



Dietary inferences from dental microwear patterns in Chalcolithic populations from the Iberian Peninsula: the case of El Portalón de Cueva Mayor (Sierra de Atapuerca, Burgos, Spain) and El Alto de la Huesera (Álava, Spain)

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Abstract

Dietary habits are inferred through dental microwear analysis in humans from two Chalcolithic sites located on the Iberian Northern Plateau: El Portalón de Cueva Mayor and El Alto de la Huesera. The pattern of dental microwear was established on the buccal surfaces of permanent and deciduous molars, on the bottom of facet 9 on the occlusal surface of lower molars and on the incisal surfaces of deciduous incisors. Our findings suggest that during the Chalcolithic, the diet of populations from the Northern Plateau is less abrasive than that at the Mediterranean coast, due mainly to high meat consumption. The differences in diet are related to environmental factors, which are more appropriate for animal husbandry on the Northern Plateau. The consumption of meat is not equivalent in sub-adult and adult individuals from our samples located on the Northern Plateau. Younger individuals show a harder diet with less meat intake than older ones.

Keywords Chalcolithic diet · Northern Plateau · Age-related dietary habits

Introduction

Diet reconstruction is an important class of analysis in archeology since it is closely related to behavioral and ecological factors (Clutton-Brock and Harvey 1977). These reconstructions have been attempted from different sources, including the archeological record, isotope chemistry, and dental microwear. Zooarcheological and arqueobotanical studies provide us with a general framework regarding diet when this

kind of record is abundant. Nevertheless, even in these cases, the interpretation of the relationship between archeological remains and diet is not always straightforward (Grine 2007). In any case, studies of this kind are necessary and can be discussed in light of the dietary information inferred from other sources. Among these sources, isotope and dental microwear analyses are the most used (Larsen 1997). Dental microwear analyses have proven to be a useful technique in characterizing subsistence strategies in both prehistoric and recent humans (Bullington 1991; Molleson and Jones 1991; Molleson et al. 1993; Pérez-Pérez et al. 1994; Ungar and Spencer 1999; Schmidt 2001; Romero et al. 2004; Teruyuki 2005; Mahoney 2006a, b, 2007; Galbany et al. 2008; Hogue and Melsheimer 2008; Krueger and Ungar 2009; Gamza and Irish 2012; Romero et al. 2013; El Zaatari and Hublin 2014). These studies have highlighted a close relationship between changes in dental microwear patterns and shifts in technical food processes (Romero and De Juan 2007; Salazar-García et al. 2016). Moreover, dental microwear analysis also has the potential to identify age-related changes, along with different infant-rearing practices, including the age at weaning and the age of acquisition of the adult diet (Bullington 1991; Mahoney et al. 2016; Scott and Halcrow 2017). Thus, this

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study has two main goals. The first goal of this study is to make dietary inferences through microwear analysis in Chalcolithic individuals recovered from the two archeological sites located on the Northern Plateau (Fig. 1). This kind of analysis in the Iberian Peninsula has been mainly restricted to the Mediterranean coast (Romero et al. 2004; Romero and De Juan 2007; Polo-Cerdá et al. 2007; Salazar-García 2009, 2011, 2014; Salazar-García et al. 2010, 2016; McClure et al. 2011). In contrast, these approaches are scarce in prehistoric populations from the interior of the Iberian Peninsula. Thus, the analysis of the dental microwear pattern of these two samples from the Northern Plateau and the comparison of these findings with those from the Mediterranean coast allow us to study the effect of the interaction between environmental and cultural factors on dental microwear patterns. The second goal addresses the difference in dietary habits between sub-adult and adult individuals from the Northern Plateau. Specifically, we focus on the weaning process.

Material

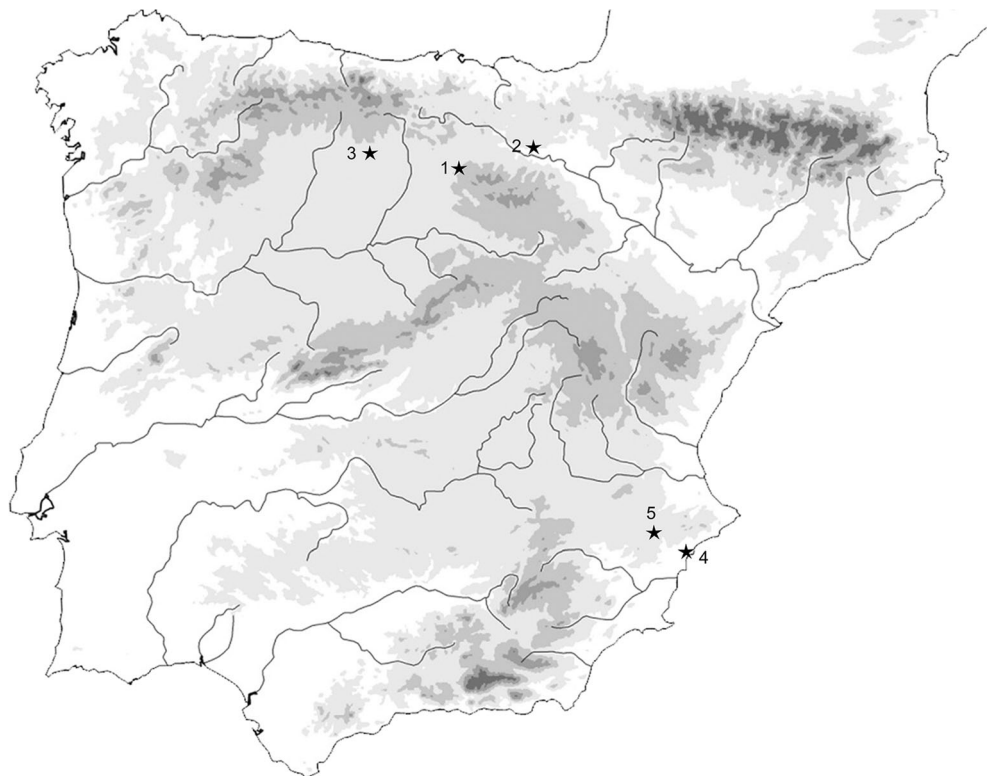
Archeological contexts of El Portalón de Cueva Mayor and El Alto de la Huesera

This study is based on the analysis of teeth from the Chalcolithic levels from two archeological sites from the

Northern Plateau (Fig. 1): El Portalón de Cueva Mayor (Sierra de Atapuerca, Burgos) and El Alto de la Huesera.

El Portalón is a Holocene archeological site located in the current entrance of the Cueva Mayor-Cueva del Silo karst system at the Sierra de Atapuerca (Spain). This site is of particular interest due to its prolonged human occupations documented from the Upper Paleolithic to historic times (Carretero et al. 2008). Its complex formation is the result of a combination of natural processes, domestic anthropogenic activities, farming, and funerary ritual practices (Alday et al. 2015; Galindo-Pellicena et al. 2014, 2017; Castilla et al. 2014; Pérez-Romero et al. 2017). The currently known stratigraphic sequence exceeds 10 m of thickness and is divided into 11 stratigraphic units grouped into two sedimentary units (Carretero et al. 2008). There is a basal sedimentary unit from the Upper Pleistocene with abundant microfauna, though evidence of macrofauna and human presence is scarce. The second sedimentary unit comprises the Holocene and is divided into ten stratigraphic units (Carretero et al. 2008). The cultural affiliation of the Holocene units, based on material records and supported by 70 radio-chronological dates, provides evidence of occupations in the Middle Ages, the Roman Age, and Iron Age I (levels 0, 1, and 2); the Final, Middle, and Early Bronze Age (levels 3/4, 5); the Chalcolithic (levels 6, 7/8); and the Neolithic/Mesolithic (level 9) (Carretero et al. 2008). The Chalcolithic stratigraphic units are divided into two phases: an Early Chalcolithic or Pre-bell beaker funerary context and a Final Chalcolithic or Campaniform phase, characterized by

Fig. 1 Archeological sites from the Iberian Peninsula mentioned in the text and whose data are used. 1: El Portalón. 2: Alto de la Huesera. 3: La Olmeda. 4: Tossal de les Basses. 5: Villena. Tossal de les Basses include sites with chronologies from Neolithic to Medieval ages. In Villena, the following archeological sites are located: Losina, Cabeza Redondo, and Molinico



herding and habitat context. A series of radiocarbon dates obtained from seeds and human and animal bones place the funerary contexts at the end of the fifth millennium BP. The funerary contexts suggest a repetitive burial activity in this cave during the Chalcolithic. This, along with a possible collapse of a large part of the roof during this period and the later habitation and stable use of the cave have contributed to the disturbances of some of the funerary structures (Pérez-Romero et al. 2017). Thus, with the exception of an unaltered burial of a complete sub-adult individual, human bones appear scattered among the limestone blocks of the tumular structure (Castilla et al. 2014). In addition to this child, the minimum number of individuals recovered from El Portalón was seven, three adults and four sub-adults (Pérez-Romero et al. 2017). Teeth object of this study represent all sub-adult individuals and two of the three adult individuals.

Among the faunal remains recovered from this burial context that have been anatomically and taxonomically identified, ovicaprines are the most dominant group (41.65%), followed by *Bos taurus* (11.15%), *Sus domesticus* (7.82%), *Canis familiaris* (1.64%), and *Equus* sp. (0.9%). In addition to these species, hunted animals (*Cervus elaphus*, *Capreolus capreolus*, *Vulpes vulpes*, Leporidae indet, and small carnivores) and very low percentages of fish and turtle shells have also been recovered (Galindo-Pellicena 2014; Pérez-Romero et al. 2017). In the habitat context, the ovicaprines (67% of the faunal remains) are the most represented livestock, followed by bovids (28%) and pigs (7%) (Galindo-Pellicena et al. 2013; 2017; Galindo-Pellicena 2014). In the case of the ovicaprine and bovids, their mortality profiles indicate that both young and elderly individuals were slaughtered (Galindo-Pellicena et al. 2013, 2014, 2017). About 50% of the ovicaprine bones show fire alteration and 90% of them show evidence of having been boiled (Galindo-Pellicena et al. 2014). Most of the fire-altered bovid (82.53%) and pig (92%) bones also show evidence of having been boiled (Galindo-Pellicena 2014). Thus, we can assert that ovicaprines, bovids, and pigs constituted the foundation of the food consumption for the inhabitants of El Portalón during both phases of the Chalcolithic (Pre-bell beaker and Campaniform).

El Alto de la Huesera, located in Laguardia, Álava (Spain), is a dolmen formed by a chamber and corridor. A minimum of 106 individuals (29 sub-adults and 77 adults) (Fernández-Crespo and de-la-Rúa 2016) were interred in a primary burial during the Final Chalcolithic (Fernández-Eraso and Mujika-Alustiza 2013). Among all these individuals, we had only access to six teeth representing a minimum number of six individuals. Neither faunal nor archeobotanical remains have been recovered from this dolmen. Nonetheless, there is a close chronological and functional relationship between El Alto de la Huesera and some archeological sites from Sierra de Cantabria (North of Spain). These sites are Peña Larga, Los Husos I and II, and San Cristobal (Fernández-Eraso and

Mujika-Alustiza 2013). The relationship between these sites was that Chalcolithic people had their stables in Peña Larga, Los Husos I and II, and San Cristobal and they are buried in El Alto de la Huesera (Fernández-Eraso and Mujika-Alustiza 2013; Alonso-Eguiluz 2012). The pollen study conducted on these sites shows that the environmental conditions were relatively dry during the Chalcolithic (Pérez Díaz et al. 2010). Agriculture is detected through the presence of pollen and grasses, though the percentages are extremely low (above 1.5% in Peña Larga and around 3% in San Cristóbal) (Pérez Díaz et al. 2010). The relatively high pollen percentages of *Chenopodiaceae*, *Plantago* sp., and *Urtica dioica*, along with some non-pollen palynomorphs, such as *Sordaria* sp. and *Sporormiella* sp., suggest an intensification of the livestock during the Chalcolithic (Pérez Díaz et al. 2010). Moreover, all these sites show levels of *fumier* (Fernández-Eraso 2010). Levels of this kind are related to animal husbandry, since they are formed as a result of piling and burning the dung in order to reduce the volume and to eliminate parasites (Angelucci et al. 2009). The *fumiers* from these sites usually contain remains of bovids and ovicaprines (Fernández-Eraso 2010). Thus, we would expect an economy mainly based on animal husbandry during the Chalcolithic in Cantabria.

Dental samples from El Portalón de Cueva Mayor and El Alto de la Huesera

In order to maximize the number of individuals analyzed, we performed the analysis of dental microwear on buccal and occlusal surfaces. We analyzed occlusal surfaces of molars and deciduous incisors. We refer as incisal surfaces these occlusal surfaces of incisors (Hillson 1996). Buccal microwear data from El Portalón were collected from a total of four teeth belonging to a minimum of two individuals. These teeth include two permanent lower right first molars, one deciduous lower right second molar, and one permanent lower third molar (Table 1). From El Alto de la Huesera, we have analyzed the buccal surface from a total of six teeth belonging to six different individuals. Five of these teeth are permanent lower first molars (three from the left side and two from the right) and one is a deciduous lower second molar (Table 1).

In the case of occlusal and incisal microwear, the data were limited to the El Portalón sample, since teeth from El Alto de la Huesera show dentine exposure over the entire occlusal surface. Data for occlusal microwear analysis were taken from the same two permanent molars from which buccal microwear was analyzed (Table 1). The sample for incisal microwear analysis consisted of six deciduous incisors belonging to a minimum of four individuals (Table 1).

The sub-adult individuals studied here were aged by assessing the stage of dental calcification and root formation (tooth mineralization). The mineralization stages of each tooth class were scored using the Moorrees et al. (1963) method.

Table 1 Specimens analyzed in this study

Specimen	Site	Tooth	Age at death	Surface analyzed
ATP11-26-H14	El Portalón	RM ₁	25–35 years ^a	Buccal and occlusal
ATP11-S304	El Portalón	RM ₃	>45 years ^a	Buccal
ATP11-635	El Portalón	Rdi ¹	14.4 months ^b	Incisal
ATP10-H2	El Portalón	Rdi ¹	27 months ^b	Incisal
ATP13-H6	El Portalón	Ldi ₁	12 months ^b	Incisal
ATP13-H7	El Portalón	Rdi ₁	12 months ^b	Incisal
ATP12-H11	El Portalón	Rdi ₁	>24 months ^b	Incisal
ATP12-65	El Portalón	Rdi ₂	15 months ^b	Incisal
ATP12-1420	El Portalón	Rdm ₂	6.6 years ^c	Buccal
ATP12-1420	El Portalón	RM ₁	6.6 years ^c	Buccal and occlusal
ADLH-4	La Huesera	Rdm ₂	5.5 ^c	Buccal
ADLH-10	La Huesera	RM ₁	11.4 years ^c	Buccal
ADLH-12	La Huesera	LM ₁	16 años ^c	Buccal
ADLH-38	La Huesera	LM ₁	>45 years ^a	Buccal
ADLH-50	La Huesera	RM ₁	>45 years ^a	Buccal
ADLH-52	La Huesera	RM ₁	35–50 years ^a	Buccal

Dental nomenclature: we employed two letters and one digit. The first letter denotes the side (R: right and L: left) and the second letter denotes the tooth and can be capital (permanent tooth) or lowercase (deciduous tooth). The digit denotes the number of tooth. If this number is a super-index, the tooth is upper and if the number is sub-index the tooth is lower

^a Dental age estimated from Lovejoy (1985) and Brothwell (1981)

^b Dental age estimated from Liversidge and Molleson (2004)

^c Dental age estimated from Moorrees et al. (1963) and Smith (1991)

The age of attainment of the different mineralization stages were interpolated from tables provided by Smith (1991). When the sex of the individual under study was unknown, we calculated the mean of age of attainment for males and for females. For the deciduous teeth, we followed the method of Liversidge and Molleson (2004).

As we have mentioned above, since neither teeth from El Portalón nor from El Alto de la Huesera are associated to skeletal remains, we could not estimate age and sex in adult individuals from classical anthropological methods. Thus, the age at death in adult individuals was estimated following the standards developed by Lovejoy (1985) and Brothwell (1981). These methods are based on the rate and patterns of dental wear, which depend on several factors besides age (White et al. 2011). When these methods have been used to test different populations, a significant correlation between known age and tooth wear has been found (Lovejoy 1985; Richards and Miller 1991). Nonetheless, we are aware that pathology or use of the teeth as tools could accelerate the wear, leading to an overestimation of the age (Milner and Larsen 1991). Thus, although we detected neither pathological signs nor evidence of using the teeth as a tool in the dental sample studied here, the age estimations for adults should be taken into account only as a gross approximation of the real age.

Comparative samples

In order to obtain a chronological and geographical perspective of the dietary habits inferred from microwear patterns in the Chalcolithic populations under study, we used several comparative obtained from Pérez-Pérez et al. (1994), Romero and de Juan (2007), and Salazar-García et al. (2016). Most samples came from the sites located in the Iberian Peninsula and span a wide chronological period ranging from the Neolithic to the Medieval ages (Table 2). Among them, seven sites are located along the Mediterranean coastline and one is on the Northern Plateau (Fig. 1).

Dietary habits have been inferred in all these samples from buccal microwear. Buccal microwear is caused by the chewing of abrasive particles along with ingested food (Pérez-Pérez et al. 1994; Romero et al. 2012). Thus, it yields a surface with scratches of different lengths and orientation but pits are rarely formed. The variables usually analyzed on buccal surfaces are the density, length and orientation of the striae. The scratch density on buccal surfaces is related to the abrasiveness of the diet of hunter-gatherer and farmer populations (Pérez-Pérez et al. 2003, 2018; Romero et al. 2013). Experimental *in vivo* reports have shown that as softer the diet the fewer microwear scratches on buccal surface (Romero et al. 2012). The length of the striations depends on several

Table 2 Microwear data for comparative purposes

Surface	Sample	Chronology	Bibliographic source
Buccal	Medieval from La Olmeda	9th–12th AC	Pérez-Pérez et al. (1994)
Buccal	Neolithic from Tossal de le Bases	4850–3800 BC	Salazar-García et al. (2016)
Buccal	Roman from Tossal de le Bases	6th–7th century AC	Salazar-García et al. (2016)
Buccal	Islamic from Tossal de le Bases	8th–9th century AC	Salazar-García et al. (2016)
Buccal	Chalcolithic from Molinico	3000–1800 BC	Romero and De Juan (2007)
Buccal	Bronze Age from Cabeza Redondo	1800–1500 BC	Romero and De Juan (2007)
Buccal	Islamic from Losina	9th–12th century AC	Romero and De Juan (2007)
Buccal	Villena	Contemporary	Romero and De Juan (2007)

AC after Christ, BC before Christ, BP before present

factors, such as the pressure applied by oral muscles, abrasiveness of particles adhered to food, or orientation of wear surface (Pérez-Pérez et al. 1994). In contrast, the relative frequency of scratches categorized by their orientation is more informative about long-term trends in dietary habits.

The sample derived from Pérez-Pérez et al. (1994) consists in adults and sub-adults from the medieval site of La Olmeda (Palencia, Spain) (Fig. 1). They studied the second deciduous molars in sub-adults younger than 12 years and permanent first molars and second premolar in older individuals. The total number of teeth analyzed was 159 (66 P4 and 93 M1). This population had an agriculturalist economy most probably based on cereals. There are differences related to age in the buccal microwear pattern. Younger individuals had higher total number of scratches than older ones, which suggest that the abrasiveness of the diet decrease with age. The decrease in the number of scratches is mainly due to a considerably decrease of horizontal striations. It could be related to a modification of dietary habits, including a higher intake of meat in older age groups.

Romero and De Juan (2007) studied buccal microwear in 80 individuals from three archeological sites, along a sample of in vivo human sample (Table 1). The teeth sample was represented by P4, M1, and M2. Although the three archeological samples had economies based on animal husbandry and agriculture, there are some differences among them in relation to the meat or vegetables that were mainly consumed. The dental sample analyzed consists of mandibular second premolars, first and second molars. Their results show a decrease in the total number of striations across the different chronological groups, which they related to a decrease on the abrasiveness of the diet due to differences in the technical processes of foodstuff.

Salazar-García et al. (2016) inferred dietary habits from buccal microwear pattern and isotope analysis of a sample from Tossal de les Bases (Alicante, Spain) (Fig. 1). Data were collected from a total of 40 individuals. Since this site had a long history of human occupation, the total sample was grouped in three different chronological sub-samples:

Neolithic, Medieval, and Late Roman. The patterns of buccal microwear were established on second premolars and first molars belonging to adult individuals. The results obtained through buccal microwear analysis showed significant statistical differences among the different chronological sub-samples: while Neolithic and Medieval had buccal surfaces with a high density of small scratches, the Late Roman group shows a low density of long scratches. Isotope values of ^{13}C and ^{15}N suggest a diet mainly based on C_3 resources during the three periods, but a higher consumption of marine resources among the Neolithic and Medieval populations than in Late Romans.

Methods

Imaging procedure

Teeth from El Portalón and La Huesera were imaged directly using an environmental scanning electron microscope (ESEM) JEOL JSM-6460LV in lower vacuum mode. The ESEM was used by García-González et al. (2015) when studying the El Mirón teeth, which indicate that the quality of images is sufficient to carry out a quantitative analysis based on dental microwear features.

Data collection

For the analysis of buccal microwear patterns, digitized micrographs were taken at a magnification of 100 \times . The number, length, and angles of all scratches were measured manually using the ImageJ software. Previously, the micrographs were first processed with the high pass filter available in GYMP software to avoid bias in the recognition of the boundaries of the different microwear features.

For methodological standardization, these measurements were taken in a selected area of 0.56 mm² (Pérez-Pérez et al. 1994, 1999). Negative angles were transformed into positive ones, by adding 180°. All observed scratches were classified by categories of orientation: vertical (67.5°–112.5°), mesio-

occlusal to distocervical (22.5° – 67.5° for right teeth and 112.5° – 157.5° for left teeth), disto-occlusal to mesio-cervical (112.5° – 157.5° for right teeth and 22.5° – 67.5° for left teeth), and horizontal (0° – 22.5° and 157.5° – 180°) (Pérez-Pérez et al. 1994, 1999). For each category, three summary variables were calculated (number, mean, and standard deviation of the length). Additionally and following the recommendations of Pérez-Pérez et al. (1994, 1999) and Lalueza et al. (1996), three indices were calculated: the number of vertical and horizontal scratches divided by the total number of striations (NV/NT and NH/NT) and the number of horizontal striations divided by the vertical ones (NH/NV).

For the analysis of occlusal and incisal microwear, these surfaces were imaged at $500\times$ (Bullington 1991 and Mahoney 2006a, b, c). In the case of occlusal surfaces, we have specifically imaged the bottom of facet 9 (Mahoney 2006a, b). Pits and scratches were measured and counted on both surfaces using the same software as was used for the buccal microwear analysis. A 4:1 ratio was chosen to distinguish between these two microwear features (Mahoney 2006a, b). The variables analyzed on the occlusal surface were the frequency or percentage of pits, as well as the mean length and width of pits and scratches (Mahoney 2006a, b, c and Mahoney 2007). On the incisal surface, we only counted the number of pits and scratches and calculated the ratio between these two microwear variables (Bullington 1991).

Analysis and comparisons of microwear patterns

The small sample size available in this study for tooth type, surface analyzed, and archeological site precludes any statistical treatment (Table 1).

Moreover, we are aware that there is a potentially high level of inter-observer error of the dental microwear analysis using scanning electron microscopy (SEM) methods (Grine et al. 2002; Galbany et al. 2005). Regarding buccal microwear measurements, the precision depends highly on both the variable definition and the researcher's expertise. Researchers tend to show low measurements error rates, whereas significant inter-observer differences appear (Galbany et al. 2005). Thus, a direct comparison of microwear densities obtained by different authors should be performed with caution. For this reason, the comparisons performed in this study have relied on the overarching trend rather than on the magnitude of the data of the studied and comparative samples.

Dietary studies performed on the comparative samples are based on the analysis of buccal microwear and, thus, our comparative analysis is limited to this surface. Our results regarding occlusal microwear patterns are only considered to complement the dietary inferences obtained from buccal surfaces. The analyses carried out on buccal surfaces provide information about dietary habits over relatively long periods of time, while analyses performed on occlusal surfaces are able to

reflect seasonality of food resource exploitation. In this sense, a recent study has demonstrated that the application of these two kinds of analyses in the same specimens offer consistent results and allow us to obtain more complete dietary conclusions (García-González et al. 2015).

The findings obtained from incisal surface are taken into account in order to establish some inferences about the weaning process and age-related dietary changes.

Results

Buccal microwear analysis

Descriptive statistics for dental microwear features collected from buccal surfaces of teeth from El Portalón and El Alto de la Huesera are provided in Table 3.

The total number of striations on the buccal surface ranged from 61 to 195, with the third molar (M3) from El Portalón as the tooth with the highest number of striations followed by both deciduous second molars (dm2), one from each archeological site included in this analysis (Table 3). The high number of scratches on the two dm2 may be due to structural differences between deciduous and permanent tooth enamel (Scott and Halcrow 2017). Deciduous dental enamel is thinner and weaker than permanent enamel (Grine 2005; Mahoney et al. 2016). It could yield differences in the rate and pattern of microwear features formation independent of the food consumed (Scott and Halcrow 2017). However, Mahoney et al. (2016) demonstrates that there were no statically significant differences in microwear texture surfaces between deciduous and permanent enamel when subjected to similar forces. Although the findings of Mahoney et al. (2016) were obtained through dental microwear texture analysis on the labial surface of deciduous incisors and on the mesial aspect of premolars, they can be extrapolated into the present study with some caution. Mahoney et al. (2016) tested the differences between some microwear texture values before and after deciduous and permanent teeth were experimentally abraded. The microwear texture variables considered by Mahoney et al. (2016) were area-scale fractal complexity (Asfc) and exact proportion length-scale anisotropy (epLsar), which show correlation with features measured in 2D microwear analyses. While high Asfc values are correlated with frequent dental pits in 2D microwear analyses, high epLsar values are correlated with frequent dental scratches (Scott et al. 2005, 2006, 2012). Thus, if the change in values of Asfc and epLsar with experimental abrasion is equivalent in deciduous and permanent teeth, we will expect that the change in frequency of both pits and scratches were also equivalent. It would imply that a higher number of scratches in deciduous teeth than in permanent ones cannot be explained exclusively by differences between the enamel structures of these two kinds of teeth.

Table 3 Number, length, and variation of microwear striations on the buccal surface of teeth from El Portalón y La Huesera

Site	Tooth	Specimen	Eruption Age (years)	DM			Horizontal			MD			Vertical			Total		
				N	X	SD	N	X	SD	N	X	SD	N	X	SD	N	X	SD
El Portalón	dm2	ATP12-1420	4.1	39	90.31	42.00	3	126.77	13.21	12	94.87	43.19	53	91.29	39.40	107	91.12	39.96
	M1	ATP12-1420	1	14	103.35	46.99	1	31.27	16.64	13	125.98	97.58	53	126.55	67.65	81	120.84	70.95
La Huesera	M3	ATP11-26-H14	19.5–23.5	24	101.73	65.43	8	99.70	60.20	25	127.91	105.03	46	114.21	73.31	103	115.02	74.84
	dm2	ATP11-S-304	> 25	38	88.38	59.83	9	108.15	53.68	43	81.70	50.39	105	95.87	57.01	195	91.72	56.58
	M1	ADLH-4	3.5	17	72.48	47.20	4	78.27	28.26	34	79.65	62.85	53	81.13	61.72	108	84.31	58.17
		ADLH-10	6	16	80.76	48.85	1	62.66	39.82	15	56.47	44.86	46	69.601	38.14	78	66.29	41.73
		ADLH-12	10	24	63.22	48.18	3	56.16	17.36	19	88.59	48.16	61	92.79	68.25	107	83.93	61.48
	ADLH-52	19.5–23.5	6	80.58	54.73	1	49.46	31.48	16	84.80	70.89	38	109.53	86.86	61	100.43	80.26	
	ADLH-38	> 39.5	7	136.73	84.97	8	161.03	128.57	26	110.07	65.59	31	145.98	85.73	72	133.53	85.33	
	ADLH-50	> 39.5	33	98.42	81.70	10	99.65	65.68	12	104.54	102.75	26	85.30	56.79	81	95.53	77.53	

Eruption age calculated as the age at death of the individual minus the average age at eruption of this tooth derived from AlQahtani et al. (2010)
N number of striations, *X* mean length of striations in micrometers, *SD* standard deviation of length of striations in micrometers, *DM* striations with a disto-occlusal to mesio-cervical orientation, *MD* striations with a mesio-occlusal to distocervical orientation

Moreover, if the enamel thickness had an effect in the number of microwear features, we would expect differences in the frequency of microwear features between permanent molars. In the mandible enamel of M1 is significantly thinner than either M2 or M3 (Grine 2005). However, we do not detect a lower frequency of striations on M3 than in the different M1s. Indeed, the only M3 analyzed presented the highest number of striations.

Taking into account the above mentioned, the high scratch densities on M3 and two dm2 may be due to the fact that the total number of scratches increases with the tooth age since eruption, given that the pattern of buccal microwear is a dynamic process with a cumulative effect throughout life (Pérez-Pérez et al. 1994; Romero et al. 2012). An increase in scratch density with the eruption age is observed in the teeth from El Portalón (Table 3). In these teeth, the older the eruption age, the higher the scratch density. Moreover, the comparison of the number of scratches between the dm2 and first molars (M1) of the same individual (ATP12-1420) also confirm this trend. The M1 shows a smaller striation density than the dm2 (Table 3), but the age of clinical eruption of these two teeth also differs. The dm2 usually is erupted around 2.5 years old, while for the M1, this occurs between 5.5 and 6.5 years of age (AlQahtani et al. 2010). This implies that the time elapsed between the eruption of dm2 and the death of the individual could be up to 4 years, while the period of time between the eruption of M1 and the death could only have lasted up to 1 year. Thus, the higher number of striations in dm2 than in M1 is due to a longer functional time.

In teeth from El Alto de La Huesera, however, there is no clear trend showing an increase in the total number of striations with an increased eruption age. From this site, teeth with a higher number of scratches (ADLH-4 and ADLH-12) have a difference in eruption ages of up to 6.5 years (Table 3). The total number of striations showed by these teeth is even higher than in those teeth belonging to fully adult individuals (Tables 1 and 3). Interestingly, the scratch densities of M1 belonging to these fully adult individuals are close to the total striation density of the M1 belonging to the adolescent ADLH-10 (Tables 1 and 3). Pérez-Pérez et al. (1994) have asserted that a definitive striation pattern is attained between 5 and 12 years from eruption. Based on the eruption age of ADLH-10 (6 years, Table 3) and the similarity in the total number of scratches present on that tooth and those of full adults, we can assert that this individual could have already attained the tooth striation pattern of the adult population. If this is true, the high number of striations shown by ADLH-12 with an age of 16 years could reflect inter-individual variability.

Regarding the scratch length, the high values for standard deviations are worth noting, which implies a high variability in the length of striations. The general trend indicated by teeth from El Portalón and El Alto de la Huesera is that the average

length of the scratches increases with age, though some exceptions were found. The teeth that do not follow this rule are the M3 from El Portalón and the dm2 and one M1 (ADLH-50) from El Alto de la Huesera. The lower average length of the scratches on the M3 from El Portalón than on the other teeth from the same site is likely independent of the tooth type, since third molars are both morphologically and functionally similar to first molars (Pérez-Pérez et al. 1994). The average length of the striations for this M3, together with the ages estimated for it and the M1 from El Alto de la Huesera, seems to suggest that the length of the scratches increases with age in younger groups and then decreases in older groups (Pérez-Pérez et al. 1994).

In sum, the analysis of buccal microwear patterns in teeth from El Portalón and El Alto de la Huesera reveals that the striation pattern is related to both the age at death of the individuals and the period of time elapsed from the eruption of teeth. Thus, in order to obtain the appropriate framework of comparison, we perform different comparisons based on the age of the individuals. In this way, Figs. 2, 3, and 4 depict the buccal microwear length against buccal microwear density for dm2, M1 belonging to sub-adult individuals and teeth belonging to fully adult individuals.

The dm2 scratch densities from El Portalón and El Alto de la Huesera are comparable to the medieval sample from La Olmeda compounded by 6–9-year-old children (Fig. 2). However, the two Chalcolithic dm2 are characterized by shorter scratches than the two age groups from La Olmeda. A similar pattern is observed when the M1s belonging to sub-

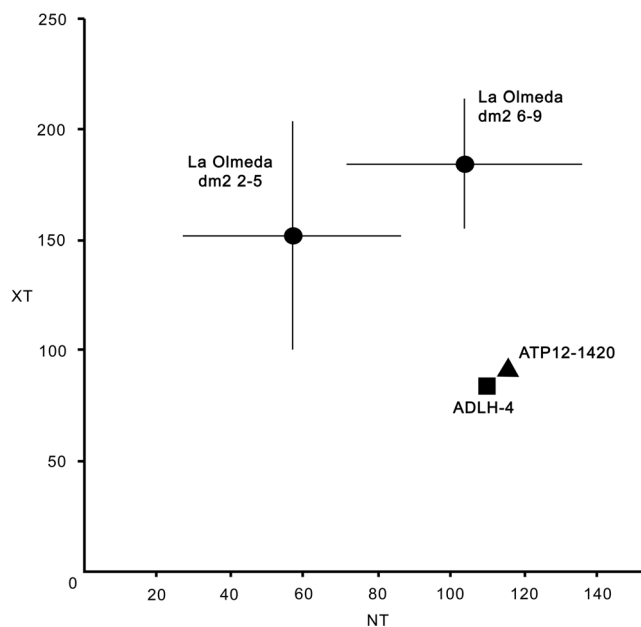


Fig. 2 Density of striations of buccal-microwear (NT) plotted versus length of striations (XT, μm) on dm2 from El Portalón, El Alto de la Huesera, and La Olmeda (Pérez-Pérez et al. 1994). Filled triangles: teeth from El Portalón; filled squares: teeth from El Alto de La Huesera; filled circles: teeth from la Olmeda. Errors bars denote ± 1 SD

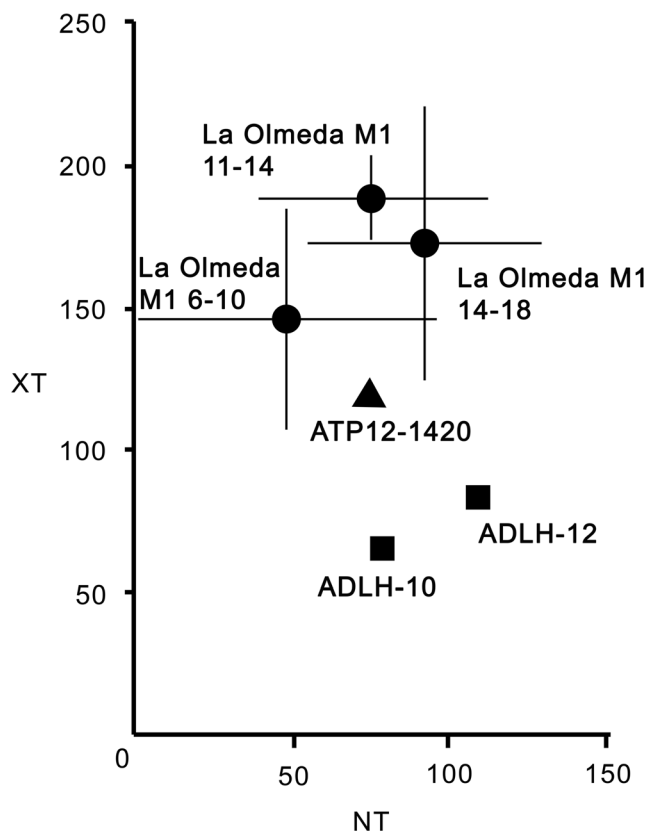


Fig. 3 Density of striations of buccal microwear (NT) plotted versus length of striations (XT, μm) on first molars belonging to sub-adult individuals from El Portalón, El Alto de la Huesera, and La Olmeda (Pérez-Pérez et al. 1994). Filled triangles: teeth from El Portalón; filled squares: teeth from El Alto de La Huesera; filled circles: teeth from la Olmeda. Errors bars denote ± 1 SD

adult individuals from El Alto de La Huesera are compared to equivalent age groups from La Olmeda (Fig. 3). These Chalcolithic individuals show shorter scratches than medieval ones. However, the scratch density and average length of striations of ATP12-1420 fall well within the range of variation of medieval children between 6 and 10 years old. Thus, Chalcolithic individuals analyzed in this study resemble each other in that they have a density of scratches similar to medieval individuals who had an equivalent age at death; however, the individuals from El Alto de la Huesera show a distinct pattern, since the scratches are shorter on average.

In Fig. 4 the buccal microwear variability among the analyzed Chalcolithic individuals is depicted, as well as the populations used for comparisons, when only the full adults are considered. In general, prehistoric populations from the Mediterranean coast of Iberian Peninsula show a higher number of striations the older the chronology of the site. Neolithic and Chalcolithic individuals are characterized by a higher number of shorter striations than Bronze (Fig. 4). It implies that there is a reduction in the abrasiveness of the diet of individuals from older sites to younger ones over the course of prehistory. Interestingly, the Islamic individuals from Tossal

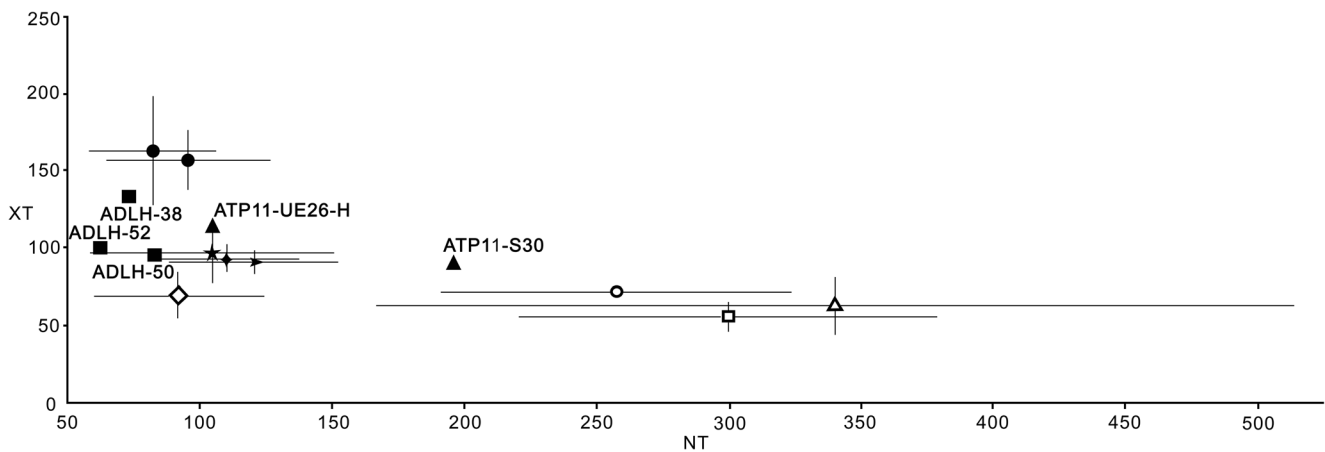


Fig. 4 Density of striations of buccal microwear (NT) plotted versus length of striations (XT, μm) on molars belonging to adult individuals from El Portalón, El Alto de la Huesera, and comparative samples. Filled triangles: El Portalón; filled squares: El Alto de La Huesera; filled circles: la Olmeda (Pérez-Pérez et al. 1994), open square: Mediterranean Chalcolithics (Romero and De Juan 2007); diamond: Mediterranean

Bronze Age (Romero and De Juan 2007); arrow: Mediterranean Islamic (Romero and De Juan 2007); open diamond: modern sample (Romero and De Juan 2007); open triangle: Neolithic (Salazar-García et al. 2016); star: Roman (Salazar-García et al. 2016); open circle: Medieval (Salazar-García, 2016). Errors bars denote ± 1 SD

de les Basses have the same number of scratches as the Neolithic sample. In contrast, medieval individuals from La Olmeda present one of the lowest values for the number of striations and the highest values for the length of the scratches. The contemporary sample from Villena and Later Roman individuals show a buccal microwear pattern characterized by few and short scratches. These findings suggest that there are differences in abrasive particles between the Mediterranean Islamic sample on the one hand and the Medieval from La Olmeda, the Contemporary, and the Later Roman individuals on the other. These differences could be due to changes in both food content and preparation methods between these samples. Individuals from El Alto de la Huesera have as few scratches as medieval individuals from La Olmeda and the contemporary sample from the Mediterranean coast. Molars from El Portalón show higher variability than those from El Alto de la Huesera. While ATP11-26-H14 resembles the molars from El Alto de la Huesera in the number and length of the striations, the number of striations found on ATP11-S304 is at the lower end of the Mediterranean Neolithic and Islamic range of variation.

In sum, the comparison of the buccal microwear variables reveals that both sub-adult and adult individuals from El

Portalón and El Alto de la Huesera show a number of striations equivalent to that of medieval individuals from La Olmeda (Figs. 2, 3, and 4). The main difference between these two distinct chronological samples is that individuals from the Chalcolithic sites display shorter scratches than the medieval ones on average. Based on the number of striations, we can assert that the diet of Chalcolithic individuals from El Portalón and El Alto de la Huesera is as abrasive as that of the medieval sample from La Olmeda. The abrasiveness of the diet depends on both dietary and non-dietary factors. The medieval population of La Olmeda had a diet mostly based on cereals, which were processed by the grinding of seeds with stone mills (Pérez-Pérez et al. 1994).

Occlusal microwear analysis

The dental microwear variables taken from the occlusal surface of the two first molars from El Portalón are provided in Table 4.

Based on these variables, we can infer that the El Portalón molars have a low frequency of pits, which may reflect a relatively soft and tough diet (Schmidt 2001; Mahoney

Table 4 Microwear measurements on the occlusal surface of teeth from El Portalón

Specimen	Number of features		% pits	Pit length (μm)		Pit width (μm)		Scratch length (μm)		Scratch width (μm)	
	Pits	Scratches		X	SD	X	SD	X	SD	X	SD
ATP11-26 H14	11	62	15.54	3.546	1.267	1.697	0.580	27.989	25.498	1.343	0.58
ATP12-1420	10	99	9.13	5.852	2.313	3.495	0.946	32.229	18.937	1.29	0.95

2007). A diet mainly based on these foods requires more shearing than compressive forces during the chewing cycle, yielding small pits and long scratches on the occlusal surface (Gordon 1982; Molleson et al. 1993; Mahoney 2006a, b). However, both molars from El Portalón show large pits. Although pitting and pit size seem to be inconsistent at first glance, it is important to note that the pit size is also related to the physical properties of the abrasive particles adhering to the food. Thus, in this case, differences in the pitting and pit size are due to different factors. The length of the scratches on two first molars is extremely variable (high SD), which implies that there are short and long scratches on these occlusal surfaces. The presence of long striations on the occlusal surface of first molars concurs with a low percentage of pits, since long scratches and few pits are associated with a chewing cycle in which shearing forces are predominant. Instead, the short scratches may reflect the chewing of soft foods, for which the lateral movements of the mandible can be less important (Luschei and Goodwin 1974).

In sum, the occlusal microwear pattern on the first molars from El Portalón seems to indicate a diet mainly consisting of soft and tough foods with large abrasive particles adhered.

Incisal microwear analysis

The microwear variables taken from the incisal surface in teeth from El Portalón are depicted in Table 5. The youngest individuals in the sample are aged 1 year (ATP13-H6 and ATP13-H7) and they show microwear. It implies that mixed feeding have commenced slightly earlier in those infants. From this age, there is not a clear trend in regard to the total incisal microwear features with both age and eruption age. Instead, an infant older than 2 years (ATP12-H1) had lesser total microwear feature frequency than individuals aged 1 year (ATP13-H6 and ATP13-H7). However, the percentage of pits increases with both age and eruption age, which suggests an increase of the abrasiveness of the diet with age. This increase in abrasiveness of the diet could reflect that older infants consumed harder and more abrasive foods than younger ones.

Discussion

The results obtained through dental microwear analysis on teeth from El Portalón and El Alto de la Huesera provide valuable information about two important topics: the age-related dietary habits in prehistoric populations and the effect of the interaction between environmental and cultural factors on the microwear pattern.

Regarding the effect of environmental factors on the dental microwear pattern, adult individuals from El Portalón and El Alto de la Huesera show a pattern of buccal microwear more similar to that found in medieval individuals from La Olmeda. Interestingly, the buccal microwear of adults from this medieval population is characterized by the lower density of shorter microwear features, indicative of a less abrasive diet than that of the other comparative samples, including historical counterparts from the Mediterranean coast (Fig. 4). Since the abrasiveness of the diet depends on both dietary and non-dietary factors, such as the foodstuff technical processes (Romero and De Juan 2007), we would expect to find differences between different chronological groups. Nonetheless, our findings suggest that differences in buccal microwear features also depend on geographical location. Thus, environmental factors, such as elevation, temperature, and precipitation, could also be correlated with the buccal microwear pattern. The environmental conditions during the Chalcolithic in the Sierra de Atapuerca were relatively dry, with a decrease in the woodlands and nitrophilous taxa and a slight increase in xeric taxa (Martínez-Pillado et al. 2014). This could have led to higher intensification of animal husbandry to the detriment of agriculture. One of the uses of the cattle herd from El Portalón during the Chalcolithic, among others, was obtaining secondary products, such as milk and meat consumption (Galindo-Pellicena 2014, Galindo-Pellicena et al. 2017). Obviously, this does not mean that agriculture was not part of the economy during the Chalcolithic in the Sierra de Atapuerca, but we hypothesized that during this prehistoric period, livestock was preferred and probably more relevant. Regarding El Alto de la Huesera during the Chalcolithic period, the studies

Table 5 Microwear measurements on the incisal surface of the deciduous incisors from El Portalón

Specimen	Eruption age (months)	Age at death (months)	Frequency			% pits
			Scratches	Pits	Total	
ATP13-H6	4.5	12	75	10	85	13.3
ATP13-H7	4.5	12	69	17	86	24.6
ATP11-635	6.9	14.4	81	10	91	12.3
ATP12-65	7.5	15	30	9	39	30.0
ATP10-H2	> 19.5	27	61	29	90	47.5
ATP12-H11	> 16.5	> 24	24	15	39	62.5

Age at death estimated from Liversidge and Molleson (2004). Eruption age calculated as the age at death of the individual minus the average age at eruption of this tooth derived from AlQahtani et al. (2010)

performed on the archeological sites related to this dolmen also reveal an intensification of animal husbandry in this area during this period. Thus, differences between Chalcolithic groups from the Northern Plateau and those from the Mediterranean coast could be due to a higher intake of meat in the former than in the latter.

The consumption of meat can be inferred from buccal microwear patterns regarding the relative frequencies of vertical and horizontal scratches. In this sense, individuals from El Portalón and El Alto de la Huesera have a higher number of vertical striations than horizontal ones. In a study of different dietary groups, Lalueza et al. (1996) established that the group with a diet mainly based on meat had a higher relative frequency of vertical scratches than horizontal ones. Thus, if we extrapolate these findings into the present study, we can assert that the relative frequency of scratches by orientation found in the teeth from El Portalón and El Alto de la Huesera suggest a high intake of meat.

A high intake of meat could also be related to the occlusal microwear pattern found in first molars from El Portalón. Although it is more difficult to establish meat consumption through occlusal microwear patterns than it is to do through buccal microwear features (Mahoney 2007), some conclusions can be drawn. Indeed, El Zaatari (2010) show that consumption of meat can lead particular microwear patterns on occlusal surfaces. In a study of seven groups of hunter-gatherers, she found differences on occlusal microwear pattern between the meat-eating hunter-gatherers and the mixed diet hunter-gatherers.

The low frequency of pits present on the first molars from El Portalón can reflect a relatively soft and abrasive diet, requiring more shearing than compressive forces during the chewing cycle (Schmidt 2001; Mahoney 2007). A diet based on this kind of food yields long scratches, a length that reflects the amount of shearing needed to comminute the foods. However, the scratches on the first molars from El Portalón are short. When meat is tough enough to require significant shearing force to cut it during chewing, this yields longer scratches and can even result in forceful tooth-on-tooth contact, leading to bigger and more abundant pits. Therefore, the relatively high frequency of short scratches on the teeth from El Portalón could be related to the consumption of soft meat. Soft meat can possibly come from immature animals or from a technical process, such as boiling, which softens the food. The data compiled with respect to the management of domestic animals support this assertion, since as we have mentioned above, most of the domestic faunal remains recovered from El Portalón show evidence of having been boiled (Galindo-Pellicena 2014).

Regarding dietary changes with age, the analysis of the microwear on buccal surfaces of teeth from El Portalón shows that the buccal microwear pattern is accumulated throughout life in this population, and sub-adult individuals attain the

characteristic striation pattern of the population in ages older than 7 years (Figs. 2, 3, and 4). It could imply a similar diet in adult and sub-adult individuals from this Chalcolithic site.

However, the analysis of microwear on incisal surfaces that belong to sub-adult individuals reveals a pattern characterized by numerous pits. This finding suggests that the diet of sub-adult individuals was harder than that of the adults. This might suggest, therefore, that meat consumption intensified after a certain age in this society. This assertion is confirmed by findings obtained from teeth found in El Alto de la Huesera. A shift in dietary habits with age in this population has been established by Fernández-Crespo and Schulting (2017). These authors analyzed the stable carbon and nitrogen isotope in teeth from El Alto de la Huesera, along with those from other mortuary monuments and caves from north-central Spain. Although the main goal of this paper was to establish differences in the burial location and treatment based on stable isotopes, they also claimed that, when data are combined from all sites, there is a clear upward trend in the values of ^{15}N with age. This increase in ^{15}N values may be attributable to skeletal growth, but could also be because of differences in diet due to a high meat intake.

Finally, the analysis of incisal microwear in deciduous teeth from El Portalón provides some insights about the weaning process. Weaning implies the introduction of supplemental foods besides maternal milk into the infant diet and it mainly depends on the availability of soft foods (Bullington 1991). In traditional societies, this process occurs during the first year of life. Infants from El Portalón aged 1 year showed microwear features on incisal surfaces, and thus, it suggest that the weaning process might be earlier in this Chalcolithic population. From the age at weaning, food consumed were either hard enough or with harder particles associated to food, to lead a pattern of microwear with numerous pits. The ingestion of hard particles adhering to food can be the result of food processing, for instance, the grinding of food with stone mills.

Conclusions

In sum, findings obtained through occlusal and buccal dental microwear analysis on teeth from El Portalón and El Alto de la Huesera suggest a mixed diet but one with a high rate of meat intake. Since the meat is unlikely to be hard enough to cause numerous microwear features (Mahoney 2007), it could be related to the less abrasive diet in Chalcolithic populations from the Northern Plateau than what was consumed by their counterparts from the Mediterranean coast.

In contrast with the archeological sites from the Northern Plateau, sites from the Mediterranean coast are located on a wetland. Accordingly, a difference in food resources must be responsible for the observed differences between the

Mediterranean site and the Northern Plateau sites in regard to microwear features.

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