Universitat Rovira i Virgili
Departament d'Història i Història de l'Art
Màster en Arqueologia del Quaternari i Evolució Humana (Erasmus Mundus)

International Master in
QUATERNARY AND PREHISTORY

Tesis de Master:

Dogs from Augusta Emerita: The stable isotopes input for diet and status evolution into human populations

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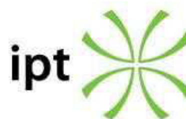
Curso académico 2020/2021



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Marta Orozco Saumell

Abstract: The possibility to infer the status of an individual over its diet is an advance in archaeological research. In this research, the quest of the status and roles of that dogs found at Almendralejo 41 st (Merida, Spain) is the purpose. The high protein intake put them in a similar human scale, enabling them a status in the top with some roles as companion or house guard. The ritual context offer a view after dead in similar way. However, the comparison with other periods does not give a similar view in pre-Roman epochs.

Key Words: Stable Isotopes, ^{13}C, ^{15}N, Diet, Dog, Roman, status, role, ritual

1. Introduction

Since the Palaeolithic, dogs were the first animal that lived with humans, and the relationship between them is special compared to other domestic animals, only comparable to cat-human relationship. This is perhaps because two reasons: the similar social structure and the admiration to them (*totem*). Hence, it is not clear if the dog introduction in human societies is due to a self-domestication (Hare et al., 2012), to an intentional pack-domestication (Morey & Jeger, 2015) or to a mutualism made by town dump cleaning (Coppinger & Coppinger, 2001).

Thus, the dog uses as packs it are important in the study of relationship among this canid and the human because once created the link, the humans used the dog raw material, work force, companion and for ritual assumptions. Regards to raw material, dogs can be used as meat, marrow or fur. Not only in Palaeolithic (Germonpré et al., 2009, 2012, 2017, 2020) or Aziliense (Pionnier-Capitan et al., 2011) but also in more recent periods as Bronze Age (Grandal-d'Anglade et al., 2019) where disarticulation marks appear together to absence of meat bones, and Iron Age (Méniel, 1984, 1991; Horard-Herbin, 1997, 2000, 2014; Lepetz, 2007) where they were used as livestock.

The work force is the most common assignation already from Palaeolithic (Germonpré et al., 2009, 2012, 2015, 2020; Bocherens et al., 2015; Shipman, 2015) as pulled and carry loads. In this case, the spondylosis deformans is the manner to assign the role (Latham & Losey, 2019) but some authors do not agree with the assignation because it can cause by multiple situations (Janssens et al., 2019). Other roles assigned to dog work force into the human societies have been natural tasks, as hunting as well as guard or protection. Nevertheless, residual food or waste elimination could be another important task given to dogs inside of human hierarchy.

We cannot forget the war role, as territorial fights have been producing since the beginning of the metallurgic societies. This is an important assignation in the classical authors as Polyaenus (*Strateg*. VII, 2 and *op.cit*., VII, 9) who describes when Cambyses attacked Pelusium in 525 BC or the dog uses in the invasion of Scythia by Darius in 513 BC leave dogs in the camp and the enemy though that Darius was there (Polyaenus,

op.cit., VII, II, 4 and Frontinus, *Strateg*. I, 5, 25; see more war uses in Froster, 1941). Of the Marathon battle, talk Eliano (*De Nat. Anim*., VII, 38). The most common names given to war dogs were *Malossus* (*op.cit.,* XI, 20)*,* similar to a mastiff, or *Canis Pugnax*, similar to a Rottweiler.

Actually, we see the dogs and other carnivores as companion pets and they can become part of the family, creating a fraternal link. Examples of this in prehistory have been related to the Magdalenian Bonn-Oberkassel interment (Napierala & Uerpmann, 2012; Street, 2013; Street et al., 2015; Janssens et al., 2016, 2018), to Natufian burials from Jordania (Valla, 1975; Davis & Valla, 1978; Tchernov & Valla, 1997) or the Mesolithic Muge's dog (Pires et al., 2019). In Roman Age, Eliano (*De Nat. Anim*., VI, 25; VI, 62; VII, 10; VII, 29; X, 41; XI, 13) talk about an intense relationship between humans and dogs as veritable fraternal relationship. References to lap dogs are also in the same author when talk about a little dog that due live inside of a house because relates the infidelity of a wife pointing out where the lover is (*op.cit*., VII, 25) or when one Milete little dog jump inside of the tomb of its owner and was buried with him (*op.cit*., VII, 40).

The Roman author also explains how in Egipt, dogs make the Nilo water grow up and for this is venerate (*Eliano, De Nat Anim*, X, 45).

Thus, we can see that there were different uses for dogs in prehistory and Antiquity, but can we differentiate these roles in archaeology? It has been seen that when it use as work force may have some pathologies in bones, but in other utilities, we have not any osteological feature, except some cut marks whether the dog has been sacrificed or intake (Martínez Sánchez et al., 2020). However, these broken bones or cut marks are not always present. One form to get closer to this differentiation is over their diets.

Ancient authors treat some aspects of the dog diets. For example, Columella in his seventh book of *De Res Rustica* (VII, XII, 10) talk about shepherded dogs say:

> *Cibaria fere eadem sunt utrique generi praebenda. Nam si tam laxa rura sunt, ut sustineant pecorum greges, omnis sine discrimine hordeacea farina cum sero commode pascit. Sin autem surculo consitus ager sine pascuo est, farreo vel triticeo pane satiandi sunt, admixto tamen liquore coctae fabae, sed tepido, nam fervens rabiem creat*. 'The rations are nearly the same for both breed. Because if the farmer are so loose that they bear the flocks of cattle, every man feeds with barley flour without distinction, and is fairly late. But if the field is planted with a twig and without pasture, they will be fed with fine wheat or wheat bread, mixed with the liquor of baked beans, but warm.' (Columella, *De Res Rustica*, VII, XII, 10)

Another clasical author, Varro in his third book of *Rerum Rusticarum* (III, 2.9.8-10, Cubero, 2010):

> *The food of the dog is more similar to that of man than that of sheep, since it eats ordinary food and bones, not herbs or leaves. You have to diligently take care that they have food, because hunger will lead them to look for food if it is not supplied, and they will be separated from the cattle "" And so they should be given barley bread better crumbled in milk so that accustomed to this food they are not separated from the cattle soon. They will not be allowed to eat dead sheep meat so that they are not attracted to the taste. They also give them bone broth and those same ground bones, because it makes the teeth firmer and the mouth wider, because the jaws separate more strongly and become more intense*

because of the taste of the marrow. They tend to eat food during the day where is grazed, in the afternoon where they are housed.

With this classical text, it is possible approximate more to the idea of how were the dog diets in Roman Empire.

1.1. Ethnography

Ethnography on pre-industrial societies is a method that can help when we want elucidate some aspect of the past. In this case, if the dogs have the hunting or guard roles as well as some ritual aspect, the Alaskan groups could show some aspect. At Northwest Alaska, dogs have some characters, companion as part of the family (Hill, 2018), that are fed better than other dogs, or working dogs, that are fed by humans specially in winter and in summer fend by themselves (Binford, 1978; Hill, 2018). Working dogs fed with 2.7 to 3.2 kg (6-7 pounds) of fish or walrus further crushed bones and offal in winter. Dogs also possess an ontological status with personal names, amulets given and *inuat* –soul-like interior persons– (Hill, 2018). Also fed meat resulting from dismemberment in kill-butchering site, heart, liver, tenderloins, some bones, some unbutchered animals and foetus (Binford, 1978). Dog guards in summer are nourished with dried meat.

In Alaskan societies, dogs also have a ritualistic consideration when it comes to a human illness or pain. Thus, the dog is sacrificed as one scapegoat (Hill, 2018).

At Nicaragua the dogs are more exactly hunting tool and contribute to 85% of the kills of mammalians (Koster, 2007). Talent hunting dogs receive better care than their less talented conspecific, and generally eat whatever has been prepared for household consumption (Koster, 2007; Tankersley & Koster, 2009; Koster & Tankersley, 2012), what in isotopical analysis seems agree (Koster & Tankersley, 2012), since carbohydrates from vegetables would be the caloric source. Puppies are nourished with milk (Koster, 2007; Tankersley & Koster, 2009), and hunting dogs sometimes with a piece of meat in kill site, while in human settlement eat human food scraps and small critters (Tankersley & Koster, 2009).

1.2. Aim

The aim of this research is to see the possible role differences of the dogs in the human society over their diets, this is, their socio-economic function throughout the human control of their food. Finally, if we know the diets and possible roles of the Merida dogs, we will attempt to compare them with the diets of other dogs around Iberian Peninsula and from other chronologies in order to see the possible evolution of the dog roles in this location with our research input.

2. Materials and methods

2.1. Archaeological site and remains

The remains come from Almendralejo 41th street (Mérida, Spain). This place belongs to the north sector of the city. It placed extramural, at the foot of the roman wall. It was the suburb, a place with polyvalence sense. In this case, the use was funerary.

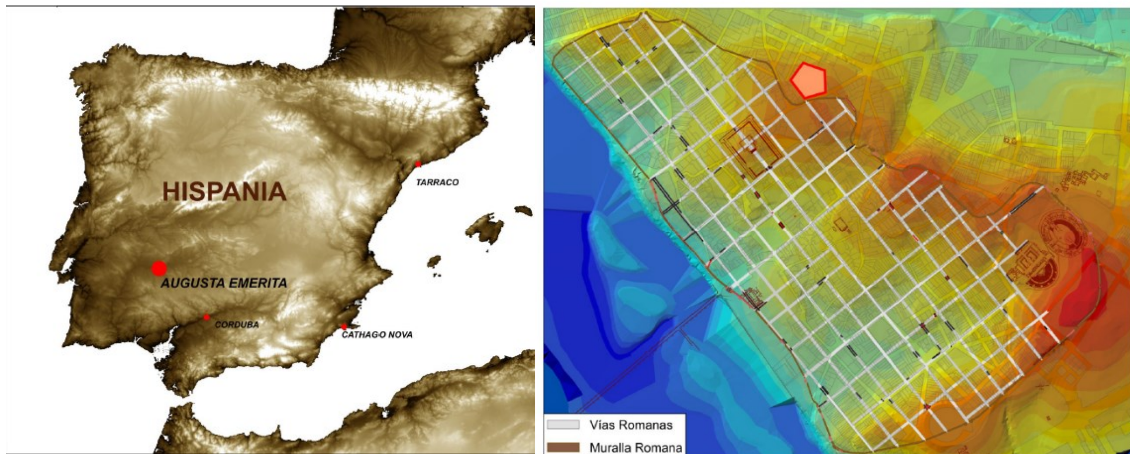

Figure 1 location of a) Augusta Emerita inside of Iberian Peninsula and b) that of Almentdralejo 41th street (images take it from Heras & Olmedo 2008)

Like almost everything in the roman world, the funerary monument from where the remains come, was in its corresponding shortening of use, to few meters from the beginning of the way (Heras & Olmedo, 2010). This way was perpendicular to the secondary road, parallel to the wall, that departed from the primary way, that came out from the monumental door placed in Cerro del Calvario and it constituted an extension of the *Cardo Maximum* since the Albarregas river (Heras & Olmedo, 2010; Heras et al., 2011).

The funerary monument is a structure turreiform, an idea that come from the Eastern Mediterranean. The structure close a gap inner space and it open at the top. It was constructed in granite ashlars of 3.60 x 3.60 x 3.85 m (Heras & Olmedo, 2010). Inside the monument, they were found four human cremation burials, three in a limestone boxes with roof-shaped cover and a third box of lead with globular form and narrow neck (Heras & Olmedo, 2010).

Figure 2 view of the funerary monument in context a) from the front monument and b) to its foot (from Heras & Olmedo 2008)

The remains come from the inside of the monument, from the open space. We have two dating, a relative dating by potter and absolute radiocarbon dating. Since the place was used as dump, we have the last deposits inside the monument dating at 60 AD. Besides, four dogs have been dating by radiocarbon in BETA Analytic between 30 BC and 40 AD (between 45 cal BC – 77 cal AD 1σ for specimen WF_38 and 22 – 170 cal AD 1σ for specimen WF_37).

Despite of we have 40 dogs (MNI), we analyse only four isotopically (WF_36, WF_37, WF_38 and WF_39; Fig S7). In order of assure the diet, we also analyse four *Equus sp.*, two *Bos sp.*, seven *Sus sp.* of which two are infantile individuals because there were large number of piglets among remains, seven ovicaprine specimens, two *Cervus sp.*, one *Accipitridae sp.*, one *Tetrax sp.*, one *Anser sp.* one *Alectoris sp.*, and two *Homo sp.* with intention of compare the dog diet regard to humans.

All individuals come from the strata of the monument inner except *Accipitridae*, *Anser* and *Alectoris*.

BETA Analytics provides also the isotopic composition of the four analysed dogs.

2.2.Isotopical analysis

The extraction of collagen has been made following the Login (1971) and improved by DeNiro & Epstein (1978, 1981) (Text S3) with some modifications.

The bone fragment between 300 and 400 mg was extracted from the whole bone with Dremel rotating tool, sawn it with a diamond-coated blade. The fragment was mechanically cleaned with diamond tip in the same Dremel tool.

During demineralization, we have followed two methods, the quick and slow protocols. The main difference is that in quick protocol the bone was grounding before soak in HCl, while in the slow protocol the bone fragment was soak completely.

The problem with the slow protocol is that the HCl may attack the organic fraction or disintegrate it. For this, the slow protocol was performed only for the bones that were very phosphates. Thus, because a bone very phosphated when it is grounding usually generates fine powder. During filtration, it is probable that fine powder be absorbed. Also that the collagen fibres may be more broken (Schoeninger et al., 1989; Jørkov et al., 2007).

In quick demineralization, once crushed in agate mortar and sieving to 355 µm, was soaking 20 minutes in 20 ml of 0.5 M HCl with rotating movement in Multistierrer 6 (velp scientifica). Slow demineralization consists in soaking the bone fragment in HCl until it becomes soft changing the HCl to the M appropriates (Table).

Once demineralized, the residue was scrubbed with distilled water flooding it five times on a MCE Membrane filter of 5.0 µm on Büchnel funnel. After this, it is plunged during 20 hours in 15 ml of NaOH, and cleaned at the same manner in Büchnel funnel. The residue was deposited in a Pyrex with 0.01 (10^{-2}) M HCl pH2 during 17 hours to 100ºC to solubilize it. This mixture was lyophilized (freeze-dry) and 0.5 g analysed in the IRMS (Isotope Ratio Mass Spectrometry).

The results are given respect to V-PDB standard for carbon and atmospheric AIR standard for nitrogen, and presented with δ notation given the next equation (Text S1):

$$\delta = \left(\frac{R_A}{R_{STD}} - 1\right) 1000 \quad (1)$$

where R_A is the ratio of one element as result of divide the heavy isotope between light isotope of one sample, R_{STD} is the ratio of the same element as result of division between

heavy and light elements of the standard, being δ showed in ‰ or per mil. This $\delta^{13}C$ isotope signals show the photorespiration plant type (C3 or C4) and the marine vs terrestrial diet (Text S2), while the $\delta^{15}N$ signal has been taken as the trophic chain position and protein consumption.

Statistics has been performed with PAST 4.07 software and Microsoft Office Excel 2016, and given that the population is Non Parametric (Shapiro Wilk W, below), this is, does not follow a normal distribution, we evaluated the differences with Kuskal-Wallis. Average values are showed with standard deviation.

3. Results

The collagen quality and amount is measured by C:N ratio, that due fall between 2.9 and 3.6 (DeNiro, 1985). By weight % of collagen, which due be between 5.7 and 28.3% if the collagen is well preserved, between 2.3 and 10.1% if the collagen is in the threshold among poorly-preserved and well-preserved, less than 1.8% is considered non-collagenous. As well as by %C and %N concentrations in collagen, which would be equal or greater than 13.0 and 4.8% respectively (Ambrose, 1990).

Our research show results for C:N ratio between 2.7 and 3.4‰, for weight % of collagen between 1.77 (only one in this limit) and 18.18%, for %C between 32.36 and 48.94%, and for %N between 13.54 and 17.80%. Then, we can conclude that the overall collagen produced gives good quality and amount (for all results see Table S1).

The $\delta^{13}C$ signals ranged in *Equus caballus* (n=4) between -21.47 and -20.81‰ with an average of -21.15±0.27‰. The range for *Bos taurus* (n=2) was between -21.31 and -20.80‰, and their average was -21.05±0.36‰. *Sus sp*. (n=7) showed a range of -21.93 to -19.61‰ and the average was -20.79±0.68‰. For *Ovis/Capra* (n=7) the results show a range between -21.06 and -19.65‰ and an average of -20.41±0.48‰. The range for *Cervus elaphus* (n=2) was -20.33 to -19.60‰ and the average was -19.96±0.52‰. For humans (n=2) the results were -20.33 to -18.83‰ and the average was -19.67±1.19‰. *Canis familiaris* showed a range between -19.00 and -18.30‰ and the average showed a value of -18.65±0.29‰. Results for *Herpestes ichneumon*, birds and other non-roman carnivores analysed for this study are showed in Table S1.

The ranges for $\delta^{15}N$ were 3.91 to 7.24‰ for *Equus caballus* (n=4) with average of 5.63±1.44‰. For *Bos taurus* (n=2) were 3.69 to 7.32‰ with an average of 5.505±2.57‰. The range for *Sus sp*. (n=7) was between 3.91 and 7.20‰, and its average was 5.18±1.3‰. *Ovis/Capra* (n=7) showed a range between 5.29 and 11.27‰ with an average of 7.84±2.04‰. For *Cervus elaphus* (n=2) the results ranged between 4.44 and 5.29‰, and its average was 4.86±0.4‰. Humans (n=2) showed a range of 8.51 to 10.39‰, and the average was 9.45±1.33‰. In *Canis familiaris* (n=4) the range was between 8.50 and 9.90‰ with an average of 9.2±0.57‰. *Herpestes ichneumon*, birds and other non-roman carnivores are showed in Table S1.

When we analyse $\delta^{13}C$ signals together the normality test shows parametric values (Shapiro-Wilk W p=0.00567). However, if we analyse each specie separately, the values are non-parametric (Shapiro-Wilk W p=0.8244 for *Equus*; p=1 for *Bos*; p=0.287 for *Sus*; p=0.637 for *Ovis/Capra*; p=1 for *Cervus*; p=1 for humans; p=0.5879 for all birds; p=0.962 for *Canis*). Thus, we prefer follow the non-parametric results.

In the case of $\delta^{15}N$, we performed the same normality test procedure. When we analysed all values together the Shapiro-Wilk W shows that are non-parametric values (p=0.1635). In this case, when we analyse each specie separately, the results are non-parametric equally.

Thus, it is preferable to use the Kruskal-Wallis in statistical analysis.

4. Discursion

4.1. Augusta Emerita site

The $\delta^{13}C$ signals seem assure a terrestrial diet baseline in C3 photorespiration plant-type. In general, all herbivores would have a mean of -20.7±0.13‰, while carnivores (humans, dogs and mongoose) would show a mean of -18.69±0.4‰. This means that enrichment of carnivores regards to herbivores occurs (see Text S2). Birds show a similar $\delta^{13}C$ value to herbivores, including vulture (*Alecctoris*). The fractionation factor among groups is $\Delta^{13}C_{her-car}$= 2.007‰ (Kruskal-Wallis' p=0.0004169). Humans have a mean of -19.67±1.19‰, being one more enriched (MD_38= -18.83‰) than the other (MD_42= -20.52‰). The average of Canis (-18.65±0.29‰) fall closest of MD_38.

If we separate among domestic herbivores –given the result of *Sus sp.*, we will include their results in the average of domestic herbivores– (-20.75±0.57‰) and wild herbivores (deer average= -19.58±1.06‰), it is possible to see an offset of 1.13‰. Although not significant (Kuskal-Walls' p=0.067), this offset could mean the difference among those humans and among *Canis* regards to MD_42. This difference in $\delta^{13}C$ values between domestic and wild herbivores may be due to water use efficiency, since in open land the water is not always available while, in agricultural lands, irrigation would cause more depleted values. Thus, although both domestic and wild herbivores eat leaves, the results are different.

In the case of *Ovis/Capra*, one $\delta^{13}C$ value (MD_27= -19.65‰) is in relation with this view. Whether these animals were in trasteminance, transhumance or fix at place, that value could means that MD_27 is a goat while the others are sheets, because the first tends to eat leaves and the other graze. This can also mean a fed type difference among farms because we can see this pattern in *Sus sp.* too. Or simply, a difference in metabolism. Values more enriched are seen in A Lanzada site (López-Costas & Müldner, 2016) but, in this case, that may be caused by sea effect.

Thus, the difference among MD_42 and MD_38 together dogs in enrichment could be due to a difference in nutritional habits where the $\delta^{13}C$ offset from herbivores to carnivores fall in the accepted 1‰.

However, if we observe the $\delta^{15}N$, the most elevated value in humans is for specimen MD_38 (10.39‰), therefore this may mean that this had a fed habit more carnic and based in more wild herbivores (below).

It is curious the case of *Herpestes ichneumon* find at Merida site as its $\delta^{13}C$ value is -16.90‰ what indicates that this specimen does not come from this place. The isotopical signal is associates with C4-plant type. That means that mongoose is exogenous specimen, likely imported from North Africa, this is an important relationship among

Mediterranean places as Iberia and Northern Africa. In addition, it was found a *Camelus sp*., what reinforces the hypothesis of connection among Mediterranean places. The isotopical signal of this last specimen was not successfully in IRMS.

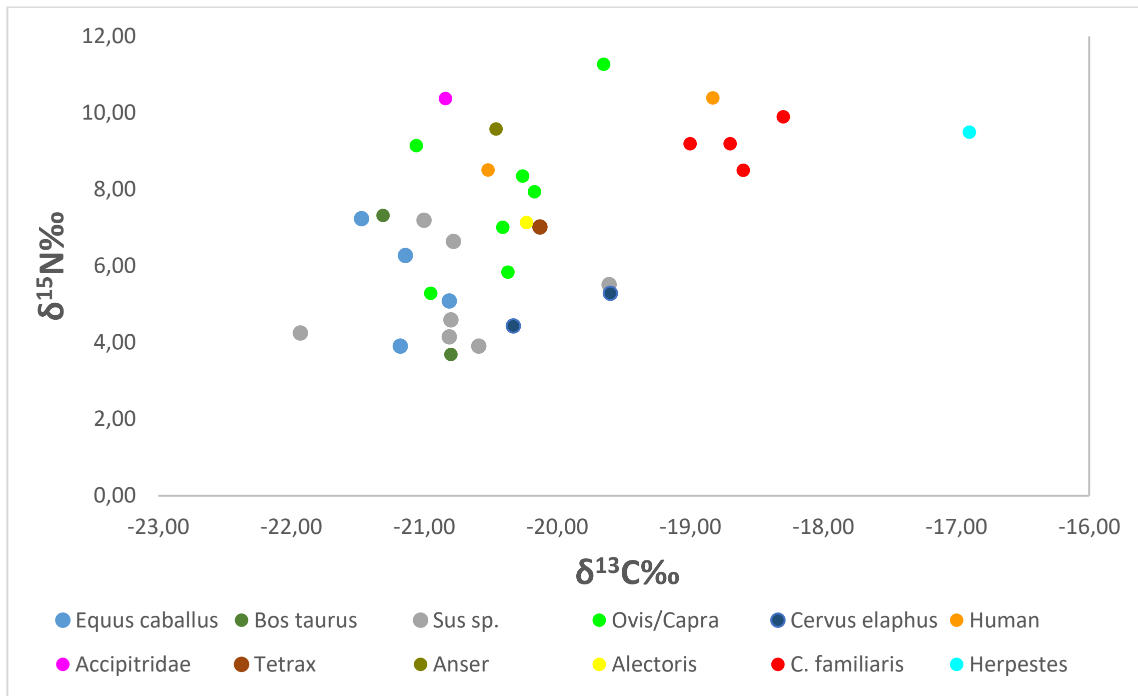

Figure 3 Isotopical analysis from funerary turreiform monument at Augusta Emerita

Respect to $\delta^{15}N$ signal, it must be necessary to take the $\delta^{15}N$ data with caution as intra-specie can vary a lot due to the differentiation caused by human or wild feeding (Text S2). Depending on the plant given to the herbivores, the ^{15}N can cause a signal or another. This is, taking into account our result of *Ovis/Capra* (Table S1), we have a range between 5.9 and 11.27‰ while carnivores are in the range of 8.5 and 11.3‰. If we take the common analysis of $\delta^{15}N$, we should say that some *Ovis/Capra* are carnivores and are in the top of the trophic chain.

Nevertheless, as Sponheimer and colleges (2003a, b) and Szpak (2014) show, there are some causes for this enrichment in herbivores. On the one hand, the high protein plant diet. On the other hand, the agriculture by-products, when cereals (seeds) and fruits are select for humans and stems and leaves (hay) are for livestock. Seeds and fruits are depleted regards to soil pool while stems and leaves are enriched. However, the herbivore enrichment would cause higher signals in humans and carnivores (if these were fed with the same food than humans).

Nodulated plants may play an important role in this discrimination, and different feeding habits among farms reflect these variances in herbivores $\delta^{15}N$ values. Although the difference among N-fixing and non N-fixing is low (Text S2), there may exist a variation of -5‰ between them. In Roman context, the legumes are a common food, especially in lower social strata. The classical authors describe the use of lupines (*lupinum*), chickpeas (*cicer*), beans (*phasoleus*, *conchis*), broad beans (*fabas*), lentils (*lens*) or green pea (*pisum indicum*) (Barrajas, 2000).

In this sense, and together the intra-plant variation due to uptake, assimilation and transportation, and the treatment of the land by husbandman (Text S2), the variation in $\delta^{15}N$ values can be large. On this way, we propose the use of enrichment factor more wide, between 1.3 and 6.9‰.

Seeing our data, $\delta^{15}N$ values of all herbivores is in average 6.11±1.95‰. If we separate domestic herbivores and wild herbivores, the results are 6.23±2‰ and 4.86±0.6‰, respectively. Besides, if we separate all species of herbivores, horses give an average of 5.63±1.44‰, cattle of 5.5±1.8‰, pigs of 5.18±1.3‰, and ovicaprines of 7.83±2.04‰. Overall results show similar patterns, the most significant are ovicaprines. Given these results among herbivores, we have compared humans and dogs with each specie group.

Humans have an average of 9.45±1.33‰, and dogs of 9.2±0.57‰. That is equal to the same trophic level (Kruskal Wallis' p= 0.6386). In the same dog level is mongoose (Kuskal Wallis' p=0.4682). When we differentiate among all herbivores and *Canis*, the result is significant (Kruskal Wallis' p=0.005554). However, if we differentiate among domestic herbivores and wild herbivores although between these groups there is no significant variance (Kruskal Wallis' p=0.7337), respect to dogs there are two views, regards domestic herbivores, with significant difference (Kruskal Wallis' p=0.006682), and regards wild herbivores (deer), without significant difference (Kruskal Wallis' p=0.06029). Within domestic herbivores, the significant difference is with *Sus sp.* (Kruskal Wallis' p=0.008007) and *Equus caballus* (Kruskal Wallis' p=0.02016).

Thus, the enrichment factor from all herbivores to dogs is 3.09‰, and from domestic herbivores to dogs is 2.967‰. In the same way, from deer to dogs is 4.34‰. Therefore, the enrichment factor fall within the accepted range, being dogs in the high position of the trophic level as they have a diet that is more proteinaceous. In this point, dogs, humans, mongoose and vulture (10.38‰, with Kruskal Wallis' p=0.1468 respect to dogs) are in the higher trophic chain level.

However, it is interesting to analyse the results of ovicaprines regards to dogs given their high $\delta^{15}N$ signals. The mean of *Ovis/Capris* is 7.836±2.04‰, with an enrichment factor ($\Delta_{O/C\text{-}D}$=1.364). Thus, the Kruskal Wallis' p is 0.08825, not having statistics significance. What is the reason for this? More data is necessary, including botanical data.

Indeed, agree with Schwarcz & Schoeninger (2011 p.738), "isotopic analysis should only be used to estimate the dietary proportions of know nutrients, and cannot by themselves be uses to tell the archaeologist what people were eating in the past".

In contrast to the other carnivores at the same site (Fig. S6), actual and mediaeval dogs would be in the high position with the most protein intake, with higher position for the Chalcolithic mongoose. Roman dogs and mongoose would be in the middle position, what means that in most recent periods the meat consume was greater. Chalcolithic dog correspond with the view that in the most pastoralist epochs the dogs would have more intake of cereal diet. As to $\delta^{13}C$‰, the Roman mongoose and actual dog had have a diet based on C4-plant type or C3-plant type with high water efficiency.

Birds are complicate because they show to have different rates, metabolism and digestive and excretory physiology that make a different collagen turnover rate (Schoeninger et al., 1983; Ambrose, 2000; Caut et al., 2009). Though Robbins et al. (2005) showed a similar discrimination factor. Additional research in this camp are precisely in order to assure how much we can use birds and the mode in human and carnivore diets. Our results show an elevated protein intake also in herbivore birds.

4.2. Dogs diet and social evolution at Iberian Peninsula

It is possible to observe an evolution in diets at least from the Chalcolithic, this with very little variation regards to Neolithic (Kruskal Wallis' p=0.1172), being the ^{15}N Δ_{N-C} equal to 1.56‰. The Neolithic mean is 8.9±1.08‰ in dog diets, and in Chalcolithic 7.26±1.17‰. In comparison with Roman Empire, the difference is not significant among that and Neolithic (Kruskal Walis' p=0.1427; Δ_{N-R}=0.74‰) and among Roman times and Chalcolithic the difference is significant (Kruskal Walis' p=0.004334; Δ_{R-C}=2.37‰).

This regression in proteins could be due to increase in starch rich diet for dogs, that is possible since Neolithic thanks to the multiplication at the pancreas of the AMY2B gen from 2 to 4-32 in the mostly dog breeds (Axelsson et al., 2013; Freedman et al., 2014; Ollivier et al., 2016, 2018). It is at this time when de locus MGAM responsible to broke the starch and the hydrolysis catalysing from maltose to glucose emerge, as well as the SGLT1 gen (Axelsson et al., 2013).

Hence, the protein diet regression could be in relationship with the use of dogs for more herd purposes, as livestock and land guard. With the increase of pastoralist societies, the hunting stopped being the most important recourse for meat entry in households, and the use of dogs for hunting would decay with its. Whether the use of dogs is for herd, the meat intake would incentivise to eat the livestock, as it is seen in ethnographic parallels (Koster & Tankersley, 2012), the meat prey serve like motivation in hunting.

However, the use of dogs in Neolithic and Chalcolithic as scavengers of human scraps would follow an important role. Then, we can argue that in these times, the social status of the dogs was as tool and no companion or fraternal idea was had for them.

Respect to Chalcolithic to Early and Middle Bronze Age (E-MBA), the variation seems significant (Kruskal Wallis' p=0.01863) with an average in this period of 8.14±0.77‰ and a difference between values of $\Delta_{C-E-MBA}$=1.38‰. An increase in protein intake regards to the immediate period. That could be due to the rise of social stratification into human society. Thus, the uses of dogs would change and diversify, not only for hunting, herd, guard and companion, but also for ritualistic purposes. Further investigations are necessary.

It is at Bronze Age when the first little dogs of less than 140 mm in cranial length emerge (Harcourt, R. A., 1974; De Grossi Mazzorin & Tagliacozzo, 1997, 2000). This would justify the diversification of the dog roles and the change in their diets. For example, the protein intake in E-MBA range between 6.5 and 9.3‰, whereas in the immediately posterior period, Late Bronze age (LBA) does it between 6.20 and 9.50‰. At LBA, the average is 8.42±0.96‰, with a variation among phases of $\Delta_{E-MBA-LBA}$ =0.28‰, having a non-significant difference (Kruskal Wallis' p=0.2495).

Also, there is not significant difference (Kruskal Wallis' p=0.5703) among LBA and Early Iron Age (EAI) when the average is 8.69±0.79‰ and the difference between two phases is $\Delta_{LBA-EIA}$=0.27‰. What means that from the social stratification into human society, the dog roles diversification do not change. Nevertheless, it is in Iron Age when the dog meat is uptake by humans, this is, the dogs are treated as livestock (Harcourt, R. A., 1974; Méniel, 1984, 1991; Horard-Herbin, 1997, 2000, 2014; Lepetz, 2007; Hambleton, 2008).

It is in Roman society when it is possible to see an increase in the protein intake, with an average of 9.64±0.62‰. An increase of Δ_{EAI-R}=0.95‰, a significant variation regarding to previous epoch (Kruskal Wallis' p=0.01101). Among Roman sites of Merida and Llanos del Pretorio there is not any significant difference (Kruskal Wallis' p=0.08143). Nevertheless, the proportion of our sample is very little in comparison with all the remains found in the funerary monument. Thus, more analysis are necessary in order to get most precision.

At this time, together iconographic remains (Fig. S1-S4), there are texts that explain the feeding habits for dogs (above) and the status of them. It is the time where the dogs rise a great size variability with wither height of Chihuahuas (near or less than 25 cm) until those of mastiffs (more than 60 cm). Thus, the roles and statuses would have great amplitude.

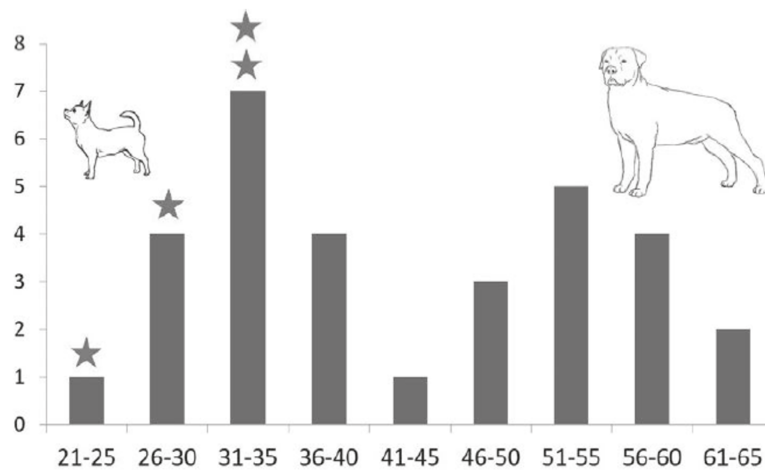

Figure 4 Dog sizes (wither height) calculate with femurs from Almendralejo Street, 41. The starts indicate remains with pathologies of broken bones with posterior fusion (from Bustamante, Heras & Detry, 2021)

4.3. Roman ritual?

Some characteristics could lead to think in the possibility of a ritual of this deposit. The remains deposition following a marked pattern (Fig. 5) starting with the deposition of four funerary urns and ending with a closure with stones (Bustamante et al., 2021). Immediately after to the funerary urns, there was a little sediment layer upon where two humans were arranged in anatomical connection. The attached humans had a torsion of their bodies (Fig. 6) and flexed legs, and were covered by a sediment layer. Follow to this, until 40 dogs together three human skulls that were covered by herbivores and ceramic remains.

Ceramics respond to an intentional and single deposition of the Nero's Empire, with a preference for vessels and plates for the preparation and presentation of foods, as well as amphorae and votive items. On the pieces there was not any presence of thermo-alteration. Animal remains did not present alteration by inclement weather. Any herbivore is complete, only dogs. Among dogs, some have pathologies in long bones, this is, healed broken bones, especially in little dogs.

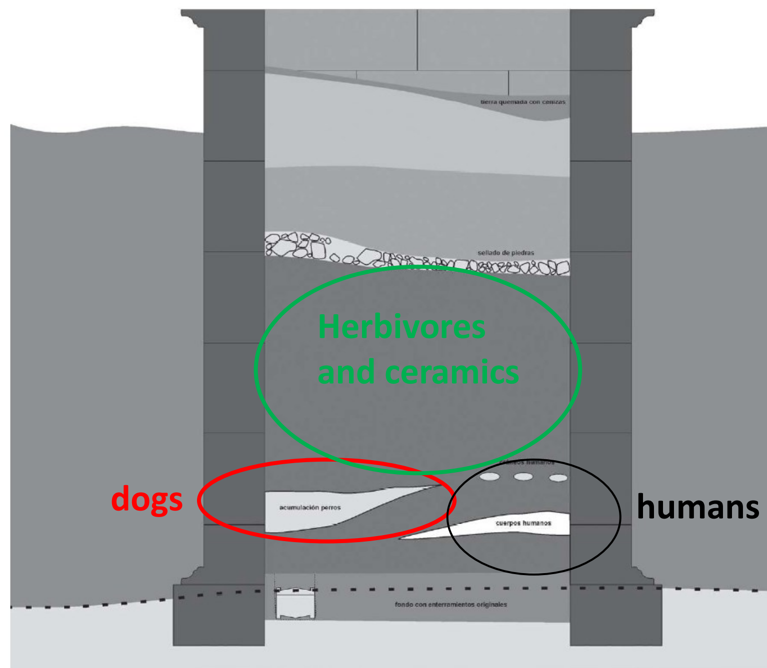

Figure 5 Funerary monument stratigraphy (from Bustamante, Heras & Detry 2021)

According to Perri (2017), the deposition of these dogs corresponds to a ritualistic context (Component). An idea already discusses by Bustamante and co-authors (2021). Other Iberian sites have presented similar pathways, Llanos del Pretorio (Cordoba) and Vila de Madrid (Barcelona). At Llanos del Pretorio (Martínez Sánchez et al., 2020), in less number than in Merida, the dog remains have a tall from 12 cm (individual of 1 to 3 months) to 51.7/52.8 cm and in one is present a hyperdislocation of the atlantoaxial joint. The isotopical analysis shows a baseline C3-plant type diet with some influence of C4-plant type, although the water efficiency is another option known the localization of the settlement.

At Vila the Madrid (Colominas, 2007, 2016) several dogs were found. They brought a wither height between 25 and 61 cm. They were not associated with any human burial, and the context has been pointed out as a *collegia funeracticia*. Also associated to other faunal remains, especially with horse.

The term *sacrificium* is a compound of the sentence *sacrum facere* ('to render sacred'), this belongs to the gods (Schultz, 2016), and it comprise a ritual with steps that culminates with the slaughtered of an animal or object. Usually, the offerings in sacrifices of the Roman world consisted in edibles with prevalence of species in diet, not being limited to those among poor people. Thus, grave goods in Roman world were foods and instrumental ablative, especially vessels that pour out liquid and plates of everyday ware used to serve food that contains a lot of liquid (Schultz, 2016 p. 65). Also votive animals

as clay animals, as well as dogs (several puppies), deer, beaver, tortoise, birds and lizards, animals that were not regularly eaten. Although it was not common, human sacrifices were done under the concept of *devotio*. An act restricted to a single family and with military connotations, a 'self-sacrifice'.

Then, the presence in the burial of at least one horse –a war animal with high status (Colominas, 2007; Schultz, 2016)–, the non-skimping in ceramic resources –especially vessels an plates for serving food–, the deposition of cattle –an animal so expensive (Schultz, 2016)–, of deer, birds and rabbits –chased animals–, a mongoose, and around 40 dogs, seem be in relation with some type of chthonic rite.

Dogs have been relaxed with Asklepious and Nodens (Colominas, 2016), the Celtic version of Mars. Mars, deity of the war, is in relationship with dogs. Accepting the risk that runs, the ritual may was the duel end where it was consecrated the deceased, he was likely an important soldier with high status social given the non-skimping in offerings. The chronology would put it in some heroic feat with Lusitania conquest. An important person who even deserved a *devotio* (Fig. 8 where human is in atypical position, as if it has been throw).

Figure 6 Human burial inside of the monument at Merida (from Bustamante, Heras & Detry 2021)

5. Conclusions

Dogs in Roman Empire had a great variety of roles, with the status connotation that each one of them had. In this case, the use with ritual meaning is very clear. However, this use was not the reason for breeding them. The presence of healed broken bones denote their fraternal link with some human as pets (MacKinnon, 2010).

Here, we present the isotopical data that put the analysed dog in the same care that humans when they were alive, both as pets, hunters or shepherds, where similar diet is shown. The scarcity of middle size dogs in the ritual context would present the sample as pets or

house guard, since middle dogs would be more related with work roles. Therefore, pets and guardhouse would have a most important link with humans, a status that would confer the same role in the Other Life. Companion and protection are the roles proposed in this research both in life and in death.

This role was not always thus. The diet evolution shows as in primary pastoralist epochs the dogs were fed in more cereal sense, likely due to livestock guard and sheepherding character, given them a tool status. It was in more advanced metallurgic periods when the dog status was diversified, with the first size variation in Bronze Age. That has been confirmed by isotopical analysis. However, further research are necessary.

Genetic analysis, besides, can put on the table if the Roman dogs slaughtered required of some phenotypical characteristics. For example, Schultz (2016) gives the detail for one red dog in the Robigalia Roman fest.

6. Acknowledges

I want to thank the 29545-02/SAICT/2017 project for bringing me the opportunity of participating in this research, to the Consorcio de Mérida for offering me the material and to IPHES for allowing me to use their installations.

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Supplemental Information

Text S1 What is an Isotope?

Isotopes of one element means that different atoms of this element have the same number of protons, it is the same charge, but different number of neutrons, it is different mass number (Busigny, 2015; Hurai et al., 2015; Hoefs, 2018; Albarède, 2015a). It should to have the same atomic number, that is, they have the same charge because their electronic shells must balance the nuclear charge, therefore, isotopes of one element have the same electronic configuration and, hence, very similar chemical properties (Albarède, 2015a). The term "isotopes" derived from Greek, means "equal places", and indicates that isotopes occupy the same position in the periodic table (Hoefs, 2018). Isotope defines the various forms of a chemical element determined to have distinct atomic weights (Busigny, 2015). The form ${}^{m}_{n}E$ is the formally isotope description, where E is the element abbreviation, the superscript "m" denotes the mass number (sum of protons and neutrons) and the subscript "n" denotes the atomic number equivalent to that of protons (Hurai et al., 2015; Hoefs, 2018), therefore, in all isotopes of the same element "m" will change and "n" will be the same in all.

There are two fundamental isotope kinds, stable and unstable (or radioactive), where about 300 stable isotopes (from 81 elements in natural environments) whilst 1200 unstable isotopes have been discovered. Only 21 elements are pure elements, in the sense that they have only one stable isotope (Busigny, 2015; Hoefs, 2018). The term "stable" is relative and depending on the detection limits of radioactive decay times (Hoefs, 2018), their stability is characterized by several important rules, of which two of them are the symmetry rule and the so-called "Oddo-Harkins" rule (1914-1917). The first one rule (Hoefs, 2018) states that in stable nuclide with low atomic number, the number of protons is approximately equal to the number of neutrons, or the neutron-to-proton ratio N/Z, that is approximately equal to unity. While in stable nuclide with 20 or more protons, the ratio N/Z is always greater than unity with a maximum value of about 1.5 for the heaviest nuclei. The N/Z ratio for hydrogen is 0 and for helium is 0.5. The other one rule (Hurai et al., 2015; Hoefs, 2018) states that, except for lithium, nuclides of even atomic number are more abundant than those with odd numbers.

Isotope abundance in a compound is a variation produced by radioactive decay and by isotope fractionation (Hoefs, 2018). Fractionation is the partitioning of a chemical species into two or more phases or compounds based on its physicochemical properties (Stern, 2015). Hence, the isotopic fractionation between two species or phases A and B represent the difference between their isotope compositions (Busigny, 2015). Thus, isotope fractionation refers to subtle variations of isotopic abundances (Albarède, 2015b) among different compounds or phases, and results from slightly different chemical properties among the isotopes of a single element. Their mass differences induce variations in terms of bound energy and chemical reactivity (Busigny, 2015). In a specific process, stable isotopes are thus fractionated according to either equilibrium or kinetic reactions (below).

Electronic structure (number and arrangement of electrons) of an element essentially determines its chemical behaviour, whereas the nucleus is more or less responsible for its physical properties (Hoefs, 2018), certain differences exist in physicochemical properties due to mass differences. For instance, the addition of one neutron can depress the rate of

chemical reaction considerably as result of quantum mechanical effects, which state that the energy of a molecule is restricted to certain discrete energy levels, but the lowest level is not at the minimum of the energy curve of the molecule. The isotopic properties of a molecule (Albarède, 2015b) reflect its share of the different sorts of energy: translational, rotational, and vibrational. Thereby, molecules of the same chemical formula that have different isotopic species will have different zero-point energies, as fundamental frequency of vibration depend on the mass of the isotopes (Hoefs, 2018): the molecule of the heavy isotope will have a lower zero-point energy than the molecule of the light isotope because it has a lower vibrational frequency. The bonds formed by the light isotopes are weaker than bonds created by heavier isotopes (Albarède, 2015b; Hurai et al., 2015; Hoefs, 2018). As consequence, during a chemical reaction, the lighter molecules react faster than heavier ones because heavy isotopes also prefer the low energy states, resulting in the enrichment of reactants in heavy isotopes. Usually, the heavier isotopes are concentrated in the ions, atoms, and molecules with a higher oxidation states and/or in more condensed state (Hurai et al., 2015).

Come back to isotope fractionation, stable isotopes suffer this process due two main phenomena: (1) isotope exchange reactions (or equilibrium isotope distribution), and (2) kinetic reactions. An isotopic exchange reaction (Charnley, 2015) is an ion-molecule reaction that can transfer heavy isotopes at low temperatures in virtue of the zero-point energy between different molecules as the vibration of a molecule or isotope depends on the temperature and their masses. The second phenomena, kinetic reaction or kinetic isotope effects (KIE) (Kobayashi, 2015; Hoefs, 2018) is an incomplete and unidirectional process due to differences in chemical reactions rates as is sensitive to atomic mass at a particular position in one of the reacting species, usually molecule with a heavier isotope relative to one containing a lighter isotope.

The fractionation factor (α) is defined as the ratio of the number of any two isotopes in one chemical compound A divided by the corresponding ratio for another chemical compound B, and can be determined experimentally or predicted from theoretical calculation (Busigny, 2015; Hurai et al., 2015; Hoefs, 2018): $\alpha_{A\text{-}B} = R_A/R_B$. In the last years, it has become regular replace the fractionation factor α by the ε-value, or isotope enrichment factor, which is defined as ε=α-1 because εx1000 approximates to the fractionation in parts per thousands (‰), similar to δ-value (Hoefs, 2018).

The delta notation (δ-value) (Pinti, 2015) expresses the variation of the isotopic ratio of an element relative to a standard and it is expressed in terms of per mil (parts per thousand, ‰). With it, it is removes the need to report absolute abundances (Pinti, 2015; Hurai et al., 2015) because is less illustrative as the absolute change in most natural samples occurs at best on the second decimal place, even in fifth place. The isotopic composition of a compound is usually measured in the laboratory by conventional mass spectrometry, and is expressed as:

$$\delta^{i}E=\left(\frac{R_{SPL}}{R_{STD}}-1\right).10^{3} \text{ or } \delta^{i}E=\left(\frac{R_{SPL}-R_{STD}}{R_{STD}}\right).10^{3} \rightarrow \delta=\left(\frac{\left[\frac{\#isotope_{rare}}{\#isotope_{abundant}}\right]_{SPL}}{\left[\frac{\#isotope_{rare}}{\#isotope_{abundant}}\right]_{STD}}-1\right).10^{3}$$

where R is the ratio of the two stable isotopes of an element (E), the superscript “i” the studied isotope, SPL is the sample of interest, and STD the standard reference material.

Negative δ-values indicate that the investigated sample is depleted in the heavier isotope of an element, meaning that it is isotopically lighter (with more abundant isotope of an element) compared to the standard, and positive values that the sample is enriched in heavy isotope. The enrichment or the depletion is caused by fractionation (above). The isotope fractionation between two compounds or phases A and B represents the difference between their isotopes and does not respect to a standard (Busigny, 2015; Hurai et al., 2015; Hoefs, 2018), and can be related using δ-notation as:

$$\Delta_{A-B} = \delta_A - \delta_B \approx 10^3 ln\alpha_{A-B}$$

According to Albarède (2015b), understanding biological and physical isotopic fractionation processes is fundamental for discriminating the isotopic signatures of elements and deciphering whether these signatures have a biological or abiotic origin. Stable isotopes can be applied to trace any type of physicochemical reaction, and in this way is very useful as biomarker. Biomarker or biosignature may be related to different meanings (Javaux, 2015), depend of the field of research. Geochemistry uses the terms to refer to different molecular fossils, or palaeontology for morphological and biosedimentary fossils. *Sensu stricto*, the term biomarker refers to any chemical compound directly derived from the activity of living organisms (Thomazo & Strauss, 2015), but geology or astrobiology has been extended to include stable isotopes measured directly on organic matter and metabolic by-products derived from anabolic and catabolic reactions. Stable isotopes are a powerful tool as they provide key information on the pertinent metabolic pathways. The relevance of using the stable isotopes as biomarker hinges on the fact that life fractionates isotopes, and this arises from the activity of enzymes which help organisms to drive desirable but thermodynamically unfavourable reactions by coupling them to a favourable one.

That is why the food isotopic compositions that metabolism of an organism digests and coupled to the same is basic for the understanding of the fractionation that the organism makes. Thus, in the trophic chain, the upper organism always tends to be the most enriched relative to the other one on who has fed itself. Without forget, in addition, that the organism location at a site tends to represent an isotopic composition relative to other site to another composition given that inorganic or abiotic fractionation is not the same in the different places should to multiple causes as the temperature or the geologic composition.

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Text S2 Dietary Isotopic Fractionation in Terrestrial Ecosystems

- **^{13}C fractionation and its archaeological interpretations**

The lecture of the isotopes in diets must be done understanding the isotopic fractionation (see Text S1) over the trophic chain. In terrestrial ecosystems, the first producers are the plants. Plants introduce the C isotope by photorespiration; this is, during CO_2 fixation (Smith & Epstein, 1971; J. C. Vogel, 1980; O'Leary, 1988; Farquhar et al., 1989; Julia A. Lee-Thorp et al., 1989; Julia A. Lee-Thorp, 2008). Thus, we can find three types of photosynthesis (O'Leary, 1988; Farquhar et al., 1989; Ambrose, 1993), besides the algae and freshwater plants. Here, we will analyse two forms of photorespiration, C3 and C4 types, as they are the principals in diets around the world.

C3 plants are composite of higher and nonvascular plants. The main difference between them is the lack of a consistent epidermis with impermeable cuticle and stomata in nonvascular plants (Farquhar et al., 1989). In general terms, C3 plants transport CO_2 through stomata into the internal gas space, where it dissolves in the cell sap and diffuses to the chloroplast by the carboxylation phase (O'Leary, 1988), an irreversible step (J. C. Vogel, 1980; O'Leary, 1988). This is the beginning of the Calvin cycle, in which CO_2 is added to RUBISCO to yield PGA (Vogel, 1980). As carboxylation is an irreversible step, the fractionation occurs in this phase (Smith & Epstein, 1971; J. C. Vogel, 1980; O'Leary, 1988), and it is the major fractionation in C3 plants due "CO2 partial pressure difference imposed by the step involved" (Farquhar et al., 1989). That pressure makes that the C3 plants select more light C during photosynthesis.

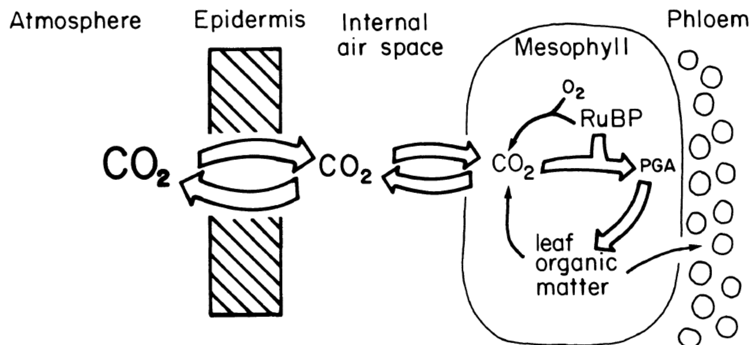

Figure 1 Steps in CO_2 fixation during C3 photosyntesis. Size of arrows show the relative fluxes through the various steps, including reverse steps. Sizes of symbols indicate the relative concentration of CO_2 at various stages (from O'Leary 1988)

On the other hand, C4 plants have two carboxylase systems in their sequential operation. CO_2 diffuses through the stomata, turned into HCO_3^- and taken up by PEP carboxylase in the mesophyll cells (O'Leary, 1988; Farquhar et al., 1989). The product is either both, malate or aspartate, and transported to the bundle sheath cells where it is cleaved to CO_2 and some other compounds (O'Leary, 1988). The CO_2 produced is taken, thus, by RUBISCO. The most important steps for fractionation in C4 plants are stomatal diffusion and PEP carboxylation. As carbon is fixed by PEP carboxylation, what discriminates against $H^{13}CO_3^-$, allows limited expression of RUBISCO discrimination by CO_2 lost (O'Leary, 1988; Farquhar et al., 1989), resulting in most enriched $\delta^{13}C$ in plants. At equilibrium, the heavier isotope concentrates in HCO_3^- (Farquhar et al., 1989).

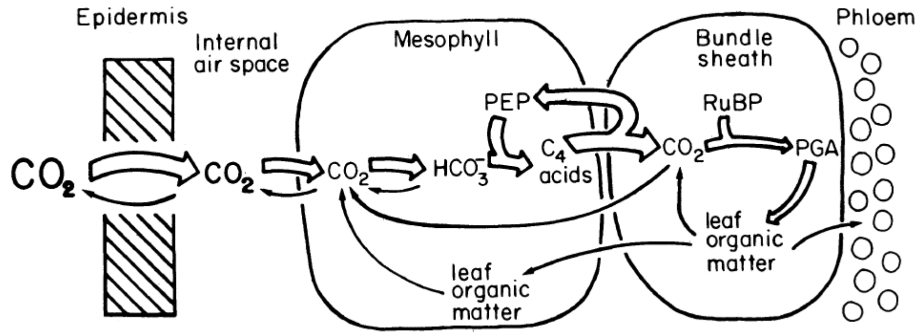

Figure 2 Important steps in CO2 fixation during C4 photosyntesis. Sizes of arrows show the relative fluxes through the various steps, including the reverse steps. Sizes of symbols reflect relative concentration of CO2 at various stages (from O'Leary 1988)

It is accepted (DeNiro & Epstein, 1978; Vogel, 1978; van der Merwe, 1982; O'Leary, 1988; Farquhar et al., 1989; Lee-Thorp, 2008) that the $\delta^{13}C$ signal range for C3 plants is -20 and -37‰ and for C4 is -6 to -20‰, in some cases may overlap. Thus, it is possible to provide the type of photosynthesis in plants, which herbivores are feeding. It has been demonstrate (DeNiro & Epstein, 1978; Vogel, 1978; DeNiro & Epstein, 1981; van der Merwe, 1982; Krueger & Sullivan, 1984; DeNiro, 1985; Lee-Thorp et al., 1989; Tieszen & Boutton, 1989; Ambrose, 1993; Lee-Thorp, 2008) that animals have an average of enrichment in isotopes regards to its diet, what allow the possibility of knowing, in general terms, the proportion of C3 or C4 plants that has been eaten. At the same way, the $\delta^{13}C$ storage in the herbivores will be incorporated in the carnivore consumers. This enrichment gradient of $\delta^{13}C$ has permitted assessing the chain trophic between producers and consumers. As it will be seen, the most complicated is to set up the omnivore diet.

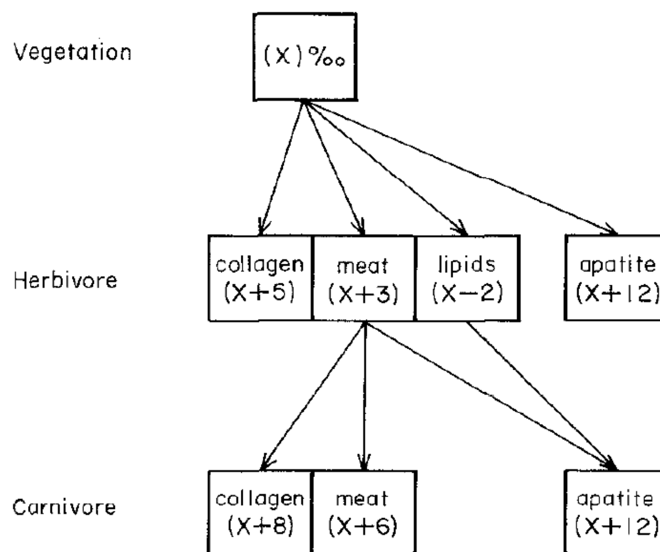

Figure 3 Foodweb model for herbivores and carnivores (from Lee-Throp 1989)

Plants do not produce the same $\delta^{13}C$ signal in all their parts (O'Leary, 1988; Farquhar et al., 1989; Tieszen & Boutton, 1989; Ambrose, 1993). The main difference between C3 and C4 plants, in order to reconstruct diets, is among trees, shrubs and grasslands, providing the classification in grazing and browser herbivores (Vogel, 1978; van der Merwe, 1982; Ambrose, 1993).

Differentiating between C3 and C4 plants, and between grazing and browser herbivores, is an important step not only to allow the paleodiets, but also to get closer to the paleoclime where these animals were moving.

Given they are not the same, first Krueger & Sullivan (1984) and after Lee-Thorp (Lee-Thorp, 1989) reconstructed the fractionation system over the trophic chain ($\Delta^{13}C_{d-c}$ and $\Delta^{13}C_{d-a}$) with the intention to differentiate between both, bioapatite and collagen isotopical signal in the same bone. Those works have been able to conclude that the herbivores have an enrichment regards to plants (X‰) of +5‰ in collagen, +3‰ in meat, -2‰ in lipids and +12‰ in apatite, while carnivores have an enrichment regards to meat's herbivores of +8‰ in collagen, +6‰ in meat and +12‰ in apatite (which also takes up from lipids) (see Text S3).

Other works (Schoeninger & DeNiro, 1984; Caut et al., 2009) state that the Δ_{d-c} is equal to 1‰, while others authors see more complicate states the Δ_{d-c} because the some aspects should take into account, as the domestic or wild context (Bocherens & Drucker, 2003) since it has values between 3.7 to 6‰ and 1.2 to 2‰, respectively. Kellner & Schoeninger (2007) fix an average in 3.6 ± 3.7‰.

- **^{15}N fractionation and archaeological applications**

On the other side, $\delta^{15}N$ has a similar process in the trophic chain, but with different fractionation in producers. While δ^{13} C is fractionated during photorespiration, $\delta^{15}N$ is during nutrition processes to make amino acids, nucleotides, chlorophyll and other nitrogenous compounds (Curtis et al., 2008). Then, plants incorporate inorganic nitrogen into organic compounds (Yoneyama et al., 2003). This is known as nitrogen autotrophy.

According to Evans (2001), it is invalid when "many studies assume that ^{15}N at natural abundances levels acts as a tracer, i.e., the isotope ratio of source nitrogen is preserved during nitrogen absorption, assimilation and translocation, and that the $\delta^{15}N$ of leaf tissues reflects that of the nitrogen source in the soil". Differences in type of N consumed, uptake mechanisms, pathways and place of assimilation, allocation and recycling of nitrogen into the plant, can discriminate and fractionate $\delta^{15}N$ and N isotopic composition (Yoneyama et al., 1991, 2003; Evans, 2001; Szpak, 2014).

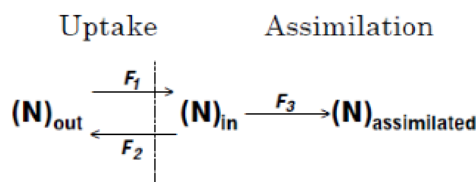

Figure 4 Discrimination observed with plant acquisition of nitrogen (from Evans 2001)

At first place, nitrogen absorption is produced from different environmental sources (Yoneyama et al., 1991, 1998, 2003; Evans, 2001), containing the plant around 1% to 6% of nitrogen in dry weight (Yoneyama et al., 1998). Although inorganic N (NH_4^+ and NO_3^-) is the most important, plants can be also catch it from manure, N_2 and/or gaseous NO_2 (Yoneyama et al., 1998, 2003), and under certain conditions as urea and amino acids (Yoneyama et al., 1998). Thus, the nitrogen may take it from soil or from atmosphere. Higher plants would take up nitrate and ammonium as sources, while nodulated plants

(mainly legumes), i.e., plants which form N_2-fixing associations, also can utilize atmospheric N_2 (Yoneyama et al., 1991).

Some experiments (Yoneyama et al., 1998, 2003; Craine et al., 2015) have demonstrated that when plant acquires nitrate (NO_3^-), this is reduced inside of the roots in ammonium (NH_4^+) and then, together exogenous ammonium, is assimilated firstly to the amide of glutamine and after to glutamic acid and aspartic acid. It is due to the coupling operation of glutamine synthetase and glutamate synthetase (GS-GOGAT) (Yoneyama et al., 1998, 2003; Evans, 2001). In each one of these assimilation steps, discrimination occurs (Yoneyama et al., 1991, 1998, 2003; Evans, 2001; Craine et al., 2015), and that increases with external concentration while decrease with plant age (Evans, 2001).

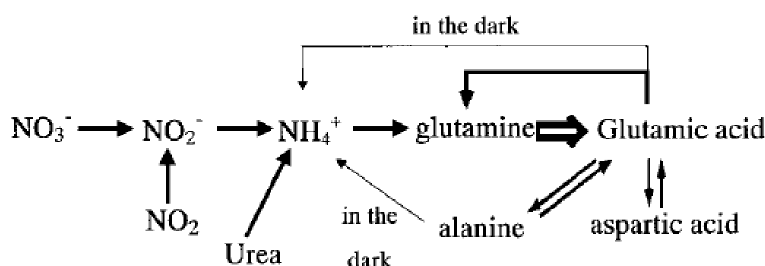

Figure 5 Nitrogen metabolism among NO_3^-, NH_4^+ and amino acids. Gaseous NO2 absorbed through stomata is largely converted into NO_2^-. Urea may be converted to NH_4^+. Transfer of glutamine amide to glutamic acid (white arrow) (from Yoneyama et al. 2003)

Regards to NO_3^-, no inherent fractionation occurs during uptake between whole plant and exogenous NO_3^- (Yoneyama et al., 1991, 1998, 2003; Evans, 2001; Craine et al., 2015). But how there are differences between roots and leaf blades, petioles and midribs (Yoneyama et al., 2003), it is assume that from the nitrate supply, the nitrate reductase and glutamine synthetase induce large fractionations (Evans, 2001; Yoneyama et al., 2003). Thus, the no assimilated nitrogen is lost through efflux and the organic ^{15}N assimilated into amino acids is transported to the plant organs.

In the case of NH_4^+, ^{15}N values of the whole plant are more negative compare with the ammonium supplies (Yoneyama et al., 1991, 2003), the same that for the different plant organs. In this case, the flux of ammonia would be the step at which fractionation occurs (Yoneyama et al., 1991) since the smaller size of ammonia gives the opportunity for isotopic discrimination regards to nitrate. It seems that NH_4^+ is assimilated into glutamine by glutamine synthetase (Yoneyama et al., 2003), and that the magnitude of the fractionation depend on the solution concentration and cultivar.

The difference between uptake and assimilation fractionation of NO_3^- and NH_4^+ is due to ammonium is taken only in roots while nitrate may be taken by roots and leaves, and this is because roots avoid the toxicity distribution of NH_4^+ (Evans, 2001), since nitrate contents in the root has been showed small (Yoneyama et al., 2003). Also, nitrate reduction has shown that this step induces large fractionation (Yoneyama et al., 1998, 2003), but only when demand is relatively low (Evans, 2001).

In nodulated plants, besides the NH_4^+ and NO_3^- fractionation pathways, we find the uptake and assimilation of N_2. In this case, the role of one type of rhizobacterium is very important. It is formed a symbiosis between root and the bacteria which look for specific flavonoids from leguminous, and recognition of these signals carries to new nodulation (*nod*) genes transcription (Stacey, 2007). Then, rhizobium makes a curl in host root and colonizes the cavity, infecting surrounding cells at the same manner. The interaction between bacteroid and cell results in a formation of a membrane-bound compartment, the nodule, which is the unit of biological N_2-fixation (*Ibid.*). Nodule incorporate fixed-N in amino acids (asparagine and glutamine) or ureides (allantoin and allentonic acid). Fractionation occurs in these nodules, being depleted in whole plant and enriched in cytosol and bacteroid (Yoneyama et al., 2003)

Mycorrhizal fungi plays an important role in the nitrogen uptake and assimilation of N_2 into plant. Many plants form symbiosis with mycorrhizae which due to their narrower diameter regards to roots are more efficient in exploring soil for nutrients (Craine et al., 2015), being capable of producing enzymes for access to N sources and make amino acids. Amino acids have lower $\delta^{15}N$ than their precursor (Evans, 2001), it is the same if is the source or the previous amino acid. Translocation of the amino acids from the fungi to the host plant seems another form of discrimination of ^{15}N (Evans, 2001; Craine et al., 2015). The reliance of the host plant to mycorrhizae increases when NH_4^+availability is low (Evans, 2001). However, even though the difference between fungi and host plant can be large (from 2‰ to 5.9‰; Evans, 2001; Szpak, 2014; Craine et al., 2015), the different $\delta^{15}N$ values between non-mycorrhizal plants and mycorrhizal-plant symbiosis are very low (Evans, 2001; Craine et al., 2015).

A very important point for archaeological interpretations is the intra-plant variations. In the first case, Yoneyama et al. (2003) show that distribution of different sources is not the same in different plant organs, coulding be 3-7‰ more enriched in leaves than in root in laboratory experiments and less than 3‰ in field studies at deciduous forest and tallgrass prairie and greater than 7‰ at warm and cold desert ecosystems (Evans, 2001). Nitrogen is incorporated into N pools for posterior transfer to growing parts and as reserve. This makes that depending of the different plant organ the ^{15}N signal may varied. Thus, the nitrogen sinks (grains) and sources (leaves and stems more than fruits and flowers) have different $\delta^{15}N$ values. The reason is that (Szpak, 2014) the different organs have different metabolic amino acids synthesis pathways, different import or export of one amino acids, or/and the catabolisms and eventual reassimilation N compounds (deamination and transamination).

Once the N pools are formed, N can be re-allocated transporting it through phloem from mature reserve organs, and it can be currently absorbed being transferred through xylem. $\delta^{15}N$ is lower in phloem than in xylem (Evans, 2001; Yoneyama et al., 2003), and $\delta^{15}N$ of xylem fluids greater than that of water-extractable soil N (Evans, 2001). Yoneyama and colleges (2003) explain that "part of the ^{15}N was effluxed in form of amino acids from mature leaves through the phloem, before its incorporation into alcohol-insoluble pool (proteins), while another part of the ^{15}N (amino acids) was effluxed after incorporation into protein and their subsequent hydrolysis". The loss of an amine group could enrich leaves but this is not likely in active growing plants (Evans, 2001). This

dynamic explain why growing leaves and fruits are dependent of the phloem import that has large content of C and N (Yoneyama et al., 2003).

It is important note that, when NO_3^- is the N source significant intra-plant variation is observed, while that when it is $NH_4^+$is observed little variation (Evans, 2001).This may be due to assimilation of the sources being roots for NH_4^+ and both roots and leaves for NO_3^- (above).

Re-growth of forage after defoliation is an important step in N re-allocation. Reserve N as well as non-structural carbohydrates in roots and stem bases are transported from the root via xylem and from the stem bases via phloem to developing shoots (Yoneyama et al., 2003).It has been suggested that 54% of N recovered in developing shoots was from stored N (*Ibid.*). Thus, the "N demand theory" explain that deficiency in plant tissues accelerates NO_3^- uptake while active synthesis and accumulation of amino acids result in repression of NO_3^-.

An important point into $\delta^{15}N$ plant fractionation is the anthropological managements of the soil. For example, the use of manure, usually enriched in ^{15}N regards animal-diet fractionation by 0.5-3‰ (Sponheimer et al., 2003a, b; Szpak, 2014), being increase during composting and storage (Szpak, 2014). This have had a correlation, but given the highly variable isotopical composition in plants, it is not possible assure a direct link between manure and elevated plant $\delta^{15}N$ values (*Ibid.*). Another important human management has been the burning/shifting cultivation, that it has been demonstrate how this practices can alter isotopical signals (+2 to +8‰) during the firsts years after burning and slowly has turned to pre-fire levels (Szpak, 2014). Treated here (for more detailed practices and mechanisms see Szpak, 2014), the last one important human isotopical modification by agricultural practices is de foddering. Due to intra-plant variation (above), the agricultural by-products (i.e. stems and leaves after grain harvest) given to husbanded animals are enriched in $\delta^{15}N$ by 1–4‰ in comparison with grains eaten by humans (Szpak, 2014).

With those all N plant pathways, we can approximate that how DeNiro & Epstein (1981) already anticipated, we can differentiate between nodulated or non-nodulated plants in $\delta^{15}N$ values because legumes have lower $\delta^{15}N$ than non-fixing N_2 plants (DeNiro & Epstein, 1981; Ambrose, 1993), although it is very low difference (above).

However, it is very common to use the $\delta^{15}N$ to know the trophic level in food webs, as well as the based diet in marine vs terrestrial foods. This last approximation has permitted see that there is a difference between 4 to 9‰ among terrestrial and marine ecosystems (Schoeninger et al., 1983; Schoeninger & DeNiro, 1984; Ambrose, 1993), thus we can approximate to differentiate the diet based in one of them, although may have an overlap of 1‰ (Schoeninger & DeNiro, 1984). However, those ranges must be taken with caution because it has been shown (Sealy et al., 1987) that terrestrial and marine ranges could overlap completely in some locations. In this case, help with $\delta^{13}C$ is complementary and required (above).

The trophic level enrichment is more discussed. According to Bocherens & Drucker (2003) there is that "emphasize the importance of using ranges of enrichment values rather than an average figure deduced" because this would compensate "for errors linked

to the uncertainties inherent to enrichment calculations, even in modern ecosystems". These uncertainties are due a several causes.

One of the most discussed causes has been the consumption of proteins. Sealy and colleges (1987) established the hypothesis that hindgut microbes can alter the $\delta^{15}N$ value in some circumstances, i.e. water and protein stress, as it is added another trophic level fractionation and urea excretion is reduced. However, these authors suggest that this is only possible in the rumen of ruminants and herbivores with hindgut fermentation and non-ruminants forestomach fermenters.

Some experiment had attempted to confirm this hypothesis. Both, water and protein stress are lead with urine excretion. Some experiments with herbivores (Sponheimer et al., 2003a, b) have demonstrate that at lower diet protein more fractionation in urine occurs, being depleted compared to diets. However, the faeces enrichment balances the urine depletion, not being different in low and high protein diet. According to Sponheimer et al. (2003a), when protein requirements of an organism are exceeded, nitrogen losses by urinary increase while faeces losses do not. However, when dietary requirements do not exceed, faecal nitrogen losses is an important part of the nitrogen efflux. Robbins et al. (2005) support this hypothesis.

Nevertheless, how is reflected the high or low protein diet in tissues? Sponheimer et al. (2003a) show that a high protein diet more enriched is $\delta^{15}N$ value in hair, which it have similar fractionation pattern as collagen (O'Connell & Hedges, 1999; Ambrose, 2000; Sponheimer et al., 2003a). However, Ambrose (2000) and Robbins et al. (2005) do not support this observation. On the other hand, heat and water stress experiments (Ambrose, 2000) gave negative results, so it is not supported by experiments. However, Fuller et al. (2005) have demonstrated that under stress conditions there are an increment of ^{15}N values. Further experiments are necessary to know the variations under protein and stress conditions.

Keeping in mind the intra-individual metabolic processes and the environmental conditions in the body offset, we can predict the $\Delta^{15}N_{coll\text{-}diet}$. The most common fractionation factor is assigned to be +3–5‰ (DeNiro & Epstein, 1981; Schoeninger et al., 1983; Minagawa & Wada, 1984; Schoeninger & DeNiro, 1984; Sealy et al., 1987; Ambrose, 1993; Hedges & Reynard, 2007; Schoeninger, 2010) while others increase the range between 1.3 and 6.9‰ (Adams & Sterner, 2000; Ambrose, 2000; Bocherens & Drucker, 2003; Caut et al., 2009; O'Connell et al., 2012), some authors fixing the fractionation factor in 2.7±0.7 (Robbins et al., 2005).

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Text S3. Collagen extraction methodologies. An overview

Actually, there does not seem to be yet any definitive method for collagen extraction that made a good quantity, and that does not harm the protein chains when delete the contaminants. However, it is possible start from the methodological knowledge more used in the laboratories for radiocarbon dating and for diets isotopical analysis in order to choose the most adequate for our study. The Jørkov's et al. (2007) study is of great help for this choice since evaluates the most employed three methods. According to them, the relationship with "the mechanisms that can alter the isotopic signal as well as with the quality indicators that are available to assess collagen preservation" is the first step (Jørkov et al., 2007:1837).

The collagen extraction departs from the Longin's (1971) method, but this methodology no removed all contaminants, both organic and inorganic. The DeNiro & Epstein's (1981) method improved when the residue treaty with hydrochloric acid (HCl) for remove mineral part is soak in sodium hydroxide (NaOH) for remove the organic part belonging to humic acids and lipids (DeNiro & Epstein, 1981; Lee-Thorp et al., 1989; Lee-Thorp, 2008; Ambrose, 1990, 1993; Liden et al., 1995). After, DeNiro & Weiner (1988) introduced the collagenase of the *Clostridium histolyticum* bacteria as collagen extracted purifier. That year, Brown et al. (1988) presented another Longin's (1971) improved method. This method was based in obtained residue ultrafiltration after submit the bone powder repeatedly in HCl for remove the insoluble residuals. Recently (Caputo et al., 2012) have been developed a method based in acetic acid extraction.

The Jørkov's et al. (2007) results show how the ultrafiltration method (Brown et al., 1988) remove great collagen proportion since only select those molecules of larger size than 30 kDa. It reduces the quantity of the resulting product. Nevertheless, the C:N rate tends to be more high than with the DeNiro & Epstein's (1981) method, independently of that this collagen extraction yields less quantity. The C and N proportions by weight regards to collagen produced also tends to be more high with Brown's (1988) method, except for N of two samples. Given the high quantity of collagen produced with the DeNiro and Epstein's (1981) method but the high elemental and isotopic proportions with Brown's et al. (1988) method, we can conclude that this is because the last method does not apply NaOH for remove the humic acids and some exogenous lipids, except phospholipids (Ambrose, 1990). So that, they are these humic acids and lipids what yield high elemental and isotopic products. Do not apply NaOH is an error that generates a false isotopic signal that affect to the diet interpretations and radiocarbon dates. The loss of the a little part of collagen by NaOH use is a risk that worth for good quality of collagen.

The isotopical signals are affected without the NaOH use by two causes. On the one hand, the lipids, insoluble organic compounds in polar organic solvents formed by a glycerol and long carbohydrates chains united by a carboxyl group, except phospholipids and glucolipids, because in the first one carbon is united to a phosphate and in the second the carbohydrates chain is shorter but they adhere monosaccharide monomers (Curtis et al., 2008). Given the high contents of carbons in lipids and the absence of nitrogen, the $\delta^{13}C$ will be affect (see Text S2). These lipids can be extracted over the methanol:clorophorm:water (Ambrose, 1990; Liden et al., 1995) and study a part (Tieszen

et al., 1983, 1989, 1993; Krueger & Sullivan, 1984; Lee-Thorp et al., 1989; Lee-Thorp, 2008), but this does not remove the humic and fluvic acids.

On the other hand, the humic and fluvic acids are organic compounds that come from the decomposition matter what can alter both carbon and nitrogen isotopic signal. Depend of the clime and weather, humic and fluvis acids may be seen affected, they producing more quantity in wet and tempered environments. The molecular weight of lipids can be remove with ultrafiltration Brown's et al. (1988) method, but the humic and fluvic acids have a molecular weight until >150,000 Da (Herrera et al., 2003), for this is important the use of NaOH to remove it.

Based on this, we have following the NaOH method (DeNiro & Epstein, 1981) improve by Schoeninger & DeNiro (1984), where it is incorporated a funnel with glass filter and a vacuum tube to do all chemical treatments of the process. Other works have demonstrate (Brown et al., 1988; DeNiro & Weiner, 1988) that the collagen extraction from a small bone fragment (20-200 mg or 10-60 mg respectively, also in bone remains of c. 10,000 ya) can be made.

A form to measure the collagen conservation of the archaeological bone remains before any "collagen" extraction is with a bone compact thin layer to plane and polarize light (Schoeninger et al., 1989) where the histologic organization will indicate the collagen fibres loss, since which organization is oriented over these fibres. Structure absence indicates a high diagenetic alteration in the collagen loss.

The extraction process consists to sawn a little fragment form the bone with a rotatory Dremel tool with a diamond-coated blade (Bocherens et al., 2015). What after will be subdued to cleaning, well soak in acetone and distilled water, and with ultrasounds (Ambrose, 1990; Bocherens et al., 2015), as well as with mechanic cleaning with a diamond tip in Dremel tool like we have done. In the first case, the acetone also will serve to remove the conservation treatments (Guiry et al., 2015) as the paraffin or the glue. Then, the samples can be rinse with distilled water, dried and grounding in maximum 0.71 mm powder (Schoeninger & DeNiro, 1984; DeNiro & Weiner, 1988; Ambrose, 1990; Bocherens et al., 1997). Seems to be that the use of virutes/chunks more than fine powder produce more "collagen" quantity (Schoeninger et al., 1989). With a mortar, we can know if there are good collagen presence (Schoeninger et al., 1989; Jørkov et al., 2007) because their absence would do more easy the fine powder production being as the apatite crystals are lost and those is the part that gives flexibility. This step is also a good indicator of conservation and diagenesis collagen.

One time done the sample cleaning, between 10 and 60 mg of the bone powder could be placed in a funnel with glass fibre filter (Schoeninger & DeNiro, 1984; Ambrose, 1990), well with Teflon stopcock of 2 mm, as with a beaker of 100 ml (Ambrose, 1990). Then, the samples can be demineralize with weak acid, among 30-40 ml of 0.1-0.3 M HCl (Brown et al., 1988; Ambrose, 1990, 1993) for 20 minutes at room temperature (Longin, 1971; DeNiro & Epstein, 1978, 1981; Schoeninger & DeNiro, 1984; DeNiro, 1985; Brown et al., 1988; DeNiro & Weiner, 1988; Ambrose, 1990; Liden et al., 1995; Bocherens et al., 1997, 2015). It is use weak acid and not hard acid (1 M HCl) because it produces more collagen quantity and remove at the same form the mineral part and fluvic

acids (Ambrose, 1990, 1993). After, the sample is soak with distilled water in a vacuum flask for neutralize (Ambrose, 1990).

Thus, the elimination of humic acid and lipids is done with the 30-40 ml of 0.125 M NaOH at room temperature for 20 minutes (DeNiro & Epstein, 1981; Schoeninger & DeNiro, 1984; DeNiro, 1985; Ambrose, 1990, 1993; Bocherens et al., 1997, 2015). After, the residue is heated at 90-96ºC while is submit in 50 ml of 10^{-3} M HCl and after 5 hours 100 µl of 1 M HCl is added in order to maintain the acidity. Then, the evaporated solution is heated for 5 hours for solubilize the “collagen” (Ambrose, 1990). The hot “collagen” broth is synthetized over glass filter in a flask of 250 ml and concentrated at 80ºC. The last step is lyophilize it by subjecting it to dry-freeze (Ambrose, 1990).

The resulting product in not pure collagen, therefore it is named “collagen” because it is different to the product extracted from the bone before the fossilization process (DeNiro & Weiner, 1988). This “collagen” will show a good conservation and low diagenesis when the C:N rate fall between 2.9-3.6 (DeNiro, 1985; Schoeninger et al., 1989; Ambrose, 1990, 1993).

Another extraction method is one that purifies with the collagenase enzyme (DeNiro & Weiner, 1988). This enzyme from the *Clostridium histolyticum* have shown that the “collagen” is susceptible to hydrolysis together him. It is a specific enzyme for native and denaturalize collagen, without relationship with other proteins. The protein substrate has the amino acid sequence proline (or hydroxiproline)-X-glicine, where X is whatever amino acid. This sequence has a high incidence in collagen and it is the request for the excision. The product of the collagenase action on the collagen are peptides of 500-700 Da (~5-7 large residual amino acids). The peptides are separate from the enzyme, from the not hydrolyzed collagen and from the organic components presents in the bone where the collagenase did not act. The peptides not dialyzable can be submit to dry-freeze, combusted, and its $\delta^{13}C$ and $\delta^{15}N$ can be determine.

The process gives them equal results in some cases follow the traditional method (DeNiro & Weiner, 1988), except for some remains of poor preservation. In this case of poor preservation, the authors propose the collagenase method due gives the same amino acids that their counterparts with the same feeding.

The Caputo’s et al. (2012) method is develop with the idea of extract whole collagen molecules. The bone fragments are cleaned by abrasion and after are heated at 100ºC by 20 hours in order to remove the superficial water and to break the covalent bonds of the collagen primary structure and the cross bonds between the collagen monomers. Thus, 25 mg of no demineralized bone powder are introduce in 2.5 ml of 0.5 M CH_3COOH. The mixture is submit to ultrasounds at 4ºC and decanted. The liquid is recover and the solid is submit of new to ultrasounds. The total liquid recovered is precipitate with 2 m NaCl, spin during 30 minutes at 4ºC. The product is analysed.

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Lab Id	Site	Chronology	Taxon	Anatomical part	Side	Col Weight (mg)	δ13C	δ15N	%C	%N	C:N	%yield
MD_01	Merida	REE	Equus caballus	Metapode	Right	0,27	-20,81	5,09	32,36	12,71	3,00	
MD_03	Merida	REE	Equus caballus	Tibia	Left	0,41	-21,47	7,24	38,09	14,11	3,10	4,14
MD_04	Merida	REE	Equus caballus	Humerus	Left	0,33	-21,14	6,28	41,28	15,24	3,20	16,83
MD_06	Merida	REE	Equus caballus	Tibia	Left	0,31	-21,18	3,91	39,79	14,13	3,30	1,77
MD_10	Merida	REE	Bos taurus	Tibia	Left	0,38	-21,31	7,32	41,87	15,85	3,10	18,18
MD_13	Merida	REE	Bos taurus	Radio	Right	0,38	-20,80	3,69	42,63	16,01	3,10	12,49
MD_18	Merida	REE	Sus sp.	Femur	Left	0,37	-21,93	4,25	41,29	15,28	3,20	2,04
MD_19	Merida	REE	Sus sp.	Tibia	Right	0,43	-20,78	6,65	35,86	13,54	3,10	15,94
MD_20	Merida	REE	Sus sp.	Femur	Left	0,28	-20,80	4,60	40,94	15,96	3,00	8,24
MD_21	Merida	REE	Sus sp.	Tibia	Left	0,36	-20,81	4,15	38,57	14,70	3,10	9,84
MD_22	Merida	REE	Sus sp.	Mandible	Left	0,36	-20,59	3,91	46,68	17,24	3,20	10,65
MD_23	Merida	REE	Sus sp.	Mandible	Right	0,32	-21,00	7,20	41,59	15,78	3,10	12,89
MD_24	Merida	REE	Sus sp.	Mandible	Right	0,33	-19,61	5,52	41,84	16,12	3,00	9,37
MD_26	Merida	REE	Ovis/Capra	Femur	Right	0,34	-21,06	9,15	37,14	15,97	2,70	15,82
MD_27	Merida	REE	Ovis/Capra	Metacarpus	Right	0,31	-19,65	11,27	39,14	15,72	2,90	16,43
MD_28	Merida	REE	Ovis/Capra	Tibia	Right	0,42	-20,95	5,29	36,19	15,61	2,70	10,11
MD_29	Merida	REE	Ovis/Capra	Humerus	Right	0,39	-20,26	8,35	37,14	14,13	3,10	14,23
MD_30	Merida	REE	Ovis/Capra	Femur	Left	0,34	-20,17	7,94	42,48	15,98	3,10	11,50
MD_31	Merida	REE	Ovis/Capra	Mandible	Right	0,33	-20,37	5,84	48,94	17,80	3,20	10,92
MD_32	Merida	REE	Ovis/Capra	Mandible	Left	0,32	-20,41	7,01	39,01	14,57	3,10	
MD_35	Merida	REE	Cervus elaphus	Humerus	Right	0,43	-19,60	5,29	40,85	15,85	3,00	16,99
MD_34	Merida	REE	Cervus elaphus	Tibia	Left	0,28	-20,33	4,44	38,84	15,22	3,00	16,52
MD_38	Merida	REE	Human	Tibia	Left	0,31	-18,83	10,39	39,35	15,08	3,00	10,52
MD_42	Merida	REE	Human	Mandible	Right	0,39	-20,52	8,51	44,19	16,88	3,10	12,61
MD_76	Merida	REE	Accipitridae	Humerus	Right	0,32	-20,84	10,38	43,02	14,76	3,40	15,84
MD_79	Merida	REE	Tetrax	Humerus	Left	0,40	-20,13	7,02	41,33	15,71	3,10	13,41
MD_80	Merida	REE	Anser	Femur	Left	0,31	-20,46	9,58	38,38	13,75	3,30	13,14
MD_81	Merida	REE	Alectoris	Femur	Right	0,34	-20,23	7,14	39,63	14,52	3,20	12,22

WF_36	Merida	REE	C. familiaris	Mandible	-18,60	8,50	40,45	14,81	3,20
WF_37	Merida	REE	C. familiaris	Mandible	-19,00	9,20	40,62	14,94	3,20
WF_38	Merida	REE	C. familiaris	Mandible	-18,70	9,20	40,92	15,01	3,20
WF_39	Merida	REE	C. familiaris	Mandible	-18,30	9,90	40,48	14,91	3,20

Lab ID	Conventional Radiocarbon	Cal 2σ	Cal 2σ BP	Cal 1σ	Cal 1σ BP	δ13C	δ15N	C:N	%C	%N
WF_36	1920±30 BP	3 - 138 cal AD	1947 - 1812 cal BP	56 - 125 cal AD	1894 - 1825 cal BP	-18,6	8,5	3,2	40,45	14,81
WF_37	1910±30 BP	22 - 170 cal AD	1928 - 1780 cal BP	68 - 126 cal AD	1882 - 1824 cal BP	-19,0	9,2	3,2	40,62	14,94
WF_38	1980±30 BP	45 cal BC - 77 cal AD	1994 - 1873 cal BP	2 - 60 cal BC	1951 - 1890 cal BP	-18,7	9,2	3,2	40,92	15,01
WF_39	1970±30 BP	45 cal BC - 85 cal AD	1994 - 1865 cal BP	2 - 68 cal AD	1948 - 1882 cal BP	-18,3	9,9	3,2	40,48	14,91
WF_28	560±30 BP			1307 - 1362 cal AD	643 - 588 cal BP	-18,4	11,1	3,2	40,81	14,94
LYEP9	110±30 BP	1978 - 1878 cal AD	29 - 30 cal BP	1978 - 1979 cal AD	29 - 30 cal BP	-16,5	10,8	3,4	41,72	14,31
LYEP11	3950±30 BP	2499 - 2346 cal BC	4448 - 4295 cal BP	2492 - 2454 cal BC	4441 - 4403 cal BP	-19,7	6,9	3,3	39,49	13,98
Merida-1 (Herp)	2020±30 BP	107 cal BC-58 cal AD	2056 - 1892 cal BP	50 cal BC - 22 cal AD	1999 -1928 cal BP	-16,9	9,5	3,2	41,63	15,2
GPF-1 (Herp)	2030±30 BP	114 cal BC - 52 cal AD	2063 - 1898 cal BP	56 cal BC - 20 cal AD	2005 - 1930 cal BP	-19,7	11,3	3,2	41,62	14,98

Lab Id	Site	Chronology	Taxon	δ13C	δ15N	%C	%N	C:N	%Yield	Paper
4/422	Llanos del Pretorio	REE	Human	-18,45	8,57	41,19	14,76	3,26	11,20	Martínez-Sánchez et al. 2020
1/419	Llanos del Pretorio	REE	Human	-20,08	9,82	29,99	11,10	3,15	12,10	Martínez-Sánchez et al. 2020
ALCF/III-I	Llanos del Pretorio	REE	Human	-19,65	9,93	39,75	14,77	3,14	18,30	Martínez-Sánchez et al. 2020
2/420	Llanos del Pretorio	REE	Human	-18,40	10,09	37,61	13,46	3,26	13,50	Martínez-Sánchez et al. 2020
3/421	Llanos del Pretorio	REE	Human	-18,84	11,15	35,15	12,33	3,33	7,10	Martínez-Sánchez et al. 2020
CAF67	Llanos del Pretorio	REE	C. familiaris	-19,25	10,33	38,54	13,24	3,20	12,60	Martínez-Sánchez et al. 2020
CAF359	Llanos del Pretorio	REE	C. familiaris	-17,08	9,88	28,10	10,21	2,91	5,50	Martínez-Sánchez et al. 2020
CAF535	Llanos del Pretorio	REE	C. familiaris	-19,32	9,84	5,81	2,52	2,72	1,90	Martínez-Sánchez et al. 2020
70UE143	Llanos del Pretorio	REE	C. familiaris	-19,15	10,24	26,92	9,84	3,15	7,40	Martínez-Sánchez et al. 2020
112	Can Roqueta	EIA	C. familiaris	-16,60	7,40	35,80	13,20	3,20	5,26	Albizuri et al. 2021
63	Can Roqueta	EIA	C. familiaris	-16,80	8,90	35,30	12,70	3,30	3,80	Albizuri et al. 2021
68	Can Roqueta	EIA	C. familiaris	-19,20	10,30	24,20	8,70	3,20	4,20	Albizuri et al. 2021
79	Can Roqueta	EIA	C. familiaris	-17,90	8,90	38,20	13,90	3,20	7,90	Albizuri et al. 2021
95	Can Roqueta	EIA	C. familiaris	-19,00	8,50	33,40	12,00	3,20	4,50	Albizuri et al. 2021
183	Can Roqueta	EIA	C. familiaris	-18,40	8,80	30,80	11,60	3,10	3,90	Albizuri et al. 2022
198-1	Can Roqueta	EIA	C. familiaris	-19,10	8,50	31,70	11,40	3,30	9,90	Albizuri et al. 2023
198-4	Can Roqueta	EIA	C. familiaris	-16,90	8,80	27,60	9,90	3,30	4,80	Albizuri et al. 2024
223-a (skull)	Can Roqueta	EIA	C. familiaris	-17,40	8,80	31,30	12,00	3,00	4,20	Albizuri et al. 2025
223-b (vertebra)	Can Roqueta	EIA	C. familiaris	-17,60	8,60	36,10	14,00	3,00	4,40	Albizuri et al. 2026
363	Can Roqueta	EIA	C. familiaris	-18,00	9,30	39,50	14,40	3,20	6,30	Albizuri et al. 2027
381-1	Can Roqueta	EIA	C. familiaris	-17,30	8,80	32,00	11,50	3,30	3,70	Albizuri et al. 2028
381-2	Can Roqueta	EIA	C. familiaris	-17,90	7,10	33,00	11,80	3,30	7,70	Albizuri et al. 2029
381-3	Can Roqueta	EIA	C. familiaris	-16,00	8,10	35,70	12,70	3,30	6,80	Albizuri et al. 2030
110	Can Roqueta	EIA	C. familiaris	-18,10	9,10	36,10	13,00	3,20	5,20	Albizuri et al. 2031
814	Can Roqueta	EIA	C. familiaris	-18,90	9,90	34,50	12,60	3,20	3,90	Albizuri et al. 2032
808	Can Roqueta	EIA	C. familiaris	-14,70	7,90	37,30	13,50	3,20	4,90	Albizuri et al. 2033
82	Can Roqueta	LBA/EIA	C. familiaris	-17,00	9,50	35,50	13,10	3,20	5,30	Albizuri et al. 2021
629	Can Roqueta	LBA/EIA	C. familiaris	-18,40	9,10	34,90	12,90	3,20	5,70	Albizuri et al. 2021

290-1	Can Roqueta	LBA	C. familiaris	-19,00	9,10	32,80	11,90	3,20	5,00	Albizuri et al. 2021
836-1	Can Roqueta	LBA	C. familiaris	-18,70	8,50	38,30	13,90	3,20	3,50	Albizuri et al. 2021
836-3	Can Roqueta	LBA	C. familiaris	-18,80	8,00	23,70	8,60	3,20	16,50	Albizuri et al. 2021
836-4	Can Roqueta	LBA	C. familiaris	-19,40	8,30	25,00	8,90	3,30	10,90	Albizuri et al. 2021
194	Can Roqueta	LBA	C. familiaris	-19,40	6,20	40,30	14,70	3,20	8,20	Albizuri et al. 2021
222	Can Roqueta	LBA	C. familiaris	-19,10	8,30	41,20	15,00	3,20	9,70	Albizuri et al. 2021
32	Can Roqueta	LBA	C. familiaris	-18,70	8,70	38,50	13,70	3,30	14,06	Albizuri et al. 2021
85	Can Roqueta	LBA	C. familiaris	-18,80	9,30	35,30	13,10	3,10	5,71	Albizuri et al. 2021
97	Can Roqueta	LBA	C. familiaris	-17,10	9,40	33,80	12,50	3,20	9,23	Albizuri et al. 2021
-	Can Roqueta	E-MBA	Canis cf. Lupus	-18,80	9,40	34,90	12,50	3,30	13,40	d'Anglade et al (2019)
474-1	Can Roqueta	E-MBA	C. familiaris	-19,60	8,00	25,80	8,60	3,50	5,90	d'Anglade et al (2019)
474-2	Can Roqueta	E-MBA	C. familiaris	-19,30	8,20	25,90	9,10	3,30	3,50	d'Anglade et al (2019)
475	Can Roqueta	E-MBA	C. familiaris	-18,70	7,50	34,90	12,70	3,20	6,10	d'Anglade et al (2019)
-	Can Roqueta	E-MBA	V. vulpes	-18,00	7,80	38,10	14,20	3,20	3,90	d'Anglade et al (2019)
481-1	Can Roqueta	E-MBA	C. familiaris	-19,10	7,00	30,60	11,00	3,30	4,30	d'Anglade et al (2019)
481-2	Can Roqueta	E-MBA	C. familiaris	-19,00	7,10	25,20	8,70	3,40	6,70	d'Anglade et al (2019)
505	Can Roqueta	E-MBA	C. familiaris	-19,20	7,80	12,20	33,20	3,20	4,70	d'Anglade et al (2019)
533	Can Roqueta	E-MBA	C. familiaris	-18,90	8,30	37,20	13,60	3,20	4,60	d'Anglade et al (2019)
558-1	Can Roqueta	E-MBA	C. familiaris	-19,40	7,00	41,40	15,20	3,20	13,50	d'Anglade et al (2019)
558-2	Can Roqueta	E-MBA	C. familiaris	-19,10	8,00	35,00	12,90	3,20	7,20	d'Anglade et al (2019)
584-4	Can Roqueta	E-MBA	C. familiaris	-19,80	9,10	24,40	9,10	3,10	3,50	d'Anglade et al (2019)
584-5	Can Roqueta	E-MBA	C. familiaris	-19,60	7,60	36,00	12,90	3,30	5,10	d'Anglade et al (2019)
588	Can Roqueta	E-MBA	C. familiaris	-19,30	7,50	39,80	14,60	3,20	7,70	d'Anglade et al (2019)
590	Can Roqueta	E-MBA	C. familiaris	-19,10	8,60	36,50	13,50	3,20	8,40	d'Anglade et al (2019)
591-1	Can Roqueta	E-MBA	C. familiaris	-19,60	6,50	12,60	35,20	3,30	5,40	d'Anglade et al (2019)
591-2	Can Roqueta	E-MBA	C. familiaris	-18,40	8,80	39,90	14,90	3,10	10,50	d'Anglade et al (2019)
591-3	Can Roqueta	E-MBA	C. familiaris	-18,30	7,20	41,10	15,10	3,20	14,40	d'Anglade et al (2019)
647	Can Roqueta	E-MBA	C. familiaris	-19,00	8,10	38,00	13,90	3,20	4,10	d'Anglade et al (2019)
162	Can Roqueta	E-MBA	C. familiaris	-19,30	7,90	30,20	11,00	3,20	6,50	d'Anglade et al (2019)

190	Can Roqueta	E-MBA	C. familiaris	-18,80	8,40	37,00	13,70	3,10	7,80	d'Anglade et al (2019)
60-1	Can Roqueta	E-MBA	C. familiaris	-20,00	7,50	15,40	5,20	3,50	6,70	d'Anglade et al (2019)
60-2	Can Roqueta	E-MBA	C. familiaris	-19,10	7,00	32,60	11,60	3,30	4,10	d'Anglade et al (2019)
2184	Minferri	E-MBA	V. vulpes	-19,70	10,00	39,60	14,40	3,20	9,30	d'Anglade et al (2019)
2220	Minferri	E-MBA	V. vulpes	-19,10	8,90	38,30	14,20	3,10	9,80	d'Anglade et al (2019)
7107	Minferri	E-MBA	C. familiaris	-18,50	9,10	42,70	15,50	3,20	32,20	d'Anglade et al (2019)
9059	Minferri	E-MBA	C. familiaris	-19,10	8,60	35,90	13,30	3,10	3,70	d'Anglade et al (2019)
9090	Minferri	E-MBA	C. familiaris	-18,80	9,00	37,20	13,70	3,20	9,10	d'Anglade et al (2019)
9078	Minferri	E-MBA	V. vulpes	-19,40	9,10	39,60	14,50	3,20	9,90	d'Anglade et al (2019)
9058	Minferri	E-MBA	C. familiaris	-19,30	8,50	40,40	14,90	3,20	16,70	d'Anglade et al (2019)
9093	Minferri	E-MBA	C. familiaris	-18,90	8,70	35,80	13,30	3,10	8,10	d'Anglade et al (2019)
9094	Minferri	E-MBA	C. familiaris	-19,20	8,50	38,30	14,20	3,10	9,00	d'Anglade et al (2019)
9095	Minferri	E-MBA	C. familiaris	-19,00	8,70	40,00	14,90	3,10	8,30	d'Anglade et al (2019)
9087	Minferri	E-MBA	C. familiaris	-18,80	9,30	39,90	14,90	3,10	8,60	d'Anglade et al (2019)
5240	Minferri	E-MBA	C. familiaris	-18,80	8,70	40,50	15,10	3,10	13,00	d'Anglade et al (2019)
5240	Minferri	E-MBA	C. familiaris	-18,40	9,30	41,30	15,40	3,10	16,10	d'Anglade et al (2019)
5246	Minferri	E-MBA	C. familiaris	-19,10	8,90	40,40	15,10	3,10	14,40	d'Anglade et al (2019)
S-UC 12	Arroyal I	LN	Human	-19,60	9,20					Jones et al 2019
S-UC 28	Arroyal I	C	Human	-20,80	10,30					Jones et al 2019
S-UC 13	Arroyal I	C	Human	-19,30	9,20					Jones et al 2019
S-UC 14	Arroyal I	C	Human	-19,50	10,90					Jones et al 2019
S-UC 15	Arroyal I	LN	Human	-19,70	9,20					Jones et al 2019
S-UC 16	Arroyal I	C	Human	-19,30	9,20					Jones et al 2019
S-UC 17	Arroyal I	C	Human	-19,60	8,70					Jones et al 2019
S-UC 18	Arroyal I	C	Human	-19,50	9,30					Jones et al 2019
S-UC 19	Arroyal I	C	Human	-19,30	9,90					Jones et al 2019
S-UC 24	Arroyal I	C	C. familiaris	-19,70	9,20					Jones et al 2019
S-UC262	El Hornazo	C	Human	-19,20	8,60					Jones et al 2019
S-UC263	El Hornazo	C	Human	-19,70	9,50					Jones et al 2019

S-UC265	El Hornazo	C	C. familiaris	-19,10	8,20				Jones et al 2019
S-UC274	El Hornazo	C	C. familiaris	-20,70	6,60				Jones et al 2019
S-UC276	El Hornazo	C	C. familiaris	-20,20	6,30				Jones et al 2019
S-UC277	El Hornazo	C	C. familiaris	-20,50	6,40				Jones et al 2019
S-UC279	Fuente Celada	C	Human	-19,30	8,90				Jones et al 2019
S-UC280	Fuente Celada	C	Human	-19,20	9,20				Jones et al 2019
S-UC281	Fuente Celada	C	Human	-19,50	8,30				Jones et al 2019
S-UC09	Abrigo de la Castañera	EBA	Human	-20,80	9,30				Jones et al 2019
S-UC10	Abrigo de la Castañera	EBA	Human	-21,30	9,50				Jones et al 2019
S-UC11	Abrigo de la Castañera	EBA	Human	-20,60	10,10				Jones et al 2019
CfN1	Perdigoes	LN	Canis familiaris	-19,30	9,80	33,40	12,10	3.2	Zalaite et al. (2016)
CfC1	Perdigoes	C	Canis familiaris	-19,80	10,00	0,90	3,80	6,50	Zalaite et al. (2016)
CfC2	Perdigoes	C	Canis familiaris	-20,40	7,50	25,20	8,90	3,30	Zalaite et al. (2016)
CfC3	Perdigoes	C	Canis familiaris	-19,20	9,10	15,00	5,30	3,30	Zalaite et al. (2016)
CfC4	Perdigoes	C	Canis familiaris	-18,60	8,10	38,30	13,40	3,30	Zalaite et al. (2016)

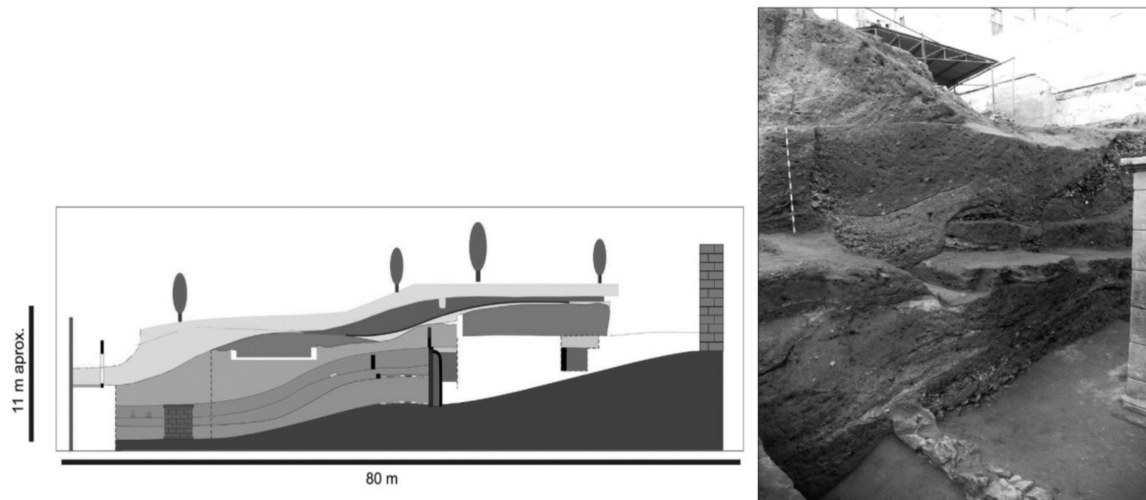

Figure S1 Stratigraphy of the trash outside of the funerary monument (from Heras & Olmedo 2011)

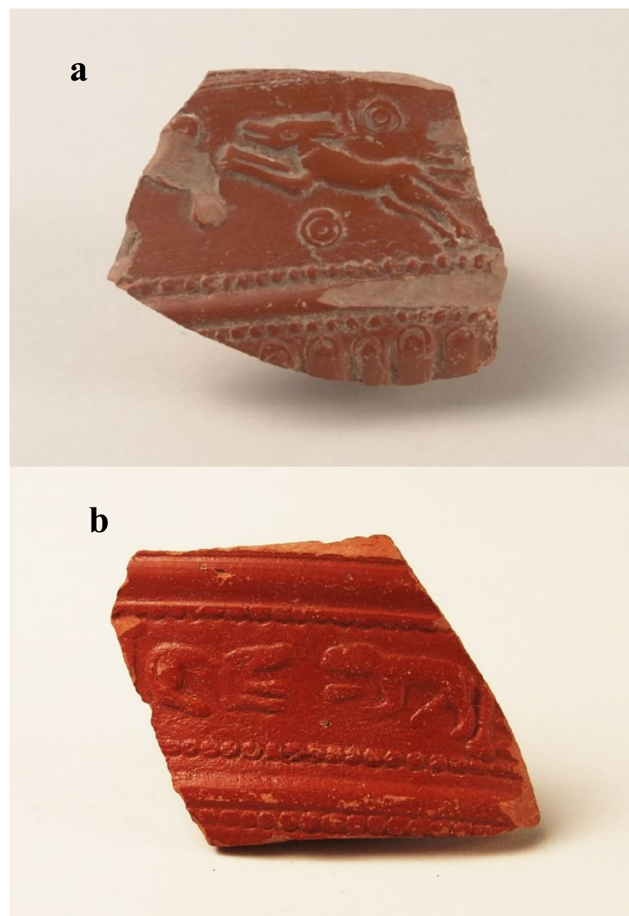

Figure S2 a) Terra Sigillata Sudgalic fragment, Merida, century I – middle II A.D. (National Museum of Roman Art, Merida); b) Terra Sigillata Sudgalic fragment, Merida, century I-middle II A.D. (National Museum of Roman Art, Merida)

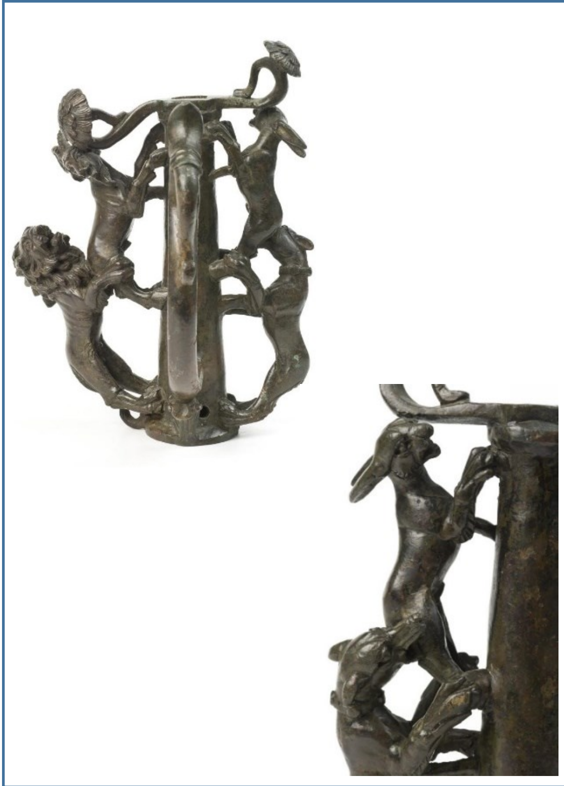

Figure S3 Bronze brake bit, Merida, century III A.D. (National Museum of Roman Art, Merida)

Figure S6 Panel 4 of the mosaic nº4 of Niebla with dog inside of red circle, Huelva, s. II A.D. Dogs also carry a belt in their neck (Huelva Museum)

Figure S5 Casa dos Repuxos, Conimbriga, Portugal, s. I A.D. (Museu Monográfico de Conimbriga – National Museum)

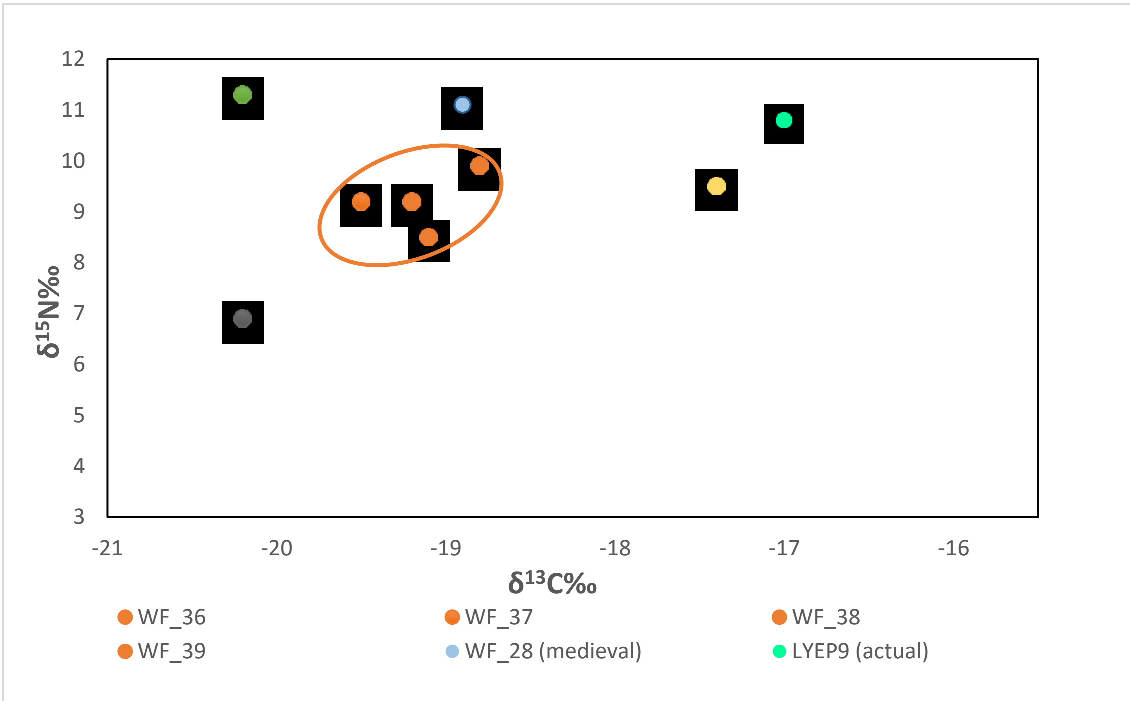

Figure S6 All canids analysed in this research

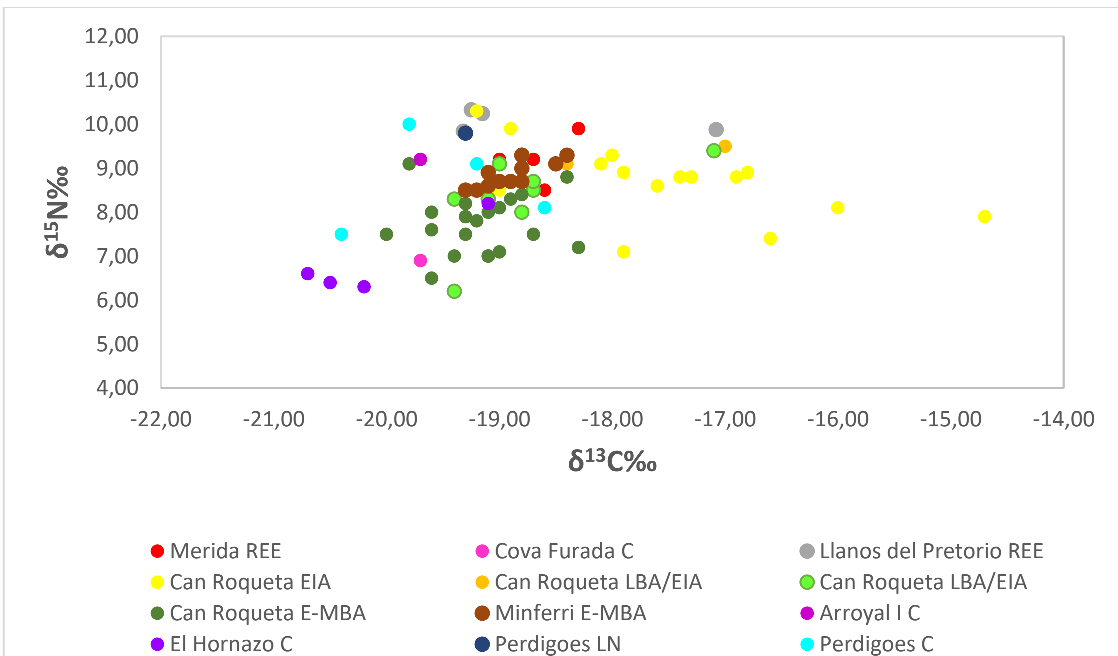

Figure S7 Comparation of the Canis familiaris in our chronologial approach

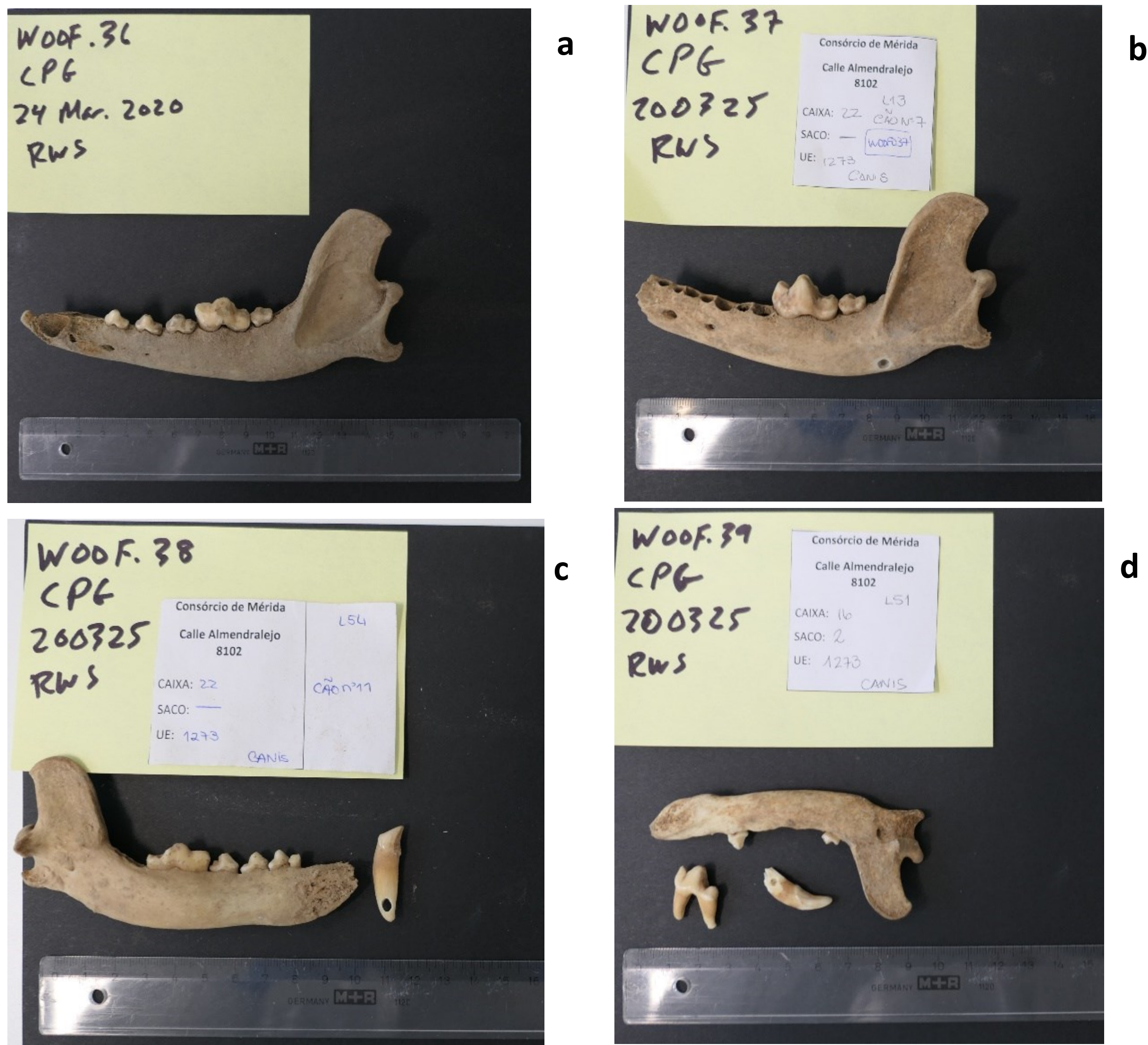

Figure S7 The four dog mandibles analysed from funerary monument at Augusta Emerita