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

Manuscript Number: FSI-D-12-00559R1

Title: Age at death estimation using bone densitometry: testing the Fernández Castillo and López Ruiz method in two documented skeletal samples from Portugal

Article Type: Forensic Anthropology Population Data

Keywords: age at death estimation; adult skeletons; bone densitometry; forensic anthropology population data

Corresponding Author: Mr. Francisco Curate, Ph.D

Corresponding Author's Institution: University of Coimbra

First Author: Francisco Curate, Ph.D

Order of Authors: Francisco Curate, Ph.D; Anabela Albuquerque, MD; Eugénia Cunha, Ph.D

Abstract: This study aims to evaluate the accuracy, precision and bias of a method for age at death estimation based in bone mineral density values assessed by dual x-ray absorptiometry at Ward's area (proximal femur). Estimated age at death was contrasted with documented age at death in two Portuguese reference samples (Coimbra Identified Skeletal Collection - CISC and Identified Skeletal Collection of the 21st Century - Santarém XXI). Mean absolute error (accuracy) varies between 10.5 years (females) and 11.6 years (males) in the CISC sample; and between 11.9 years (males) and 12.7 years (females) in the Santarém XXI study base. The precision of the method varies between 13.0 (females) and 14.5 (males), in the CISC sample, and between 8.4 (females) and 9.5 (males), in the Santarém XXI sample. Mean error values (bias) suggest that this method tends to overestimate age in younger individuals, and to underestimate it in older individuals, regardless of sex or sample. Nonetheless, the method seems to perform as well as, or better than, other widely tested age estimation techniques, making it a suitable option when more accurate tests are not feasible in any given situation.

Suggested Reviewers:

Title Page (with authors and addresses)

Age at death estimation using bone densitometry: testing the Fernández Castillo and López Ruiz method in two documented skeletal samples from Portugal

Curate, Francisco

Research Centre for Anthropology and Health – University of Coimbra
Apartado 3046
3001-401 Coimbra, Portugal

Forensic Sciences Centre - National Institute of Legal Medicine
Largo da Sé Nova, s/n
3000-213 Coimbra, Portugal

E-MAIL: fcurate@uc.pt

TEL: + 351 968 071 779 / FAX: + 351 239 823 491

Albuquerque, Anabela

Nuclear Medicine Service – University of Coimbra Hospitals
Praceta Professor Carlos Mota Pinto
3030 Coimbra, Portugal

Cunha, Eugénia M.

Forensic Sciences Centre - National Institute of Legal Medicine
Largo da Sé Nova, s/n
3000-213 Coimbra, Portugal

Department of Life Sciences – University of Coimbra
Apartado 3046
3001-401 Coimbra, Portugal

Age at death estimation using bone densitometry: testing the Fernández Castillo and López Ruiz method in two documented skeletal samples from Portugal

Abstract

This study aims to evaluate the accuracy, precision and bias of a method for age at death estimation based in bone mineral density values assessed by dual x-ray absorptiometry at Ward's area (proximal femur). Estimated age at death was contrasted with documented age at death in two Portuguese reference samples (Coimbra Identified Skeletal Collection – CISC, and Identified Skeletal Collection of the 21st Century – Santarém XXI). Mean absolute error (accuracy) varies between 10.5 years (females) and 11.6 years (males) in the CISC sample; and between 11.9 years (males) and 12.7 years (females) in the Santarém XXI study base. The precision of the method varies between 13.0 (females) and 14.5 (males), in the CISC sample, and between 8.4 (females) and 9.5 (males), in the Santarém XXI sample. Mean error values (bias) suggest that this method tends to overestimate age in younger individuals, and to underestimate it in older individuals, regardless of sex or sample. Nonetheless, the method seems to perform as well as, or better than, other widely tested age estimation techniques, making it a suitable option when more accurate tests are not feasible in any given situation.

Keywords age estimation; adult skeletons; bone densitometry; forensic anthropology population data

Introduction

Establishing an accurate age at death on the basis of skeletal remains is a prerequisite to determine a comprehensive biological profile, and a pivotal step for the identification of individual skeletal remains [1,2]. Unfortunately, biological aging shows great variation, both within and between populations [3,4,5]; and the assessment of age at death in adult skeletal remains usually renders mediocre to poor estimates of both biological and chronological age [2,3,6]. Hence, it is appropriate to consider as many techniques as possible to assess age at death in adult skeletons, although recognizing that the available aging methodologies are not equivalent, with different accomplishments in the issues of reliability and validity [2,5,7].

Dual x-ray absorptiometry (DXA) has seldom been applied in the forensic sciences [e.g., 8-10] but it is widely acknowledged as the gold-standard methodology to assess bone mineral density (BMD) and to diagnose osteoporosis in clinical and epidemiological settings [11]. DXA calculates the quantity of hydroxyapatite in bone, conveying it in grams of mineral per unity of area. The technology uses radiation from two X-ray beams with different energy levels. The radioactive beams are collimated and directed into a radiation detector, located opposite to the mensuration area, where the X-ray attenuation by bones and soft tissues is used to define the bone mineral content (BMC). Bone mineral density is then computed as the ratio between BMC and the measured area. Fundamentally, DXA produces a linear

measurement of BMC (in grams) that is subsequently converted into an area of bone density (g/cm^2) [8,11,12].

BMD declines with age in all populations, especially in females [12,13]. Hence, theoretically BMD can be a useful indicator of biological age in skeletal remains. Following this assumption, Fernández Castillo and López Ruiz [14] developed an aging method based in BMD measurements at the Ward's triangle area. The authors found a very high correlation between BMD values measured at the Ward's area region of interest (ROI) of the proximal femur and documented age in a Spanish hospital population. As such, they proposed two regression formulae (for men and women) to infer chronological age based on BMD values at the ROI "Ward's area".

In forensic anthropology, the continuous re-testing of existing age at death estimation methods in adult skeletons perseveres as the superlative trial to ascertain their reliability [5]. Such validation trials are also intended to test the uniformity of biological aging patterns, clarifying which aging methods can be validated across populations [15]. In this study we aimed to test the accuracy, precision and bias of the Fernández Castillo and López Ruiz method [14] for age at death estimation, by applying it in two documented samples from Portugal. Our specific purposes were to determine if this new aging technique could be endorsed across populations, and applied to skeletonized bodies, both in forensic and archaeological contexts.

Materials & methods

The "Coimbra Identified Skeletal Collection" (CISC) was assembled between 1915 and 1942 and comprises individuals born between 1822 and 1921, and dead between 1904 and 1936. The collection of 505 skeletons with known sex and age at death (among other biographical information) consists mainly of Portuguese nationals, mostly manual workers with low socioeconomic status. Individuals exhumed from shallow graves in the Municipal Cemetery of Conchada (Coimbra, Portugal) compose the bulk of the collection (N=198). These individuals were buried for at least five years – after that it was common to perform the exhumation of the bodies [16]. The "Identified Skeletal Collection of the 21st Century" (Santarém XXI) is the latest Portuguese osteological reference collection. It includes 77 identified individuals, of Portuguese nationality, born between 1905 and 1968, and dead between 1995 and 2001. All individuals from the Santarém XXI skeletal collection were recovered from the Municipal Cemetery of Capuchos (Santarém, Portugal), where they were interred from five to seven years.

The testing samples consist of 100 individuals (50 ♀; 50 ♂) from the CISC and 40 individuals (20 ♀; 20 ♂) from the Santarém XXI, randomly chosen from the two identified skeletal assemblages. The CISC sample included individuals born between 1831 and 1914; and dead between 1910 and 1936. Recorded ages at death varied between 20 and 95 years (Mean=54.6; SD=18.2; 95%CI: 51.0 – 58.2). The sampled Santarém XXI individuals were born between 1906 and 1968 and died between 1995 and 2001. The youngest individual of this sample died at 33 years, the oldest at 96 years (Mean=75.2; SD=14.8; 95%CI: 71.0 – 80.2).

BMD at ROI “Ward’s area” was measured in the left femur of each individual with a Hologic QDR 4500C densitometer. Femurs were placed anteroposteriorly, with the diaphysis parallel to the central axis of the scanner, in a low-density paper box with dry rice (10 cm depth) standing for a soft-tissue proxy [17,18].

Age at death was estimated following the regression equations proposed by Fernández Castillo and López Ruiz [14]:

Men: *Age* = 100.558-79.124(*BMD Ward’s area*) ± 4.149

Women: *Age* = 94.488-66.391 (*BMD Ward’s area*) ± 4.855

Linear Pearson correlation was used to associate documented age at death with estimated age at death. The mean difference between estimated ages at death and documented ages at death was evaluated with a paired sample *t*-test (the normal distribution of the variables was evaluated with a Kolmogorov-Smirnov test and the homogeneity of variances with a Levene test). Accuracy was expressed as the mean absolute error (MAE) [19], as follows:

The precision of the method was measured as the standard deviation (SD) of the mean difference between estimated age and documented age. Bias (*i.e.*, systematic error) was computed using the mean error (ME) [19]:

All statistical analyses were performed with IBM® SPSS® (version 19.0.0), and Microsoft® Excel® (version 14.2.1).

Results

There was a strong positive linear dependency between documented age at death and estimated age at death in both samples and sexes. In the CISC sample, females showed a higher Pearson product-moment correlation coefficient (*Pearson’s r*=0.732; *p*≤0.001) when compared to males (*Pearson’s r*=0.574; *p*≤0.001). On the contrary, in the Santarém XXI study base, the estimated correlation coefficient in men (*Pearson’s r*=0.803; *p*≤0.001) exceeded that of the women (*Pearson’s r*=0.704; *p*≤0.001).

The paired sample t-tests showed a significant difference between the means of documented versus estimated ages in the CISC women (*t*=-2.860, *df*=49,

p=0.006), the Santarém XXI women (*t*=6,145; *df*=19; *p*≤0.001), and the Santarém XXI men (*t*=4.821; *df*=19; *p*≤0.001). There was no significant difference between estimated and documented values in the CISC male group (*t*=-1,172; *df*=49; *p*=0.247).

Mean absolute error (expressing the accuracy of the method) in the CISC sample varied between 11.1 years in the female group and 12.9 years in the male group. In both sexes there was a decrease of mean inaccuracy in older age categories. In the younger age category (20-39 years), MAE values ranged between 14.8 (males) and 17.1 (females). In the intermediate age group (40-59 years), MAE was 11.4 in men and 10.5 in women, whilst in the older age category (60+ years); the mean absolute error was 9.8 in men and 8.2 in women. In the males group, across all age categories, 28% of age estimates were within ± 5 years of documented age; 52% within ± 10 years of documented age; and 76% within ± 15 years of documented age. In the female set, across all age groups, 30% of age estimates felled within ± 5 years of acknowledged age; 60% within ± 10 years of known age; and 78% within ± 15 years of documented age (Table 1).

Accuracy in the Santarém XXI sample ranged from 11.9 years in men and 12.7 years in women. Overall, in women, 20% of age estimates were within ± 5 years of known age; 35% within ± 10 years of documented age; and 55% within ± 15 years of documented age. In the men's group, across all age categories, 35% of age estimates felled within ± 5 years of acknowledged age; 45% within ± 10 years of documented age; and 65% within ± 15 years of documented age (Table 1).

The standard deviation (precision) of the mean difference between estimated age at death and documented age at death varied between 13.0 (women) and 14.5 (men), in the CISC sample; and between 8.4 (women) and 9.5 (men), in the Santarém XXI sample. As shown in Table 2, the precision of the method increased in the older age categories (for the CISC sample).

In the CISC sample, mean error (bias, incorporating the direction of the error) was 2.6 years in the male group of CISC; and 5.4 years in the female group. That is to say, the method overestimates age in both sexes in the sample as a whole (age range of the sample: 20 – 95 years). The difference between estimated age at death and documented age at death for each individual is expressed in Table 3 and Figures 1-4. The figures show that, in both sexes, the Fernández Castillo and López Ruiz method [14] tended to overestimate age in the younger (20-39 years; Female's ME= 16.2/ Men's ME= 14.8) and intermediate age groups (40-59 years; Female's ME= 9.2/ Men's ME= 5.9). In the older age category (60+ years) there was an underestimation of age at death (Female's ME= -4.0/ Men's ME= -8.0). Mean error in the Santarém XXI sample was -11.1 years in the men's group, and -11.9 years in the women's group. Older individuals prevail in this sample. As such, an overall propensity for underestimation of age at death was discernible. Nonetheless, the underestimation of age at death also occurred in the few younger male individuals of the sample.

Discussion

Results show that projected age (estimated with the Fernández Castillo and López Ruiz regression formulae) and known age are linearly correlated in both testing samples, ultimately reflecting the universal pattern of BMD decline with age [12,13]. Nevertheless, results also express a statistical difference between the means of the documented versus the estimated ages in all but one group (men in the CISC study base). Moreover, the mean absolute difference between estimated age and documented age always surpasses 10 years. Mean absolute difference conveys how well a method performs on average [23] but it is important to remind that, in a substantial fraction of individuals of both samples, estimated age fall outside ± 15 years of documented age. In fact, the precision (a proxy of the statistical variance of an estimation [19]) of the method, considered as the standard deviation of the mean difference, was modest in both samples and sexes – although there is a tendency to an increase of the precision in older age categories.

Accurate estimation of human adult age at death has always been a difficulty for anthropologists or forensic scientists, and the high variability of physiological age indicators is the primary contributing factor for this issue [3,20,24]. Bone mass at any time in adult life reflects the peak investment in bone mineral at skeletal maturity minus that which has been subsequently lost [25]. Although BMD declines with age in all populations [12,13], the nature of the decline displays an undeniable amount of variation, both within and amongst populations, being influenced by genetic and environmental factors [25,26]. Therefore, the observed differences between documented age at death and predicted age at death were expected to reflect the inter- and intra-population variation in BMD decline.

In the CISC sample (which has a more thorough age range than the Santarém XXI study base), the accuracy of the method is somewhat improved in older individuals – an uncommon outcome in other age estimation techniques [e.g., 7,27,28]. Peak bone mass – the maximum quantity of bone acquired during growth – is affected by genetics and also by sociocultural behaviors (including nutrition and physical activity) while bone loss later in life is mostly controlled by genetic factors [25,32].

This method tends to overestimate age at death in younger individuals, and to underestimate it in the older individuals – a phenomenon previously observed in a vast array of age estimation techniques [e.g., 27-31]. Masset [29] christened this trend as ‘‘the attraction of the middle.’’ He attributed it to the particular age distribution of the reference sample used to construct any age estimation methodology, but it has been suggested that this error is, to a degree, the result of the statistical procedure used to estimate chronological age from biological age predictors, viz. linear regression with age as the dependent variable [24,33,34]. The underestimation of age at death in the few younger individuals of the Santarém sample (mostly males) is probably just an outcome of individual variation of BMD expression.

Another potential source of error is related to the assessment of BMD at Ward’s area. In the realm of densitometry, Ward’s area is defined as a computed region of low density and does not refer to a specific anatomical region [35]. It is not used for osteoporosis diagnosis since the modest precision of BMD measurements at this location seriously hampers its

diagnostic power [36,37]. We can extrapolate that this low precision of BMD measurements at Ward's area will generate further problems to the estimation of age using bone densitometry in this proximal femur region. Also, Fernández Castillo and López Ruiz [14] used a hospital sample (i.e., living subjects) to acquire BMD values. Ordinarily, in forensic or archaeological settings, the femur lacks both marrow and covering soft tissues, which slightly complicates comparisons between dead and living individuals [38]. Also, the possible influence of diagenesis (e.g., chemical or microstructural modifications of bone) on BMD can hinder the use of DXA to estimate age at death both in archaeological and forensic contexts [39]. Notwithstanding, there is some evidence that, even in bones with established diagenetic change, bone mineral content is inconsequentially affected [39,40].

The application of the Fernandez Castillo and López Ruiz method [14] on two Portuguese skeletal samples produced a mean absolute error always superior to 10 years – this level of accuracy may not be suitable for a forensic analysis – and also revealed a modest precision. Furthermore, the technique consistently overages younger individuals and underestimates age in older ones. Notwithstanding, the method seems to perform as well as, or better than, other extensively tested techniques [see, e.g., 7,27,28,30,31,41] making it an alternative to the seemingly more accurate dental age estimation methods [28,42-45]. Different parts of the skeleton show differential survival rates, which may limit the effectiveness of some age estimation techniques [20,21], but the femur is often well preserved [22]. As such, this methodology can be successfully applied when other parts of the body, which act as better age predictors, are absent. This situation is not infrequent, such as in cases of mass disasters or even in contexts of mass graves with commingled remains.

Conclusion

The reliability of any age determination technique depends on the correspondence between biological age and chronological age [3,6] and although BMD shows a good correlation with age, it also displays a definite degree of intra- and inter-population variability. The application of the Fernandez Castillo and López Ruiz methodology [14] is simple (albeit somewhat constrained by the availability of a densitometer), and reproducible in skeletal remains. In a way these results elaborate the well-known method proposed years ago by Acsadi and Nemeskeri [46], but uses a much more reliable and precise technology for bone mass measurement.

No single age predictor reflects accurately the multiplicity of factors that affect biological age [27]. Therefore, when estimating age at death it is appropriate to use as many indicators of age as possible, including BMD decline.

References

[1] S. Ritz-Timme, C. Cattaneo, M.J. Collins, E. R. Waite, H. W. Schütz, H.-J. Kaatsch, H. I. M. Borrman, Age estimation: the state of the art in relation to the specific demand of forensic practice, Int. J. Legal. Med. 113 (2000) 129–136.

[2] E. Cunha, E. Baccino, E. Martrille, F. Rahmstaler, J. Prieto, Schuliar, Y, N. Lynnerup, C. Cattaneo. The problem of aging human remains and living individuals: A review, Forensic Sci. Int. 193 (2009) 1-13.

[3] E. Baccino, A. Schmitt, Determination of adult age at death in the forensic context, in: A. Schmitt, E. Cunha, J. Pinheiro. (Eds.), Forensic anthropology and medicine, Humana Press, Totowa, 2006, pp. 259-280.

[4] R.D. Hoppa, Paleodemography: looking back and thinking ahead, in: R.D. Hoppa, J.W. Vaupel (Eds.), Paleodemography: age distribution from skeletal samples, Cambridge University Press, Cambridge, 2002, pp. 9-28.

[5] A. Kemkes-Grottenthaler, Historical perspectives on age indicator methods, in: R.D. Hoppa, J.W. Vaupel (Eds.), Paleodemography: age distribution from skeletal samples, Cambridge University Press, Cambridge, 2002, pp. 48-72.

[6] M. Jackes, Building the bases for paleodemographic analysis: adult age determination in: A. Katzenberg, S. Saunders, S (Eds.), Biological Anthropology of the human skeleton. Wiley Liss, New York, 2000, pp. 417-466.

[7] L. Martrille, D.H. Ubelaker, C. Cattaneo, F. Seguret, M.Tremblay, E. Baccino, Comparison of four skeletal methods for the estimation of age at death on white and black adults, J. Forensic Sci. 52 (2007) 302-307.

[8] B.P. Wheatley, An evaluation of sex and body weight determination from the proximal femur using DXA technology and its potential for forensic anthropology, Forensic Sci. Int. 147 (2005) 141–145.

[9] U. Chinappen-Horsley, G.M. Blake, I. Fogelman, T.D. Spector, A method for determining skeletal lengths from DXA images, BMC Musculoskelet. Disord. 8 (2007) 103–113.

[10] A. Galloway, P. Willey, L. Snyder, Human bone densities and survival of bone elements: a contemporary sample, in: W. Hagland, M. Sorg (Eds.), Forensic taphonomy: the postmortem fate of human remains, CRC Press, Boca Raton, FL, 1997, pp. 295-317.

[11] E. Lewiecki, N. Watts, M. Mcclung, S. Petak, L. Bachrach, J. Shepherd, R. Downs JR., Official Positions of the International Society for Clinical Densitometry, J. Clin. Endocrinol. Metab. 89 (2004) 3651-3655.

[12] T. De Villiers, Bone health and osteoporosis in postmenopausal women. Best Pract. Res. Cl. Ob. 23 (2009) 73-85.

[13] S. Kaptoge, J. Silva, K. Brixen, D. Reid, H. Kröger, T. Nielsen, M. Andersen, C. Hagen, R. Lorenc, S. Boonen, M-C. Vernejoul, J. Stepan, J. Adams, J-M. Kaufman, J. Reeve, Geographical variation in DXA bone mineral density in young European men and women. Results from the Network in Europe on male osteoporosis (NEMO) study. Bone. 43 (2008) 332-339.

[14] R. Fernández Castillo, M.C. López Ruiz, Assessment of age and sex by means of DXA bone densitometry: Application in forensic anthropology, Forensic Sci. Int. 209 (2011) 53-58.

[15] B.M. Usher, Reference samples: the first step in linking biology and age in the human skeleton, in: R.D. Hoppa, J.W. Vaupel (Eds.), Paleodemography: age distribution from skeletal samples, Cambridge University Press, Cambridge, 2002, pp. 29-47.

[16] E. Cunha, S. Wasterlain, The Coimbra identified osteological collections, in: G. Grupe, J. Peters (Eds.), Skeletal series and their socio-economic context, Rahden/Westf., Verlag Marie Leidorf GmbH, 2007, pp. 23-33.

[17] S. Bréban, C. Benhamou, C. Chappard, Dual-Energy X-ray absorptiometry assessment of tibial mid-third bone mineral density in young athletes, J. Clin. Densitom. 12 (2009) 22-27.

[18] J. McEwan, S. Mays, G. Blake, measurements of bone mineral density of the radius in a medieval population. Calcified Tissue Int. 74 (2004) 157-161.

[19] B.A. Walther, J.L. Moore, The concepts of bias, precision and accuracy, and their use in testing the performance of species richness estimators, with a literature review of estimator performance, Ecography 28 (2005) 815-829.

[20] S. Purves, L. Woodley, L. Hackman, Age determination in the adult, in: S. Black, E. Ferguson (Eds.), Forensic Anthropology – 2000 to 2010, CRC Press, Boca Raton, FL, 2011, pp. 29-59.

[21] C. Rougé-Maillart, B. Vielle, N. Jousset, D. Chappard, N. Telmon, E. Cunha, Development of a method to estimate skeletal age at death in adults using the acetabulum and the auricular surface on a Portuguese population, Forensic Sci. Int. 188 (2009) 91-95.

[22] S. Mays, Taphonomic factors in a human skeletal assemblage. Circaea 9 (1992) 54-58.

[23] S.P. Nawrocki, The nature and sources of error in the estimation of age at death from the skeleton, in: K.E. Latham, M. Finnegan (Eds.), Age estimation of the human skeleton, CC Thomas, Springfield, IL, 2010, pp. 79-101.

[24] R.G. Aykroyd, D. Lucy, A.M. Pollard, T. Solheim, Technical note: regression analysis in adult age estimation, Am. J. Phys. Anthropol. 104 (1997) 259–265.

[25] R. Marcus, M. Bouxsein, The nature of osteoporosis. in: R. Marcus, D. Feldman, D. Nelson, C. Rosen (Eds.), Osteoporosis. Academic Press, San Diego, 2007, pp. 27-36.

[26] A. Woolf, K. Akesson, An atlas of investigation and management of osteoporosis. Clinical Publishing, Oxford, 2008

[27] S.R. Saunders, C. Fitzgerald, T. Rogers, C. Dudar, H. McKillop. A test of several methods of skeletal age estimation using a documented archaeological sample. Can. Soc. Forensic Sci. J. 2 (1992) 97–118.

[28] A. Meinl, C.D. Huber, S. Tangl, G.M. Gruber, M. Teschler-Nicola, G. Watzek, Comparison of the validity of three dental methods for the estimation of age at death. Forensic Sci. Int. 178 (2008) 96–105.

[29] C. Masset, Age estimation based on cranial sutures, in: İşcan (Ed.), Age markers in the human skeleton, CC Thomas, Sprinfield, IL, 1989, pp. 71-103.

[30] S.E. Calce, T.L. Rogers, Evaluation of age estimation technique: testing traits of the acetabulum to estimate age at death in adult males, J. Forensic Sci. 56 (2011) 302-311.

[31] S.M. Hens, E. Rastelli, G. Belcastro, Age estimation from the human os coxa: a test on a documented Italian collection, J. Forensic Sci. 53 (2008) 1040-1043.

[32] J. Plochocki, Mechanically-induced osteogenesis in the cortical bone of pre- to peripubertal stage and peri- to postpubertal stage mice. J. Orthop. Surg. Res. 4 (2009) 22-26.

[33] D. Lucy, A.M. Pollard, Further comments on the estimation of Error Associated with the Gustafson dental Age Estimation Method, J. Forensic Sci. 40 (1995) 222-227.

[34] D. Lucy, R.G. Aykroyd, A.M. Pollard, Nonparametric calibration for age estimation, Appl. Statist. 51 (2002) 183-196.

[35] S. Bonnick, Bone densitometry in clinical practice - Application and interpretation, Humana Press, Totowa, 2010.

[36] C. Njeh, J. Shepherd, Absorptiometric measurement, in: C. Langton, C. Njeh (Eds.), The physical measurement of bone, Institute of Physics Publishing, Bristol & Philadelphia, 2004, pp. 267-307.

[37] V. Tavares, H. Canhão, J. Gomes, E. Simões, J. Romeu, P. Coelho, R. Santos, A. Malcata, D. Araújo, C. Vaz, J. Branco, Recomendações para o diagnóstico e terapêutica da osteoporose. Acta Reumatol. Port. 32 (2007) 49-59.

[38] S. Mays, G. Turner-Walker, U. Syversen, Osteoporosis in a population from medieval Norway. Am. J. Phys. Anthropol. 131 (2006) 343-351.

[39] S. Mays, Radiography and allied techniques in the paleopathology of skeletal remains, in: R. Pinhasi, S. Mays (Eds.), Advances in human palaeopathology, John Wiley & Sons, Chichester, 2008, pp. 77-100.

[40] G. Turner-Walker, U. Syversen, Quantifying histological changes in archaeological bones using BSE-SEM image analysis. Archaeometry. 44 (2002): 461-468.

[41] C. Rissech, J. Wilson, A.P. Winburn, D. Turbón, D. Steadman, A comparison of three established age estimation methods on an adult Spanish sample, Int. J. Legal Med. 126 (2012) 145-155.

[42] S. Ohtani, Studies on age estimation using racemization of aspartic acid in cementum, J. Forensic Sci. 40 (1995) 805–807.

[43] R. Cameriere, L. Ferrante, M.G. Belcastro, B. Bonfiglioli, E. Rastelli, M. Cingolani, Age estimation by pulp/tooth ratio in canines by peri-apical X-rays, J. Forensic Sci. 52 (2007) 166–170.

[44] R.C. Griffin, A.T. Chamberlain, G. Hotz, K.E. Penkman, M.J. Collins, Age estimation of archaeological remains using amino acid racemization in dental enamel. Am. J. Phys. Anthropol. 140 (2009) 244-252.

[45] U. Wittwer-Backofen, J. Gampe, J.W. Vaupel, Tooth cementum annulations for age estimation: results from a large known-age validation

study, Am. J. Phys. Anthropol. 123 (2004) 119–129.

[46] G. Acsadi, J. Nemeskeri, History of human lifespan and mortality, Akademiai Kiado, Budapest, 1970.

Figure captions:

Figure 1: Difference in years between documented age at death and estimated age at death for each female in the CISC sample.

Figure 2: Difference in years between documented age at death and estimated age at death for each male in the CISC sample.

Figure 3: Difference in years between documented age at death and estimated age at death for each female in the Santarém XXI sample.

Figure 4: Difference in years between documented age at death and estimated age at death for each male in the Santarém XXI sample.

Figure 1

Click here to download high resolution image

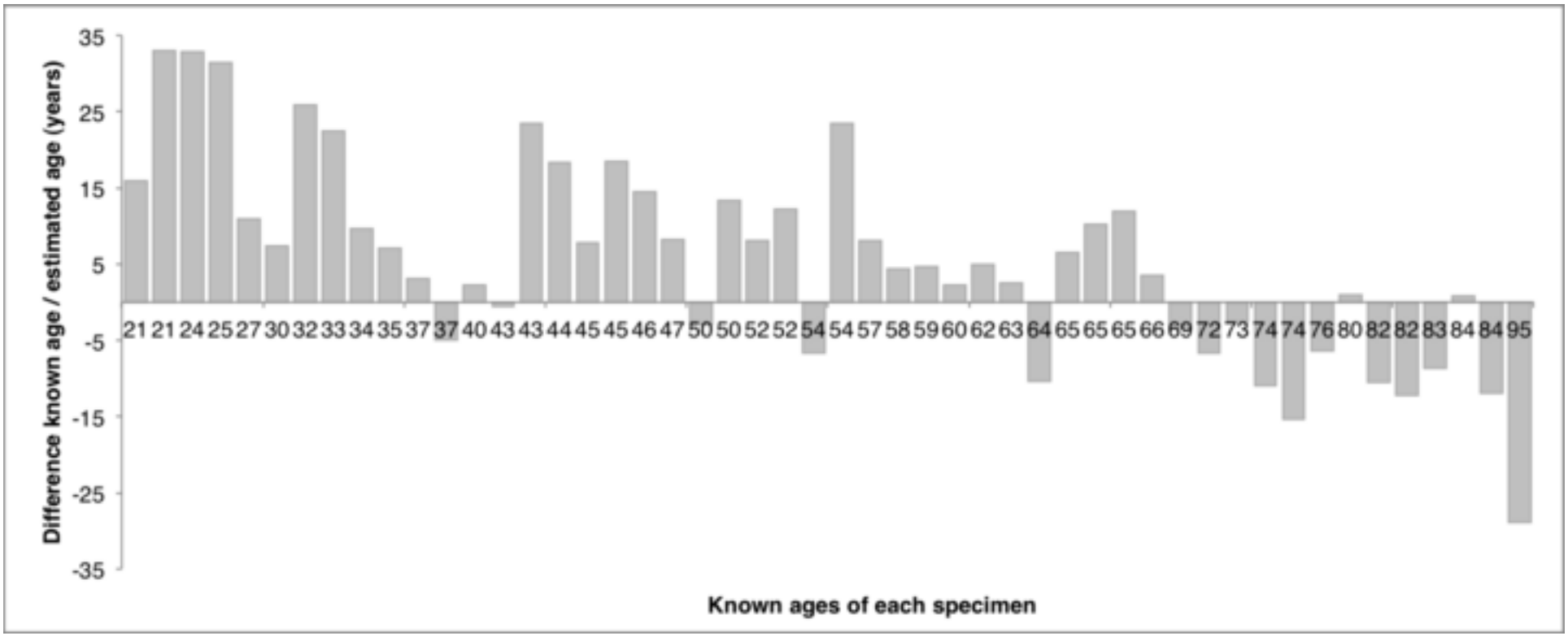

Figure 2

Click here to download high resolution image

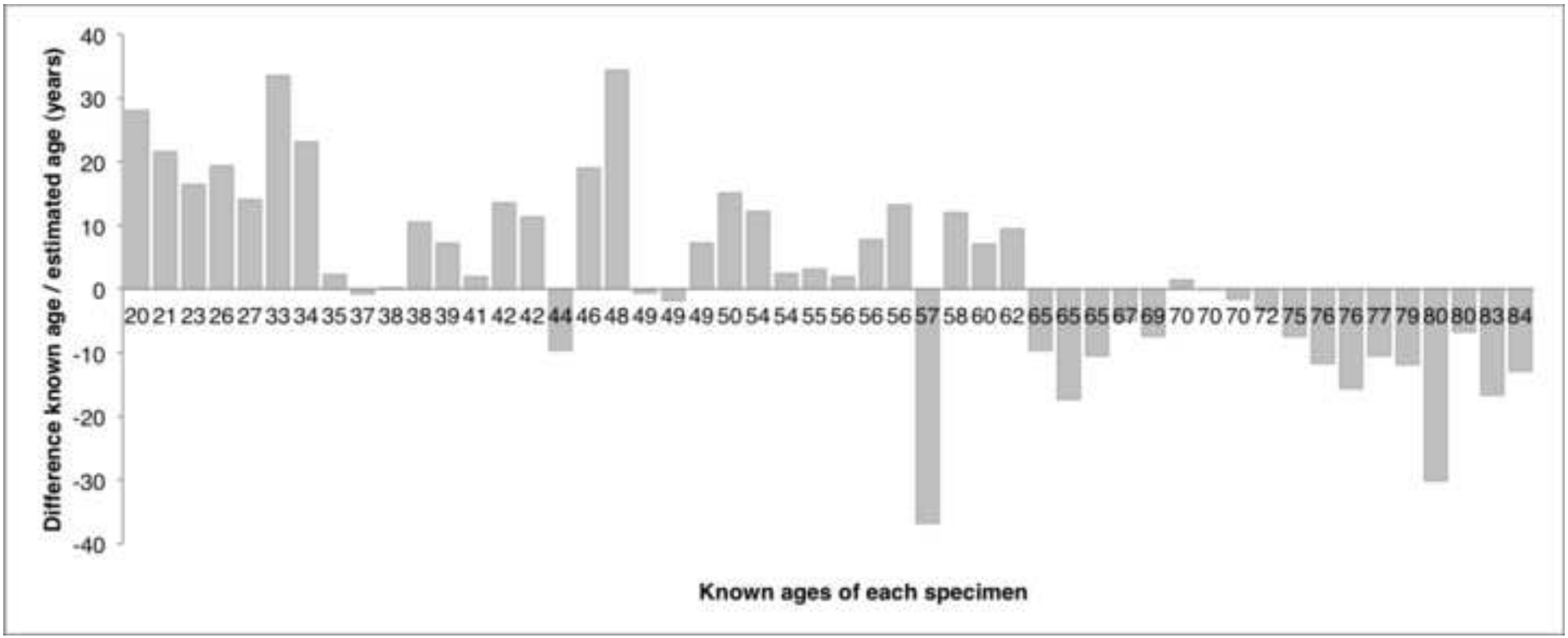

Figure 3

Click here to download high resolution image

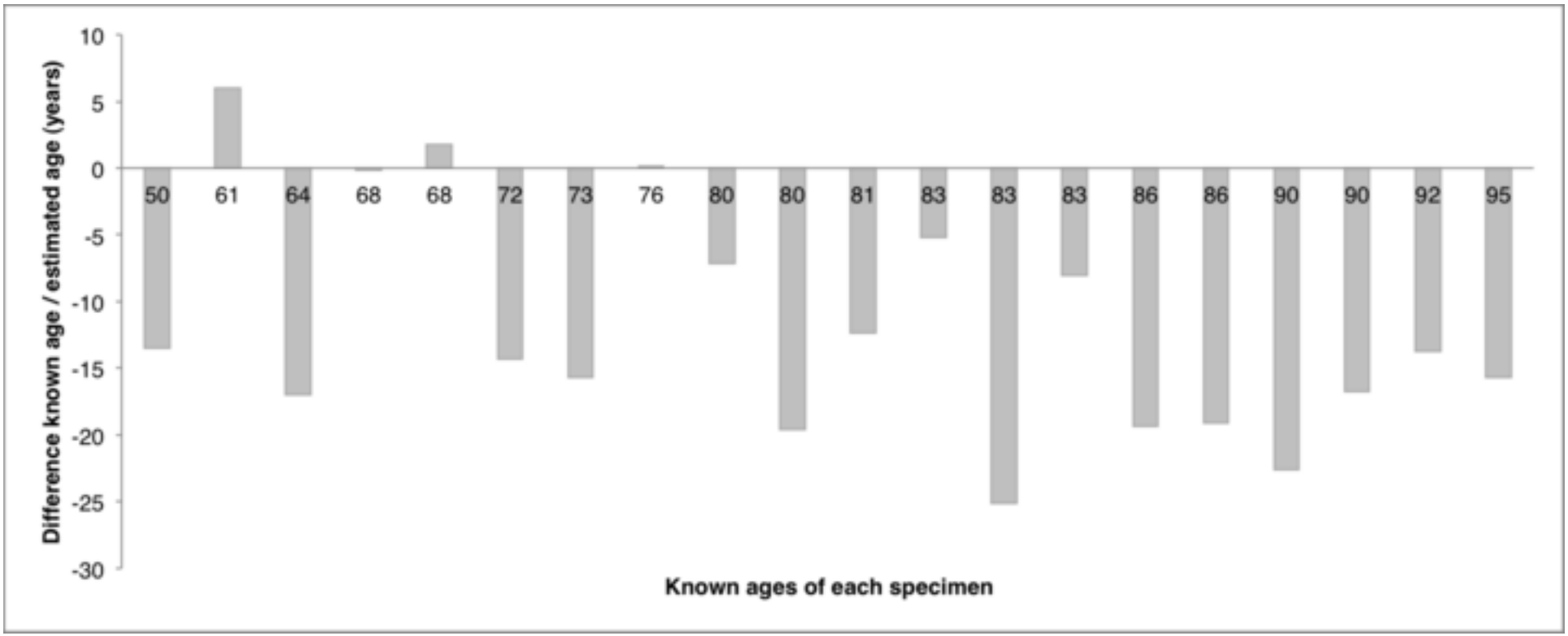

Figure 4

Click here to download high resolution image

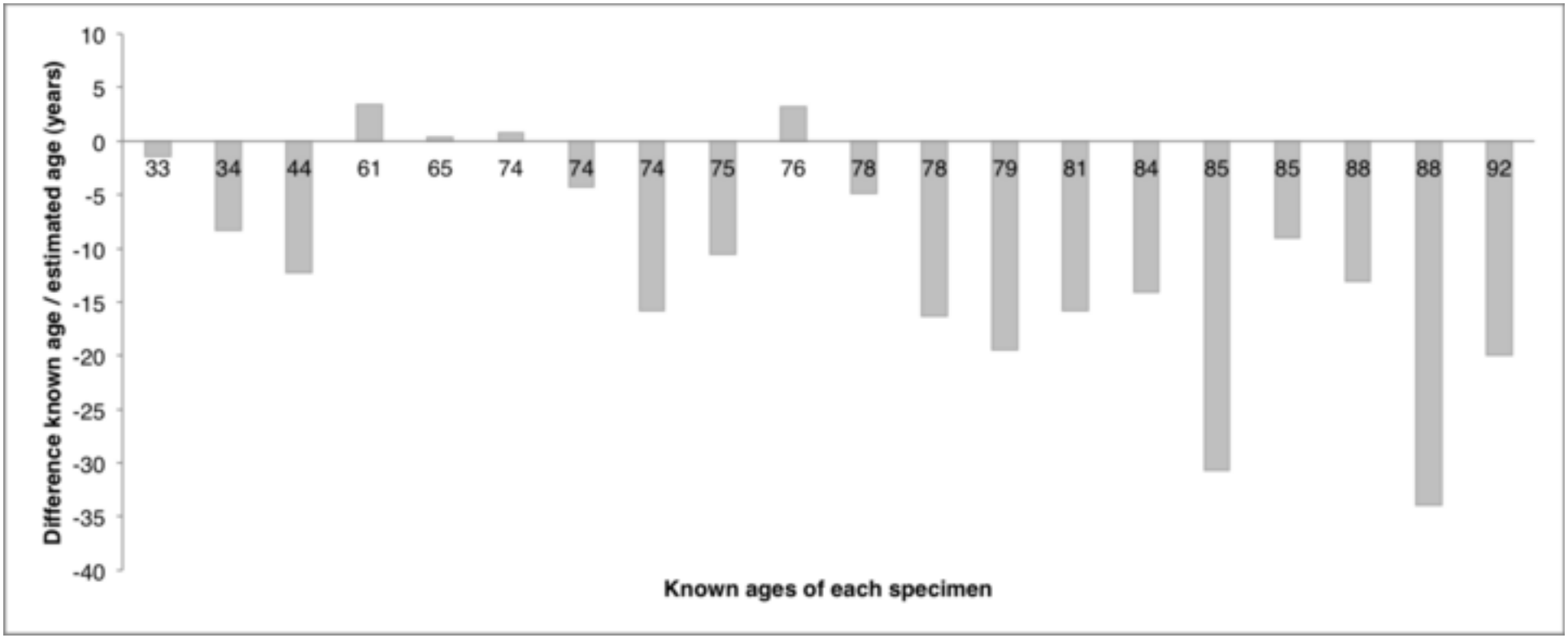

Table 1

Table 1: Mean absolute error (accuracy) in the younger, middle, older age categories and all ages (CISC and Santarém XXI).

Accuracy		MAE (males/females)
Age groups		
20-39	CISC	14.8 / 17.1
	Santarém XXI	---
40-59	CISC	11.4 / 10.5
	Santarém XXI	---
60+	CISC	9.8 / 8.2
	Santarém XXI	---
All ages	CISC	12.9 / 11.1
	Santarém XXI	11.9 / 12.7

Table 2

Table 2: Precision in the younger, middle, older age categories and all ages (CISC and Santarém XXI).

Precision		SD (males/females)
Age groups		
20-39	CISC	10.4 / 11.0
	Santarém XXI	---
40-59	CISC	10.1 / 6.9
	Santarém XXI	---
60+	CISC	8.8 / 6.2
	Santarém XXI	---
All ages	CISC	14.5 / 13.0
	Santarém XXI	9.5 / 8.4

Table 3

Table 3: Mean error (bias) in the younger, middle, older age categories and all ages (CISC and Santarém XXI).

Bias		ME (males/females)
Age groups		
20-39	CISC	14.8 / 16.2
	Santarém XXI	---
40-59	CISC	5.9 / 9.2
	Santarém XXI	---
60+	CISC	- 8.0 / - 4.0
	Santarém XXI	---
All ages	CISC	2.6 / 5.4
	Santarém XXI	- 11.1 / - 11.9

***Revision Notes**

REPLY TO THE REVIEWERS

Dear Reviewer #1,
Thank you for the kind remarks and helpful comment. We have included a short characterization of the DXA method in the Introduction (pp. 1-2, second paragraph), hoping to best cope with your appeal.
Our best regards,
The authors

Dear Reviewer #2,
Thank you for the insightful commentaries and advises. To account your first concern, we added more detail to the description of both the skeletal collections, namely the place of interment and the probable number of years that the bodies were buried (section Materials and Methods, p. 2, first paragraph). We also briefly discussed diagenetic changes in relation to BMD (section Discussion, p.6, first paragraph). We abstained to comment the observed underaging in the Santarém group since the youngest (age at death: 33 and 34 years) in this sample are just two individuals and the age range is much more restricted than in the CISC sample. As such, the observed “pattern” is probably just the consequence of individual variation of BMD values. Anyway, we highlight this situation in page 5 (last paragraph before the Discussion) and succinctly discuss the matter in page 6, first paragraph (section Discussion). Finally: Table 1 obviously does not belong to this paper. We are very sorry for this lapse, which has been corrected. We hope that our answers can dismiss your concerns.
Our best regards,
The authors