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Title Testing times: identifying puberty in an identified skeletal sample

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Abstract

Background: Identifying the onset of puberty in skeletal remains can provide evidence of social changes associated with the onset of adulthood.

Aim: This paper presents the first test of a skeletal method for identifying stages of development associated with the onset of puberty in a skeletal sample of known age and cause of death.

Materials and Methods: Skeletal methods for assessing skeletal development associated with changes associated with puberty were recorded in the identified skeletal collection in Coimbra, Portugal. Historical data on the onset of menarche in this country are used to test the method.

Results: As expected, females mature faster than their male counterparts. There is some side asymmetry in development. Menarche was found to have been achieved by an average age of 15.

Conclusions: Asymmetry must be taken into account when dealing with partially preserved skeletons. Age of menarche is consistent, although marginally higher, than the age expected based on historical data for this time and location. Skeletal development in males could not be tested against historical data, due to the lack of counterpart historical data. The ill health known to be present in this prematurely deceased population may have delayed skeletal development and the onset of puberty.

Keywords

menarche, skeletal development, Portugal

Introduction

Puberty, and development leading up to it, is an important socio-cultural as well as biological period for the human body with the visible and audible changes defining a change from the child to the adult world. Importantly society itself impacts on the timing of these changes, with lower socio-economic status and those growing up in rural environments achieving menarche later than those of higher socio-economic status or those in urban environments (Henneberg and Louw, 1995; Jenicek and Demirjian, 1974; Łaska-Mierzejewska et al., 1982; Padez, 2003, 2007; Qamra et al., 1991). Secular changes have also occurred, which may in part be related to improving living conditions and better nutrition, with a consequent reduction in age of menarche from an average of 15 years in the early and mid-twentieth century to 11.6 years in modern Portugal (Reis et al., 2016). Reduction in age of menarche increases later life chances of certain diseases, including cardiovascular disease, which are significant health problems in an increasingly elderly population (Qiu et al., 2013). It is therefore important to characterise past patterns of skeletal development associated with the onset of puberty to interpret current trends, particularly in the onset of menarche.

Skeletal changes associated with puberty follow general trends in growth. Growth accelerates until it reaches peak height velocity, the point at which growth increases most by time period (Tanner and Whitehouse 1976). After this growth slows again although it continues until long bone epiphyses fuse at which point height no longer increases. Long and short bone epiphyses fuse at different times generally following the same sequence in all normally developing individuals and from this, as well as tooth and flat bone growth, stages of development associated with puberty have been created (Shapland and Lewis, 2013, 2014). During this adolescent growth spurt pubic hair develops and increases in extent, testes volume and penis size increase in males and breasts develop in females. Development of male genitalia occurs approximately 6 months after female breasts begin to develop and are almost or completely adult at the point of peak height velocity (Marshall and Tanner 1970). Peak height velocity in females occurs about 1.3 years prior to menarche, with breasts developing from age 9 and continuing into the late teens (Marshall and Tanner 1969).

Peak height velocity for British boys in the mid-twentieth century occurred at a mean age of 14.1 years and for girls at 12.1 years, 1.1 years prior to menarche (Tanner et al., 1966). In the US at the same period peak height velocity was at 14.0 for boys and 11.8 for girls (Frisch and Revelle 1969). However, there is limited data from which to study longitudinal secular changes (Hauspie et al., 1997). Rather secular changes have been predominantly studied using changes in age of menarche (ibid.). Current research has not only demonstrated a decreasing trend in the age of menarche, it has also highlighted factors which affect the age of onset (Padez, 2003, 2007; Reis et al., 2016). Genetic factors are a key factor in age of onset. While socio-economic status and education are factors, these are not so important in modern industrial societies as birth order and the environment, e.g. urban versus rural living (Padez, 2003; Padez and Rocha, 2009). Obesity is also an important factor with increased body mass index being associated with earlier menarche (Biro et al., 2012). Behaviour is also known to be affected by onset of menarche. Age of first sexual intercourse is lower for those with earlier menarche (Reis et al., 2016), this could be an important factor in the past where the lack of contraceptives may have led to more young pregnancies. Such early pregnancies have been found, in modern societies, to lead to more preterm births (Jolly et al., 2000) which may have been less likely. In modern (2010) Portugal, girls experiencing early menarche were found to engage in riskier behaviours, including fights, than their later developing counterparts (Reis et al., 2016).

Recent work on skeletal remains from medieval and Roman Britain has characterised the timings of the initiation of the growth spurt, its acceleration, deceleration and end (Shapland and Lewis, 2013; Arthur et al., 2016; Lewis et al., 2016). In males only the deceleration phase seems to be widely affected by the urban setting, while in females it is apparent in all but the initiation of the growth spurt. In boys peak height velocity was found to have occurred by age 15 in a rural setting, but

increased to 18 in an urban setting, while in girls it occurred on average age 14 in the rural setting but was the same as males in the urban setting (Shapland and Lewis, 2013; Arthur et al., 2016; Lewis et al., 2016). These ages were based on dental development and not the chronological age of the individuals. An increase in age for the stages was also found in those who had evidence for nutritional deficiencies and other chronic pathological conditions (Lewis et al., 2016). No comparable data have been published for other periods or countries.

The aims of this paper are twofold: firstly, to describe for the first time, the relationship between skeletal indicators of puberty and age in a known age-at-death human skeletal sample. Secondly, to characterise puberty in the early twentieth century in Portugal in terms of contemporaneous data.

Material and Methods

All male and female skeletons under the age of 21 curated in the Coimbra identified skeletal collection were studied. This collection includes skeletal remains as well as documentary evidence on age-at-death, gender, occupation and cause of death for individuals dying in the early twentieth century (Rocha, 1995).

Skeletal development (for brevity “puberty stages”) and onset of puberty were identified using standard methods (Shapland and Lewis, 2013, 2014). Four puberty stages were defined, rather than the normal five due to the small sample size and to reduce mis-categorisation. Stages included were initiation, acceleration and end of growth spurt, while peak height velocity (PHV) and deceleration were combined into “after PHV” as there are fewer indicators available to differentiate these two stages (Shapland and Lewis, 2013). Puberty stage for each side was taken from the highest stage reached from all the observations for each side, unless more than four observations were unobservable or the mandibular canine or hook of hamate could not be observed and the remaining bones were unfused (Table 1). Stages were combined first into a stage of puberty for the left and right sides of the skeleton, based on the highest stage of attainment. Subsequently, these were pooled into an overall attainment score (for which cervical vertebrae development was also included), by using the maximum stage of attainment.

Menarche occurs during the deceleration phase of the growth spurt and is correlated with the commencement of fusion of the distal phalangeal epiphysis and ossification of the iliac crest (Shapland and Lewis, 2013). Therefore, in this study, menarche will have commenced by the time that the distal phalangeal epiphyses are fused and the iliac crest is fused to the ilium and age at commencement will be overestimated. For this reason this study will subtract one year from the mean age of distal phalangeal epiphysis fusion which occurs at least one year after PHV (Shapland and Lewis, 2013).

All statistical analysis was undertaken in R version 3.1.2. Boxplots were drawn for each variable by side to study medians and detect outliers. Hedge's *g* was used to calculate effect size, as this takes into account sample size. It should be noted, however, that this is an approximation of differences, because these variables are not normally distributed. Package *psych* version 1.5.1 was used to calculate descriptive statistics by groups (Revelle, 2015), while *compute.es* version 0.2-4 was used to calculate the effect size, Hedge's *g*, for comparison of asymmetry between left and right sides, as well as sexual differences, and for ease of interpretation the common language effect size (CLES) is also provided which indicates the probability that a score from one group will be higher than the other (del Rey, 2014). Statistical analysis was performed on each bone change based on overall puberty attainment to provide meaningful mean ages: otherwise means of stages which are completed early are biased by the older individuals. This also highlights those features (or sides) which were at a lower puberty stage than the skeletal maximum stage.

Results

For the 56 individuals included in this study, a combined puberty score could not be ascertained for one individual whose mandibular canines and hamates were unobservable (a key developmental feature for the first three stages of puberty) and whose remaining observable features were all unfused. This female was seven years old and, because she had no overall puberty score, she has been removed from further analysis.

Table 1 provides the descriptive statistics for each puberty stage by side, for the cervical vertebrae and for maximal attainment. Eleven individuals were represented in the first puberty stage divided approximately in half between females ($n=5$) and males ($n=6$). For the majority in this stage the only indicator available was the cervical vertebrae and five of these individuals remained in the prepubertal growth spurt, based on the hook of hamate development with only one individual, a sixteen year old male exhibiting signs of the start of the growth spurt in this bone. This individual's other bony changes indicated that he remained in the first stages of pubertal development at the time of his death. With this individual removed the mean age for the maximal puberty score for males in the initiation phase is 9 ($n=5$, $sd=1.58$). It should be noted that the individual side puberty scores do not include the cervical vertebrae which has the initiation phase as its lowest category and thus underestimating the age of individuals in this category.

Those in the deceleration phase of cervical vertebrae development are between the ages of 14 and 20, while fusion of the distal hand phalanges has occurred in one 12 year old and one 14 year old. This indicates that menarche may be occurring in girls as young as 12. The mean age for those in the deceleration phase is, however, 17.86, while for those with fused distal phalanges it is 15.3. This is also the mean age of the after PHV category: the period during which menarche occurs.

Asymmetry

There was very little asymmetry in the mean ages for each bony developmental change in either sex. For females the largest difference was in the "after PHV" phase for the intermediate hand phalanges some of which had yet to fuse at death (but for which other indicators had progressed to the "after PHV phase) and some of which had. Table 1 presents the differences between left and right sides for both of these stages neither of which were large (unfused difference $g=0.34$, 95% CI [-1.33, 20.1], CLES=59.5%, fused $g=-0.27$, 95% CI [-.24, 1.86], CLES=42.48%). For males there are more differences, but again these are small. The largest difference is for the distal hand phalanx fusion feature ($g=0.41$, 95% CI [-0.86, 1.68], CLES=61.4%). It must be noted that a sixteen year old male has no epiphyses in the hand phalanges, distal radius or iliac crests which are fused and is therefore skewing the age of the initiation sample upwards. Without this individual the mean age for all features is 9 ($n=5$, $SD = 1.58$), except for the left and right distal radius and left proximal hand phalanges (mean= 9.25, $n=4$, $SD = 1.71$) all of which are missing in the same individual.

Sexual differences

Sex differences are readily apparent for puberty stage on the left side for the final two phases, but no large differences are seen on the right (Table 1). There is a large difference for the acceleration phase of the cervical vertebrae development, but for no other phase. For the maximal puberty attainment the results show a higher mean age for every puberty stage in males, compared to females (Fig. 1). Age ranges overlap for females for the initiation and acceleration stages, as well as for the after PHV and end of growth spurt phases. For males there is only an overlap for the latter stages. Notably male and female age ranges overlap for the final two phases. Nevertheless there are

statistically significant differences for the mean values for all except the first stage of puberty (Table 1). If the sixteen year old male outlier from the initiation phase is removed the differences between males and females for this stage are reduced for the majority of variables (total $n = 9$, $g=0.27$, 95% CI [-1.15, 1.69], CLES=57.6%), but the average age of the males remains higher than that of females (mean=9 and mean=8.5, respectively).

Discussion

The aims of this paper were to test a method for identifying puberty based on skeletal development on a known age sample and to contextualise these results based on contemporaneous data. Contemporaneous data have little to say about the ages at which males develop, but there are data on the average age of menarche in girls which is at 15 (Padez, 2003), based on the average age of the after PHV category, 15, these skeletal results concur with the historical evidence. The lack of historical data on puberty in males demonstrates the value of using skeletal analysis on historical samples to plug gaps in our understanding of the past.

Side asymmetry in age is present in some of the puberty stages, but is typically low and never statistically significant. Differences are predominantly caused by the small sample sizes, but there are numerous instances of bony features being a stage of development behind on one side compared to the other and this is most apparent in the later stages where features which should be fused are not. This is also clearly apparent in the sample sizes for the after PHV category for left and right sides compared to overall attainment: many of those who are in the after PHV category on the right side have already completed their growth spurt according to features on the left side and vice versa. This demonstrates a clear problem with this methodology: if elements are missing puberty is likely to be miscategorised particularly in the later stages.

As expected, based on archaeological evidence, this study shows that in this sample females begin and complete puberty earlier than males. In previous skeletal studies on samples from mediaeval Britain, the average age of males in the initiation phase is 11 at all sites as are females. For males this is comparable to this study's results, but the Coimbra females are considerably younger based on the overall phase average although the data conforms better with the left and right side averages (which do not include cervical vertebral development which appears to include those in the pre-pubertal growth spurt). In contrast the average age of acceleration is higher in this study's males than in mediaeval Britain, whereas for females both provide similar results. The difference between males between these studies is most likely caused by the small sample size which has also made it impossible to remove any potential outlier from that group. For the later stages males seem closer to their urban male counterparts of London than the rural or semi-rural northerners, based on average ages for after PHV and end of growth spurt. The average ages for the females, in contrast, falls between the rural and semi-rural sites of Barton-on-Humber and York (Lewis et al., 2016). Sample sizes are a clear issue for this study, but it is possible that males are more affected by the urban environment, because they have longer during development for it to disturb their biological system, than females.

Historical data on skeletal development to contextualise this Portuguese data are lacking. British data from 1965 – 70 indicate that boys reached peak height velocity on average at 14.5 (Tanner and Whitehouse 1976). In this study acceleration was found to be occurring on average aged 15.7 and ended with an average age of 18.4, indicating that boys had delayed maturity compared to British males living approximately half a century later. Historical and current data on age of menarche in Portugal are available (Fig. 2). The skeletal evidence is unable to pinpoint the exact age of menarche, but indicates the age by which it has occurred thus over-estimating that actual age of menarche. In this study menarche was found to have occurred, on average, by 15.3 years of age.

The individuals in this study were born in the period between 1892 and 1922 and died between 1910 and 1933 and, assuming they attained menarche at 15, provides menarche data for the period 1907 to 1931 (average 1920). Historical evidence indicates that menarche occurred at the average age of 15 in the 1890s and by 1910 had decreased to 14.7 (Padez 2003). By the 1940s this had decreased to 13.4 years and has subsequently continued in this trend to the current average age of 11.9 (Padez 2003, Reis et al., 2016). Average age of menarche does not provide the complete picture as it occurs at varying ages for many reasons (Reis et al., 2016). The youngest girl in this study to begin menarche was 12, similar to the modern average age.

Factors which influence skeletal development and the age of menarche are many, with nutrition and socio-economic status both playing a major role (Lewis et al. 2016; Padez 2003; Reis et al., 2016). Previous attempts at defining socio-economic status in this skeletal sample have been problematic, with occupation primarily used as an indicator (Alves Cardoso et al., 2015). Parental socio-economic status cannot be ascertained from the available data and neither can nutritional status. Ill health, in contrast, can be tentatively identified based on cause of death; this provides only a short window on the course of sickness at the time before death. However, previous studies have indicated that disease does slow development of the indicators used in this study (Lewis et al., 2016). A large proportion of the individuals in this study had a chronic disease, typically tuberculosis ($n=17$, female $n=8$, male $n=9$). However, this sample size is too small to consider its impact on skeletal development and age of menarche. Yet these results may explain some of the disparity between this study and the archaeological results from the UK.

Conclusions

This study of early twentieth century Portuguese skeletons aimed, for the first time, to test a method for identifying stages of development leading up to and following puberty in a known age-at-death skeletal population and to determine whether the results correspond to contemporaneous documentary data. The results of this study show that the method for identifying developmental stages is consistent with expectations from previously published results as well as consistent with age at menarche in contemporaneous records. Importantly it highlights asymmetrical differences in stages which must be considered when missing elements are present in the archaeological record. Future work testing the method radiographically (where possible) *in vivo* would provide better data on how these stages relate to Tanner stages and the onset of puberty in boys, for which contemporaneous Portuguese data were lacking.

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Tables

Table 1. Descriptive statistics for combined puberty stages, n =sample size, sd =standard deviation, g = Hedge's g , CLES = common language effect size, CI=confidence interval (95%). Italics indicates CLES over 80%, bold indicates a p value for Hedge's g of 0.05 or less.

Table 2. Descriptive statistics and effect sizes comparing male and female ages for stages of attainment (bony features with no indicators observable). n =sample size, sd =standard deviation, CLES = common language effect size, g = Hedge's g , CI=confidence interval (95%)Italics indicates CLES over 80%, bold indicates a p value for Hedge's g of 0.05 or less.

Figure 1. Maximal puberty attainment by age (in years) with trimmed means (removing 16 year old male outlier). Males and females compared (CLES).

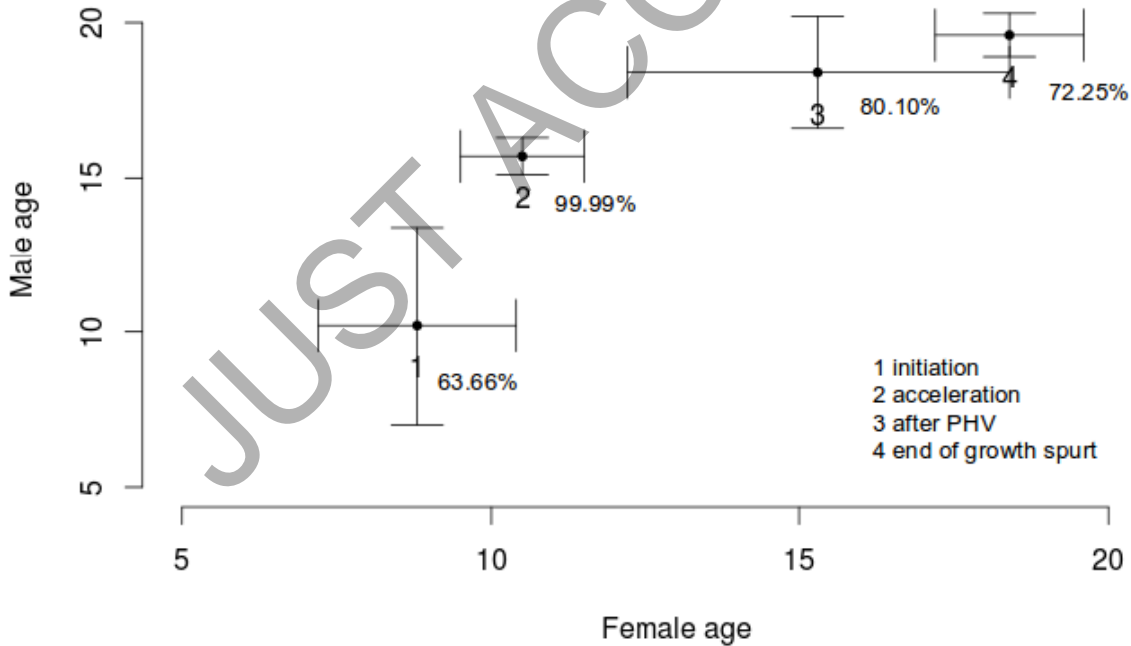
Figure 2. Age (in years) of menarche in Portugal (Padez 2003, 2007; Reis et al. 2016). This study is plotted on the mean year of death for the females of 1923.

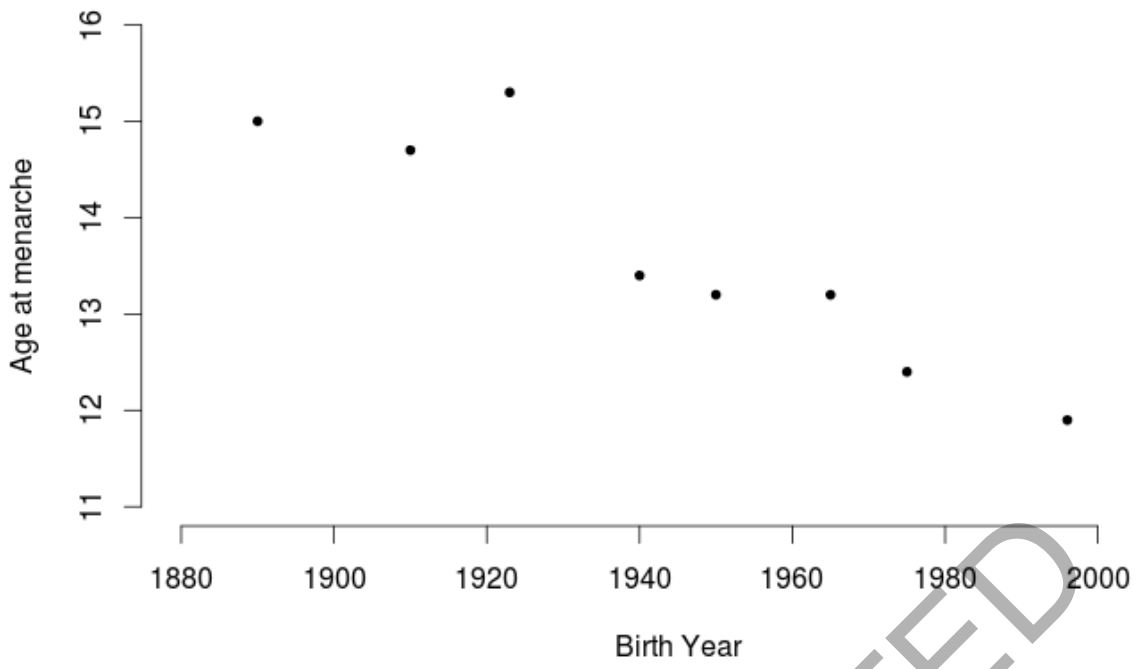
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Puberty stage	vars	n	Male			Female			Age by sex comparison						
			mean	median	sd	n	mean	median	sd	g	CI: lower	CI: upper	CLES	p	
Left	Initiation	4	12.8	13	3.30	6	10.3	10	-0.44	1.03	1.00	-0.44	2.44	76.10	0.15
	acceleration	1	16.0	16	NA	1	11.0	11	NA	NA	NA	NA	NA	NA	NA
	after PHV	10	18.5	19	1.65	7	18.6	17	2.88	1.25	0.15	2.35	81.16	0.03	
	end of growth spurt	8	19.6	20	0.52	10	18.6	19	1.17	1.03	0.01	2.06	76.69	0.05	
Right	Initiation	4	12.5	12.5	3.51	4	9.5	10	1.91	0.92	-0.68	2.53	74.31	0.21	
	acceleration	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	after PHV	8	18.4	19	1.77	11	18.6	17	2.73	0.70	-0.27	1.66	68.91	0.15	
	end of growth spurt	10	19.5	20	0.71	5	18.6	19	1.52	0.82	-0.34	1.98	71.96	0.15	
Cervical vertebrae (only)	Initiation	5	9.0	9	1.58	5	8.8	10	1.64	0.11	-1.21	1.43	53.16	0.65	
	acceleration	2	15.5	15.5	0.71	7	10.7	11	1.11	4.01	1.21	6.81	99.77	0.01	
	after PHV	6	19.3	19.5	0.82	7	17.9	19	2.04	0.85	-0.34	2.05	72.66	0.15	
	end of growth spurt	3	18.3	19	1.83	7	17.3	18	2.50	0.45	-0.96	1.46	62.52	0.36	
Maximal puberty stage	Initiation	6	10.2	9.5	3.19	5	8.8	10	1.64	0.48	-0.79	1.75	63.25	0.42	
	acceleration	3	15.7	16	0.58	6	10.5	10.5	1.05	4.89	1.79	7.99	99.97	0.01	
	after PHV	8	18.4	19	1.77	6	15.3	15.5	3.08	1.19	-0.02	2.39	79.95	0.05	
	end of growth spurt	10	19.5	20	0.71	11	18.5	19	1.21	1.00	0.07	1.94	76.10	0.04	

Puberty Stage	Bone	Stage of bone	Left			Right			Left			Right			Age by sex comparison										
			N	Mean	Sd	N	Mean	Sd	N	Mean	Sd	N	Mean	Sd	g	CI: lower	CI: upper	CLES	p						
Initiation	Hamate Hook	Prepubertal growth spurt	2	10	1.41	2	9.5	0.71	1	10.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA					
	Distal hand phalanges	Unfused	5	9.0	1.6	6	10.2	3.2	4	8.5	1.7	4	8.5	1.7	0.27	-1.15	1.69	57.57	0.67	0.55	-0.82	1.93	65.17	0.38	
	Intermediate hand phalanges	Unfused	6	10.2	3.2	6	10.2	3.2	4	8.5	1.7	4	8.5	1.7	0.55	-0.82	1.93	65.17	0.38	0.55	-0.82	1.93	65.17	0.38	
	Proximal hand phalanges	Unfused	6	10.2	3.2	5	10.6	3.4	4	8.5	1.7	4	8.5	1.7	0.55	-0.82	1.93	65.17	0.38	0.67	-0.79	2.13	68.25	0.31	
Acceleration	Distal radius	Unfused	5	10.6	3.4	5	10.6	3.4	4	8.5	1.7	4	8.5	1.7	0.67	-0.79	2.13	68.25	0.31	0.67	-0.79	2.13	68.25	0.31	
	Ilac crest	Unfused	6	10.2	3.2	6	10.2	3.2	4	8.5	1.7	4	8.5	1.7	0.55	-0.82	1.93	65.17	0.38	0.55	-0.82	1.93	65.17	0.38	
	Hamate Hook	Growth spurt starting	1	15.0	NA	1	15.0	NA	4	10.8	1.0	2	10.0	1.4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Distal hand phalanges	Unfused	3	15.7	0.6	2	15.5	0.7	6	10.5	1.1	6	10.5	1.1	4.89	1.79	7.99	99.97	0.01	4.34	1.17	7.52	99.89	0.02	
After PHV	Intermediate hand phalanges	Unfused	2	15.5	0.7	2	15.5	0.7	6	10.5	1.1	6	10.5	1.1	4.34	1.17	7.52	99.89	0.02	4.34	1.17	7.52	99.89	0.02	
	Proximal hand phalanges	Unfused	2	15.5	0.7	3	15.7	0.6	6	10.5	1.1	5	10.6	1.1	4.34	1.17	7.52	99.89	0.02	4.46	1.32	7.59	99.92	0.01	
	Distal radius	Unfused	3	15.7	0.6	3	15.7	0.6	6	10.5	1.1	6	10.5	1.1	4.89	1.79	7.99	99.97	0.01	4.89	1.79	7.99	99.97	0.01	
	Ilac crest	Unfused	3	15.7	0.6	3	15.7	0.6	6	10.5	1.1	6	10.5	1.1	4.89	1.79	7.99	99.97	0.01	4.89	1.79	7.99	99.97	0.01	
End	Hamate Hook	Acceleration	2	17.5	2.1	2	17.5	2.1	1	17.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Distal hand phalanges	After PHV	7	18.6	1.8	7	18.7	1.6	5	16.0	2.9	4	15.3	2.8	1.02	-0.27	2.31	76.53	0.11	1.54	0.04	3.03	86.17	0.04	
	Intermediate hand phalanges	Unfused	3	18.3	2.1	3	18.3	2.1	4	15.0	3.6	3	13.7	2.9	0.92	-0.85	2.69	76.18	0.24	1.48	-0.69	3.65	85.24	0.13	
	Proximal hand phalanges	After PHV	5	18.4	1.8	5	18.4	1.8	2	16.0	2.8	3	17.0	2.7	0.98	-0.95	2.91	75.59	0.25	0.97	-1.02	2.16	85.67	0.41	
End	Distal radius	Unfused	3	18.3	2.1	3	18.3	2.1	2	12.0	0.0	2	12.0	0.0	2.71	-0.74	6.16	97.24	0.09	2.71	-0.74	6.16	97.24	0.09	
	Proximal hand phalanges	After PHV	5	18.4	1.8	5	18.4	1.8	3	16.3	2.1	3	16.3	2.1	0.94	-0.72	2.6	74.73	0.21	0.94	-0.72	2.6	74.73	0.21	
	Distal radius	Unfused	6	18.0	1.9	6	18.0	1.9	6	15.3	3.1	5	15.6	3.4	0.96	-0.3	2.23	75.21	0.12	0.83	-0.49	2.14	72.09	0.19	
	Ilac crest	Unfused	4	18.9	1.9	4	19.0	2.0	6	15.3	3.1	6	15.3	3.1	1.15	-0.32	2.61	78.11	0.11	1.22	-0.27	2.7	80.51	0.1	
End	Ilac crest	After PHV	4	18.0	1.8	4	17.8	1.5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Distal radius	End	7	19.6	0.5	8	19.5	0.8	10	18.6	1.2	10	18.6	1.2	0.95	-0.1	2.01	74.98	0.07	0.85	-0.16	1.85	72.55	0.09	
End	Ilac crest	End	8	19.6	0.5	10	19.5	0.7	5	18.2	1.1	5	18.6	1.5	1.69	0.31	3.06	88.37	0.02	0.82	-0.34	1.98	71.96	0.15	

Puberty stage by age and sex (trimmed means, 1 sd error bars)





JUST ACCEPTED