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Age Estimation From Stages of Epiphyseal Union in the Presacral Vertebrae

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ABSTRACT The presacral vertebrae have various secondary centers of ossification, whose timing of fusion can be used for age estimation of human skeletal remains up to the middle to the latter third decade. However, detailed information about the age at which these secondary centers of ossification fuse has been lacking. In this study, the timing of epiphyseal union in presacral vertebrae was studied in a sample of modern Portuguese skeletons (57 females and 47 males) between the ages of 9 and 30, taken from the Lisbon documented skeletal collection. A detailed photographic record of these epiphyses and the age ranges for the different stages of epiphyseal union are provided. Partial union of epiphyses was observed from 11 to 27 years of age. In general, centers of ossification begin to fuse first in the cervical and lumbar vertebrae, followed by centers of ossification in the thoracic region. The first center of ossification to complete fusion is usually that of the mammillary process in lumbar vertebrae. This is usually followed by that of the transverse process, spinous transverse process, and annular ring, regardless of vertebra type. There were no statistically significant sex differences in timing of fusion, but there was a trend toward early maturation in females for some vertebra or epiphyses. Bilateral epiphyses did not show statistically significant differences in timing of fusion. This study offers information on timing of fusion of diverse epiphyseal locations useful for age estimation of complete or fragmented human skeletal remains. Am J Phys Anthropol 000:000–000, 2010. ©2010 Wiley-Liss, Inc.

This study documents the age variation in the timing of fusion of the secondary centers of ossification in the cervical, thoracic, and lumbar vertebrae and provides comparative data and readily available information to aid in the age estimation of adolescent and young adult skeletons. We were motivated by the relative paucity of detailed information concerning the age at which the various epiphyses of the spine fuse, as well as by the opportunity to use a relatively large collection of fully identified human skeletons of the relevant age range. Beyond the study of other age indicators from the presacral spine, such as the morphometrics of the fetal vertebra (Kósa and Castellana, 2005) or the size and shape of osteophytes in the mature and old adult spine (Stewart, 1958; Snodgrass, 2004; Watanabe and Terazawa, 2006), the literature has shown few studies dealing with the fusion of secondary ossification centers in the vertebral column. Apart from anatomical texts, which report a basic outline of the age ranges and broad patterns of development and fusion (e.g., Scheuer and Black, 2000), the only studies that provide fusion times are those carried out by McKern and Stewart (1957) for the thoracic vertebrae, Buikstra et al. (1984) for the cervical vertebrae, Albert and Maples (1995), and Albert et al. (2010) for thoracic and first two lumbar vertebrae. One other study (Veschi and Facchini, 2002) also provides fusion timing for vertebral epiphyses, but here no detailed data can be found for each individual vertebra and secondary center of ossification since they have all been pooled.

McKern and Stewart (1957) document the timing of union in the annular rings of the vertebral bodies and the spinous process, while Buikstra et al. (1984), Albert and Maples (1995), and Albert et al. (2010) provide fusion times for the annular rings of the vertebral bodies. Due to limitations and specificities in their own samples, union data provided by McKern and Stewart (1957) and Buikstra et al. (1984) are truncated inferiorly at the age of 17 years, and these studies included only males and females, respectively. Because Albert and Maples (1995) utilized vertebral specimens mostly collected during autopsy, their study was necessarily restricted to annular rings. Although McKern and Stewart's (1957) study included the entire vertebral column, data were pooled from different vertebrae, and thus fusion times are not discriminated by vertebra number. Albert et al. (2010) have also pooled data from the differ-

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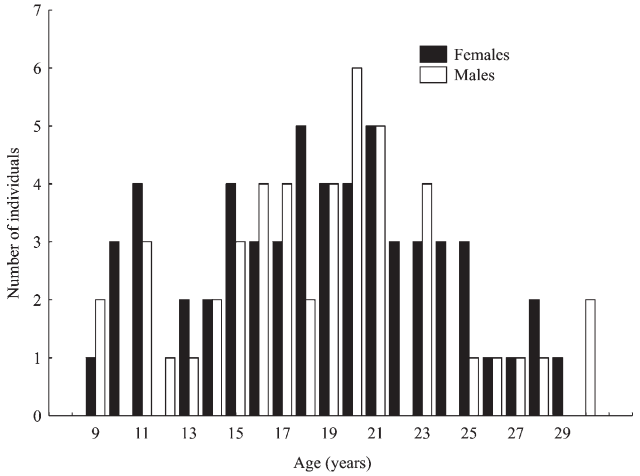

Fig. 1. Sex and age distribution of the sample.

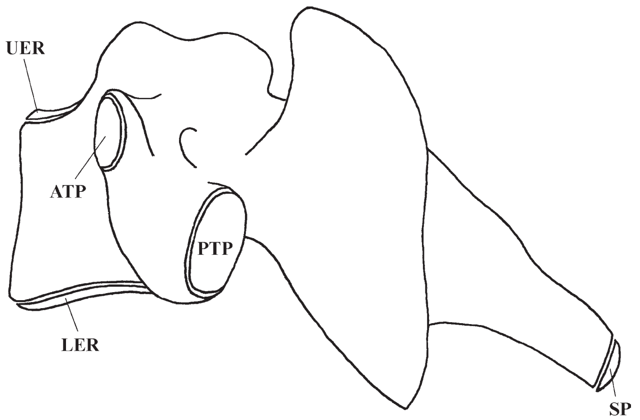

Fig. 2. Epiphyses of a typical cervical vertebra. UER, upper epiphyseal ring; LER, lower epiphyseal ring; ATP, anterior portion of the transverse process; PTP, posterior portion of the transverse process; SP, spinous process.

ent vertebrae. In addition, because large series of documented skeletons of adolescents and young adults are hard to obtain, data in some of these studies are necessarily influenced by relatively small samples (Buikstra et al., 1984; Albert and Maples, 1995). Regrettably, this problem is hard to overcome. As a consequence, there is a large need to fill in or complete the missing information.

Although collections of documented skeletons are relatively rare, a series of recent studies have documented the variation in chronological age of epiphyseal union in the infra-cranial skeleton using known sex and age skeletal samples from contemporary Bosnia (Schaeffer and Black, 2005), 20th century Portugal (Coqueugniot and Weaver, 2007; Cardoso, 2008a,b; Rios et al., 2008), and 20th century Italy (Veschi and Fachinni, 2002). These works provide a geographically and temporally diverse perspective to variation in bone maturation from dry bone observations. In addition, some of these studies provide detailed information for poorly documented epiphyses, such as the ones in the ribs (Ríos and Cardoso, 2009), or for epiphyses rarely documented in dry bone, such as in the hand and foot (Cardoso and Severino, 2009). Collectively, these studies provide data that aid age estimation for a diverse age range, which can be easily applied to incomplete and fragmentary remains. We wish to contribute to this growing knowledge of bone maturation for age estimation purposes in human remains by documenting the stages of fusion of the epiphyses of the presacral spine.

MATERIALS AND METHODS

Sample description

This study utilized a sample of 104 skeletons from the collection of identified human skeletons curated at the National Museum of Natural History in Lisbon, Portugal (Cardoso, 2006). All individuals between the ages of 9 and 30 years were selected, of which 57 are females and the remaining 47 are males. The sample's age range was established during data collection. The skeletal remains represent middle to low social class individuals, who lived in the city of Lisbon at the time of their death. Their births occurred between 1887 and 1960, whereas deaths occurred between 1903 and 1975. Gross pathological cases were eliminated. This same sample supported other similar studies (Cardoso, 2008a,b; Rios et al., 2008; Cardoso and Severino, 2009), but its composition varies slightly because of differences in age range and preservation. Reported ages at death are considered accurate (Cardoso, 2005), and in most cases ages were confirmed by comparison with birth and death dates obtained from civil records. The age and sex distribution of the sample is depicted in Figure 1.

Anatomical description and scoring system

The different segments of the presacral spine have unique sets of secondary ossification centers and this section provides a brief description of these epiphyses. On the cervical vertebrae, different epiphyses were scored depending on vertebra number (Scheuer and Black, 2000). Typical cervical vertebrae (C3–C7) have five epiphyses (Fig. 2): one epiphyseal ring for the lower and one for upper plates of the body (Supporting Information Fig. S1, plates A and B); one epiphysis for the tip of each transverse process, which can be split in two portions for the anterior and posterior tubercles of the transverse process (Supporting Information Fig. S1, plates C and D); and one epiphysis for the spinous process, sometimes divided in two separate portions paralleling the bifid shape of the spinous process (Supporting Information Fig. S2). The first two vertebrae are the most atypical. Two epiphyses are present on the atlas, one for each of the transverse process (Supporting Information Fig. S3, plates A and B). The axis shows four epiphyses (Supporting Information Fig. S3, plates C, D, and E): one epiphyseal ring for the lower plate of the body; one epiphysis for the spinous process, which sometimes can be split in two portions; and one epiphysis for each of the tips of the transverse process.

On the thoracic vertebrae, Scheuer and Black (2000) describe five typical epiphyses in each vertebra (Fig. 3), namely two epiphyseal rings (Supporting Information Fig. S4, plates A, B, and C); one epiphysis for each of the tips of the transverse processes (Supporting Information Fig. S5); and one epiphysis for the spinous process (Supporting Information Fig. S4, plates D–G). In addition to these epiphyses, the epiphyseal rings can show thin flake-like projections to cover the costal demi-facets in the upper half of the thoracic vertebrae, which become

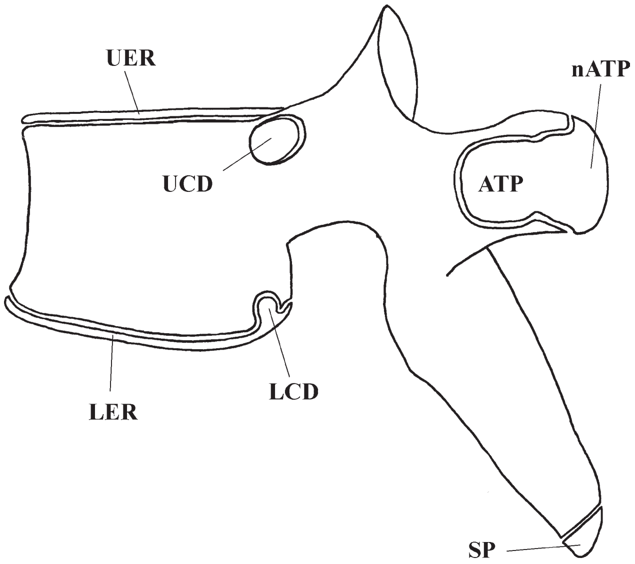

Fig. 3. Epiphyses of a typical thoracic vertebra. UER, upper epiphyseal ring; LER, lower epiphyseal ring; UCD, upper costal demi-facet; LCD, lower costal demi-facet; ATP, articular portion of the transverse process; nATP, non-articular portion of the tranverse process; SP, spinous process.

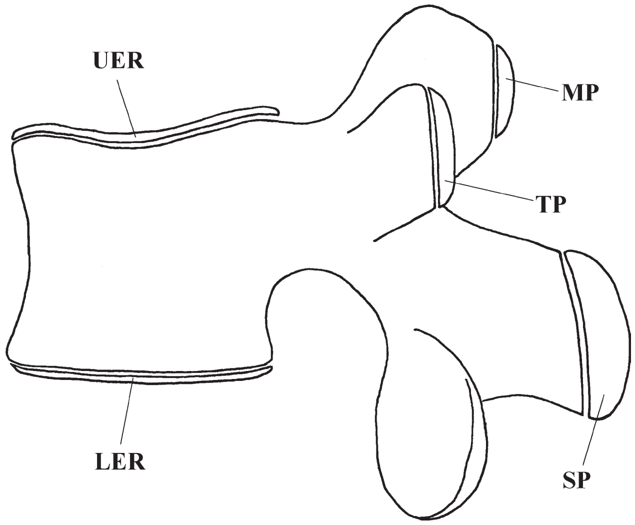

Fig. 4. Epiphyses of a typical lumbar vertebra. UER, upper epiphyseal ring; LER, lower epiphyseal ring; MP, mammillary process; TP, transverse process; SP, spinous process.

completely separate epiphyseal flakes in the lower half of the segment (Fig. 3 and Supporting Information Fig. S6, plates A, B, and C). The transverse process may also show a common epiphysis for the articular and nonarticular regions of the transverse process (Fig. 3 and Supporting Information Fig. S5, plate D), or it may show two distinct centers, one articular and one nonarticular (Supporting Information Fig. S5, plate E) which, in some cases, may be difficult to distinguish as separate identities. This distinction is complicated by the fact that, starting from about T9, the nonarticular facets start to disappear, mirroring the disappearance of the nonarticular tubercle in ribs (Rios and Cardoso, 2009). Finally, the transition from T10 to T12 also entails a reduction of the transverse process, which may disappear all together, and a mammillary-like process (Supporting Information Fig. S5, plate F), typical of lumbar vertebra, may develop (Pal and Routal, 1999). In addition to the five typical epiphyses, we scored separately the fusion of the articular and nonarticular sides of the transverse process, as well as both the upper and lower flakes for the costal demi-facets as separate epiphyses. In total, eleven epiphyseal locations were initially included.

The lumbar vertebrae have been described as having seven typical epiphyses (Scheuer and Black, 2000) (Fig. 4), namely two epiphyseal rings (Supporting Information Fig. S7, plates A, B, and C), two epiphyses for each of the tips of the transverse processes (Supporting Information Fig. S8), two epiphyses for each mammillary process (Supporting Information Fig. S9), and one epiphysis for the spinous process (Supporting Information Fig. S7, plates D, E, and F).

During the initial collating of the data, we decided to reduce the number of epiphyseal locations as follows. Epiphyses for the superior and inferior costal facets in the bodies of thoracic vertebrae were excluded from the analysis because it was difficult to distinguish a completed union from an unfused state, as well as to establish these facets as a separate entity from the annular ring in the upper vertebral segment. In addition, these epiphyses do not seem to provide significantly different information from that of the annular rings themselves. This elimination reduced the number of epiphyses in the thoracic segment from eleven to seven, which were further reduced by pooling data from the articular and nonarticular epiphyses of the transverse process, as well as by pooling the left and right side in the transverse process and pooling the upper and lower annular rings. The articular and nonarticular epiphyses of the transverse process were pooled together as one single site because these two epiphyses are seldom completely separated, and in various occasions the two cannot be identified separately and this causes scoring problems. These observations were pooled by always assigning the partially fused stage to a site which showed only one partially fused epiphysis on either the articular or nonarticular side. There were no cases of differences between the articular and nonarticular side greater than one stage. Furthermore, because no statistically significant bilateral asymmetry was observed (see below), the left and right transverse processes were also combined. The same rationale applies to combining the upper and lower annular rings. In those cases where there was bilateral or superior–inferior asymmetry, the vertebrae was scored unfused (if neither epiphysis was fusing) or partially fused (either epiphysis partially fused or one epiphysis and the other unfused).

Following the absence of significant bilateral asymmetry observed in the sample, the number of epiphyses in the cervical and lumbar segments was reduced by pooling the left and right transverse processes, as well as combining the upper and lower annular rings. In lumbar vertebrae, the number of epiphyses was further reduced by pooling the left and right mammillary processes.

We followed a three-stage scheme for scoring the degree of fusion of these epiphyses: 1) no union, 2) partial union, and 3) completed union (Supporting Information Figs. S1–S9 for illustrations of stages). In every single vertebra each epiphysis was scored independently. An additional stage was initially added to observations on the epiphyseal rings of the centra when a completed union was seen but a scar of recent fusion was still present (Supporting Information Fig. S6 plate D and Fig. S7 plate C), but was later collapsed into Stage 3 (complete union). The presence of this scar was found to persist several years after complete union in the remaining epiphyses.

Replicability and repeatability of fusion scores assigned to each epiphysis was assessed by repeating the observations on four individuals and comparing them with those of the same or other author, respectively. Percentage of agreement and the κ statistic (or Cohen's κ) was used to quantify the amount of intra- and inter-observer error. When comparing the two sets of observations, data from different epiphyses were combined.

Summaries of age of union were established for each vertebral epiphysis and for the sex-pooled sample. For each individual epiphysis and vertebra, the oldest individual at Stage 1 (not fused) provides the upper age limit for this stage's age interval. The youngest individual at Stage 3 (completely fused) provides the lower age limit for this stage's age interval. The youngest and oldest individuals at Stage 2 (partial union) provide the upper and lower age limits for these stage's age range. Posterior probability tables of age for a given stage of fusion, assuming uniform prior probability of age, were also generated to provide more detailed information about the age variation in fusion of secondary ossification centers of the vertebra. Given the problem that age distributions for skeletons tend to mimic the underlying age distribution of reference samples (Bocquet-Appel and Masset, 1982), such as our own, we have used a uniform prior distribution. A uniform prior, which has a flat age distribution, assumes that the unidentified individual has an equal probability of being any age. However, because the calculation of posterior probabilities for all epiphyses would entail a large amount of tabular information, tables were only provided for those transitional vertebrae—C7, T12, and L5—and epiphyses where data were more complete, and for the sex-pooled sample. This has the advantage of providing more detailed information for the most easily identified vertebra, which can be crucial in cases of an incomplete or poorly preserved spine.

Sex differences and bilateral asymmetry

Because of previous findings regarding the possible presence of asymmetry in the degree of fusion in bilateral epiphyses (Albert and Greene, 1999; Ríos and Cardoso, 2009), a separate record was kept for the left and right epiphyses of the transverse process for cervical, thoracic, and lumbar segments as well as for the mammillary process from the lumbar vertebrae. A variable was created, defined as the sum of the absolute values of the differences in fusion between asymmetrical transverse process for each skeleton. The distribution of this variable (total asymmetry, AST) was compared with the Poisson distribution to assess whether asymmetric cases (AST > 0) were just rare events in comparison with symmetric cases (AST $= 0$). The Kolmogorov–Smirnov test was used to assess the goodness of fit.

For assessing sex differences in the age at which the various epiphyses fuse, data for each epiphysis were dichotomized into "fusion not-attained" versus "fusion attained", and an overall logistic regression model was calculated with age and sex as the covariates. The significance of sex differences in timing of fusion was obtained using the Wald statistic by testing whether the coefficient for the variable "sex" is statistically different from zero (no statistically significant sex differences).

TABLE 1. Cohen's kappa (κ) and percentage of agreement (%A) for intraobserver and interobserver error in repeated observations of four individuals

	Intraobserver error				Interobserver error	
	Observer 1		Observer 2			
Segment	%A	κ	%A	κ	%A	κ
Cervical	95.2	0.72	87.0	0.63	79.8	0.63
Thoracic	89.6	0.78	88.1	0.74	89.6	0.81
Lumbar	95.4	0.88	95.8	0.88	92.7	0.88

Data from the different epiphyses is pooled for each vertebral segment.

RESULTS

Observer error results reveal substantial to almost perfect agreement as measured by Cohen's κ (Table 1). Intra-observer agreement showed κ values between 0.63 and 0.88, with a percentage of agreement varying from 87.0 to 95.8%. Similar error was obtained for inter-observer comparisons, with κ values varying from 0.63 to 0.88 and percentage agreement between 79.8 and 92.7%. Observations on the cervical vertebrae seem to be the least repeatable and least replicable. However, these results compare favorably with those obtained on the ribs by our previous study (Rios and Cardoso, 2009).

The assessment of bilateral asymmetry in epiphyseal union at the transverse and mammillary processes was carried out using only those cases in the age range 11–21 years old ($N = 74$), which include the range of partial fusion for those epiphyses. In the transverse process, the number of cases with at least one asymmetric vertebra was three for the cervical segment, seventeen for the thoracic vertebrae, and ten for the lumbar segment. Ten out of the seventeen asymmetric cases at the thoracic segment showed asymmetry in fusion in just one vertebra, and seven out of the ten asymmetric cases at the lumbar segment showed asymmetry in fusion just in one vertebra. When the Kolmogorov–Smirnov goodness of fit test was applied to the variable AST (total asymmetry score), the result showed that the distribution of cases from the three vertebral regions did not differ from the Poisson distribution, thus indicating that the presence of asymmetric cases can be considered a rare and random event (cervical $P = 1.000$; thoracic $P = 0.786$; lumbar $P = 0.995$). Similarly, in the mammillary process, nine cases showed asymmetry in epiphyseal union in at least one vertebrae, but the Kolmogorov–Smirnov test failed to show a significant asymmetry ($P = 0.940$).

The ranges of age for fusion of the various secondary centers of ossification in cervical, thoracic, and lumbar vertebrae are summarized by sex in Tables 2–4. The last row in each table (C*, T*, L*) represents the age ranges for epiphyseal union in a generic vertebra, based on the oldest age at Stage 1, widest age range for Stage 2, and youngest age at Stage 3, obtained from all the vertebrae from the respective segment. In the cervical vertebrae, the annular epiphysis is the first to initiate fusion at about 11–21 years, followed by the transverse process (14−19 years), and spinous process (15–21 years).

TABLE 2. Summary of ages of union for the annular epiphysis, spinous, and tranverse process in cervical vertebrae

	Annular epiphysis			Spinous process			Transverse process		
Vertebra	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
C1	–	–	–	–	–	–	≤18	–	≥15
C2	≤18	14–21 (14)	≥15	≤18	19 (1)	≥17	≤16	14–15 (2)	≥15
C3	≤18	14–21 (17)	≥15	≤15	–	≥15	≤16	14–15 (2)	≥16
C4	≤18	11–21 (16)	≥15	≤15	15–19 (2)	≥15	≤16	14 (1)	≥15
C5	≤18	11–21 (22)	≥16	≤15	–	≥15	≤16	–	≥14
C6	≤18	11–21 (19)	≥16	≤21	17–19 (2)	≥15	≤18	–	≥15
C7	≤18	14–21 (21)	≥15	≤20	15–21 (4)	≥17	≤16	17–19 (2)	≥15
C*	≤18	11–21	≥15	≤21	15–21	≥15	≤18	14–19	≥14

Ages are in years, and sexes are pooled.
Number of observations for stage 2 are in brackets.
C* is a generic cervical vertebra

TABLE 3. Summary of ages of union for the annular epiphysis, spinous, and transverse process in thoracic vertebrae

	Annular epiphysis			Spinous process			Transverse process		
Vertebra	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
T1	≤18	14–21 (25)	≥18	≤21	15–20 (7)	≥17	≤18	14–20 (7)	≥16
T2	≤20	15–21 (24)	≥18	≤19	16–20 (4)	≥18	≤18	14–19 (5)	≥15
T3[a]	≤18	16–24 (32)	≥15	≤21	15–18 (3)	≥18	≤17	11–18 (10)	≥17
T4	≤18	15–24 (35)	≥18	≤20	15–20 (3)	≥17	≤17	14–18 (7)	≥16
T5	≤18	15–27 (29)	≥18	≤20	18 (1)	≥15	≤18	14–16 (5)	≥15
T6	≤21	14–27 (33)	≥18	≤21	–	≥17	≤18	14–16 (4)	≥15
T7[b]	≤18	15–24 (40)	≥18	≤21	20 (1)	≥17	≤18	11–16 (5)	≥15
T8[b]	≤18	15–24 (26)	≥18	≤19	18–20 (3)	≥17	≤18	11–21 (6)	≥14
T9[b]	≤18	14–21 (30)	≥18	≤19	19–20 (4)	≥16	≤18	11–17 (8)	≥15
T10[a,b]	≤18	14–23 (29)	≥18	≤21	19 (2)	≥15	≤18	11–17 (4)	≥15
T11[a]	≤18	14–21 (31)	≥18	≤20	21 (1)	≥17	≤18	15–17 (2)	≥14
T12	≤18	14–21 (33)	≥18	≤19	16–20 (5)	≥18	≤18	15–17 (2)	≥14
T*	≤21	14–27	≥18	≤21	15–21	≥16	≤18	11–21	≥14

[a] Sexes different at $P < 0.05$ for the spinous process.
[b] Sexes different at $P < 0.05$ for the transverse process.
T* is a generic thoracic vertebra.
Ages are in years, and sexes are pooled.
Number of observations for stage 2 are in brackets.

TABLE 4. Summary of ages of union for the annular epiphysis, spinous, transverse, and mammillary process in lumbar vertebrae

	Annular epiphysis			Spinous process			Transverse process			Mammillary process		
Vertebra	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
L1	≤18	15–23 (28)	≥18	≤21	19–21 (2)	≥17	≤21	16–18 (2)	≥16	≤21	11–19 (5)	≥15
L2	≤18	14–22 (29)	≥17	≤21	15–20 (6)	≥17	≤21	19–20 (4)	≥17	≤18	15–19 (4)	≥11
L3	≤18	14–21 (26)	≥18	≤21	15–20 (3)	≥17	≤20	19–21 (2)	≥17	≤18	–	≥11
L4	≤17	14–22 (29)	≥17	≤16	16–21 (6)	≥16	≤21	–	≥17	≤18	11–17 (4)	≥11
L5	≤17	14–20 (21)	≥17	≤17	15–20 (4)	≥17	≤19	15–20 (6)	≥17	≤18	14–17 (5)	≥11
L*	≤18	14–23	≥17	≤21	15–21	≥16	≤21	15–21	≥16	≤21	11–19	≥11

Ages are in years, and sexes are pooled.
Number of observations for stage 2 are in brackets.
L* is a generic lumbar vertebra.

Comparatively, in the thoracic vertebrae, the first epiphysis to initiate fusion is that of the transverse process at 11 years, followed by the annular at 14 years, and the spinous process at 15 years. It is important to note that for the epiphysis of the thoracic transverse process, partial union initiates in several vertebra at the age of 11 years. In these cases, an 11-year-old male and an 11-year-old female have pushed the lower limit of the age range down. Partial union in these individuals is represented by a very small epiphyseal flake (Supporting Information Fig. S5, plate B), which is not typical of the partial union scored in other individuals. If these cases were not considered, the youngest age for partial union would increase to 14 years of age. It is also important to indicate that the upper age limit of 27 for partial fusion in the annular epiphyses of vertebrae T5 and T6 is because of a single female case, and if this case was not considered, the oldest age for partial union would drop to 24 years of age. For comparative purposes, Table 5 shows the percentage of complete fusion in annular rings of thoracic vertebra.

The first epiphysis to fuse in the lumbar vertebrae is that of the mammillary process, with an age range for partial union of 11–19 years, followed by the annular epiphyses (14–23 years) and the spinous (15–21 years), and transverse processes (15–21 years). In the lumbar segment, a partial union is observed from 11

TABLE 5. Summary table for percentage of complete fusion of the annular epiphysis in thoracic vertebra for the sex-pooled sample

Age	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
15	–	–	14.3	–	–	–	–	–	–	–	–	–
16	–	–	–	–	–	–	–	–	–	–	–	–
17	–	–	–	–	–	–	–	–	–	–	–	–
18	28.6	28.6	42.9	14.3	14.3	14.3	28.6	28.6	28.6	85.7	85.7	42.9
19	–	14.3	12.5	–	–	–	–	–	12.5	–	–	–
20	55.5	50.0	10.0	20.0	20.0	20.0	20.0	20.0	10.0	40.0	30.0	33.3
21	70.0	66.6	50.0	37.5	33.3	22.2	12.5	55.6	77.8	77.8	62.5	66.7
22[a]	100	100	33.3	33.3	33.3	33.3	66.6	66.6	100	100	100	100
23	100	100	83.3	71.4	71.4	28.6	57.1	71.4	100	83.3	100	100
24[a]	100	100	50.0	50.0	50.0	–	–	50.0	100	100	100	100
25	100	100	100	100	100	100	100	100	100	100	100	100
26	100	100	100	100	100	100	100	100	100	100	100	100
27	100	100	100	100	50.0	75.0	100	100	100	100	100	100
28	100	100	100	100	100	100	100	100	100	100	100	100
29	100	100	100	100	100	100	100	100	100	100	100	100

[a] Include observations from females only.

TABLE 6. Posterior probabilities of age given a certain stage of epiphyseal union for the annular epiphysis and transverse process of the seventh cervical vertebra (sexes pooled, uniform priors)

Annular epiphysis				Transverse process			
Age	Stage 1	Stage 2	Stage 3	Age	Stage 1	Stage 2	Stage 3
<13	0.67	0.00	0.00	<14	0.72	0.00	0.00
14	0.11	0.20	0.00	15	0.16	0.00	0.02
15	0.07	0.20	0.01	16	0.12	0.00	0.03
16	0.07	0.20	0.01	17	0.00	0.53	0.06
17	0.03	0.12	0.04	18	0.00	0.00	0.07
18	0.04	0.07	0.05	19	0.00	0.47	0.06
19	0.00	0.10	0.06	>20	0.00	0.00	0.76
20	0.00	0.05	0.07	–	–	–	–
21	0.00	0.05	0.07	–	–	–	–
>22	0.00	0.00	0.69	–	–	–	–

The spinous process is not depicted because of missing data at 14 years of age.

(mammillary process) to 23 (annular epiphysis) years of age.

Tables 6–8 document the sex-pooled posterior probabilities of age given the stages of fusion of the annular epiphyses, transverse, and spinous processes in C7, T12, and L5, respectively. Table 8 also documents sex-pooled posterior probabilities of age for the mammillary epiphysis in L5. In Table 6, posterior probabilities for the spinous process are not provided because there are no observations at age 14. This results in a posterior probability of zero of showing any stage at any location for a 14-year-old individual, which is unrealistic.

Statistically significant sex differences in age of fusion are identified in Table 3. Most epiphyses in the three segments show statistically insignificant sex differences. No sex differences were detected in cervical and lumbar vertebrae, whereas in the thoracic segment only T10 and T11 show statistically significant sex differences in the fusion of the spinous process, along with T7 to T10 in the transverse process. Despite the lack of significant differences, there seems to be a slight trend toward early fusion in females in several epiphyses. In those cases where females are significantly ahead of the males, the difference between the sexes is about 2.5 years.

DISCUSSION

This is the first study to systematically document the age variation in the fusion of the secondary centers of ossification in the cervical, thoracic, and lumbar vertebrae. Although sample size may be considered insufficient to accurately document the true range of variation in fusion, fully identified skeletons of the relevant age are difficult to obtain, and we believe the information provided here can be used in aiding the age estimation of pubertal and young adult skeletons in archaeological and forensic contexts. Data are also particularly scarce for females and some epiphyses, and here is also where this study is most useful.

Besides the number of available skeletons for study, preservation problems and fragility of secondary ossification centers were important issues in scoring stages of fusion in vertebral epiphyses. Most of the epiphyses are composed of small bone flakes located at the extremities of each vertebra, which can be easily detached and broken off. The annular rings, in particular, are the largest secondary ossification centers, but are probably also the most fragile. As a consequence, an epiphysis may have been scored as absence of fusion when in fact it had begun to fuse and subsequently broken away. This means that our age ranges for no union in the annular epiphyses may actually overestimate age. One of the areas with greatest postmortem destruction was that of the spinous process, mainly because of taphonomic factors. Although some of the epiphyses may have been lost during recovery, curation of most of these skeletons was carried out by an expert in human osteology and special care was taken to preserve them. We also believe that we took advantage of the good preservation of most of the Lisbon collection specimens, as well as from the fact that these subadult and young adult skeletons have only been studied on a few occasions and thus bone extremities and edges have not been destroyed by storage and excessive handling over the years.

In addition to sampling and preservation problems, some concerns were raised when assessing intra- and inter-observer error. The scoring of epiphyseal fusion in the vertebrae presented the occasional difficulty of recognizing fusion. The majority of observer error lies in the scoring of the epiphyses, which cover the costal demi-facets on the thoracic vertebral bodies. These epiphyses cannot be easily distinguished as a separate epiphysis from that of the annular ring. However, since we have

TABLE 7. Posterior probabilities of age given a certain stage of epiphyseal union for the epiphyses of the twelfth thoracic vertebra (sexes pooled, uniform priors)

Annular epiphysis				Transverse process				Spinous process			
Age	Stage 1	Stage 2	Stage 3	Age	Stage 1	Stage 2	Stage 3	Age	Stage 1	Stage 2	Stage 3
<13	0.55	0.00	0.00	<13	0.67	0.00	0.00	<14	0.63	0.00	0.00
14	0.12	0.10	0.00	14	0.11	0.00	0.06	15	0.21	0.00	0.00
15	0.13	0.08	0.00	15	0.13	0.58	0.02	16	0.00	0.45	0.00
16	0.15	0.05	0.00	16	0.06	0.00	0.09	17	0.11	0.23	0.00
17	0.03	0.25	0.00	17	0.00	0.42	0.10	18	0.00	0.09	0.13
18	0.03	0.08	0.05	18	0.03	0.00	0.10	19	0.05	0.00	0.12
19	0.00	0.29	0.00	>19	0.00	0.00	0.61	20	0.00	0.23	0.08
20	0.00	0.13	0.05	–	–	–	–	>21	0.03	0.00	0.67
21	0.00	0.04	0.08	–	–	–	–	–	–	–	–
>22	0.00	0.00	0.82	–	–	–	–	–	–	–	–

TABLE 8. Posterior probabilities of age given a certain stage of epiphyseal union for the epiphyses of the fifth lumbar vertebra (sexes pooled, uniform priors)

Annular epiphysis				Transverse process				Spinous process				Mammillary process			
Age	Stage 1	Stage 2	Stage 3	Age	Stage 1	Stage 2	Stage 3	Age	Stage 1	Stage 2	Stage 3	Age	Stage 1	Stage 2	Stage 3
<13	0.61	0.00	0.00	<13	0.55	0.00	0.00	<14	0.70	0.00	0.00	<10	0.33	0.00	0.00
14	0.14	0.10	0.00	14	0.12	0.28	0.00	15	0.09	0.44	0.00	11	0.12	0.00	0.02
15	0.10	0.15	0.00	15	0.12	0.28	0.00	16	0.17	0.00	0.00	12	0.16	0.00	0.00
16	0.12	0.13	0.00	16	0.15	0.00	0.01	17	0.04	0.22	0.04	13	0.16	0.00	0.00
17	0.03	0.20	0.01	17	0.03	0.12	0.05	18	0.00	0.00	0.08	14	0.08	0.42	0.00
18	0.00	0.13	0.05	18	0.03	0.00	0.06	19	0.00	0.22	0.06	15	0.07	0.17	0.03
19	0.00	0.20	0.03	19	0.00	0.21	0.06	20	0.00	0.11	0.07	16	0.05	0.28	0.02
20	0.00	0.11	0.05	20	0.00	0.09	0.07	>21	0.00	0.00	0.76	17	0.00	0.14	0.06
<21	0.00	0.00	0.85	<21	0.00	0.00	0.75	–	–	–	–	18	0.02	0.00	0.06
–	–	–	–	–	–	–	–	–	–	–	–	>19	0.00	0.00	0.82

not presented age variation data for these epiphyses, they are of no concern here. Other errors resulted from a nonunited (Stage 1) epiphysis being mistaken for a united (Stage 3) epiphysis and vice-versa. This occurred on a few occasions, particularly on the transverse and spinous processes, especially at the cervical segment, whenever the epiphyses are too small or poorly defined. In rare cases, a partially united (Stage 2) epiphysis was mistaken for a united (Stage 3), when a very small open metaphyseal space was undetected at the edge of a small epiphyseal flake. Overall, special attention should be paid to unfused epiphyses which can simulate the appearance of a united epiphysis (especially in small epiphyses such as those from the cervical transverse and spinous processes), as well as to small open metaphyseal spaces which indicate partly fused epiphyses.

The most reliable information about bone maturation for use in age estimation is, perhaps, better obtained from the observation of a partial union. However, even the scoring of a partial union can present problems. For example, the epiphyses of the spinous and transverse processes in cervical vertebra have been described as difficult to detect since they are "flake-like structures that probably do not exist as separate entities but fuse directly with the process as it forms" (Scheuer and Black, 2000). This fact is, probably, reflected in the small number of cases where partial union of these epiphyses was detected in this study (Table 2). Therefore, we recommend caution when assigning an age-range solely based on the stage of fusion of these epiphyses. However, as illustrated in Supporting Information Figures S1–S3, after careful examination of the anatomical location, these epiphyses and their stage of fusion can be scored with relative confidence. As for the epiphyses of the transverse process in thoracic and lumbar vertebrae, as well as for the epiphyses of the mammillary process in lumbar vertebrae, our opinion is that these epiphyses and the three stages of fusion can be easily identified even in a fragmentary vertebra (Supporting Information Figs. S4, S5, S7, S8, and S9), and therefore they can be confidently used to assign an age-range (Tables 3 and 4). Caution is recommended when considering the eleventh and twelfth thoracic vertebrae because of the reduction of the transverse process and the presence of mammillary-like process (Pal and Routal, 1999), where the epiphyses are smaller (Supporting Information Fig. S5 plate F).

Information summarized in Tables 2–4 indicates that the fusion of vertebral epiphyses is most useful for age estimation in pubertal and young adult skeletons. Since observations of a partial union provide the most reliable information, vertebral epiphyses can be utilized to estimate age in the range of 11–27 years. Age ranges of epiphyseal fusion for the generic vertebra can be particularly useful in cases of fragmentary or incomplete remains, where vertebra number cannot be easily identified. In the probability tables (Tables 6–8), posterior probabilities can be determined for any specific age interval using epiphyseal union in C7, T12, and L5 by adding probability values for those ages. For example, using the fusion of the spinous process in T12, the probability of an individual being between 16 and 18 years of age given Stage 2 of fusion is the sum of the probabilities for the ages 16, 17 and 18, which is 0.77 (0.45 + 0.23 + 0.09). No fusion or fusion complete can also provide the probability of an individual being younger or older than a certain age. For example, the probability of an individual being older than 24 years of age, given Stage 3 of fusion in the annular epiphyses of C7, is 0.67. Information in these probability tables is particularly helpful in fragmentary cases where sex cannot be determined.

Comparative data for epiphyseal union in presacral vertebrae are scarce or inexistent for some epiphyses, but some comparisons are possible and our results show an overall similarity. Because epiphyseal union did not show significant sex differences, comparisons are made with sex-pooled samples. For example, Buikstra et al. (1984) report on the timing of epiphyseal rings fusion for the cervical region (C2, C3, and C4) in a sample of "black" females of the Terry Collection. In their study, a partial union occurs in individuals under 19 years of age, and complete fusion with a visible scar between the ages of 17 and 25 years. These results are comparable with the age ranges obtained for the Lisbon sex-pooled sample, where partial union (Stage 2) occurs for the age range 11–21 years (Table 2).

When comparing our results for the thoracic annular epiphyses with those obtained by McKern and Stewart (1957; MS) and Albert and Maples (1995; AM), it can be seen that absence of fusion is present at any vertebra until 18 years in the MS sample and until 20 years and 8 months in the AM sample. Comparatively, in the Lisbon sample absence of fusion occurs until 21 years old. Complete fusion in thoracic vertebrae occurs by 17 years in the MS sample (the sample is truncated at this age), and in the AM sample by 18 years and 9 months, whereas the earliest case of complete fusion in any thoracic vertebra in the Lisbon sample occurs at the age of 15 years. Although no detailed information is given for the MS sample, partial union occurs until 23 years, which is slightly younger than in the Lisbon sample (27 years). However, a comparison of age ranges for partial union between the Lisbon and AM samples was not possible because the scoring system used was different. Albert and Maples' (1995) Stage 2 included vertebrae with almost complete fusion (Stage 2 in our study) and in the state of recent fusion (Stage 3 in our study). In females, the Albert and Maples (1995) sample (AM) showed absence of fusion for the thoracic annular epiphyses at any vertebra until 17 years and 3 months and complete fusion at any vertebra by 18 years. Comparatively, in our sample absence of fusion is still present by the age of 21 years, but complete fusion also occurs by 18 years, with the exception of one case. In this single case, a 15-year-old female shows complete epiphyseal fusion. Fusion times from Albert et al. (2010) do not differ from our own data as well. For example, in the Lisbon sample absence of fusion in the thoracic and lumbar vertebrae generally occurs until 18–21 years old, which is in general agreement with the Albert et al. (2010) study (<18 years old in females and <22 years in males). As for partial and complete fusion, data cannot be compared because the scoring system used by Albert et al. (2010) was different. Although fusion times seem similar in both studies, in their study a partial fusion has been assigned to two different stages, which hampers a direct comparison between both studies.

With regard to epiphyseal union in the spinous process, there is no specific information except the male data offered by McKern and Stewart (1957). According to this study, absence of fusion is observed until 20 years old, partial union occurs between 17 and 23 years of age, and complete fusion is already present by the age of 17 years. However, one needs to keep in mind that McKern and Stewart's (1957) sample is truncated at the age of 17. Nonetheless, these results are overall similar to the findings of our own study.

Although Veschi and Facchini (2002) have published ages of fusion in the vertebra, data on the various secondary centers of ossification and on the entire vertebral column have been pooled in one single table. This only permits the general observations that the age ranges for fusion in Veschi and Facchini (2002) are within the limits of our own data.

Although there are some differences in age ranges for epiphyseal union between the Lisbon collection and the other samples, we cannot identify a clear and consistent pattern of advancement or delay in fusion between the Portuguese and the US samples utilized by McKern and Stewart (1957), Buikstra et al. (1984), Albert and Maples (1995), and Albert et al. (2010). The differences in age ranges found when the samples are being compared could be assigned to population differences in bone maturation (genetic or environmental factors) or to methodological problems. Diverse authors have observed differences in dental development and bone maturation at different skeletal locations by comparing large samples of temporally, geographically, and socioeconomically diverse groups (Schmeling et al., 2000; Schaefer and Black, 2005; Schmeling et al., 2006; Meijerman et al., 2007; Heuzé and Cardoso, 2008; Shirley and Jantz, 2010). The differences observed between samples have been mostly attributed to the impact of environmental variables such as nutrition and disease on maturation rates (Schmeling et al., 2000, 2006; Meijerman et al., 2007; Heuzé and Cardoso, 2008). On the other hand, comparing samples can be complicated by methodological issues, namely insufficient sample sizes that document poorly the range of variation in bone maturation, the use of different scoring systems, or the lack of detailed documentary information on the health and socioeconomic status of the skeletal samples, beyond a general knowledge of the living standard of the population from which it is derived. In spite of these limitations, whatever the causes of the differences in maturation observed between samples, the overall pattern is one of great similarity. Nonetheless, it is likely that socioeconomic status and secular trend effects within the same population and different levels of social and economic development between populations may influence the timing of epiphyseal union. Therefore, when performing age estimations, forensic anthropologists and bioarchaeologists should pay special attention to these variations to establish the most probable age range (Schaefer and Black, 2005; Schmeling et al., 2006).

There is also scarce information regarding the chronological sequence in which the various epiphyses fuse. The only information available has been published by McKern and Stewart (1957) and Albert and Maples (1995) for annular epiphyses in thoracic and lumbar vertebra, and by Buikstra et al. (1984) for the annular epiphyses in cervical vertebrae. In the earlier study, a clear pattern in the maturation of the annular epiphysis is described. According to McKern and Stewart (1957), "until the age of 24, there is a definite and consistent lag throughout the age groups in the region between T-2 and T-7 but, especially in segments T-4 and T-5. Thus it is important to examine these segments for the last signs of union in the presacral spine." On the other hand, Albert and Maples (1995) could not replicate this finding since they did not observe a clear pattern in the order of fusion, although they suggested that union may begin in T1 and the lower thoracic region (T8–T12) in comparison with the middle thoracic vertebrae (T2–T7). Buikstra et al. (1984) have also found that the more cranially oriented vertebrae tend to be more advanced than that of the more caudal ones.

As shown in Table 5, our findings are consistent with those of McKern and Stewart (1957): complete union of the annular epiphysis is first achieved in the T1–T2 and T9–T12 segments by 24 years, considering the whole sample, and both segments are clearly ahead in percentage of complete fusion at earlier ages in comparison with the T3–T8 segment. A clear pattern in the chronological sequence of fusion in the remaining epiphyses could not be detected, except on the annular and spinous epiphyses of the lumbar vertebrae (data not shown). An earlier beginning of fusion and earlier achievement of complete fusion for both epiphyses were observed in the lower lumbar vertebrae (L5) in comparison with the upper ones (L1). An insufficient sample size and preservation issues may have prevented a clear pattern from emerging.

Compared with observations on the timing of epiphyseal union in ribs (Ríos and Cardoso, 2009), the presacral vertebrae show similar age ranges. In particular, the head epiphyses in ribs fuses at about the same time as the annular epiphyses in vertebrae, and the articular tubercle in ribs shows a similar age-range than that of the remaining epiphyses in the vertebra. The fusion of the nonarticular tubercle in ribs seems to occur earlier than that of most vertebral epiphyses. Perhaps it is no surprise that the annular epiphyses of the thoracic vertebrae show similar ages of fusion to that of the heads of the ribs, since in the upper vertebrae, the annular epiphyses are topographically connected to the costal demifacets, with which the heads of the ribs articulate (Scheuer and Black, 2000). When comparing the sequence of fusion of the head epiphyses of the ribs with that of the vertebral epiphyses, data from the annular rings indicate an overall agreement, where fusion occurs first in the more cranial and caudal vertebrae and ribs (Ríos and Cardoso, 2009). Similarly, the epiphyses from the vertebral transverse processes and the epiphyses from the articular tubercles of the ribs are anatomically connected and also show similar ages of fusion. This pattern of fusion is consistent with what is reported in the literature (Scheuer and Black, 2000).

CONCLUSIONS

The age variation in fusion of secondary centers of ossification in the vertebra described here can provide important information for aiding the estimation of age of adolescent and young adult skeletons, increasing the available information from previously published works. The data provide additional information which can be useful in a variety of contexts. Besides reconstructing age distributions in archaeological settings, examples include forensic cases of unknown identity as illustrated by Albert (1998), where epiphyseal union of the annular rings of the vertebra was important to narrow the estimated age range and the identification process of victims of human rights violations and genocide recovered from mass graves. In these last cases, the demographic profile of the individuals interred usually include a large number of young males in their late teens and early twenties (e.g. Schaeffer and Black, 2005), precisely the age range for which skeletal maturation of the spine is most useful.

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LITERATURE CITED

Albert AM. 1998. The use of vertebral ring epiphyseal union for age estimation in two cases of unknown identity. Forensic Sci Int 97:11–20.

Albert AM, Greene DL. 1999. Bilateral asymmetry in skeletal growth and maturation as an indicator of environmental stress. Am J Phys Anthropol 110:341–349.

Albert AM, Maples WR. 1995. Stages of epiphyseal union for thoracic and lumbar vertebral centra as a method of age determination for teenage and young adult skeletons. J Forensic Sci 40:623–633.

Albert M, Mulhern D, Torpey MA, Boone E. 2010. Age estimation using thoracic and first two lumbar vertebral ring epiphyseal union. J Forensic Sci 55:287–294.

Bocquet-Appel JP, Masset CL. 1982. Farewell to paleodemography. J Hum Evol 11:321–333.

Buikstra JE, Gordon CC, St. Hoyme L. 1984. The case of the severed skull. Individuation in forensic anthropology. In: Rathbun TA, Buikstra JE, editors. Human identification. Case studies in forensic anthropology. Springfield, IL: Charles C Thomas. p 121–135.

Cardoso HFV. 2005. Patterns of growth and development of the human skeleton and dentition in relation to environmental quality. Ph.D. thesis. Hamilton: McMaster University.

Cardoso HFV. 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 129:173–176.

Cardoso HFV. 2008a. Epiphyseal union at the innominate and lower limb in a modern Portuguese skeletal sample, and age estimation in adolescent and young adult male and female skeletons. Am J Phys Anthropol 135:161–170.

Cardoso HFV. 2008b. Age estimation of adolescent and young adult male and female skeletons. II. Epiphyseal union at the upper limb and scapular girdle in a modern Portuguese skeletal sample. Am J Phys Anthropol 137:97–105.

Cardoso HFV, Severino RSS. 2009. The chronology of epiphyseal union in the hand and foot from dry bone observations. Int J Osteoarchaeol (DOI 10.1002/oa. 1097).

Coqueugniot H, Weaver TD. 2007. Brief communication: Infracranial maturation in the skeletal collection from Coimbra. Portugal: new aging standards for epiphyseal union. Am J Phys Anthropol 134:424–437.

Heuzé Y, Cardoso HFV. 2008. Testing the quality of nonadult Bayesian dental age assessment methods to juvenile skeletal remains: the Lisbon collection children and secular trend effects. Am J Phys Anthropol 135:275–283.

Kósa F, Castellana C. 2005. New forensic anthropological approachment for the age determination of human fetal skeletons on the base of morphometry of vertebral column. Forensic Sci Int 147(Suppl):S69–S74.

McKern TW, Stewart TD. 1957. Skeletal changes in young American males. Analyzed from the standpoint of age identification. Environmental Protection Research Division (U.S. Army Quartermaster Research and Development Command), Technical Report EP-45, Natick, US Army.

Meijerman L, Maat GJR, Schulz R, Schmeling A. 2007. Variables affecting the probability of complete fusion of the medial clavicular epiphysis. Int J Legal Med 121:463–468.

Pal GP, Routal RV. 1999. Mechanism of change in the orientation of the articular process of the zygapophyseal joint at the thoracolumbar junction. J Anat 195:199–209.

Ríos L, Cardoso HF. 2009. Age estimation from stages of union of the vertebral epiphyses of the ribs. Am J Phys Anthropol 140:265–274.

Ríos L, Weisensee K, Rissech C. 2008. Sacral fusion as an aid in age estimation. Forensic Sci Int 180:111–117.

Schaefer MC, Black SM. 2005. Comparison of ages of epiphyseal union in North American and Bosnian skeletal material. J Forensic Sci 50:777–784.

Scheuer L, Black S. 2000. Developmental juvenile osteology. London: Academic Press.

Schmeling A, Reisinger W, Loreck D, Vendura K, Markus W, Geserick G. 2000. Effects of ethnicity on skeletal maturation: consequences for forensic age estimations. Int J Legal Med 113:253–258.

Schmeling A, Schulz R, Danner B, Rösing FW. 2006. The impact of economic progress and modernization in medicine on the ossification of hand and wrist. Int J Legal Med 120:121–126.

Shirley NL, Jantz RL. 2010. A Bayesian approach to age estimation in modern Americans from the clavicle. J Forensic Sci 55:571–583.

Snodgrass JJ. 2004. Sex differences and aging of the vertebral column. J Forensic Sci 49:458–463.

Stewart TD. 1958. The rate of development of vertebral osteoarthritis in American whites and its significance in skeletal age identification. Leech 28:144–151.

Veschi S, Facchini F. 2002. Recherches sur la collection d'enfants et d'adolescents d'âge et de sexe connus de Bologne (Italie): diagnose de l'âge sur la base du degré de maturation osseuse. Bull Mém Soc Anthropol Paris 14:263–294.

Watanabe S, Terazawa K. 2006. Age estimation from the degree of osteophyte formation of vertebral columns in Japanese. Legal Med 8:156–160.