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35	Abstract	<p>The goal of the present study is to examine cross-sectional information on the growth of the humerus based on the analysis of four measurements, namely, diaphyseal length, transversal diameter of the proximal (metaphyseal) end of the shaft, epicondylar breadth and vertical diameter of the head. This analysis was performed in 181 individuals (90 ♂ and 91 ♀) ranging from birth to 25 years of age and belonging to three documented Western European skeletal collections (Coimbra, Lisbon and St. Bride). After testing the homogeneity of the sample, the existence of sexual differences (Student's <i>t</i>- and Mann–Whitney <i>U</i>-test) and the growth of the variables (polynomial regression) were evaluated. The results showed the presence of sexual differences in epicondylar breadth above 20 years of age and vertical diameter of the head from 15 years of age, thus indicating that these two variables may be of use in determining sex from that age onward. The growth pattern of the variables showed a continuous increase and followed first- and second-degree polynomials. However, growth of the transversal diameter of the proximal end of the shaft followed a fourth-degree polynomial. Strong correlation coefficients were identified between humeral size and age for each of the four metric variables. These results indicate that any of the humeral measurements studied herein is likely to serve as a useful means of estimating sub-adult age in forensic samples.</p>	
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Humeral development from neonatal period to skeletal maturity—application in age and sex assessment

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**Abstract** The goal of the present study is to examine cross-sectional information on the growth of the humerus based on the analysis of four measurements, namely, diaphyseal length, transversal diameter of the proximal (metaphyseal) end of the shaft, epicondylar breadth and vertical diameter of the head. This analysis was performed in 181 individuals (90 ♂ and 91 ♀) ranging from birth to 25 years of age and belonging to three documented Western European skeletal collections (Coimbra, Lisbon and St. Bride). After testing the homogeneity of the sample, the existence of sexual differences (Student's *t*- and Mann–Whitney *U*-test) and the growth of the variables (polynomial regression) were evaluated. The results showed the presence of sexual differences in epicondylar breadth above 20 years of age and vertical diameter of the head from 15 years of age, thus indicating that these two variables may be of use in determining sex from that age onward. The growth pattern of the variables showed a continuous increase and followed first- and second-degree polynomials. However, growth of the

transversal diameter of the proximal end of the shaft followed a fourth-degree polynomial. Strong correlation coefficients were identified between humeral size and age for each of the four metric variables. These results indicate that any of the humeral measurements studied herein is likely to serve as a useful means of estimating sub-adult age in forensic samples.

**Keywords** Immature · Infants · Skeletal development · Sub-adult age · Sex determination · Forensic science

Introduction

The diagnosis of age and sex are two of the basic steps in any osteological study, whether archaeological or forensic. In the archaeological domain, for instance, both estimations are essential for reconstructing the demographic profiles of past populations and are key to the assessment and interpretation of many factors, including indicators of health status and life conditions. Likewise, in the forensic domain, the age and sex diagnosis are fundamental in order to be able to identify victims, especially in cases of murder or mass disasters where the bodies are grossly mutilated or in advanced stages of decomposition [1]. For identification purposes, apart from sex (which excludes almost half of the population), age is one of the most important criteria for excluding large portions of the population [1]. The reliability and accuracy of methods for estimating the age and sex of sub-adult individuals often depend upon the availability of appropriate data relating to the growth and maturation of skeletal elements with regard to the population of origin and thus genetic, environmental and cultural influences [2]. In addition, in order for both growth and maturity standards and methods of age estimation to be suitable for the analysis

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of skeletal remains, it is essential that they be based on direct measurements and observations obtained from documented osteological material (known age and sex), which have a similar development to the sample under study. However, despite the existence of numerous growth-related studies ([3–17], among others), there is a notable lack of information regarding the development of many elements of the human skeleton based on direct measurements from documented osteological material, especially from Western European populations. Indeed the vast majority of maturation and growth standards currently available for osteological studies are based on radiographic images of North American Caucasian children [7, 8, 11, 13, 14, 17, 18]. Although it is true that direct studies of osteological material also exist, most of these are based on archaeological individuals (for which age and sex have been estimated in the laboratory) and are restricted to children of Slavic [19], Germanic [20], Eskimo [21], Amerindian [20–22] or Egyptian [23] descent. Likewise, many of the few studies that have considered children of Western European ancestry are also based on archaeological material [24–28] or are restricted to specific skeletal elements such as the scapula [29], innominate [30–33], sacrum [34], femur [2] and tibia [35]. For this reason, some authors have recently highlighted the need to increase and diversify the number of growth models based on direct measurements from documented osteological material [30, 36], to elaborate reference data concerning the development of each skeletal element [29, 35] and to deepen our understanding of the development of the different populations [23, 35].

With the intention of furnishing more information regarding post-natal skeletal growth and completing the studies initiated by Rissech and colleagues on the lower [2, 30–33, 35] and upper [29] extremities of the skeleton, this paper examines cross-sectional information concerning humeral growth from documented skeletal material of Western European origin covering the full developmental spectrum, in other words, from birth to the attainment of adult form. The study has a twofold aim, namely: to analyse the growth of the humerus in order to describe the changes that accompany post-natal ontogeny in this bone and to develop algorithms that facilitate accurate evaluation of sub-adult age at death of Western European skeletal remains, specifically from the Iberian Peninsula.

## Materials and methods

Three documented (known age, sex and biological origin) and contextualized skeletal collections from Western Europe were used in this study. A contextualized collection is one where the maximum amount of information concerning the demographic, socio-economic and temporal context has

been extracted from the basic information about the individuals (age, sex, year of birth, geographical location, etc.) [37]. The three series in question are:

- St. Bride's collection, which is kept in the crypt of St. Bride's church in London (UK). This collection consists of 227 adults and sub-adults born between the late eighteenth and nineteenth centuries, originating from the church cemetery [38].
- Esqueletos Identificados from the Coimbra collection in the Anthropological Museum at Coimbra University (Portugal). This collection consists of 505 adult and sub-adult individuals from the local cemetery at Conchada [39]. All these individuals were born between the late nineteenth and late twentieth centuries.
- The Lisbon collection, which is kept in the Bocage Museum in Lisbon (Portugal). This collection arose from the accumulation of adult and sub-adult skeletons from three local cemeteries (Alto de S. João, Prazeres and Benfica) [40]. It comprises 1,400 individuals born between the late nineteenth and late twentieth centuries.

All individuals in these series under 26 years of age were analysed. This age range was selected in order to cover the entire post-natal growth period in all individuals and to determine the age of growth cessation for each of the variables analysed. Those individuals displaying any type of pathology or anatomical deformation that could affect the analysis were excluded. A total of 181 individuals (90 ♂ and 91 ♀) aged 0 to 25 years were analysed. Table 1 presents detailed information regarding the age and sex of the individuals selected for this study.

Four measurements taken on the humerus that enabled documentation of growth from birth to adult age were recorded:

- Diaphyseal length (Fig. 1a)—the maximum distance between the proximal and distal ends (metaphyseal) of the humeral shaft, excluding any epiphyses. This

**Table 1** Distribution of specimens by sex, age and population

Age	Sb		Co		Lb		Total	
	m	f	m	f	m	f	m	f
0–4	6	1			9	7	15	8
5–9	4		3	3	5	3	12	6
10–14	1	1	3	10	2	4	6	15
15–19	1	2	13	12	7	7	21	21
20–25	7	5	22	22	7	14	36	41
Total	19	9	41	47	30	35	90	91

*m* males, *f* females, *Sb* St Bride's collection (London), *Co* collection of Esqueletos Identificados of Coimbra, *Lb* Lisbon collection



measurement can no longer be recorded once the distal epiphysis has begun to unite [41].

2. Transversal diameter of the proximal end (metaphyseal) of the humeral shaft, excluding the epiphysis (Fig. 1b)—this measurement can no longer be recorded once the proximal epiphysis has united completely.
3. Epicondylar breadth, whether the different elements (capitulum, trochlea, lateral and medial epicondyles) of the distal epiphysis have fused or not (Fig. 1c)—this measurement is a modification of the width of the distal end of the humeral diaphysis used previously by Fazeakas and Kósa [41].
4. Vertical diameter of the head—direct distance between the most superior and inferior points on the border of the articular surface of the humeral head. It is a maximum distance in a coronal plane and perpendicular to the anteroposterior diameter (horizontal diameter) of the head [42].

Left bones were used whenever possible, although the right side was used if the left was damaged, pathologic or unavailable.

#### Statistical analysis

Analysis was carried out in four stages. First of all, the inter-series homogeneity was determined using the graphical Lowess method in the young group and the Kruskal–Wallis test in the adult group. The Lowess method is an iterative locally weighted least-squares method for fitting a curve to a set of points. It was used in the young group because of the different composition of the samples in several age groups and derived differences due to growth [see 2, 29–32]. The Kruskal–Wallis test is a non-parametric method identical to a one-way analysis of variance (ANOVA) which is useful

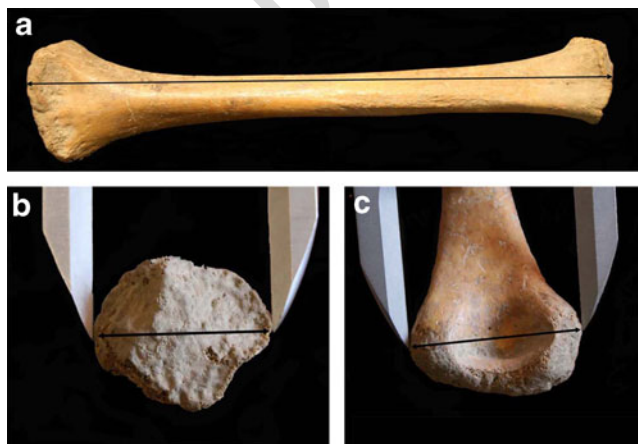
when the sample size is smaller or when the sample does not have a normal distribution. The Kruskal–Wallis test was applied in the adult group because of the small size of some of the series analysed in this age range.

The age of the fusion of the proximal epiphysis was used to determine whether individuals were still growing (young group) or no longer growing (adult group) as the humerus ceases to grow at that point [43] and the graph of the variables analysed becomes constant. This age was found to be 20 years in males and 19 years in females in the present study (see “Results” section).

Secondly, in order to make an initial approximation of sexual dimorphism, the means and standard deviations for each humeral variable in each age group were calculated and Student's *t*-test was applied to each age category. However, Mann–Whitney's *U*-test was applied if there were fewer than 15 individuals in either of the two sexual series for one age group. The series used in this study are not very large and their age and sex composition is unequal; a similar problem is encountered in the few documented series that contain juvenile remains. For this reason, and in line with current methodological practice, each series was divided into 5-year intervals in order to carry out the second analysis. The results of this analysis must therefore be viewed with caution due to the lack of homogeneity in the age distribution of the younger groups, the rhythm of growth within and among different age groups and the small size of the sample.

Thirdly, the growth behaviour of each of the four variables was analysed using polynomial regression up to the fifth degree, treating age as a continuous variable. Only individuals still growing were used. Polynomial regression analysis was selected based on the assumption that growth dynamics can be described by an incremental continuous function [3, 15]. The most appropriate statistical model was then selected on the basis of three factors, namely: the strength of the correlation coefficient ( $R^2$ ), the significance of the function expressed by the *F* value and the significance of the coefficients of the function obtained using the ANOVA test.

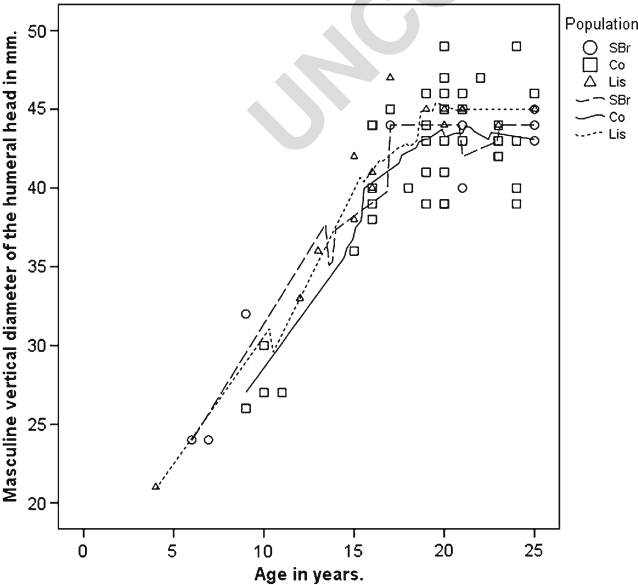
Finally, to enable predictions of age at death, inverse regression analysis was performed for age and each metrical variable of the humerus, using age as the dependent variable. In other words, each metrical variable of the humerus (*x*) was regressed on age (*y*). A Bayesian approach is the method of choice when the objective is to obtain a methodology for sub-adult age estimation that can be applied to any population without exception [44]. However, in order to develop a Bayesian approach with these characteristics, a relatively broad sample containing the variability of several populations is required [45, 46]. As stated above, the main difficulty facing any sub-adult study based on documented osteological material is the scarcity of such collections.



**Fig. 1** Dorsal view of a sub-adult humerus (a) showing the proximal (b) and the distal end of the diaphysis (c). Arrows indicate the diaphyseal length, transversal diameter of the proximal end and epicondylar breadth

Therefore, due to the type of sample used in the present study, this paper focuses on the Western European population, specifically on that from the Iberian Peninsula. An inverse regression analysis, which is the method of choice when there is some a priori reason for presuming that the case in question comes from the same distribution as represented within the reference sample, was selected for this reason [44]. Such a presumption is generally warranted in forensic settings [44]. There is, of course, some biological differentiation within Western European populations. This differentiation does not, however, seem to greatly affect the variability observed during the growth of the skeletal elements analysed here. Indeed their biological proximity is indicated by the closeness of the lines for the different samples (Fig. 2) during the homogeneity test and the low standard deviations (Table 2). French and Portuguese populations are biologically similar to Spanish populations as a result of their shared biological population history and geographical proximity [37, 47]. Furthermore, in her study based on several documented skeletal collections from various Western European countries (Spain, Portugal and England), Rissech [48] found that these collections could be considered as a single series due to the observed homogeneity.

The inverse regression was calculated considering the age range in which the series displayed no sexual differences, and calculations were performed using the data as a whole (males and females combined). Considering males and females together markedly improves the utility of the function by increasing the sample in which the calculus will be applied, which is statistically good, and facilitating the



**Fig. 2** Vertical diameter of the humeral head of the humerus of the masculine series considering the three populations from 0 to 25 years of age. Curves were calculated using Lowess method

**Table 2** Descriptive statistics of the four variables of the humerus classified according to each age category and sex. Sexual differences by Mann–Whitney’s *U*-test

Variables	Age				
	0–4	5–9	10–14	15–19	20–25
Diaphyseal length					
♂ <i>n</i>	7	11	6	1	
$\bar{x}$	112.86	183.09	223.83	252.00	
SD	35.25	18.15	23.28	–	
Mean range	5.00	8.82	11.17	–	
♀ <i>n</i>	3	5	10	–	
$\bar{x}$	137.33	179.60	203.00	–	
SD	14.15	29.69	14.48	–	
<i>t</i>					
Mean range	6.67	7.80	6.90	–	
<i>U</i>	7.000	21.000	14.000	–	
<i>p</i>	0.425	0.730	0.093	–	
Transversal Ø of the proximal end					
♂ <i>n</i>	8	11	6	18	8
$\bar{x}$	19.75	28.45	32.17	38.72	42.88
SD	4.77	2.62	2.32	2.54	4.26
Mean range	6.00	9.18	4.08	–	–
♀ <i>n</i>	5	5	15	18	–
$\bar{x}$	22.20	26.80	30.07	37.44	–
SD	5.40	3.49	3.26	2.15	–
<i>t</i>				1.630	–
Mean range	8.60	7.00	9.77	–	–
<i>U</i>	12.000	20.000	26.500	–	–
<i>p</i>	0.770	0.390	0.146	0.112	–
Epicondylar breadth					
♂ <i>n</i>	3	6	6	18	23
$\bar{x}$	24.67	34.33	40.67	53.83	59.52
SD	3.79	1.21	2.81	6.84	3.54
Mean range	2.17	6.33	11.33	–	–
♀ <i>n</i>	3	5	13	19	30
$\bar{x}$	31.33	33.60	40.46	50.68	50.93
SD	2.52	6.43	5.74	5.42	3.33
<i>t</i>			0.82	1.557	9.05
Mean range	4.83	5.60	9.38	–	–
<i>U</i>	0.500	13.00	–	–	–
<i>p</i>	0.077	0.792	0.935	0.128	0.000*
Vertical diameter of the head					
♂ <i>n</i>	1	4	5	20	33
$\bar{x}$	21.00	26.50	30.60	41.80	43.79
SD	–	3.79	3.91	3.04	2.60
Mean range	4.50	4.13	7.00	–	–
♀ <i>n</i>	4	3	12	20	39
$\bar{x}$	17.00	24.67	33.17	39.05	39.08
SD	3.65	7.10	4.90	2.35	2.52
<i>t</i>				3.203	7.801

t2.49

**Table 2** (continued)

Variables	Age				
	0–4	5–9	10–14	15–19	20–25
t2.51 Mean range	2.63	3.83	9.83		
t2.52 <i>U</i>	0.500	5.500	20.000		
t2.53 <i>p</i>	0.400	0.857	0.289	0.003*	0.000*

Asterisk indicates significance, ♂ males, ♀ females

estimation of age in those individuals where the sex is unknown. In other words, for applying the technique, it will not be necessary to know the sex of the individuals a priori. All calculations were performed using SPSS 15, except for the polynomial regression, which was calculated using “R”.

Results

The homogeneity test in young (Lowess method) and adult individuals (Kruskal–Wallis test) for the three series, according to each sex and metrical variable, showed similar inter-series growth patterns in the young group for the four variables analysed (i.e. Fig. 2) and no significant inter-series differences in the adult group for epicondylar breadth ( $X_{\text{♂}}^2=3.103$ ,  $p=0.212$ ;  $X_{\text{♀}}^2=0.630$ ,  $p=0.730$ ) and the vertical diameter of the head ( $X_{\text{♂}}^2=1.694$ ,  $p=0.429$ ;  $X_{\text{♀}}^2=2.137$ ,  $p=0.343$ ). The diaphyseal length of the humerus and the transversal diameter of the proximal end of the shaft were not considered during adult homogeneity testing as fusion of the epiphyses prevents these variables from being measured. The results indicated the homogeneity between the three series analysed in terms of both adult and sub-adult individuals, thus meaning that they cannot be considered as different series. For this reason, the individuals from all three series were analysed together as a single series. For the sake of clarity, the results for each variable will be related separately.

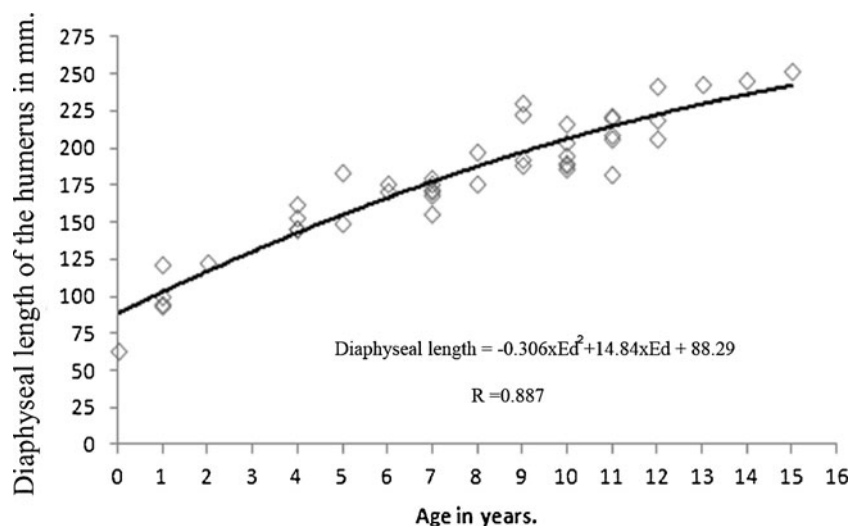
Diaphyseal length of the humerus

Application of the Mann–Whitney *U*-test to each age range showed that the average length of the diaphysis of the humerus is greater in females than in males (Table 2) from birth up until 4 years of age. Beyond this age, the average value for males is always greater, although neither of these differences is statistically significant (Table 2). Table 2 shows that the diaphyseal length of the humerus increases until the 10–14 years age range in both males and females. As mentioned above, this variable can no longer be

measured once fusion of the distal epiphysis has begun, thus preventing analysis for subsequent age groups. In the sample analysed, fusion of the distal epiphysis occurred before the age of 15 in both sexes (with the exception of one boy aged 15; see Table 2). These results are consistent with the standard age range for the fusion time of the distal epiphysis of the humerus in the current population. Although a complex fusion pattern for the distal epiphysis is found when the separate centres of the distal epiphysis (capitulum, medial epicondyle, trochlea and lateral epicondyle) coalesce, it is possible to distinguish two components with two different fusion times [43]. These two components are: (1) the combined capitulum, trochlea and lateral epicondyle, which is usually fused by about 15 years of age in the current population [43], and (2) the medial epicondyle, which is the last component of the distal epiphyses of the humerus (and the elbow) to fuse (14–19 years in boys and 11–16 years in girls [43, 49]). In the present study, the fusion time of the combined capitulum, trochlea and lateral epicondyle (first component of the distal epiphysis) marks the limit of the measurement of the diaphyseal length of the humerus, which is before 15 years of age in both sexes of the sample analysed, thus coinciding with the age given for the current population. As no significant differences were found between the diaphyseal lengths of the humerus between the two sexual series, boys and girls were combined to calculate one growth model up to 14 years of age. The results of this study indicated that the diaphyseal length of the humerus' growth behaviour can be approximated to a second-degree polynomial (Fig. 3), with an explained variability of 87 %. This model is in agreement with the constant rhythm of the rate of growth in longitudinal measurements [2, 30, 31, 50], and, as a result, it was not possible to observe the growth spurt in this variable. The lack of sexual differences in any of the age groups indicates that diaphyseal metrics of the humerus are not useful for sex diagnosis in sub-adults, but it is interesting for the estimation of age at death in sub-adult skeletal remains before the fusion of the first component of the distal epiphysis in individuals of both known and unknown sex. To obtain a function for determining age in sub-adult individuals, the inverse regression between diaphyseal length of the humerus and age was calculated considering a single sexual series (Table 3). The selected function was a first-degree polynomial, with an explained variability of 87 % for the unisex model. Transversal diameter of the proximal end (metaphyseal) of the humeral shaft Table 2 shows that the average of the transversal diameter of the proximal end of the humeral shaft is greater for females



**Fig. 3** Polynomial regression line and equation for diaphyseal length of the humerus considering a unisex series from 0 to 15 years of age.  $R^2$  means explained variability



than for males from birth to 4 years of age. The average for males is always greater after this age, although neither of these differences is statistically significant (Table 2). The transversal diameter of the proximal end of the shaft increases as a result of growth until 20 years of age in males and 19 years in females. In the analysed sample, the age limit for complete fusion of the proximal epiphysis is 20 years in males and 19 years in females. However, it was possible to measure this variable in male individuals of 20 years of age and in female individuals of 19 years of age because in these individuals the fusion line between the shaft and the proximal epiphysis was visible. These age limits for complete fusion of the proximal epiphysis observed in the analysed sample are in accordance with current standards for the fusion time of the proximal humerus in boys (15.75–20 years) and girls (12–19 years) in the living population [43, 49]. Complete fusion of the humeral proximal epiphysis coincides with the growing end of the bone and is responsible for 80 % of the length growth of the humeral shaft [43].

The absence of significant sexual differences for the transversal diameter of the proximal end of the humerus allowed the calculation of a single growth model for this variable to describe developmental traits that included both

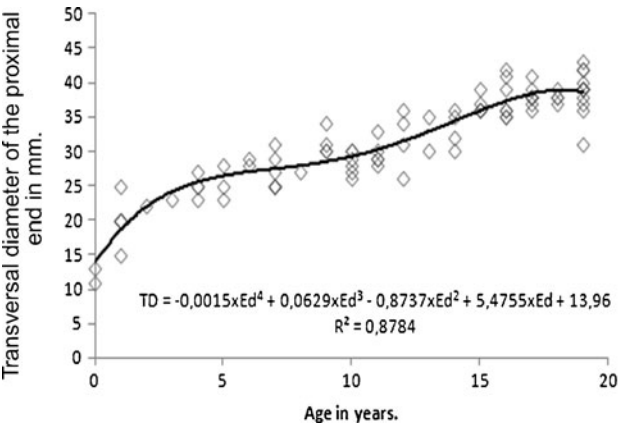
males and females up until 19 years of age. This limit was used because it is the age of complete fusion of the humeral head in the female series and represents the end of growth in these individuals. The best growth model for this variable was a four-degree polynomial (Fig. 4) with an explained variability of 87 %. As is the case for horizontal variables [29, 32, 50], a growth restraint is observed before the growth spurt in the fitted curve (Fig. 4). In this variable, the growth spurt begins at about 11–12 years of age. Usually, the masculine growth spurt occurs later in males than in females due to the later maturation of boys [15]. However, as the growth curve has been calculated using a unisex series, we only have one unisex age for the growth spurt. This age is the result of combining the ages of the growth spurts for both females and males. Nevertheless, this age range for the unisex age of the growth spurt falls within the standard age ranges for the puberal growth spurt for boys (10.5–17.5 years) and girls (9.5–14.5 years) in the current population [5, 15].

The transversal diameter of the proximal end of the humeral shaft is not suitable for sex diagnosis due to the lack of significant sexual differences, although it could be useful for sub-adult age estimation. The inverse relationship between the transversal diameter of the proximal end of the

**Table 3** Inverse functions for age prediction: the variable is introduced in millimetres and age is given in years.  $R^2$  means coefficient of correlation of the function. Regressing age on bone dimensions violates the assumption of equal variance of bone dimensions at different ages. The variance of bone dimensions is low at low ages and increases

as age increases. Therefore, the standard error is an unreliable indication of age range at low ages. When the estimated age is about 8 or more, the standard error can approximately indicate a variation in the estimate

Single sexual series (boys and girls combined)	Standard error	$R^2$	Age limit of application
Age = $0.081 \times \text{diaphyseal length} - 6,874$	1.399	0.869	Up to 15 years
Age = $0.764 \times \text{transversal } \emptyset \text{ of the proximal end} - 12.553$	2.326	0.841	Up to 19 years
Age = $-0.012 \times \text{epicondylar breadth}^2 + 1,536 \times \text{epicondylar breadth} - 29,251$	2.149	0.830	Up to 19 years
Age = $0.471 \times \text{vertical diameter of the head} - 4.053$	1.336	0.869	Up to 15 years



**Fig. 4** Polynomial regression line and equation for transversal diameter of the proximal end of the humerus considering a unisex series from 0 to 19 years of age.  $R^2$  means explained variability

shaft and age (Table 3) is a first-degree polynomial with an explained variability of 84 %.

#### Epicondylar breadth

As was the case for the variables discussed earlier, the girls in this study showed higher average epicondylar breadth values than boys from birth to 4 years of age. Likewise, boys showed higher average values than girls from 5 years of age onwards (Table 2). These differences are, however, only statistically significant in adults (Table 2). These results for the adult age group agree with the current literature on sexual dimorphism in adults [51–54]. The increase in size of the epicondylar breadth continues up until the 20–25 years age range in males and 15–19 years in females, thus indicating the end of growth of this variable (Table 2) in each sex. However, the increase in the male series between the age ranges 15–19 and 20–25 years is much smaller than that between the age ranges 10–14 and 15–19 years, thus indicating that the end of growth of the epicondylar breadth in the male series occurs early in the 20–25 years age range, possibly around 20 years.

Due to the lack of sexual differences during growth for the epicondylar breadth, the growth behaviour of this variable was analysed on the basis of a single sexual series (boys and girls combined) up until 19 years of age, which is the age limit of growth for the female series. The best growth model was a first-degree polynomial with an explained variability of 76 %. This indicates a rapid maturation of the distal epiphysis of the humerus in relation to the maturation of the proximal epiphysis, thereby reflecting the earlier formation of the elbow with respect to the shoulder [43].

Based on the data from this study, the epicondylar breadth may be of value for sex diagnosis in adults. As far as the estimation of age at death is concerned, this variable

could be useful for osteological remains between 0 and 19 years of age. The inverse regression between epicondylar breadth of the humerus and age was calculated to assess age at death (Table 3), and a second-degree polynomial with an explained variability of 83 % was selected.

#### Vertical diameter of the head

The results obtained upon applying the Mann–Whitney’s  $U$ -test and Student’s  $t$ -test to the vertical diameter of the humeral head (Table 2) indicate that the average value for males is always greater than that for females with the exception of the 10–14 years age range, where the opposite is found. However, these differences only have statistical significance from 15 years of age upward (Table 2). These sexual differences agree with the well-defined sexual dimorphism of the humeral head in adults [43, 51–53] and are related to the sexual dimorphism found in the shoulder in post-pubescent individuals [29]. According to Table 2, growth in the vertical diameter of the humeral head continues until the beginning of the 15–19 years age range in females and the beginning of the 20–25 years age range in males.

The most appropriate growth model for the vertical diameter of the humeral head was a second-degree polynomial in both males and females (Fig. 5). The explained variability of the models is 87 % in males and 89 % in females. Due to the constant rhythm of the rate of growth in both functions, it was not possible to observe the growth spurt of this variable within the fitted curves. The maximum for the male and female curves (Fig. 6), which indicates the end of growth, occurs at approximately the end of the 15–19 years



**Fig. 5** Polynomial regression line and equation for the feminine and masculine vertical diameter of the humeral head from 0 to 19 years of age.  $R^2$  means explained variability

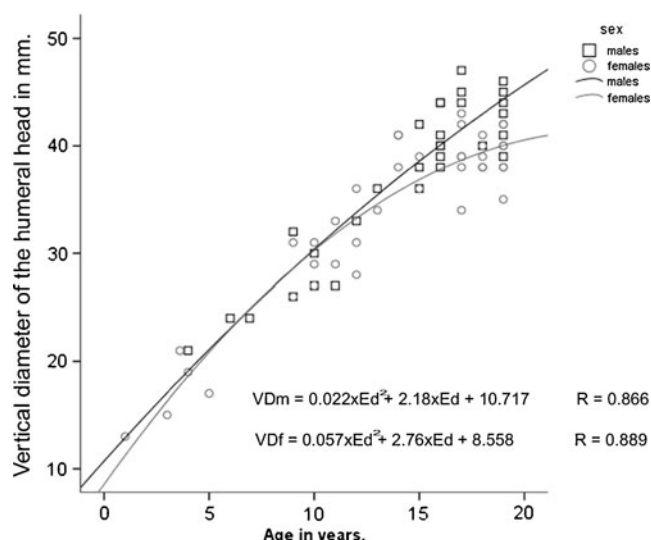


Fig. 6

age range in females and slightly later in males (around the beginning of the 20–25 years age range). This age coincides with the approximate age at which linear growth of the humerus ceases within this sample and corresponds to the fusion times of the humeral head (15.75–20 years in boys and 12–19 years in girls) in the current population [43, 48].

On the basis of the data from this study, the vertical diameter of the humeral head may be of value for diagnosing sex from 15 years of age and throughout the adult period. As far as the estimation of age at death is concerned, this variable could be useful for osteological remains of known and unknown sex from birth until 15 years. For age estimation, the inverse relationship between the vertical diameter of the humeral head and age (Table 3) was a first-degree polynomial for the unisex series containing all subjects under 15 years of age. The explained variability in this model was 87 %.

## Discussion

This study has presented a cross-sectional interpretation of the longitudinal growth of four variables of the humerus (diaphyseal length, transversal diameter of the proximal end of the humeral diaphysis, epicondylar breadth and vertical diameter of the head) based on the evidence from many individuals (documented skeletons) at different ages. As a result, it is not a true representation of growth per se of the bone but a populational perspective.

According to the findings presented herein, the growth pattern for the humerus-related variables shows a continuous increase and follows either a first- or second-degree polynomial. The only exception is the transversal diameter of the proximal end of the humerus, which follows a fourth-degree

polynomial and displays a non-growth stage before the growth spurt. This constant growth rhythm is characteristic of vertical variables, which are known to show regular growth, whereas horizontal variables exhibit a non-growth stage before the growth spurt [2, 29, 31, 32, 50]. The curves have a good fit with no significant amounts of scatter as can be seen from the consistently high correlation and the significance of the functions and coefficients achieved in the models.

In order to evaluate the utility of the growth models and functions obtained for sub-adult age estimation in forensic cases, it is necessary to assess whether the analysed sample is representative of modern samples. For this reason, its growth parameters will be discussed in relation to those of the current population. The growth parameters considered are: the growth spurt, the timing of maturation and the cessation of growth in height.

*Growth spurt* The transversal diameter of the proximal end of the shaft is the only variable in the present study that shows an upturn in its growth curve as an indication of the growth spurt for the series analysed. This upturn occurs approximately between 11 and 12 years of age. These values were obtained based on the single sexual series (the combination of boys and girls from the sample analysed) and fall within the standard age ranges for the male (10.5–17.5 years) and female (9.5–14.5 years) growth spurt in the current population [15]. These results are in accordance with the results obtained for the age of the growth spurt in the ischiopubic area (pubis and ischiopubic index) [32] and in the ilium width [31] for this sample (which is around 14–15 years of age in males and 10–11 years of age in females), which clearly fall within the standard age ranges for the puberal growth spurt in the current population (10.5–17.5 years of age in males and 9.5–14.5 years in females [15]).

*Timing of maturation* The mean ages for fusion of the epiphyses in the analysed sample [2, 30–32, 35] are consistent with the age ranges reported previously for the current population. For example, the age of fusion of the acetabulum (acetabulum maturity) in the analysed sample is 16 years in males and 12 years in females [30–32], both of which fall within the standard ranges for the current population (14–17 and 11–15 years in males and females, respectively [43]). Furthermore, the age of fusion of the proximal (19 years in boys and around 17 years in girls) and distal (17 years in boys and 16 years in girls) epiphyses of the tibia (tibial maturity) in the analysed sample [35] fall within the standard ranges for the current population (16–19 years in boys and 13–17 years in girls for the proximal epiphysis of the tibia [43] and 15–18 years in boys and 14–16 years in girls for the distal epiphysis of the tibia [43]). The fusion time of the femoral epiphyses is also consistent with the age ranges for the current population. Thus, the femoral head fuses in



544	males at 17 years of age and in females at 16 years of age in	stature between the modern Spanish series and the series of	595
545	the sample analysed, both of which agree with the normal	the present study. In general, it can be stated that the humerus	596
546	fusion times of the proximal femur in males (14–19 years)	of the analysed series shows no evidence of secular	597
547	and females (11–16 years) in the current population [43]. As	change, malnutrition or delays in growth or osseous maturation	598
548	far as the age of fusion of the distal and proximal epiphysis	and therefore corresponds to a modern Western European sample,	599
549	of the humerus is concerned, these concur perfectly with the	specifically from the Iberian Peninsula.	600
550	age ranges for the current population:		
551	(a) In the studied sample, fusion of the first component of	Sexual dimorphism	601
552	the distal epiphysis of the humerus (combined capitulum,		
553	trochlea and lateral epicondyle) occurs before	According to the results of this study, female growth cessation	602
554	15 years of age in boys and 14 years of age in girls,	is the main cause of the sexual differences in both epicondylar	603
555	which corresponds to the normal fusion times for the	breadth and vertical diameter of the head. This can be explained	604
556	first component of the distal epiphysis of the humerus	by the earlier maturation of females and the longer male growth	605
557	in the current population (this process starts at around	period, which is consistent with existing literature reports [43].	606
558	12.5 years old in boys and 11.5 years old in girls and is	According to the results of the present study, epicondylar	607
559	usually complete by about 15 years of age [43, 49]).	breadth is useful for sexual diagnosis in adult ages and vertical	608
560	(b) The fusion of the proximal epiphysis in the analysed	diameter of the humeral head is useful for sexual diagnosis after	609
561	sample occurs at the latest age of 20 years in males and	15 years of age. These results are in accordance with the	610
562	19 years in females, both of which are consistent with	importance of breadth measurements of the joint regions for	611
563	the normal age ranges for fusion of the proximal epiphysis	adult [51–54] and post-pubescent (the vertical diameter of the	612
564	of the humerus in boys (15.75–20 years) and girls	humeral head) [29] sexual diagnosis. Breadth measurements of	613
565	(12–19 years) in the current population [43, 49].	the elbow and knee joints are of special interest [54] in cases of	614
		fractured bones and incomplete remains.	615
566	<i>Cessation of growth in height</i> As noted previously, the age	Age estimation	616
567	limit for fusion of the proximal humeral epiphysis found in the		
568	sample studied herein concurs with the standard values for	The rate of growth for all the absolute measurements of the	617
569	cessation of growth of the humerus for the current population.	humerus proved to be valuable for sub-adult age estimation,	618
570	However, the lower extremities provide a better indication of	where the four functions obtained from the inverse regression	619
571	growth in height than the upper extremities [43, 55]. In the	were of interest. The utility of the diaphyseal length of the	620
572	sample analysed here, the ages of fusion of the proximal	humerus is interesting because of the tendency of this skeletal	621
573	epiphysis of the tibia [36] and the distal epiphysis of the femur	element to be well preserved in osteological remains and also	622
574	[2] for both boys (19 years) and girls (17 years) fall within the	because it can be applied in sub-adult remains of unknown sex	623
575	standard ranges for cessation of growth in height for males	until fusion of the first component of the distal epiphysis,	624
576	(17.75 years; SD, 13 months) and females (16.25 years; SD,	which occurs at around 15 years of age. The transversal	625
577	10 months) in the current population [15]. This is due to the fact	diameter of the proximal end of the shaft and the epicondilar	626
578	that the end of the longitudinal growth of these two skeletal	breadth are interesting because they can be applied in sub-	627
579	elements (tibia and femur) is determined by the fusion of their	adult remains of unknown sex from birth until 19 years of age,	628
580	proximal (in the case of the tibia) and distal epiphysis (in the	at which point fusion of the humeral head (in the transversal	629
581	case of the femur) [43], and the fusion of the epiphyses that	diameter of the proximal end of the shaft) has occurred and	630
582	constitute the knee coincides with the cessation of growth in	sexual dimorphism (in the epicondylar breadth) has appeared.	631
583	height [43].	Both of these variables, together with the vertical diameter of	632
584	All these facts indicate no delayed growth in the present	the humeral head (applicable until 15 years of age), are of	633
585	series. This conclusion is corroborated by the homogeneity	interest as they are especially useful for incomplete remains.	634
586	observed in the maximum length of the adult femur between	The functions obtained in the present study, which were	635
587	these analysed series and the Spanish documented and contextualized	obtained from a modern Western European population, therefore	636
588	modern skeletal collection at the Universitat Autònoma de Barcelona	allow us to estimate the age of modern sub-adult skeletal	637
589	(UAB) [2]. The UAB collection consists exclusively of modern individuals	remains from Western Europe, specifically from the Iberian	638
590	from the industrial city of Granollers (an industrial, commercial and	Peninsula, in a reliable and easy manner.	639
591	trade city situated 25 km northeast of Barcelona) [37] and the femur	Establishment of the identity of an individual is of utmost	640
592	is one of the bones that best correlates with stature [55], thus indicating	medico-legal significance for both living and dead individuals.	641
593	the similarity in	For identification purposes, sex and age are the two	642
594			

most important criteria for excluding large portions of the population [1]. Forensic age estimation of unidentified corpses and skeletons for the purpose of identification has been a traditional feature of forensic science. Successfully determining the identity of a decedent is of considerable significance from the ethical, legal and criminal perspective; not only is it the prerequisite for officially declaring an individual dead, but it is also the basis for investigating crimes, mass disasters or war crimes. There is a pressing need for accuracy and reliability of the methods in the Iberian Peninsula and Mediterranean area in the field of forensic anthropology. Since 2000, forensic archaeologists have worked to recover the historical memory of the Spanish Civil War era by exhuming the skeletal remains of the victims [cf 56–58]. Forensic anthropologists develop a biological profile of the individuals for identification purposes, but most of the skeletal ageing and stature standards that are available were developed from USA reference samples. The magnitude of error involved in applying these methods to Spanish individuals who were probably born around the beginning of the twentieth century is unknown, and great errors have been observed when USA reference standards have been applied to Spanish samples. For example, the method for calculating adult stature based on USA reference samples fails in the estimation of living height in Spain and Italy. In these populations, the formulae proposed by Pearson [59] at the end of the nineteenth century, which were based on a French sample, perform better than that of Trotter and Gleser [55, 60, 61] because of the closely linked biological population history of French, Spanish and Italian populations [55, 60, 61] and because they are all populations of medium stature [55, 61]. In contrast, the equations of Trotter and Gleser for Whites systematically overestimate stature in both female and male skeletons of Spanish and Italian origin [55, 60, 61]. In fact, there is a need to abandon the notion of the ‘universality’ of osteological methodology and to promote the standardization of methods instead [62]. In this respect, methodology should not be applied to skeletal material without regard to the secular and regional origin of the reference collection(s) used to create the method. In this way, the data presented in this study are of great importance for forensic anthropologists analysing modern skeletal human remains from Western Europe, specifically from Iberian Peninsula and the Western Mediterranean area. It will be very useful for application to the analysis of twentieth century sub-adult skeletal remains from this area.

In general, calculated curves fit well with our mixed European series and also correspond to known adult bone behaviour. Although more research is necessary to strengthen the validity of the results presented here, forensic scientists can nevertheless use them to broaden the range of methods used for the age estimation and sexual determination of human skeletal remains.

## Conclusion

This cross-sectional study of humeral growth, based on three documented skeletal collections from Western Europe, has provided researchers with information pertaining to a populational perspective of the humeral growth profile. Calculations were performed using the values for four humeral variables as a basis to derive four formulae that may prove valuable in sub-adult age estimation of osteological remains. This analysis has also generated information regarding the timing at which sexual differences in humeral measurements occur, thus providing indications as to when these variables may be useful for sex diagnosis. The results and formulae obtained from this study should prove to be useful for the diagnosis of age and sex, especially during forensic work and in some paleoanthropological cases when there is reason to suppose a strong similarity between the case and the sample on which the model was based. Further research on the growth and development of the humerus is, however, necessary to obtain better information for skeletal diagnosis, especially within sub-adults.

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- Q1. Figure 6 citation was changed to Figure 5 (previously uncited)  Please check if appropriate.
- Q2. Please provide the missing caption  for Figure 6.
- Q3. "Trotter and Gleser" were mentioned twice but the references indicated did not reflect both as authors. Please check if appropriate modification  necessary.

UNCORRECTED PROOF