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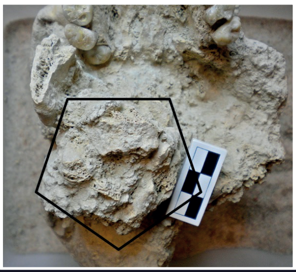


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Tomb 17 at the cemetery of Los Millares (Santa Fe de Mondújar, Almería).
Photo: Miguel A. Blanco de la Rubia.

STABLE ISOTOPE ANALYSIS OF HUMAN REMAINS FROM LOS MILLARES CEMETERY (ALMERÍA, SPAIN, *C*. 3200-2200 CAL BC): REGIONAL COMPARISONS AND DIETARY VARIABILITY

Anna J. Waterman[1], Jess L. Beck[2], Jonathan T. Thomas[3] and Robert H. Tykot[4]

Abstract:

The fortified site of Los Millares in southeastern Spain is one of the best-known Copper Age sites in southern Europe and has been studied extensively, improving our understanding of the development of political centralization and social stratification during the 3rd millennium cal BC in the Iberian Peninsula. A unique feature of Los Millares is its expansive cemetery complex, from which human remains of dozens of individuals have been recovered. While burial patterns and grave goods have been key to interpretations of the site, bioanthropological data from the skeletal remains are limited. This study uses stable isotope analysis from human bone to discern dietary patterns from 12 individuals buried at Los Millares and compares these data with other contemporary burial populations in Spain and Portugal. The goal of this study was to evaluate the dietary importance of aquatic resources and domesticated animals during this period. The results of this study point to diets mainly composed of terrestrial proteins with little marine input, despite the site's proximity to the Mediterranean Sea. While these findings are based on a small sample size and more data are needed to clarify these results, larger than expected standard deviations suggest some dietary heterogeneity within this population, with variations in protein sources and plant intake.

Keywords: Los Millares, Copper Age, Diet, Stable Isotopes, Social Complexity.

ANÁLISIS DE ISÓTOPOS ESTABLES DE RESTOS HUMANOS PROCEDENTES DE LA NECRÓPOLIS DE LOS MILLARES (ALMERÍA, ESPAÑA, *C*. 3200-2200 AC): COMPARACIONES REGIONALES Y VARIABILIDAD DIETÉTICA

Resumen:

El sitio fortificado de Los Millares en el sureste de España es uno de los yacimientos más conocidos de la Edad del Cobre en el sur de Europa y ha sido estudiado extensivamente, mejorando nuestra comprensión del desarrollo de la centralización política y la estratificación social durante el tercer milenio cal AC en la Península Ibérica. Una característica única de Los Millares es la compleja estructura del cementerio, del que se han recuperado restos humanos de numerosos individuos. Mientras que los patrones de enterramiento y los ajuares funerarios han sido claves en la interpretación del sitio, los datos bioantropológicos de los esqueletos todavía son muy limitados. Este estudio utiliza análisis de isótopos estables de restos óseos humanos para discernir los patrones dietéticos de 12 individuos enterrados en Los Millares y compara estos datos con otras muestras contemporáneas de España y Portugal. El objetivo de este estudio ha sido evaluar la importancia dietética de los recursos acuáticos y de los animales domésticos durante este período de transición. Los resultados de este estudio apuntan a dietas compuestas principalmente de proteínas terrestres con poco aporte marino, a pesar de la relativa proximidad del sitio con el Mar Mediterráneo. Existen algunas desviaciones estándar que son más grandes que las esperadas lo cual sugieren una heterogeneidad dietética dentro de esta población, con variaciones en los recursos de proteínas y en la consumición de plantas.

Palabras Clave: Los Millares, Edad del Cobre, Dieta, Isótopos Estables, Estratificación Social.

[1] Department of Natural and Applied Sciences, Mount Mercy University, IA, USA. [awaterman@mtmercy.edu]

[2] Center for Comparative Archaeology, University of Pittsburgh, PA, USA.

[3] Department of Rhetoric, University of Iowa, IA, USA.

[4] Department of Anthropology, University of South Florida, FL, USA.

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1. INTRODUCTION

The archaeological record for Los Millares provides evidence of increasing political centralization and social stratification during the 3rd millennium cal BC before the eventual abandonment of the site in the Early Bronze Age. Despite its importance as a center of political and economic activity in this region and the expansive cemetery complex, little is known about the biological histories of Los Millares' inhabitants. This study uses stable isotope analysis from human bone to discern dietary patterns from 12 individuals buried at Los Millares and compares these data with other contemporary burial populations in Spain and Portugal. The goal of this study is to evaluate the dietary importance of aquatic resources and domesticated animals during this time as information about dietary patterns and breadth may be useful for understanding the relationships between subsistence patterns and social differentiation during this dynamic time period.

2. LOS MILLARES BACKGROUND

Los Millares, interpretations of which have ranged from an egalitarian (Almagro and Arribas, 1963) to a state-level society (Molina *et al.*, 2004), is one of the most significant Copper Age sites in Europe not only because of its sheer size and well-documented archaeological record, but because of the central theoretical role that it plays in discussions of the evolution of social inequality and complex societies in Late Prehistoric southern Iberia (Gilman, 1976, 1981, 1987, 1996, 2001; Chapman, 1981, 2003; Arribas and Molina, 1984; Díaz-del-Río, 2004; Molina *et al.*, 2004; Esquivel and Navas, 2007; Navas *et al.*, 2008). First excavated in 1891 by Luis Siret (see Siret, 1893a, 1893b), the site is a seven ha walled settlement located at the confluence of the Andarax and Huéchar rivers in Santa Fe de Mondújar in the province of Almería, 20 km from where the Andarax meets the Mediterranean Sea (Fig. 1).

Los Millares was maintained and occupied between 3200-2200 cal BC, with several distinct phases of construction and habitation. The citadel was occupied during Phases I and II, and the three walls and forts were constructed later, during Phase III (Esquivel and Navas, 2007). Over time the site slowly declined and by 2500 BC each of the walls except the citadel had been abandoned. At this point in time, defensive forts (fortines) were erected on the hilltops surrounding the settlements, and by the beginning of the Bronze Age the entire site was abandoned (Díaz-del-Río, 2011).

While Los Millares is similar to other Late Neolithic and Copper Age political centers with large-scale defensive fortifications in Iberia (e.g., Zambujal, Vila Nova de Sao Pedro, Leceia, Santa Justa, and Monte da Tumba), it is unique in its association with an expansive megalithic cemetery complex. The cemetery is located outside of the settlement area, and extends 13 ha beyond the walls of the settlement. It is comprised of dozens of circular burial chambers

Fig. 1. Map of Iberian Peninsula showing location of Los Millares.

(*tholoi*) containing numerous rare imported burial goods such as ostrich eggshell, ivory, amber, and greenstone (Almagro and Arribas, 1963; Gilman, 1976; Harrison and Gilman, 1977). The site also encompasses 13 forts scattered on the hilltops to the southwest of the town, which overlook the course of the Huéchar River.

The inhabitants of Los Millares were mixed agriculturalists who devoted substantial effort to livestock management. The higher number of female animals recovered from the site indicates a strong reliance on secondary products, and Navas *et al*. (2008) suggest that the focus on animal husbandry at Los Millares likely also affected the political and economic organization of the settlement, with domestic husbandry organized by individuals or groups responsible for administering cattle and their resources and assigning and organizing labor.

Settlement architecture includes an inner citadel surrounded by four lines of fortifications, all built of local limestone. The exterior-most wall line contains both internal structures and external bastions, as well as an impressive barbican entrance that admits only one person at a time, limiting access to the interior of the settlement. These physical fortifications may have been bolstered by symbolic fortifications –Oliveira Jorge highlights that the location of the cemetery immediately outside of the exterior–most wall likely acted as a line of preliminary defense, with potential trespassers forced to confront a dangerous mortuary space charged with symbolic potency before even reaching the walls (Oliveira Jorge, 2003: 114). The cemetery itself was one of the first areas of the site to be excavated. Pedro Flores, the foreman for Louis Siret, first excavated 24 tombs in 1892, with more following in later years. In 1943, George and Vera Leisner published information on 75 tombs, and their grave goods, that had been excavated by the Siret team. Later, between 1953 and 1954, Almagro and Arribas exposed the interior of the settlement and the wall lines, re-excavated 21 tombs, and published a detailed map of the site (Chapman, 1981).

While tombs did not vary markedly in size, the cemetery was well-studied in part due to the wealth of grave goods uncovered during early excavations. There is evidence of arsenical copper metallurgy (Harrison and Craddock, 1981; Arribas *et al*., 1987), the production and use of undecorated ceramics, Bell Beaker ceramics, and distinctly Millaran pottery incised with ocular or 'eyed' images (*symbolkeramik*). About 43 of the tombs investigated in Chapman's 1981 mortuary analysis contained 'prestige' goods of some kind, including ivory, ostrich egg shell, jet, amber, callaïs, greenstone, sea shells, fine pottery, and copper objects. Chapman underscored that prestige goods were carefully recorded by both Flores and the Leisners. His analysis revealed a marked disparity in the frequency and range of grave goods included in the sample of tombs, with some graves containing low frequencies of exotic artifacts, and others containing a greater range and frequency of such materials. In particular, he pointed to Tomb 40, that "contained the highest numbers of ivory (over a dozen) and copper (ten) objects of any tomb, as well as one example each of painted pottery and *symbolkeramik*" (Chapman, 1981:402). Likewise, Tomb 12 contained "the second largest number of ivory grave goods (eleven), 800 ostrich eggshell beads (one of only two tombs in which they occur) and the highest numbers of jet (fifteen) and amber (five) beads" (Chapman, 1981: 402).

The differential inclusion of prestige grave goods within the tombs represents one line of evidence suggesting some form of mortuary status differentiation at Los Millares. When compared to population estimates for the settlement, the limited number of burials also indicates some form of restricted mortuary treatment. Using the burial numbers reported by Siret and the Leisners of approximately 1,980 individuals, Chapman (1981) estimated a burial rate of either 1.5–2 burials per year, depending on how long the site was occupied. Thus, we can expect to only view a small sample of the population who occupied the site over time.

3. STABLE ISOTOPE ANALYSIS

Stable isotope analyses on human and animal remains are an integral part of archaeological and bioanthropological research because stable isotope data can provide a record of dietary and migration patterns. Published literature reviews of isotopic research in archaeology, including Ambrose and Krigbaum, 2003; Katzenberg, 2008; Makarewicz and Sealy, 2015; and Schoeninger and Moore, 1992, provide in depth coverage of the importance and scope of this work. In prehistoric studies, researchers are primarily concerned with stable isotopic accumulations in teeth and bone as these hard tissues are taphonomically durable and are more likely to survive in the archaeological record. When recovering stable isotope ratios from bone or tooth enamel it is important to recognize that isotopic data reflect the biological processes related to tissue formation. Dental enamel does not remodel over time and thus reflects the isotopic input during the limited window of tooth formation. In contrast, bone is constantly undergoing remodeling processes as it responds to physiological growth, stress and strain, and calcium homeostasis. While different parts of the skeleton may turn over at slightly different rates, it is generally accepted that isotopic values in bone reflect approximately the last ten years of an individual's life (Manolagas, 2000).

Bone is composed of organic protein (collagen) and inorganic minerals (apatite). Past experimental studies have demonstrated that that dietary proteins contribute to the stable isotope ratios in bone collagen (Ambrose and Norr, 1993; Richards and Hedges, 1999; Tykot, 2004), while, in contrast, bone apatite rations reflect whole diet (proteins, carbohydrates, and lipids) (Ambrose and Norr, 1993; Schwarcz, 2000; Tykot, 2002, 2004). In humans and animals $\delta^{13}C$ and $\delta^{15}N$ values from bone collagen are used to calculate the dietary protein input of C_3, C_4 (and CAM) plants and marine and terrestrial animal proteins (Schoeninger and DeNiro, 1984; Ambrose and Norr, 1993; Chisholm *et al.*, 1982; Norr, 1995; Smith and Epstein, 1971; Tykot, 2002), while in bone apatite, $\delta^{13}C$ values are used to evaluate patterns of C_3, C_4 and CAM plant consumption and marine protein input within the whole diet. When $\delta^{13}C$ data on both collagen and apatite are available, calculations of $\delta^{13}C$ collagen-apatite spacing can provide additional information about dietary patterns. In particular, comparisons of $\delta^{13}C$ in collagen and apatite can help to quantify the dietary intake of marine versus terrestrial protein and C_3 versus C_4 plants (Kellner and Schoeninger, 2007).

Investigations of oxygen isotope values ($\delta^{18}O$) from human bones are also informative about mobility and diet in prehistoric populations. In bone apatite, $\delta^{18}O$ can vary according to water sources and can therefore be used to recognize plants and animals from different geographic locations (Stuart-Williams *et al.*, 1996; White *et al.*, 2004). Additionally, $\delta^{18}O$ values can be enriched via breastmilk consumption due to the oxygen isotope fractionation between mother and offspring, and, thus, these values can be used to distinguish suckling young and weaning time periods (Fricke and O'Neil, 1996) and animal milk consumption (Lai, 2008).

4. MATERIALS AND METHODS

For this study, samples of human bone from 12 individuals from four tombs (55, 57, 74, and 63) were obtained. These remains are from collections from tombs excavated at the turn of the 20th century and housed at the Museo Arqueológico Nacional (MAN) in Madrid. Due to the fragmentary nature of the collection, and the need to select from bone fragments in accordance with museum protocol, consistent sampling from one skeletal element was not possible. Additionally, only a small number of samples were permitted to be taken for analysis due to the destructive nature of this type of bone chemistry work. The bone samples used in this study include the remains from 2 young children (3-7 years-old), 2 adolescents, and 8 adults. No fauna was available for comparative isotopic analysis, and, at the time of this study, the Los Millares human remains from these tombs had not been fully analyzed. However, in 2011, an analysis of the collection was published (Peña Romo, 2011). For the tombs used in this analysis, this publication reported the following demographic data: Tomb 55 contained 8 adults, 8 nonadults, Tomb 57 contained 25 adults, 15 nonadults, Tomb 63 contained one adult and one nonadult, and Tomb 74 contained 5 adults and 2 nonadults. It was not possible to assess biological sex for any of the individuals interred in these tombs (Peña Romo, 2011).

Each of the bone fragments were prepared and analyzed at the Laboratory for Archaeological Science at the University of South Florida. Bone collagen was extracted by demineralizing whole bone using 2% hydrochloric acid for 72 hours, dissolving base-soluble contaminants using 0.1 M sodium hydroxide (24 hours before and after demineralization), and separating residual lipids with a mixture of methanol, chloroform and water for 24 hours. Collagen pseudomorphs were analyzed for carbon and nitrogen isotopes using a CHN analyzed coupled with a Finnigan MAT Delta Plus stable isotope ratio mass spectrometer using continuous flow. Along with visual analyses and data from the sample preparation, C:N ratios of the analyzed gases were calculated to determine the preservation of collagen and the reliability of the isotope results. Apatite samples were also extracted using established techniques, with removal of organic components using sodium hypochlorite (24 hrs for enamel, 72 hrs for apatite), and of non-biogenic carbonates using buffered 1 M acetic acid (24 hrs). Apatite and enamel samples were analyzed with a second Finnigan MAT Delta Plus instrument using a Kiel III device with 100% phosphoric acid at 90° C. For both collagen and apatite results, carbon and nitrogen isotope ratios are reported using the delta (*) notation, in parts per mil (‰) relative to the PDB and AIR standards respectively. The precision of the results is approximately ±0.1‰ for δ^{13}C and ±0.2‰ for δ^{15}N. Further information on isotope analysis methods has been published elsewhere (see Tykot, 2006 and references therein).

5. RESULTS AND DISCUSSION

The results from the collagen and apatite analyses are presented in Table 1. No reliable collagen yields were obtained for individuals 57.25 and 74.1. Collagen yields for the $\delta^{13}C_{co}$ ranged from -20.2 to -18.9‰ (average -19.5 ± 0.4‰). The δ^{15}N range was 8.8 to 10.5‰ (average 9.8 ± 0.6‰). For the apatite, $\delta^{13}C_{ap}$ ranged from -13.7 to -8.4‰ (average -11.3 ± 2.0‰) and the δ^{18}O ranged -6.4 to -2.8‰ (average -4.8 ± 1.2‰). Collagen-apatite spacing ranged from 6.5 to 11.2‰ (average 8.4 ± 1.9‰). For three samples the collagen-apatite spacing could not be calculated because of missing collagen or apatite data.

5.1. COLLAGEN RESULTS

When considering the collagen data, the $\delta^{13}C_{co}$ and the δ^{15}N ranges suggest a diet based on C_3 plants and terrestrial animals (Fig. 2). In general, δ^{15}N values are much higher in fish (~14‰) than in terrestrial animals, and marine predators can exhibit values as high as 20‰ (Schoeninger and DeNiro 1984; Richards and Hedges 1999). Thus, humans consuming large amount of marine proteins can be expected to exhibit much higher δ^{15}N ranges (>12 ‰), whereas the Los Millares individuals exhibited δ^{15}N ranges from 8.8 to 10.5‰. Research on terrestrial protein versus marine protein diets has found that, in general, entirely terrestrial protein diets will result in bone $\delta^{13}C_{co}$ values of -20 to -21‰, while entirely marine protein diets will result in much higher values in the -12‰ range (Schulting and Richards 2001). Collagen yields for $\delta^{13}C_{co}$ in the Los Millares individuals ranged from -20.2 to -18.9‰ (average -19.5 ± 0.4‰). Thus, we conclude that, despite the settlement's proximity to the sea (~20km), there is little evidence that marine proteins were dietary staples. However, in individuals 55.1 and 57.4, slight enrichments of $\delta^{13}C_{co}$ and δ^{15}N values, in combination with the apatite results, suggest consumption of at least some seafood. Low standard deviations for isotopic markers (< 0.3‰) point to fairly homogenous diets (Lovell *et al.*, 1986). Standard deviations at Los Millares are 0.4‰ for δ^{13}C and 0.6‰ for δ^{15}N, suggesting some variation in protein intake for its individuals. Enriched nitrogen values may be attributable to freshwater fish intake or to the consumption of omnivores, such as pigs, as these animals occupy higher trophic levels in the food web and pass this enrichment onto the consumer. Zooarchaeological analyses conducted by Navas *et al.* (2008) show high levels of exploitation

Sample	Age	USF #	$\delta^{13}C_{co}$	$\delta^{15}N$	C:N	USF #	$\delta^{13}C_{ap}$	$\delta^{18}O$	$\delta^{13}C_{co-ap}$
Ind. 55.0	Adult	12469	-19.6	9.5	3.2	12481	-13.0	-5.6	6.6
Ind. 55.1	Adult 20-35 yr	12470	-18.9	10.5	3.3	12482	-8.4	-2.8	10.5
Ind. 55.6	Adolescent	12471	-20.2	8.9	3.3	12483	-9.0	-4.7	11.2
Ind. 55.15	Child 3-7	12472	-20.0	8.8	3.1	12484	-12.6	-5.6	7.4
Ind. 57.1	Adult	12473	-19.8	9.7	3.3	12485	-12.3	-5.7	7.5
Ind. 57.4	Adult 25-35	12474	-19.2	10.4	3.3	12486	-8.5	-4.7	10.7
Ind. 57.25	Adolescent	12475	*	*	*	12487	-13.3	-4.5	*
Ind. 57.31	Child 3-7	12476	-19.6	9.5	3.2	12488	-10.7	-4.3	8.9
Ind. 74.1	Adult	12477	*	*	*	12489	-13.7	-6.4	*
Ind. 74.3	Young Adult	12478	-19.6	10.1	3.3	12490	-13.1	-5.2	6.5
Ind. 74.0	Adult	12479	-19.5	10.4	3.3	12491	-11.5	-5.9	8.0
Ind. 63.0	Adult	12480	-18.9	9.7	3.2	12492	*	*	*
		ave	-19.5	9.8			-11.5	-5.0	8.6
		sd	0.4	0.6			2.0	1.0	1.8

Table 1. Bone collagen and apatite results for Los Millares individuals

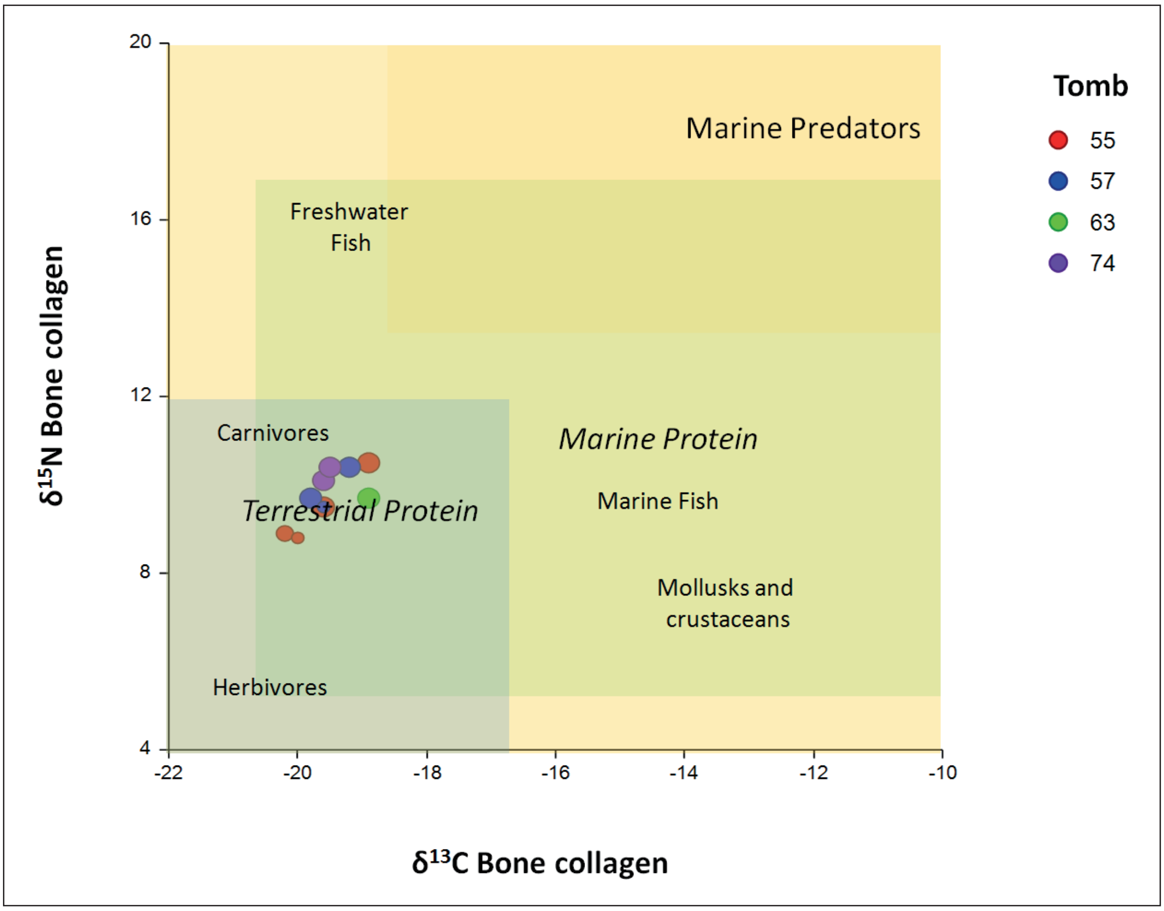

Fig. 2. $\delta^{15}N$ and $\delta^{13}C$ collagen values from the sampled humans by tomb. Large circles = adults, medium circles = adolescents and small circles = children. Generalized values for protein sources in Western Europe (after Lai, 2008).

of swine (*Sus domesticus*) in both the wall lines and the hillforts, thus the isotope values may relate to pork consumption.

Published dietary studies on prehistoric Iberian populations which use stable isotopic data have increased in the last several years, allowing for geographic and temporal comparisons with the Los Millares data (Díaz-del-Río *et al.*, 2017; Díaz-Zorita Bonilla, 2017; Fontanals-Coll *et al.*, 2015; Garcia Guixé *et al.*, 2006; McClure *et al.*, 2011, Salazar García, 2009; 2011; Waterman *et al.*, 2016a). The level of isotopic variability in the diets of the sampled humans from Los Millares is similar to other contemporaneous groups in the Iberian Peninsula and is likely indicative of mixed farming economies with supplementation from local wild resources.

The three nonadults exhibit the lowest $\delta^{15}N$ values of the sample, suggesting that they consumed slightly less meat or fish than the adults. This may indicate culturally mediated differences in adult and nonadult diet. Differences in meat distribution between adults and children are well known in the ethnographic literature (*cf.* Dettwyler, 1989). However, these lower $\delta^{15}N$ values may also represent physiological differences related to skeletal growth and development. Delayed growth has been found to lead to isotopic enrichment (Warinner and Tuross, 2010), and conversely, rapid growth during childhood may lead to isotope depletion. However, these relationships are still ill-defined. The recovery of a high percentage (30-50%) of nonadult skeletal remains is common in Neolithic and Copper Age burial throughout the Iberian Peninsula (Beck, 2016), which likely indicated a combination of high fertility and high childhood mortality rates (Waterman and Thomas, 2011). Insufficient protein intake can contribute to morbidity in juveniles, and may reflect a sampling bias as the nonadult individuals in the Iberian prehistoric mortuary record represent the children that did not survive to adulthood. However, we must also underscore that the $\delta^{15}N$ values for the nonadults in the sample are only slightly lower than adult values (1-2‰) and are on the high range of $\delta^{15}N$ for comparative populations in other parts of the Iberian Peninsula (Waterman *et al.*, 2016a).

There are clear differences between Mesolithic and Neolithic diets throughout Western Europe, with a strong reliance on marine protein shown in the former, and a near complete switch to terrestrial proteins demonstrated for the latter (Schulting and Richards, 2002; Tauber, 1983; Richards and Hedges, 1999; Stoddart *et al.*, 2009; Schulting, 2011). While we would expect that this change would be less pronounced at settlements near the sea, this shift is also found in island and coastal contexts. (Lai *et al.*, 2007; Van Strydonck *et al.*, 2002). These dietary changes were likely heavily influenced by increased availability of terrestrial protein sources which resulted from animal domestication. However, this dietary pattern could also point to a cultural shift in food valuation in these growing agricultural communities (Thomas, 2003). It is important to note that when marine protein consumption equals < 20% of protein intake, terrestrial proteins isotopic signatures may "swamp" the marine signatures due to processes of isotopic fractionation during collagen formation (Milner *et al.*, 2004). Therefore it is also possible that marine proteins were of a larger dietary importance than these data suggest.

In the Iberian Peninsula the dietary divergence between Mesolithic and Neolithic populations is strongly apparent in some regions, such as in southwestern Portugal (Carvalho and Petchey, 2013; Lubell *et al.*, 1994; Waterman *et al.*, 2016a), but not in others. For example, at the Mesolithic site of El Collado (Valencia), located Northeast of Los Millares on the Balearic coast, little evidence of dietary transition is found. Here isotopic signatures from human remains have $\delta^{13}C_{co}$ values that range from -17.6 to -19.5‰ and $\delta^{15}N$ values that range from 8.9 to 12.8‰ (N=13) (García Guixé *et al.*, 2006), which are similar to Neolithic patterns in the region. For some individuals in the El Collado sample, carbon and nitrogen values are slightly higher than those found at Los Millares, but still do not indicate that marine protein was a major dietary staple. These studies suggest that on the Mediterranean coast we may see more continuity in diets from the Mesolithic to the Copper Age, with Mesolithic populations similarly relying on terrestrial resources. The few stable isotope studies on humans from other Chalcolithic settlements on the Southeast coast show stable isotope ratios from bone collagen that are consistent with those from Los Millares (McClure *et al.*, 2011, Salazar García, 2009; 2011). Thus, while there was likely some limited marine input into the diet of populations interred at Los Millares, these data are consistent with the mixed farming economies that are suggested by the archaeological and bioarchaeological record in coastal Spain.

5.2. APATITE RESULTS

The $\delta^{13}C_{ap}$ values for the Los Millares samples exhibited more variability than the collagen values (Fig. 3). Because collagen values reflect the protein component of diet and apatite values are reflective of whole diet, this variability likely reflects differences in carbohydrate and fat intake. In strictly C_3 ecosystems, $\delta^{13}C$ apatite or enamel values should be roughly -14‰, while in C_4 ecosystems the values should be close to ~0, and intermediate values suggest mixed diets (Lai, 2008; Tykot *et al.*, 2009; Kohn and Cerling, 2002). For Los Millares three of the sampled individuals have values under -10‰, indicating the likely consumption of some carbon enriched foods. For individuals 55.1 and 57.4, slight corresponding collagen enrichment may indicate some marine protein consumption. However, the Kellner and Schoeninger (2007) model suggests that this $\delta^{13}C_{ap}$ enrichment may also be related to a C_4 energy source (carbohydrates) (Fig. 4). Thus, there is a possibility of some C_4 or CAM plant intake, or the carbon enrichment of C_3 plants due to environmental or anthropogenic factors. There are few terrestrial C_4 or CAM plants that are native to Spain, and most are inedible for humans. We know that millet, a C_4 plant, was introduced to Europe and consumed as human food and animal fodder by the Middle Bronze Age (mid 2nd millennium BC) (Rovira Buendía, 2007). However, direct evidence of millets is largely absent from the archaeological record before the 3rd millennium BC in Spain (Moreno Larrazabal *et al.*, 2015; Díaz-del-Río *et al.*, 2017). Another possibility is that humans consumed seaweed, which is also a C_4 plant, or consumed a local wild yet unidentified C_4 plant.

Other isotopic studies of Neolithic and Copper Age human bone and enamel apatite from Southwest Portugal (Waterman *et al.*, 2016a) and Central Spain (Díaz-del-Río *et al.*, 2017), have similarly found unexpected $\delta^{13}C_{ap}$ enrichment. Other possible explanations for these results may relate to natural or anthropogenic environmental alterations. Fertilizing

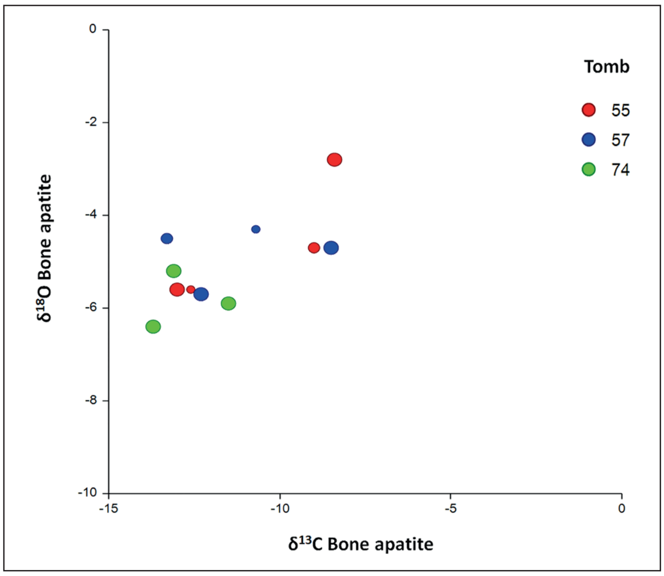

Fig. 3. Scatter plot of bone apatite values. Large circles = adults, medium circles = adolescents and small circles = children.

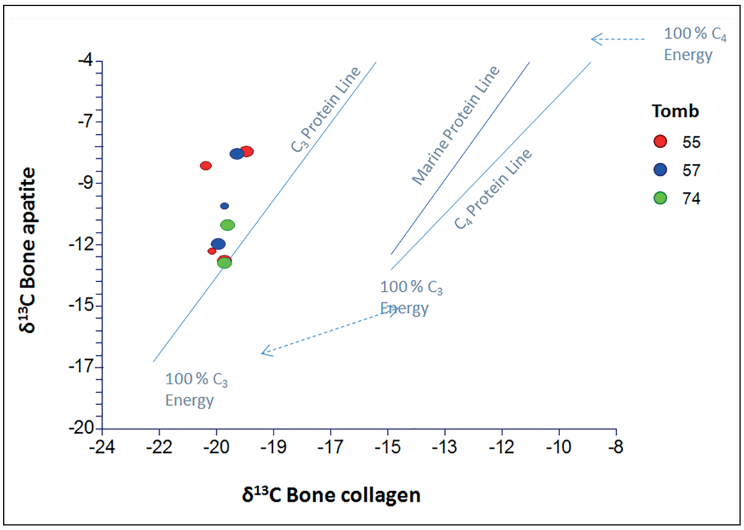

Fig. 4. Scatter plot comparing $\delta^{13}C_{co}$ and $\delta^{13}C_{ap}$ values for humans by tomb. Large circles = adults, medium circles = adolescents and small circles = children. Linear dietary model for carbon isotopes proposed by Kellner and Schoeninger (2007).

fields may lead to slight $\delta^{13}C$ enrichment in C_3 plants by increasing soil salinity (Farquhar *et al.*, 1989). Increasing aridity (Fernández-Crespo *et al.*, 2017: 331) may also lead to isotopic enrichments as plants undergo more water and heat stress. In instances of the "canopy effect," forest canopies produce more $\delta^{13}C$ depleted plants on forest floors, which are then consumed by animals. Deforestation leads to higher $\delta^{13}C$ values in some terrestrial herbivores and their human consumers as they begin to feed in more open landscapes and/or fallow agricultural fields (van der Merwe and Medina, 1991). Thus, this enrichment may be a general trend across the Iberian Peninsula due to more intensive agricultural production and climatic changes with led to increasing aridity across the region (García *et al.*, 2006; López-Sáez *et al.*, 2014; Waterman *et al.* 2016b). Alternatively, the enriched $\delta^{13}C_{ap}$ values may represent non-local individuals in these burials, as long distance trade and cultural contact is well documented at this time (*cf.* Asian ivory artifacts recovered from Los Millares [Schuhmacher *et al.*, 2009]), and recent research has identified migrant individuals in some Late Prehistoric burials in the Iberian Peninsula using $^{87}Sr/^{86}Sr$ ratios (Díaz-del-Río *et al.*, 2017; Díaz-Zorita Bonilla *et al.*, 2009; Waterman *et al.* 2014).

Concerning the $\delta^{18}O$ values, for the city of Almería, a weighted mean of -5.5‰ for $\delta^{18}O$ values in meteoric precipitation collected from 2000-2006 was reported by the Spanish Monitoring Network of Isotopes in Precipitation (REVIP) Díaz-Teijeiro *et al.* (2009). The range of $\delta^{18}O$ values for the Los Millares humans was -6.4 to -2.8‰ (average -4.8 ± 1.2‰) (Fig. 3). Differences between the modern and ancient data could relate to several factors including: isotope fractionation from water source to consumer, variability in water source, and/or climatic fluctuations over time. One individual, 55.1, exhibits strong enrichment in both $\delta^{13}C_{ap}$ and $\delta^{18}O$ values. Since oxygen isotopes reflect water intake and are dependent on local environmental conditions, these divergences may indicate that this individual was a recent immigrant into the region. The $\delta^{18}O$ enrichment in some individuals may also relate to the consumption of animal milk. As with the consumption of breast milk, consumption of animal milk may also result in higher $\delta^{18}O$ values (Lai 2008). The gene mutation for lactose persistence had spread throughout populations in Europe by this time (Gerbault *et al.* 2011) and faunal evidence suggests that secondary products, like milk, would have been an important part of the human diet at Lost Millares. However, more data on current environmental and prehistoric values in humans and animals are needed in this region to clarify these findings.

Because of the process of isotope fractionation in food webs, enrichments of $\delta^{15}N$, $\delta^{13}C_{ap}$ and $\delta^{18}O$, when found in tandem, likely relate to the consumption of higher trophic-level animals, which may include suckling young, omnivores, or marine animals. However, in the case of the sampled Los Millares individuals, the relationships of these values are less strongly patterned, suggesting more variability in the consumption of animal proteins and in plant-based carbohydrates and fats. This dietary variability fits well with the archaeological record which suggests a variety of domesticated plants and animals made up the regional subsistence base (McClure, *et al.*, 2006; Navas *et al.*, 2008). Based on the data presented in this study, in general, no clear dietary patterns are distinguishable by tomb. However, the small sample size constrains the scope of such comparisons, and, as dating on human remains from other contemporaneous tombs in Spain and Portugal has demonstrated that tombs were reused from long periods of time, the dietary differences may reflect temporal changes as well as dietary heterogeneity within the population (Aranda and Lozano, 2014, 2017; Díaz-Zorita *et al*, 2016; Waterman *et al*. 2016a). Subsequent research involving isotopic analysis of domesticated animal remains from Los Millares is being considered to further investigate these matters.

6. CONCLUSION

In order to examine dietary patterns, stable isotope values in bone collagen and apatite from 12 individuals buried at Los Millares were examined and compared with data from other contemporaneous burial populations in Spain and Portugal. Results from this study point to a diet mainly composed of terrestrial proteins with little if any marine input, despite the proximity of the site to the sea. The larger than expected standard deviations suggest dietary heterogeneity within this population with variations in both protein sources and plant intake. Dietary energy (carbohydrate) sources, as reflected in the $\delta^{13}C_{ap}$ values, are more diverse, with some of the sampled individuals exhibiting enriched $\delta^{13}C_{ap}$ values which suggest that a portion of their dietary energy may have come from C_4 or CAM plants. Alternately, these data may reflect anthropogenic environmental changes and/or climatic fluctuations. The diets of the people from Los Millares that were studied appear to be similar to those who are found at other Late Prehistoric sites in the Iberian Peninsula. This likely reflects a regional pattern of mixed agricultural production, supplemented with limited wild and aquatic resources. The obtained $\delta^{18}O$ values exhibited a broader range, suggesting some variability in water source or perhaps milk consumption. The more strongly divergent $\delta^{18}O$ values for individual 55.1 may indicate that this individual was a migrant into the region. The findings of this study are based upon a small sample size and more data are needed to clarify these results. Further investigations which include isotopic data from domesticated and wild animal remains recovered from Los Millares will help clarify these results and add to our knowledge of subsistence patterns in Late Prehistoric southeastern Spain.

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