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Application of the recent SanMillán-Rissech acetabular adult aging method in a North American sample

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Abstract:	Recently, a renewed acetabular aging methodology was published by San-Millán et al. (Int J Leg Medicine, 2017a, 131: 501-525), refining the variables associated with acetabular fossa aging in different populations. Due to its novelty, this method has not yet been examined in any other population, other than it was developed and originally tested on. Therefore, the main goals of this study are two-fold: 1) to evaluate the accuracy of SanMillán-Rissech's method in a North American sample made up of 826 white (456 males and 370 females) and 54 black (46 males and 8 females) individuals from the Bass Collection and, 2) to determine whether the revised methodology shows higher rates of accuracy than the original methodology (J Forensic Sci, 2006, 51(2): 213-229). Scores obtained by both methodologies were analyzed via a Bayesian statistical program (IDADE2) that estimates a relative likelihood distribution for the target individuals, produces age-at-death estimates and provides 95% confidence intervals. Even though the revised method was developed using a Western European collection, the results demonstrate that it is also applicable to North American samples with reasonable accuracy results, i.e. an average absolute error of 7.19 years in white males and 9.65 years in white females. However, accuracy in females is significantly lower than in males, likely due to their higher morphological variability associated with different factors other than age. The significantly better performance of the revised methodology compared with the original, is also been confirmed by the current findings from this North American sample, supporting the renewed system as a better aging methodology. Although work on further populations is needed, previously and current results should encourage professionals to include the acetabular method in forensic and archaeological laboratories routines.
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	He has recently published interesting papers regarding acebular methodology, aging and factors other than age. He has a good undestanding of the topic together with a wide experience in academic and field contexts.
	Allysha Winburn University of Florida apwinburn@gmail.com Her actual affiliation is University of West Florida. She have explored different age markers, including the acetabulum in detail. She knows the original and the revised methodologies and we believe she could give an interesting feed back to enhance the publication.
	Molly Miranker mm6184@nyu.edu Her current affiliation is New York University, Department of Anthropology. She have recently published acetabular data estimations so she trully knows the methodology and the challenging estimation based on acetabulum.
	Bridget Algee-Hewitt SENIOR RESEARCH SCIENTIST, Stanford University bridget.algee-hewitt@stanford.edu She is an experienced biological anthropologist focused on skeletal and genetic information with an emphasis on understanding patterns of human variation for uses in human identification. Also she is forensic practitioner and most recently she has been leading a project on computational methods for age-at-death estimation from laser scans. We believe she could give a wide, useful and innovative perspective to the current paper on age estimation.

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Dear Dra. Pfeiffer Editor of International Journal of Legal Medicine,

enclose you can find the manuscript entitled APPLICATION OF THE RECENT SANMILLÁN-RISSECH ACETABULAR ADULT AGING METHOD IN A NORTH AMERICAN SAMPLE to be considered for publication in the International Journal of Legal Medicine as a research paper.

This manuscript has not been published or submitted elsewhere for publication, nor has the data any overlap with other material published or in press. The authors, Marta San Millán, Carme Rissech and Daniel Turbón are aware that the manuscript is now being submitted for publication in this Journal.

Sincerelly,

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Application of the recent SanMillán-Rissech acetabular adult aging method in a North American sample

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Abstract

Recently, a renewed acetabular aging methodology was published by San-Millán et al. (Int J Leg Medicine, 2017a, 131: 501-525), refining the variables associated with acetabular fossa aging in different populations. Due to its novelty, this method has not yet been examined in any other population, other than it was developed and originally tested on. Therefore, the main goals of this study are two-fold: 1) to evaluate the accuracy of SanMillán-Rissech's method in a North American sample made up of 826 white (456 males and 370 females) and 54 black (46 males and 8 females) individuals from the Bass Collection and, 2) to determine whether the revised methodology shows higher rates of accuracy than the original methodology (J Forensic Sci, 2006, 51(2): 213-229). Scores obtained by both methodologies were analyzed via a Bayesian statistical program (IDADE2) that estimates a relative likelihood distribution for the target individuals, produces age-at-death estimates and provides 95% confidence intervals. Even though the revised method was developed using a Western European collection, the results demonstrate that it is also applicable to North American samples with reasonable accuracy results, i.e. an average absolute error of 7.19 years in white males and 9.65 years in white females. However, accuracy in females is significantly lower than in males, likely due to their higher morphological variability associated with different factors other than age. The significantly better performance of the revised methodology compared with the original, is also been confirmed by the current findings from this North American sample, supporting the renewed system as a better aging

methodology. Although work on further populations is needed, previously and current results should encourage professionals to include the acetabular method in forensic and archaeological laboratories routines.

Keywords Acetabulum, aging, age-at-death estimation, North American population, accuracy

Introduction

Due to the high variability in aging within and between populations, age-at-death estimation is one of the most difficult tasks of forensic or archaeological studies. Particularly in forensics, having documented collections representing the current living population is extremely important, as this is the population from which forensic cases are derived. Through these types of diverse collections, which at a minimum provide data on sex, age and geographical origin, researchers have been able to better understand the biological processes that change the morphology of age markers during the aging process. These collections have also provided better methodologies for estimating the age-at-death of adult individuals. Although different anatomical areas have been explored to create diverse aging methodologies over time [1-13], the pelvis, and specifically its three joint surfaces, have been the most analyzed, studied, used and accepted by forensic anthropologists and bioarchaeologists. More specifically, the two most analyzed pelvic joints are the pubic symphysis [1, 14-22] and the auricular surface [23-26. The hip joint, or acetabulum, was not investigated as an age marker until the beginning of the current century [27-29].

Based on this previous, the research published by Rissech *et al.* (2006) is currently the most established and well-known acetabular male-specific methodology for age estimation within the scientific community [30-31]. Since this first published methodology, new researchers explored the acetabulum, tested in different populations and created and published new methodologies [32-45]. Due to the high variability aging has on joint surfaces, there are concerns about the applicability of these new age estimation techniques being used on ancestral populations different from the one used to create the methodology. For instance, researchers have tested Rissech's acetabular methodology on different populations with mixed results. Some researchers have found the method to be fair [31, 33, 39], while others have documented poor and weak age estimation results [34-37]. With the intention of extending the application of the acetabular method to both sexes and in response to some critical reviews regarding Rissech's variables 5, 6 and 7, which were related to acetabular fossa [35-37], San-Millán and colleagues have recently revised Rissech's methodology [42]. They provided novel data and a renewed system to determine age, called the SanMillán-Rissech's method [42].

The SanMillán-Rissech's method was created from the analysis of 611 Portuguese individuals, from the Lisbon Collection [46]. The results of this first study demonstrated the suitability of combining acetabular traits and a Bayesian approach to estimate age in adults of both sexes in a Western European sample, and extended the acetabular method's applicability to females. Approximately 74 % of the sample studied had an absolute error less than 10 years, with averages of 7.28 years and 7.09 years for males and females respectively. Also, with better defined and more detailed descriptions and images, good levels of consistency were demonstrated in every renewed variable, with 75% representing an almost perfect agreement between repetitions or observers for the different traits considered [42]. Further, corroborating results from Mays [36], the study revealed that even though the acetabular aging process follows similar trends in both sexes, acetabular traits in males age faster comparable to females, who have a slower aging rate. This supports the recommendation that the sexes be analyzed separately, with sex-specific reference samples.

Due to its novelty, SanMillán-Rissech's method has not been applied or tested beyond the Lisbon Collection where it was developed. Therefore, the main goal of the present study is to extend our research to non-European populations and expanding its applicability to different samples representing diverse continents and ancestors. Therefore, the present study evaluated the SanMillán-Rissech's age estimation methodology on the Bass Collection, a forensic sample population from the United States.

The purpose of this study is twofold: 1) To evaluate the revised method's applicability in a sample from the United States of America (USA); and 2) To verify how differently the revised method [42] performs, in relation to the original [30]. The first goal was achieved by investigating possible differences in accuracy between sexes, ancestors (i.e. individuals who identify as black or white) and geographical origin (implemented by the comparison of the current results obtained in the USA sample with previous findings on Lisbon Collection [42]) while the second goal investigated possible differences, in terms of accuracy, between the revised [42] and the original [30] methodologies applied to the same USA sample.

Material and methods

Material

The osteological sample comes from a unique documented skeletal collection: the William Bass Donated Skeletal Collection housed in the Forensic Anthropology Center of University of Tennessee (Knoxville, Tennessee, United States). The William Bass collection has an established body donation program and it now consists of more than 1000 individuals, the largest collection of modern human skeletons in the United States. This collection also has the advantage of including individuals with different ancestries, i.e. European (Caucasians, referred as *whites*), African (Negroid, referred as *blacks*) and some Asian (Mongoloid) individuals. All the individuals analyzed in this study died in the late 20th, and the first years of 21th century, specifically between the years 1977 and 2013 [47].

From this collection, male and female individuals with completely fused acetabulums, were chosen for the analysis. Specimens with evident pathologies affecting the acetabulum were not included. However, individuals with non-inflammatory osteoarthritis or diffuse idiopathic skeletal hyperostosis (DISH) were included, as both of these conditions are related to age [48-49]. Under these criteria, 826 white (456 males and 370 females from 15 to 101 years of age) and 54 black (46 males and 8 females from 23 to 99 years of age) North American individuals were analyzed. North Americans with Asian ancestry were not included because the sample size available was not sufficient for statistical analyses. Thus, the sample has an age range of 15 to 101 years. This wide age range was used to illustrate all the morphological changes that occur in the acetabular area during the human life span. Information regarding sex, age and ancestor distribution in this sample, is displayed in Table 1. From the whole sample, preferably the left *os coxae* were analyzed. The right side was evaluated when the left was damaged, pathological, or unavailable.

Methods

To carry out this study and achieve the goals, both the classic Rissech [30] and the new revised acetabular SanMillán-Rissech [42] methods were applied. The variables of Rissech's and SanMillán-Rissech's methods were described, illustrated and evaluated extensively by Rissech et al. [30-31] and San-Millán et al. [42], respectively. Any repeatability analysis was conducted here, because only minimal intra and inter observer variability were detected in the previous studies [30-31, 42], with very good to excellent levels of consistency. In addition, in both studies, the present study and the study of San-Millán et al. [42], the person who took the measurements was the same, first author of both papers, a fact that greatly reduces possible errors.

The seven variables of the original/classical method are: 1) acetabular groove, 2) acetabular rim shape, 3) acetabular rim porosity, 4) apex activity, 5) activity on the outer edge of the acetabular fossa, 6) activity of the acetabular fossa, and 7) porosities of the acetabular fossa. The revised system, besides incorporating some small points in the first four variables, has fully renewed variables from 5 to 7, and has changed the designation of variables 6 and 7 to "*texture and bone density in the center of the acetabular fossa*" and "*activity in the acetabular fossa*", respectively (for a detailed description see [30] and [42]). Therefore, in total, ten combined variables from the classic and renewed methods, were scored in both males and females, because, as previously explained, a similar acetabular aging pattern was observed in both sexes in previous analyses (43, 50).

After visual assessment, every individual was described by placing it into one of the several morphological states of each variable of both systems; i.e. since both methodologies roughly share variables 1 to 4, each acetabulum was assessed and classified in each of the total of 10 variables, without time lapse between systems. Age-at-death estimates for every test specimen were calculated by entering data in the custom specific software IDADE2, using frequencies in a Reference collection and the Bayesian inference methodology used by Rissech et al. [30] and described in detail by Lucy et al. [51]. The *a priori probability* of any 5-year age-at-death class was taken to be the fraction of individuals in the Reference collection in that age-at-death class. It was assumed that each individual whose age-at-death was estimated, is a sample of the population represented by the Reference collection and that each of the seven acetabular variables were independent of one another. An estimation of age-at-death takes the form of a probability distribution over 5-year wide age-at-death ranges: 15–19, 20–24, etc. A single year estimate of age-at-death was calculated as the expected value of this distribution, attributing to each age class its central age.

Males and females were analyzed separately with sex-specific Reference samples. As previously mentioned, although males and females share the same acetabular aging pattern, which allow us to use the same specific acetabular variables, rates of male acetabular aging is higher than that of females [36, 42, 50]. In other words, males age faster than females. Following San-Millán et al. [42], the white American sample was divided randomly into a Reference sample and *Test* sample. Black Americans were not separated, due to their small sample size. The Reference sample was always higher in number than the Test sample, in order to contain the maximum variability possible. Hence, white North Americans, 456 males were distributed into a Reference sample of 300 individuals and a *Test* sample of 156 individuals, while the 370 females were separated into a *Reference* sample of 220 individuals and a *Test* sample of 150 individuals.

To estimate the age of the black North American sample, due to the small sample size, the Reference sample of the white North Americans was used; specifically, the 300 white North American male Reference sample was used to age black males and the 220 white North American female Reference sample was used to age black females. Although it is known that to use a more biologically related sample would had been much better to estimate the age of this group of black North American individuals, it was not possible due to the scarcity of black individuals in the Bass collection. The white USA Reference sample defined in this study, 300 white males and 220 white females, were chosen to use as reference of the black USA sample, because it was the culturally and geographically closest available sample to the black USA sample. The results of this analysis are interesting, and provide valuable information on the use of different ancestral reference samples, for individuals who share a geographical area, state and culture.

Although the collected sample contains both black and white individuals, because of the low sample size of the black North American sample, the statistical results focus primarily on the white sample.

Statistical analyses

To investigate the success of the performance of the age estimation by the classical and the revised methods, average values of bias and absolute error were calculated and evaluated. Both parameters are considered good indicators of a method's inaccuracy [52]. Bias is the statistical measure that identifies the direction of the difference between the estimated and chronological ages [53-55], i.e. whether the age is over- (positive value) or underestimated (negative value). Bias was calculated as the average difference between estimated age and chronological age (∑ (estimated age - chronological age)/n). Absolute error is the statistical measure that evaluates the degree of the method's inaccuracy. Absolute error was calculated as the average absolute difference between estimated age and chronological age (∑(estimated age - chronological age)/n). This parameter does not take into account the sign (positive or negative) of the difference between estimated age and chronological age [53-55]. To analyze possible sex and ancestor-related differences in bias and absolute error, U Mann-Whitney tests were applied in each sample for each age group (<40 years, 40-64 years and > 65 years). These age intervals were used to follow the same methodological process as the previous study based on the Lisbon Collection [42], in order to make both studies comparable. In addition, these specific age groups were chosen to ensure reasonable sample size to statistical analyses, particularly in the youngest age group. We used a non-parametric test, because of the low sample size in some age intervals and the fact that not all of the intervals follow a normal distribution.

In the aim of investigating if the SanMillán-Rissech's method can maintain a similar accuracy in a North American sample, comparable to the Lisbon Collection, whose individuals were used to develop the revised methodology and whose results were previously published [42], accuracy data and bias values were compared through U Mann-Whitney statistical analyses. In this case, for comparison purposes, age estimations were made in white American individuals by using a white American Reference sample and comparing the results of the age estimation obtained in our previous study on Portuguese individuals [42], by using Portuguese Reference sample, in both sexes separately. The distribution of Lisbon individuals between the Reference and Test samples was the same that was used and described in San-Millán et al. [42].

Lastly, to evaluate how differently the revised method performed on a North American population, in relation to the original methodology, possible differences in bias and absolute error, when both methods were applied in the white North American Test sample, were tested in males and females separately by a Wilcoxon test of related samples. The age estimations of these two sexual series were based on their respective Reference samples: the white male North American Reference sample and the white female North American Reference sample, respectively, as was described previously in this section. The Wilcoxon test of related samples was used, since exactly the same individuals where evaluated in both methodologies and a normal distribution could not be assumed for some variables.

All the statistical analyses were performed by SPSS 21.0 software.

Results

Table 2 shows detailed accuracy values of each 10-year age interval, specifically bias and absolute error measurements for the white USA Test sample, when SanMillan-Rissech's method was performed using the white USA Reference sample. As mentioned in previous sections, the small Black North American sample size available and analyzed made it impossible to investigate accuracy with the same detail as white American samples. First age groups, especially in females, are either not represented or underrepresented in Table 2, due to the scarcity of individuals with this specific age-at-death in documented collections. Results indicate that while 75.3 % of the males analyzed were estimated with an absolute error lower than 10 years, only 59.7 % of the females had a similar absolute error range (Table 2). With respect to the number of individuals with an absolute error lower than 5 years, 45.5 % of the males and 31.5 % of the females were estimated with this specific accuracy (Table 2).

In relation to bias, both sexes had an average close to zero, specifically males -0.17 (underestimation on average) and females 0.96 (overestimation on average). Results show that age was overestimated in males until the 50-59 decade, and underestimated after 60 years, while females' breakpoint was positioned around 70 years of age, overestimating on average before this age and underestimating after it (Table 2). In concordance with results regarding 5 and 10-years-error percentage, the mean absolute error is lower in males than in females. Therefore, the revised methodology performs better in males compared to females, with 7.19 years vs 9.65 years of absolute error average respectively (Table 2). The best estimated males were between 20 and 40 years old and also in the seventh decade, while the worst were older than 80, especially in individuals older than 90 years of age (Table 2). In females, the mean absolute error did not differ as much as in males, with the best results in the third decade and between 60 and 80 years old and the worst values in the fourth decade and over 90 years of age (Table 2).

Statistical analyses of the possible bias and absolute error differences between sexes, in the revised acetabular method applied to whole North American Test samples, are shown in Table 3, separated by ancestry. In the white sample and following with the same pattern as in the previous tables, results indicate that in general for the bias and the absolute error, females had less accurate age estimations than males. However, these differences were significant only in the 40-64 age interval for both males and females (Table 3). In addition, these significant sexual differences were also observed in the absolute error, when the global sample was taken into account (Table 3). In the case of the black sample, and taking into account the small sample size available for the statistical analysis, no significant differences were found in any analyzed case.

Additionally, to determine the relevance of the ancestry of the Reference collection in the age-at-death estimation, white and black American accuracies, based on the same white American Reference sample, were compared in Table 4, separated by sex. Concerning this comparison, when possible, results of both sexes found no significant differences in any case, nor bias or absolute difference (Table 4). However, in the age interval of 40-64 years in males, the "p" value is near the significance value, indicating that in this age interval, black individuals were estimated with a higher error than whites.

In order to evaluate the differences in bias and absolute error between the white USA sample and the Lisbon Collection (white Portuguese Caucasians) using the revised method, a Mann-Whitney U test was conducted to compare the current results on white North American sample and our previous findings for Lisbon Collection [42]. Table 5 shows the comparison of the obtained accuracies when the revised method is performed in both white North American and Portuguese Test samples by using as reference the white North American Reference sample and the Portuguese Reference sample, respectively. As previously mentioned, both Test and Reference samples from Portugal are the same as those used in the past study [42]. The results related to bias demonstrated no significant differences between both populations, nor in males or in females, with the exception of females younger than 40 years of age. In this specific age interval, Portuguese bias is almost zero, while age estimation in North Americans was overestimated on average (Table 5). Results regarding absolute error were quite different (Table 5). While no significant differences were found in males, the revised methodology performed significantly better in Portuguese females than in American females (9.65 vs 7.09 years of difference on average between estimated and chronological age). This occurred in the same manner along all age intervals considered, with the exception of 40-64 years group, where the revised technique also performed better, but the differences were not significant (Table 5).

Finally, after having delved into the study of the revised method [42] applied to the whole North American sample, the effectiveness when compared with the original was verified [30]. For this reason, both methods (revised and original) were performed on the same individuals in the white North American Test sample, which was based on the same white North American Reference sample. Results are displayed in Table 6. Only the white North American sample was shown due to its suitable sample size. No significant differences were found in bias, males or females, the global sample or in the different age intervals; however, absolute error was significantly higher in the original method comparable to the revised method, when the global sample is taken into account, both in males and females independently (Table 6). Furthermore, in males older than 65 years old, the revised method performed significantly better than the original (Table 6).

Discussion

The results have demonstrated that, despite the fact that the renewed method was created based on a Western European skeletal sample, the age-at-death estimation by SanMillán-Rissech’s method in North American individuals were still reasonably accurate, demonstrating the suitability of the variables and descriptions of the method for distant populations. These findings were expected for the white North American sample, since they have a European origin. However, regarding the black population, whose origin is African, this study has confirmed the reasonable usefulness of this age marker to age diagnosis even given the inter-population differences in the aging process of the acetabulum found here and in other publications [31, 35, 36, 38]. A further analysis of the biology, behavior and applicability of this age marker in other rarely studied populations, would be highly recommended.

Although mean chronological age of the female white Test USA sample is older than corresponding males (65.82 vs 59.74 years, respectively), the revised method tends to underestimate age-at-death in males and overestimate it in females. The bias values give important information regarding the direction of errors in estimation, helping to understand the methodology limitations and the aging process itself. As other authors have demonstrated in different age markers [e.g. 54, 56, 57], bias in age estimation through life span tends to show positive values (overestimation trend in younger individuals) to negative values (underestimation trend in older specimens). The age-point at where individuals change from positive to negative values is younger in males, and is possibly linked to their faster aging rate [36, 42, 50].

Compared with the Lisbon bias analysis [42], there are no significant differences between the global bias values, as shown in Table 5. The positive to negative value transition is comparable in Lisbon males, but not in Lisbon females, where the pattern was more irregular with no discernable trend (Fig. 1). These results should be regarded with caution, due to the low simple size of some age groups and the lack of individuals in others. Consequently, the low sample size, specifically of the younger and older age groups available in the documented collections, may be biasing and determining these previous patterns. Further, if some pattern would be consistent along enough different studies, different methodologies could be revised to add some value to correct or balance the bias committed during the estimation in different age intervals.

The percentages whose absolute error is less than 5 or 10 years are similar in Lisbon [42] and in white North American male samples (44 % vs. 45.5 % and 73.4 % vs. 75.3 %, respectively). Due to the lower reliability in the white North American female sample, previous percentages are significantly higher in the Lisbon collection than in the white North American female sample (48.3 % vs. 31.5 % and 75 % vs. 59.7 %, respectively). Following these absolute error results, while estimation in males is statistically similar in the white North American sample and in the Lisbon collection, accuracy is significantly higher in Lisbon compared to the white USA sample in females, both in the general sample and in the younger and older age groups. As far as sexual dimorphism relative to accuracy is concerned, neither bias or absolute error was significantly different between sexes in the white or black North American samples, with the exception of absolute error between USA white males and females. Because the low accuracy in females, likely due to high morphological variability [43], the application of the SanMillán-Rissech's method was more precise for males in both the black and white samples; but, it is only significant in the white North American sample. It is worth remembering caution about black sample results due to its low sample size, especially in females. These results are in accordance with different authors [26, 39, 54, 55, 57-60] who have already shown this imbalance in accuracy that favors males when applying some of the age-at-death methodologies based on pubic symphysis, auricular surface and acetabulum.

The reported lower precision in the revised method applied on females, compared with the original results from the Lisbon collection, may be related to differences in genetics, nutrition, physical activity, lifestyle, drug use, chronic disease, and/or the aging pattern itself. Skeletal age indicators for adults are only imperfectly correlated with chronological age [61], since more than a half [62] or more than a 70 % [63] of the variability in age estimation is associated with factors other than age. Joint mobility generally accelerates degenerative changes [64], so the ageing process might be accelerated in females of high parity [65] or from stress injuries in physically active groups, given that the frequencies of osteoarthritis seem to be generally higher in USA females comparable to males [41]. However, some authors have published that acetabular variables appeared resistant to mechanical loading and relatively resistant to the effects of occupational and habitual physical activity [41], while others have concluded the opposite [36]. This suggests that the interactions between activity, osteoarthritis and age are more complicated than previously thought. Further, studies have shown that obesity did not have a significant effect on acetabular changes [41], on the pubic symphysis, or on auricular surface age indicators [66]. This indicates the relevance of the acetabulum for age estimation, even in today's increasingly obese populations.

Inter-individual differences in obesity, bone density, mechanical loading, and hormonal levels affect skeletal degeneration in ways that are poorly understood. Recently, authors are focusing on researching the influence of osteoarthritis [40, 41] or bone loss [45] on age-at-death estimations, highlighting the necessity of analyses involving large documented samples to improve our control over lifestyle co-variates [67]. Further research in this line is strongly needed to better understand the relationships among acetabular changes and osteoarthritis, age, activity, and obesity and will contribute to forensic anthropology, bioarchaeological research and the practice of medicine. Besides, the impact of an inherent tendency toward bone formation, energy balance, vitamin D status, biomechanical and reproductive factors and genetic influences on bony age markers could potentially affect age timing at pelvic joints and are worth investigating empirically [61].

Scientific institutions should recognize the critical importance of having documented research collections that researchers could increase the current knowledge of the aging process and consequently enhance the reliability and accuracy of age estimation methodologies. As an example, since 1999, the Forensic Anthropology Center curating the Bass Collection, has added extensive information on the donors contained within the collection, including health, occupation, socio-economic status, birth information, and habitual activities. This additional information provides new opportunities to determine how modern populations are changing and to study specific disease processes that focus on the different bone effects caused by obesity, alcoholism, diabetes, and trauma patterns. Essentialy, the better the documented collections, the better the understanding of the factors affecting the relationship between age indicators and age, ultimately facilitating better controlled studies.

Even with worse precision results for females, general reliability values for the acetabulum are reasonable enough for both archaeological and forensic contexts compared to other pelvic age marker research findings from documented collections in the United States [39, 54, 68], England [26, 52], Italy [55, 60], Spain [56, 57], Greece [69] and Thailand [58]. It is remarkable that even applied to a population other than the one used for renewing the original methodology and taking into account the existence of inter-population differences, the estimation based on acetabular morphology provides higher accuracy values, compared to other pelvic age markers, frequently applied in anthropological routines [70].

The lack of precise age estimates in older individuals and the often poor preservation of the pubic symphysis in adverse depositional environments undermine the utility of the pubic symphysis as an age indicator. Similarly, the auricular surface methods classify individuals into broad phases that result in imprecise age estimates [71]. In contrast, the acetabulum has the potential to improve adult age estimation due to: 1) higher accuracy and narrower age ranges leading to precise age estimates, even for elderly individuals [30, 41, 42]; 2) more resistance to postmortem damage [32], since most acetabular changes are resistant to the effects of previous injury and surgical intervention, with the exception of the variables of the acetabular fossa [41]; and 3) evidence that the changes observed in this joint are metamorphic rather than merely degenerative, and are therefore, believed to be more relevant to age estimation. The recent results and those reported here should be a good argument to increase the use of the acetabulum to the daily routines in forensic and archaeological laboratories that estimate the age of an individual.

Regarding the validity of SanMillán-Rissech's method tested on a sample of varied ancestries, no significant differences were found in bias or in absolute difference values. This extends its applicability to samples with different geographical origins where, results do not reveal significant differences, even when taking into account inter-population differences. These results agree with Martrille et al.'s [54] results, as they did not find significant differences in the application of the four age estimation methods on blacks and white North American individuals from the Terry collection [20, 23, 72-74]. In addition, Mulhern and Jones [68] found, in general, the revised auricular surface method [26] was equally applicable to blacks and whites. However, Schmitt [58] applied age estimations based on pubic symphysis [20] and auricular surface [23] to an Asian population and found greater error comparable to the reported results in the literature based on white or black North American samples. Katz and Suchey [75] showed that the Suchey-Brook's method [20] overestimated age in blacks, whereas Işcan et al. [76] tested the fourth rib method on the Terry collection and found that it gave different results for blacks compared with whites. Such scientific controversy would need to be solved through further research, and testing the available methodologies on different documented collections with different geographical and ancestral background.

Lastly, the study reported here, does reveal that the revised method [42] performs significantly better than the original [30], with inaccuracy values significantly lower in both males and females. The advantages of the revised methodology are not only a more accurate age-at-death estimation, but also the newly-defined variables are easier to apply and have higher repeatability than those from the original method. This enhancement and ease in scoring the variables, with more detailed descriptions and pictures, will hopefully be replicated in future studies and different populations. Further, the acetabular methodology provides the user the possibility of choosing the Reference sample, allowing a choice of standards which, as far as possible, resemble the material under study in terms of the environmental and genetic factors that may affect the indicator-age association. Thus, the methodology lends the method flexibility and greater applicability to diverse populations.

Conclusions

The SanMillán-Rissech's revised acetabular method of age estimation [42], was applied to a large sample of 880 individuals from the Bass Collection with known age, sex, and ancestry.

The original acetabular method of age estimation developed by Rissech et al. [30] was also tested on the same sample population, to compare the applicability and accuracy of the two methodologies. Present results indicate the following:

1. Despite lower precision in age estimation of North American females, the revised method is reasonably applicable to males and females who identify as black and white, in terms of accuracy. The method provided better age estimates than other pelvic age markers currently used in anthropological laboratories.

2. The SanMillán-Rissech's method provides a useful tool for age-at-death estimation for different populations, including Western Europeans and North Americans. Therefore, with the method's proven wider applicability, it should be included as a powerful age reference indicator.

3. The revised acetabular method [42] is significantly more accurate than the original method [30] in the analyzed sample. These results demonstrate that the revised system has actually enhanced the age estimation methodology, in terms of understanding the acetabular aging process , increasing its applicability as an age estimation marker, and increased the overall accuracy of the original method.

Multidisciplinary research, prompted by a need to understand the aging process, will further enhance the current knowledge of the biology behind the physiological aging processes. Acetabular changes are valid age indicators and appropriate method refinements will serve to improve precision and accuracy sufficiently to enable the use of acetabular aging methods in forensic anthropological and bioarchaeological research.

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Figure 1

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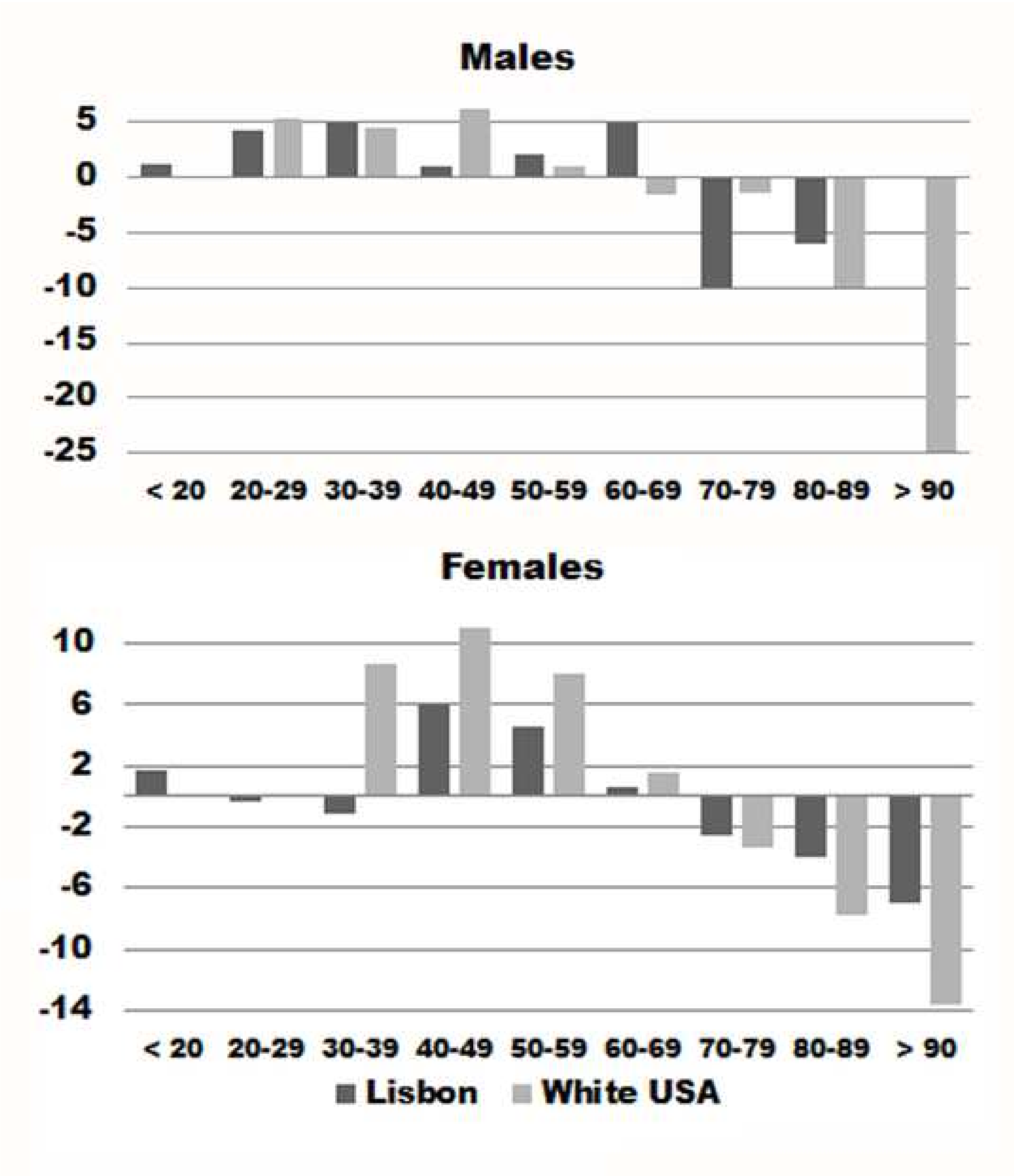

Figure 1 caption

Fig 1 Difference between chronological and estimated age-at-death (bias) for each 10-year interval in males (top) and females (bottom) obtained upon application of the SanMillán-Rissech´s method both in Lisbon (dark grey) and white North American (light grey) samples

Table 1. Distribution of analyzed individuals by sex, age group, and ancestry.

Age group	American whites		American blacks		Total
	Male	Female	Male	Female	
< 20 years	1	0	0	0	1
20-29 years	9	2	3	1	15
30-39 years	40	14	1	1	56
40-49 years	74	38	16	0	128
50-59 years	84	75	9	2	170
60-69 years	101	98	7	1	207
70-79 years	89	78	8	2	177
80-89 years	51	48	1	0	100
≥ 90 years	7	17	1	1	26
Total	456	370	46	8	880
	American whites		**American blacks**		**Total**
	Male	**Female**	**Male**	**Female**	
Reference	300	220	-	-	520
Test	156	150	46	8	360
Total	826		54		880

Table 2. Values depicting the accuracy of the revised acetabular method obtained in the white North American test sample, by using the white North American Reference sample as reference. Number of individuals in each 10-year age group and their percentage of the total number of males and females, with absolute error (|e|) less than specific amounts (< 5 years and < 10 years), tabulated within age classes, and over all specimens, mean bias and mean absolute error. Number of individuals underestimated (-), overestimated (+) and perfectly estimated (0). 'Perfectly estimated' means that the estimated age coincides exactly with the chronological age of the individual.

Sample	Age group	|e| < 5				|e| < 10				n	Mean bias	Mean |e|	Individuals not estimated
			-	+	0		-	+	0				
Males	< 20 years	-	-	-	-	-	-	-	-	-	-	-	-
	20-29 years	2 (50 %)	0 (0 %)	2 (100 %)	0 (0 %)	3 (75 %)	3 (100 %)	0 (0 %)	0 (0 %)	4	5.38	5.38	0
	30-39 years	8 (61.5 %)	2 (25 %)	6 (75 %)	0 (0 %)	12 (92.3 %)	2 (16.7 %)	10 (83.3 %)	0 (0 %)	13	4.57	5.55	2
	40-49 years	11 (40.7 %)	7 (63.6 %)	4 (36.4 %)	0 (0 %)	19 (70.4 %)	4 (26.7 %)	11 (73.3 %)	0 (0 %)	27	6.79	7.75	0
	50-59 years	13 (44.8 %)	8 (61.5 %)	5 (38.5 %)	0 (0 %)	22 (75.9 %)	10 (45.5 %)	12 (54.5 %)	0 (0 %)	29	0.94	7.43	0
	60-69 years	14 (43.8 %)	7 (50 %)	7 (50 %)	0 (0 %)	23 (71.9 %)	13 (56.5 %)	10 (43.5 %)	0 (0 %)	32	-1.69	6.81	0
	70-79 years	19 (63.3 %)	8 (42.1 %)	11 (57.9 %)	0 (0 %)	28 (93.3 %)	15 (53.6 %)	13 (46.4 %)	0 (0 %)	30	-1.45	4.66	0
	80-89 years	3 (17.6 %)	1 (33.3 %)	2 (66.7 %)	0 (0 %)	9 (52.9 %)	7 (77.8 %)	2 (22.2 %)	0 (0 %)	17	-9.87	10.55	0
	> 90 years	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	2	-26.05	26.05	0
	Total	70 (45.5 %)	33 (47.1 %)	37 (52.9 %)	0 (0 %)	116 (75.3 %)	54 (46.6 %)	62 (53.4 %)	0 (0 %)	154	-0.17	7.19	2
Females	< 20 years	-	-	-	-	-	-	-	-	-	-	-	-
	20-29 years	-	-	-	-	-	-	-	-	-	-	-	-
	30-39 years	-	-	-	-	3 (75 %)	3 (100 %)	0 (0 %)	0 (0 %)	4	8.63	8.63	0
	40-49 years	1 (7.1 %)	0 (0 %)	1 (100 %)	0 (0 %)	5 (35.7 %)	2 (40 %)	3 (60 %)	0 (0 %)	14	11.02	14.98	0
	50-59 years	12 (36.4 %)	4 (33.3 %)	8 (66.7 %)	0 (0 %)	20 (60.6 %)	4 (20 %)	16 (80 %)	0 (0 %)	33	8.09	9.28	0
	60-69 years	10 (27 %)	8 (80 %)	2 (20 %)	0 (0 %)	27 (73 %)	17 (63 %)	10 (37 %)	0 (0 %)	37	1.55	8.38	1
	70-79 years	14 (42.4 %)	3 (21.4 %)	11 (78.6 %)	0 (0 %)	19 (57.6 %)	6 (31.6 %)	13 (68.4 %)	0 (0 %)	33	-3.30	8.66	0
	80-89 years	8 (38.1 %)	5 (62.5 %)	2 (25 %)	1 (12.5 %)	13 (61.9 %)	10 (76.9 %)	2 (15.4 %)	1 (7.7 %)	21	-7.85	9.18	0
	> 90 years	2 (28.6 %)	0 (0 %)	2 (100 %)	0 (0 %)	2 (28.6 %)	0 (0 %)	2 (100 %)	0 (0 %)	7	-13.67	14.04	0
	Total	47 (31.5 %)	20 (42.6 %)	26 (55.3 %)	1 (2.1 %)	89 (59.7 %)	39 (43.8 %)	49 (55.1 %)	1 (1.1 %)	149	0.96	9.65	1

Table 3. Sex differences in accuracy, specifically in bias and absolute error values obtained in the black and white North American test samples, by using white North American Reference sample as reference. Mann-Whitney U test were performed in the global sample and in each age group separately. Significant differences are marked in bold.

Bias								
	American white males			American white females			U Mann-Whitney	p
	n	Mean	SD	n	Mean	SD		
15-39 years	17	4.76	4.87	4	8.63	2.75	15	0.089
40-64 years	74	2.62	9.35	64	6.97	10.76	1852	**0.028**
> 65 years	63	-4.77	8.23	81	-4.16	11.16	2456	0.701
Global	154	-0.17	9.32	149	0.96	12.18	10835.5	0.403
	American black males			American black females			U Mann-Whitney	p
	n	Mean	SD	n	Mean	SD		
15-39 years	4	5.53	8.63	1	9.90	-	-	-
40-64 years	30	3.41	13.15	2	-4.30	11.60	21	0.483
> 65 years	12	-5.08	7.43	4	-8.63	13.56	21	0.716
Global	46	1.38	12.06	7	-4.74	12.66	131	0.431
Absolute error								
	American white males			American white females			U Mann-Whitney	p
	n	Mean	SD	n	Mean	SD		
15-39 years	17	5.51	3.94	4	8.63	2.75	15	0.089
40-64 years	74	7.36	6.28	64	10.35	7.51	1776	**0.011**
> 65 years	63	7.45	5.87	81	9.14	7.58	2290.5	0.293
Global	154	7.19	5.89	149	9.65	7.46	9265	**0.004**
	American black males			American black females			U Mann-Whitney	p
	n	Mean	SD	n	Mean	SD		
15-39 years	4	5.88	8.32	1	9.90	-	-	-
40-64 years	30	10.75	8.10	2	8.20	6.08	25	0.697
> 65 years	12	6.29	6.33	4	12.03	9.51	13	0.182
Global	46	9.16	7.84	7	10.63	7.40	136	0.511

Table 4. Differences in bias and absolute error between white and black American individuals, estimated both by using the extensive white North American Reference sample, for males and females separately. Mann-Whitney U test was performed in the whole sample and in each age group independently. Significant differences are marked in bold.

Bias								
	American white males			American black males			U Mann-Whitney	p
	n	Mean	sd	n	mean	sd		
15-39 years	17	4.76	4.87	4	5.53	8.63	30	0.720
40-64 years	74	2.62	9.35	30	3.41	13.15	1092.5	0.900
> 65 years	63	-4.77	8.23	12	-5.08	7.43	374.5	0.960
Global	154	-0.17	9.32	46	1.38	12.06	3468.5	0.831
	American white females			American black females			U Mann-Whitney	p
	n	Mean	SD	n	Mean	SD		
15-39 years	4	8.63	2.75	1	9.90	-	-	-
40-64 years	64	6.97	10.76	2	-4.30	11.60	-	-
> 65 years	81	-4.16	11.16	4	-8.63	13.56	-	-
Global	149	0.96	12.18	7	-4.74	12.66	396	0.283
Absolute error								
	American white males			American black males			U Mann-Whitney	P
	n	Mean	SD	n	Mean	SD		
15-39 years	17	5.51	3.94	4	5.88	8.32	25	0.420
40-64 years	74	7.36	6.28	30	10.75	8.10	839.5	0.052
> 65 years	63	7.45	5.87	12	6.29	6.33	294	0.225
Global	154	7.19	5.89	46	9.16	7.84	3236	0.374
	American white females			American black females			U Mann-Whitney	P
	n	Mean	SD	n	Mean	SD		
15-39 years	4	8.63	2.75	1	9.90	-	-	-
40-64 years	64	10.35	7.51	2	8.20	6.08	-	-
> 65 years	81	9.14	7.58	4	12.03	9.51	-	-
Global	149	9.65	7.46	7	10.63	7.40	465	0.629

Table 5. Differences in bias and absolute error between white North American individuals and the Lisbon Collection, when the revised acetabular method was performed. Age was estimated using their respective Reference samples, taking into account sex (see the Material and Methods section). Mann-Whitney U test was performed in the whole sample and in each age group independently. Significant differences are marked in bold.

Bias								
	American white males			Portuguese males			U Mann-Whitney	p
	n	Mean	SD	n	Mean	SD		
15-39 years	17	4.76	4.87	21	3.83	6.19	162	0.628
40-64 years	74	2.62	9.35	47	2.15	8.8	1646	0.621
> 65 years	63	-4.77	8.23	41	-4.61	9.82	1267.5	0.873
Global	154	-0.17	9.32	109	-0.07	9.41	8360	0.957
	American white females			Portuguese females			U Mann-Whitney	p
	n	Mean	SD	n	Mean	SD		
15-39 years	4	8.63	2.75	19	0.01	8.62	5	**0.007**
40-64 years	64	6.97	10.76	30	2.78	11.52	795	0.181
> 65 years	81	-4.16	11.16	67	-2.31	8.48	2442.5	0.296
Global	149	0.96	12.18	116	-0.61	9.55	7876	0.216
Absolute error								
	American white males			Portuguese males			U Mann-Whitney	p
	n	Mean	SD	n	Mean	SD		
15-39 years	17	5.51	3.94	21	5.83	4.11	172.5	0.860
40-64 years	74	7.36	6.28	47	6.72	5.99	1150.5	0.348
> 65 years	63	7.45	5.87	41	8.67	6.42	1615.5	0.511
Global	154	7.19	5.89	109	7.28	5.91	8378	0.980
	American white females			Portuguese females			U Mann-Whitney	p
	n	Mean	SD	n	Mean	SD		
15-39 years	4	8.63	2.75	19	5.27	6.7	13	**0.042**
40-64 years	64	10.35	7.51	30	9.46	6.95	933.5	0.830
> 65 years	81	9.14	7.58	67	6.54	5.82	2179.5	**0.040**
Global	149	9.65	7.46	116	7.09	6.39	6760.5	**0.002**

Table 6. Differences in bias and absolute error obtained between the revised and the original methods, when performed in the white North American sample, for males and females separately. Due to the same individuals being evaluated, the Wilcoxon test for related samples was performed on the whole sample and for every age group independently. Significant differences are marked in bold.

Bias										
	American white males					American white females				
	Revised	Original	n	Wilcoxon	p	Revised	Original	n	Wilcoxon	p
15-39 years	4.76	5.00	17	-0.398	0.691	8.63	10.95	4	-0.730	0.465
40-64 years	2.62	2.12	74	-1.005	0.315	6.76	7.88	63	-1.543	0.123
> 65 years	-4.77	-5.68	63	-1.143	0.253	-4.16	-4.78	81	-0.784	0.433
Global	-0.17	-0.75	154	-1.376	0.169	0.84	1.03	148	-0.553	0.580
Absolute error										
	American white males					American white females				
	Revised	Original	N	Wilcoxon	P	Revised	Original	N	Wilcoxon	P
15-39 years	5.51	5.53	17	-0.170	0.865	8.63	10.95	4	-0.730	0.465
40-64 years	7.36	7.37	74	-0.226	0.821	10.19	10.71	63	-0.838	0.402
> 65 years	7.45	9.74	63	-3.422	**0.001**	9.14	10.38	81	-1.740	0.082
Global	7.19	8.14	154	-2.348	**0.019**	9.58	10.53	148	-1.967	**0.049**