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Title: Puberty in the Bronze Age: first application of a puberty estimation method to a prehistoric population

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ABSTRACT

Puberty and adolescence represent a significant period of physical growth and maturation and a critical life stage in which children transition into adults within their societies. Numerous studies have observed a secular trend and have determined that puberty is now occurring earlier than in the past. This investigation represents the first application of a methodology for assessing the pubertal status of osteological remains to a prehistoric skeletal sample. Six Bronze Age adolescent skeletons from the *Cerro de La Encantada* archaeological site (Ciudad Real, Spain) were analyzed. Pre-pubescence was observed at age 9 and the transition phase of the pubertal growth spurt at 15 years of age. These results were similar to those obtained from medieval, Industrial Revolution, and modern populations, both within and outside of the Iberian Peninsula. The similarity in the development of the Bronze Age adolescents to that of other past and contemporary populations suggests that the pubertal process has remained essentially unchanged across millennia until recent times. However, other interpretations, including the influence of a subpar developmental environment and potential methodological artifacts, are possible. Nevertheless, studies of this type provide important information about a crucial transitory period in human development.

INTRODUCTION

Puberty, the process by which complicated hormonal interactions bring about internal and external changes in sexual characteristics and eventual reproductive ability (Dorn & Biro, 2011), represents one of the most important periods of physical growth and maturation in the human life cycle. The pubertal growth spurt, a hallmark of this process, is associated with skeletal growth and results in the achievement of adult height; following onset of the spurt, growth accelerates until peak height velocity (PHV) is reached, at which point it decelerates and finally ceases with epiphyseal fusion of the long bones (Rogol et al., 2000). Menarche, the onset of menstruation, is achieved approximately one year after PHV in the deceleration phase (Hägg & Taranger, 1982). During adolescence, a term that includes both the biological and social development that results in reproductive ability and social maturity (Hochberg and Belsky, 2013), individuals typically occupy new roles and take on new responsibilities within their communities. Accordingly, puberty is understood, valued, and experienced differently depending on the cultural and religious standards in place in a given society (Muuss, 1970; Weisfeld, 1997).

The timing and tempo of puberty have been investigated in depth in clinical studies of modern populations, many of which have suggested that puberty is now occurring earlier than in the past (eg: Aksglaede et al., 2008; Biro et al., 2013). Within the Iberian Peninsula, for example, the average age at menarche decreased by 0.22 years in one decade in both rural and urban Madrid (Marrodán et al., 2000). It has been suggested that following the Industrial Revolution, improvements in hygiene, medicine, and nutrition resulted in decreasing age at menarche compared with medieval highs (Gluckman & Hanson, 2006). Thereafter, starting at the end of the 19th century, age at menarche has been decreasing and adult heights increasing

(Padez, 2007). However, as reliable records of pubertal development are limited, studies investigating this secular trend can only investigate a narrow time period.

A novel way to study puberty in the past has been made possible with the proposal of an osteological methodology by Shapland and Lewis (2013, 2014). This method, which has opened up a new branch of bioarchaeology, evaluates skeletal and dental development to determine an individual‟s placement on the pubertal growth spurt at the time of death. A wide variety of individuals and populations from various geographic, temporal, cultural, and religious backgrounds including Medieval (Lewis et al., 2016a) and Roman British (Arthur et al., 2016), 20th century Portuguese (Henderson & Padez, 2017), and medieval Hispano-Muslim (Doe et al., 2019) populations have been studied. These studies have all indicated that the pubertal growth spurt began at ages similar to those observed in modern adolescents, but delays in reaching the later stages of development caused a prolonged puberty. These delays were exacerbated in individuals with evidence of chronic illness (Lewis et al., 2016b) and a recent study of non-adult health from rural and urban England during the Industrial Revolution also found delays in pubertal development (Gowland et al., 2018).

While attempts have been made to approximate puberty and age at menarche in prehistory (eg: Gluckman & Hanson, 2006; Papadimitrious, 2016), this study represents the first application of a comprehensive osteological methodology to a prehistoric skeletal collection. A better understanding of the pubertal process in the Bronze Age in the Iberian Peninsula would permit for the secular trend to be studied across millennia. Moreover, any additional applications would help to verify the potential use of the methodology.

The objectives of this study were to apply the Shapland and Lewis (2013, 2014) methodology to the adolescent individuals of the *El Cerro de La Encantada* archaeological site and to compare the results obtained with those from previous puberty studies on past populations.

MATERIALS AND METHODS

Materials

The adolescent sample includes the remains belonging to six individuals that were excavated from the Bronze Age archaeological site *El Cerro de La Encantada*, located in the province of Ciudad Real, Spain (Figure 1).

[Figure 1 here]

El Cerro de La Encantada is located in the town of Granátula de Calatrava in Ciudad Real, a region in central Spain. Strategically located for visual control of the local communication routes and surrounding areas, the site is difficult to access and takes advantage of the natural defense provided by the topography. The archaeological site dates from 2500-1450 BC, placing it in the Spanish, specifically *La Mancha*, Bronze Age (Sánchez Meseguer & Galán Saulnier, in press).

The *El Cerro de La Encantada* archaeological site is a rich complex with remains of houses, defensive constructions, and assorted funerary areas including a necropolis. Various types of tombs have been discovered including slab tombs, stone tombs, *pithoi* burials and pit graves. Simple and double burials were identified. In double inhumations, an individual in a hyper flexed position represented the primary burial and the remains of a second individual were found piled together near the waist of the primary. Grave goods were associated with some burials and include ceramics, weapons such as daggers and arrowheads made of silver and copper, and jewelry made of stone, bone, and metal (Romero Salas, 1987). This site has been the subject of various anthropological studies due to the favorable state of preservation

displayed by the skeletons recovered (eg: González Martín et al., 1992; González Martín et al., 1994; Molina et al., 2016). More than eighty individuals have been excavated from the site, of which, 39 were placed in a non-adult category (Molina et al., 2016).

Methods

Preservation was analyzed using the "State of Taphonomical Alteration" method (abbreviated EAT for "*Estado de Alteración Tafonómica*" in Spanish), which provides a numerical value from one, optimal preservation, to nine, very poor preservation (Rascón et al., 2011). Age-at-death was estimated according to the eruption and calcification of the mandibular and maxillary dentition (Ubelaker, 1978) and sex assignments were attempted using features of the distal humerus (Rogers, 1999, 2009) and pelvis (Phenice, 1969; Bruzek, 2002).

Pubertal status was estimated using the Shapland and Lewis (2013, 2014) methodology, which takes into consideration the development of six osteological markers: 1) calcification of the permanent mandibular canine, 2) cervical vertebrae maturation (CVM), 3) development of the hamate hook and the epiphyseal fusion of the 4) distal radius, 5) hand phalanges, and 6) the iliac crest. Each of the aforementioned markers was examined macroscopically and the degree of development was analyzed on both the left and right side for paired indicators. If asymmetry was observed, the more advanced stage was used. Epiphyseal fusion was graded as "unfused", "partially fused" and "fused" according to Buikstra and Ubelaker (1994). With regards to the cervical vertebrae, the general shape of the vertebral body and the concavity in the inferior border were recorded and a CVM stage was assigned only when a decisive identification of the second, third and fourth vertebrae was possible.

Six pubertal stages corresponding to specific points on the growth spurt were used following Lewis et al. (2016a): (1) pre-pubescence, (2) acceleration, (3) transition/PHV, (4) deceleration, (5) maturation and (6) post-pubescence. These stages were assigned based on the development of the aforementioned pubertal indicators (Table 1).

Pathological and non-pathological characters of interest were evaluated in the entire skeleton based on the method of elementary alterations proposed by Thillaud and Charon (1994). Dental enamel hypoplasia (DEH) represents disrupted growth of tooth enamel during dental calcification possibly associated with poor diet and/or disease (Lewis, 2017), two factors which have also been shown to influence pubertal development (eg: Kaplowitz, 2010; Soliman et al., 2014). For this reason, the presence or absence of DEH was recorded for all individuals and when present, an approximate age at which the defect(s) formed was determined using the age ranges of dental crown formation following Ubelaker (1978).

RESULTS

Although seven potential adolescent skeletons from the osteoarchaeological population of *El Cerro de La Encantada* were initially identified, six were included in the following study; one skeleton presented without dental remains making an age-at-death estimation impossible. Age-at-death estimations of the six individuals in the subsample range from 9 to 15-years. It was only possible to assign a sex to one individual, a probable male, while a pubertal stage was determined for four individuals (Table 2). Hereafter, each skeleton will be considered separately.

EN-2

This individual is considered “well preserved” as the EAT was determined to be type 2. Bone quality was “partially altered” and all anatomic units (AU) were present (AU=8), although the pelvic region was badly damaged.

Age-at-death was estimated at 15 ± 3 years based on dental development and indeterminate sex was assigned as the distal humerus displayed both male and female characteristics and a less than ideal preservation of the pelvis made any assessment of the pubis impossible.

EN-2 presented five of the six osteological markers of the transition stage: fully calcified left permanent mandibular canine, completely developed hook on the right hamate, unfused epiphyses on the right hand phalanges, an unfused right distal radius, and CVM stage 3. Given the state of preservation of the pelvic region, an evaluation of the iliac crest epiphysis was impossible. It was determined that this individual was in the transition stage of the pubertal growth spurt and had probably already reached PHV at the time of death (Table 2).

This individual also presented with various pathological and non-pathological characteristics worth highlighting. Fusion was observed between two vertebrae, most likely T5 and T6, completely affecting the transverse processes and posterior arches (Figure 2). This pathology is compatible with a posterior congenital vertebral block. Congenital vertebral fusion, or block vertebra, usually occurs in the lumbar and cervical segments, but when the block vertebra occurs at the thoracic level, as in this case, it typically impacts the mid-thoracic region (Kumar et al., 1988; Barnes, 1994). The lack of evidence of pathology in the remainder of the vertebral column and in the base of the cranium rules out the possibility of Klippel-Feil syndrome (Barnes, 1994; Campo Martín, 2003). Porosity was also noted in the

vertebral bodies. Additionally, DEH lines on the mandibular left canine and central and lateral incisors and canines of the maxilla were observed. These lines have been associated with disrupted growth of tooth enamel that would have occurred in the calcification period of the incisors (between 6 ± 3 months and 4 ± 1 year) and of the canines (between 6 ± 3 months and 5 ± 1.5 years) (Figure 3). Lastly, the mastoid process presented with a green stain, probably resulting from the degradation of a copper adornment, possibly an earring.

EN-5

This individual is considered to be "highly altered" with an EAT type 6. Bone quality was determined to be "altered" and, with only a fragment of the occipital bone, one cervical vertebra, two proximal foot phalanges, and various loose teeth preserved, the skeleton is incomplete.

Age-at-death was estimated to have been 10 ± 2.5 years based on the calcification of the dentition. Due to the absence of the necessary skeletal elements, indeterminate sex was assigned to this individual and an assessment of the pubertal development was impossible (Table 2). EN-5 presented with DEH lines on the upper left and right central incisors, which would have formed roughly between 6 months and 4 years of age.

EN-14.1

EN-14.1 was determined to have an intermediate degree of preservation; although all AU were represented, the quality of the bone was very altered indicating a final EAT of type 3.

This individual was estimated to have died at 15 ± 3 years of age based on eruption and calcification of the dentition. Indeterminate sex was assigned as neither coxal bones nor the distal portions of the humeri were preserved.

With regards to pubertal development, remains of the left arm include unfused epiphyses of the hand phalanges and the distal radius, which indicate maximum development of stages 3 and 4, respectively. The cervical vertebrae were sufficiently preserved to decisively identify C2, C3, and C4 and the degree of CVM was determined to be stage 3. These three osteological markers indicate that this individual died during the transition phase of the pubertal growth spurt (Table 2).

As characters of interest, DEH lines have been identified; maxillary left central and lateral incisors would have formed approximately between 6 months and 4 years of age while lines on both maxillary and mandibular left canines would have formed between 6 months and 5 years of age.

EN-14.2

This individual is represented by only the mandible and mandibular dentition and is therefore considered to be "scarce remains". A final EAT of type 8 reflects the partially altered bone quality observed. It was possible to estimate an age-at-death using the eruption and calcification of the mandibular dentition and this skeleton was determined to have died at 9 ± 1 years of age. Understandably, sex assignment was impossible. Mandibular canines were not preserved and nothing could be determined about pubertal status (Table 2). Lastly, the pre-mortem loss of the mandibular left M1 was observed evidenced by a partial alveolar reabsorption and significant dental wear in the remaining right M1.

EN- 22.1

This individual is “well preserved” with an EAT type 2 as all AU are represented (AU=8) and bone quality is considered to be partially altered.

Age-at-death was estimated to be 15 ± 3 years. This individual was determined to be a probable male based on characteristics of the distal humerus. Moreover, although the Phenice (1969) method is less than ideal for individuals with age-at-death estimations under 23 years, certain masculine characteristics were seen in the pubis, such as the absence of the ventral arc and subpubic concavity and a rounded pubic tubercle. Lastly, the sciatic notch was also observed to have a masculine appearance following the method of Bruzek (2002).

This individual presented with four pubertal indicators enabling the assignment of a pubertal stage. Unfused epiphyses on the right hand phalanges and a fully developed right hamate hook suggested that PHV had been achieved. The epiphysis of the left distal radius was preserved but fusion had not yet begun. Both coxal bones were sufficiently preserved to observe that neither left nor right iliac crests had any signs of fusion of the epiphyses. All dentition was maintained in the mandible making observation of the mandibular canine roots impossible and poor preservation of the cervical vertebrae did not permit for CVM stage determination. The stages of four pubertal markers indicate that this individual died during the transition phase of the pubertal growth spurt, after reaching PHV (Table 2).

DEH lines were observed on the maxillary central and lateral incisors, which would have formed around between 6 months and 4 years, and canines, forming between 6 months and 5 years of age.

EN- 61.1

The EAT of this individual was determined to be type 3 as all AU were represented (AU=8), but the bone tissue was significantly altered.

Age-at-death was estimated at 9 ± 1 years and indeterminate sex was assigned as the significant alteration of the bone tissue did not permit for a sex assignment.

This skeleton was determined to have been pre-pubescent at the time of death. The left permanent mandibular canine of this individual had yet to reached Demirjian stage F (Demirjian et al., 1985), indicating pre-pubescence. Unfused epiphyses on the right hand phalanges and an unfused right distal radius were observed, two marker stages also observed in pre-pubescence. Unfortunately, neither the left nor right hamate, whose development is associated with the early phases of the growth spurt, were preserved. While C2 was intact, C3 and C4 could not be definitely determined making analysis of the CVM stage impossible (Table 2).

DISCUSSION

This study represents the first application of the Shapland and Lewis (2013, 2014) methodology for assessing pubertal development of osteological remains to a prehistoric skeletal collection. Of the seven potential adolescent skeletons belonging to the Bronze Age *El Cerro de La Encantada* archaeological population, it was possible to estimate an age-at-death for six; ages range from 9-years to 15-years. Only one skeleton, EN-22.1, a probable male, was assigned a sex due to the less than ideal preservation of the subsample. Lastly, it was possible to determine a stage of pubertal development for four skeletons (Table 2). In

spite of a limited sample size, the results obtained were similar to those of other archaeological populations.

Degree of preservation is undeniably linked to the amount information that can be obtained in an osteological investigation. With regards to EAT values and the applicability of the Shapland and Lewis (2013, 2014) methodology, a pubertal stage assignment was possible for four of six adolescents. These skeletons presented with either EAT type 2, EN-2 and EN-22.1, or type 3, EN-14.1 and EN-61.1. Both EAT types 2 and 3 require a complete skeleton with 7 or 8 AU preserved. As the methodology for determining pubertal status takes into consideration the development of six osteological indicators representing various parts of the body, it is unsurprising that its application was possible in "complete" skeletons. Conversely, the two individuals in whom a pubertal assessment was impossible, skeletons EN-5 and EN-14.2, presented with only three and one AU, respectively, greatly hindering an investigation of puberty. While other researchers have commented on the necessity for well-preserved remains in studies of this type (eg: Henderson & Padez, 2017), future investigations on larger populations should aim to study preservation and method applicability specifically. Nonetheless, it is important to take all individuals into consideration including those that are less well preserved, as valuable information can always be obtained (Molina et al., 2016).

One skeleton from *El Cerro de La Encantada*, EN-61.1, was determined to have been pre-pubescent at 9-years, an age similar to previous archaeological populations and modern Spaniards (Carrascosa et al., 2004, 2012). In historic British skeletons, pre-pubescence was observed between 8 and 9 years of age in Roman adolescents (Arthur et al., 2016) and between 10-11 years in both medieval (Lewis et al., 2016b) and Industrial Revolution (Gowland et al., 2018) individuals. Within the Iberian Peninsula, similar trends were observed; medieval Hispano-Muslims between 9 and 10 years of age were placed into the pre-pubescence phase (Doe et al., 2019) while the range was 8 to 10 years in early 20th

century Portuguese adolescents (Henderson & Padez, 2017). Unfortunately, neither onset nor the acceleration phase of the growth spurt were observed in the prehistoric adolescents.

The transition phase, which includes PHV, was observed in three skeletons from the *El Cerro de La Encantada* population, all with estimated ages-at-death of 15 years. These results are similar to those obtained from other osteological collections from the Iberian Peninsula; the acceleration (Henderson & Padez, 2017) and transition (Doe et al., 2019) phases of the growth spurt were observed in individuals with ages-at-death of up to 16 years. The results do, though, reflect a delay compared with modern Spaniards, in whom PHV is typically achieved at 12 years of age in girls and 14 years in boys (Carrascosa et al., 2004, 2012). Delays in reaching PHV and the later pubertal stages appear to be a hallmark of osteological puberty trends.

While studies of prehistoric puberty are few and far between, most focus on the achievement of menarche and female reproductive competence. Onset of menstruation is highly variable and has been shown to be greatly influenced by the environmental conditions (eg: Boynton-Jarrett & Harville, 2012) making it an ideal indicator of developmental environments in the past. Osteological markers linked with the achievement of menarche do exist, namely the epiphyseal fusion of the hand phalanges and the ossification and fusion of the iliac crest epiphysis (Lewis et al., 2016a), however as no probable females were identified among the *El Cerro de La Encantada* adolescents, it was impossible to obtain any data about the age at menarche for this population.

Regrettably, the later pubertal stages of maturation and deceleration and post-pubescence were not observed in this sample. Although the data is limited and it was not possible to make all indicator assessments, taking the results that were obtained into consideration, the general pubertal trends appear quite similar to those of other archaeological populations, despite significant temporal differences. This suggests that there has been little

to no change in the biological pattern of pubertal development in thousands of years within the Iberian Peninsula, leading one to question why this might be.

It is possible that the "non-survivors" (Wood et al., 1992) included in this study suffered disease or illness which could have caused delayed growth and premature death. The ability of illness to delay pubertal development has been thoroughly studied in modern adolescents (eg: Pozo & Argente, 2002; Kaplowitz, 2010) and has been observed in archaeological populations as well (Lewis et al., 2016b). With regards to the *El Cerro de La Encantada* individuals, previous studies have identified various characteristics with a possible pathological origin in the non-adult skeletons, including porosity in the *pars basilaris* (González Martín et al., 2018) and osteological markers related with rickets (González Martín et al., 1999). The majority of individuals in this study (4/6), specifically skeletons EN-2, EN-5, EN-14.1 and EN-22.1, presented with DEH. This indicator of stress, which has been associated with illness and nutritional deficiency (Lewis, 2017), has been identified in the Bronze Age population of *Motilla del Azuer* (Nájera Colino et al., 2006), also from the Spanish province of Ciudad Real, and other Neolithic populations from the northeastern Iberian Peninsula (Gibaja et al., 2010). Similarly, Arthur et al. (2016) noted that indicators of stress including DEH were present in the majority of adolescents included in an investigation of puberty in Roman Britain. Modern clinical studies have suggested various possible causes for DEH including celiac disease and infections such as mumps (Rashid et al., 2011), both of which have been linked to delayed puberty, albeit mumps only in males (Kaplowitz, 2010). Moreover, it has been suggested that stress early in life, indicated by the presence of DEH, reduces the ability to survive future stresses thereby increasing mortality (Temple, 2019). The prehistoric individuals in this study could have experienced delayed development due to less than ideal environmental conditions, failed to overcome additional hardship, and died prematurely during puberty.

In view of the results obtained, the possibility for delayed puberty in the *El Cerro de La Encantada* adolescents, and other osteological populations thus far studied, could be due to the inherent nature of investigations which analyze unidentified skeletal remains rather than a consequence of illness or poor nutrition; with the exception of the early 20th century Portuguese individuals who come from a documented skeletal collection, age-at-death is unknown. Although dental development is more reliable than ageing based on skeletal maturation as it is less impacted by external environmental stimuli (Smith, 1991; Cardoso, 2007), there is still a significant degree of uncertainty associated with these estimations. In this study, age ranges of up to ± 3 years were included and their impact could be significant; a 12-year-old individual in the transition phase would be on par with modern developmental trends (Carrascosa et al., 2004, 2012) while an 18-year-old in the same phase would be severely delayed, even in comparison with other historic individuals. Evidently, these ranges could significantly skew the developmental trends observed. Age-at-death estimations are a limitation confronted by all those studying skeletal remains, but when studying a phenomenon with such a short duration as puberty, the importance of the associated ranges is paramount. The uncertainties associated with age-at-death estimations could represent a significant methodological artifact that cannot be overcome.

In spite of the aforementioned limitations, puberty studies must be carried out on more populations representing different time periods, geographic locations, and cultural traditions. When studying development in prehistory, the only indicators available are skeletal, and osteological investigations are the first and only tool. For this reason, the Shapland and Lewis (2013, 2014) method is of the utmost importance; it permits for an understanding of puberty and adolescence in the past when there are no written sources. Additionally, only with more data will the significance of this methodology be further supported. The authors look forward to future osteological studies of prehistoric populations

with special attention paid to preservation and method applicability and age at menarche, as it is an ideal indicator of the developmental environment in the past.

CONCLUSIONS

This study represents the first application of the Shapland and Lewis (2013, 2014) methodology for assessing pubertal development of adolescent remains in a prehistoric population. Of the six individuals included in the study, it was possible to assign a pubertal stage to four skeletons, highlighting the importance of preservation state in the applicability of this method. The Spanish Bronze Age adolescents of *El Cerro de La Encantada* displayed pubertal development trends comparable to other historic populations; pre-pubescence was observed at 9 years of age, similar to both contemporary adolescents and archaeological adolescent skeletons from within and outside of the Iberian Peninsula. The achievement of PHV was observed in three individuals with estimated ages-at-death of 15 years. While this does represent a significant delay compared with modern developmental tendencies, similar results were observed in Roman (Arthur et al., 2016), medieval (Lewis et al., 2016b), and Industrial Revolution British skeletons (Gowland et al., 2018) and medieval Hispano-Muslims (Doe et al., 2019). The similarity in the results of the *El Cerro de La Encantada* adolescents and all other historic populations is striking and implies that there has been little to no change in the biological pattern of pubertal development in thousands of years, although other explanations are possible.

It has been suggested that the delayed puberty observed in archaeological adolescents could be due to a less than ideal developmental environment (eg: Arthur et al., 2016) and the *El Cerro de La Encantada* skeletons do show evidence of generalized stress, most likely due to nutritional deficiencies or illness. While the puberty process would have been more

significantly impacted in those suffering from a chronic condition rather than an acute illness, as cause of death is unknown, the assumption is made that these skeletons are representative of their environment. However, as all adolescents included in bioarchaeological studies are "non-survivors" (Wood et al., 1992), their remains may simply reflect an increased mortality that could have lead to premature death. Alternatively, the ranges associated with age-at-death estimations have the ability to skew the developmental trends observed, especially as the pubertal process takes place over a brief period of time. Nevertheless, the study of puberty from osteological material enables an understanding of this crucial transitory phase in the human life cycle in individuals and populations in whom this would otherwise be impossible. The authors look forward to additional applications of the Shapland and Lewis (2013, 2014) methodology in other prehistoric populations.

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REFERENCES

Aksglaede, L., Olsen, L.W., Sørensen, T.I.A., & Juul, A. (2008). Forty years trends in timing of pubertal growth spurt in 157,000 Danish school children. *PLoS One*, 3, 2728. doi: 10.1371/journal.pone.0002728

Arthur, N.A., Gowland, R.L., & Redfern, R.C. (2016). Coming of age in roman Britain: osteological evidence for pubertal timing. *Am J Phys Anthropol*, 159(4), 698-713. doi: 10.1002/ajpa.22929

Barnes, E. (1994). *Developmental Defects of the Axial Skeleton in Paleopathology*. Niwot, Colorado: University Press of Colorado.

Biro, F.M., Greenspan, L.C., Galve, M.P., Pinney, S.M., Teitelbaum, S., Windham, G.C., Deardorff, J., Herrick, R.L., Succop, P.A., Hiatt, R.A., & Kushi, L.H. (2013). Onset of breast development in a longitudinal cohort. *Pediatrics*, 132, 1019–1027. doi: 10.1542/peds.2013-3773

Boynton-Jarrett, R. & Harville, E.W. (2012). A prospective study of childhood social hardships and age at menarche. *Ann Epidemiol*, 22(10), 731-737. doi: 10.1016/j.annepidem.2012.08.005

Buikstra, J.E. & Ubelaker, D. (1994). *Standards for data collection from human skeletal remains*. Arkansas: Arkansas Archaeological Survey.

Bruzek, J. (2002). A method for visual determination of sex, using the human hip bone. *Am J Phys Anthropol*, 117(2), 157-168. doi: 10.1002/apja.10012

Campo Martín, M. (2003). Paleopatología de la columna vertebral. In A. Isidro, A. Malgosa (Eds.), *Paleopatología. La enfermedad no escrita* (pp. 163-193). Barcelona: Masson Ed.

Cardoso, H.F. (2007). Environmental effects on skeletal versus dental development: using a documented subadult skeletal sample to test a basic assumption in human osteological research. *Am J Phys Anthropol*, 132, 223-233. doi: 10.1002/apja.20482

Carrascosa, A., err ndez, A., Audí, L., & S nchez, E. (2012). Pubertal growth and adult height according to age at pubertal growth spurt onset: data from a Spanish study including 540 subjects (281 boys and 259 girls). In V.R. Preedy (Ed.), *Handbook of growth and growth monitoring in health and disease* (pp. 1525-1544). New York: Springer.

Carrascosa, A., Yeste, D., Copil, A., & Gussinyé, M. (2004). Aceleración secular del crecimiento. Valores de peso, talla e índice de masa corporal en niños, adolescentes y adultos jóvenes de la población de Barcelona. *Med Clin (Barc)*, 123(12), 445-451. doi: 10.1016/S0025-7753(04)74552-2

Demirjian, A., Buschang, P.H., Tanguay, R., & Kingnorth Patterson, D. (1985). Interrelationships among measures of somatic, skeletal, dental and sexual maturity. *Am J Orthod*, 88, 433-438.

Doe, D.M., Rascón Pérez, J., Cambra-Moo, O., Campo Martín, M., & González Martín, A. (2019). Assessing pubertal stage in adolescent remains: an investigation of the San Nicolás maqbara burial site (Murcia, Spain). *Archaeol Anthropol Sci*, 11(2), 541-554. doi: 10.1007/s12520-017-0543-0

Dorn, L.D. & Biro, F.M. (2011). Puberty and its measurement: a decade in review. *J Res Adolesc*, 21(1), 180-195. doi:10.1111/j.1532-7795.2010.00722.x

Gibaja, J.F., Majó, T., Chambon, P., Ruiz Ventura, J., & Subirá, M. (2010). Prácticas funerarias durante el Neolítico. Los enterramientos infantiles en el noreste de la Península Ibérica. *Complutum*, 21(2), 47-68.

Gluckman, P.D. & Hanson, M.A. (2006). Evolution, development and timing of puberty. *Trends Endocrinol Metab*, 17(1), 7-12. doi: 10.1016/j.tem.2005.11.006

González Martín, A., Campo Martín, M., Robles Rodríguez, F.J., & Pastor Abascal, I. (1999). Evidencias paleopatológicas de raquitismo en España. n . A. S nchez (Ed.), *Actas V Congreso Nacional de Paleopatología* (pp. 139-145). Jaén, Spain: Ayuntamiento de Alcalá la Real.

González Martín, A., Robles Rodríguez, F.J., Cambra-Moo, O., Rascón Pérez, J., & Campo Martín, M. (2018). Comment upon „Basilar portion porosity: A pathological lesion possibly associated with infantile scurvy". *Int J Paleopathol*, 20, 114-115. doi: 10.1016/j.ijpp.2017.09.003

González Martín, A., Robles Rodríguez, F.J., & González, V.M. (1992). *Analysis of the early childhood from Spanish Bronze Age. Cerro de La Encantada site*. Poster presented at the VIII Congress European Anthropological Association, Madrid, Spain.

González Martín, A., Robles Rodríguez, F.J., & Vlasáková, M. (1994). La Encantada: una población del Bronce español. In C. Bernis, C. Varea, F. Robles, & A. González A (Eds.), *Biología de las poblaciones humanas: problemas metodológicos e interpretación ecológica* (pp. 135-145). Madrid: Ediciones de la Universidad Aut noma de adrid.

Gowland, R.L., Caffell, A., Newman, S., Levene, A., & Holst, M. (2018). Broken childhoods: rural and urban non-adult health during the industrial revolution in Northern England (eighteenth-nineteenth centuries). *Bioarchaeology International*, 2(1), 44-62. doi: 10.5744/bi.2018.1015

Hochberg, Z. & Belsky J. (2013). Evo-devo of human adolescence: beyond disease models of early puberty. *BMC Med*, 11, 113. doi: 10.1186/1741-7015-11-113

Hägg, U. & Taranger, J. (1982). Maturation indicators and the pubertal growth spurt. *Am J Orthod*, 82, 299-309. doi: 10.1016/0002-9416(82)90464-X

Henderson, C.Y. & Padez, C. (2017). Testing times: Identifying puberty in an identified skeletal sample. *Ann Hum Biol*, 44(4), 332-337. doi: 10.1080/03014460.2016.1250949

Kaplowitz, P.B. (2010). Delayed puberty. *Pediatr* Rev, 31(5), 189-195. doi: 10.1542/pir.31-5-189

Kumar, R., Guinto, F.C., Madewell, J.E., Swischuk, L.E. & David R. (1988). The vertebral body: radiographic configurations in various congenital and acquired disorders. *Radiographics*, 3(3), 455-458.

Lewis, M. (2017). *Paleopathology of Children. Identification of pathological conditions in the human skeletal remains of non adults*. London: Academic Press.

Lewis, M.E., Shapland, F., & Watts, R. (2016a). On the threshold of adulthood: a new approach for the use of maturation indicators to assess puberty in adolescents from medieval England. *Am J Hum Biol*, 28, 48–56. doi: 10.1002/ajhb.22761

Lewis, M.E., Shapland, F., & Watts, R. (2016b). The influence of chronic conditions and the environment on pubertal development. An example from medieval England. *Int J Paleopathol*, 12, 1-10. doi: 10.1016/j.ijpp.2015.10.004

Marrodán, M.D., Mesa, M.S., Aréchiga, J., & Pérez-Magdaleno, A. (2000). Trend in menarchal age in Spain: rural and urban comparison during a recent period. *Ann Hum Biol*, 27, 313-319. doi: 10.1080/030144600282190

Molina, M., Herrero, A., & González, A. (2016). Pequeños pero visibles: el estudio osteológico de los individuos no-adultos como fuente de información arqueológica. *Revista Otarq: Otras arqueologías*, 1, 121-135.

Muuss, R.E. (1970). Puberty rites in primitive and modern societies. *Adolescence*, 5(17), 109-128.

Nájera Colino, T., Molina González, F., Sánchez Romero, M., & Aranda Jiménez, G. (2006). Un enterramiento infantil singular en el yacimiento de la Edad del Bronce de la Motilla del Azuer (Daimiel, Ciudad Real). *Trabajos de Prehistoria*, 63(1), 149–156. doi: 10.3989/tp.2006.v63.i1.9

Padez, C. (2007). Secular trend in Portugal. *J Hum Ecol*, 22(1), 15-22. doi: 10.1080/09709274.2007.11905993

Papadimitriou, A. (2016). The evolution of the age at menarche from prehistorical to modern times. J *Pediatr Adolesc Gynecol*, 29(6), 527-530. doi: 10.1016/j.jpag.2015.12.002

Rascón, J., Cambra-Moo, O., Pimentel de Francisco, G., González Martín, A., & Campo Martín, M. (2011). Influencia del estado de preservación de los restos óseos en el diagnóstico paleopatológico. In A. González Martín, O. Cambra-Moo, J. Rascón Pérez, M. Campo Martín, M. Robledo, E. Labajo, & J.A. Sánchez Sánchez (Eds.), *Paleopatología: ciencia multidisciplinar (X Congreso Nacional de Paleopatología)* (pp. 45-59). Madrid: Sociedad Española de Paleopatología, Universidad Autónoma de Madrid, Universidad Complutense de Madrid. Retrieved from https://repositorio.uam.es/handle/10486/11406

Rogol, A.D., Clark, P.A. & Roemmich, J.N. (2000). Growth and pubertal development in children and adolescents: effects of diet and physical activity. *Am J Clin Nutr*, 72, 521S-528S. doi: 10.1093/ajcn/72.2.521S

Phenice, T.W. (1969). A newly developed visual method of sexing the os pubis. *Am J Phys Anthropol*, 30, 297–302. doi: 10.1002.ajpa.1330300214

Pozo, J. & Argente, J. (2002). Delayed puberty in chronic illness. *Best Pract Res Clin Endocrinol Metab*, 16, 73-90. doi: 10.1053/beem.2002.0182

Rashid, M., Zarkadas, M., Anca, A. & Limeback, H. (2011). Oral Manifestations of Celiac Disease: A Clinical Guide for Dentists. *J Can Dent Assoc*, 77(b39), 1-6.

Rogers, T.L. (1999). A visual method of determining the sex of skeletal remains using the distal humerus. *J Forensic Sci*, 44, 57-60. doi: 10.1520/jfs14411j

Rogers, T.L. (2009). Sex determination of adolescent skeletons using the distal humerus. *Am J Phys Anthropol*, 140, 143-148. doi: 10.1002/ajpa.21060

Romero Salas, H. (1987). a personalidad del "horizonte" e cr polis del Cerro de la Encantada. *CuPAUAM (1984 – 1985)*, 11–12, 143 – 151.

Sánchez Meseguer, J.L. & Galán Saulnier, C. (In Press). La Cronología del Cerro de La Encantada: estratigrafía, dataciones radiocarbónicas y paleoclimatología. *Calatrava Estudios*, 1.

Shapland, F. & Lewis, M.E. (2013). Brief communication: a proposed osteological method for the estimation of pubertal stage in human skeletal remains. *Am J Phys Anthropol*, 151, 302-310. doi: 10.1002/ajpa.22268

Shapland, F. & Lewis, M.E. (2014). Brief communication: a proposed method for the assessment of pubertal stage in human skeletal remains using cervical vertebrae maturation. *Am J Phys Anthropol*, 153, 144-153. doi: 10.1002/ajpa.22416

Smith, B.H. (1991). Standards of human tooth formation and dental age assessment. In M.A. Kelly & C.S. Larsen (Eds.). *Advances in dental anthropology* (pp. 143-168). New York: Wiley-Liss.

Soliman, A., De Sanctis, V. & Elalaily R. (2014). Nutrition and pubertal development. *Indian J Endocrinol Metab,* 18(7), 39-47. doi: 10.4103/2230-8210.145073

Temple, D.H. (2019). Bioarchaeological evidence for adaptive plasticity and constraint: Exploring life-history trade-offs in the human past. *Evol Anthropol*, 28(1), 34-46. doi: 10.1002/evan.21754

Thillaud, P. & Charon, P. (1994). - *cueil et identification*. Sceaux, France: Kronos BY Editions.

Ubelaker, D.H. (1978). *Human skeletal remains. Excavation, analysis, interpretation*. Chicago: Aldine.

Weisfeld, G. (1997). Puberty rites as clues to the nature of human adolescence. *Cross Cult Res*, 31(1), 27-54.

Wood, J.W., Milner, G.R., Harpending, H.C., & Weiss, K.M. (1992). The osteological paradox: problems of inferring prehistoric health from skeletal samples. *Curr Anthropol*, 33(4), 343–370. doi: 10.1086/204084

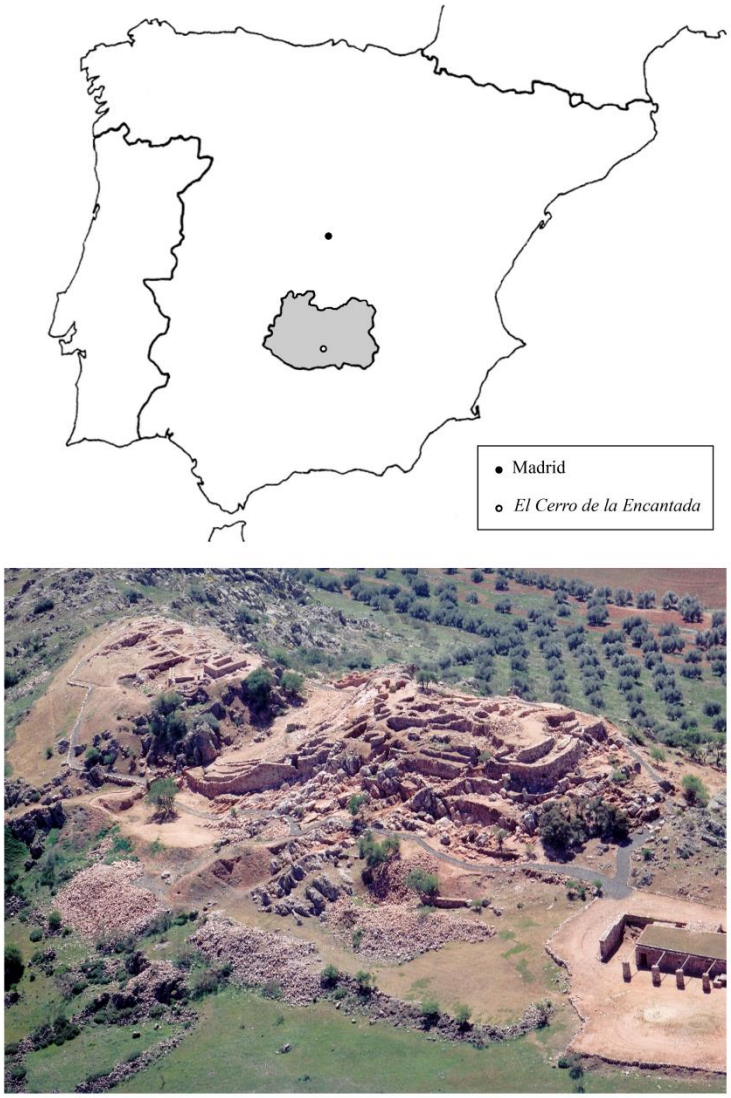

Figure 1 Top, location of *El Cerro de la Encantada* archaeological site, indicated by a white point, within the province of Ciudad Real, in grey. For reference, Madrid is indicated by a black point. Bottom, aerial view of *El Cerro de la Encantada*. Photo courtesy of Dr. J.L. Sánchez Meseguer.

Figure 2 Fusion of T5 and T6 vertebrae in individual EN-2. Left, posterior view. Right, right lateral view. Scale is 1 cm.

Figure 3 Detailed view of the defects in the enamel of the right maxillary canine of individual EN-2.

TABLE 1 – Six pubertal stages and the associated dental and skeletal marker criteria, based on Arthur et al. (2016) and Lewis et al. (2016a).

Pubertal stage	Osteological marker criteria
Pre-pubescence (1)	Mandibular canine stage F or earlier; undeveloped hamate hook; hand phalanges, distal radius, and iliac crest epiphyses unfused; CVM stage 1
Acceleration (2)	Mandibular canine stage G/H; appearing or developing hamate hook; hand phalanges, distal radius, and iliac crest epiphyses unfused; CVM stage 2
Transition/PHV (3)	Mandibular canine stage H; developed hamate hook; hand phalanges, distal radius, and iliac crest epiphyses unfused; CVM stage 3
Deceleration (4)	Mandibular canine stage H; developed hamate hook; hand phalanges fusing; distal radius and iliac crest epiphyses unfused; CVM stage 4/5
Maturation (5)	Mandibular canine stage H; developed hamate hook; hand phalanges fusing/fused, distal radius fusing; iliac crest epiphyses fusing; CVM stage 5/6
Post-pubescence (6)	Mandibular canine stage H; developed hamate hook; hand phalanges and distal radius fused; iliac crest epiphyses fusing/fused; CVM stage 6

TABLE 2– Anthropological, pubertal indicator, and pubertal stage assignment data obtained for the six adolescent individuals from the *El Cerro de la Encantada* archaeological population, ordered by age-at-death estimation.

Individual	EAT type	Age group	Sex	Pubertal Indicators						Pubertal stage
				Mandibular canine	Hamate hook	Hand phalanges	Distal radius	Iliac crest	CVM stage	
EN-14.2	8	9±1	Indeterminate	-	-	-	-	-	-	Indeterminate
EN-61.1	3	9±1	Indeterminate	F	-	unfused	unfused	-	-	Pre-pubescent
EN-5	6	10±2,5	Indeterminate	-	-	-	-	-	-	Indeterminate
EN-2	2	15±3	Indeterminate	H	developed	unfused	unfused	-	3	Transition
EN-14.1	3	15±3	Indeterminate	-	-	unfused	unfused	-	3	Transition
EN-22.1	2	15±3	Probable male	-	developed	unfused	unfused	unfused	-	Transition