

Infant and childhood diet at the passage tomb of Alto de la Huesera (north-central Iberia) from bone collagen and sequential dentine isotope composition

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Running title: Early dietary life histories in Late Neolithic Iberia

Keywords: nursing, weaning, infancy, childhood, carbon and nitrogen stable isotopes, megalithic tombs, Late Neolithic, Iberia.

Abstract: The Rioja Alavesa region of north-central Spain witnessed great demographic pressure and social unrest, manifested as widespread violent conflict, during the Late Neolithic (ca. 3500-2900 cal BC). Drawing upon the ethnographic literature, it is possible that this situation impacted upon child-rearing practices, both through food shortages and differential parental investment, favoring male infants. Here, carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope measurements from bone collagen of 17 juveniles and from 163 serial microsamples of dentine from first and second molars of seven adults from the site of Alto de la Huesera are used to examine breastfeeding, weaning and childhood diets. Bone and dentine collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values both decrease from infancy to early childhood and increase slightly towards adolescence, but dentine provides a more time-sensitive means of monitoring dietary changes. High $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values compatible with exclusive breastfeeding are detected up to ca. 1 year, with a significantly shorter duration among males, suggesting differential sex-related parental strategies from infancy. This is tentative given the small number of individuals being compared, but does suggest that further work would be worthwhile. A gradual decline in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, compatible with the weaning process, is then observed up to ca. 4 years in both sexes. This delayed cessation of nursing is interpreted as a possible response to food shortage. With regard to post-weaning patterns, shifts to lower $\delta^{13}\text{C}$ and

$\delta^{15}\text{N}$ values in females at around age 9-11, and a general progressive increase in both isotope values from childhood to adolescence, are detected. These could be linked either with differential protein intake due to social age-related nutritional practices or to physiological demand. The comparison between bone and dentine values shows differences between survivors and non-survivors in both isotopes, so that assessments based on deceased children may be biased by their potentially compromised health status.

Introduction

Breastfeeding, weaning and childhood dietary practices are essential milestones in an individual's life history. They are to some extent conditioned by basic human physiological needs, but also strongly influenced by cultural understandings of nutrition and health (Howie et al., 1990), by child and parental social and economic identities and relationships (Fildes, 1995), and by religious beliefs and lifeways (Fouts, 2004; Ineichen et al., 1997). In addition, subsistence regimes, mobility and availability of suitable infant foods all play key roles (Schurr and Powell, 2005). Thus, childhood foodways can provide valuable information for the understanding of past cultural behaviors and immaterial aspects of human lives, for which explicit evidence is almost inexistent in the prehistoric archaeological record.

Additionally, infant- and child-rearing practices permit some inferences on population dynamics, as they shape fertility, morbidity and mortality (Halcrow and Tayles, 2011). For example, breastmilk is a source of essential nutrients and easily digestible calories, contains important antibodies and generally provides a long-term assurance of health into adulthood (Lönnerdal, 2000). However, its production involves a significant energetic investment and temporarily reduces female reproductive capacity (i.e. lactation amenorrhea) (Borgerhoff-Mulder, 1992; Sellen, 2006). Thus, early weaning enables females to invest in their next offspring sooner, while prolonged nursing implies longer birth intervals and greater parental effort to try to maximize each offspring's survival chances. Parental decisions depend on several cultural and environmental factors, including the extent to which parental care benefits offspring (Quinlan, 2007). The timing of weaning, the intensity of the process (e.g. gradual or abrupt cessation of nursing), and the quality of complementary foods used are all socially and culturally mediated aspects. The declining immunological benefit of breastmilk and the increasing exposure to environmental pathogens are often associated with an increased incidence of illness, particularly intestinal and immune system dysfunctions (Martin and Sela, 2013), and so the weaning process is usually related to complex beliefs and occasionally special

weaning foods (Fouts, 2004). Finally, childhood diets and more specifically food allocation and age-related dietary strategies shed light on cultural constructions such as social age (i.e. norms of appropriate behavior for individuals of a given biological age), vulnerability and gender (Messer, 1997).

Stable isotope studies of breastfeeding and weaning are essentially based on raised $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in infants between the beginning and the cessation of breastfeeding as demonstrated by studies made on nail clippings and hair of modern human mother-infant pairs (Fogel et al. 1989; Schurr, 1997; Bourbou et al., 2013). Typically, infant $\delta^{15}\text{N}$ values increase promptly with the onset of nursing, raising them a trophic level (~ 2 to 3‰) above the mother (de Luca et al., 2012; Herrscher et al., 2017). As complementation with other food begins, $\delta^{15}\text{N}$ values start to decrease, generally falling to a level similar to the mother's (assuming both are consuming the same food) after the cessation of nursing (Reynard and Tuross, 2015; Beaumont et al., 2015). A comparable but smaller effect ($\sim 1\text{‰}$) may be seen in $\delta^{13}\text{C}$ values (Fuller et al., 2006). In archaeological research, however, where nails and hair are rarely preserved, the duration and rapidity of breastfeeding and weaning practices have generally been estimated using bone collagen from infants and children that was forming at the time of death (e.g. Pearson et al., 2010; Tsutaya et al., 2015; Waters-Rist et al., 2011). More recently, the much higher resolution possible with the sequential sampling of primary dentine has come to dominate studies on this topic (e.g. Beaumont et al., 2013; Eerkens et al., 2011; Henderson et al., 2014), since it grows incrementally and, unlike bone, does not remodel once formed.

Isotopic approaches to past childhood diets mainly focus on determining whether the isotope values of fully weaned children differ from those of adolescent and adults (Halcrow and Tayles, 2011; Schurr and Powell, 2005). In a similar vein, comparison between isotope values from those individuals who did not survive childhood (through bone or dentine collagen from non-adults) and those who did (through dentine collagen from adults) has also been a recurrent research topic (Fuller et al., 2003; Sandberg et al., 2014; Pfeiffer et al., 2017). Despite not being consistently observed, the existence of differences between survivors and non-survivors' diets in some studies has called into question the validity of dietary assessments based on data from children dying before reaching adulthood (i.e. the 'Osteological Paradox' (Wood et al., 1992; Katzenberg et al., 1996)).

The Alto de la Huesera megalithic grave, located in the Rioja Alavesa region of north-central Iberia, serves as an important case study for exploring Late Neolithic (3500-2900 cal. BC) infant and childhood dietary practices. The presence in the region of at least 16 Late Neolithic funerary sites in

an area of only 250 km² and the large mortuary assemblages they hold (>800 individuals), together with the regular spacing of the megalithic graves (6.8 ± 3.6 km), suggests high demographic pressure (De Carlos, 1988; Fernández-Crespo and de-la-Rúa, 2016), which was probably the result of the co-occurrence of a degree of territorial circumscription and resource concentration here (Carneiro, 2012). This could have led to the high levels of conflict that Rioja Alavesa witnessed during this period, supported by the considerable evidence for interpersonal violence, primarily involving males, and the presence of what have been interpreted as mass war graves (Vegas et al., 2012). The region provides the largest absolute number of arrowhead injuries in prehistoric Europe, as well as so-called ‘parry fractures’, cranial trauma and indirect evidence of projectile injuries in soft tissues (Fernández-Crespo, 2017). Thus, it is an interesting context for the study of the potential influence of endemic conflict on parental investment and child-rearing practices.

To explore this question, we analyze bone collagen carbon and nitrogen stable isotope data of 17 non-adults and intra-tooth stable carbon and nitrogen isotope profiles in the dentine collagen of the permanent first and second molars of seven adults. Specifically, our study aims at assessing a) the existence of potential differences in infant and childhood feeding practices by sex, as warlike societies may place higher value on male over female infants (Divale 1970; Divale and Harris 1976), and b) the possibility that surviving and non-surviving individuals show isotopic divergences, for example, due to differential feeding patterns or parental investment in a context of potential food scarcity (Quinlan, 2007). While our sample size here is too small for formal hypothesis testing, the data presented will ultimately contribute towards these goals.

Material

Alto de la Huesera (Laguardia, Álava) is a passage tomb (Fig 1a) located in the Rioja Alavesa region of north-central Iberia (Fig 1b). It was discovered in 1948 by D. Fernández Medrano, who together with J. M. Barandiarán excavated the upper layer of the chamber, corresponding to the Late Chalcolithic and Bronze Age (ca. 2900-1450 cal. BC) (Barandiarán and Fernández Medrano, 1958), holding a minimum of 80 individuals (20 non-adults and 60 adults) (Fernández-Crespo and de-la-Rúa, 2015). New excavations in 2010-2014 targeted the lower layer of the chamber (which was sealed beneath a stone paving) and the corridor, used during the Late Neolithic (ca. 3250-2900 cal. BC) (Fernández-Eraso and Mujika, 2013; Fernández-Eraso et al., 2016) for interring at least 56 individuals (18 non-adults and 38 adults) (Fernández-Crespo and de-la-Rúa, 2016) (Fig 1c). Osteological investigations show a low frequency of oral pathologies together with skeletal

evidence for non-specific stress indicators, such as linear enamel hypoplasias affecting at least five individuals from the lower layer between age 10.5 months and 4.5 years, and for nutritional deficits through 11 cases of cribra orbitalia possibly related to anaemia (e.g. vitamin B₁₂ or iron deficiency, malabsorption, high parasite loads, etc. (Walker et al., 2009; Oxenham and Cavill, 2010; McIlvaine, 2015)) and three long bone deformities attributable to rickets (i.e. vitamin D and/or calcium deficiency (Mughal et al., 1999)). There are several cases of degenerative disease, enthesal changes and, at least seven cranial depressions and eight postcranial fractures (Etxeberria, 1991; Fernández-Crespo, 2012). Particularly, two blunt force cranial injuries and two parry fractures, affecting different individuals, are clearly attributable to episodes of interpersonal violence. Stable isotope analysis on bone collagen suggests that Late Neolithic dietary practices were based on the consumption of C₃ plants and terrestrial animals ($\delta^{13}\text{C}$: $\bar{X} = -19.9 \pm 0.3\text{‰}$; $\delta^{15}\text{N}$: $\bar{X} = 9.0 \pm 0.6\text{‰}$; n = 46) (Fernández-Crespo and Schulting, 2017).

The material selected for study comes exclusively from the Late Neolithic contexts of Alto de la Huesera (i.e. the lower layer of the chamber and the corridor). Bone samples were taken from the mandibles of 17 non-adult individuals, whose ages at death (from 0.6 to 15.5 years) were estimated through tooth development and eruption (AlQahtani et al., 2010). The permanent first and second molars were sampled from the mandibles of seven young (20-39 years) and mature adults (40-59 years) (three females and four males) (for details on age and sex estimation methods, see Fernández-Crespo and de-la-Rúa, 2016). First molars (M1s) begin forming around birth, with complete closure of the root tips by ca. age 10. Permanent second molars (M2s) partially overlap first molars, forming between approximately age 2.5 and 16 (AlQahtani et al., 2010). The selected molars were minimally worn and free of caries, as many studies recommend (e.g. Beaumont et al., 2013; Fuller et al., 2003; Henderson et al., 2014).

Methods

Bone samples were shot-blasted with aluminium oxide pellets before collagen extraction, which was carried out following a modified Longin method (Login, 1971) as described by Richards and Hedges (Richards and Hedges, 1999).

Teeth were recorded using high resolution photogrammetry, shot-blasted to remove surface debris and then embedded in Herculite II (a high-strength gypsum molding material) and sectioned longitudinally using a Buehler Isomet low-speed diamond saw with a micrometer gauge, an

abrasive wafering blade and a cooling water bath (cf. Beaumont et al., 2013; Sandberg et al., 2014). Collagen extraction was carried out following a modified University of Cape Town method (Sealy et al., 2014), with sequential microsampling undertaken following the method developed by Czermak et al. (this issue). One complete tooth half was demineralized in 0.5 M hydrochloric acid (HCl) solution at 4 °C for ca. two weeks, rinsed three times with deionized water until pH neutral. The demineralized tooth half was then cut longitudinally and the posterior side removed in order to minimize incremental growth overlapping as dentine formation is S-shaped rather than linear and increments bend concentrically around the pulp (for more details, see Czermak et al., this issue). The thin cementum layer was removed by mechanical cleaning of the tooth root surface, and any identified secondary and tertiary dentine was avoided by reaming out the pulp cavity before sampling. The remaining ~2 mm wide longitudinal tooth slice, still retaining its original shape, was sampled sequentially from crown to apical root (mesial side) using a 1 mm diameter KAI Medical biopsy punch with plunger, obtaining ca. 12 samples on average per tooth. This method is more anatomically sensitive than linear slicing (Czermak et al., this issue). Resulting dentine microsamples were labeled according to a numerical sequence of dentine segments and their approximate age assigned by comparing the dentine sections' anatomical location to the stage of dental development, following AlQahtani et al. (2010).

Bone and dentine samples were freeze-dried before being weighed into tin capsules and loaded into a SERCON 20/22 continuous flow isotope ratio mass spectrometer coupled with an elemental analyzer at the Research Laboratory for Archaeology and the History of Art (RLAHA), University of Oxford. Analytical precision is $\pm 0.2\text{‰}$ (1σ) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ based on repeated analyses of internal (alanine, marine seal, cow) and international standards (caffeine IAEA 600). Bone samples were measured in duplicate. Dentine samples were generally not measured in duplicate, due to the small yields resulting from the sampling technique and to the impossibility of precisely replicating a sample elsewhere on the same tooth due to the complexity of dentine growth (Dean and Scandrett, 1995). However, a comparison was conducted on 10 samples with sufficient yields for duplicate measurement, resulting in a mean difference of $0.0 \pm 0.1\text{‰}$ ($\delta^{13}\text{C}$) and $0.1 \pm 0.3\text{‰}$ ($\delta^{15}\text{N}$) between the two runs. This is within measurement error and, moreover, the comparison between runs shows no significant differences (paired *t*-test; $\delta^{13}\text{C}$: $t = 0.110$, $df = 9$, $p = 0.915$; $\delta^{15}\text{N}$: $t = 0.934$, $df = 9$, $p = 0.375$).

Collagen preservation quality was checked according to standard criteria, including collagen yield, atomic weight C:N ratio, and %C and %N (Ambrose, 1990; DeNiro, 1985; van Klinken, 1999). With regard to atomic weight C:N ratios, in a conservative approach given the lack of duplicate

dentine measurements, a more constrained C:N range of 2.9-3.4 was used rather than the oft-cited 2.9-3.6 (van Klinken, 1999). Finally, only samples weighing ≥ 0.4 mg were considered reliable, since lower weights may affect mass spectrometer measurements (e.g. Burt and Amin, 2014).

Statistical analysis was performed using IBM SPSS software for Windows v17. Shapiro-Wilk tests were used to assess whether or not the data were normally distributed. For two-sample comparisons, Student's *t*-tests were employed when the data did not depart significantly from a normal distribution; when they did, non-parametric Mann-Whitney *U*-tests were used. Two-level mixed model nested ANOVA tests were used to assess variation in mean dentine collagen isotope ratio by sex (i.e. males vs. females), since the comparison involves one measurement variable (e.g. $\delta^{13}\text{C}$ values) and two nominal variables (individuals and sex/tissue), the latter being nested (forming subgroups within groups) (McDonald, 2014). Due to unequal group sample sizes, the Satterthwaite formula was applied, (using modified mean squares at each level to provide a better estimate of the effective degrees of freedom and, therefore, more accurate *p* values (Satterthwaite, 1946). Z-scores were also calculated to detect the presence of outliers where appropriate, and to compare dentine collagen to bone collagen values. A significance level of $\alpha = 0.05$ was used for all tests.

Results

All non-adult bone collagen samples included in the study ($n = 17$) show good-quality collagen (Table S1) and are used for analysis. Six of 169 dentine samples obtained from the M1s and M2s failed to meet the criteria set out above, leaving 163 samples for analyses (Table S2).

Results obtained on non-adult bone collagen are limited by the small sample size. The only measurement available for a young infant (LHUE 11c) provides high $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values consistent with a nursing signal (cf. Table S1), showing a trophic enrichment of 0.9‰ for $\delta^{13}\text{C}$ and 3.5‰ for $\delta^{15}\text{N}$ relative to all the adult female bone collagen values available for the site ($\delta^{13}\text{C}$: $\bar{X} = -19.9 \pm 0.2\text{‰}$; $\delta^{15}\text{N}$: $\bar{X} = 9.0 \pm 0.7\text{‰}$; $n = 12$) (Fernández-Crespo and Schulting, 2017). Those individuals whose ages at death correspond to years following the cessation of breastmilk consumption (i.e. 4-7 years) show some of the lowest values in the dataset for both isotopes. Finally, older children show more enriched $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as they approach adolescence.

Dentine samples from all individuals show the predicted elevated $\delta^{15}\text{N}$ values in the first M1 crown samples, generally ca. 12-14‰, indicative of high trophic level protein (the adult average at the site

is $9.0 \pm 0.6\text{‰}$). The exception is individual LHUE2i, an adult male whose M1 crown $\delta^{15}\text{N}$ value is only 9.8‰ (Fig. 2). Unfortunately, attrition of the cusps removed dentine corresponding to the period prior to age 1 in a number of individuals, despite having selected those specimens with the least dental wear. This probably explains why only in three (LHUE34, LHUE 52 and LHUE53) of the seven individuals a ‘plateau’ in the earliest, highest values can be clearly related to an exclusive breastfeeding signal, which is seen until age 1.1-1.5 (Table 1). In any case, the data indicate that exclusive nursing would extend not long after age 1 in the remaining cases. During the period interpreted as exclusive nursing, the three aforementioned individuals show a trophic enrichment of 0.4‰ for $\delta^{13}\text{C}$ and 3.2‰ for $\delta^{15}\text{N}$ relative to their adult bone collagen values (cf. Table S2), and of 0.6‰ and 3.4‰ , respectively, relative to all the adult female bone collagen values available for the site (Fernández-Crespo and Schulting, 2017). This is consistent with the trophic level offset expected between fully breastfed infants and their mothers (Schurr 1997).

Weaning is isotopically characterized by a noticeable decrease in $\delta^{15}\text{N}$ values corresponding with a full drop in trophic level (here ca. 3‰ - 4‰), except in the case of LHUE2i (with a drop of 1.4‰). Following other studies (Eerkens et al., 2011; Nitsch et al., 2011; Sandberg et al., 2014), we place its beginning at the point when $\delta^{15}\text{N}$ starts decreasing and its end at the last lowest $\delta^{15}\text{N}$ value after the sustained drop. Weaning is detected in all individuals and ends around 3.9 ± 0.6 years, suggesting a prolonged and gradual transition from breastmilk to solid food lasting at least 2.8 ± 0.6 years. It is after this process that the lowest $\delta^{15}\text{N}$ values are usually reached in the isotope profiles (mean $\delta^{15}\text{N}$: $8.7 \pm 0.8\text{‰}$), being ca. 0.35‰ lower on average than the adult bone collagen values of the same individuals (cf. Table 1) and of all the adults analysed at the site (Fernández-Crespo and Schulting, 2017), respectively. The $\delta^{13}\text{C}$ results also follow the same pattern, dropping simultaneously by 0.5‰ - 1‰ in all cases except for LHUE52 and perhaps LHUE19c (cf. Fig. 2).

Concerning childhood diets, there are two main observations to be made. Firstly, there are clear dips in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values around age 9-11 in at least three individuals, all females (LHUE34, LHUE36 and LHUE52), which result in a fairly consistent mean decrease of ca. 0.5‰ in $\delta^{13}\text{C}$ and 1.0‰ in $\delta^{15}\text{N}$ over a period of 1.5-2 years. And secondly, there is slight but statistically significant increase in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from childhood to adolescence ($\delta^{13}\text{C}$: $r^2 = 0.090$; $p = 0.001$ / $\rho = 0.251$; $p = 0.005$; $\delta^{15}\text{N}$: $r^2 = 0.086$; $p = 0.001$ / $\rho = 0.291$; $p = 0.001$) (Fig. 3), which may start to reverse at age 12-13 onwards in at least two cases (LHUE34 and LHUE 2i).

Comparison between the sexes is limited by sample size. Statistical tests reveal no significant differences in the isotope profiles of females and males overall or within specific age categories

(Table S3), which is consistent with the absence of sex-related divergences in adult bone collagen data (Fernández-Crespo and Schulting, 2017). However, female infants appear to have been exclusively breastfed for longer (1.4 ± 0.2 years) than males (1.0 ± 0.2 years) ($t = 2.748$, $df = 5$, $p = 0.040$) (cf. Table 1). No differences are seen in the timing of weaning. The aforementioned presence of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ dips in the isotope profiles of the three female individuals analysed (and not in those of males) is also worth mentioning.

Finally, comparing the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of survivors (i.e. dentine) and non-survivors (i.e. bone) shows that mean bone collagen isotope values are significantly more depleted than those of dentine formed at the same age (paired t -test: $\delta^{13}\text{C}$: $t = 5.884$, $df = 11$, $p < 0.001$; $\delta^{15}\text{N}$: $t = 4.158$, $df = 11$, $p = 0.002$) (Table S4; Fig. 4).

Discussion

The first relevant finding to be addressed is the estimated prolonged period of exclusive nursing documented in Alto de la Huesera. Ethnographic studies have described prolonged breastfeeding (beyond 2 and occasionally even beyond 4 years) as a common response to food shortages in Africa (Lindstrom and Berhanu, 2000), which may also have been a strategy followed by past populations (Sandberg et al., 2014). In this respect, there is evidence that the Late Neolithic Rioja Alavesa region witnessed demographic pressure and social unrest lasting at least a few generations (Fernández-Crespo, 2017; Vegas et al., 2012) and, so, some extent of deprivation and malnutrition is not implausible.

This response, however, has its cons. Several studies have found a link between protracted exclusive breastfeeding and increased mortality in children (Sandberg et al. 2014: 289), suggesting that infant feeding regimes that introduced infants to weaning foods in the first year of life had a positive effect on their survival, while later weaned infants, although capable of some immunological response, were probably undernourished, putting them at a disadvantage when encountering bacteria in their weaning food (Pearson et al., 2010). In 2003 the World Health Organization reported that ca. 4.5 million children die every year of weaning-related disorders, where timing plays a critical role (together with food quality and safety), because infant foods should not be introduced too early or too late (WHO, 2003). While breastmilk is by far the most appropriate food for supporting growth and development of infants providing a reliable and easily digestible source of calories and nutrients, and buffering against pathogens, alone it is insufficient to

meet nutritional requirements beyond ca. 6 months of age (Kramer and Kakuma, 2004). In this regard, it is estimated that breastmilk only provides as much as a half of a child's energy needs between the ages of 6 and 12 months, and up to one third of energy needs between 12 and 24 months (WHO, 2005). Thus, lactation needs to be complemented with food (after 6 months). Otherwise, it will lead to nutritional stress, some extent of immunological depression and, occasionally, some metabolic disorders (e.g. rickets, anemia). The identification of three cases of child and adolescent long bone deformities at Alto de la Huesera compatible with rickets (Etxeberria, 1991; Fernández-Crespo, 2012) may be evidence of the pernicious effects of prolonged exclusive nursing (Mughal et al., 1999) identified here.

In this scenario, the apparently shorter exclusive breastfeeding (though still prolonged past the recommended 6 months) seen in the males at Alto de la Huesera, may be understood as a parental investment strategy in which boys preferentially receive complementary foods earlier in a context of possible food scarcity. The reason behind such a differential investment by sex may be the higher value placed on males in warlike societies (Divale and Harris, 1976). The incidence of conflict has been associated with a preference for rearing male children, along with female infanticide or the benign neglect of female infants (Divale, 1970; Divale and Harris, 1976), which might be supported by the systematic demographic bias favoring males in the region's mortuary monuments (Fernández-Crespo and de-la-Rúa, 2015). Simultaneous or alternatively, earlier weaning among males may also have been intended to limit body contact with their mothers, as a means to foster aggressive, belligerent personality among potential future warriors (Ember and Ember, 1994).

Similarly, weaning seems to be an extended process in Alto de la Huesera. This could also be related with problems with food availability and supply during an extended period of even low-level conflict, due to perceived danger and insecurity. The presence of caries in deciduous dentition among some coeval megalith populations of the region has been interpreted as the result of the common use of sticky texture and high carbohydrate/sugar content for early childhood diets (de-la-Rúa and Arriaga, 2004). It is possible that the initial complementary food was a C₃ cereal gruel. As fully weaned individuals have isotope values similar to those of adults, it is likely that dairy products and meat (e.g. sheep/goat, cattle, pig) would have complemented early childhood diets, contributing to the observed gradual decrease in both stable isotope ratios (Sandberg et al., 2014).

The second main finding is the observation of age-related divergences among fully weaned child and adolescent dentine isotope ratios. Firstly, there are clear dips in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values around age 9-11 in three of the seven individuals studied here, all females. While some studies have

suggested that metabolic processes associated with normal growth may affect nitrogen isotope fractionation (e.g., D'Ortenzio et al., 2015), the fact that others have not found this correlation (Waters-Rist and Katzenberg, 2009) suggests that age-related subsistence practices, particularly supplementation by children of the parentally-provisioned diet (Greenwald et al., 2016) or changes in the amount and/or type of dietary protein (Turner et al., 2007), may be behind this pattern. Considering that this is only seen in females in our study around the time of puberty, it may involve certain food restrictions associated with their preparation for fully adult living. Secondly, there is a progressive increase in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from childhood to adolescence, a tendency that may reverse at the beginning of adolescence (i.e. progressive decrease in both ratios). The absence of age-based isotope correlations in the vast majority of the published paleodietary studies in Neolithic Europe (e.g. Hedges et al., 2013) supports the idea that the underlying explanation should be sought in behavioral rather than purely physiological factors (Messer, 1997).

Finally, an important finding is that of a significant difference between the dentine and the bone isotope collagen values of those who reached adulthood and those who did not. Physiological stressors, such as high rates of skeletal growth, disease or starvation, can alter isotope discrimination factors in metabolic pathways, most notably in the catabolic recycling of nitrogen in tissues when protein is limited, increasing $\delta^{15}\text{N}$ values (Katzenberg and Lovell, 1999; D'Ortenzio et al., 2015). However, what has been found here is a systematic depletion in both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of non-survivors compared to survivors. This suggests that non-adults who consumed less protein (e.g. low-quality post-weaning diets and/or dietary protein sources from lower trophic level foods) had a higher mortality risk (Waterman, Tykot and Silva, 2016:143). Complicating this interpretation, it is likely that in this context of potential food deprivation the non-survivor sample is dominated by health-compromised children, as malnourished children have a greater risk of infection (Lewis, 2007:100). Thus, children may have been cared for and fed differently depending on their health status, since they may have had dissimilar physical needs and nutritional requirements (Kyle et al., 2012). Parents may also have made strategic decisions concerning their offspring in times of food shortage. In any case and independently of the rationale behind it, it is probable that insights into the duration of breastfeeding, onset and duration of weaning and many other practices in a number of isotope studies are biased towards an isotopic characterization of samples that are potentially dominated by unhealthy children as they did not reach adulthood (Fuller et al., 2003; Katzenberg et al., 1996).

Conclusion

Understanding variability in early dietary practices is one of the key aims of paleodietary studies due to their ability to inform on cultural behaviors, health and population dynamics. The results of the present paper provide evidence of prolonged exclusive breastfeeding and protracted weaning, which may be related to possible food shortage due to great demographic pressure and social unrest in the Rioja Alavesa region during the Late Neolithic. Potential differences between males and females in the duration of exclusive breastfeeding are provisionally interpreted as a result of the higher value placed on males in warlike societies. Analyses of other contemporary sites in the region are underway to confirm these observations. Moreover, age-related divergences among fully weaned child and adolescent dentine isotope ratios are identified. Finally, the data support the existence of potential isotope differences between dentine and isotope values from individuals who reached adulthood and those who did not, suggesting lower protein intake among the latter and further confirming the importance of life-history approaches based on sequential dentine collagen from adult individuals.

Acknowledgements

We are grateful to Javier Fernández-Eraso and José Antonio Mujika (University of the Basque Country) for providing unpublished information, Peter Ditchfield (University of Oxford) for Mass Spectrometry technical support, Javier Ordoño (Arkikus) for graphic assistance and J. Aguirre (Museo de Arqueología de Álava - Bibat) for the facilities rendered for collecting samples. This research has been funded by the John Fell OUP Research Fund (Project title: *Social differentiation and life histories in Late Neolithic/Early Chalcolithic Northern Spain (EBD10940 - 151/102)*), and supported by the Basque Government (POS_2015_2_0001; IT542/10) and the University of the Basque Country (UPV/EHU) (UFI11/09).

Supporting information

Supplementary data related to this article can be found in the online edition. These include:

Table S1. Bone collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from the 17 non-adult individuals selected for comparison with dentine values.

Table S2. Sequential dentine collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from the seven individuals selected for the early life-histories approach.

Table S3. Comparison between male and female dentine collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

Table S4. Comparison between non-adult bone and dentine collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values by age.

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Table 1. Duration of exclusive breastfeeding and weaning process, age at complete weaning, and pre-weaning, post-weaning and adult $\delta^{15}\text{N}$ values for the seven individuals selected for the early life-histories approach.									
ID	Inventory no.	Age at death (years)	Sex	Estimated duration of exclusive breastfeeding (years)	$\delta^{15}\text{N}$ (‰) (AIR) pre-weaning (dentine)	Estimated duration of weaning process (years)	Age at complete weaning (years)	$\delta^{15}\text{N}$ (‰) (AIR) post-weaning (dentine)	$\delta^{15}\text{N}$ (‰) (AIR) adulthood (bone)
LHUE34	AH.2111	35-40	Female	1.5	12.4	3.3	4.8	7.9	8.7
LHUE36	AH.2157	30-35	Female	< 1.5	indet.	> 2.0	3.5	10.2	9.8
LHUE52	AH.2170	24-30	Prob. female	1.1	12.0	2.5	3.6	9.1	9.1
LHUE53	AH.2169	45-55	Male	1.1	12.9	2.7	3.8	8.9	9.8
LHUE2i	AH.2075	24-30	Male	< 1.1	indet.	> 2.2	3.3	8.4	8.3
LHUE17c	AH.2383.2	40-45	Male	≤ 0.8	indet.	≥ 2.8	3.6	8.4	8.8
LHUE19c	AH.2386.2	40-55	Male	≤ 0.9	indet.	≥ 3.8	4.7	8.2	9.5
Mean				≤ 1.1	≈ 12.4	≥ 2.8	3.9	8.7	9.1
σ				0.3	0.5	0.6	0.6	0.8	0.6