological heterogeneity of the human remains, which ideally should be all directly radiocarbon dated to ensure the attribution of each individual to one or another chronological period (e.g. Salazar-García et al. 2016a). However, this is not always possible due to budgetary constrictions, and only a few specimens can be dated (e.g. Villalba-Mouco et al. 2017). Secondly, it is difficult to know if the humans buried together belonged to the same community/settlement or if they came from different ones (potentially from environments with different availability of resources). In this case, the isotopic baselines from the different populations buried in a same burial enclosure might be different. These two limitations are applied to both human and faunal remains. Furthermore, faunal remains are normally poorly represented in the burial spaces and very possibly do not represent all species consumed. All these limitations are considered in the following discussion for both sepulchral sites.

# Cova de la Guineu

All fauna  $\delta^{13}$ C and  $\delta^{15}$ N values from Cova de la Guineu group together by species and suggest a similar environment, as well as discarding a big shift in isotope values due to potential chronological differences. Domestic (*Ovis aries/Capra hircus*) and wild ungulates (*Cervus elaphus*) are in the same range for  $\delta^{13}$ C and  $\delta^{15}$ N values, probably related to the use of shared areas for grazing (Villalba-Mouco et al. 2018).

Different isotopic values between wild and domestic fauna can be also due to feeding from different ecosystems (e.g. aquatic resources in terrestrial ecosystem) (Schulting et al. 2017; Müldner et al. 2014) or to the introduction of manuring practices that would imply an enrichment in  $\delta^{15}N$  of domestic plants potentially consumed by domestic animals (Bogaard et al. 2007). But in this case, only one sheep/goat falls into the pig range (higher  $\delta^{13}C$  and  $\delta^{15}N$  values) suggesting a distinctive diet and/or provenance for this specimen, or even a different chronological period attribution (Fig. 4).

The average  $\delta^{15}$ N difference between herbivores and domestic omnivores (*Sus domesticus*) is of 1.8%. This slight increment suggests the omnivore attribution of the pigs, pointing out at a small input of animal protein in their diet (Fig. 4).

The only carnivore data available was a mustelid. It shows  $\delta^{13}C$  and  $\delta^{15}N$  values slightly higher than human mean but in the same trophic level according to Bocherens and Drucker (2003). This little increase could be in agreement with mustelid generalist and opportunistic diet, which can include carrion feeding and another species normally non-consumed by humans as micromammals, reptiles and different kind of invertebrates (Barja 2017; Mangas 2017) (Fig. 4).

Human samples show homogeneous isotopic values, especially the adult group where values are probably not influenced by physiological factors related to growth and/or development (Beaumont et al. 2013). The  $\delta^{13}$ C human values indicate a quite homogeneous animal protein diet based on  $C_3$  terrestrial resources, with no evidence of regular aquatic nor  $C_4$  resource consumption. Otherwise, the  $\delta^{15}$ N human values suggest a high importance of animal proteins in human diet, showing a  $\delta^{15}$ N value increase of 4.9% higher than herbivores



and 2.4% higher than omnivores. If we analyse the possible species consumed one by one, we can also suggest that  $\delta^{15}$ N values from rabbits and horse do not fit with ca. 3-5% the theoretical enrichment (Bocherens and Drucker 2003). In this sense, we could suggest animal protein consumed by humans could come mainly from ovicaprids. This is despite the fact that red deer consumption could also fit isotopically, because the archaeozoological data for this period in the region points out to a decrease in hunting followed by the rise of husbandry practices (Saña 2013). The consumption of other domestic animals like pig could be less regular, as well as those from rabbit and horse, according to  $\delta^{15}N$  enrichment (Bocherens and Drucker 2003) (Fig. 4). Due to the limitation of stable isotope analysis on bone collagen, we cannot discuss the amount of plant foods consumed (Hedges and Reynard 2007). However, if a high proportion of proteins were coming from plant resources, we would not expect the observed increase in  $\delta^{15}N$  human values (Fahy et al. 2013). A nonparametric statistical test (Mann-Whitney) reveals differences between adult and subadult groups, showing subadults lower δ<sup>15</sup>N values, possibly due to the physiological process associated to the increase of collagen turnover during the growth and/or a changes in the diet (Hedges et al. 2007; Beaumont et al. 2013).

# Cueva de Abauntz

The domestic herbivores recovered from Cueva de Abauntz group together in their  $\delta^{13}C$  and  $\delta^{15}N$  values, which could be associated to a common environment and chronological period. The average of  $\delta^{15}N$  isotopic enrichment between herbivores and domestic omnivores (*Sus domesticus*) in this case is 2.1‰, similar as at Cova de la Guineu. Assuming that all the pig remains were contemporary, the household model could be proposed since the  $\delta^{15}N$  values are very different among pigs (Madgwick et al. 2012), showing a variety of types of diet: values compatible with an herbivore diet (e.g. S-UCT 19086), a carnivore diet (e.g. S-UCT 18989), even so a mixed omnivore diet (e.g. S-UCT 19087). This heterogeneous feeding pattern seem to be more frequent in a household management model, where the number of pigs is small and they are fed with human leftovers (Halley and Rosvold 2014) (Fig. 5).

Carnivore values were represented by felids (*Felis* sp.). Their elevated  $\delta^{13}C$  values in comparison with humans suggest they were feeding on different resources, perhaps a diet based on micromammals, reptiles and birds (Lozano 2017), which are not commonly consumed by humans.

Most of the human samples from Cueva de Abauntz show homogeneous isotopic values except for one individual outlier that will be discussed separately. The  $\delta^{13}C$  human values show that human dietary protein was mainly coming from  $C_3$  terrestrial resources, with no evidence of regular aquatic or  $C_4$  resource consumption. The  $\delta^{15}N$  human values suggest

a high importance of animal proteins in human diet, showing a  $\delta^{15}$ N enrichment of 4.7% compared to herbivore average, and 2.6% compared to omnivore average (Fig. 5). Looking into the possible species consumed, we cannot rule out any herbivore intake based on the faunal and human isotopic values (Bocherens and Drucker 2003). On the other hand, a 2.6%  $\delta^{15}$ N enrichment between humans and omnivores is too small to conclude that pig was the preferential intake. Our results would be consistent with a mixed diet that could include ovicaprids and bovids preferably and pig and plant protein potentially in lower amount.

As mentioned before, there is only one outlier adult human (S-UCT 19029) shifted to a higher position of the plot. This human shows an enrichment of 2.3% in  $\delta^{15}N$  values and 0.9% in  $\delta^{13}$ C values regarding to human mean values. The consumption of enough freshwater resources as to be reflected in the isotopic signature could be one possibility. This type of dietary outlier has already been reported in other Iberian sites for other chronologies, and they are usually linked to fish consumption (Guiry et al. 2015). The detection of intake of freshwater resources is not straightforward, but it seems that high  $\delta^{15}$ N values are a common characteristic when freshwater resource exploitation is present (Lillie et al. 2011). The Ultzama River goes a few metres away from Cueva de Abauntz, making it plausible that riverine fish could have been consumed even if not knowing where was the location of the settlement/s where the individuals buried in Cueva de Abauntz lived. Otherwise, the consumption of marine resources is unlikely because the increase with respect to the other individuals is mainly in  $\delta^{15}$ N values but not in  $\delta^{13}$ C, which would be less negative if the individual showed a marine diet (Richards and Hedges 1999). The consumption of estuarine resources, which have lower  $\delta^{13}$ C and  $\delta^{15}$ N values than marine ones (Salazar-García et al. 2014), could be another explanation given that Cueva de Abauntz is only ca. 80 km away from Atlantic sea lochs and marine marshes. Finally, a preference consumption of pig livestock could also be suggested for this individual (4.9%o enrichment in  $\delta^{15}N$  between this human sample and the pig mean) (Fig. 5).

The direct radiocarbon date of this human sample is contemporary to another human sample analysed (S-UCT 19048) whose isotopic values fit well within the human mean (Fig. 2). This probably discards the isotopic difference due to a different chronological attribution of the remains and could be reflecting that some humans have different access to some types of resources due to several reasons. Carbon and nitrogen have been explored in respect to hierarchy in medieval societies, finding high protein diet in higher status burials (Trautmann et al. 2017). During Prehistory, and especially in the case of collective burial caves, assessing different social positions is difficult to test. The association of specific individuals to grave goods could shed light on this (Blasco and Ríos 2010), but unfortunately in the case of Cueva de Abauntz



having all skeletal remains and grave goods comingled makes this impossible (Utrilla et al. 2015). Another way to approach the study of hierarchy in prehistoric burial sites could be the study of funerary structures (Fernández-Crespo and de-la-Rúa 2015). Cueva de Abauntz presents some particular burials in pits and cists inside the cave (Utrilla et al. 2007), but none include these two earlier individuals (S-UCT 19029 and S-UCT 19048). Both of these directly dated individuals came from a small rock niche, without any specific burial structure nor grave goods.

Other non-dietary explanations could be argued to justify these differences too. A non-local origin of this individual could be one option that should be further studied through strontium isotope analysis of the population (Waterman et al. 2014). In this sense, the combination of carbon and nitrogen together with strontium isotope ratio analysis could give us more information about this unequal protein intake, as it is already done for the same chronology in nearby territories (Sarasketa-Gartzia et al. 2017; Villalba-Mouco et al. 2017).

# Inter-population analysis

The human isotope values from both sites portray a quite homogeneous overall diet among humans. This homogeneous pattern of diet based on C3 terrestrial resources seems to be general along the entire Iberian Peninsula during the Late Neolithic and Chalcolithic (e.g. Alt et al. 2016; Díaz-Zorita 2014; Fernández-Crespo et al. 2016; Fontanals-Coll et al. 2015; García-Borja et al. 2013; López-Costas et al. 2015; McClure et al. 2011; Sarasketa-Gartzia et al. 2017; Villalba-Mouco et al. 2017; Salazar-García 2011; Salazar-García et al. 2013b; Salazar-García 2014; Waterman et al. 2016). The reason of this homogeneity could be the consolidated economy based on agriculture and livestock, together with a higher mobility among the different communities and the increase of trade networks, not only in prestigious objects (Schuhmacher and Banerjee 2012) but also in food products. Isotopic analyses in fauna remains could give us more clues about animal trade, as happens in other chronologies (Salazar-García et al. 2017).

In any case, and even if the dietary interpretation does not vary, it is noteworthy to mention that there are significant differences between  $\delta^{13}$ C human values from Cova de la Guineu and  $\delta^{13}$ C human values from Cueva de Abauntz (Mann-Whitney test,  $p=1.05\times 10^{-12}$ ) (Fig. 6). This observed  $\delta^{13}$ C differences among humans is also present among herbivores (Mann-Whitney test, p=0.0004), which define the baseline of each ecosystem. This suggests that the observed human difference between sites should not be attributed to diet, but most possibly to the existence of enough environmental differences to be recorded in the collagen  $\delta^{13}$ C values along the food web. Plants are very sensitive to different environmental factors (altitude, temperature, luminosity or water

availability) and their physiological adaptation to its factors can generate a variation in their isotopic values as happens with C<sub>3</sub> and C<sub>4</sub> adaptations (O'Leary 1981; Ambrose 1991). This spectrum of values has been used to assess several aspects about past environmental conditions when studying the  $\delta^{13}$ C and  $\delta^{15}$ N isotopic values of a species with a fixed diet over time (e.g. Stevens et al. 2008; González-Guarda et al. 2017). Moreover, this gradual  $\delta^{13}$ C and  $\delta^{15}$ N variation among different environments is very helpful to discriminate altitudinal movements in herbivores with a high precision method based on serial dentine analysis (Tornero et al. 2016b). In our case, results reflect the influence of environment from at least two areas in Iberia (the Western Prepyrenees and the Northeastern coast of Iberia). These differences demand caution when interpreting human diets from different sites that are not contemporary and/or not in a same area, as it is possible that the environmental influence is responsible for changes otherwise attributed to different subsistence patterns and social structures (Fernández-Crespo and Schulting 2017), as has been demonstrated in neighbouring territories (Herrscher and Bras-Goude 2010; Goude and Fontugne 2016).

#### **Conclusions**

This study represents a comparative isotopic analysis between two burial caves dated back to the Late Neolithic-Chalcolithic Period from the Nestern Prepyrenees and the Northeastern coast of Iberia. The isotopic dietary approach shows a diet based on C<sub>3</sub> terrestrial resources, common for the whole Iberian Peninsula during this period. A preferential herbivore intake as main protein source, probably domestic, has been proposed for both sites. Moreover, isotopic human values also suggest that the consumption of domestic pig was not very frequent, at least not enough to leave a clear sign in human bulk collagen values. The present study also reveals a significantly different carbon isotope signature in humans and herbivore baselines from both sites, suggesting an environmental effect between these two Iberian areas, and suggests caution when interpreting diet in isotopic studies comparing human values from different regions and/or chronologies.

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