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HUMERAL DEVELOPMENT FROM THE NEONATAL PERIOD TO SKELETAL MATURITY - APPLICATION IN AGE AND SEX ASSESMENT

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HUMERAL DEVELOPMENT FROM THE NEONATAL PERIOD TO SKELETAL MATURITY – APPLICATION IN AGE AND SEX ASSESMENT.

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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 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.

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 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 elements [29,35], and to deepen our understanding of the development of the different populations [23,35].

With the intention of furnishing more information regarding postnatal 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 postnatal 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.

MATERIAL 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:

(a) 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 18th and 19th centuries, originating from the church cemetery [38].

(b) 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 19th and late 20th centuries.

(c) 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. Joaõ, Prazeres and Benfica) [40]. It comprises 1400 individuals born between the late 19th and late 20th centuries.

All individuals in these series under 26 years of age were analysed. This age range was selected in order to cover the entire postnatal 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 study.

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

1- Diaphyseal length (Fig. 1, a). The maximum distance between the proximal and distal ends (metaphyseal) of the humeral shaft, excluding any epiphyses. This 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. 1, b). 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.1, c). This measurement is a modification of the width of the distal end of the humeral diaphysis used previously by Fazekas and Kosa [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 the growth [see 2,29,30-32]. 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 applied to each age category. However, the 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 five-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. 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 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. Figure 2) and no significant inter-series differences in the adult group for epicondylar breadth ($X^2_{♂} = 3.103$, $p = 0.212$; $X^2_{♀} = 0.630$, $p = 0.730$) and the vertical diameter of the head ($X^2_{♂} = 1.694$, $p = 0.429$; $X^2_{♀} = 2.137$, $p = 0.343$). 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 are 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 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 are 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 ages limit 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 [15,5].

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 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 above, 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 breath 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. 6). 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 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 a 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 are 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 [2,30,31,32,35] 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 [30,31,32], 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 males at 17 years of age and in females at 16 years of age in the sample analysed, both of which agree with the normal fusion times of the proximal femur in males (14–19 years) and females (11–16 years) in the current population [43]. As far as the age of fusion of the distal and proximal epiphysis of the humerus is concerned, these concur perfectly with the age ranges for the current population:

(a) In the studied sample, fusion of the first component of the distal epiphysis of the humerus (combined capitulum, trochlea and lateral epicondyle) occurs before 15 years of age in boys and 14 years of age in girls, which corresponds to the normal fusion times for the first component of the distal epiphysis of the humerus in the current population (this process starts around 12.5 years old in boys and 11.5 years old in girls and is usually complete by about 15 years of age [49,43]).

(b) The fusion of the proximal epiphysis in the analysed sample occurs at the latest age of 20 years in males and 19 years in females, both of which are consistent with the normal age ranges for fusion of the proximal epiphysis of the humerus in boys (15.75–20 years) and girls (12–19 years) in the current population [43,49].

Cessation of growth in height. As noted previously, the age limit for fusion of the proximal humeral epiphysis found in the sample studied herein concurs with the standard values for cessation of growth of the humerus for the current population. However, the lower extremities provide a better indication of growth in height than the upper extremities [43,55]. In the sample analysed here, the ages of fusion of the proximal epiphysis of the tibia [36] and the distal epiphysis of the femur [2] for both boys (19 years) and girls (17 years) fall within the standard ranges for cessation of growth in height for males (17.75 years; SD: 13 months) and females (16.25 years; SD: 10 months) in the current population [15]. This is due to the fact that the end of the longitudinal growth of these two skeletal elements (tibia and femur) is determined by the fusion of their proximal (in the case of the tibia) and distal epiphysis (in the case of the femur) [43], and the fusion of the epiphyses that constitute the knee coincides with the cessation of growth in height [43].

All these facts indicate no delayed growth in the present series. This conclusion is corroborated by the homogeneity observed in the maximum length of the adult femur between these analysed series and the Spanish documented and contextualized modern skeletal collection at the Universitat Autònoma de Barcelona (UAB) [2]. The UAB collection consists exclusively of modern individuals from the 20th century from the industrial city of Granollers (an industrial, commercial and trade city situated 25 km north-east of Barcelona) [37] and the femur is one of the bones that best correlates with stature [55], thus indicating the similarity in stature between the modern Spanish series and the series of the present study. In general, it can be stated that the humerus of the analysed series show no evidence of secular change, malnutrition or delays in growth or osseous maturation and therefore correspond to a modern Western European sample, specifically from the Iberian Peninsula.

Sexual dimorphism

According to the results of this study, female growth cessation is the main cause of the sexual differences in both epicondylar breadth and vertical diameter of the head. This can be explained by the earlier maturation of females and the longer male growth period, which is consistent with existing literature reports [43]. According to the results of the present study, epicondylar breadth is useful for sexual diagnosis in adult ages and vertical diameter of the humeral head is useful for sexual diagnosis after 15 years of age. These results are in accordance with the importance of breadth measurements of the joint regions for adult [51-54] and post-pubescent (the vertical diameter of the humeral head) [29] sexual diagnosis. Breadth measurements of the elbow and knee joints are of special interest [54] in cases of fractured bones and incomplete remains.

Age estimation

The rate of growth for all the absolute measurements of the humerus proved to be valuable for sub-adult age estimation, where the four functions obtained from the inverse regression were of interest. The utility of the diaphyseal length of the humerus is interesting because of the tendency of this skeletal element to be well preserved in osteological remains, and also because it can be applied in sub-adult remains of unknown sex until fusion of the first component of the distal epiphysis, which occurs at around 15 years of age. The transversal diameter of the proximal end of the shaft and the epicondilar breadth are interesting because they can be applied in sub-adult remains of unknown sex from birth until 19 years of age, at which point fusion of the humeral head (in the transversal diameter of the proximal end of the shaft) has occurred and sexual dimorphism (in the epicondylar breadth) has appeared. Both of these variables, together with the vertical diameter of the humeral head (applicable until 15 years of age) are of interest as they are especially useful for incomplete remains. The functions obtained in the present study, which were obtained from a modern Western European population, therefore allow us to estimate the age of modern sub-adult skeletal remains from Western Europe, specifically from the Iberian Peninsula, in a reliable and easy manner.

Establishment of the identity of an individual is of the utmost medico-legal significance for both living and dead individuals. For identification purposes, sex and age are the two 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 19th 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 standardisation 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 is 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 20th 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.

ACKNOWLEDGEMENTS

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We are grateful to the curator of the Museum of London, Jelena Bekvalac; the rector of St Bride's, David Meara; Professor Luisa Santos from the University of

Coimbra; and Professor Hugo Cardoso from the Museu Bocage of Lisbon, for providing access to human skeletal collections.

REFERENCES

1. Modi NJ (1988) *Modi's Medical Jurisprudence and Toxicology*. N.M. Tripathi Private Ltd. Bombay. pp. 28-43.

2. Rissech C, Schaefer M, Malgosa A (2008) Development of the femur - implications for age and sex determination. Forensic Sci Int 180:1–9.

3. Coleman WH (1969) Sex differences in the growth of the human bony pelvis. Am J Phys Anthropol 31:125–152.

4. Garn SM (1962) X-linked inherence of developmental timing in man. Nature 196**:**695–696.

5. GasserT, Keneip A, Ziegler P, Largo R, Molinari L, Prader A (1991) The dynamics of growth of width in distance, velocity and acceleration. Ann Hum Biol 18:449–461.

6. Gasser T, Müler HG, Köhler W, Prader A, Largo R, Molinari L (1985) An analysis of the mid-growth spurt and the adolescent growth spurt of height based on acceleration. Ann Hum Biol 12**:**129–148.

7. Ghantus MK (1951) Growth of the shaft of the human radius and ulna during the first two years of life. Am J Roentgenol 65**:**784–786.

8. Gindhart PS (1973) Growth standards for the tibia and radius in children aged one month through eighteen years. Am J Phys Anthropol 39**:**41–48.

9. Greulich WW (1960) Skeletal features visible on roentgenogram of hand and wrist which can be used for establishing individual identification. Am J Phys Anthropol 83**:**756–764.

10 Greulich WW, Thoms H (1938) The dimensions of the pelvic inlet of 789 white females. Anat Rec 72**:**45–52.

11. Maresh MM (1955) Linear growth of the long bones of extremities from infancy through adolescence. Am J Dis Chil 89:725–742.

12. Pyle SI, Waterhouse AM, Greulich WW (1971) Radiographic Standard of Reference for Growing Hand and Wrist, Press of Case Western Reserve University, Chicago.

13. Reynolds EL (1945) The bony pelvic girdle in early infancy. A roentgenometric study. Am J Phys Anthropol 3:321–354.

14. Reynolds EL (1947) The bony pelvis in prepuberal childhood. Am J Phys Anthropol 5:165–200.

15. Tanner JM (1962) Growth at Adolescence, second ed. Blackwell Scientific Publications, Oxford.

16. Tanner JM, Whitehouse RH, Marubini E, Resele LF (1976) The adolescent growth spurt of boys and girls of the Harpenden Growth Study. Ann Hum Biol 3:109–126.

17. Hoffman JM (1979) Age estimations from diaphyseal lengths: two months to twelve years. J Forensic Sci 24:461-469.

18. Smith SL, Buschang P (2004) Variation in longitudinal diaphyseal long bone growth in children three to ten years of age. Am J Hum Biol 16:648-657.

19. Stloukal M, Hanáková H (1978) Die Länge der Längsknochen altslawischer Belvö kerungen, unter besonderer Berö cksichtigung von wachstumsfragen. Homo 29:53–69.

20. Sundick RI (1978) Human skeletal growth and age determination. Homo 29:228–249.

21. Stewart TD (1976) Identification by the skeletal structures. In: Camps FE (ed.). Gradwohl's Legal Medicine. John Wrigh, Bristol, pp 123-154.

22. Merchant VL, Ubelaker DH (1977) Skeletal growth of the protohistoric Arikara. Am J Phys Anthropol 46:61–72.

23. Boccone S, Micheletti-Cremasco M, Bortoluzzi S, Moggi-Cecchi J, Rabino-Massa (2010) Age estimation in subadult Egyptian remains. Homo, 61:337-358.

24. Saunders S, Hoppa R, Southern R (1993) Diaphyseal growth in a nineteenth-century skeletal sample of subadults from St. Thomas' Church, Belleville, Ontario. Int J Osteoarchaeol 3 :265–328.

25. Alduc-le Bagousse A (1988) Estimatión de l'âge des non-adultes: maturation dentaire et croissance osseuse, Données comparatives pour deux nécropoles médiévales basnormandes. Proceedings of the 3dr Journées Anthropologiques. Notes et Monographies techniques no. 24. Éditions du CNRS, Paris, pp 81-103.

26. Hoppa RD (1992) Evaluating human skeletal growth: and Anglo-Saxon example. Int J Osteoarchaeol 2:275–288.

27. Miles AE, Bulman JS (1994) Growth curves of immature bones from Scottish island population of sixteenth to mid-nineteenth century: limb-bone diaphyses and some bones of the hand and foot. Int J Osteoarchaeol 4:121–136.

28. Miles AE, Bulman JS (1995) Growth curves of immature bones from a Scottish island population of sixteenth to mid-nineteenth century: shoulder, girdle, ilium, pubis and ischium. Int J Osteoarchaeol 5:15–27.

29. Rissech C, Black S (2007) Scapular development from the neonatal period to skeletal maturity: a preliminary study. Int J Osteoarchaeol 17:451–464.

30. Rissech C, García MM, Malgosa A (2003) Sex and age diagnosis by ischium morphometric analysis. Forensic Sci Int 135:188–196.

31. Rissech C, Malgosa A (2005) Ilium growth study: applicability in sex and age diagnosis. Forensic Sci Int 147:165–174.

32. Rissech C, Malgosa A (2007) Pubis growth study: applicability in sexual and age diagnosis. Forensic Sci Int 173:137–145.

33. Rissech C, Sañudo JR, Malgosa A (2001) The acetabular point: a morphological and ontogenetic study. J Anat 198:743–748.

34. Rios L, Weisensee K, Rissech C (2008) Sacral fusion as an aid in age estimation, Forensic Sci Int 180:111.e1–111.e7.

35. López-Costas O, Rissech C, Trancho G, Turbón D (2011) Postnatal growth of the tibia. Implications for age and sex estimation. Forensic Sci Int. doi:10.1016/j.forsciint.2011.07.038.

36. Humphrey LT (1998) Growth patterns in the modern human skeleton. Am J Phys Anthropol 105:57-72.

37. Rissech C, Steadman DW (2010) The demographic socio-economic and temporal contextualization of the Universitat Autònoma de Barcelona Collection of identified human skeletons (UAB Collection). Int J Osteoarchaeol 21:313-322.

38. Scheuer L, Black S (1995) The St. Bride's Documented Skeletal Collection. Unpublished Archive Held at the Biological Anthropology Research Centre, Department of Archaeological Sciences, University of Bradford.

39. Rocha MA (1995) Les collections ostéologiques humaines identifiées du Musseé Anthropologique de l'Université de Coimbra, Antropologia Port. 13:17–38.

40. Cardoso H (2006) Brief communication: the collection of identified human skeletons housed at the Bocage Museum (National Museum of Natural History), Lisbon, Portugal. Am J Phys Anthropol 129:173–17.

41. Fazekas IG, Kósa F (1978) Forensic foetal osteology. Académiai Kiadó, Budapest.42. Steele DG, Brambeltt CA (1988) The anatomy and biology of the human skeleton. Texas A&M University Press, Texas.

43. Scheuer L, Black S (2000) Developmental Juvenile Osteology. Academic Press, London.

44. Konigsberg LW, Hens SM, Jantz LM, Jungers WL (1998) Stature estimation and calibration: Bayesian and Maximum likelihood perspectives in Physical Anthropology. Yearb Phys Anthropol 41:65–92.

45. Rissech C, Estabrook GF, Cunha E, Malgosa A (2006) Using the acetabulum to estimate age at death of adult males. J Forensic Sci 51:213–229.
46. Rissech C, Estabrook GF, Cunha E, Malgosa A (2007) Estimation of age-at-death for adult males using the acetabulum. Applied to four Western European populations. J Forensic Sci 52:774–778.
47. Márquez-Grant, C. Rissech, O. López-Costas, L. Caro-Dobón, Spain/España, in: N. Márquez-Grant, L. Fibiger (Eds.), The Routledge Handbook of Archaeological Human Remains and Legislation: An International Guide to Laws and Practice in the Excavation, Study and Treatment of Archaeological Human Remains, Routledge editorial, London, 2011, pp. 423–438.
48. Rissech C (2001) Anàlisis del creixement del coxal a partir de material ossi i les seves aplicacions en la MedicForense i l'Antropologia. Ph.D Dissertation, Universitat Autònoma de Barcelona.
49. Hansman CF (1962) Appearance and fusion of ossification centres in the human skeleton. AJR 88:476-482.
50. Twiesselmann F(19699 Développement biomètrique de l'enfant á l'adulte. Presses Universitaires de Bruxelles-Librairie Maloine, Paris.
51. Dwight T (1905) The size of the articular surfaces of the long bones as characteristics of sex as an anthropological study. Am J Phys Anthropol 4:19-31.
52. Thieme FP, Schull WJ (1957). Sex determination from the skeleton. Hum Biol 29: 242-273.
53. Işcan MY, Loth SR, King CA, Shihai D, Yoshino M (1998) Sexual Dimorphism in the humerus: a comparative analysis of Chinese, Japanese and Thais. Forensic Sci Int 98:17-29.
54. Sakaue K (2004) Sexual determination of long bones in recent Japanese. Anthropol Sci 112:75-81.

55. Formicola V, Franceschi M (1996) Regression equations for estimating stature from long bones of early Holocene European samples. Am J Phys Anthropol 100:83–88.

56. Gassiot E, Oltra J, Sintes E, Steadman DW. 2007. Archaeology of the Spanish Civil War: rescue of the memory and historic justice. In Archaeology and Capitalism: From Ethics to Politics, Y, Hamilakis P Duke (eds). Left Coast Press: Walnut Creek, CA; 235–245.

57. Gassiot E, Steadman DW. 2008. The political, social and scientific contexts of archaeological investigations of mass graves in Spain. Archaeologies 4(3): 429–444.

58. Rios L, Martinez B, Garcia-Rubio A, Jime´nez J. 2008. Death in captivity during the early Franco regime: exhumation of the cemetery of Valdenoceda prison (1938–1943). Complutum 19: 139–160.

59. Pearson K (1899) Mathematical contributions to the theory of evolution. On the reconstruction of the stature of prehistoric races. Philos Trans Soc Lond 192:169–244.

60. Lalueza-Fox C (1998) Stature and sexual dimorphism in ancient Iberian populations. Homo 49:260–272.

61. Formicola V Stature reconstruction from long bones in ancient population samples: an approach to the problem of its reliability. Am J Phys Anthropol 90:351–358.

62. Komar D, Grivas C (2008) Manufactured populations: what do contemporary reference skeletal collections represent? A comparative study using the maxwell museum documented collection. Am J Phys Anthropol 137:224–233.

TABLE AND FIGURE CAPTIONS

Table 1 Distribution of specimens by sex, age and population. Sb: St Bride's collection, London. Co: collection of Esqueletos Identificados of Coimbra. Lb: Lisbon collection. Males are indicated by m and females are indicated by f.

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. The significance is indicated by asterisk (*). Males are indicated by ♂. Females are indicated by ♀.

Table 3 Inverse functions for age prediction, the variable is introduced in mm 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 eight or more, the standard error can approximately indicate variation in the estimate.

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

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

Figure 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.

Figure 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.

Figure 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.

Figure 1
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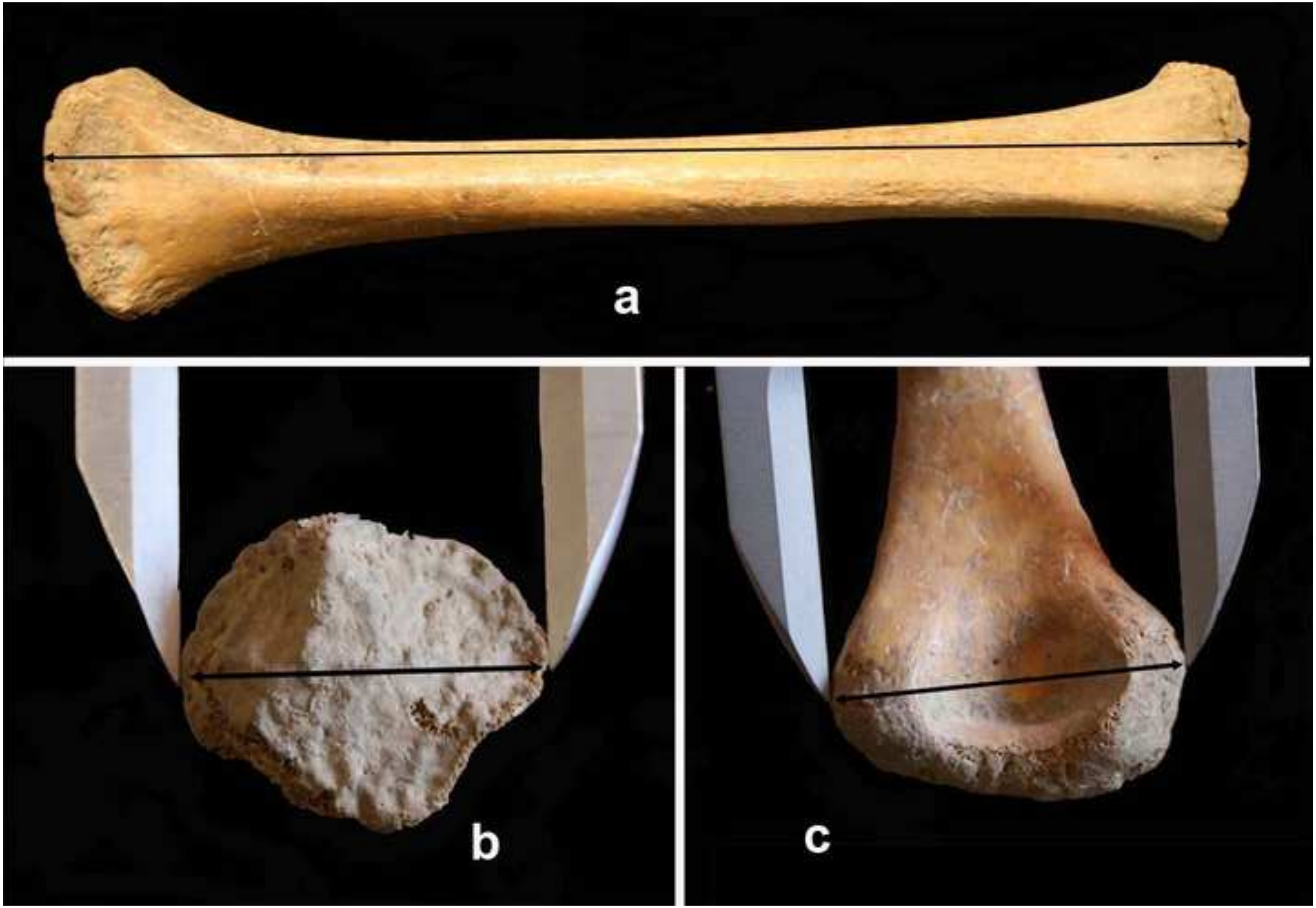

Figure 2
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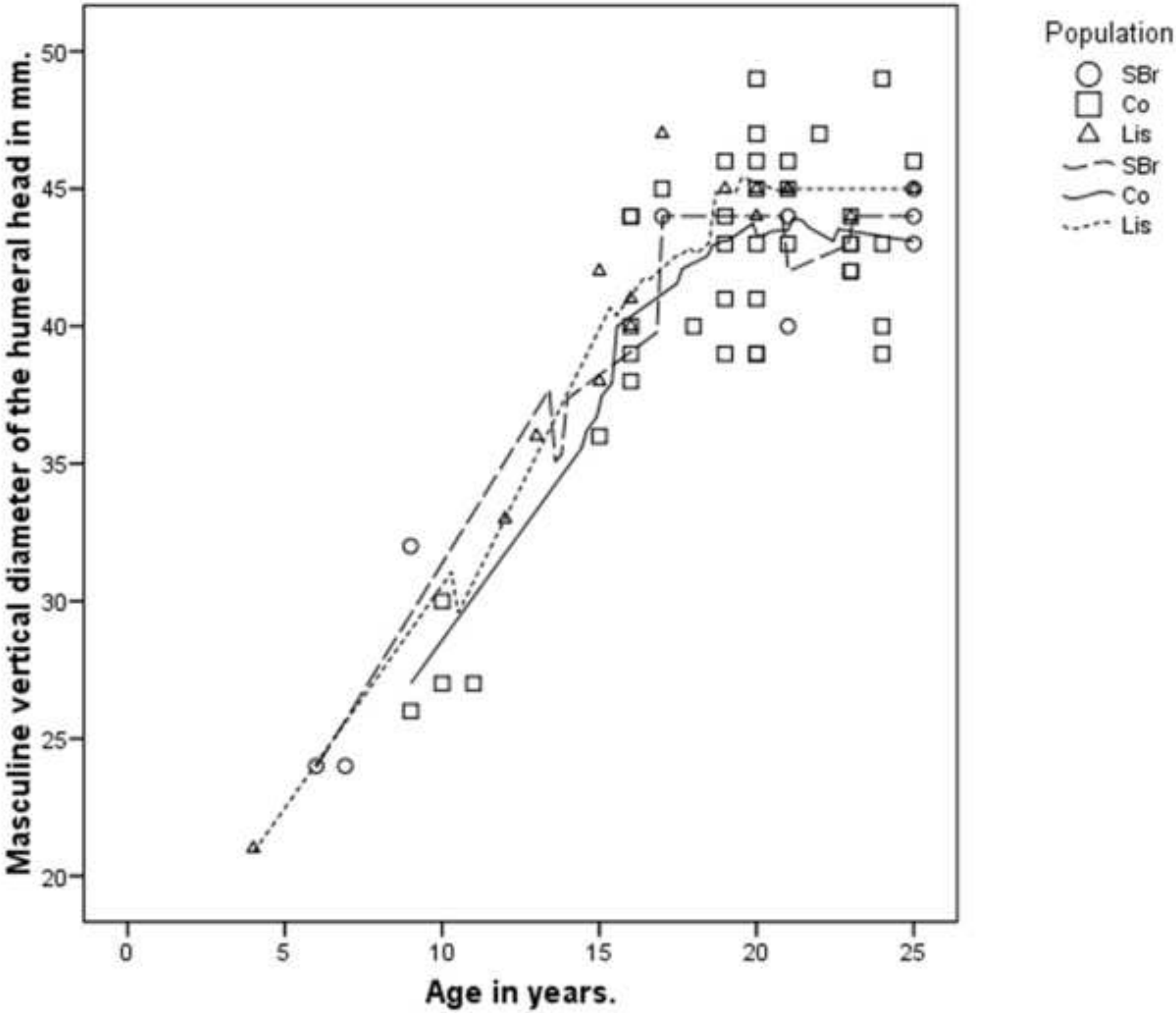

Figure 3
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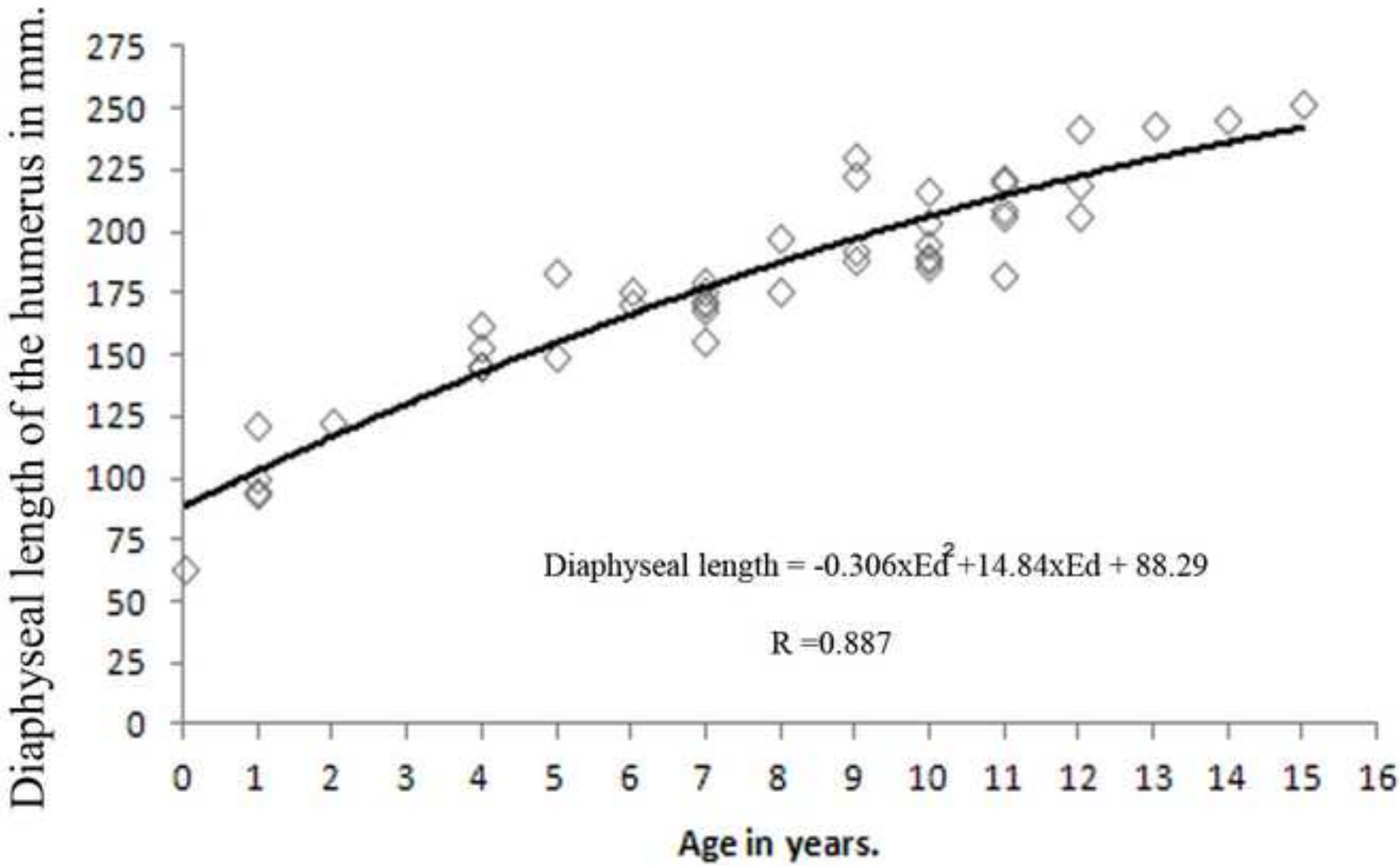

Figure 4

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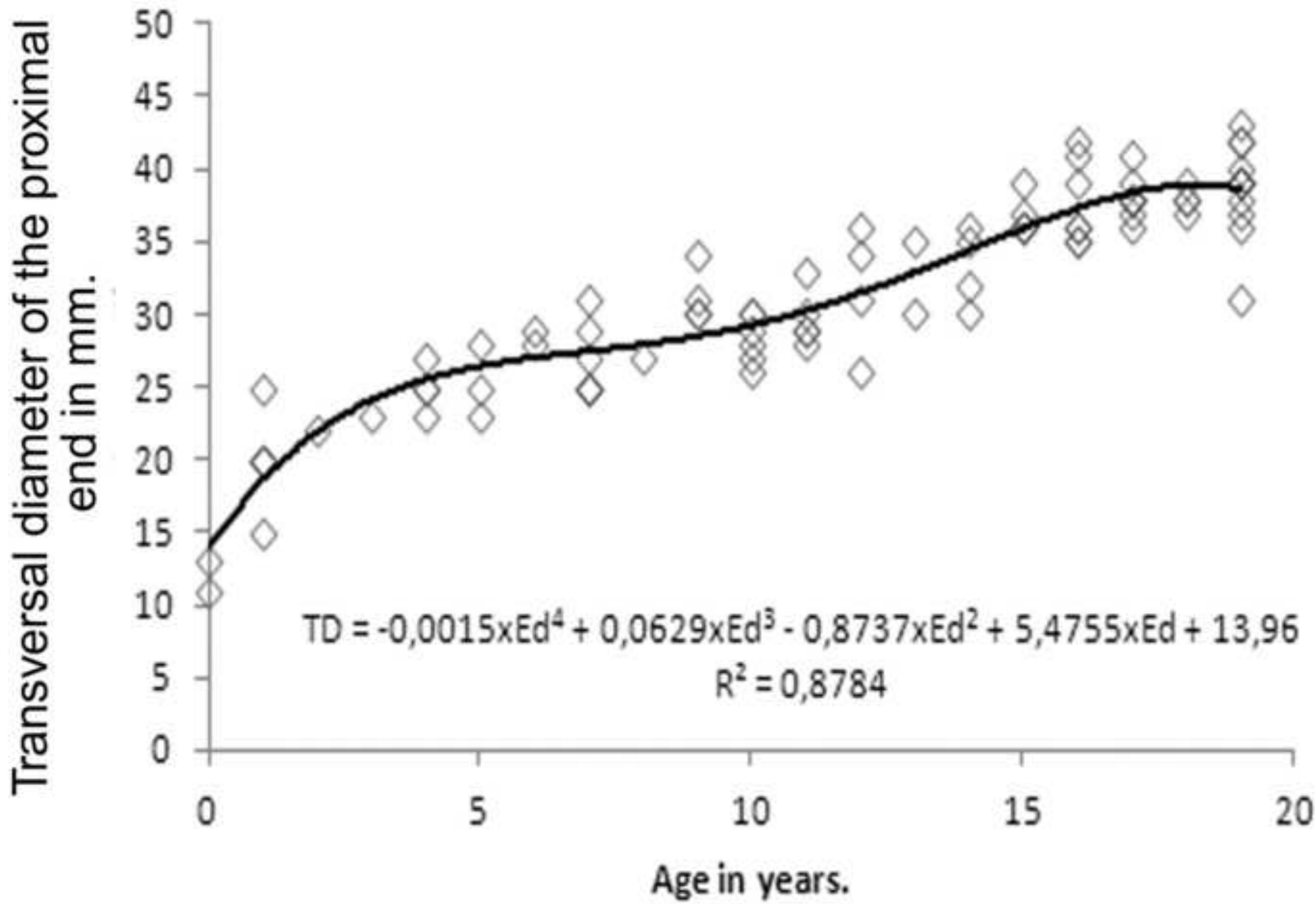

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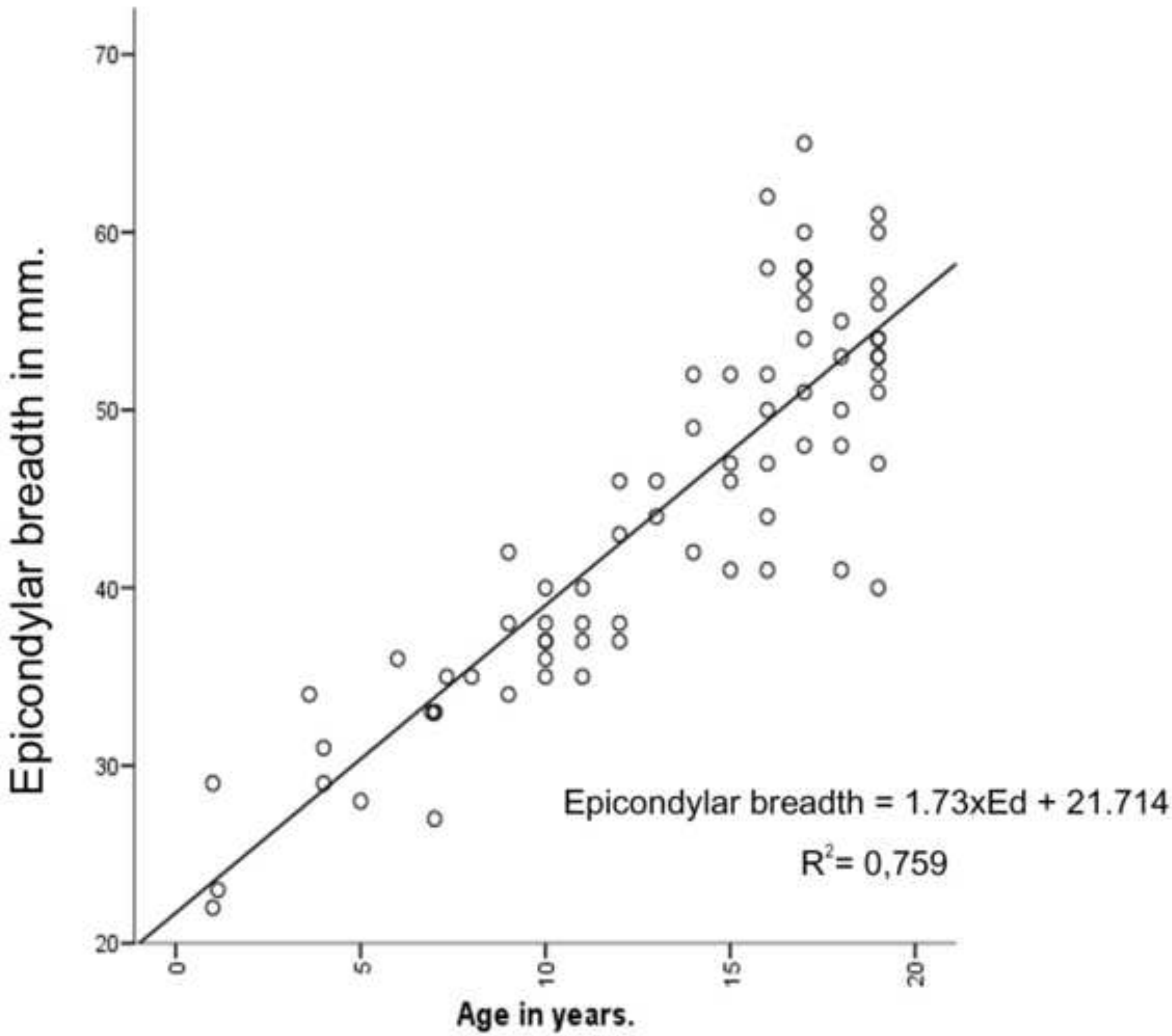

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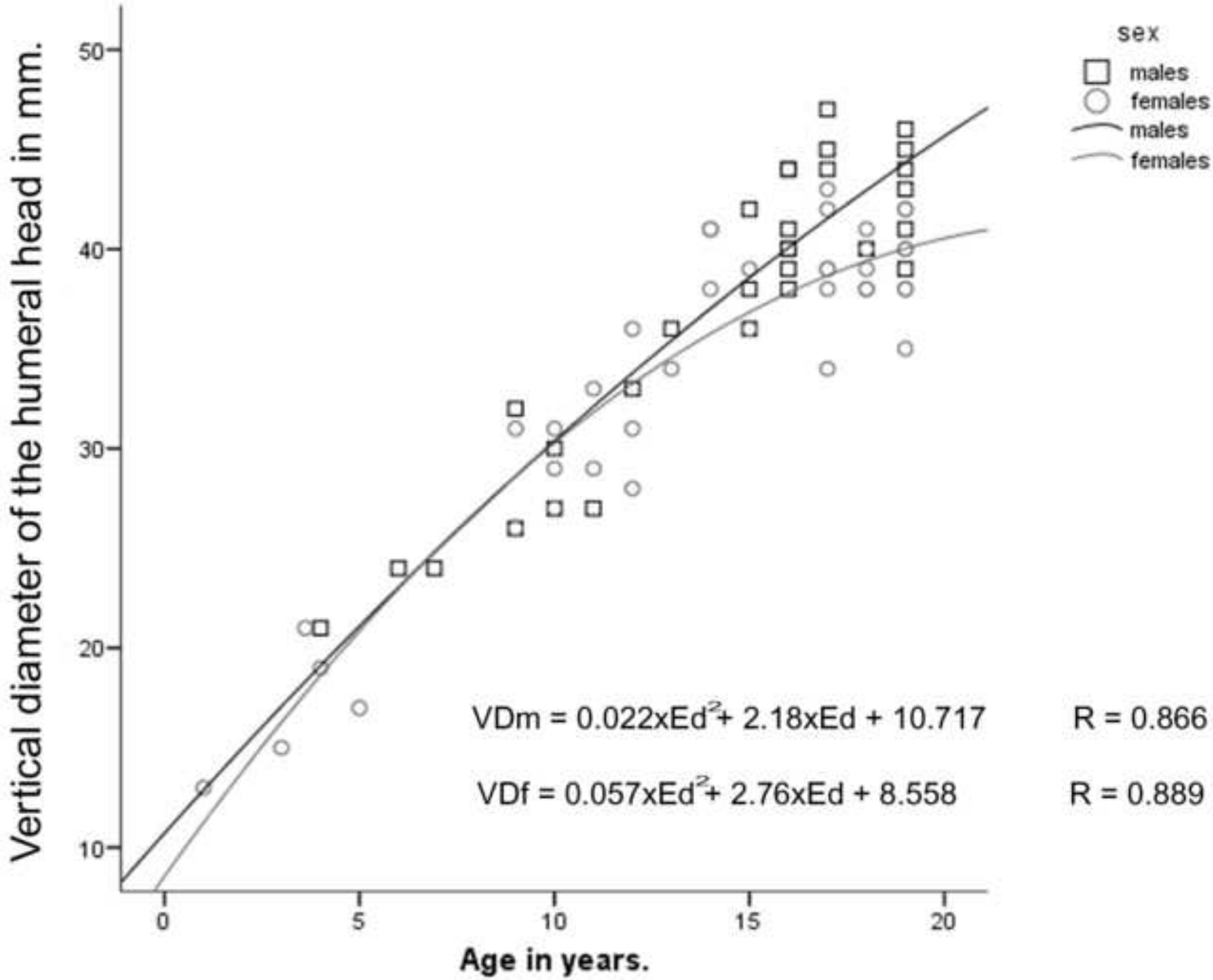

TABLES

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

Table 1

Variables	Age				
	0-4	5-9	10-14	15-19	20-25
Diaphyseal length					
♂ n	7	11	6	1	
$\bar{x}$	112,86	183,09	223,83	252,00	
SD	35,25	18,15	23,28	-	
Mean Rang	5,00	8,82	11,17	-	
♀ n	3	5	10	-	
$\bar{x}$	137,33	179,60	203,00	-	
SD	14,15	29,69	14,48	-	
t					
Mean Rang	6,67	7,80	6,90	-	
U	7,000	21,000	14,000	-	
p	0,425	0,730	0,093	-	
Transversal Ø of the proximal end					
♂ n	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 Rang	6,00	9,18	4,08		-
♀ n	5	5	15	18	-
$\bar{x}$	22,20	26,80	30,07	37,44	-
SD	5,40	3,49	3,26	2,15	-
t				1,630	-
Mean Rang	8,60	7,00	9,77		
U	12,000	20,000	26,500		
p	0,770	0,390	0,146	0,112	
Epicondylar breadth					
♂ n	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 Rang	2,17	6,33	11,33		
♀ n	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
t			0,82	1,557	9,05
Mean Rang	4,83	5,60	9,38		
U	0,500	13,00			
p	0,077	0,792	0935	0,128	0,000*
Vertical diameter of the head					
♂ n	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 Rang	4.50	4.13	7.00		
♀ n	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
t				3.203	7.801
Mean Rang	2.63	3.83	9.83		
U	0.500	5.500	20.000		
p	0.400	0.857	0.289	0.003*	0.000*

Table 2

Single sexual series (boys and girls combined)	Standard Error	R^2	Age limit of application
Age = 0.081 x **Diaphyseal length** – 6,874	1,399	0.869	Up to 15 y.
Age = 0.764 x **Transversal Ø of the proximal end** – 12.553	2,326	0.841	Up to 19 y.
Age = - 0.012 x **Epicondylar breadth** ^{2} + 1,536 x **Epicondylar breadth** – 29,251	2,149	0.830	Up to 19 y.
Age = 0.471x **Vertical diameter of the head** – 4.053	1.336	0.869	Up to 15 y.

Table 3

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Revision of: *Humeral development from the neonatal period to skeletal maturity - application in age and sex assesment*. (IJLM-D- 12-00077)

23rd of April, 2012. Barcelona

Dear Dr H. Pfeiffer,

Thank you for the attention that you and the reviewers have personally given to our manuscript. We revised and modified the manuscript in accordance with the suggestions of your reviewers. Please find enclosed a list of the changes made in the new version of the paper and our reply to the comments made by the reviewers.

Yours Sincerely,

Carme Rissech

REVIEWER #1:

I recommend publication of this excellent manuscript. My only suggestion for change is to eliminate/reduce the discussion of growth in height on page 16 since this relates to data on the femur and tibia not reported in this manuscript.

The discussion of growth in height on page 16 have been eliminated. Please, see discussion section.

REVIEWER #2:

Thank you for the opportunity to review this communication. I have very few comments to make.

1- The authors need to make it clear (first appears in the abstract) that of course this is not a longitudinal study of growth but is a cross sectional interpretation of growth based on evidence from many participants at different ages. As a result, it is not a true representation of 'growth' per se of the bone but a populational perspective.

Although this information existed in the objectives when it is said "this paper examines cross-sectional information concerning humeral growth from documented skeletal material of Western European origin covering the full developmental spectrum" , we have added some clarifications about it in the paper:

- In the abstract, we have changed the first sentence "**The goal of the present study is to provide information <u>regarding the longitudinal growth</u> of the humerus based on…**." to "**The goal of the present study is <u>to examine cross sectional information on</u> the growth of the humerus based on…**". We have also added the word skeletal in order to clarify the type of material used in the study. We changed "**documented Western European collections**" to "**documented Western European skeletal collections**"

- In the discussion, we have added a new paragraph at the beginning of this section. "**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**."

- In the conclusion, we added "**a populational perspective of**". Now the conclusion starts with "**This cross-sectional study of humeral growth, based on three documented skeletal collections from Western Europe, has provided researchers with information pertaining to <u>a populational perspective of</u> the humeral growth profile.**"

2- Is the vertical diameter of the head a maximum dimension? I suspect not as it talks about it being perpendicular to the anterposterior diameter - but I do not know what this is.

The vertical diameter of the humerus is a classical mearurement in osteology which is defined as the distance between the most superior and inferior points on the border of the articular surface of the humeral head. It is the maximum distance in a coronal plane, and for this reason it is perpendicular to the anteroposterior diameter (horizontal diameter) of the head. You can find this information in most of the Anatomical and Anthropological books and papers on the human skeleton such as Steele and Brambeltt, (1988), Buikstra and Ubelaker (1994), Charisi et al (2011). We have added this information in the description of the variables (See material and methods section)

3- The only other issue I would raise is the validity of the methodology as it is prescribed. The authors talk about the US standards not being applicable for Iberian material but this argument falls apart when we talk about forensic specimens which is purported to be the thrust of the article. The Iberian Peninsula has a very high influx rate of foreign visitors and therefore an Iberian standard may not be any more applicable than a US standard. I absolutely agree that it is of greater value when looking at archaeological material but this is not a valid arguement for forensic cases and this paper has after all been submitted to the International Journal of Legal Medicine. I would like to see this aspect of the discussion tidied up.

Of course, historically the Iberian Peninsula had had multiple waves of immigration and conquest (such as Greeks, Romans, Muslims) which have impacted in the modern population structure of Spain and Portugal, and because of this we consider them Mediterranean populations. They are related biologically with the other populations around the Mediterranean Sea, with which they share a lot of physical characteristics. Much more recently, as from 20 years ago, the Iberian Peninsula and more specifically, Spain have had some number of visitors, which now are returning to their countries of origin due to the current economical crisis, and of course this number of visitors to Spain is absolutely not comparable with the very high influx of different ethical groups in U.S., which in fact colonized North America.

The Mediterranean population, as it is well known between the European Anthropologists, differs from that of Northern Europe, and of course, differs from the population of U.S. Furthermore, not all the populations of Europe have the same morphology and the same stature. For example, it is well known that the North Europeans (Germans, Danes, Swedes) are taller than Western Europeans (Spanish, French, English, Italians). To assume that all the populations are similar is not correct. For example, it is known that the U.S. population tends to be taller than most European populations (Komlos, 2001; Komlos et al, 2004; Smith and Norris, 2004). It is nevertheless true that the stature of Europeans has increased in recent years due to improvements in living conditions, although some differences still exist (Pebles and Norris, 1998). Due to the differences between the different populations and in order to have reliable methods (which is fundamental in Forensic Anthropology), nowadays, there is a tendency to test any standard before its systematic application in a specific population and to develop specific standards for any population (Cameriere and Ferrante, 2008; Boccome et al., 2010; Charisi, 2011; Rissech et al., 2012).

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 (Gassiot et al., 2007; Gassiot y Steadman, 2008; Rios, 2008). 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, as we say in the paper, 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 19th century, which were based on a French sample performs better, because of the biological population history of French, Spanish and Italian populations (Formicola, 1993; Formicola and Franceschi, 1996; Lalueza-Fox, 1998) and because they are populations of medium stature (Formicola, 1993; Formicola and Franceschi, 1996). In contrast, the equations of Trotter and Gleser systematically overestimate stature, both in female and male skeletons of Spanish and Italian origin (Formicola, 1993; Formicola and Franceschi, 1996; Lalueza-Fox, 1998). This differences have strong consequences in the forensic remains.

Following this we have changed and improved the discussion of the paper.

References

Boccome S, Cremasco MM, Bortoluzzi S, Moggi-Cecchia J, Massa ER. 2010. Age estimation in subadult Egyptian remains. Homo, 61:337-358.

Buikstra JE, Ubelaker DH, editors. 1994. Standards for data collection from human skeletal remains. Proceedings of a seminar at the Field Museum of Natural History, organized by Jonathan Haas. Fayetteville, Ark: Arkansas Archeological Survey Research Series.

Cameriere R, Ferrante L. 2008. Age estimation in children by measurement of carpals and epiphyses of radius and ulna and open apices in teeth: A pilot study. Forensic Science International 174:59–62.

Charisi D, Eliopoulos C, Vanna V, Koilias Ch, Manolis S (2011) Sexual Dimorphism of the Arm Bones in a Modern Greek Population. Journal of Forensic Sciences, 56:10-18.

Gassiot E, Oltra J, Sintes E, Steadman DW. 2007. Archaeology of the Spanish Civil War: rescue of the memory and historic justice. In Archaeology and Capitalism: From Ethics to Politics, Y, Hamilakis P Duke (eds). Left Coast Press: Walnut Creek, CA; 235–245.

Gassiot E, Steadman DW. 2008. The political, social and scientific contexts of archaeological investigations of mass graves in Spain. Archaeologies 4(3): 429–444.

Formicola V. 1993. Stature reconstruction from long bones in ancient population samples: an approach to the problem of its reliability. American Journal Physical Anthropology 90:351-358.

Formicola V, Franceschi M. 1996. Regression equations for estimating stature from long bones of early Holocene European samples. American Journal Physical Anthropology 100:83-88.

Komlos J, Baur M (2004) From the Tallest to (One of) the Fattest: The Enigmatic Fate of the Size of the American Population in the Twentieth Century. Econ Hum Biol 2:57-74.

Komolos J. 2001. On the biological standard of living of Eighteenth-Century Americans: taller, richer, healthier. Resech Econ Hist 20:223-248.
Smith SA, Norris BJ. 2004. Changes in the body size of UK and US children over the past three decades. Ergonomics 47:1195-1207.
Lalueza-Fox C. 1998. Stature and sexual dimorphism in ancient Iberian populations. Homo. 49:260-272.
Pearson K. 1899. Mathematical contributions to the theory of evolution. On the reconstruction of the stature of prehistoric races. Phil. Trans. R. Soc. Lond. 192:169-244.
Pebles L, Norris B. 1998. Adult data: The handbook of adult anthropometric and strength measurements: data for desing safety. Department of Trade Industry, Governement Consume Research.
Rissech C, Wilson J, Winburn AP,l Turbón D, Steadman D. 2012. A comparison of three established age estimation methods on an adult Spanish sample. International Journal of Legal Medicine 126:145-155.
Rios L, Martinez B, Garcia-Rubio A, Jiménez J. 2008. Death in captivity during the early Franco regime: exhumation of the cemetery of Valdenoceda prison (1938–1943). Complutum 19: 139–160.
Steele DG, Brambeltt CA (1988) The anatomy and biology of the human skeleton. Texas A&M University Press, Texas.