Elsevier Editorial System(tm) for Forensic Science International
Manuscript Draft

Manuscript Number: FSI-D-10-00666R1

Title: POSTNATAL GROWTH OF THE TIBIA. IMPLICATIONS FOR AGE AND SEX ESTIMATION

Article Type: Original Research Paper

Keywords: Growth of the tibia; immature; sex determination; age estimation

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Results indicate that all five variables exhibit linear behaviour during growth, which can be expressed by a first-degree polynomial function. Sexual significant differences were observed from age 15 onward in the two epiphysis measurements and condylo-malleolar length, suggesting that these three variables could be useful for sex determination in individuals older than 15 years. Strong correlation coefficients were identified between the five tibial variables and age. These results indicate that any of the studied tibial measurements is likely to serve as a useful source for estimating sub-adult age in both archaeological and forensic samples.

Suggested Reviewers:

POSTNATAL ONTOGENESIS OF THE TIBIA. IMPLICATIONS FOR AGE AND SEX ESTIMATION

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Short title: Tibia Development

KEYWORDS: femur growth, immature, sex determination, age estimation.

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ABSTRACT

The growth of five variables of the tibia (diaphyseal length, diaphyseal length plus distal epiphysis, condylo-malleolar length, sagittal diameter of the proximal epiphysis, maximum breadth of the distal epiphysis) were analyzed using polynomial regression in order to evaluate their significance and capacity for age and sex determination during and after growth. Data were collected from 181 (90♂ and 91♀) individuals ranging from birth to 25 years of age and belonging to three documented collections from Western Europe.

Results indicate that all five variables exhibit linear behaviour during growth, which can be expressed by a first-degree polynomial function. Sexual significant differences were observed from age 15 onward in the two epiphysis measurements and condylo-malleolar length, suggesting that these three variables could be useful for sex determination in individuals older than 15 years. Strong correlation coefficients were identified between the five tibial variables and age. These results indicate that any of the studied tibial measurements is likely to serve as a useful source for estimating sub-adult age in both archaeological and forensic samples.

INTRODUCTION

Estimation of age and sex from the skeleton are on the basis of any osteological study, whether anthropological or forensic. In the osteoarchaeological domain, accurate age and sex estimation are fundamental in order to make accurate reconstructions of the demographic profiles of past populations and they also provide information that is useful for interpreting the funerary context. In forensic anthropology, accurate age and sex estimation are key elements of victim identification. The accuracy of age and sex assessment may depend upon the availability of appropriate data relating to the growth and maturation of the skeletal elements with regard to the population of origin and thus genetic, environmental and cultural influences. Data of this nature should therefore be based on osteological material that is well documented (i.e. of known sex and age) in order to avoid inappropriate circular arguments linked to the establishment of methods derived from a pre-existing profile [1-6]. Despite the existence of numerous studies on growth [7-21, among others], there is a notable lack of information about the development of many elements of the human skeleton based on direct measurements from documented osteological material, especially from Western European populations. Of the growth standards that are currently available for osteological studies, many are based on radiographic images of North American Caucasian children [11,12,15,17,18,21]. Direct studies on osteological material also exist, but most are based on archaeological specimens (for which age and sex have been estimated in the laboratory) and are restricted to children of Slavic [22], Germanic [23], Eskimo [24] or Amerindian [23-25] descent. Of the few studies that have considered children of Western European ancestry, many are also based on archaeological material [26-30] or they are restricted to specific skeletal elements such as the scapula [5], the innominate [3,31-34], the sacrum [35] and the femur [4]. Despite the anatomical and anthropological importance of the tibia [36,37] and the large volume of research pertaining to this bone [12,15,38-43], we have encountered no tibial growth studies based on documented osteological material from Western European collections.

To bridge the gap in the literature and with the intention of completing the growth studies on the lower extremities of the skeleton, which have been initiated by Rissech and colleagues with the innominate bone [3,31-34,44] and the femur [4], this paper examines cross-sectional information on tibial growth from documented skeletal material of Western European origin through the full development spectrum, from birth to the attainment of adult form. The study has a twofold aim: to analyse the growth of the tibia, with the intention of describing 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 contextualised skeletal collections from Western Europe were used for the study. A contextualised collection is one where the maximum amount of information about 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.) [6]. The three series in question are:

a) St. Bride's collection (Sb) kept in the crypt of St Bride's church in London (UK). This collection consists of 227 adults and sub-adults born between late 18th and 19th centuries, originating from the church cemetery [45].

b) Esqueletos Identificados (Co) kept in the Museu Antropologico at Coimbra University in Coimbra (Portugal). This collection consists of 505 adult and sub-adult individuals from the local cemetery of Conchada [46]. All the individuals were born between late 19th and late 20th centuries.

c) Lisbon collection (Lb) kept in the Museu Bocage in Lisbon (Portugal). This collection arose from the accumulation of adults and sub-adults skeletons from three local cemeteries of Alto de S. Joaõ, Prazeres and Benfica [47]. It comprises 1400 individuals, who were born between late 19th and late 20th centuries.

All the individuals under 26 years of age were selected from these three series. This age was selected in order to cover the entire growth period in all individuals and to determine the age of growth cessation for each of the analysed variables. Individuals displaying pathologies that could affect the analysis were excluded, leaving a total of 181 individuals (90♂ and 91♀) aged 0 to 25 for analysis. Table 1 presents detailed information about the age and sex of the individuals selected for the study.

Three classical measurements were used for this study (diaphyseal length, condylo-malleolar length, sagittal diameter of the proximal epiphysis and maximum breadth of the distal epiphysis) along with a new measurement: diaphyseal length plus distal epiphysis. The left tibia was always used for the measurements. If the left tibia was lost, or displayed pathological or taphonomic abnormalities, the right was used.

Definitions of the measurements:

(1) Diaphyseal length of the tibia. Maximum distance between the proximal and distal ends of the tibia shaft minus both epiphyses. This measurement could no longer be recorded once the proximal epiphysis had begun to unite [48].

(2) Diaphyseal length plus distal epiphysis of the tibia. Maximum distance between the proximal end of the diaphysis and the distal end of the distal epiphysis. The unfused epiphysis was included in the measurement by securing its position with adhesive tape. This measurement could no longer be recorded once the proximal epiphysis had begun to unite. Documentation of growth following this developmental period was possible using the next variable, condylo-malleolar length.

(3) Condylo-malleolar length of the tibia (no. 1 of Martin). Maximum distance from the lateral condyle at the proximal end, to the tip of the medial malleolus [49]. If the epiphyses were not fused, they were included in the measurement by securing their position with adhesive tape.

(4) Sagittal diameter of the proximal epiphysis. Anteroposterior distance of the proximal epiphysis between medial and lateral condyles, at the mid-plane level (Fig. 1) [42].

(5) Maximum breadth of the distal epiphysis (no. 6 of Martin). Maximum distance between the two most laterally projecting points on the medial malleolus and the lateral surface of the distal epiphysis (Fig. 2) [49].

Statistical analysis

Statistical analyses were carried out in four parts:

(1) First, the homogeneity between series was observed by Graphic Lowess method in the young group and by ANOVA 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 [3-5,32-33,44]).

To decide whether individuals were still growing (young group) or not still growing (adult group), we used the age of the fusion of both epiphyses, which is, in the sample of the present study, 19 years in males and 17 years in females (see results). From that point the tibia is no longer growing and the graph of the analysed variables becomes constant.

(2) Second, in order to make an initial approximation of sexual dimorphism, the means and standard deviations for each tibial variable in each age group were calculated and Student's t-test was applied to each age category. However, if there were less than 15 individuals in one of the two sexual series for one age group, the Mann-Whitney's U-test was applied. The series used in this study are not very large and their age and sex composition is unequal; this is the same problem for all the documented series, of which there are few, that contain juvenile remains. For this reason and in line with current methodological practice, each series was divided using 5-year intervals in order to carry out the second analysis. Results from this analysis must be viewed with caution due to the lack of homogeneity in the age distribution of the younger groups, the rhythm of growth within and amongst different age groups and the small size of the sample.

(3) Third, the growth behaviour of each of the five variables was analysed using polynomial regression up to the fifth degree, treating age as continuous. Only individuals still growing were used. Polynomial regression analysis was selected based on the assumption that the dynamics of growth can be described by an incremental continuous function [7,19]. The most appropriate statistical model was then selected on the basis of three factors: (1) the strength of the correlation coefficient (R^2); (2) the significance of the function expressed by the F value; and (3) the significance of the coefficients of the function obtained using the ANOVA test.

(4) Finally, to enable predictions of age at death, Inverse Regression analysis for age and each metrical variable of the tibia was calculated by using age as dependent variable. That is to say, each metrical variable of the tibia (x) was regressed on age (y). When the objective is to obtain a methodology for sub-adult age estimation, which can be applied to any population without exception, a Bayesian approach is the best [50]. However, in order to develop a Bayesian approach with these characteristics, a relatively broad sample, containing the variability of several populations, is required [51,52]. As stated above, the main difficulty facing any sub-adult study based on documented osteological material is the scarcity of any such collections. Therefore, due to the type of sample used in the present study, the main objective is focused on the Western European population, specifically on the Iberian Peninsula. Inverse regression analysis was selected for this reason, being 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 [50], and this presumption is generally warranted in forensic settings [50]. There is, of course, some biological differentiation within Western European populations, however this differentiation does not seem to

greatly affect the variability observed during the growth of the analysed skeletal elements. Their biological proximity is indicated by the closeness of the lines for the different samples (Fig. 3) during the homogeneity test, and the low standard deviations (Table 2). French and Portuguese populations are biologically similar to the Spanish, because of their shared biological population history and geographical proximity [6,53]. Furthermore, Rissech [44], in her study based on several documented skeletal collections from various Western European countries (Spain, Portugal and England), found that they could be considered as a single series due to the observed homogeneity.

Inverse Regression was calculated separately for each sex. However, in series that displayed no sexual differences, calculus was applied to the data as a whole (males and females combined). This equation makes it possible to apply the technique in situations where sex is unknown.

The statistical packages were sourced via PASW Statistics 18.

RESULTS

Homogeneity testing by Lowess method in young individuals and ANOVA test in adult individuals between the three series, according to each sex and metrical variable revealed: 1) similar patterns of growth between the series in the young group (i.e. Fig. 3); and 2) no significant differences between the series in the adult group for condylo-malleolar length (F♂ = 1.249, p = 0.300; F♀ = 2.137, p =0.133), sagittal diameter of the proximal epiphysis (F♂ =0.240, p = 0.788; F♀ = 2.394, p=0.107) and maximum breadth of the distal epiphysis of the tibia (F♂ =2.705, p =

0.082; F♀ = 1.520, p=0.233). The diaphyseal length of the tibia and the diaphyseal length of the tibia plus distal epiphysis were not considered in the adult homogeneity testing because of the fusion of the epiphyses, which impede the measurement of these two variables. Results indicated the homogeneity between the analysed series in sub-adult individuals and adult individuals; therefore they cannot be considered as different series. Individuals from the three samples were therefore analysed together as a single series.

For the sake of clarity, the results for each variable will be explained separately.

Diaphyseal length of the tibia

Table 2 shows the descriptive statistics of the diaphyseal length of the tibia and the results obtained by applying the Mann-Whitney's U-test to each age interval. The female average is greater than the male within the 0–4 year interval, although from 5 years upward the male average is greater than the female. However, none of these differences is statistically significant (Table 2). The length of the diaphysis increases due to growth up until the 15-19 age interval. In the analysed sample, the union of the distal epiphysis occurs by the age of 17 in boys and 16 in girls. These values are within the range of current standard values (15-18 years in boys and 14-16 in girls [36]). However, the sample contained a boy and a girl whose maximum limits were situated at 19 and 17 years respectively. Given that there are authors who place this limit slightly later, at around 19 years [54,55], these individuals can also be considered as falling within the expected variability range for fusion of the distal epiphysis.

Due to the absence of significant sexual differences in diaphyseal length of the tibia, growth analysis for this variable was carried out considering all individuals as one single series (boys and girls combined). Results indicate that the growth behaviour of the diaphyseal length of the tibia can be approximated to a first-degree polynomial (Figure 4). Its coefficients have statistical significance and the F value indicates the significance of the function. The explained variability of the model is 87%. As the diaphyseal length of the tibia has lineal behaviour, which also occurs in the majority of vertical variables [3,4,56], it was not possible observe the growth spurt of this variable. Neither was it possible to observe the age of growth cessation of this variable due to the lineal progression of the curve.

The lack of sexual differences in any of the age groups indicates that the diaphyseal length of the tibia is not a useful variable for sexual determination, but it can be of use for age estimation before the fusion of the proximal 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 tibia was calculated and age (Table 3). The selected function was a first-degree polynomial in both sexual series and in the unisex series (boys and girls combined). The explained variability of the male, female and unisex models is 89%, 83% and 87% respectively.

Diaphyseal length of the tibia plus distal epiphysis

The Mann-Whitney's U-test applied to each interval shows that the average length of the diaphysis is greater in females than in males (Table 2) from birth to the age of 19, although none of these differences is statistically significant. In Table 2, diaphyseal length of the tibia plus distal epiphysis increases in size until the 15–19 age interval for both males and females. In the studied sample, the age limit for fusion of the proximal epiphysis is 19 years in boys and 17 in girls. These ages agree with the current standard data for union time of the proximal tibia (16-19.5 ♂ and 13-17 ♀ [36]).

As no significant sexual differences were found for the diaphyseal length of the tibia plus distal epiphysis, males and females were combined to calculate one growth model up to the age of 17. The best growth model for this variable was a first-degree polynomial (Fig. 5). Its coefficients are significant, and the F value indicates the significance of this function. The explained variability of the model is 88 % (Fig. 5). This model mirrors the constant rhythm of the growth rate in longitudinal measurements [56] and consequently, the curve does not display an adolescent upturn.

Diaphyseal length of the tibia plus distal epiphysis is not an adequate measurement for sex diagnosis due to the lack of sexual significant differences in any of the age groups, but it is useful for sub-adult age estimation in osteological remains of known and unknown sex. The Inverse Regression between diaphyseal length of the tibia plus distal epiphysis and age (Table 3) is a first-degree polynomial for the male, female and unisex series with 88%, 89% and 88% of explained variability, respectively.

Condylo-malleolar length of the tibia

Results obtained by applying the Mann-Whitney's U-test and Student's t-test to the condylo-malleolar length of the tibia (Table 4) indicate that the male average is greater than female average with the exception of the 10-14 age interval, where the female average is slighter greater than the male (Table 4). However, these differences are not significant until 15 years and above (Table 4). The increase in size of the condylo-malleolar length due to the growth continues up until the 15-19 age category for both males and females.

The most appropriate growth model for the condylo-malleolar length of the tibia was a first-degree polynomial in both the male (Fig. 6) and female (Fig. 7) series. The coefficients have significance in the two sexual series and the F values indicate the significance of the function. The explained variability of the models are 88% in males and 82% in females. The growth spurt cannot be visualized from the fitted curves due to the linear increase of this variable. The maximum for both male and female curves is approximately 19 years, indicating the end of tibial growth (Fig. 8); but this seems to be a little earlier in girls, although with our data it is not possible to be more precise. The cessation of tibial growth coincides with the fusion of its proximal epiphysis and with the cessation of growth in height, since the epiphyses around the knee are the growing end of the lower limb bone. The obtained ages for cessation of tibial growth and the age of fusion of the proximal epiphysis are consistent with the standard age range for the end of tibial growth, union of the proximal epiphysis (16-19.5 years ♂ and 13-17 years ♀ [36]) and the end of growth in height (17.75 years, SD: 13 months for males and 16.25 years, SD: 10 months for females) in the current population [19].

Female and male growth curves move closer to one another until the point of female growth cessation (Fig. 8). The female curve has a steeper slope than the male, indicating that the female growth rate for this variable is slightly greater than the male. Significant differences between the sexes appear after the cessation of growth in girls, due to the longer male growth period. This fact is related to females reaching maturity earlier, as is also reported in the existing sources in the literature [36]. Using our data as a base, maximum tibial length can be used to diagnose sex from 15 years of age and throughout the adult period. These results agree with existing literature on the adult tibia [41,57-61].

With regard to age diagnosis, tibial length can be useful for estimating the age of sub-adult osteological remains of known sex, as well as remains of unknown sex between the ages of 0 and 15. The absence of sexual significant differences up to 15 years of age allows the male and female series to be combined to create a unisex sub-adult model. To assess age at death, the Inverse Regression between condylo-malleolar length and age was calculated. A first-degree polynomial from male, female and unisex series was selected, with an explained variability of 88%, 82% and 85% respectively (Table 3).

Sagittal diameter of the proximal epiphysis

Table 4 shows that the female average for the sagittal diameter of the proximal epiphysis of the tibia is slightly greater than the male average from birth until the age of 4. Between 5 and 9 years of age the male average is the same as the female, and after 9 years of age boys have the higher values, although these differences are significant only from the age of 15 upwards (Table 4). These sexual differences are in accordance with the well-defined sexual dimorphism on the tibial proximal epiphysis in sub-adults [36,43] and adults [37]. In Table 4 it can be observed that the sagittal diameter of the proximal epiphysis of the tibia increases in size due to growth until 15 years of age in both sexes. From 15 years onward, the values are constant, indicating growth cessation for this variable.

The best growth model for the sagittal diameter of the proximal epiphysis of the tibia was a first-degree polynomial in both the male (Fig. 9) and female (Fig. 10) series, expressing a linear progression of this variable in both sexes. In both models the coefficients are significant and F values indicate the significance of the functions (Fig. 9 and 10). The explained variability of the models is 93% in males and 87% in females. The maximum in the male curve is at approximately 17 years of age, and around 15 in the female (Fig. 11), indicating the growth cessation for this variable. This age is earlier than the approximate age at which linear growth of the tibia ceases within this sample and corresponds to the early formation of the epiphysis. Early maturation of the epiphysis seem desirable, in order to enable the joints to withstand the considerable forces they are required to bear, as body mass and weight increase during puberty [4,36].

According to the data obtained in this study, the sagittal diameter of the proximal epiphysis of the tibia is useful for sexual determination of individuals over the age of 15 (Table 4). Regarding age estimation, this variable can be useful for osteological remains of known sex and also for remains of unknown sex between 0-15 years of age. The absence of sexual differences until the age of 15 made it possible to use the combined series to calculate a unisex juvenile model. To assess age at death, the Inverse Regression between the sagittal diameter of the proximal epiphysis of the tibia and age was calculated (Table 3). A first-degree polynomial model was selected for the male, female and unisex series, with an explained variability of 93.2%, 88.2% and 88.8% respectively.

Maximum breadth of the distal epiphysis

From 0 to 25 years of age, boys from this study have a higher mean value than girls for the maximum breath of the distal epiphysis of the tibia (Table 4). However, these differences are significant only from age 15 onwards, which agrees with the current literature [37]. The increase in size of the maximum breath of distal epiphysis due to growth continues up until 15 years in both sexes. After this age the values are constant, indicating the end of the growth of this variable (Table 4).

The best growth model for the maximum breadth of the distal epiphysis of the tibia was a first-degree polynomial in both males (Fig. 12) and females (Fig. 13), with an explained variability of 92% in males and 90% in females. The maximum for the male and female curves (Fig. 14) indicates the end of the growth of this variable. This occurs at approximately 15 years in males and a little earlier in females, although with our data it is not possible to be more precise. As in the proximal epiphysis, rapid maturation is observed in the distal epiphysis in relation to the longitudinal maturation of the tibia. However, the approximate age of growth cessation in the distal epiphysis is earlier than the approximate age at which linear growth of the proximal epiphysis ceases within this sample, reflecting the early formation of the ankle in relation to the knee [36].

Based on the data from this study, the maximum breadth of the distal epiphysis of the tibia may be of value for sex diagnosis from 15 years of age and throughout the adult period. Regarding the estimation of age at death, this variable can be useful for osteological remains of known sex and for individuals of unknown sex between 0 to 14 years of age. The absence of significant sexual differences between 0 and 14 years of age allows us to group the two sexual series into a single, unisex series. To assess age at death, the Inverse Regression between maximum breadth of the distal epiphysis and age was calculated (Table 3). A first-degree polynomial was selected for the male, female and unisex series with an explained variability of 91%, 89, and 88% respectively.

DISCUSSION

According to our data, the growth behaviour of the five tibial variables is essentially linear. The main characteristic that distinguishes between horizontal and vertical variables is the behaviour of the growth curves. While the horizontal variables display a decrease in growth rate before the growth spurt, the longitudinal variables show more continuous linear growth [3,33,56]. The curves are, in general, a good fit and there is little scatter, as evidenced by the consistently high correlation and significance of the functions and their coefficients achieved in the models.

In order to evaluate the utility of the growth models and obtained functions for sub-adult age estimation in forensic cases, it is necessary to assess whether the analysed sample is representatives of modern samples. For this reason its growth parameters will be discussed in relation to the growth parameters 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- Due to the continuous increase during growth of the analysed variables, none of the curves shows an adolescent upturn and consequently, it was impossible to determine the age of the growth spurt for the analysed sample in the present study. However, the ages of the growth spurt found in the ischiopubic area (pubis and ischiopubic index) [33] and in the ilium width [32] of this sample (which are around 14-15 years of age in males and 10-11 years of age in females) fall into the standard age intervals for the pubertal growth spurt in the existing population: 10.5–17.5 years of age in males and 9.5–14.5 in females [19].

Timing of maturation- The mean age of fusion of the epiphyses of the analysed sample are consistent with the given age intervals for the current population. For example, the age of fusion of the acetabulum (acetabulum maturity) in the analysed sample is 16 in males and 12 in females [3,32,33], which fall into the standard intervals for the current population: 14–17 and 11–15 in males and females respectively [36]. With regard to the age of fusion of the distal and proximal epiphysis of the tibia, these concur perfectly with the age intervals for the current population:

a) In the studied sample, fusion of the distal epiphysis of the tibia occurs at the age of 17 in boys and 16 in girls, which corresponds to the normal union times for the distal epiphysis of the tibia in boys (15-18 years) and girls (14-16 years) in the current population [36].

b) The fusion of the proximal epiphysis in the analysed sample occurs at 19 years in males and little earlier in females (around 17 years), which are consistent with the given age intervals for the normal fusion of the proximal epiphysis of the tibia in boys (16-19 years) and girls (13-17 years) in the current population [36].

Cessation of growth in height- The ages of fusion of distal epiphysis found in the studied sample (19 years ♂ and 17 years ♂) also concur with the standards of 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 [19], because the end of the longitudinal growth of the tibia is determined by the fusion of its proximal epiphysis [36], and the fusion of the epiphyses that constitute the knee coincides with the cessation of growth in height [36].

All of 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 contextualised modern skeletal collection of the Universitat Autònoma de Barcelona (UAB) [4]. The UAB collection is made up exclusively of modern individuals from the 20th century from the industrial city of Granollers (it is an industrial, commercial and trade city, situated 25 km north-east of Barcelona) [6] and the femur is one of the bones that best correlates with stature [62], indicating the similarity in stature between the modern Spanish series and the series of the present study. In general, it can be said that the tibiae of the analysed series do not show evidence of secular change, malnutrition, or delays in growth or osseous maturation and 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 the condylo-malleolar length of the tibia, sagittal diameter of the proximal epiphysis and maximum breadth of the distal epiphysis. This can be explained by earlier female maturation and the longer male growth period, which is consistent with existing sources in the literature [36]. Results in sexual dimorphism for the condylo-malleolar length of the tibia is to be expected, since sexual dimorphism in vertical variables is related to the cessation of female growth rather than the spurt itself and it is a fact that characterises the vertical variables [20]. Condylo-malleolar length of the tibia, sagittal diameter of the proximal epiphysis and maximum breadth of the distal epiphysis are useful for sexual diagnosis from 15 years onward. The latter two variables can be of special interest for sexual diagnosis when the studied osteological material is incomplete. The statistical significance of the three above-mentioned variables agrees with the importance of these variables in adult sexual diagnosis [37].

Age Estimation

Tibial growth rate is useful for age estimation in sub-adult individuals, using Inverse Regression functions of the absolute variables of the tibia. The functions obtained from the five variables analysed in this study are useful for sub-adult age estimation of skeletal remains. One of the most interesting functions for use in estimating sub-adult age in osteological remains is the diaphyseal length of the tibia because of its tendency to be well preserved and also because it can be applied up until the moment of fusion of the distal epiphysis, which is approximately 17 years of age, in remains of unknown sex. The functions of the sagittal diameter of proximal epiphysis and the maximum breadth of distal epiphysis are especially useful for incomplete remains, and can also be applied up to 15 years of age in remains of unknown sex. The functions obtained in this study, taken from a Western European population, allow us to estimate the age of modern sub-adult skeletal remains in a reliable way in individuals from Western Europe, specifically from the Iberian Peninsula.

The establishment of the identity of an individual is of the utmost medico-legal significance, both in living and dead, especially in cases of murder or mass disasters, where the bodies are grossly mutilated or in advanced stages of decomposition. 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 [63]. There is a pressing need for accuracy and reliability in sub-adult age estimation in the Iberian Peninsula and Mediterranean area in the field of forensic anthropology. Physical anthropologists develop a biological profile of the individuals for identification purposes but most of the sub-adult skeletal ageing and stature standards that are available were developed from US reference samples. The magnitude of error involved in applying these methods to Iberian and Mediterranean individuals is unknown. For example, the method for the calculating adult stature based on US reference samples fails in the estimation of living height in Spain and Italy. In these populations, the formulae proposed by Pearson [64] at the end of 19th century, based on a French sample, performs better, because of the intertwined biological population history of French, Spanish and Italian populations [62,65,66] and because they are all populations of medium stature [62,66]. In contrast, the equations of Trotter and GIeser systematically overestimate stature, in both female and male skeletons of Spanish and Italian origin [63,65,66]. In fact, there is a need to abandon the notion of the "universality" of osteological methodology and rather, promote the standardisation of methods [67]. Methodology should not be applied to skeletal material without regard for the secular and regional origin of the reference collection(s) used in the creation of the method. In this way, the data presented in this study is of great importance for physical anthropologists of Western Europe, especially those of Iberian Peninsula. They will be very useful for application to the analysis of 20th century 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 reinforce the obtained results, in the meantime forensic scientists can take advantage of the results of this study for widening the range of methods for age estimation and sexual determination of human skeletal remains.

CONCLUSIONS

This cross-sectional study on five tibial measurements collected from three documented skeletal series of Western European descent has provided researchers with information pertaining to the tibial growth profile. Using the data as a basis, calculus was performed to derive formulae that may prove valuable in age-at-death diagnosis of the skeleton. The analysis has also generated information regarding the timing at which sexual differences in tibial measurements are present, thus offering indications as to when the variables may be useful for sex diagnosis. The results and formulae obtained from this study are useful tools in the diagnosis of age and sex, as applied to the forensic tasks and in some paleoanthropological cases when there was some reason for presuming a strong prior of similitude between the case and the sample in which was based the model. Further research on the growth and development of the tibia is necessary to obtain better information for skeletal diagnosis, especially within sub-adults.

ACKNOWLEDGEMENTS

This work was funded by the *Ministerio de Ciencia e Innovación* research project no. CGL2006-02170/BTE and the *Generalitat de Catalunya* research project 2009SGR884GRQ-Grup d'Estudis d'Evolució d'Homínids i d'Altres Primats.

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.

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TABLE 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 diaphyseal length and diaphyseal length plus distal epiphysis 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. Error means the error of the function.

Table 4: Descriptive statistics of the condylo-malleolar length, sagittal diameter of the proximal epiphysis, maximum breadth of the distal epiphysis classified according to each age category and sex. Sexual differences by Mann–Whitney's U-test and Student's t-test. The significance is indicated by asterisk (*). Males are indicated by ♂. Females are indicated by ♀.

FIGURE CAPTIONS

Figure 1: Proximal epiphysis of the adult tibia. Arrow indicates the sagittal diameter of the proximal epiphysis.

Figure 2: Distal epiphysis of the sub-adult tibia. Arrow indicates the maximum breadth of the distal epiphysis.

Figure 3: Sagittal diameter of the proximal epiphysis of the tibia of the masculine series considering the three populations from 0 to 25 years of age. Curves were calculated using Lowess' method.

Figure 4: Polynomial regression line with 95% confidence intervals and equation for diaphyseal length of the tibia (DL) considering a unisex series from 0 to 17 years of age. Coefficient = coefficients of the function; p_1 mean the statistical significance of the coefficients; F and p_2 mean the significance of the function; and R^2 the explained variability.

Figure 5: Polynomial regression line with 95% confidence intervals and equation for diaphyseal length of the tibia plus distal epiphysis (DLD) considering a unisex series from 0 to 17 years of age. Coefficient = coefficients of the function; p_1 mean the statistical significance of the coefficients; F and p_2 mean the significance of the function; and R^2 the explained variability.

Figure 6: Polynomial regression line with 95% confidence intervals and equation for the masculine condylo-malleolar length of the tibia (TL) from 0 to 19 years of age. Coefficient = coefficients of the function; p_1 mean the statistical significance of the coefficients; F and p_2 mean the significance of the function; and R^2 the explained variability.

Figure 7: Polynomial regression line with 95% confidence intervals and equation for the feminine condylo-malleolar length of the tibia (TL) from 0 to 17 years of age. Coefficient = coefficients of the function; p_1 mean the statistical significance of the coefficients; F and p_2 mean the significance of the function; and R^2 the explained variability.

Figure 8: Comparison between the feminine and masculine polynomial regression lines obtained for condylo-malleolar length of the tibia.

Figure 9: Polynomial regression line with 95% confidence intervals and equation for the masculine sagittal diameter of the proximal epiphysis (SDP) of the tibia from 0 to 17 years of age. Coefficient = coefficients of the function; p_1 mean the statistical significance of the coefficients; F and p_2 mean the significance of the function; and R^2 the explained variability.

Figure 10: Polynomial regression line with 95% confidence intervals and equation for the feminine sagittal diameter of the proximal epiphysis (SDP) of the tibia from 0 to 15 years of age. Coefficient = coefficients of the function; p_1 mean the statistical significance of the coefficients; F and p_2 mean the significance of the function; and R^2 the explained variability.

Figure 11: Comparison between the feminine and masculine polynomial regression lines obtained for sagittal diameter of the proximal epiphysis of the tibia.

Figure 12: Polynomial regression line with 95% confidence intervals and equation for the masculine maximum breadth of the distal epiphysis of the tibia (DEB) from 0 to 15 years of age. Coefficient = coefficients of the function; t and p_1 mean the statistical significance of the coefficients; F and p_2 mean the significance of the function; and R^2 the explained variability.

Figure 13: Polynomial regression line with 95% confidence intervals and equation for the femenine maximum breadth of the distal epiphysis of the tibia (DEB) from 0 to 13 years of age. Coefficient = coefficients of the function; p_1 mean the statistical

significance of the coefficients; F and p_2 mean the significance of the function; and R^2 the explained variability.

Figure 14: Comparison between the feminine and masculine polynomial regression lines obtained for maximum breadth of the distal epiphysis of the tibia.

Figure 1
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Figure 2

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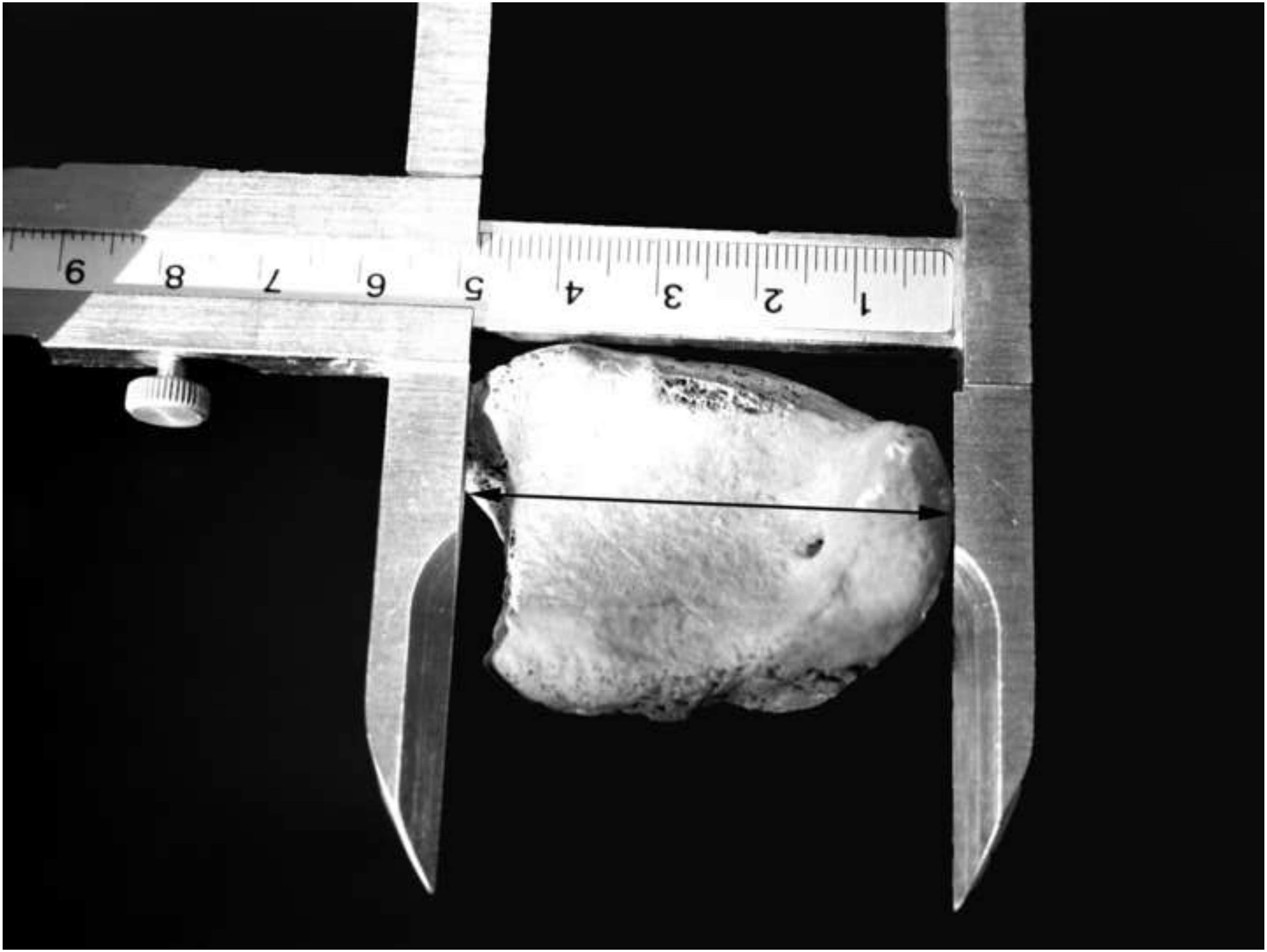

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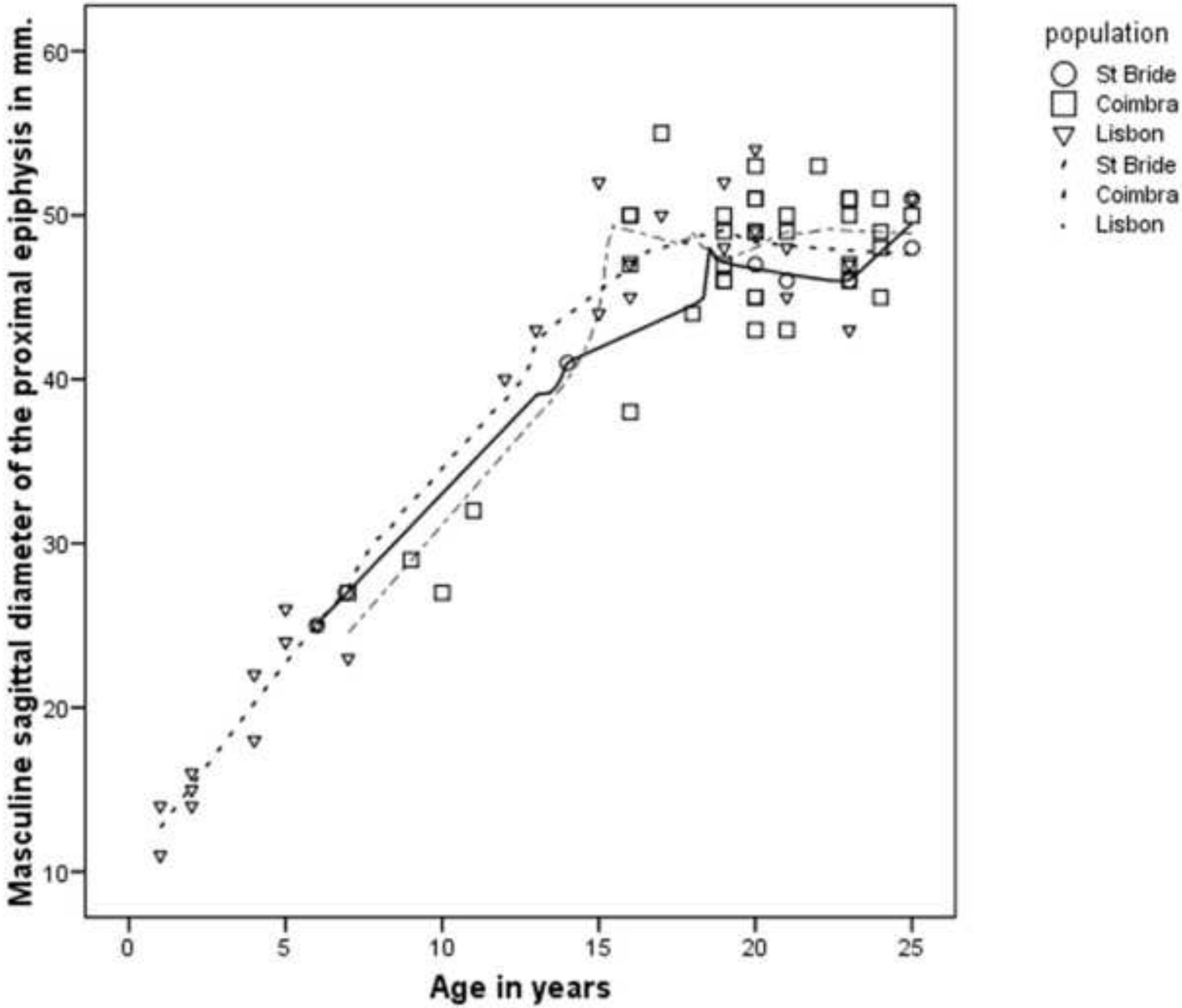

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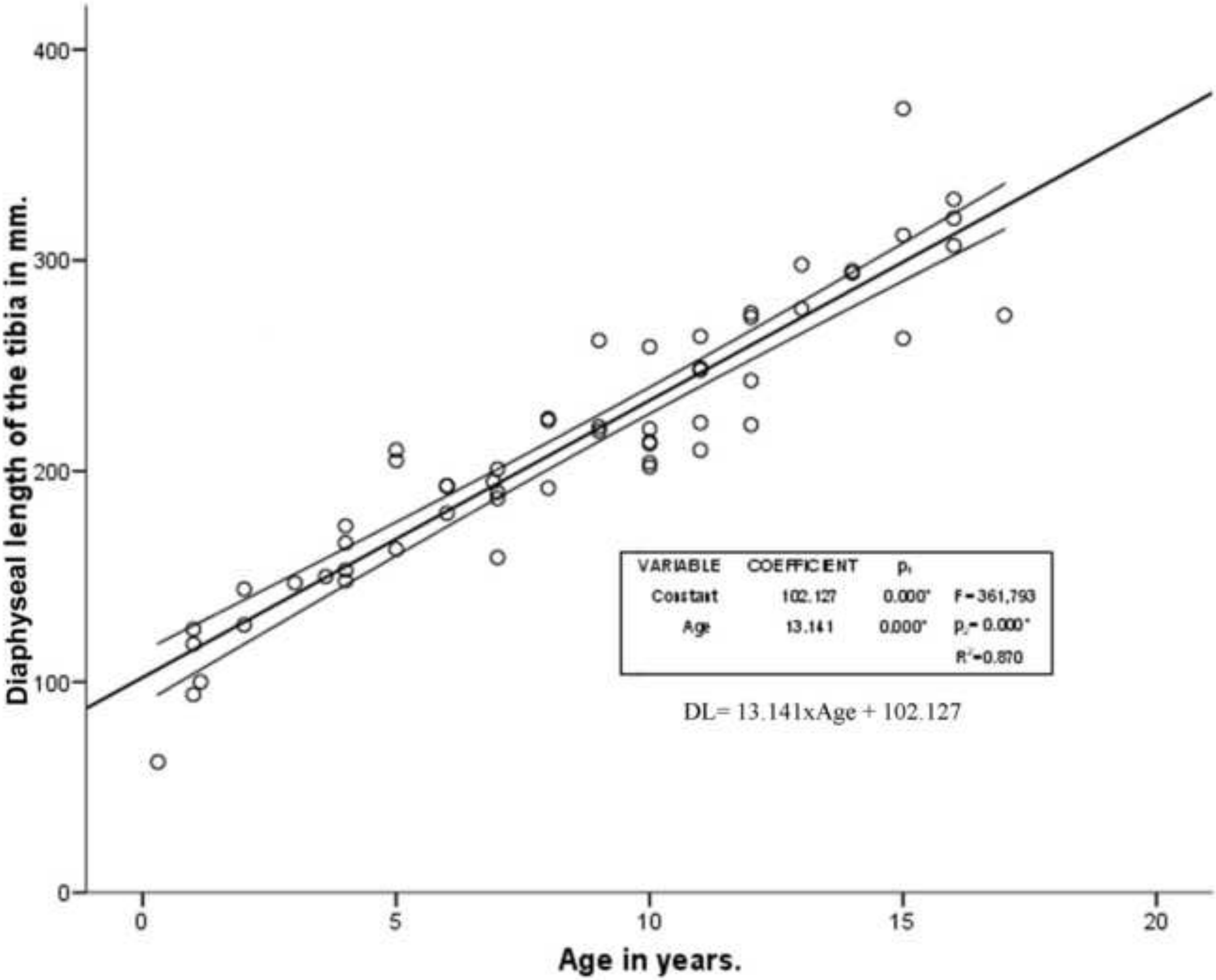

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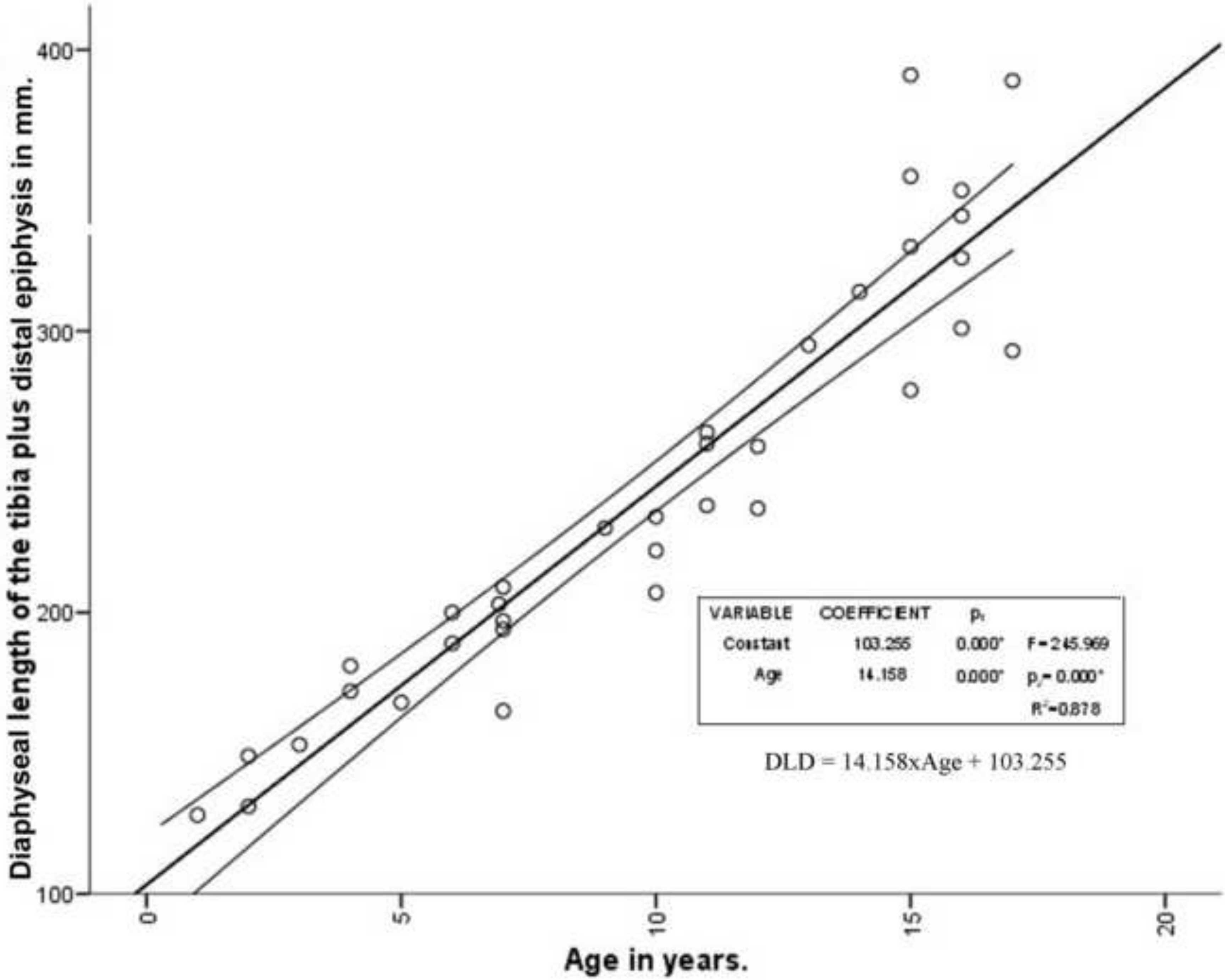

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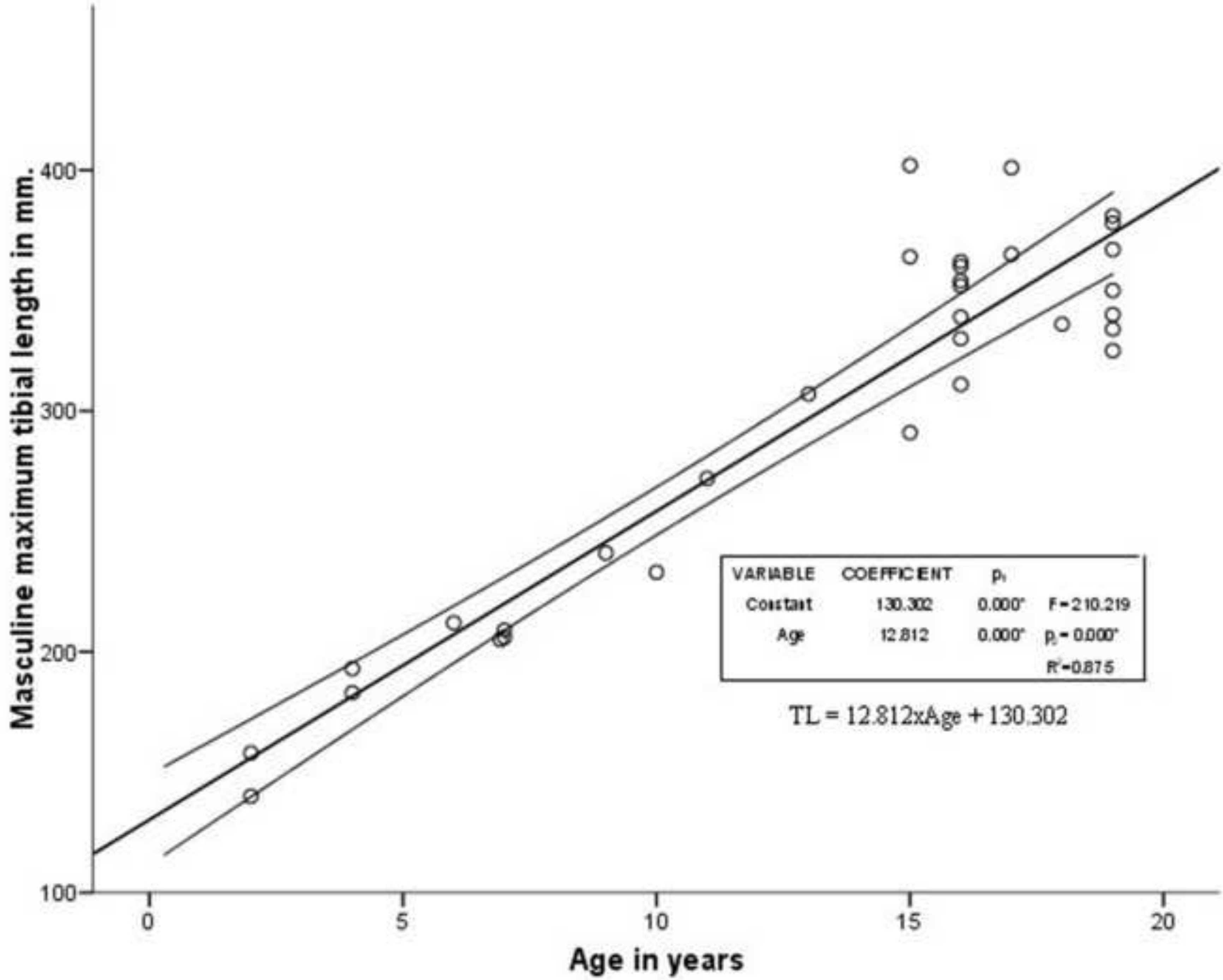

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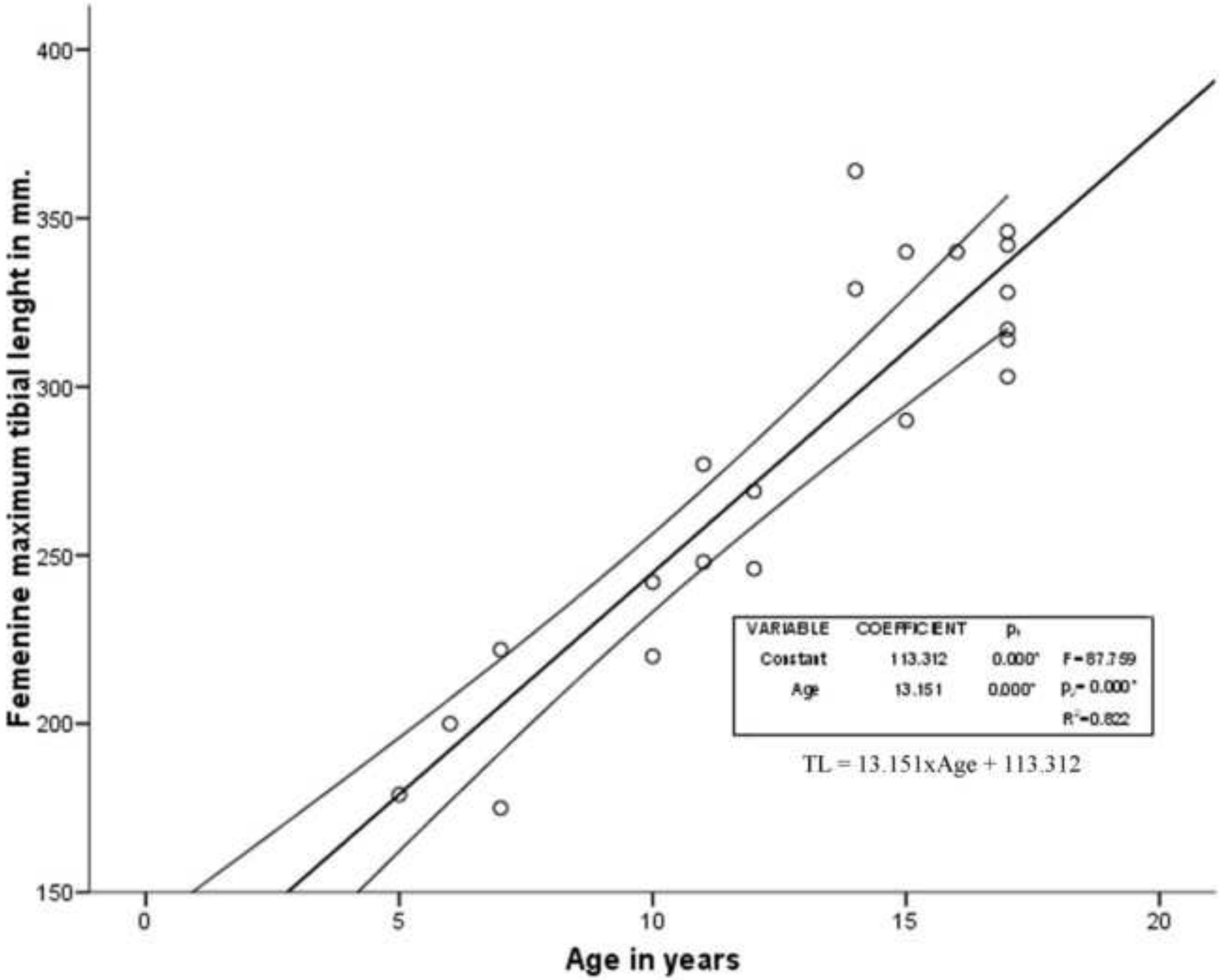

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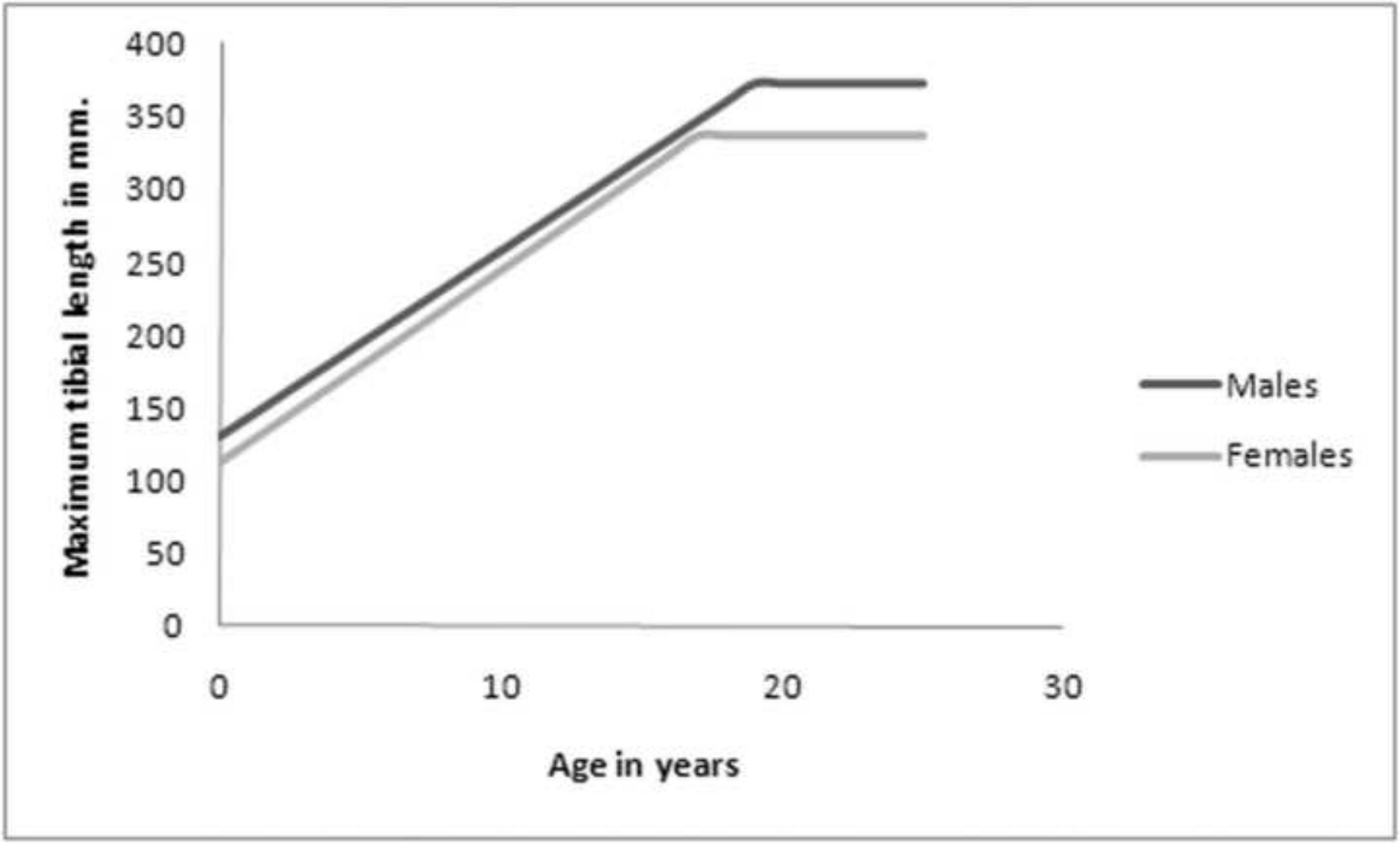

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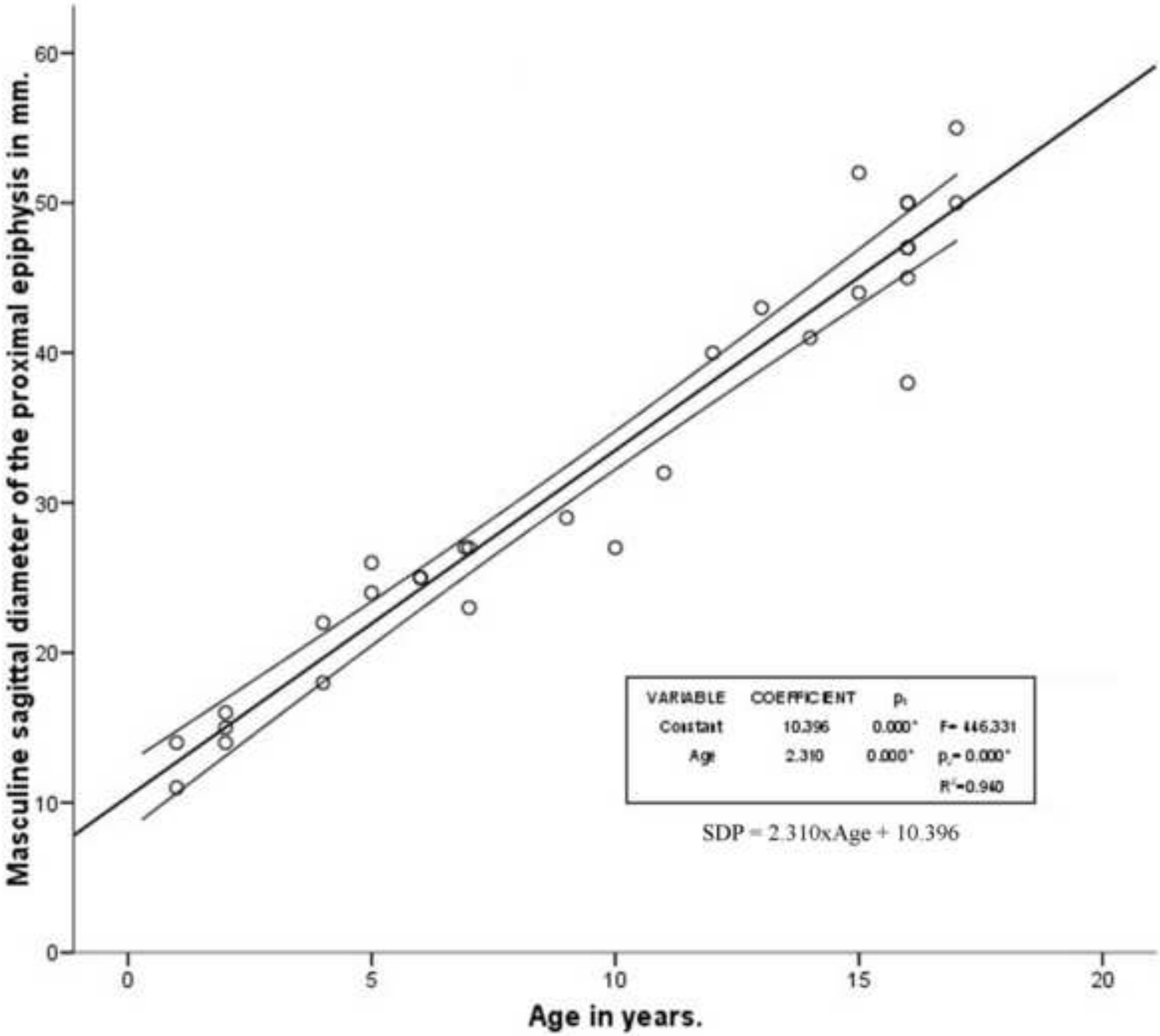

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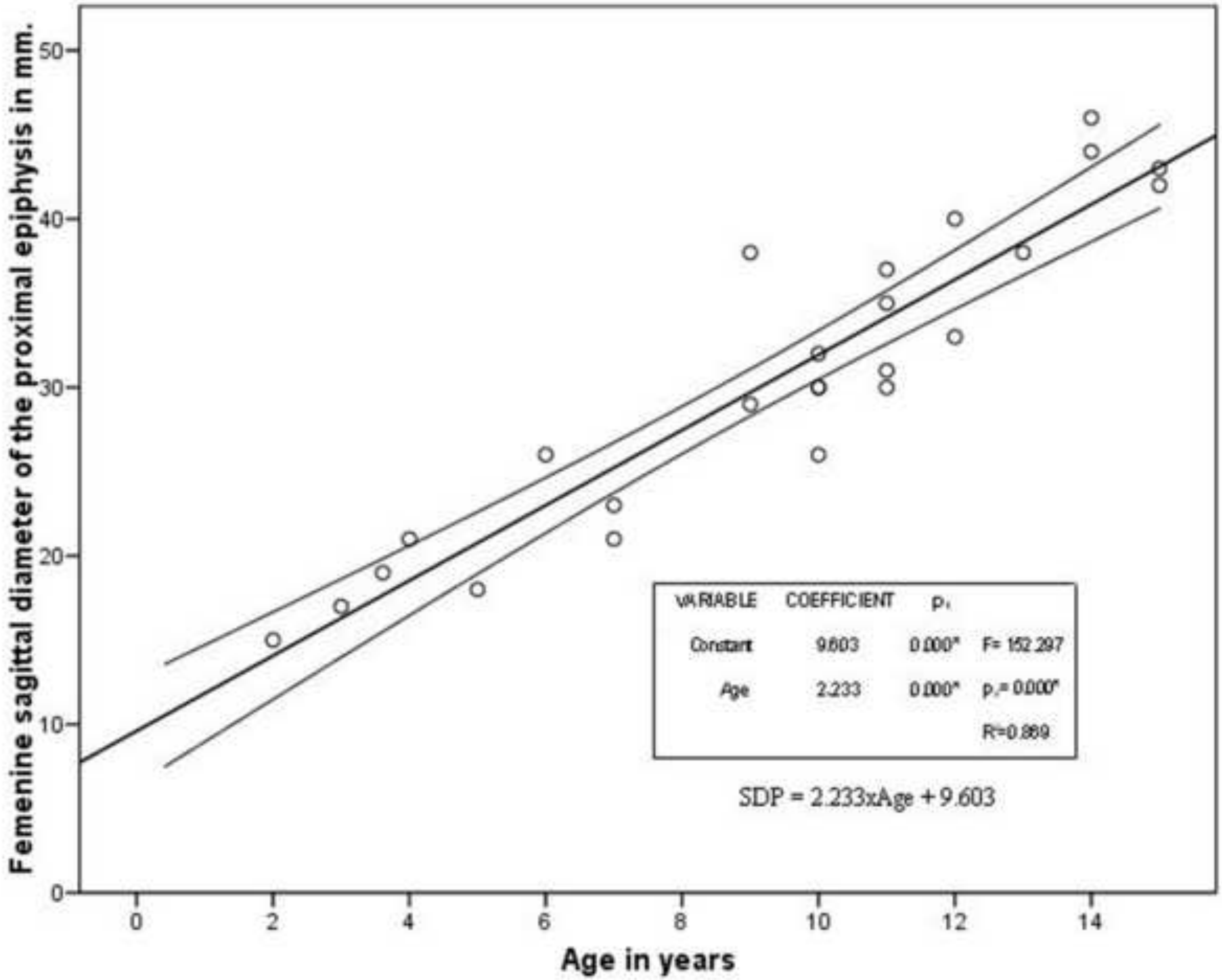

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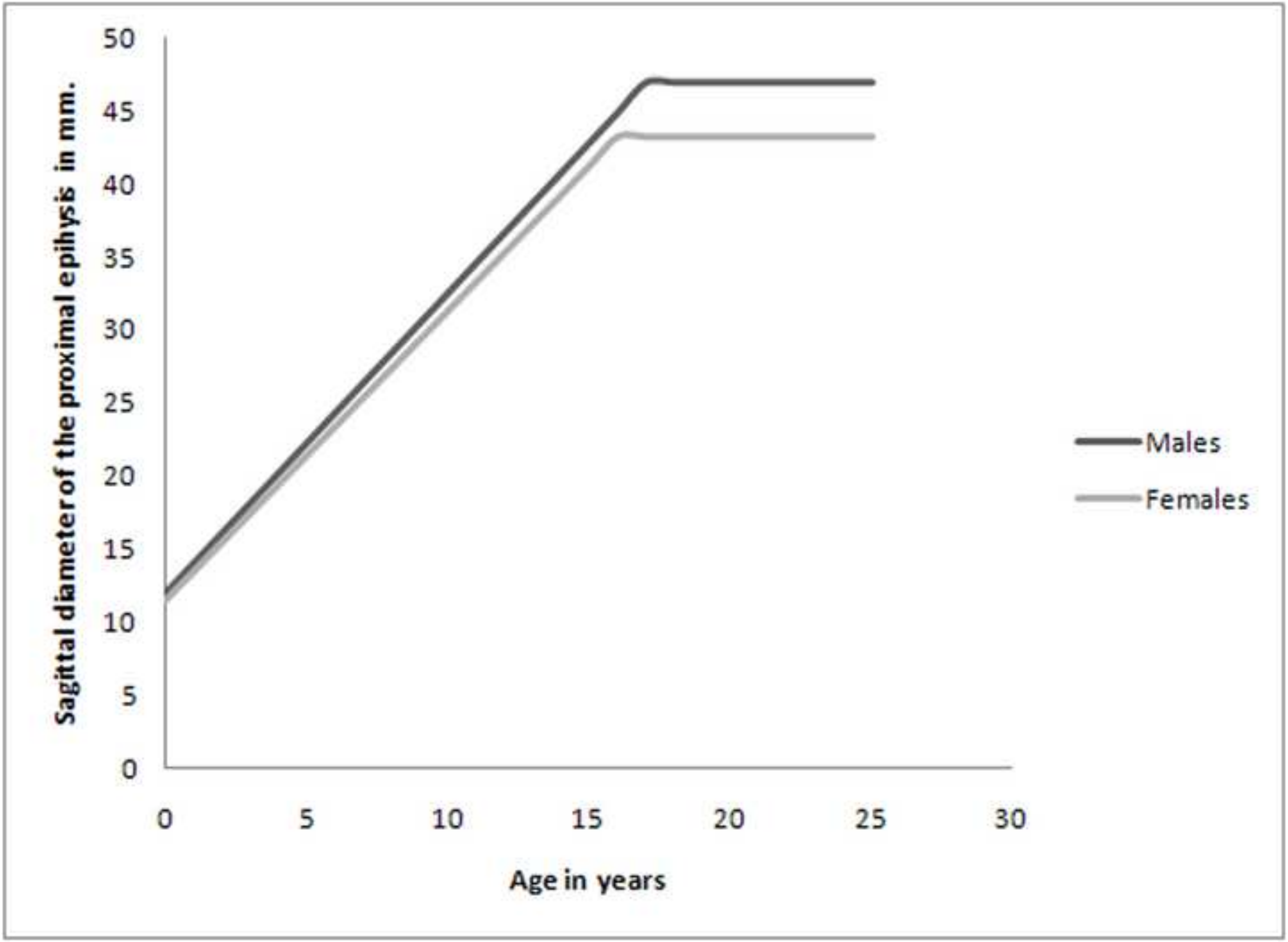

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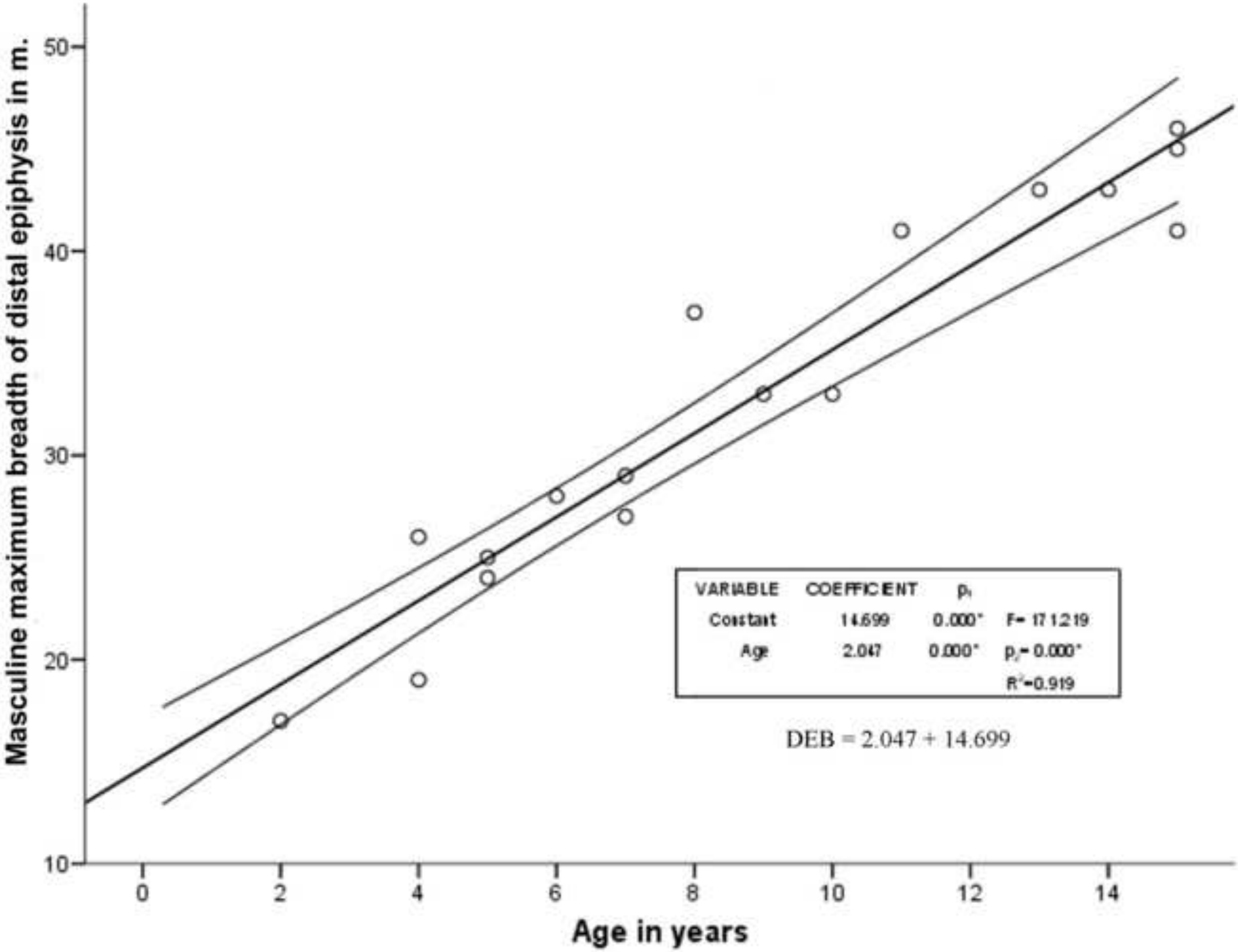

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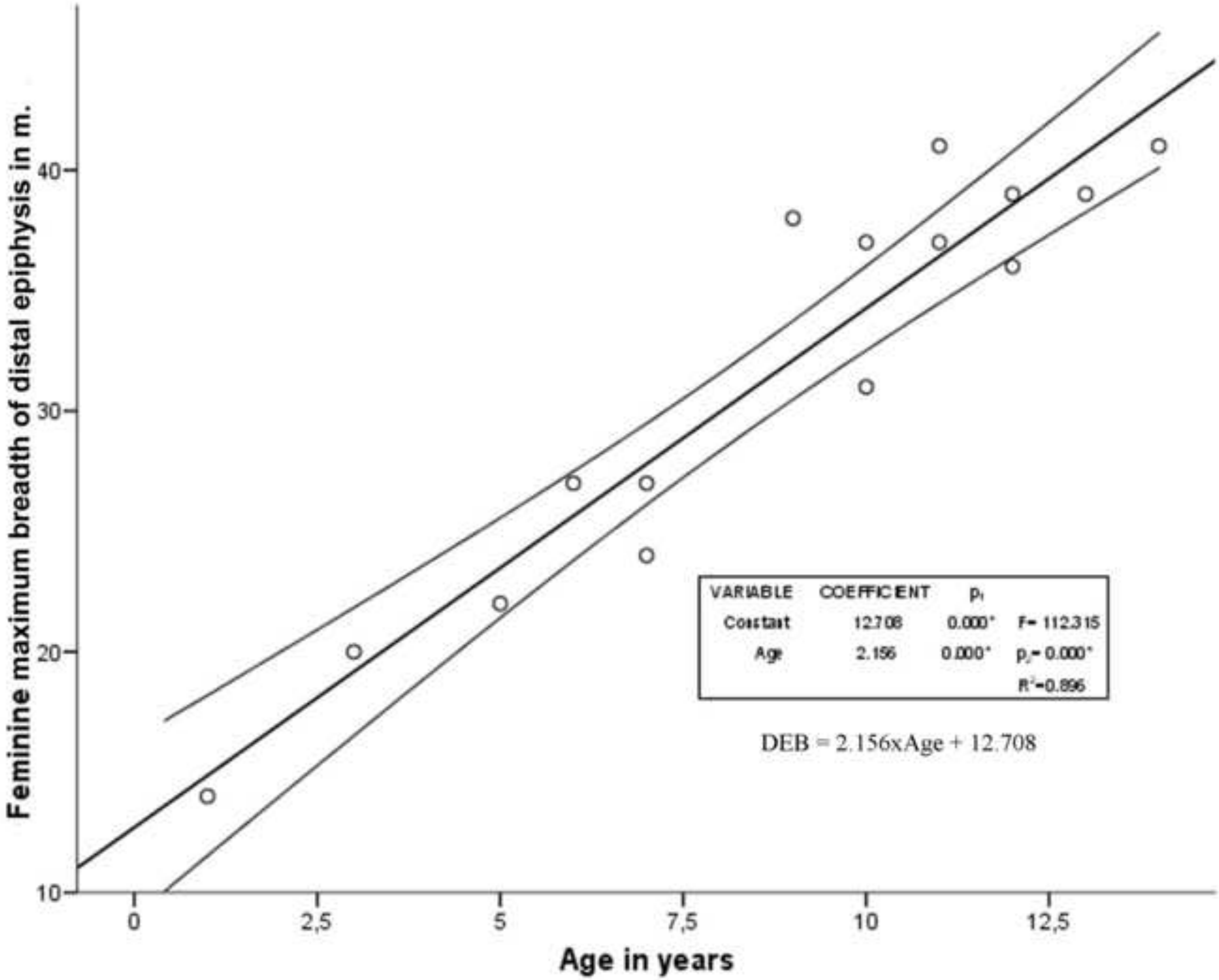

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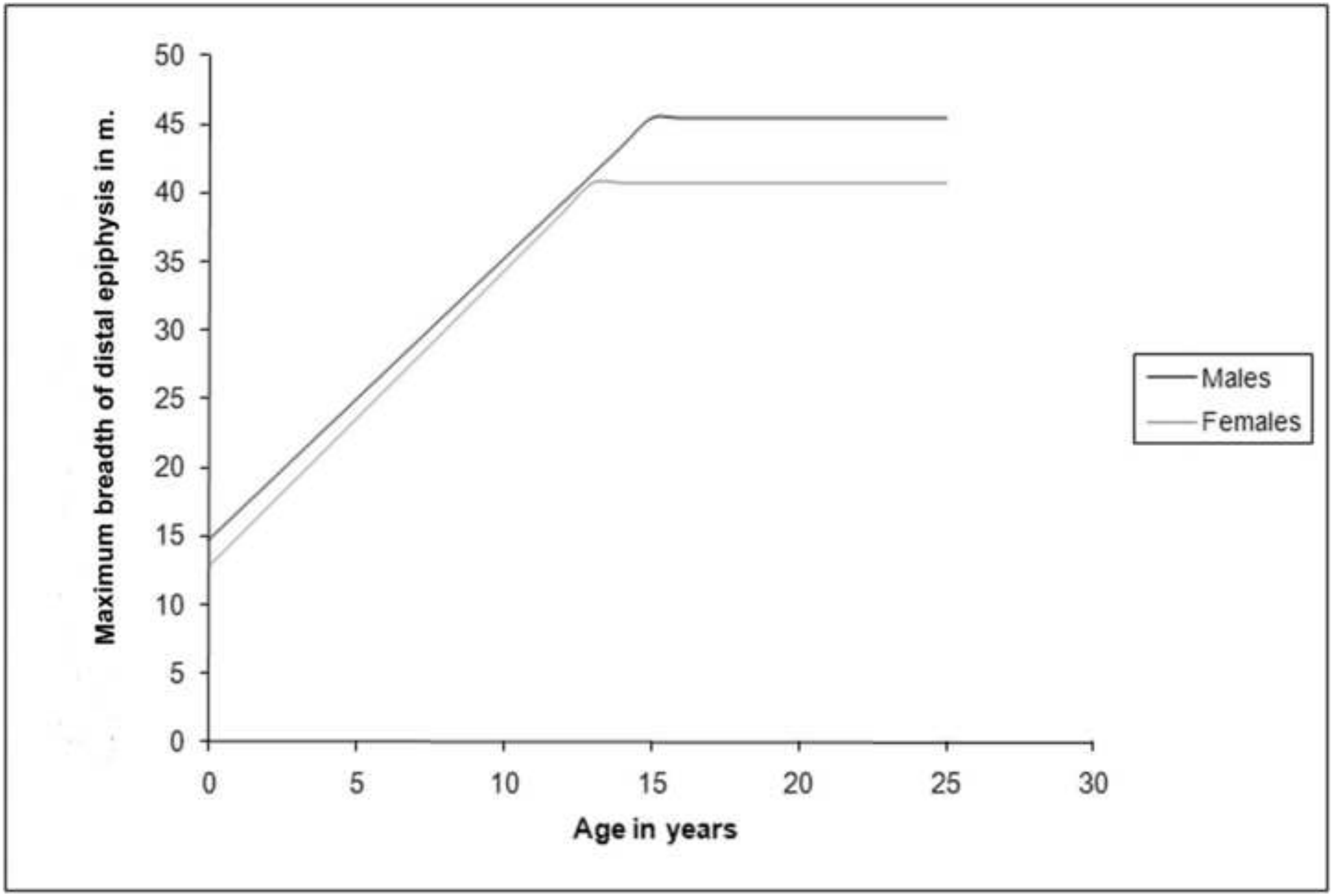

Table

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
Diaphysial length						
♂	n	8	11	6	6	
	Mean	123.13	203.18	261.50	315.67	
	DS	37.84	14,53	28.15	35.72	
	Mean rank	6.00	9.73	12.83	4.83	
♀	n	5	6	13	2	
	Mean	144.60	197.33	239.54	293.00	
	DS	11.19	39.02	33.41	26,87	
	Mean rank	8.60	7.63	8.69	3.50	
	t					
	U	12.000	29.000	22.000	4.000	
	p	0.242	0.421	0.136	0,505	
Diaphysial length + distal epiphysis						
♂	n	4	5	4	9	
	Mean	158.25	204.80	272.75	339.00	
	DS	22.62	14.48	40.56	37.413	
	Mean rank	4.00	6.20	7.00	6.44	
♀	n	2	4	6	2	
	Mean	140.50	182.75	239.83	311.50	
	DS	17.68	20.50	20.37	26.163	
	Mean rank	2.50	3.15	4.50	4.00	
	t					
	U	2.000	4.000	6.000	5.000	
	p	0.355	0.142	0.201	0.346	

TABLE 2

	Error	R^2	Age limit
Males			
Age = 0.067 x diaphyseal length – 6.172	1.777	0.891	Up to 19 years
Age = 0.063 x diaphyseal length plus distal epiphysis – 5.717	1.911	0.878	Up to 19 years
Age = 0.068 x condylo-malleolar length – 2.246	2.013	0.875	Up to 19 years
Age = 0.407 x sagittal Ø of the proximal epiphysis – 3.668	1.397	0.932	Up to 17 years
Age = 0.405 x maximum distal epiphysis breadth – 4.791	1,122	0.908	Up to 15 years
Females			
Age = 0.069 x diaphyseal length – 5.838	1.676	0.831	Up to 17 years
Age = 0.075 x diaphyseal length plus distal epiphysis – 7.325	1.549	0.890	Up to 17 years
Age = 0.063 x condylo-malleolar length – 4.82	1.748	0.822	Up to 17 years
Age = 0.393 x sagittal Ø of the proximal epiphysis – 2.610	1.377	0.882	Up to 17 years
Age = 0.402 x maximum distal epiphysis breadth – 4.032	1,205	0.890	Up to 14 years
Unisex series			
Age = 0.066 x diaphyseal length – 5.656	1.656	0.871	Up to 17 years
Age = 0.062 x diaphyseal length plus distal epiphysis – 5.251	1.706	0.882	Up to 17 years
Age = 0.062 x condylo-malleolar length – 5.806	1.409	0.850	Up to 15 years
Age = 0.409 x sagittal Ø of the proximal epiphysis – 3.462	1.330	0.888	Up to 15 years
Age = 0.396 x maximum distal epiphysis breadth – 4.246	1.193	0.882	Up to 14 years

TABLE 3

Variables	Age				
	0-4	5-9	10-14	15-19	20-25
Condylo-malleolar length					
♂ n	4	5	3	20	37
Mean	168.50	214.60	270.67	352.1	363.14
DS	24.035	15.01	37.02	27.75	21.46
Mean rank	2.50	6.20	6.00		
♀ n	0	4	8	21	38
Mean	-	194.00	274.38	329.76	327.21
DS	-	21.65	48.63	16.24	15.87
Mean rank	-	3.50	6.00		
t				3.164	8.257
U	-	4.000	12.000		
p	-	0.142	1.000	0.003*	0.000*
Sagittal diameter of the proximal epiphysis					
♂ n	7	8	5	18	34
Mean	15.71	25.75	36.60	47.78	48.35
DS	3.50	1.91	6.80	3.83	2.95
Mean rank	5.07	7.94	10.80		
♀ n	4	6	13	21	37
Mean	18.00	25.83	34.77	42.82	41.92
DS	2.58	7.08	5.95	2.48	3.39
Mean rank	7.63	6.92	9.00		
t				4.876	8.500
U	7.500	20.500	26.000		
p	0.217	0.650	0.520	0.000*	0.000*
Maximum distal epiphysis breadth					
♂ n	3	7	4	19	35
Mean	20.67	29.00	40.00	45.05	45.77
DS	4.73	4.58	4.76	2.84	2.21
Mean rank	3.33	7.21	8.50		
♀ n	2	5	8	19	37
Mean	17.00	27.60	37.63	41.26	41.05
DS	4.24	6.19	3.25	2.13	2.41
Mean rank	2.50	5.50	5.50		
t				4.655	8.632
U	2.000	12.500	8.000		
p	0.564	0.413	0.169	0.000*	0.000*

TABLE 4

***Revision Notes**

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Revision of: *Postnatal growth of the tibia. Implications for age and sex estimation*

(FSI –D-10-00666).

16th of March, 2011. Barcelona

Dear Professor Cattaneo,

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. We have: 1) increased the introduction to clearly show the necessity of this type of studies that we present on the tibia; 2) read the statistical literature recommended by the referee and discussed the reason for selecting the statistical methods used in the paper; and 3) discussed the utility and application of the results obtained in the tibia paper within the discussion section. Furthermore we have added a new author, Professor GonzaloTrancho.

Please find enclosed a list of the changes made in the new version of the paper and our comments to the commentaries made by the reviewers.

Yours Sincerely,

Carme Rissech

1- **Pp. 4-5.measurement definitions. Maximum diaphyseal lengths are self explanatory, but in those instances where Martin is cited as the measurement reference, the Martin number should be given. It appears that #3, maximum length of the tibia, is what Martin calls condylar malleolar length. It is not actually a maximum length since the intercondylar eminences are excluded.**

We have changed "*maximum length of the tibia*" to "*condylar-malleolar length*" and in those instances where Martin is cited the number of the measurement of Martin is given. See Material and Methods section.

Figure 1 is a photograph that shows clearly what is measured, but Figure 2 is a drawing that doesn't look especially like the distal end of a tibia. Why not provide a photograph of the distal breadth measurement?

We have changed the drawing of Figure 2 to a photograph showing the distal breadth measurement.

2- **P. 5, rather than use age 19 as the cut point separating the still growing group from the adult group, why not use fusion of both epiphyses? If both are fused the tibia is no longer growing, regardless of whether the individual is < 19. Consequently the young group will contain some individuals that have stopped growing.**

We have changed the cut point for separating the still growing group from the adult group from "*19 years of age*" to "*the age of fusion of both epiphyses*". See the second paragraph in Statistical Analysis of Material and Methods section.

3- **It isn't clear to me exactly what the purpose of the paper is. In the introduction the importance of estimating sex and age is mentioned in connection with reconstructing demographic profiles of earlier populations, and in identifying forensic remains. Since the paper was submitted to FSI, the latter goal is presumably what the author is aiming at. However, I question whether these data are appropriate for modern forensic cases. The samples are 18th, 19th or early 20th century.**

The main purpose of this study is to analyse the growth of the tibia, with the intention of describing the changes that accompany postnatal ontogeny in this bone and develop algorithms that facilitate accurate evaluation of sub-adult age at death principally of modern Western European skeletal remains, specifically from Iberian Peninsula. We understand as modern individuals, the individuals who lived from the later 19^{th} century to the present. For this reason at the end of the introduction we have introduced our main purpose "*The main purpose of this study is to analyse the growth of the tibia, with the intention of describing the changes that accompany postnatal ontogeny in this bone and develop algorithms that facilitate accurate evaluation of sub-adult age at death of Western European skeletal remains, specifically from Iberian Peninsula*"

The first submitted manuscript of the tibia said that "*the individuals from Lisbon and Coimbra collection belonging from the 19^{th} and early 20^{th} centuries*". But **it was an error**. **The paper should say that *"All the individuals were born from the late 19^{th} century until the end of the 20^{th} century"***. We have changed it. See the Material and Methods section.

It is true that some of the individuals from St. Bride collection are from 18th century; however the vast majority of the individuals of the sample are from the 20th century and they form the vast majority of the individuals analyzed in the tibia paper. You can see it in our previous papers about growth published in FSI (Rissech et al., 2003, 2008, Rissech and Malgosa, 2006, 2008) with the same sample. However, if you have any more doubts about the dating of the collections you can see the papers specifically published for the demographic, socio-economic and temporal contextualisation of the collections (Scheuer and Black, 1995; Rocha, 1995; Cardoso, 2006).

Apart from the correction of these errors of dating about the collections, in the discussion section we discuss about the suitability of the analyzed sample as a modern sample and its applicability in sub-adult age estimation. See the discussion section.

The mean tibia length of adults in the present sample is about 30 mm shorter than modern Americans. Comparing the present results for diaphyseal length to Hoffman's analysis of modern Americans from the Denver growth study (not cited in the present paper, see ref. below), shows that these mostly pre-20th century Europeans are growing almost 2 standard deviations below the Americans. Modern Europeans are as tall, if not taller, than Americans, so the equations in Table 3 will overestimate age by around 2 years in modern populations.

The Hoffman's reference has been included in the study.

As we show before the individuals of this study are not pre-20th century.
It is true that in recent years European populations have increased their stature due the improvement of life conditions. However, the different populations of Europe do not have 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). It is also well known between European Anthropologists that the method for calculating adult stature based on U.S. reference samples fails in the estimation of living height in Spain and Italy. In Spain and Italy, the formulae proposed by Pearson (Pearson, 1899) at the end of 19th century 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 GIeser systematically overestimate stature, both in female and male skeletons of Spanish and Italian origin (Formicola, 1993; Formicola and Franceschi, 1996; Lalueza-Fox, 1998).

All of this is corroborated by the homogeneity observed in maximum length of the adult femur between the three series of the tibia paper and the Spanish documented and contextualised modern skeletal collection of the Universitat Autònoma de Barcelona (UAB) (Rissech and Sheafer, 2008). UAB collection is constituted exclusively by modern individuals from the 20th XX century from the industrial city of Granollers, which is an industrial, commercial and trade city, situated 25 km north-east of Barcelona (Rissech and Steadman, 2010); and femur is one of the bones which closest correlate with stature (Formicola and Franceschi, 1996). This indicates the similarity in stature between the modern Spanish series and the three series of the tibia paper.

All of these points have been explained extensively in the discussion section of the paper. Please see it.

4- **Even if we accept there are appropriate uses, the regression equations in Table 3 are not useful because they contain no error term so one doesn't know what the +/- around the estimate might be. Furthermore, assumptions of linear regression are violated because the variance of tibia length increases with age. That is readily apparent from the graphs of mean and standard deviations to be seen in the Hoffman paper already mentioned. The authors should consult Konigsberg et al. (Ref below), which discusses various regression approaches in forensic anthropology. Although the paper is mainly concerned with stature, the general principals apply in the present situation.**

We have read the paper of Konigsberg et al., and selected the best regression approach for our purposes, which is Inverse Regression, the analyzed variables as independent variable and the age as dependent variable. We have also taken into account the error of the estimation. See the Material and Method section and table 3.

5- **The main thing the author should do in the general sense is present a clear purpose for this paper, discuss limitations of the sample, and where and how the results can be used. I also found the paper somewhat repetitious. That should be eliminated.**

We have increased the introduction in order to show with clarity the necessity of the present study. At the bottom of the Introduction section we presented a clear purpose for this paper. See Introduction section.

We have discussed the limitations of the sample and where and how the results can be used. See Discussion section.

We have removed some sentences which were repetitious.

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